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Basic Science

Osteogenic Differentiation Potential of Human Bone Marrow and Amniotic Fluid-Derived Mesenchymal Stem Cells *in Vitro* & *in Vivo*

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Abstract

BACKGROUND: Cell therapies offer a promising potential in promoting bone regeneration. Stem cell therapy presents attractive care modality in treating degenerative conditions or tissue injuries. The rationale behind this is both the expansion potential of stem cells into a large cell population size and its differentiation abilities into a wide variety of tissue types, when given the proper stimuli. A progenitor stem cell is a promising source of cell therapy in regenerative medicine and bone tissue engineering.

AIM: This study aimed to compare the osteogenic differentiation and regenerative potentials of human mesenchymal stem cells derived from human bone marrow (hBM-MSCs) or amniotic fluid (hAF-MSCs), both *in vitro* and *in vivo* studies.

SUBJECTS AND METHODS: Human MSCs, used in this study, were successfully isolated from two human sources; the bone marrow (BM) and amniotic fluid (AF) collected at the gestational ages of second or third trimesters.

RESULTS: The stem cells derived from amniotic fluid seemed to be the most promising type of progenitor cells for clinical applications. In a pre-clinical experiment, attempting to explore the therapeutic application of MSCs in bone regeneration, Rat lumbar spines defects were surgically created and treated with undifferentiated and osteogenically differentiated MSCs, derived from BM and second trimester AF. Cells were loaded on gel-foam scaffolds, inserted and fixed in the area of the surgical defect. X-Ray radiography follows up, and histopathological analysis was done three-four months post- operation. The transplantation of AF-MSCs or BM-MSCs into induced bony defects showed promising results. The AF-MSCs are offering a better healing effect increasing the likelihood of achieving successful spinal fusion. Some bone changes were observed in rats transplanted with osteoblasts differentiated cells but not in rats transplanted with undifferentiated MSCs. Longer observational periods are required to evaluate a true bone formation. The findings of this study suggested that the different sources; hBM-MSCs or hAF-MSCs exhibited remarkably different signature regarding the cell morphology, proliferation capacity and osteogenic differentiation potential

CONCLUSIONS: AF-MSCs have a better performance *in vivo* bone healing than that of BM-MSCs. Hence, AF derived MSCs is highly recommended as an alternative source to BM-MSCs in bone regeneration and spine fusion surgeries. Moreover, the usage of gel-foam as a scaffold proved as an efficient cell carrier that showed bio-compatibility with cells, bio-degradability and osteoinductivity *in vivo*.

Introduction

Mesenchymal stem cells (MSCs) are multipotent stem cells that can self-renew and differentiate into different cell types including osteoblasts, chondrocytes, tenocytes, adipocytes, and hepatocytes which make them a promising tool in tissue engineering and regenerative medicine applications. MSCs can be derived from different

human sources including; bone marrow tissue, fetal amniotic fluid, adipose tissue cord blood, umbilical cord, placenta and dental pulp [1], [2], [3].

MSC originating from the stroma of the bone marrow (BM) was one of the first known MSC, and are also more advanced in clinical trials. BM-MSCs generally serve as the “gold standard” against which other MSC sources are compared. It is well known that bone marrow stroma contains progenitor cells with osteogenic potential, generally referred to as mesenchymal stem cells or bone marrow stromal cells (BM-MSCs) [4]. Human BM-MSCs have been demonstrated to differentiate toward the osteoblastic lineage *in-vitro* and to form bone tissue upon ectopic implantation [5].

Bone marrow-derived mesenchymal stem cells (BM-MSCs) have shown a great promise in animal studies and even in a few clinical trials for skeletal tissues regeneration [6]. Harvesting BM-MSCs from a patient is an invasive and rather painful procedure. Furthermore, the number, proliferative capacity, and differentiation potential of BM-MSCs decline with age suggesting that tissue-engineering strategies based on these cells might not be feasible in older patients [7].

Fetal Amniotic Fluid Stem cells (AF-MSCs) seems a very promising type of cells and its application is rapidly growing in regenerative research. Almost ten years ago, the first suggestion of human amniotic fluid as a new putative source for stem cells was reported [8]. The first evidence for the existence of AF-MSCs was demonstrated by the discovery of a highly proliferative cell type in human amniotic fluid expressing the pluripotent stem cell marker Oct4 [9]. AF-MSCs have been applied to critically sized femoral bone defects of a nude rat in combination with biomaterial scaffold and shown the bone formation in rat femoral defect [10].

AF-MSCs cells demonstrated high potential in differentiation into hematopoietic [11], neurogenic [9], [12], [13], [14], osteogenic [13], [14], chondrogenic [14], adipogenic [13], [14], renal [15], hepatic [16], and various other lineages [9], [13]. The biological properties and markers expression pattern of AF-MSCs appears to be more similar to that of embryonic stem (ES) cells [17]. They express many but not all of the markers of embryonic stem cells (ESCs) [18]. However, they require no feeder layers for culture, they have not been observed to form teratomas *in-vivo* and are capable of > 300 population doublings in culture [19]. It is also possible to generate monoclonal genomically stable AF-MSC lines, harbouring high proliferative potential without raising ethical issues [20].

Both BM- and AF-derived MSCs offer a very promising and much more abundant potential cell-source for repair of bone defects, particularly the vertebral spines defects. The vertebral spine (or backbone) plays an important role in the stability of the upper body and the protection of the Spinal Cord [21]. Vertebral spines underwent pathological degeneration, or developed cancerous tumours or exposed to accidents are treated by surgical intervention, which employs autologous bone graft transplantation or substitutes for non-union defects and replacement of damaged tissue [22], [23]. However, several inconveniences are likely to occur with surgical interventions. It includes extra surgery to remove grafted bone from the patient's body, increased potential operations’ complications [23]. Additionally, the amount of bone available for grafting might be insufficient. Moreover, the cost is very high [24].

In this study, we have isolated human MSCs (hMSCs) from two sources; bone marrow and amniotic fluid to compare the proliferation and osteogenic differentiation potentials *in-vitro* and evaluate the ability of transplanted human fetal amniotic fluid and adult bone marrow stem cells in the enhancement of functional repair in "rats" with induced spine defect.

Subjects and Methods

The Ethics Committee of the National Research Center, Cairo, Egypt, approved the study protocol and all participants gave informed consent.

Mesenchymal stem cells were isolated from two different human sources; Bone Marrow and Amniotic fluid. Bone marrow (BM) samples were aspirated from the sternum of healthy human subjects, following their consent, at Cairo-Nasser Institute. BM aspiration was done as a clinical care procedure in the process of doing the cross-matching procedure for donor BM transplantation. The subjects age range was between 6-33 years old (n = 6). The third-trimester AF samples were collected during two deliveries of the elective cesarean section. Eight women’ AF samples were collected, whereas seven samples only (n = 7) were successfully isolated. The maternal age range was from 21 to 38 years. Second trimester AF samples (collected between the 14th and 18th weeks of gestation) were obtained by amniocentesis from five women, three (n = 3) were successfully isolated. The age range was between 21 to 38 years old.

Bone marrow cells were isolated by ficoll gradient separation of mononuclear cells [25]. Second and third-trimester amniotic fluid cells were isolated by centrifugation and pelleting of cells [19]. We didn’t study the effect of the parameters; sex and age on the MSCs isolation protocol, since isolation of AF only from pregnant female but BM isolation could be from both sex.

Isolation of human Bone Marrow-derived Mesenchymal Stem Cells was done by RosetteSep® Human Mesenchymal Stem Cell Enrichment Cocktail (RosetteSep, Stem Cell Technologies, Canada) was applied to the bone marrow to get rid of the unwanted cells by negative selection. Bone marrow mononuclear cells were isolated from the bone marrow sample by Ficoll-Paque™ PLUS density gradient solution (Stem Cell Technologies, GE Healthcare, Canada). Mononuclear cells were plated at a density of 10,000 cells/cm^2 in 60 mm plastic tissue culture dishes in complete culture media containing Alpha-MEM (Lonza, Belgium), 10% FBS (Lonza, Belgium), 1% glutamax (Gibco, Invitrogen, Life Technologies, USA), 1% 10,000 U/ml penicillin and 10,000 U/ml streptomycin (Pen-Strep, Lonza, Belgium), (3 ng/ml) fibroblast growth factor basic (Sigma-Aldrich, USA) and incubated in a humid 5% CO2 atmosphere at 37ºC in a CO2 incubator. Mesenchymal stem cells adhered to the culture plates, meanwhile the other non-adherent cells were discarded upon the first replaced media. Media was replaced twice a week.

Isolation of amniotic fluid-derived Mesenchymal Stem Cells was done by centrifugation and pelleting of cells [19]. Cells were cultured in α-MEM media (Lonza) containing 20% FBS (Lonza), glutamax (Invitrogen), penicillin-streptomycin (Lonza) and bFGF (Sigma). Media was exchanged two times /week and passage was done when the cells reached the confluence of about 75%.

Culture Expansions were maintained to reach about 80% confluence, and then cells were passage and reseed. Manual scraping technique using cell scraper (Corning incorporated, Costar, Mexico) and collection of the cells, followed by centrifugation and re-suspension in the complete medium then re-seeding of cells in culture plates.

Differentiation of MSCs into bone-forming cells was initiated at third passage. Cells at 70% confluence were used for osteogenic differentiation, cultured in DMEM containing 20% FBS, 100 µg/ml penicillin & streptomycin and 1% glutamax, L-ascorbic acid 2-phosphate, β-glycerol phosphate and dexamethasone [26]. A comparable control culture maintained in proliferation media was generated for each sample. Two similar simultaneous sets were created at the same time; first set: plates in differentiation media, as well as plates in proliferation media, were kept in the corresponding culture media for 14 days before being passed for characterisation protocols. The second set: plates in differentiation media and plates in proliferation media were kept in its culture media for 29 days before characterisation.

Characterisation of osteogenic differentiation was done using Alizarin Red staining. Alizarin red S (Sigma) staining for the detection of mineralized nodules in the differentiated cultures was done according to the manufacturer's instructions.

Cultures maintained in osteogenic and control media for 14 days were used for early alizarin staining, whereas the second similar set remained in its corresponding media for 29 days were used for late alizarin staining.

Alizarin Red staining, Monolayers in the plates were washed three times with PBS and fixed in 70% ethanol at room temperature for one hour. Monolayers were then washed three times with dH_2O before the addition of 1.3 g% Alizarin Red S (Sigma) (pH 4.2). The plates were incubated at 37ºC for one hour with gentle shaking. After aspiration of the unincorporated dye, the plates were washed with dH_2O till colour disappears. The plates were then washed with PBS for 5 min. Distal H_2O was added to the plate to prevent cell dryness, and the plates became ready for visual inspection using an inverted microscope where calcium deposits were stained orange-red.

The *in vivo* study was performed by transplantation of human stem cells (MSC at passage 3 and differentiated osteogenic cells after 29 days of osteogenic induction) into rat spines. Six male Sprague Dawley rats weighed 200-300 gm were divided into two groups; each group consists of three rats.

All animals experiments were conducted after animal ethics approvals with approval numbers 16/263, and all animals were treated humanely.

BM Group: this group is being transplanted with BM-derived stem cells. Transplantation of BM-MSCs was done in duplicate: Two rats labeled as Osteogenic 1 and Osteogenic 2 had received MSCs differentiated into osteogenic progenitors (experimental group). Cells used for implantation underwent osteogenic induction for 29 days. A third rat (control one), had received matched but undifferentiated BM-derived MSCs.

AF Group: this group is transplanted with second-trimester AF-derived stem cells. Two rats; Osteogenic 1 and Osteogenic 2 had received MSCs differentiated into osteogenic progenitor cells. Transplanted cells were derived from second-trimester pregnancy and completed 30 days in osteogenic induction media. The third rat (control one), had received AF-undifferentiated MSCs.

An absorbable gelatin sponge, gel foam (Cutanplast®, Italy), was used as a scaffold to load the cells on.

The rats were anesthetized by being injected intraperitoneally with 200 µl (10 mg) freshly prepared Thiopental Sodium and 200 µl (0.093 mg) Xyla-Ject® (23.3 mg/ml Xylazine Hydrochloride).

Induction of rat spine bone defects in both AF-MSCs and BM-MSCs groups: Spines "decortication" defects have been created in the transverse processes of spines at the lumbar region, consists of

5 vertebrae that have a larger bed to work on.

Scaffold Implantation loaded with approximately 2 million cells, Cell-loaded gel foams, were applied and fitted in the decorticated areas of the lumbar spines in the backbone of the rats.

The X-ray photographing of the rat's backbone was done for both groups at different time intervals to follow up the healing process in the osteogenic versus control rats.

In the BM transplanted group: X-ray filming was done after 4, 9, 13 and 17 weeks from the first day of cells transplantation. For the AF transplanted group: X-ray filming was done after 4, 8 and 12 weeks (as this AF group had started later than the BM group).

Histological analysis was performed for qualitative evaluation of new bone formation in the defected lumber spine. Three-four months post-implantation rats were sacrificed according to the recommendation of the ethical committee of the National Research Centre for Animal Experiment. The specimens of rat defected lumber spines were cut in blocks 0.5-1 cm apart from the defect, decalcified in 4% formic acid at room temperature and routinely prepared to be embedded in paraffin blocks. Lateromedial 5 µm thick sections were obtained and stained with hematoxylin and eosin to be examined by a light microscope [26].

Results

Proliferative potential and morphological criteria of BM-MSCs and AF-MSCs, human Mesenchymal Stem Cells (hMSCs) were compared. The primary BM-MSCs and AF-MSCs cultured cells took a similar period (10-14 days) to reach confluence and showed comparable plating efficiency (Figure 1A and 1E), respectively. The BM-MSCs showed a higher proliferation rate than the second-trimester AF-MSCs at the first Passage (Figure 1B) and (Figure 1F) respectively. After subsequent passages (the 2nd and 3rd passages), both BM-MSCs and AF-MSCs showed spindle-shaped fibroblast morphology (Figure 1C and 1G) (Figure 1D and 1H), respectively. The proliferation capacity of BM samples differed from one sample to another, but generally, the younger age derived sample showed a higher proliferation rate and more typical spindle cell morphology than the older age sample (data not shown).

Proliferative potential and morphological criteria of 2nd and 3rd trimester AF: The cells can form colonies (clonogenic ability) at the primary culture that appeared mostly in the 2nd trimester AF cultures (Figure 1E). However, the adherent cells of 3rd-trimester cultures appeared in a scattered manner with the detection of a few aggregates of no more than ten cells (Figure 1I).

The primary AF cell cultures of 2nd and 3rd trimesters had a heterogeneous morphology consisting of fibroblastoid-like morphology and small rounded resembling epithelial cells (Figure 1E) and (Figure 1I), respectively. During the subsequent passages, the cultures exhibited only typical fibroblastic cells. The epithelioid ones had no longer observed in cases of 2nd trimester (Figure1F). While in some of 3rd trimester AF cultures both morphologies, the fibroblastic and rounded epithelial, were shown (Figure 1J). Cultures at P3 in all AF samples had a homogenous typical fibroblast-like MSCs morphology (Figure 1H and Figure 1 L).

The cells acquire senesce-like morphology (Figure 1L). The culture period in osteogenic media was 17 days from the third passage to osteogenic induction. The cell morphology showed elongated flattened cells with widened inter-cellular spaces (Figure 1L).

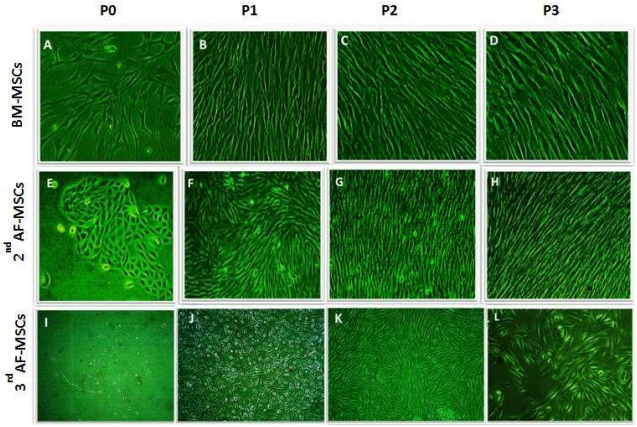

Figure 1: Proliferation and culture expansion of BM-MSCs, 2nd trimester AF- MSCs & 3rd trimester AF-MSCs; A-D) represented Bone marrow (BM) Proliferation & culture expansion at different passage; A) P0, B) P1, C) P2, D) P3 by x10 magnification; E-H) showed 2nd trimester AF-MSCs Proliferation & culture expansion at different passage; E) P0; F) P1; G) P2; H) P3 by x 10 magnification; I-L) represented 3rd trimester AF-MSCs Proliferation & culture expansion at different passage; I) P0; J) P1; K) P2; L) P3 by x 10 magnification

The BM-MSCs, 2nd and 3rd-trimester AF-MSCs after the first 14 days in osteogenic media are shown in Figure 2. There were three sets of plates, one set, in which cells continue in proliferation growth media (control), two sets in which, cells cultured in osteogenic media extended for two-time intervals, 14 and 29 days.

The BM-MSCs after 14 days in osteogenic medium were stained by Alizarin Red stain. The control plate showed a negative Alizarin stain (Figure 2A), while the osteogenic plate showed weak to moderate staining intensity (Figure 2B).

The 2nd-trimester AF-MSCs plates after 14 days in osteogenic medium and staining by Alizarin

Red stain showed no calcium stained spots across the control plate (Figure 2C). The osteogenic plate showed multiple orange-red spots scattered all over the plate, including the periphery (Figure 2D).

The 3rd-trimester AF-MSCs on day 14th of osteogenic induction: the control plate showed multiple stained patches of variable sizes (Figure 2E) and the osteogenic plate showed multiple stained spots and patches that were of more enhanced when compared to the control plate (Figure 2F).

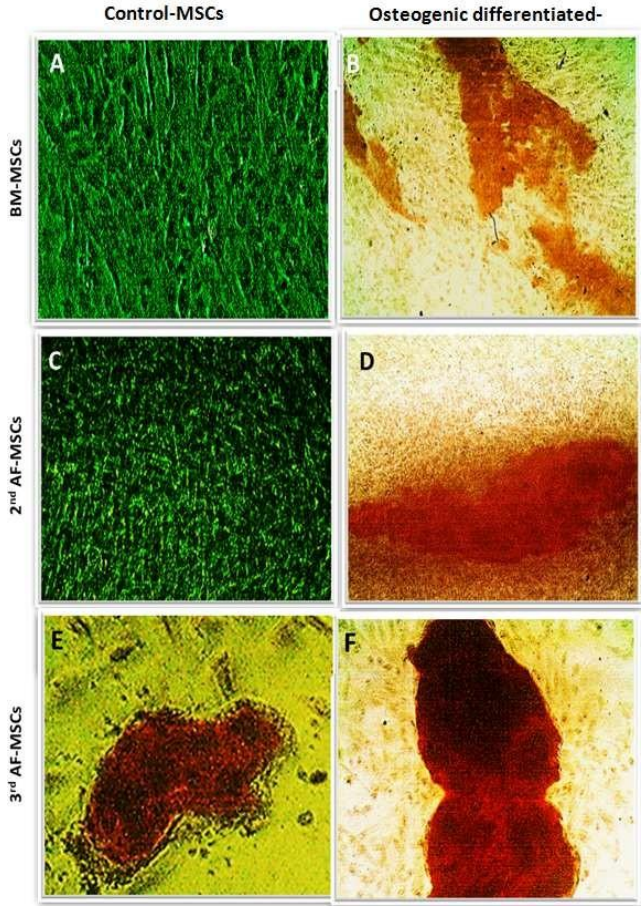

Figure 2: Osteogenic differentiation of BM-MSCs, 2nd trimester AF-MSCs and 3rd trimester AF-MSCs after 14 days; Osteogenic differentiation of BM cells on day 14; A) Control; B) Osteogenic differentiation stained by Alizarin; Osteogenic differentiation of 2nd trimester AF cells; C) Control; D) Osteogenic differentiation stained by Alizarin after 14 days; Osteogenic differentiation of 3rd trimester AF cells; E) Control; F) Osteogenic differentiation stained by Alizarin after 14 days

Cultured plates after the 28 days: BM-MSCs, 2nd & 3rd-trimester AF-MSCs in osteogenic media are shown in Figure 3. BM-MSCs cultures in the osteogenic medium stained with by Alizarin Red stain: The control plate, showed negative Alizarin stain (Figure 3A), while the osteogenic plate showed strong red staining intensity spots all over the plate (Figure 3B).

The 2nd-trimester AF-MSCs in osteogenic medium stained by Alizarin Red stain: The Control plate showed no stained spots across the plate (Figure 3C), while the osteogenic plate showed multiple orange-red stained spots (Figure 3D).

The 3rd-trimester AF-MSCs in osteogenic medium stained by Alizarin Red stain: Control plate showed red-orange stained spots in few areas of the plate (Figure 3E), while the osteogenic plate showed strong red stained spots in multiple different parts across the plate (Figure 3F).

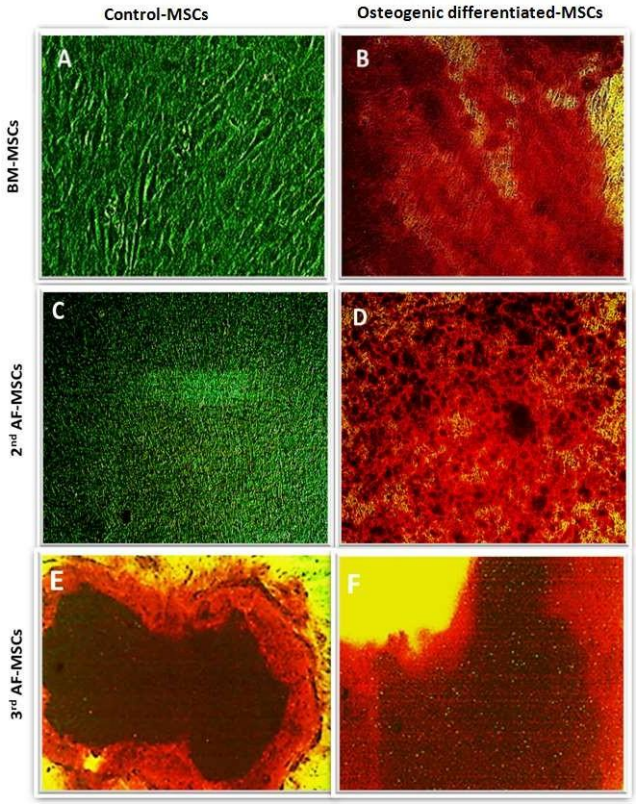

Figure 3: Osteogenic differentiation of BM-MSCs, 2nd AF-MSCs & 3rd AF-MSCs at 28 days; Osteogenic differentiation of BM cells on day 28th; A) Control; B) Osteogenic differentiation stained by Alizarin; Osteogenic differentiation of 2nd trimester AF cells; C) Control; D) Osteogenic differentiation stained by Alizarin; Osteogenic differentiation of 3rd AF cells; E) Control; F) osteogenic differentiation stained by Alizarin

In this study, we suggested that MSCs cultured in osteogenic differentiation medium may induce new bone formation in experimental spinal fusion rats. In pre- clinical experiments testing the therapeutic application of MSCs in bone regeneration, rat lumbar spines defects were surgically created and treated with undifferentiated and osteogenic differentiated MSCs, derived from BM and 2nd trimester AF. Cells were loaded on gel-foam scaffolds. X-ray radiography follows up, and histopathological analysis showed that AF-MSCs offer better healing effect and have a higher potential of achieving successful spinal fusion.

The BM–rats group, after four months, the control rat, transplanted with undifferentiated MSCs showed no change at the lumbar spine, while one of the two osteogenic rats, who transplanted with

osteogenic differentiated MSCs, showed bone mottling, and a shadow area at the lumbar spine (Figure 4).

The AF–rats group, after three months, the control rat (undifferentiated MSCs) showed no bone changes, whereas the two osteogenic rats (transplanted with osteoblastic 2nd trimester AF-derived MSCs showed bone mottling (Figure 4).

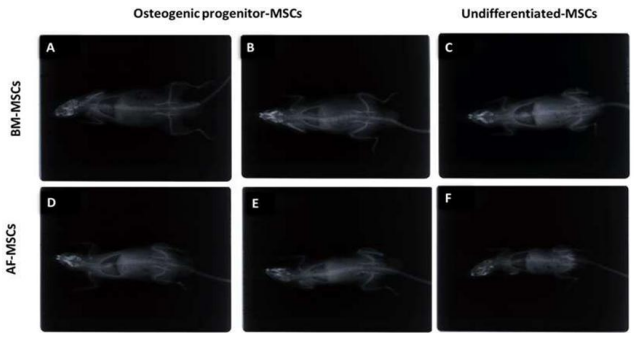

Figure 4: X-ray for AF-MSCs and BM-MSCs transplantation in rats. AF- and BM-derived undifferentiated MSCs, and differentiated osteogenic cells transplanted on decorticated lumber rat-spine beds. Among the BM group, after 4 months, the control rat, transplanted with undifferentiated MSCs showed no change at the lumbar spine. While, one of the two osteogenic rats transplanted with osteogenic differentiated MSCs, showed bone mottling, a shadow area at the lumbar spine. For the AF– group, after 3 months, the control rat (undifferentiated MSCs) showed no bone changes. However, the two osteogenic rats (transplanted with osteoblastic 2nd trimester AF- derived MSCs) showed bone mottling

Histopathological examination of spinal-decorticated rat lumber specimens that were transplanted with different grafts has been performed. Grafts used in this experiment were the osteogenic differentiated BM-MSCs versus undifferentiated BM-MSCs as well as undifferentiated AF-MSCs versus osteogenic differentiated AF-MSCs (Figure 5).

At four months post-transplantation, histopathological examination of sections of the rat transplanted with BM-MSCs differentiated into osteogenic progenitors showed the peripheral cartilage area that was followed by calcified cartilage and area of newly formed bone in contact with the host bone (Figure 5A). While the control rat received undifferentiated BM-MSCs exhibited irregular calcified areas that associated with few osteocytes and blood vessels (Figure 5B).

At three months post-transplantation, microscopic examination of the defect section of the rat transplanted with matched AF-MSCs differentiated into osteogenic progenitor cells, showed calcified cartilage, newly formed bone, host bone and bone marrow (Figure 5C), while the control rat received matched undifferentiated AF-MSCs displayed calcified areas with few osteogenic foci at the top of the host bone (Figure 5D).

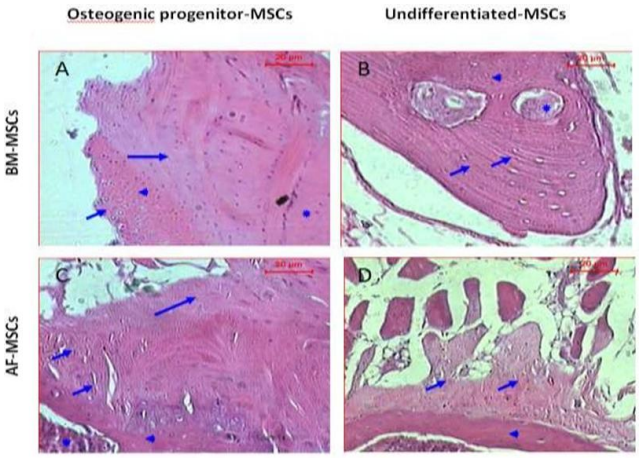

Figure 5: Histopathological examination of sections of experimental and control rats, 4 months after transplantation with (BM-MSCs) & 3 months after transplantation with (AF-MSCs) stained with hematoxylin and eosin dye. (A & B) Are sections of rat spinal defect transplanted with BM-MSCs, A) section of rat spinal defect transplanted with BM- MSCs differentiated into osteogenic progenitors shows cartilage area (short arrow), calcified cartilage (arrowhead) and formed new bone (long arrow) and host bone (asterisk). B) Section of rat spinal defect transplanted with undifferentiated BM-MSCs shows irregular calcified areas (short arrows) that associated with few osteocytes (arrowhead) and blood vessels (asterisk). (C & D) Represented sections of rat spinal injury transplanted with AF-MSCs, C) Section of rat spinal defect transplanted with AF-MSCs differentiated into osteogenic progenitors shows calcified cartilage (short arrow), newly formed bone (long arrow), host bone (arrowhead) and bone marrow (asterisk). D) Section of rat spinal defect transplanted with undifferentiated AF-MSCs shows calcified area with few osteogenic (short arrow) on the top of the host bone (arrowhead) (H&E, Scale bar: 20 µm)

Discussion

MSCs have a powerful ability to differentiate into a range of multi-cell types, including osteoblasts, adipocytes, chondrocytes, myoblasts, and neurons [27], [28]. The findings of Wang et al., (2018) reveal that the tumour antigen 15-leucine-rich repeat containing membrane protein (LRRC15) is an essential regulator for osteogenesis of MSCs through modulating p65 cytoplasmic/nuclear translocation [29].

The classic method of osteogenic differentiation of MSCs *in vitro* involves incubating a confluent monolayer of MSCs with ascorbic acid, beta-glycerophosphate, and dexamethasone for 2-3 weeks. The MSCs form aggregates or nodules and increase their expression of alkaline phosphatase, and calcium accumulation can be seen over time. These bone nodules stain positively by alizarin red [30]. In our experiments, the standard osteogenic differentiation media and protocol worked very effectively and osteogenic differentiation indicated by alizarin nodules was obviously detected in cell cultures applying osteogenic differentiation media, whereas, no mineralized nodules were seen in comparable cultures with normal proliferation media

except in plates of 3rd trimester AF, which might indicate the presence of shaded differentiated cells at that advanced gestational age.

BM-MSCs generally serve as the „gold standard' against which other MSCs sources are compared. Previous studies demonstrated that populations of bone marrow-derived MSCs from human, canine, rabbit, rat, and mouse can develop into terminally differentiated mesenchymal phenotypes both *in vitro* and *in vivo*, including bone and cartilage [31], [32].

Amniotic fluid (AF) of second-trimester pregnancy has been used, many years ago, for prenatal diagnosis of genetic and chromosomal disorders. Research reports have shown that AF contains in addition to committed and differentiated cells a subpopulation with stem cell characteristics of multi-lineage differentiation, and expression of stem cell markers [33].

Amniotic fluid cells are heterogeneous, including cells shed from embryonic and extra-embryonic tissues. Ectodermal, mesodermal and endodermal cells can be found in amniotic fluid. Placental amnion may be derived from the epiblast as early as eight days after fertilisation. Thus, amnion epithelial cells are thought to retain the pluripotent properties of early epiblast cells, and these found in amniotic fluid and may serve as a source for mesenchymal stem cells [34]. The osteogenic commitment of AF-MSCs could be enhanced by using appropriate osteoconductive scaffolds and osteoinductive growth factors [35].

Amniotic fluid-derived stem cells (AF-MSCs) display high self-renewal capacity and plasticity, the second trimester derived cells, in particular, are rapidly expanded cells. Age of females and the stage of gestations constitute very important contributing factors marking AF properties. AF-MSCs showed the capacity to engraft and to contribute lineage-specific progeny in animal models. Therefore, AF-MSCs show great promise for use in cell-based therapeutic application in regenerative medicine. In this study, there was a significant difference in both the proliferation rate and osteogenic differentiation potentials of MSCs derived from the AF at different gestational ages a higher proliferation capacity and osteogenic potential compared to that of BM-derived cells. In a previous study, BM-MSCs showed a stronger osteogenic differentiation potential compared to adipose tissue-derived MSCs (AT-MSCs) [36].

Other investigators concluded similar findings are informing that the osteogenic differentiation potential of AF-MSCs is highly promising at both 2nd and 3rd trimester when incubated in osteogenic media for 2 or 4 weeks [31]. The striking finding in our study was detecting osteoblastic progenitor cells in the cellular content of 3rd trimester AF-MSCs, as clearly shown by detection of orange-red stained mineralised patches in control plates in which MSCs were continuously fed with MSCs proliferation media only.

In general, cell attachment depends on the intrinsic characteristics of the cells and is modulated by choice of medium and supplements, the type of culture surface used as well as the interaction with heterotypic cell types. The combination of these factors influences the activation of the required signal transduction pathways leading to isolation and proliferation of the desired cell type [37].

In our *in vivo* study, transplantation of both osteogenic differentiated and undifferentiated MSCs, derived from bone marrow or AF (2nd trimester) loaded on gel-foam and directly applied to the decorticated spines into rat-spines showed potential promising results. The x-rays, 3-4 months from the day of transplantation, showed a shadow (described as potential bone mottling) in one of the BM-groups, the rats transplanted with osteoblastic cells. In the AF-MSCs group, the two rats transplanted with osteoblastic cells showed potential bone mottling on X-ray filming. Additional 3-4 months may be required to confirm that this mottling is a true bone-forming tissue.

The use of AF-MSCs in cell therapy and tissue repair applications is yet in its beginning; however, several studies indicated that human amniocytes obtained by amniocentesis could be largely expanded and have the capacity to attach and proliferate on biodegradable scaffolds [38]. Osteogenically differentiated human CD117+ AFS (isolated by FACS separation) gave rise to tissue-engineered bone grafts after subcutaneous transplantation into immune-deficient mice [19]. The therapeutic potential of human CD117+ AFS was also shown after subcutaneous implantation in athymic nude rats [38]. They produced mineralized structures and supported the functional repair of large bone defects. Unsorted human AFS, as in our case, were cultured under osteoblastic differentiation condition. They formed osteoblasts with detectable mineralization on titanium surfaces indicating a possible application in osteosynthesis.

In this study, we suggested that MSCs cultured in osteogenic differentiation medium may induce the formation of new bone in experimental spinal fusion rats. X-ray radiography follows up, and histopathological analysis done three months post-operation showed that AF-MSCs have a better healing effect and increasing the future likelihood of achieving successful spinal fusion.

The present histopathological analysis was performed at three months post-cells transplantation of spinal-decorticated rat lumber specimens transplanted with AF-MSCs and at four months post-cells transplanted with BM-MSCs grafts. Results observed represented some differences in bone formation between the AF-MSCs and BM-MSCs rat groups. The experimental rats transplanted with osteogenically differentiated BM-MSCs showed

cartilage area, calcified cartilage (ossified) and newly formed bone, while the control rats that received matched undifferentiated BM-MSCs exhibited irregular calcified areas that associated with few osteocytes and blood vessels representing the beginning of bone repair healing phase. The experimental rats transplanted with osteogenically differentiated AF-MSCs showed a more advanced stage than that of BM-MSCs rats involving calcified area with new bone structures contained osteocyte-like cells. The control rat received matched undifferentiated AF-MSCs showed calcified area with little osteogenic formed bone at the top of the host bone, which presents a more advanced stage of healing compared to that of BM-MSCs control rats.

In conclusion, there was a significant difference noted between the 2nd-trimester AF-MSCs which have a greater proliferation capacity and typical fibroblast morphology compared to that of the 3rd-trimester AF-MSCs which showed limited proliferation capacity *in vitro*. However, assessment of osteogenic differentiation of the AF-MSCs at early pregnancy 2nd trimester, versus late pregnancy 3rd trimester, by Alizarin Red Staining has shown that the 3rd trimester AF-MSCs contains osteoblast committed progenitor cells at both 14th and 28th days of osteogenic differentiation induction. However, both 2nd and 3rd-trimester AF-MSCs have greater proliferation capacity and osteogenic differentiation potentials *in vitro* compared to that of BM-MSCs. The *in vitro* osteogenic induction of MSCs derived from the two human sources; BM or 2nd trimester AF before they're *in vivo* implantation, promotes their osteogenic/bone forming capacity and raising their healing effect. AF-MSCs, have a higher potential of inducing bone healing *in vivo* than that of BM-MSCs. Hence, they can be used as an alternative source to BM-MSCs in bone regeneration and spine fusion surgeries. Our findings suggested AF-MSCs a promising and safe alternative to BM-MSCs for cell therapy of bone defects and injury.

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Evaluation of *APOE* Genotype and Vascular Risk Factors As Prognostic and Risk Factors for Alzheimer's Disease and Their Influence On Age of Symptoms Onset

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Keywords: Alzheimer's disease; APOEε4 allele; hypertension; dyslipidemia

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Abstract

BACKGROUND: Alzheimer's disease (AD), the most common cause of dementia, is evolving to become a threatening epidemy of the 21st century. Only 21% of the predicted number of AD patients in Macedonia have been diagnosed and treated, which means that almost 80% are underdiagnosed or misdiagnosed. Apolipoprotein E gene (*APOE*) is recognised as the strongest genetic risk factor for sporadic AD. Whether and when Alzheimer's disease develops, depends on the very complex interaction between genetic and modifiable risk factors. It has been known that vascular factors like hypertension, diabetes mellitus, hypercholesterolemia and obesity increase the risk of developing both AD, vascular dementia and mixed AD and vascular pathology

AIM: This study aims to evaluate the influence of *APOEε4* allele presence and modifiable vascular risk factors (hypertension, diabetes mellitus and dyslipidemia) as prognostic and risk factors for AD and their influence on the age of onset of AD symptoms among 144 AD patients from Macedonia.

MATERIAL AND METHODS: Study group of a total of 144 patients diagnosed with AD was evaluated. APOE genotyping was performed using *APOE* haplotype-specific sequence specific-primer (SSP)-PCR (Polymerase Chain Reaction) methodology. The non-standardized questionnaire was used to obtain information about demographics, lifestyle and modifiable risk factors that could influence disease onset and phenotype.

RESULTS: Statistically significant association was found between the presences of *APOEε4* allele in AD group versus controls. The presence of *APOEε4* allele increases the risk of developing AD in a 3-fold manner. The average age of disease onset in the ε4 carrier group was 67.2 ± 8.3 and in the ε4 non-carrier group 69.7 ± 9.4. This confirms that the presence of *APOEε4* allele shifts towards earlier disease onset, though the difference is not statistically significant. Out of the vascular risk factors, only hypertension was significantly associated with earlier AD onset. Out of total 144 patients, in 22.9% the first symptom onset was before the age of 65, that can be considered as early onset Alzheimer's Disease (EOAD), which is much higher than 5% for EOAD as most of the studies report.

CONCLUSIONS: The average age of disease onset of 68.4 years could be considered earlier than the average age of AD onset worldwide. Out of all the vascular risk factors analysed in this study, only hypertension and dyslipidemia were found to significantly increase the risk for developing AD and only the presence of hypertension influences the age of onset, shifting towards earlier disease onset. Public awareness campaigns should be organised to influence general population knowledge about Alzheimer's disease, early recognition and the influence of modifiable vascular risk factors.

Introduction

Alzheimer's disease (AD), the most common cause of dementia, is evolving to become a threatening epidemy of the 21st century, even though not an infectious disorder. In 2015, 47 million people had dementia worldwide, with predictions that this number would at least triple by the year of 2050, estimating that there will be around 131 million living with dementia, mostly AD [1]. A disease that was in

details described by Alois Alzheimer in 1906 and was considered to be a rare disease at that time is about to become a global medical and social problem, mostly affecting people living in low- and middle-income countries. Even though the exact aetiology and pathogenesis of Alzheimer's disease is still an unrevealed challenge for the neuroscience, it is well known that old age is a major risk factor for AD. With better medical treatment for cardiovascular, cancer and infectious disorders and a growing number of ageing populations worldwide, the rapid expansion of Alzheimer's disease shortly is expected.

The standardized age prevalence for dementia worldwide is 5-7% for aged 60 years and more [1], that assumes that there are around 20,000 people living with AD in Macedonia (according to State Statistical Office, R. Macedonia 2.4.15.10/821, there are around 400,000 people aged 60 and more living in Macedonia). According to the data from Health Insurance Fund of Macedonia for donepezil (acetylcholinesterase inhibitor, registered for treatment of AD) prescriptions in 2017, only 4,200 patients were treated. This assumes that only 21% of the predicted number of AD patients in Macedonia have been diagnosed and treated, which means that almost 80% are underdiagnosed or misdiagnosed.

Two forms of Alzheimer's Disease are recognised according to the age of onset. Early-onset Alzheimer's disease (EOAD) that begins before the age of 65 and presents only 5% of overall AD patients. EOAD patients usually have positive family history for the disease, and in half of the EOAD, that is in only 1- 2% of the total AD patients, the disease develops due to a monogenic deterministic mutation in one of the three known genes amyloid precursor protein (*APP*), prseniline1 (*PSEN1*) or presenilin2 (*PSEN2*) gene.

According to another study, the percentage of autosomal dominant inherited forms of EOAD is even smaller, only around 7% of all early-onset cases [2]. The other 95% of AD patients, have disease onset at, or after the age of 65, late-onset Alzheimer's disease (LOAD), and present the majority of AD patients [1]. LOAD is sporadic, occurring in individuals with genetic susceptibility in complex interaction with environmental and lifestyle modifiable risk factors.

Apolipoprotein E gene (*APOE*) is recognised as the strongest genetic risk factor for LOAD. It has three allelic variants ε2, ε3 and ε4. It is the presence of *APOEε4* allele that increases the risk for LOAD. According to several studies, the presence of *APOEε4* allele in LOAD patients is 50-60% compared to 20-25% in healthy older adults respectively. The presence of ε4 allele increases the risk of developing AD in a dose-dependent manner. *APOEε4/ε4* homozygosity increases the risk for developing AD 14-fold, and APOE ε3/ε4 heterozygosity increases the lifetime risk for AD, 4-fold in comparison to ε3 homozygosity [3], [4], [5]. The presence of *APOEε4* allele shifts the age of disease onset approximately 5 to10 years earlier in heterozygosity, and up to 10-20 years earlier in homozygosity [2]. *APOEε4* allele frequency is highly variable in different population and ethnic groups [6]. The worldwide frequency of ε2, ε3 and ε4 alleles is 8.4%, 77.9% and 13.7%, respectively, but in AD patients the ε4 frequency increases up to 40% [7]. When discussing the APOE gene as a risk factor for AD, it must be stressed that it only influences the individual's genetic susceptibility, but it is not deterministic as the previously mentioned three gens. That means, that even if homozygosity for ε4 is present, it only increases lifetime risk, but does not mean that AD would certainly develop. Whether and when Alzheimer's disease develops depends on the very complex interaction between genetic and the modifiable risk factors. It has been known that vascular risk factors like hypertension, diabetes mellitus, hypercholesterolemia and obesity increase the risk of developing both AD, vascular dementia and mixed AD and vascular pathology [6].

This study aims to evaluate the influence of APOEε4 allele presence and modifiable vascular risk factors (hypertension, diabetes mellitus and dyslipidemia) as prognostic and risk factors for AD and their influence on age of symptoms onset among 144 AD patients from Macedonia.

Material and Methods

The study group includes 144 subjects that were diagnosed in the dementia outpatient clinic at the University Clinic of Neurology-Skopje and dementia centre at the University Clinic of Psychiatry-Skopje within the period from 2016 to 2018. All subjects fulfilled criteria for probable Alzheimer's dementia according to standard diagnostic criteria [8]. A standard procedure of blood sample collection was performed for DNA isolation. APOE genotyping was performed in the genetic laboratory "Prof. Dr Georgi Efremov", Macedonian Academy of Arts and Sciences. *APOE* haplotype-specific sequence specific-primer (SSP)-PCR (Polymerase Chain Reaction) methodology was used to determine the three main APOE isoforms. The non-standardized questionnaire was used to obtain information about demographics, lifestyle and modifiable risk factors that could influence disease onset and phenotype. We used an age-matched control group to evaluate ε4 allele frequency.

Written informed consent was obtained from all subjects included in the study group and from the control group.

Statistical analysis in STATISTICA 7.1, SPSS 20.0 were done, using chi-square test, t-test and univariate and multiple logistic regression analyses.

Results

A study group of a total of 144 patients diagnosed with AD was evaluated. *APOEε4* allele carriers were found to be 72 out of 144 AD patients, that is 50.0%, compared to the control group where *APOEε4* allele carriers were 21 out of 90 individuals, which makes 23.3%. Statistically, a significant association was found between the presence of *APOEε4* allele and AD, $p < 0.05$ (Pearson Chi-square = 16.8103, $p = 0.000041$). 9% of all AD patients in our study group were *APOEε4/ε4* homozygotes, versus only 1 subject (1.1%) in the control group. Calculated *APOEε4* allele frequency was 30.14% in the AD patients' group, compared to 12.22% in the control group, which is statistically significant ($p < 0.05$).

APOE genotype/AD patients	Count	%
e3-e3	67	46.5
e3-e4	59	41.0
e2-e3	4	2.8
e4-e4	13	9.0
missing	1	0.7
total	144	100.0
APOE genotype/controls		
e3-e3	60	66.7
e3-e4	19	21.1
e2-e3	9	10.0
e2-e4	1	1.1
e4-e4	1	1.1
total	90	100.0

Figure 1: APOE genotype distribution among AD patients and controls

According to the analysis in our study group, the presence of *APOEε4* allele increases the risk of developing AD in a 3-fold manner [OR-3.3320 (1.8502-6.0005)]. *APOEε4* undoubtedly has influenced the genetic susceptibility in sporadic AD patients and has influenced the disease manifestation.

The average age of onset of Alzheimer's disease in our study group, which makes the first AD research till now in this geographic region and among this ethnic population is 68.4 years. This can be considered lower in comparison to Nussbaum reports that the average age of dementia onset is around 80 years [2].

One of the explanations could be that younger patients are referred to the dementia outpatient clinic at the University Clinic of Neurology and dementia centre at the University Clinic of Psychiatry, and older patients with Alzheimer's disease are under or miss diagnosed. Unfortunately, the public awareness about Alzheimer's disease in Macedonia is still low, and forgetfulness is still considered as a part of normal ageing.

Even though this might seem like a logical explanation, it is unlikely that only younger AD patients were referred to tertiary level, as this research lasted for 2 years, and in the meantime, a lot of educational workshops for family doctors were organised influencing the professional awareness about AD. If we conclude that sporadic AD in Macedonia has a somewhat earlier onset than in other population, it is important to identify the causes. As the presence of *APOEε4* allele not only increases the genetic susceptibility for AD in a dose-dependent manner but also influences the age of dementia onset, shifting to earlier onset in ε4 carriers, this might be one possibility.

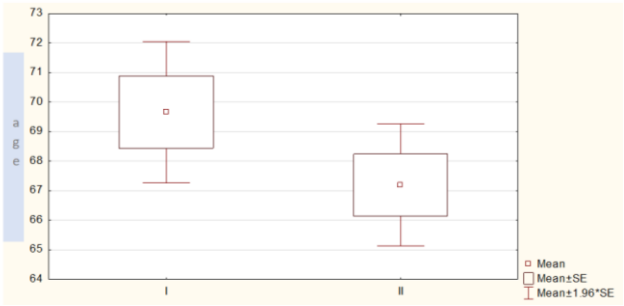

Figure 2: Mean age distribution among APOEε4 non-carriers-I and APOEε4 carriers-II

When we divided AD patients into two groups according to their *APOEε4* status, ε4 carriers and ε4 non-carriers, we found that average age of disease onset in the ε4 carrier group was 67.2 ± 8.3, and in the ε4 non-carrier group 69.7 ± 9.4. This confirms that the presence of *APOEε4* allele shifts towards earlier disease onset, though the difference is not statistically significant, $p > 0.05$ (t-test = 1.533270, $p = 0.127864$).

Discussion

In our AD patients study group, out of total 144 patients, 22.9% had the first symptom onset before the age of 65, that can be considered as EOAD, which is much higher than 5% as most studies report [1]. Out of those EOAD patients, *APOEε4* allele was present in 54.5% (in 94.4% as heterozygotes, and 5.6% as homozygotes).

Excluding the influence of *APOEε4* on the age of onset we looked for the presence and influence of the modifiable risk factors.

When analyzed for the association of modifiable vascular risk factors (hypertension,

diabetes mellitus and dyslipidemia) for AD in our study group compared to the control group, a statistically significant association was found for hypertension and $p < 0.05$ (Pearson Chi-square = 4.5302, $p = 0.033015$) and dyslipidemia $p < 0.05$ (Pearson Chi-square = 6.1103, $p = 0.013439$), but not for diabetes mellitus $p < 0.05$ (Pearson Chi-square = 0.0377, $p = 0.845696$). Having hypertension was found to be associated with 1.5-fold increased risk for developing AD [OR = 1.8767 (1.0479-3.3615)] and dyslipidemia was found to be associated with 2-fold increased risk for AD [OR = 2.2656 (1.1749-4.3688)] in our study.

Using multivariant logistic regression only the presence of *APOEε4* allele was confirmed to be a predictor for Alzheimer's disease.

Correlation among age of AD onset and hypertension, diabetes mellitus, obesity, dyslipidemia and physical activity was done, showing that only hypertension was significantly associated with earlier AD symptoms onset.

This confirms what previous studies reported by identifying hypertension, especially midlife hypertension to be one of the main modifiable risk factors for AD and as Winblad et al. suggest, pharmacological control of hypertension, implemented in middle-aged or younger old adults, might be effective in reducing the incidence of dementia [1]. Other population-based studies have shown that having multiple cardiovascular risk factors (smoking, hypertension, diabetes mellitus, hypercholesterolemia) in middle age or several years before dementia onset, increases the risk for developing AD [1], [9].

In conclusion, in our study group of 144 AD patients, a statistically significant association was found between the presences of *APOEε4* allele in AD group versus controls. The presence of *APOEε4* allele increases the risk for developing AD in a 3-fold manner and is the only confirmed to be a predictor for Alzheimer's disease. The average age of disease onset of 68.4 years could be considered earlier than the average age of AD onset worldwide. The presence of *APOEε4* allele shifts towards earlier disease onset, though the difference is not statistically significant.

Out of all the vascular risk factors analysed in this study, only hypertension and dyslipidemia were found to significantly increase the risk for developing AD and only the presence of hypertension influences the age of onset, shifting towards earlier disease onset.

Public awareness campaigns should be organised to influence general population knowledge about Alzheimer's disease, early recognition and the influence of modifiable vascular risk factors.

As the autosomal dominant inherited forms of EOAD are extremely rare, we should continue our research in evaluating potentially modifiable risk factors that influence the age of disease onset. Other design protocols might reveal other risk or lack of protective factors (such as higher levels of education, socialisation) that also influence the age of disease onset. Aggressive treatment of especially midlife hypertension could delay AD onset in genetically susceptible individuals that would aid more years to their life and more quality lifetime.

Further genetic research is needed especially in the EOAD subgroup to evaluate the presence of the three deterministic monogenic mutations in *APP, PSEN1* and *PSEN2* genes as potential genetic factors for EOAD, even though there is no family history that indicates autosomal dominant trait of transmitting. Only two decades ago, the diagnose of Alzheimer's disease in Macedonia was extremely rare and the term "sclerosis" was used to describe the dementia syndrome, which is why the general population does not report if someone from the family had forgetfulness, got lost (symptoms and signs that might be indicative for AD), which can mislead us in taking the family history. GWAS (Genome-wide associated study) might identify other than *APOE* genes, some of them specifically for geographic region or ethnicity, that might also influence genetic susceptibility, increase the individual risk for AD and decrease the age of symptoms onset.

In times when there is no etiological treatment that would stop or even slow down Alzheimer's disease progression, identifying individuals at risk and developing preventive strategies is more than necessary if we want to cut down the predicted numbers of affected population with Alzheimer's dementia till the year of 2050.

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Basic Science

The Effect of Puguntano Leaf Extract (*Curanga Fel - Terrae Merr.*) On P38 Mapk Levels and Glut-4 Expression in Type 2 Diabetic Rat Muscle

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Abstract

BACKGROUND: Puguntano (*Curanga feel-terrae Merr*.) contains flavonoids, saponins, tannins, and steroids/ terpenoids which improved post-receptor insulin signalling in rats model of type 2 diabetes mellitus (T2DM).

AIM: This study aimed to determine the effect of puguntano leaf extract on p38 mitogen-activated protein kinase (MAPK) levels and glucose transporter-4 (GLUT-4) expression in diabetic rats muscle.

METHODS: Forty-eight male Wistar rats had T2DM induced using a combination of feeding a high-fat diet for 5 weeks and multiple intraperitoneal injections of low-dose streptozotocin (30 mg/kg). The diabetic rats were randomly divided into control and treatment groups, and 200 mg/kg/day puguntano extract was administered orally for 10 days to treatment group. Subsequently, p38 MAPK levels were measured by Sandwich Elisa and plasma membrane GLUT-4 expression was evaluated by Immunohistochemistry in their gastrocnemius muscles.

RESULTS: There were significantly higher p38 MAPK levels and GLUT-4 expression in the treatment group than in the control group.

CONCLUSION: These data suggest that a puguntano leaf extract can improve post-receptor insulin signalling by enhancing p38 MAPK levels and GLUT-4 expression in a rat model of T2DM.

Introduction

Diabetes mellitus (DM) is a chronic condition characterised by high blood glucose concentration resulting from insufficient insulin production and/or ineffective insulin action [1]. The International Diabetes Federation (IDF) reported in 2017 that there were 10.3 million people with diabetes in Indonesia, and this number was estimated to increase to 16.7 million by 2045. Indonesia is in the 6th rank of the top ten countries for some people with diabetes of all the countries of the world [2].

Insulin is an anabolic hormone secreted by pancreatic β-cells that regulate a wide range of physiological processes. Normal metabolism requires the coordinated secretion and action of insulin, but in type 2 diabetes (T2DM), both its action and secretion

are impaired [3].

Insulin resistance in the insulin-sensitive tissues, such as liver, muscle, and fat, is the principal feature of T2DM [4]. Skeletal muscles play an important role in insulin-mediated glucose uptake in the post-prandial state (80%) [5]. In the initiation stage of the insulin signalling pathway, binding of the insulin to its receptor is followed by intracellular signalling via two main downstream pathways, i.e. mitogen-activated protein kinase (MAPK) pathway and phosphatidylinositol-3 kinase (PI3K) pathway [6]. Activation of PI3K pathways promotes glucose uptake by increasing the translocation of glucose transporter-4 (GLUT-4) from intracellular vesicles to the plasma membrane of skeletal muscle, facilitating glucose uptake [5]. p38 MAPK has also been shown to be necessary for regulation of insulin-stimulated glucose uptake through GLUT-4 in response to insulin, but findings regarding the potential role of p38 MAPK in the regulation of glucose transport in skeletal muscle remain controversial [7]. GLUT-4 plays an important role in the maintenance of glucose homeostasis via its translocation and expression in skeletal muscle [5]. A great deal of evidence implicates defects in post-receptor signalling as the major cause of insulin resistance in target tissues [8], including defects in GLUT-4 expression and function [9].

Puguntano (*Curangafel-terrae* Merr), a medicinal plant from Scrophulariaceae family, grows in Asia especially in China, India, Indonesia, Philippines, Malaysia and Myanmar. Puguntano leaves from the Dairi area of North Sumatera Province have long been used empirically to control blood glucose levels. Puguntano leaves contain flavonoids, saponins, tannins, and steroids/terpenoids, which have anti-diabetic activity [10], [11]. A study in diabetic mice demonstrated that blood glucose levels were reduced by 44.47% after a 10-day treatment with n-hexane-extracted puguntano [12].

Tannins promote PI3K and p38 MAPK activity and GLUT-4 translocation [13], while flavonoids promote GLUT-4 translocation [9], terpenoids increase GLUT-4 expression and translocation through *proliferator-activated receptor gamma* (PPAR-Y) activation [14], [15], [16], and saponins increase GLUT-4 expression [17], [18]. Also, a previous study showed that quercetin from berry extract with flavonoid compound increases insulin receptor substrate 1 (IRS1), IRS2, AKT, p38 MAPK, adenosine monophosphate-activated protein kinase (AMPK) and GLUT-4 expression in skeletal muscle cells [19]. Furthermore, Lindarto et al., Reported that insulin resistance is ameliorated in newly diagnosed T2DM patients after treatment with puguntano leaf extract for 12 weeks, illustrated by the significant reduction in fasting blood glucose (FBG) levels, homeostasis model assessment-insulin resistance (HOMA-IR), and glycated haemoglobin (HbA1c) [20].

The present study aimed to determine the effect of puguntano leaf extract *(Curanga feel-terrae* Merr.*)* on p38 MAPK levels and GLUT-4 expression in a rat model of T2DM.

Material and Methods

Forty-eight male 8-week-old Wistar rats weighing 180-200 g were housed in stainless steel cages under environmentally controlled conditions. The ambient temperature was 22-25°C, and the light/dark cycle was 12/12 hours. The animals had free access to water and standard diet. After 3 days' acclimatisation, the rats commenced consumption of a high-fat diet (HFD), which continued for 5 weeks and was followed by two intraperitoneal injections of low-dose streptozotocin (STZ; 30 mg/kg), 1 week apart [21]. STZ was dissolved in 50 mM sodium citrate solution (pH 4.5) containing 150 mM NaCl [22]. After the induction of diabetes using HFD and STZ, fasting blood glucose (FBG) levels were measured in the blood from the tail vein using a glucometer. Rats with FBG level > 200 mg/dL were considered to be diabetic [21].

Diabetic rats were then randomly divided into control and treatment groups, each containing 24 rats. The treatment group was administered with an ethanolic extract of puguntano leaves in carboxyl methyl cellulose-Na (CMC-Na; 0.5% solution; 200 mg/kg/day) using an orogastric cannula for 10 days. The extract was prepared by maceration in Department of Biological Pharmacy, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia [23].

At the end of the experiment, blood was obtained from the left ventricle, left undisturbed at room temperature for 15–30 min, then centrifuged at 1-2,000 × *g* for 10 min. FBG levels were determined using spectrophotometry and fasting insulin using sandwich ELISA. The rats were euthanised using ketamine and decapitated, and then gastrocnemius muscles were dissected for examination of p38 MAPK levels and GLUT-4 expression.

p38 MAPK levels was evaluated from a slice of muscle that was placed in round bottom microfuge tube sand than either snap frozen or kept on ice for immediate homogenization. For a ~5 mg piece of tissue, ~300 µL complete extraction buffer (100 mM Tris, pH 7.4, 150 mM NaCl, 1 mM EGTA, 1 mM EDTA. 1% Triton X-100, and 0.5% Sodium deoxycholate) was added to the tube and homogenized using an electric homogenizer. The blade was rinsed twice using 300 µL complete extraction buffer; then the homogenate was agitated for 2 hr at 4°C and centrifuged for 20 min at 13,000 x rpm at 4°C then the supernatant was transferred to a fresh, chilled tube and store samples at -80°C. The

cell extraction was supplemented with phosphatase, protease inhibitor cocktails and PMSF to 1 mM, immediately before use. After thawing, samples were centrifuged before use at 10,000 rpm for 5 min at 4°C to remove any precipitate.

GLUT-4 expression was evaluated in paraffin-embedded sections of rat skeletal muscle tissue. Four-millimetre-thick paraffin sections were dewaxed, rehydrated, and microwaved for 10 minutes. The endogenous peroxidase activity of the investigated specimens was blocked using 3% H_2O_2 for 10 minutes, followed by 25 minutes washing with phosphate-buffered saline (PBS). The tissue sections were incubated with normal rabbit serum for 10 minutes, and then the slides were incubated at room temperature with rabbit polyclonal anti-Glucose Transporter GLUT-4 rat antibody (b33780). Sections were then washed with PBS and incubated with a secondary antibody goat anti-rabbit polyclonal IgG for 30 minutes, washed twice with PBS, counterstained with haematoxylin, and mounted using DPX. A positive signal for GLUT-4 in muscle tissue was semi-quantitatively estimated by recording the distribution of positively stained cells and the intensity of the staining at the plasma membrane. Cell counting was performed using a light binocular microscope, and the data were presented as immunohisto score.

This experimental protocol was approved by the Institutional Ethics Committee of Universitas Sumatera Utara, Medan, Indonesia (Reference 42/TGL/KPEK FK USU-RSUP HAM/2018).

Biochemical analysis

STZ was purchased from Sigma Aldrich (Munich, Germany). FBG was measured using a commercially available enzymatic kit. Fasting insulin and p38 MAPK levels were determined using commercial kits supplied by Qayeebio (China). GLUT-4 expression was determined using a kit supplied by Abcam (Cambridge, UK). FBG, fasting insulin, p38 MAPK levels were quantified in the Molecular Genetics Laboratory of the Medical Faculty of Universitas Padjajaran, and GLUT-4 expression was determined in the Immunopathology Laboratory of the Department of Anatomical Pathology, Hasan Sadikin Hospital Bandung. The HOMA-IR equation, which estimates the degree of insulin resistance using fasting insulin and glucose levels, was used as previously described [24].

Statistical Analysis

Values were expressed as the median (minimum-maximum). Data were analysed using the Wilcoxon test in SPSS version 22.0 to identify statistically significant differences between groups, which were accepted when $p < 0.05$.

Results

As shown in Table 1, FBG, fasting insulin, and HOMA-IR were significantly lower in the treatment group than in the control group.

Table 1: FBG, fasting insulin and HOMA-IR in the control and treatment group

Variable	Group		
	Control (n = 24) Med (min-max	Treatment (n = 24) Med (min-max)	P
FBG (mg/dl)	384 (207-490)	122 (95-213)	0.001*
Fasting insulin (μlU/ml)	56.56 (49.63-73.67)	51.31 (47.77-59.00)	0.001*
HOMA-IR	2.77 (2.60-3.71)	0.77 (0.59-1.29)	0.001*

Wilcoxon test. Significant if $p < 0.05$.

As shown in Table 2, p38 MAPK and GLUT-4 protein expression were higher in the treatment group than in the control group.

Table 2: p38 MAPK levels and GLUT-4 expression in the control and treatment group

Variable	Group		
	Control (n = 24) Med (min-max	Treatment (n = 24) Med (min-max)	p
p38MAPK (ng/mL)	19.70 (17.37-27.34)	23.24 (17.74-34.41)	0.001*
GLUT-4 (score)	2 (1-6)	4 (2-9)	0.001*

Wilcoxon test. *Significant if $p < 0.05$.

Figure 1 showed the effects of a puguntano leaves extract on the histological features of GLUT-4 expression in T2DM diabetic rat muscle.

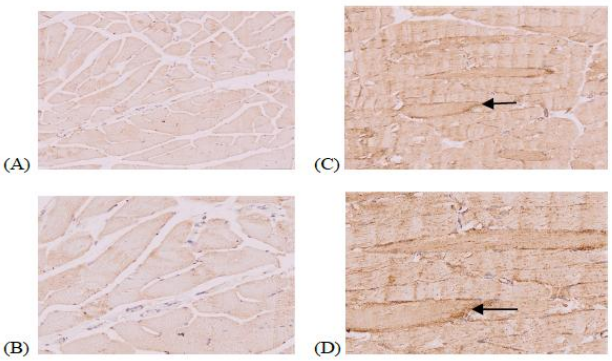

Figure 1. Effects of a puguntano leaves extract on the histological features of skeletal muscle in a rat model of T2DM. GLUT-4 expression in the control rats (A and C; 100X and 200X magnification, respectively) and treated rats (B and D; (100X and 200X magnification, respectively) demonstrating higher GLUT-4 expression in the plasma membrane of T2DM rats that had been treated with puguntano extract (Arrow)

Discussion

The rat model of diabetes induced by HFD feeding and low-dose STZ closely simulates the natural pathogenesis of T2DM and is widely used in studies of the efficacy of anti-diabetic drugs. Induction with HFD/STZ will exhibit hyperglycemia and insulin

resistance [25]. This study is the first study to evaluate the effect of puguntano leaf extract *(Curanga feel-terrae* Merr.*)* on p38 MAPK levels and GLUT-4 expression in such a rat model of T2DM.

Insulin binding to the α-subunits of the insulin receptor (IR) increases the receptor β-subunit tyrosine kinase activity, which results in phosphorylation of IR substrates (IRS; IRS-1 and IRS-2 in skeletal muscle). These IRS proteins then interact with the specific SH2 domains of downstream molecules, including PI3K, Grb2, and phosphotyrosine phosphatase (SHP2). IRS-1-Grb2 binding initiates a cascade that activates the protein Ras and MAPK, and consequently nuclear transcription factors. IRS-PI3K binding generates phospholipids that activate further downstream kinases and ultimately induced physiologic response such as glucose transport, and protein and glycogen synthesis [26], [27]. These kinases are the serine/threonine kinases [AKT/protein kinase B (PKB) and protein kinase C (PKC)]. Phosphorylation of AKT initiates the translocation of GLUT4 from its intracellular storage location to the surface of the cell to facilitate glucose transport into the cell [28], [29].

Fasting insulin concentration is a significant indicator of insulin resistance and is increased in obesity [24]. In this study, FBG, fasting insulin levels, and HOMA-IR were significantly lower in the treatment group than in the control group. This may be explained by an effect of one or more secondary metabolites the tannins, flavonoids, triterpenoids, and saponins present in puguntano leaf extract to insulin sensitivity. The results of the present study are consistent with those of Lindarto et al., who showed the anti hyperglycemic and insulin-sensitizing effect of puguntano leaf extract in decreasing FBG and HOMA-IR in newly diagnosed T2DM patients [20].

Muscle p38 MAPK levels and GLUT-4 expression were significantly higher in the treatment group than in the control group in this study. These effects of puguntano are similar to those reported for quercetin, a citrus flavonoids present in berry extract that caused increases in p38 MAPK and GLUT-4 expression in L6 myotubes. Indeed, quercetin was reported to have anti-diabetic effects through activation of both the PI3K/AKT and MAPK pathways, inducing glucose uptake through the increasing of GLUT-4 expression and translocation [19].

The significant increase in GLUT-4 expression noted in the treatment group was also consistent with that in previous studies of other plant extracts containing tannins, flavonoids, triteropenoids, and/or saponins. A study by Xiong et al., showed that Entada phaseoloides (L.) Merr. With the major secondary metabolite triterpenoid saponin Entagenic acid (EA) promoted glucose uptake into the skeletal muscle of T2DM rats by enhancing the translocation and expression of GLUT-4 [5]. An ethanolic extract of *Vernonia amygdalina Del.* (VA) which contains a high concentration of flavonoid polyphenols also caused a significant increase in GLUT-4 expression (24%) and translocation (35.7%) to the plasma membrane of skeletal muscle in diabetic treatment group [30]. Furthermore, there was higher GLUT-4 expression after the administration of other herbal products containing triterpenoids saponins [31], flavonoids [32], [33], and tannins, flavonoids and triterpenoids [34].

In conclusion, puguntano leaf extract improved post-receptor insulin signalling by increasing p38 MAPK levels and GLUT-4 expression in a rat model of T2DM. Further studies should be undertaken to establish whether it may represent a novel therapy for T2DM in people.

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Basic Science

Dimensional Analysis of $CD44^{High}$ $CD24^{Low}$ and Ki67 in Triple Negative Breast Cancer

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Keywords: Triple-negative breast cancer; Stemness; Differentiation; EMT; CD44; CD24

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Abstract

AIM: To study the dimensional analysis $CD44^{high}$ $CD24^{low}$ and Ki67 in triple negative breast cancer (TNBC).

METHODS: This cross-sectional study was performed on patients with breast cancer in Haji Adam Malik Hospital Medan from 2013 to 2016 to determine the frequency and pathologic features of TNBC by immunohistochemistry stained.

RESULTS: By using immunohistochemistry staining panel of CD44, CD24, Twist, Claudin 7, CK5, CK8/18, EMA, E-Cadherin, Ki-67, a total 67 breast tumour samples with TNBC were classified as 9 stem-cells like 1 basal, 22 baso-luminal, and 23 luminal subtypes

CONCLUSION: By using immunohistochemical staining panel, TNBC can be differentiated into stem cells like basal, baso-luminal and luminal subtypes. Didifferentiation and EMT can produce heterogeneity in TNBC subtypes and this will affect in handling TNBC. Stemness in stem cells- like subtypes are resistant to therapy. Therefore, TNBC needs special attention in order to assist in more optimal handling.

Introduction

Breast cancer is thought to derive from the stem or progenitor cells having abnormalities in the self-renewal process [1]. Mammary stem cells (MaSCs) play an important role in the growth and development of breast cancer, resistance to therapy, and metastasis [2]. Various stem cell markers are used to identify and isolate CSC from various solid tumours, such as CD44 and CD24 [3]. CD24 is a little more expressed in progenitor cells compared to differentiated cells [4]. Therefore, for therapy to be effective, CSC must be recognised and must be differentiated from normal breast stem cells.

The increasing level of Ki-67 shows aggressiveness of tumour growth and indicates a poor prognosis. Triple-negative breast cancer (TNBC) is also correlated with high Ki-67 level [5]. Hence, we were interested in studying about the dimensional analysis $CD44^{high}$ $CD24^{low}$ and Ki67 TNBC.

We aimed to study the dimensional analysis $CD44^{high}$ $CD24^{low}$ and Ki67 in triple negative breast cancer.

Material and Method

This descriptive study with the cross-sectional design was conducted from March to October 2017 and was carried out after getting permission from the Ethical Committee of Medical Faculty USU Medan.

The population was patients diagnosed as breast cancer based on histopathology (mastectomy/biopsy) at RSUP Haji Adam

Malik/Departement of Anatomical Pathology Medical Faculty of USU Medan. TNBC tumours were further stained with CD44 (DF1485, Novocastra Laboratories Ltd., dilution 1:100), CD24 (C-20, Santa Cruz Biotechnology, dilution 1:100), TWIST-1 (H-81, Santa Cruz Biotechnology, dilution 1:100), CK5 (XM26, Novocastra-Vision Biosystems, dilution 1 : 25), CK8/18 (5D3, Lab Vision, dilution 1:300), Claudin-7 (NBPI-35677, Rabbit polyclonal antibody, Novus Biological, dilution 1:100), E-Cadherin (NCH-38; M3612, monoclonal primary antibody, DakoCytomation, Denmark, dilution 1:50), EMA (E29, monoclonal antibody, DAKO, dilution 1:400), and Ki-67 (clone SP6, biomarkers, dilution 1:100).

CD44, CD24 and Twist were stained in membrane cells, with score 0 if < 10% positive tumour cells; 1 if 10-25%; 2 if 25-50%; and 3 if > 50%. Intensity was scored as 0 if unstained, 1 if weakly stained, 2 if intermediate, and 3 if strong. Interpretation of CD44, CD24, and Twist staining was determined based on multiplication of the percentage of positive cells and the intensity of staining. CD44 and CD24, were scored as 0 if (-), 1-3 (+1), 4-6 (+2), and 7-9 (+3) [6]. While Twist was considered weak if total score < 6 and strong ≥ 6 [7]. Claudin-7 staining was scored as 0 if no membranous staining; 1+ (1-10% tumour cells); 2+ (10-30%); and 3+ (> 30%) [6].

For E-cadherin staining, the interpretation of staining is divided into 0 if (-); +1 if weakly and heterogenous stained; +2 if weakly but homogenous stained; +3 if moderately stained, or if strongly but heterogenous stained; and +4 if strongly and homogenous stained. Percentage of tumour cells were scored as 0 if (-); 1 if < 10% membranous stained; 2 if 10-50%; and 3 if > 50%. Interpretation of E-cadherin was determined based on multiplication of percentage of positive cells and intensity of staining, which is negative (scored as 0); weakly stained (total score 1-4); moderately stained (5-8); strongly stained (9-12) [8]. CK5 and CK8/18 were stained in the cytoplasm and positive if ≥ 10% tumour cells. EMA was stained positive in membranous/ cytoplasm cells.

Intensity of staining was scored as 0 if < 25%, 1 if 26-50%, and 2 if 51-100%. Score 0 and 1 was considered low and scored 2 as high [9]. For Ki-67, 300 cells were counted (include proliferating and non-proliferating cells), and the percentage of proliferation were counted with cut-off point 10% positively nuclear cells [10]. After that, molecular classification of TNBC was done and classified as Claudin low (stem cell-like) subtypes if $CD44^{+}$ $CD24^{-}$, Claudin-low, Twist-1^{high}; basal-like subtypes if $CK5^{+}$ and EMA^{+}, and luminal subtypes if $CK8/18^{+}$ and $E\text{-cadherin}^{+}$.

The results of this study were processed using statistical software and displayed in frequency distribution in tables.

Results

To determine the ontogeny and differentiation of TNBC subtypes in stem cells stages, we used CD44 and CD24 immunohistochemical stains. The classifications of stem cells-like type into SC-1 to SC-3 were arbitrary (Figure 1).

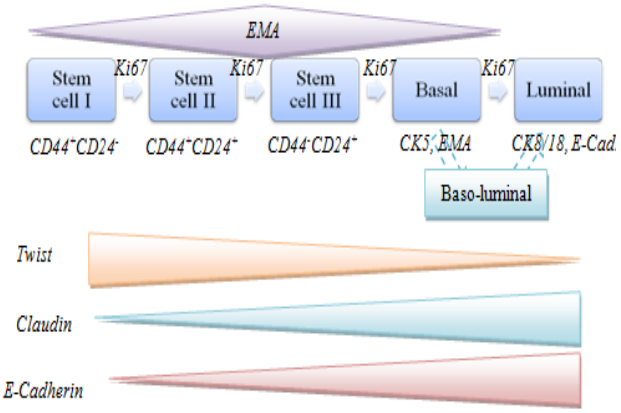

Figure 1: Schema of ontogeny differentiation breast epithelial from stem cells to luminal cells associated with a panel of various molecular markers for TNBC

From 67 TNBCs in this study, there were 10 cases of $CD44^{+}CD24^{-}$, 36 cases of $CD44^{-}CD24^{+}$, 9 cases of $CD44^{+}CD24^{+}$, and 12 cases of $CD44^{-}CD24^{-}$. With Twist, Claudin 7, Cytokeratin 5 dan 8/18 (CK5 dan CK8/18), EMA, E-Cadherin, and Ki67 immunohistochemical stain panel, TNBC was classified as 9 cases of stem-cell-like, 1 basal, 22 baso-luminal and 23 luminal subtypes. After classified, Tumours would then be categorised as low and high proliferation based on Ki67 staining. Both low and high Ki67 were more commonly found in TNBC with $CD44^{-}CD24^{+}$ (both 18 cases, 50%).

Discussion

MaSCs are marked with high CD44 and negative/low CD24 ($CD44^{+}CD24^{-/low}$) adhesion molecule expressions. $CD44^{+}CD24^{-/low}$ phenotype is often related to poor prognosis [11]. In this study, CD44, CD24, Claudin-7 and Twist-1 were used as molecular markers of TNBC stem cell-like subtypes; CK5, EMA for basal-like sub-types; and CK8/18 for luminal sub-types. Results from 67 TNBC cases showed marked heterogeneous and overlapping profiles.

After immunohistochemistry staining of 67 TNBC cases was seen, we concluded that the clinical application of dividing stem cell-like subtypes into SC-1, SC-2, and SC-3 would not be useful. The importance of this study was the identification of stemness which will influence therapy. The

classification of SC-1 to SC-3 can only help to facilitate understanding these complicated problems of ontogeny. In this study, $CD44^{-}CD24^{+}$ groups were very heterogeneous.

In conclusion, by using immunohistochemical staining panels, TNBC can be classified into stem cell-like basal, baso-luminal, and luminal subtypes. Differentiation signs (EMT) in basal, baso-luminal and luminal subtypes can be recognised with CD44, CD24 and Twist. Differentiation and EMT can cause heterogeneity in TNBC subtypes, and this influence in TNBC therapy. Stemness behaviour in stem cell-like subtypes is resistant to therapy. Besides that, Ki-67 expression shows the aggressiveness of tumours. In controlling the aggressiveness of tumours, effective medicines must be used to manage the cell cycle. Therefore, TNBC needs special attention to assist in more optimal handling.

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Basic Science

The Effect of Mesenchymal Stem Cell Wharton's Jelly on Matrix Metalloproteinase-1 and Interleukin-4 Levels in Osteoarthritis Rat Model

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Keywords: Matrix Metalloproteinase-1; Mesenchymal Stem Cell Wharton Jelly; Interleukin-4; Osteoarthritis

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Competing Interests: The authors have declared that no competing interests exist

Abstract

BACKGROUND: Osteoarthritis (OA) is generally considered a degenerative joint disease caused by biomechanical changes and the ageing process. In OA pathogenesis, the development of OA is thought to be regulated largely by excess matrix metalloproteinase (MMP), which contributes to the degradation of extracellular matrices such as MMP-1 and Interleukin-4.

AIM: This study aims to prove the influence of Mesenchymal Stem Cell Wharton Jelly on decreasing MMP-1 levels and increasing IL-4 which is a specific target as a target component in cases of osteoarthritis in vivo.

MATERIAL AND METHODS: This research is an experimental study with the design of Post-Test-Only Control Group Design. The sample consisted of 16 OA rats as a control group and 16 OA rats treated with MSC-WJ as a treatment group. OA induction is done by injection of monosodium iodoacetate (MIA) into the intra-articular right knee. Giving MSC-WJ is done in the third week after MIA induction. The serum MMP-1 and IL-4 levels were measured after 3 weeks treated with MSC-WJ using the ELISA method. The statistical test used is an independent t-test. The value of $p < 0.05$ was said to be statistically significant.

RESULTS: The result showed that serum MMP-1 levels were higher in the group treated with MSC-WJ than in the control group ($p < 0.05$). Serum IL-4 levels were higher in the group treated with MSC-WJ than in the control group ($p < 0.05$).

CONCLUSION: This study concluded that MSC-WJ increased MMP-1 levels and IL-4 levels in serum OA rats. MSC-WJ showed a negative effect on MMP-1 in the serum of OA rats.

Introduction

Osteoarthritis (OA) is considered a cumulative result of mechanical and biological events caused by an imbalance between catabolic and anabolic processes in articular joint tissue [1]. At present, the development of OA is thought to be regulated largely by excess matrix metalloproteinase (MMP), which contributes to the degradation of extracellular matrices, such as MMP-1 and MMP-3 which play an important role in the development of OA by decreasing extracellular matrix [2], where this MMP is induced by inflammatory mediators, such as interleukin-1-beta (IL-1β) and tumour necrosis factor alpha (TNF-α) in tissue and OA joint fluid [3]. So far there are no drugs available to guarantee a complete cure and the possibility of recurrence from OA.

Mesenchymal stem cells (MSCs) are promising candidates for cartilage regeneration and OA therapy because they have a chondrogenic potential and the ability to form extracellular matrices [4]. Also, MSC has an immunomodulatory and trophic capacity by secreting anti-inflammatory factors and growth factors [5], which might improve the inflammatory and catabolic aspects of OA.

Monosodium iodoacetate injection (MIA) to intra articular has been studied extensively as a model for OA in animals [6], [7] and is regarded as a suitable model and resembles a phenomenon observed in human OA [8].

Matrix metalloproteinase-1 is one of the protease enzymes that acts to degrade the components of the main cartilage matrix, such as collagen, aggrecan, link protein, and cartilage oligomer proteins [9], [10] while IL-4 and other cytokines are secreted in large amounts to counter the inflammatory response when Th2 dominates inflamed tissue because these immunomodulatory cytokines can reduce the production and activity of proinflammatory cytokines which are classified as inhibitors [11].

This study aims to prove the influence of Mesenchymal Stem Cell Wharton Jelly on decreasing MMP-1 levels and increasing IL-4 which is a specific target as a target component in cases of osteoarthritis in vivo.

Material and Methods

Animal and Experimental Design

Male, white rats (*Rattus novergicus*) with a weight ranging from 200-250 grams as experimental animals placed in clean, disinfected and pathogen-free cages and given standard food in the form of pellets and drinking in ad libitum. Trial animals adapted first for 1 week before treatment. Induction of osteoarthritis conducted with 300 μg intra-articular injection of monosodium iodoacetate (MIA) (Sigma Aldric, USA) in 50 μl of saline solution (0.9% NaCl) sterile (12) single into the right knee joint rats anaesthetized by intraperitoneal injection of xylazine 10 mg/kg and ketamine 20 mg/kg uses insulin syringe with a needle (*needle*) 27G. 32 osteoarthritis male, white rats (three weeks after MIA induction) were divided into 2 treatment groups (n = 16): Control group and MSC-WJ group. MSC group-WJ is given 50 μl MSC-WJ with a dose of 1 x 10^6 cells into the right knee joint and a control group given 50 μl complete medium after anaesthetized.

Mesenchymal Stem Cell Wharton Jelly was obtained from the Indonesian Medical Education and Research Institute (IMERI) Faculty of Medicine, University of Indonesia. Based on the analysis of flow cytometry, MSC-WJ used for this therapy had CD73-APC cell surface expression 99.8%, CD105-PerCP-Cys5.5 95% and CD90-FITC 99.9%. Rats were sacrificed after 3 weeks of treatment. Serum and knee joint were taken and then analysed.

Analysis of Flow Cytometry

Mesenchymal Stem Cell Wharton Jelly was obtained from the Indonesian Medical Education and Research Institute (IMERI) Faculty of Medicine, University of Indonesia. Based on the analysis of flow cytometry, MSC-WJ used for this therapy had CD73-APC cell surface expression 99.8%, CD105-PerCP-Cys5.5 95% and CD90-FITC 99.9%. The photocell was taken use Nikon Ti-S microscope. Scale bar: 500 μm.

Histological Analysis

The right knee joint from the two groups and the left knee joint (normal) was cut and fixed in 4% formalin for 1 weekend calcified with formic acid (5%) for 3 days. The specimen then underwent automatic network processing for 24 hours. The tissue planted in paraffin and cutting using a microtome with a thickness of 5 μm. Ribbon cutting results were placed on the surface of warm water with a temperature of 45°C to remove folds on the ribbon due to cutting. Every ribbon was stained with Hematoxylin and Eosin (H&E).

Measurement of serum MMP-1 and IL-4 by ELISA

Blood was taken from sinus periorbital and centrifuged at 3000 rpm for 15 minutes. The collected serum was stored at -80°C until measurement. Serum MMP-1 and IL-4 levels were measured by an ELISA kit (Bioassay Technology Laboratory, China). All samples are measured in duplicate.

Examination of MMP-1 Levels **(*Work protocol based on rat MMP-1 ELISA Kit)***

Prepare all reagents, standard solutions and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature. Determine the number of strips required for the assay. Insert the strips in the framers for use. The unused strips should be stored at 2-8°C. Add 50 μL standard well. Add 40 μL sample to sample wells and then add 10 μL anti-MMP-1 antibody to sample wells, then add 50 μL streptavidin-HRP to sample wells and standard wells (Not blank control well). Mix well. Cover the plate with a shaker. Incubate 60 minutes at 37°C. Removed the sealer and wash the plate 5 times with wash buffer. Soak wells with at least 0,35 ml wash buffer for 30 seconds to minute for each wash. For automated washing, aspirate all wells and wash 5 times with wash buffer, overfilling wells with wash buffer. Blot the plate onto paper towels or other absorbent material. Add 50 μL substrate solution A to each well and then add 50 μL substrate solution B to each well. Incubate plate covered with a new sealer for 10 minutes at 37°C in the dark. Add 50 μL

stop solution to each well; the blue colour will change into yellow immediately. Determine the optical density (OD value) of each well immediately using a microplate reader set a 450 nm within 30 min after adding the stop solution.

Examination of IL-4 Levels (Work protocol based on rat IL-4 ELISA Kit)

Prepare all reagents, standard solutions and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature. Determine the number of strips required for the assay. Insert the strips in the framers for use. The unused strips should be stored at 2-8°C. Add 50 µL standard well. Add 40 µL sample to sample wells and then add 10 µL anti-MMP-1 antibody to sample wells, then add 50 µL streptavidin-HRP to sample wells and standard wells (Not blank control well). Mix well. Cover the plate with a shaker. Incubate 60 minutes at 37°C. Removed the sealer and wash the plate 5 times with wash buffer. Soak wells with at least 0,35 ml wash buffer for 30 seconds to minute for each wash. For automated washing, aspirate all wells and wash 5 times with wash buffer, overfilling wells with wash buffer. Blot the plate onto paper towels or other absorbent material. Add 50 µL substrate solution A to each well and then add 50 µL substrate solution B to each well. Incubate plate covered with a new sealer for 10 minutes at 37°C in the dark. Add 50 µL stop solution to each well; the blue colour will change into yellow immediately. Determine the optical density (OD value) of each well immediately using a microplate reader set a 450 nm within 30 min after adding the stop solution.

Research Ethics

This study was already passed the ethics clearance and has been approved by the Ethics Committee of the Faculty of Medicine, Andalas University, Padang with registration number: 549/KEP/FK/2017.

Statistical analysis

Data is presented in mean and elementary forms. The statistical analysis used is SPSS 18.0. The statistical test used is an independent t-test. The value of $p < 0.05$ was said to be statistically significant.

Result

A study of 32 osteoarthritis rats induced with monosodium iodoacetate (MIA) for 3 weeks was carried out. OA rats were divided into 2 groups, namely the control group and the group treated with MSC-WJ (Figure 1). Three weeks after MSC therapy, termination was done at the expense of experimental animals. Then a histopathological examination of mouse right knee (OA) was carried out. Examination of the levels of MMP-1 and IL-4 was carried out in the serum of rats by ELISA.

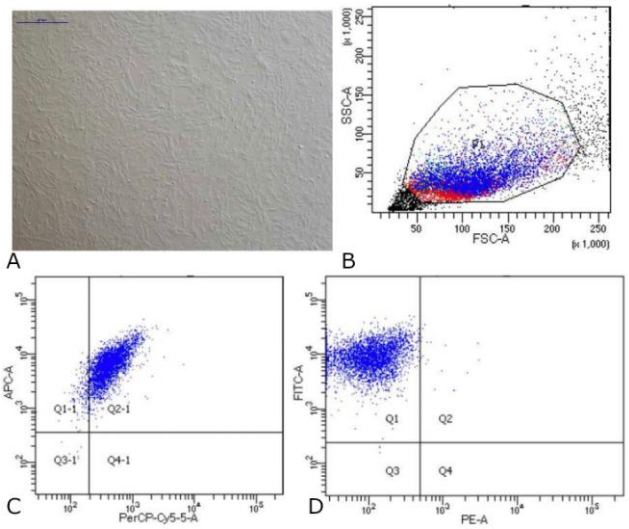

Figure 1: Data on Characteristics of Mesenchymal Stem Cells Wharton Jelly. (A) Cells MSC-WJ reach confluence. Scale bar: 500 µM. Photographs of cells taken using a Nikon Ti-S microscope; (B) Data flow cytometry. Forward scatter (FCS) plot&side scatter (SSC) plot. Population gated events (P1): 20,000; (C) Cell surface markers expression: CD73-APC 99.8% and CD105- PerCP-Cy5.5 95%; (D) Cell surface markers expression: CD90-FITC 99.9% and Lin (-) - PE 0.4%

Histopathology examination

After the bone portion of the rat's knee is obtained preserved with formalin buffer, Histopathological examination was carried out using Hematoxylin-Eosin staining; the results are shown in figure 2. The results we can see in tissue reactions are changes in the thickness of cartilage and the number of chondrocytes.

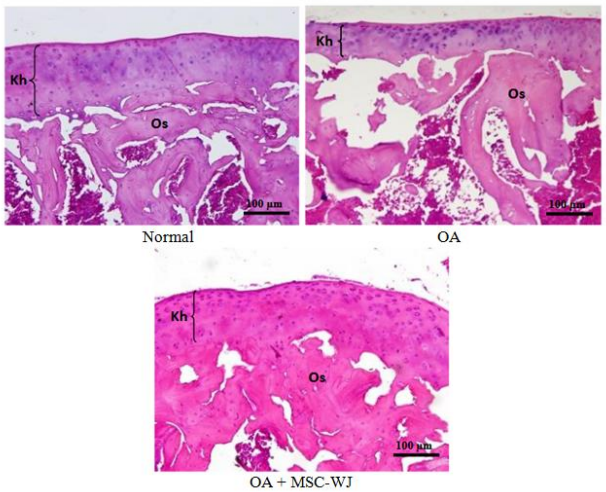

Figure 2: Microscopic joint tissue of experimental animals, showed joint fragility (Kh), bone tissue (Os) in normal rats, osteoarthritis (OA), and OA + MSC-WJ. Objective hematoxylin-eosin

Histopathological results show joint tissue with the surface consisting of cartilage with chondrocyte cells. In the OA group, there were areas with a thickness of cartilage that was thinner than normal animals and OA animals injected by MSC-WJ (Figure 2).

In addition to changes in the thickness of the cartilage, histopathological results also showed an increase in chondrocyte cell density in the OA mouse group compared to normal rats. In the OA group, of rats given MSC-WJ, the density of chondrocyte was close to the mean of normal rats.

ELISA examination

The blood obtained from the centrifuged animal is then obtained serum. Serum before analysis was stored in a refrigerator temperature of -80°C. The serum obtained was analysed for MMP-1 and IL-4 levels carried out in the Biomedical laboratory FK Unand.

The measurement of MMP-1 and IL-4 levels were carried out in normal rat, and the mean levels of MMP-1 and IL-4 were 1.62 ng/ml and 34.27 ng/ml. Based on the results of the normality test the data showed that the two research variables namely MMP-1 and IL-4 were normally distributed ($p > 0.05$). Thus, furthermore, parametric tests (free t-test) can be carried out.

Effect of MSC-WJ on serum MMP-1 levels in OA rats treated with MSC-WJ

The measurement of MMP-1 levels by ELISA method showed that the serum MMP-1 levels of OA rat treated with MSC-WJ were higher than those not treated which can be seen in Figure 3.

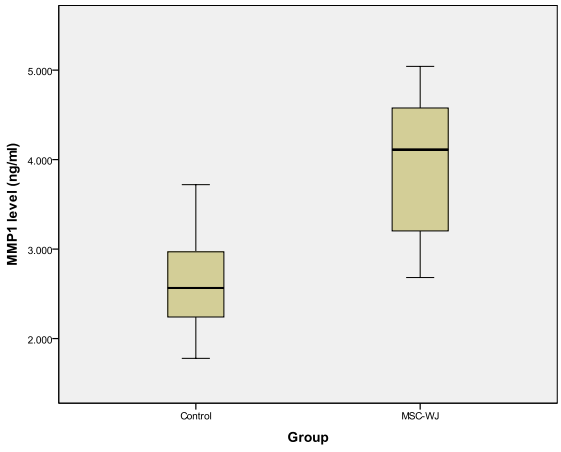

Figure 3: Boxplot graph of rat serum MMP-1 levels

The difference in MMP-1 levels between serum of rats treated with MSC-WJ and control can be seen in Table 1.

Table 1: Differences in mean levels of MMP-1 by group

Groups	MMP-1 levels (ng/ml) (Mean ± SD)	P value
Control	2.63 ± 0.55	0.001
MSC-WJ	3.96 ± 0.81	

Table 1 showed that there are differences in MMP-1 levels based on treatment. Increased levels of MMP-1 in the group treated with MSC-WJ from the control group. There were significant differences, between MSC-WJ with control ($p < 0.05$).

Effect of MSC-WJ on IL-4 levels in serum of OA rats

The results of measurement of IL-4 levels by ELISA method showed that the serum IL-4 levels of OA rats treated with MSC-WJ were higher than those not treated with bivariate tests which can be seen in Figure 4.

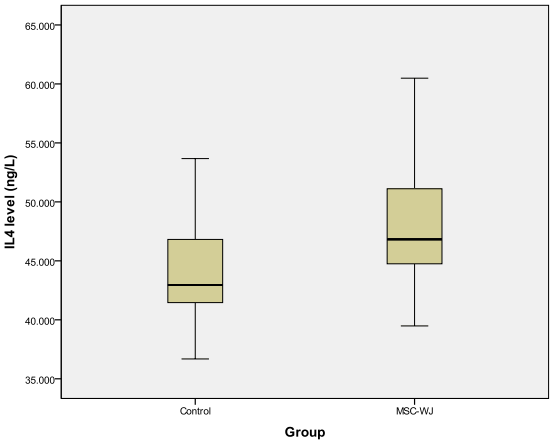

Figure 4: Boxplot graph of rat serum IL-4 levels

The difference in IL-4 levels between serum of rats treated with MSC-WJ and control can be seen in Table 2.

Table 2 Mean differences in IL-4 levels by group

Groups	IL-4 Levels (ng/l) (Mean ± SD)	P value
Control	43.90 ± 4.99	0.027
MSC-WJ	47.95 ± 4.88	

Table 2 showed that there are differences in IL-4 levels based on treatment. Increased IL-4 levels in the group treated with MSC-WJ from the control group. There were significant differences, between MSC-WJ with control ($p < 0.05$).

Discussion

The results showed that MIA induction in rats with a single dose of 300 μg after 3 weeks showed the

reduced thickness of cartilage. The results of this study are also the same as those conducted by Janusz *et al.,* (2001) regarding the effect of MIA on rat cartilage, wherein the study there was also reduced of cartilage in the surrounding area after one week of MIA induction [13].

This reduced cartilage occurs due to the loss of proteoglycans that build up the matrix. Also, the results of this study also showed a form of reactive chondroblast cell proliferation, so that cell densities were higher than those of OA rat cartilage that was not treated with MSC-WJ.

The results of the serum analysis using the ELISA method showed a tendency to increase serum MMP-1 and IL-4 levels compared to the serum of non-induced (normal) rat. This increase is due to chondrocyte cells and immune cells stimulated by inflammatory cytokines (IL-β and TNF-α) whose levels increase when inflammation occurs due to MIA induction.

Increased matrix-degrading enzymes and anti-inflammatory cytokines after MIA induction showed that rats experienced osteoarthritis. Induction of MSC-WJ in OA rat for 3 weeks showed a thickness of joint cartilage close to the thickness of normal cartilage and showed lower cell density closer to normal compared to OA rat. This shows the appearance of tissue repair in osteoarthritis by MSC-WJ.

Matrix metalloproteinase-1 (MMP-1)

Matrix metalloproteinase-1 is one of the protease enzymes that act to degrade the components of the main cartilage matrix, such as collagen, aggrecan, link protein, and cartilage oligomer proteins [14], [15]. This enzyme also functions to increase the proliferation and migration of MSCs [16]. MMP-1 increases production in synovial membranes, synovial fluid, and human cartilage that undergo OA [17], also in the serum of OA rat significantly [2]. The release of MMP-1 protein by human articular chondrocyte is stimulated by IL-1β [18].

This study showed that the serum MMP-1 levels of OA rat treated with MSC-WJ were higher than those not treated. Research by Saulnier *et al.,* (2015) showed that the administration of MSC in rabbits OA had was not effective in reducing the expression of MMP-1 after 2 weeks of injection with MSC, but effectively reduced MMP-1 expression after 8 weeks of injection [19].

The results of this study indicate that MSC-WJ therapy has not been effective in reducing MMP-1 levels within 3 weeks after injection. Possibly in this period, MSC-WJ was still synthesising and releasing MMP-1 which is needed for the apoptosis of chondrocyte and synovial cells [20], the process of MSC migration and proliferation [16] and differentiation. Ho *et al.,* (2009) showed that MMP-1 plays an important role in the MSC migration function, which operates through MMP1-PAR1 axis signalling [21]. According to Voronkina *et al.,* (2017) that the involvement of MMP-1 is in the process of MSC differentiation, namely the increase in MMP-1 activity during the differentiation process [22].

The high levels of MMP-1 in OA mice treated with MSC-WJ were compared with OA mice that were not treated because in this period MSC expressed MMP-1 constitutively [23]. It is possible that MSC-WJ requires MMP-1 in repairing cell damage because it involves the process of apoptosis, migration and MSC differentiation and proliferation.

Interleukin-4 (IL-4)

Interleukin-4 (IL-4) is an anti-inflammatory cytokine that plays a role in stimulating the proliferation of B cells and T cells and encourages differentiation of CD4 + T cells into Th2. IL-4 also plays a key role in regulating humoral and adaptive immunity. IL-4 and other cytokines are secreted in large amounts to counter the inflammatory response when Th2 dominates inflamed tissue. Because these immunomodulatory cytokines can reduce the production and activity of proinflammatory cytokines which are classified as inhibitors [11].

Research by Hui *et al.,* (2005) found that IL-4 was reported to play a role in the regulation of ADAMTS-4 in chondrocyte, although the exact mechanism has not been explained [24]. The proteins secreted by rat MSC and humans are chemokines, cytokines, growth factors and protease inhibitors [25], including IL-4, IL-10, and IL-13 which are anti-inflammatory cytokines [26].

In this study, it was found that IL-4 levels were higher in the serum of OA rat treated with MSC-WJ compared with those not treated. The same thing was found in the study of Kay *et al.,* (2017) which showed that IL-4 expression was higher in arthritis rats given MSC compared to those not given [27]. Whereas Yan *et al.,* (2017) research found that serum IL-4 levels of arthritis rats treated with MSC were compared with serum of non-treated arthritis rats, but the difference was not significant [28]. Chai *et al.,* (2016) also found the same thing that UC-MSC can increase IL-4 in liver fibrosis both in vitro and in vivo [29].

Increased levels of IL-4 in the serum of OA mice show that MSC-WJ can increase the immunosuppressive activity of Th2 cells. MSC increases anti-inflammatory cytokines such as IL-4 which have anti-inflammatory effects through inhibition of the NF-κB cascade, which contributes to the regulation of proinflammatory cytokines [30]. MSC plays an important role in cartilage repair through direct differentiation into chondrocyte and paracrine effects [31], [32].

Information about the increase in expression in the form of serum IL-4 proteins in this study is important for the development of MSC-WJ as a therapy for osteoarthritis. The development of MSC-WJ as a therapy for osteoarthritis is quite promising. Differences in IL-4 levels between serum of rats treated and those not treated with MSC-WJ proved that MSC-WJ had a therapeutic effect. Thus, the information obtained encourages further studies of MSC-WJ as a treatment for OA.

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Basic Science

Hepatoprotective Activity and Nephroprotective Activity of Peel Extract from Three Varieties of the Passion Fruit (*Passiflora Sp.*) in the Albino Rat

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Abstract

BACKGROUND: The Passion Fruit (*Passiflora sp.*) that grows in the Indonesian region generally has three varieties, namely purple passion fruit (*Passiflora edulis Sims*.), red passion fruit (*Passiflora ligularis Juss*.), and yellow passion fruit (*Passiflora verrucifera Lindl*.). The passion fruit peel is an economic waste that has not been utilised optimally, but has many efficacious phytochemical contents.

AIM: The objectives of this research are to examine scientifically hepatoprotective activity (with paracetamol-induced hepatotoxic) and nephroprotective activity (with gentamicin-induced nephrotoxic) from three varieties of the passion fruit (purple passion fruit peel extract, red passion fruit peel extract and yellow passion fruit peel extract) in the albino rat (*Rattus norvegicus*).

METHODS: Three varieties of passion fruit peels were extracted by maceration method. The experimental animals used were the albino rat (*Rattus norvegicus*). Hepatoprotective activity was done by the liver biochemical (alanine transaminase and aspartate transaminase) analysis with paracetamol (hepatotoxic compound) induced after 10 days of treatment with extract. Nephroprotective activity was done by the kidney biochemical (urea and creatinine) analysis with gentamicin (nephrotoxic compound) induced after 10 days of treatment with extract.

RESULTS: The hepatoprotective activity for positive control was similar to the 250 mg of purple passion fruit peel extract per kg of body weight, 250 mg of red passion fruit peel extract per kg of body weight, and 500 mg of yellow passion fruit peel extract per kg of body weight. The nephroprotective activity for positive control (50 mg of silymarin per kg of body weight) was similar to the 250 mg of purple passion fruit peel extract per kg of body weight, 500 mg of red passion fruit peel extract per kg of body weight, and 500 mg of yellow passion fruit peel extract per kg of body weight.

CONCLUSIONS: The extracts were shown hepatoprotective activity and nephroprotective activity with a dose-dependent activity. The hepatoprotective activity and nephroprotective activity of purple passion fruit peel extract were the best compared to red passion fruit peel extract and yellow passion fruit peel extract.

Introduction

The liver is an important organ in the body that has an important function, which is: bile secretion, bilirubin metabolism, nutritional metabolism, producing immune agents to control infections, and metabolism of foreign molecules (exogenous chemical and endogenous chemical). The liver is the metabolic centre in the body, especially those given orally. Drug metabolism in the liver occurs in microsome cells through an enzyme system that is very complex through two phases. The first phase includes oxidation reactions, reduction reactions, and hydrolysis reactions. The second phase includes conjugation reactions. Liver damage can be caused by a viral of hepatitis or cirrhosis; drugs can cause toxic effects on the liver resulting in liver damage [1].

The kidney is an important organ that has an important role in the body for blood filtration to excrete

waste products, balance electrolytes in the body, control blood pressure, stimulate the production of red blood cells, and regulate the balance of water and metabolites in the body and maintain acid-base balance in the blood. Drugs and metabolic results of drugs that are in the blood and are no longer used in the body will be excreted by the kidneys through urine. Urine will leave the kidneys to the urinary tract to be removed from the body. Most drugs are excreted by the kidneys, so the use of drugs that exceed the therapeutic dosage can damage the kidneys [2].

Paracetamol is a drug with analgesic and antipyretic effects that is widely used by the wider community. Paracetamol is an over the counter class drug that can be traded freely without supervision, but this drug causes liver damage in the use of high doses. Paracetamol is predicted to be a major factor in the cause of acute liver damage [3], [4]. Gentamicin is an antibiotic derived from aminoglycosides, which is generally an option in the treatment of infections that are needed immediately if a condition that can endanger life is found. Gentamicin is a broad-spectrum antibiotic that has bactericidal activity against gram-positive bacteria and gram-negative bacteria. Gentamicin can cause kidney damage because it rapidly induces extensive renal cortical necrosis with renal dysfunction [5], [6].

The passion fruit is a plant that originates from Brazil and spreads to all countries of the world. The passion fruit can grow in subtropical regions or highland in tropical regions [7]. The passion fruit can be found in several regions in Indonesia; one region in Indonesia that can cultivate the passion fruit is North Sumatera region (Karo district, Simalungun district, Dairi district, and North Tapanuli district). The passion fruit has many varieties that cultivate in all countries of the world, but the passion fruit that grows and develops in Indonesia are three varieties, namely: purple passion fruit, red passion fruit, and yellow passion fruit [8], [9]. Figure 1 shows the physical appearance of purple passion fruit, red passion fruit, and yellow passion fruit.

Figure 1: Physical appearance of purple passion fruit, red passion fruit, and yellow passion fruit

The passion fruit is generally consumed on the inside of the fruit, because the inside of the fruit is the edible portion, and the skin is a waste that has not been utilised optimally [10]. The passion fruit peel has many different phytochemical contents, including alkaloids, flavonoids, steroids, triterpenoids, saponins, tannins, glycosides, and phenolic [11], [12], [13]. The passion fruit peel has also been tested for pharmacological activity as an antioxidant [12], [14], [15], [16], an antimicrobial [13], [15], [16], a neuroprotective [15], [17], a cardioprotective [18], a gastroprotective [19], an analgesic [20], an anti-inflammation [21], an antihypertriglyceridemic [21], and an antihyperglycemic [21], [22].

The hepatoprotective activity and nephroprotective activity of the passion fruit peel extract have never been reported in the research article. But with the abundance of phytochemical content found in the passion fruit peel extract, an extract that is likely to provide hepatoprotective activity [23] and nephroprotective activity [24].

This study aims to examine scientifically hepatoprotective activity (with paracetamol-induced hepatotoxic) and nephroprotective activity (with gentamicin-induced nephrotoxic) from three varieties of the passion fruit (*Passiflora sp.*) (purple passion fruit peel extract, red passion fruit peel extract and yellow passion fruit peel extract) in the albino rat (*Rattus norvegicus*).

Methods

Research

This research was conducted by experimental research. The independent variables were type of the extract (purple passion fruit peel extract, red passion fruit peel extract, and yellow passion fruit peel extract) and dose of the extract (100 mg extract per kg of body weight, 200 mg extract per kg of body weight, and 400 mg extract per kg of body weight). The dependent variables were level of the hepatotoxic or hepatoprotective biochemical marker (alanine transaminase and aspartate transaminase) in the serum and nephrotoxic or nephroprotective biochemical marker (urea and creatinine) in the serum. These research samples were purple passion fruit peel and yellow passion fruit peel obtained from the passion fruit farmer. The passion fruit growth in Gundaling, Berastagi, Karo, Sumatera Utara, 22152, Indonesia. The growth condition was a suitable condition for the passion fruit growth with 1,375 meters above sea level for the average altitude, 16°C to 26°C for the temperature, 60% to 100% for the relative humidity, 2100 mm to 3200 mm for the rainfall, 5 to 6 for the soil pH, 1009 HPa to 1015 HPa for the atmospheric pressure, 4 km/hr to 11 km/hr for the wind speed. Harvest of purple passion fruit, red passion fruit, and yellow passion fruit was done in 85th days to 90th days after the flowers bloom.

Materials and Tools

The materials used in this research were purple passion fruit peel, red passion fruit peel, yellow passion fruit peel, ethanol (E-Merck), ethylene diamine tetraacetic acid (E-Merck), alanine transaminase activity assay kit (Sigma Aldrich), aspartate transaminase activity assay kit (Sigma Aldrich), urea assay kit (Sigma Aldrich), creatinine assay kit (Sigma Aldrich), sodium carboxymethyl cellulose (Tokyo Chemical Industry), and demineralized water (Brataco).

The tools used in this research were cutter (Kenko), drying cabinet (Alumex), blender (Miyako), balance (Mettler Toledo), analytical balance (Mettler Toledo), filter paper (Whatman), dropper (Iwaki), evaporating dish (Iwaki), maceration vessel (Iwaki), beaker glass (Iwaki), measuring glass (Iwaki), volumetric flask (Iwaki), test tube (Iwaki), hot plate stirrer (Thermo), water bath (Memmert), incubator (Memmert), rotary evaporator (Buchi), thermometer (Lutron), test sieve 10 (Retsch), stopwatch (Casio), spectrophotometer (Agilent).

Samples Identification and Samples Extraction

Samples used in this research were collected and identified at Herbarium Medanense (MEDA), Faculty of Mathematics and Natural Sciences, University of Sumatera Utara, Padang Bulan, Medan Baru, Medan, Sumatera Utara, 20155, Indonesia. The extraction process was based on a modification of the maceration method from Irawan et al., 2018 and Zhang et al., 2018. The passion fruit peel is washed, cut, dried, and powdered. One kilogram of dried passion fruit peel powder was weighed, soaked with 10 L ethanol for 5 days (stirred every day), and filtered. The residue was squeezed, soaked with 5 L ethanol for 3 days (stirred every day), and filtered (the maceration procedure was repeated several times until clear extract was obtained). The aqueous extract was collected and evaporated with a rotary evaporator until a viscous extract was obtained [25], [26]. The extracts obtained were used for hepatoprotective activity test and nephroprotective activity test.

Hepatoprotective Activity Test and Nephroprotective Activity Test

The method used for nephroprotective activity and hepatoprotective activity is a modification of the method from Abel-Hady et al., 2018; Fatima and Sultana, 2018; Tung et al., 2017; Okokon et al., 2017; and Thuwaini et al., 2016. The animals used were the Albino Rat (*Rattus norvegicus*) with Wistar strain which was 8 weeks old-12 weeks and weighed 250 g ± 10 g. The animals were kept for 5 days before the experiments. The animals were kept in standard conditions (12 hours light and 12 hours dark cycle), with temperatures of 23°C ± 2°C, and with relative humidity 50% ± 10%. Experimental animals were divided into 12 groups for testing hepatoprotective activity and 12 groups for testing nephroprotective activity with each group consisting of 10 animals. Table 1 shows the treatment given to each group of experimental animals for 10 days and after 10 days [27], [28], [29], [30], [31].

Table 1: Treatment given to each group of experimental animals for 10 days and after 10 days

Number	Given	Group											
		1	2	3	4	5	6	7	8	9	10	11	12
1	Protection with Standard Protector for 10 Days (mg of Curcumin or mg of Silymarin per kg of body weight) by Oral	-	-	✓	-	-	-	-	-	-	-	-	-
2	Purple Passion Fruit Peel Extract for 10 Days (mg of Purple Passion Fruit Peel Extract per kg of body weight) by Oral	-	-	-	✓	✓	✓	-	-	-	-	-	-
3	Red Passion Fruit Peel Extract for 10 Days (mg of Red Passion Fruit Peel Extract per kg of body weight) by Oral	-	-	-	-	-	-	✓	✓	✓	-	-	-
4	Yellow Passion Fruit Peel Extract for 10 Days (mg of Yellow Passion Fruit Peel Extract per kg of body weight) by Oral	-	-	-	-	-	-	-	-	-	✓	✓	✓
5	Induction with Toxic Inducer after 10 Days (mg of Paracetamol or mg of Gentamicin per kg of body weight) by Intraperitoneal	-	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

All groups (group 1 to group 12) were given food and drink ad libitum. The protector used were 250 mg of curcumin per kg of body weight (for hepatoprotective activity) and 50 mg of silymarin per kg of body weight (for nephroprotective activity). The inducer used were 500 mg of paracetamol per kg of body weight (for hepatotoxic activity) and 125 mg of gentamicin per kg of body weight (for nephrotoxic activity). The samples used were purple passion fruit peel extract, red passion fruit peel extract and yellow passion fruit peel extract. Each extract was used with various concentrations (125 mg of extract per kg of body weight, 250 mg of extract per kg of body weight, and 500 mg of extract per kg of body weight). The animals from all groups were sacrificed under diethyl ether anaesthesia, and then the blood samples were collected after 24-hour induction with toxic inducer by cardiac puncture by using 21 gauge needles mounted on a 5 ml syringe into ethylene diamine tetraacetic acid.

The blood samples obtained were centrifuged at 3000 rotations per minute for 15 minutes. Serum obtained were used for the biochemical analysis with hepatoprotective activity parameters (alanine transaminase and aspartate transaminase) and nephroprotective activity parameters (urea and creatinine) [27], [28], [29], [30], [31]. The biochemical analysis of hepatoprotective activity parameters (alanine transaminase and aspartate transaminase) and the biochemical analysis of nephroprotective activity parameters (urea and creatinine) were carried out by the standard method from Sigma Aldrich.

Results

Increasing the dose of administration will increase the hepatoprotective activity and the nephroprotective activity. This can be seen from the results that increase treatment dose will improve the liver function through a decrease in the alanine transaminase value and aspartate transaminase value and improve the kidney function through a decrease in the urea value and creatinine value so that it can be seen that purple passion fruit peel extract, red passion fruit peel extract, and yellow passion fruit peel extract have a dose-dependent hepatoprotective activity and dose-dependent nephroprotective activity. Table 2 shows the results of treatment of hepatoprotective activity test (alanine transaminase and aspartate transaminase) and treatment of nephroprotective activity test (urea and creatinine).

Table 2: Results of treatment of hepatoprotective activity test (alanine transaminase and aspartate transaminase) and treatment of nephroprotective activity test (urea and creatinine)

Number	Treatment		Alanine Transaminase (IU/L)	Aspartate Transaminase (IU/L)	Urea (mg/dL)	Creatinine (mg/dL)
1	Control	Normal	27.45 ± 2.84 $^{\#\dagger}$	33.19 ± 3.11 $^{\#\dagger}$	13.12 ± 1.51 $^{\#\dagger}$	0.441 ± 0.024 $^{\#\dagger}$
2		Negative	95.84 ± 9.87 *‡	116.17 ± 10.94 *‡	77.64 ± 9.12 *‡	7.419 ± 0.409 *‡
3		Positive	32.34 ± 3.41 $^{*\#}$	38.09 ± 3.61 $^{*\#}$	17.57 ± 2.02 $^{*\#}$	0.521 ± 0.029 $^{*\#}$
4	Purple Passion Fruit Peel Extract (mg of Purple Passion Fruit Peel Extract per kg of body weight)	125	40.97 ± 4.18 $^{*\#\ddagger}$	54.07 ± 4.94 $^{*\#\ddagger}$	24.14 ± 3.24 $^{*\#\ddagger}$	0.559 ± 0.031 $^{*\#\ddagger}$
5		250	32.59 ± 3.32 $^{*\#}$	38.33 ± 3.71 $^{*\#}$	17.67 ± 2.25 $^{*\#}$	0.518 ± 0.028 $^{*\#}$
6		500	30.84 ± 3.22 $^{*\#\dagger}$	33.55 ± 3.09 $^{*\#\dagger}$	13.19 ± 1.95 $^{*\#\dagger}$	0.500 ± 0.026 $^{*\#\dagger}$
7	Red Passion Fruit Peel Extract (mg of Red Passion Fruit Peel Extract per kg of body weight)	125	41.15 ± 4.19 $^{*\#\ddagger}$	53.57 ± 4.91 $^{*\#\ddagger}$	31.99 ± 4.08 $^{*\#\ddagger}$	0.675 ± 0.042 $^{*\#\ddagger}$
8		250	32.13 ± 3.30 $^{*\#}$	38.69 ± 3.76 $^{*\#}$	24.56 ± 3.13 $^{*\#\ddagger}$	0.595 ± 0.036 $^{*\#\ddagger}$
9		500	30.34 ± 3.18 $^{*\#\dagger}$	33.99 ± 3.12 $^{*\#\dagger}$	17.69 ± 2.52 $^{*\#}$	0.534 ± 0.032 $^{*\#}$
10	Yellow Passion Fruit Peel Extract (mg of Yellow Passion Fruit Peel Extract per kg of body weight)	125	66.21 ± 6.94 $^{*\#\ddagger}$	75.04 ± 7.14 $^{*\#\ddagger}$	32.24 ± 4.10 $^{*\#\ddagger}$	0.671 ± 0.041 $^{*\#\ddagger}$
11		250	43.75 ± 4.49 $^{*\#\ddagger}$	49.74 ± 4.71 $^{*\#\ddagger}$	24.22 ± 3.07 $^{*\#\ddagger}$	0.591 ± 0.035 $^{*\#\ddagger}$
12		500	32.94 ± 3.53 $^{*\#}$	37.88 ± 3.48 $^{*\#}$	18.01 ± 2.59 $^{*\#}$	0.530 ± 0.030 $^{*\#}$

Note: value represents as mean ± standard deviation; $^{*}p < 0.05$, a significant difference compared with the normal control group (n = 10); $^{\#}p < 0.05$, a significant difference compared with the negative control group (n = 10); $^{\dagger}p < 0.05$, a significant difference (lower) compared with the positive control group (n = 10) $^{\ddagger}p < 0.05$, a significant difference (higher) compared with the positive control group (n = 10).

The normal control group that was not given a protector and was not given an inducer showed normal liver biochemical value (alanine transaminase value < 35.0 IU/L and aspartate transaminase value < 45.0 IU/L) and normal kidney biochemical value (urea value < 24.0 mg/dL and creatinine value < 1.20 mg/dL). The negative control group that was not given a protector and was given an inducer showed significantly increased in liver biochemical value (alanine transaminase value > 35.0 IU/L and aspartate transaminase value > 45.0 IU/L) and significantly increased in kidney biochemical value (urea value > 24.0 mg/dL and creatinine value > 1.20 mg/dL) compared to the normal control group. This indicates that the hepatotoxic inducer given and nephrotoxic inducer given have been able to cause hepatotoxic in the liver organ and nephrotoxic in the kidney organ.

The positive control group that was given a protector and was given an inducer showed significantly decreased in liver biochemical value (alanine transaminase value and aspartate transaminase value) and significantly decreased in kidney biochemical value (urea value and creatinine value) compared to the normal control group and the negative control group. Although the positive control group results showed significantly different from the normal control group, but the results obtained were within the normal range of the biochemical value (alanine transaminase value < 35.0 IU/L and aspartate transaminase value < 45.0 IU/L) and normal range of kidney biochemical value (urea value < 24.0 mg/dL and creatinine value < 1.20 mg/dL).

The test group that was given a passion fruit extract and was given an inducer showed significantly decreased in liver biochemical value (alanine transaminase value and aspartate transaminase value) and significantly decreased in kidney biochemical value (urea value and creatinine value) compared to the negative control group. This result means that the passion fruit peel extract has hepatoprotective activity and has nephroprotective activity. But only several treatment doses of the passion fruit peel extract were given a similar (not significantly different) to the positive control group.

The hepatoprotective activity for positive control (250 mg of curcumin per kg of body weight) was similar (not significantly different) to the 250 mg of purple passion fruit peel extract per kg of body weight, 250 mg of red passion fruit peel extract per kg of body weight, and 500 mg of yellow passion fruit peel extract per kg of body weight. The nephroprotective activity for positive control (50 mg of silymarin per kg of body weight) was similar (not significantly different) to the 250 mg of purple passion fruit peel extract per kg of body weight, 500 mg of red passion fruit peel extract per kg of body weight, and 500 mg of yellow passion fruit peel extract per kg of body weight.

Discussions

Transaminase is a type of intracellular enzyme that is involved in nutrition metabolism. Transaminase enzymes are present in the cells of several organs such as the heart, liver, kidneys and pancreas. The alanine transaminase enzyme and the aspartate transaminase enzyme are present in hepatocytes cell and are released into the bloodstream in response to hepatocyte injury or death (hepatitis). Elevation of alanine transaminase enzyme or aspartate transaminase enzyme on the blood test for liver profile indicates the abnormality in the liver

organ. The good liver function will give a low value for alanine transaminase parameters and aspartate transaminase parameters [32], [33], [34], [35].

Both urea and creatinine are metabolic waste products that are excreted by the kidneys through urine, and only a small amount is left in the blood. If there is a disruption in kidney function, then there is an increase in these two parameters. Certain medical conditions can cause an increase in these two parameters, for example, chronic uncontrolled hypertension, uncontrolled diabetes mellitus, kidney stones, kidney inflammation, kidney infection, dehydration, and others [36], [37], [38], [39].

The hepatoprotective activity and the nephroprotective activity of an extract, in general, is a dose-dependent activity [40]. Purple passion fruit peel extract has the best hepatoprotective activity and the best nephroprotective activity compared to red passion fruit peel extract and yellow passion fruit peel extract. Although the purple passion fruit has the same genus with the red passion fruit and has the same species with the yellow passion fruit, due to genetic differences (differences in species or differences in varieties) there are morphological differences (colors and sizes) and genetical differences (sequences) [41], [42]. Also, due to differences in species or differences in varieties, it can cause qualitative differences in phytochemical contents and/or quantitative differences in phytochemical contents, also pharmacological differences and toxicological differences [43], [44].

The hepatoprotective activity possessed by the passion fruit peel extract may be due to the phytochemical content in the passion fruit peel. The phytochemical content in an extract is a good prospect for the development of phytotherapy [45], [46], [47]. The greater the content of alkaloids, flavonoids, and saponins in an extract, the higher the hepatoprotective activity possessed by the extract. Alkaloids are found in abundance in almost all parts of plants and have activities in scavenging the reactive oxygen species which can damage hepatocytes and are often useful compounds in medicinal chemistry for the development of new drugs. Flavonoids are polyphenol compounds that have been proven for hepatocytes protection from free radical scavenging activity. Saponins can directly protect hepatocytes from apoptosis through a mechanism of inhibition of the production of Tumor Nuclear Factor Alpha (TNFα) [48], [49], [50].

The nephroprotective activity of the passion fruit peel extract is produced by the complex phytochemical content and varied phytochemical content contained in the passion fruit peel. An extract is thought to have nephroprotective activity through the mechanism of its antioxidant activity [51]. High flavonoid content extracts are reported to have high nephroprotective activity through an antioxidant mechanism [52]. The nephroprotective mechanism is also shown by tannins compounds and which have good antioxidant activity [53]. Antioxidant compounds can protect the kidneys from alteration of kidney proteins and prevented the medical histopathological change of kidney tissue [54].

In conclusion, the extracts were shown a dose-dependent activity in hepatoprotective activity and nephroprotective activity. Purple passion fruit peel extract and red passion fruit peel extract have better hepatoprotective activity than yellow passion fruit peel extract. Purple passion fruit peel extract has better nephroprotective activity than red passion fruit peel extract and yellow passion fruit peel extract. The best hepatoprotective activity and nephroprotective activity showed by purple passion fruit peel extract compared to red passion fruit peel extract and yellow passion fruit peel extract.

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Basic Science

Analysis of the Relationship between *RELA* Gene Expression and *MMP-13* Gene Expression in Synoviocyte Cells after Mesenchymal Stem Cell Wharton Jelly

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Abstract

BACKGROUND: Therapy that can cure osteoarthritis with satisfactory results has not been found to date. In the pathogenesis of osteoarthritis, the genes involved in cartilage degradation include the *RELA* gene which plays an important role in modulating the occurrence of cartilage damage, which involves activation of pro-inflammatory cytokines. One of the cytokines involved in the cartilage degradation process is Matrix Metalloproteinase (MMP) - 13 which is also modulated by NFκβ.

AIM: This study aims to look at the expression of the *RELA* gene and expression of the MMP-13 gene and analyse the relationship of *RELA* gene expression with MMP-13 gene expression after administration of Mesenchymal Stem Cell Wharton Jelly in synoviocytes in vitro.

MATERIAL AND METHODS: This research is pure experimental research. The samples used derived from synovial tissue in osteoarthritis patients who underwent surgery for Total Knee Replacement (TKR). This study was divided into 6 treatment groups with 4 replications. Group I was the synoviocyte OA cell control group which was incubated 24 hours, group II was control of synoviocyte OA cell which was incubated 48 hours, group III was a group of Mesenchymal Stem Cell Wharton Jelly (MSC-WJ) which was incubated 24 hours, group IV was a Mesenchymal Stem Cell Wharton Jelly (MSC-WJ) cell group incubated 48 hours, group V was the co-culture group of synoviocyte-MSC-WJ cells incubated 24 hours and group VI was the co-culture of synoviocyte-MSC-WJ cells which were incubated 48 hours. Observation of *MMP-13* gene expression and *RELA* gene in each group was carried out using qPCR.

RESULT: The results showed that the analysis of the relationship between *RELA* gene expression and MMP-13 gene expression in osteoarthritis synoviocytes cells after Mesenchymal Stem Cell Wharton Jelly as big as (r = 0.662).

CONCLUSION: The conclusion of this study is there was a strong correlation between *RELA* gene expression and MMP-13 gene expression in osteoarthritis synoviocytes after Mesenchymal Stem Cell Wharton Jelly (r = 0.662).

Introduction

Knee osteoarthritis is a degenerative disease, in the form of a "wear and tear" process in the joints as a result of the ageing process and is local [1]. At the molecular level, the imbalance between catabolic and anabolic activities where the primary injury response occurs in joint cartilage resulting in osteoarthritis [2]. The expression of several genes involved in the inflammatory response and cartilage degradation, such as IL-1 and TNF-α is regulated predominantly by Nuclear Factor Kappa Beta (NFκβ). NFκβ stimulates the cytokines of TNF-α and IL-1β which contribute to the inflammatory process in osteoarthritis. NFκβ is also needed for the transcription of the Matrix Metalloproteinase (*MMP-13*) gene [3]. *RELA* is a subunit of the NFκβ p65 gene which plays an important role in the pathogenesis of osteoarthritis.

The rapid development of stem cell science has broadened the picture of potential stem cells in the world of research and the medical world, a number of characteristics that stem cells have proven to provide great hope for healing many people who suffer from diseases that are no longer possible to be

treated conservatively or operatively, especially degenerative diseases and abnormalities such as trauma, malignancy and so on which also increase dramatically [4].

This study aims to look at the expression of the *RELA* gene and expression of the *MMP-13* gene and analyse the relationship of *RELA* gene expression with *MMP-13* gene expression after administration of Mesenchymal Stem Cell Wharton Jelly to synoviocytes in vitro.

Material and Methods

This research is a pure experimental study which is divided into 6 treatment groups with 4 number of replications. Group I was a control group of Osteoarthritis (OA) synoviocytes (incubated for 24 hours), group II was a control group of OA synoviocyte cells incubated for 48 hours, group III was a Mesenchymal Stem Cell Wharton Jelly (MSC-WJ) cell group incubated for 24 hours, group IV is a cell group Wharton's Jelly Mesenchymal Stem cell (MSC-WJ) were incubated for 48 hours, the group V is a group of co-culture synoviocyte-MSC-WJ cells were incubated for 24 hours and the group VI is a group of co-culture cell synoviocyte-MSC-WJ were incubated for 48 hours. The number of cells used in each treatment group was 105cells, each for synoviocytes and MSC-WJ cells. Mesenchymal Stem Cell Wharton Jelly comes from IMERI (Indonesian Medical Education and Research Institute), Faculty of Medicine, University of Indonesia. Synoviocyte cells are derived from synovial tissue of patients with grade IV Osteoarthritis who undergo knee joint surgery (Total Knee Replacement) at Dr Hospital. M. Djamil Padang, Indonesia. The synoviocyte cells taken for treatment were the result of 3rd phase cell culture. The samples taken did not use informed consent because the samples used were stored biologically after post-knee joint surgery Osteoarthritis Grade IV. Samples were taken from six patients with male sex aged 40-70 years.

Isolation of OA Primary Cells

Synovial tissue and search are obtained from OA patients after Total Knee Arthroplasty. Ten samples were used for experiments. Synovial tissue is planted in the well plate with 10% Fetal Bovine Serum (FBS), 1% penicillin/streptomycin and 1% fungizone in Dulbecco's Modified Eagle's Medium (DMEM, Life Technologies) which is planted with an explant planting system. Cells were sub-cultured three times, and and the result of 3rd sub-culture was used for treatment. Each experiment was repeated for three times.

Culture with stem cells with OA primary cells

OA primary cells were cultured to 50–60 % confluence, then cultured together with mesenchymal stem cells Wharton jellies. These cells are observed for 24 hours and 48 hours. Cells are calculated by Haemocytometer with 105cells/well.

Primary design

No. Primer Nucleotide Sequence NM Amplicon, NCBI Accession Size, Number Gene.

1. MMP-13F 5’-CACTTTATGCTTACTGATGACG-3’ NM_002427.3 154 bp

2. MMP-13R 5’-TCCTCGGAGACTGGTAATGG-3’ NM_002427.3 154 bp

3. RELA F 5’-CGCATCCAGACCAACAACAA-3’ NM_001243984.1 154 bp

4. RELA R 5’-AGATGGGATGAGAAAGGACAGG-3’ NM_001243984.1 154 bp

5. HPRT1 5’-CCTGGCGTCGTGATTAGTGAT-3’ NM_000194.2 158 bp

6. HPRT1 5’-CCCATCTCCTTCATCACATCTC-3’. NM_000194.2 158 bp

PCR Gradient Amplification

Each gene was replicated with the SYBR Green amplification kit. The PCR program is as follows: Predenaturation 95.0°C for 30 seconds, 5 seconds denaturation, gradient annealing at 55°C for 5 seconds for 50 cycles, additional melting curve 65.0°C-95.0°C with an increase of 0.5°C every 5 seconds.

RNA extraction and cDNA synthesis

RNA was extracted from synovial tissue isolates of grade IV Osteoarthritis patients. RNA isolation using TRIzol®, Invitrogen Life Technologies. Synthesis of cDNA was performed by using iScript cDNA Synthesis Kit (BioRad, USA) on thermal cycler C1000 (BioRad, USA) Reverse Transcriptase PCR (RT-PCR) devices.

RNA Isolation

Cells that have been treated with stem cells trizol as much as 500 µl. Then, homogenized and put in a 1.5 ml PCR tube. After that, cells that were given trizol, added 100 µl of chloroform, then homogenized. Once homogeneous, incubation for 5 min and centrifuged at a speed of 12.000 xg, for 15 minutes at 4°C. Take a clear layer, then move it to a new tube. Add 250 µl isopropanol, shake back and forth and let stand for 10 minutes. Followed by 12.000 xg speed centrifuge, for 10 minutes at 4°C. Pour on a dry, airy

tissue. After that, add 500 µl of ethanol 75%, shake, and centrifuge 7.500 xg speed, for 5 minutes at 4°C. Dispose of ethanol, dry all for 15 minutes. After that add 25 Rna-se free water, dilute it. Then adjust the concentration of RNA using NanoDrop.

cDNA synthesis

The synthesis composition of total cDNA was 5 µg total RNA, 1x RT buffer 20 pmol oligodT, 4 mM dNTP, 10 mM DTT, 40 U enzyme SuperScript TMII RTase and H_2O-DEPC with a total volume of 20 µl. Total cDNA synthesis was carried out at 52°C for 50 minutes with the work protocol by the manual kit BioRad, USA).

PCR Gradient Amplification

Each gene was replicated with the SYBR Green amplification kits. The PCR program is as follows: Predenaturation 95°C for 30 seconds, 5 seconds denaturation, gradient annealing at 55°C for 5 seconds for 50 cycles, additional melting curve 65-95°C with an increase of 0.5°C every 5 seconds. Measurement of gene concentration can use two methods, namely the absolute quantification method and relative quantification method. The method used in this study is the relative quantification method of Livak-Schmittgen (2001) or the comparison of Treshhold deltas or plafl methods [5].

ΔCT experimental = CT target in experiment-CT housekeeping on experiments

ΔCT control = CT target on control-CT housekeeping on control

ΔΔCT = ΔCT experimental-ΔCT control

The comparison of expression levels is obtained by using the equation: Comparison of gene expression levels = 2ΔΔCT. The measurement of gene concentration in this study uses the LightCycler® software program which refers to the quantification of the automated Livak formula so that the concentration value of gene expression is obtained in picogram size. HPRT1 gene was a housekeeping gene, and calibrator gene was from the control group.

Data analysis

Data will be presented in the form of table and graph, as well as the results of expressions of the RELA gene and MMP-13. P is no data of gene expression of MMP-13 and RELA normality test by using the Shapiro-Wilk test and homogeneity of data with the Levene test. The test decision criteria in the Shapiro-Wilk Test are if the value of $p > 0.05$ then it is said the data is normally distributed, while the test decision criteria in the Levene Test are if the value of $p > 0.05$, then the data is said to be homogeneous. For MMP-13 gene expression or normally distributed homogeneously, then continued with ANOVA Test and Tukey's HSD Post Hoc Test, but for RELA gene expression, the data were not normally distributed and homogeneous, then non-parametric Kruskal Wallis test was carried out and followed by Mann Test Whitney [6]. Data is processed using SPSS 15 statistical analysis.

Research Ethics Requirements

Research permits will be submitted to the International Committee of the ethics Faculty of Medicine, the University of Andalas which later if approved will receive a registration number as the approval of the Research Ethics Committee of the Faculty of Medicine, University of Andalas. By the research problems, the research object used is a network sample stored in BioBank's FK Unand network. Before submitting to the Ethics Committee, researchers submitted a letter of approval from the Cancer Study Center and Stem Cell as the rightful owner of a sample of knee osteoarthritis sufferers. In this study, researchers will involve competent experts in the field of orthopaedic surgery.

Result

Sample Characteristics

The result of synoviocyte isolation from synovial tissue was a fibroblast-shaped cell, cultured in a plate. The morphology of synoviocyte and MSC-WJ presented in Figure 1. while the morphology of synoviocyte co-culture MSC-WJ 24 hour and 48 hour and data on Characteristics of Mesenchymal Stem Cells Wharton Jelly presented in Figure 2 and Figure 3.

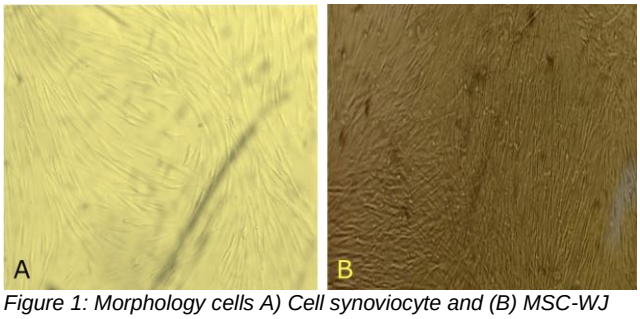

Figure 1: Morphology cells A) Cell synoviocyte and (B) MSC-WJ

Expression of RELA and MMP-13 Genes

From the results of the research obtained, before the analysis, a preliminary test was carried out for the basic assumptions of normality and homogeneity of data. The results of the normality test

with the Shapiro Wilk Test obtained a significant value of ≥ 0.05 in all treatment groups, meaning that data was normally distributed.

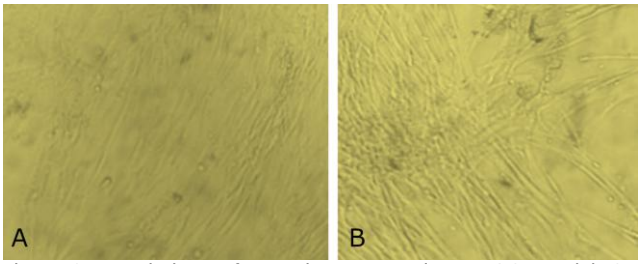

Figure 2: Morphology of synoviocyte co-culture MSC-WJ (A) Co-culture 24 hour and (B) Co-culture 48 hour

This is supported by descriptive analysis with the Skewness ratio value < 2 in all treatment groups. To test the variance homogeneity based on the Levene Test is 0.18 ≥ 0.05, this means data on gene research RELA and MMP-13 have the same variant (homogeneous).

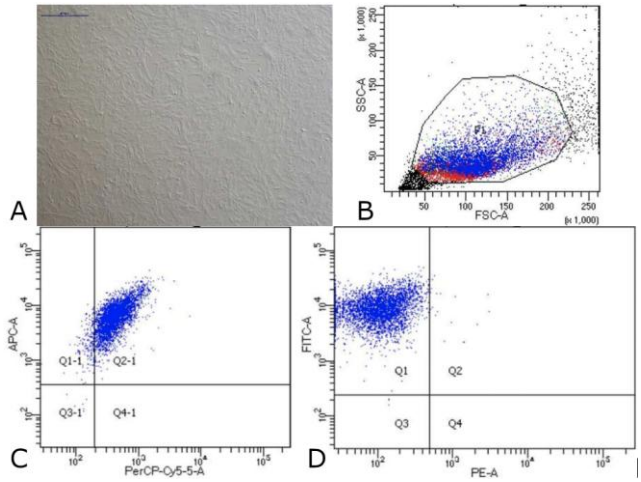

Figure 3: Data on Characteristics of Mesenchymal Stem Cells Wharton Jelly; (A) Cells MSC-WJ reach confluence. Scale bar: 500 µM. Photographs of cells taken using a Nikon Ti-S microscope; (B) Data flow cytometry. Forward scatter (FCS) plot&side scatter (SSC) plot. Population gated events (P1): 20,000; (C) Cell surface markers expression: CD73-APC 99.8% and CD105- PerCP-Cy5.5 95%; (D) Cell surface markers expression: CD90-FITC 99.9% and Lin (-) - PE 0.4%

From RELA gene expression data and MMP-13 gene expression data, the Pearson correlation test was conducted to see the relationship between RELA gene expression and MMP-13 gene expression after Mesenchymal Stem Cell Wharton Jelly administration.

Table 1: Analysis of the relative expression of MMP-13 target genes using the Livak-Schmittgen method (2001) [5]

Groups	C_T MMP13 average	C_T average HPRT1	ΔC_T = MMP13-HPRT1	$\Delta\Delta C_T$ = ΔC_T treatment - ΔC_T control	$2^{-\Delta\Delta C_T}$
Synoviocyte control 24 hours	46.89	35.89	11.00	0.00	1
MSC-WJ 24 hours	45.89	34.39	11.50	0.50	0.70
Co-culture 24 hours	47,18	33.34	13,84	2.84	0.14
Synoviocyte control 48 hours	43.09	33.95	9.14	0	1
MSC-WJ 48 hours	43.41	33.44	9.97	0.83	0.56
Co-culture 48 hours	46.55	32.26	14.29	5.15	0.03

The relationship between RELA gene expression and MMP-13 gene expression

To see the direct relationship between RELA gene expression and MMP-13 gene, Pearson correlation test was conducted which can be seen in Table 3.

Table 2: Analysis of relative RELA target gene expression using the Livak-Schmittgen method (2001)

Groups	C_T MMP13 average	C_T average HPRT 1	ΔC_T = MMP13-HPRT1	$\Delta\Delta C_T$ = ΔC_T treatment - ΔC_T control	$2^{-\Delta\Delta C_T}$
Synoviocyte control 24 hours	35.72	33.29	2.43	0	1
MSC-WJ 24 hours	35,64	32.50	3.14	0.71	0.61
Co-culture 24 hours	36.35	33.34	3.01	0.58	0.67
Synoviocyte control 48 hours	35.94	33.95	1.99	0.00	1
MSC-WJ 48 hours	38,56	33.90	4.66	2.67	0.1 6
Co-culture 48 hours	37.09	32.41	4.68	2.69	0, 15

From Table 3 there is a positive relationship, where the lower the level of RELA gene expression, the lower the expression of MMP-13 genes. From the results of the Pearson correlation, RELA gene expression was found to be strongly related to the expression of the MMP-13 gene with r = 0.662 and p = 0.01.

Table 3: Relationship between RELA gene expression and MMP-13 gene expression

Variables	RELA	MMP-13	r^2	*p*
RELA	1	0,662	0,438	0,01
MMP-13	0,662	1		

From Figure 4 it can be seen that the lower the RELA gene expression, the lower the expression of the MMP-13 gene.

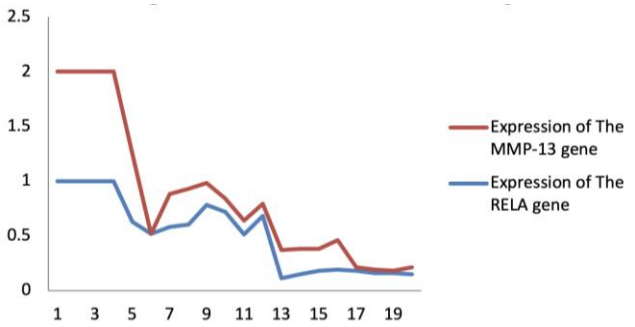

Figure 4: Expression of the RELA and MMP-13 Gene

Discussion

The relationship between RELA gene expression and MMP-13 gene expression

The results showed a positive relationship between *RELA* gene expression (NFκβ p65) and

MMP-13 gene expression, where the lower the level of *RELA* gene expression, the lower the expression of the *MMP-13* gene. This positive relationship absolutely occurs in synoviocytes affected by osteoarthritis.

This is in line with several related research results that can explain the results of this study. Over *MMP-13* gene expression is very high in pathological conditions such as in disease rheumatoid arthritis, osteoarthritis and carcinoma. Clinical trials in patients with cartilage damage were found in patients who had high *MMP-13* expression [7]. The results of this study can be used as a new therapeutic target for OA by inhibiting enzymes that play a role in the process of cartilage degradation [8]. Another study also showed that overexpression of *MMP-13* in GMO mice spontaneously would cause cartilage damage; MMP-13 can prevent erosion in joint-prone [9].

At the time of the inflammatory process, the *RELA* gene, which is one of the families of NFκβ, is involved in the expression of several genes that play a role in the inflammatory response. The transcription process of NFκβ is stimulated by pro-inflammatory cytokines and chemokines. Activation of NFκβ will trigger the expression of genes that induce articular joint damage resulting in osteoarthritis. *RELA* is also needed to modulate the immune response. *RELA* is expressed in various cell types, including epithelial cells, endothelial cells and nerve tissue. In general, *RELA* plays a role in the adaptive immune system and destroys pathogens through activation of NFκβ.

RELA plays a key role in regulating immune responses to infections; incorrect RELA regulation has been linked to cancer, inflammation and autoimmune diseases. RELA is a transcription factor in mammals that controls some important genes in the process of immunity and inflammation. RELA is involved in the expression of several genes that play a role in the inflammatory response, cartilage degradation, cell proliferation, angiogenesis and are predominantly regulated by RELA. The RELA transcription process is stimulated by pro-inflammatory cytokines and chemokines. Activation of RELA will trigger the expression of genes that induce articular joint damage resulting in OA. Besides that RELA controlling the expression of many adaptive genes such as Major Histocompatibility Complex (MHC) and genes important for the regulation of apoptosis. Besides NFκβ controlling the expression of many adaptive genes such as Major Histocompatibility Complex (MHC) and genes important for the regulation of apoptosis [10].

RELA is needed in the transcription process of MMP-3 and MMP-13, activation of MMP-3 and MMP-13 requires the RELA gene. Interleukin-1 induces RELA and MMP-13 transcription processes [3]. RELA is one of the NFκβ units which plays an important role in NFκβ activity [11]. The MMP-13 target is not only in type II collagen, but also damages proteoglycans, type IV and IX collagen, osteonectin in the cartilage. During embryonic development, MMP-13 is expressed in the skeleton [12].

Based on the results of the research that has been done, it can be concluded that there is a strong correlation between *RELA* gene expression and MMP-13 gene expression in osteoarthritis synoviocytes after Mesenchymal Stem Cell Wharton Jelly.

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Clinical Science

Suboccipital Muscles Injection for Management of Post-Dural Puncture Headache After Cesarean Delivery: A Randomized-Controlled Trial

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Keywords: Suboccipital muscles injection; management; post-dural puncture headache

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Abstract

INTRODUCTION: Post-dural puncture headache (PDPH) is a common complication following neuraxial anaesthesia that increases the duration of hospital stay.

AIM: This study aims to evaluate the effectiveness of injection of the dexamethasone-lidocaine mixture in suboccipital muscles treatment of PDPH after cesarean section.

PATIENT AND METHODS: A group of 90 females with PDPH following cesarean section under spinal anaesthesia were randomly allocated into two equal groups: study group (Group S) and control group (group C). All patients received bilateral intramuscular (in the suboccipital muscle) (Group S) (n = 45) patients received lidocaine 40 mg (2 mL of 2% solution) and dexamethasone 8mg in a total volume of 4 mL; whilst, patients in the control group (group C) (n = 45) received 4 mL normal saline. The primary outcome is the Visual Analogue Score for a headache at 24 hours after injection.

RESULTS: Demographic data and the baseline, headache score, neck muscle spasm, and nausea were comparable in both groups. Group S showed lower headache score compared to group C at all the post-injection time points. All patients in group S showed resolution of nausea after the intervention; while none of the control group showed any improvement. All patients of group C needed rescue analgesia; while only 6 (13.3%) patients in group S asked for an analgesic. Time to the first analgesic request was longer in group S compared to group C (10.17 ± 7.96 hours versus 1.00 ± 0.00 hours, P < 0.001).

CONCLUSION: Ultrasound-guided injection of the dexamethasone-lidocaine mixture in suboccipital muscles is effective management of PDPH after CS.

Introduction

Post-dural puncture headache (PDPH) is a common complication following neuraxial anaesthesia that increases the duration of hospital stay [1], [2], and is considered a significant cause of increased anaesthetic workload and often interferes with maternal-infant interaction [3].

The incidence of PDPH after cesarean delivery has been reported to be high up to 38% [4].

Conservative therapy, pharmacotherapy and interventional procedures have been used in the management of PDPH. Conservative measures such as hydration and bed rest [5] Pharmacotherapy such as gabapentin [6], hydrocortisone [7], cosyntropin (ACTH) [8], sumatriptan [9] and caffeine [10].

An epidural blood patch is an effective intervention for management of PDPH; however, it is relatively invasive [11]. Till now, a standard, evidence-based protocol for management PDPH is still lacking.

The exact cause of PDPH is not clearly understood, however, CSF leakage through dural puncture appears to be the most accepted explanation of PDPH [12].

PDPH is commonly associated with neck

stiffness and muscle spasm. Presence of myodural bands a connective tissue band between the spinal dura mater and suboccipital muscles) in the back of neck might play a role in PDPH [13].

Local anaesthetic injection in neck muscles has been reported to relieve some types of chronic headache [14], [15], [16], [17], [18], but to our knowledge, no one has tried to use this technique in the treatment of PDPH.

Intramuscular (neck muscles) injection of local anaesthetic- steroid combination may improve both neck muscles spasm and subsequently PDPH.

This study aims to evaluate the effectiveness of injection of the dexamethasone-lidocaine mixture in suboccipital muscles treatment of PDPH after cesarean section.

Patient and Methods

A prospective double-blinded randomised controlled trial was conducted from January to June 2018 at Cairo University hospital after approval of the Research Ethics Committee. Written informed consent was obtained from all participants before enrollment. Randomisation was achieved using a computer-generated sequence of numbers. Opaque sealed envelopes were used for concealment.

The study 90 female, ASA I&II patients, aged between 18 and 40 years, with PDPH following cesarean section under spinal anaesthesia. Patients with pre-spinal chronic headache or migraine, hypertensive patients, patients who cannot comply with Visual Analogue Score (VAS) were excluded from the study.

Patients were randomly allocated into two equal groups: study group (Group S) and control group (group C). All patients received bilateral intramuscular (in the suboccipital muscle) injection of either local anaesthetic-steroid mixture or saline (according to the group allocation).

(Group S) (n = 45) patients received lidocaine 40 mg (2 mL of 2% solution) and dexamethasone 8 mg in a total volume of 4 mL; whilst, patients in the control group (group C) (n = 45) received 4 mL normal saline.

Details of the technique

The drug was prepared by a research assistant, and the syringe was delivered to another anesthesiologist who was blinded to the study group. Suboccipital muscle injection was performed blindly while the patient is sitting on chair and neck maximally flexed on the edge of a bed. Patient follow-up was performed by a research assistant who was also blinded to the study group Mention what are the other routes of management.

Outcomes

Primary outcome

Visual Analogue Score (VAS) for headache: VAS assessed at 24 hours after injection.

Secondary outcomes:

1. Visual Analogue Score (VAS) for headache: VAS was assessed at the baseline before injection, 1 hour, 6 hours, and 12 hours, after injection during rest.

2. The need for rescue analgesia (the frequency of patients who requested additional analgesic). In patients with VAS more than 3, rescue analgesic in the form of ketorolac 30 mg was taken intramuscular up to 120 mg max per day.

3. Time to 1st rescue analgesia.

4. Nausea pre and post injection

Sample size calculation

In a pilot study on 8 patients, we reported a postoperative headache score of 4.2 ± 1.3 after cesarean delivery. Using MedCalc Software version 14.10.2 (MedCalc Sofware bvba, Ostend, Belgium), we calculated a conservative sample size that could detect 20% difference in headache score (i.e. 0.84) between the two study groups. A minimum number of 78 patients (39 patients per group) was calculated to have a study power of 80% and an alpha error of 0.05. The number was increased to 90 patients (45 patients per group) to compensate for possible drop-outs.

Statistical analysis

Data were presented in terms of mean ± standard deviation (± SD), median and range, or frequencies (number of cases) and percentages when appropriate. Comparison of numerical variables between the study groups was made using Student t-test for independent samples or Mann Whitney test as appropriate. For comparing categorical data, Chi-square (χ^2) test was performed. The exact test was used instead when the expected frequency is less than 5. p values less than 0.05 was considered statistically significant. Repeated measures were compared using two-way Analysis of Variance (ANOVA) test with Post Hoc Bonferroni test. All statistical calculations were done using computer program SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) release 15 for Microsoft Windows (2006).

Results

One hundred and four patients with PDPH were assessed for legibility. Ten patients were excluded as they did not meet inclusion criteria; four patients refused to sign the consent, and 90 patients received the intervention, and all of them completed the study and were available for final analysis (Figure1).

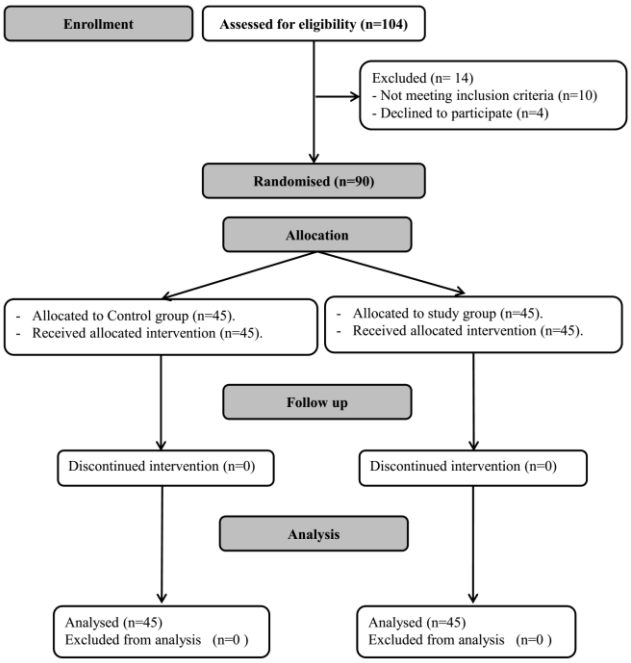

Figure 1: Consort flow diagram

Demographic data were comparable in both groups (Table 1).

Table 1: Demographic data

	Group c (n = 45)	Group s (n = 45)	P value
Age (years)	27.82 ± 3.58	25.87 ± 5.20	
BMI (kg/m^2)	29 ± 3	29 ± 4	0.8

Data expressed as mean ± SD; *Denotes significance between both groups.

At the baseline, both groups were comparable about headache score, neck muscle spasm, and nausea (Table 2, and 3).

Group S showed lower headache score compared to group C at all the post-injection time points (Table 2).

Table 2: Headache score

	Group c (n = 45)	Group s (n = 45)	P value
Baseline	6.40 ± 1.42	6.51 ± 1.82	0.75
Post intervention 1st hour	5.82 ± 1.11	1.69 ± 0.49	0.00*
Post intervention 6th hour	2.20 ± 1.06	1.69 ± 1.10	0.03*
Post intervention 12th hour.	2.87 ± 1.69	1.67 ± 1.24	0.00*
Post intervention 24th hour	3.22 ± 2.14	1.69 ± 1.10	0.00*

Data expressed as mean ± (SD); *Denotes significance between both groups.

All patients in group S showed resolution of nausea after the intervention; while none of the control group showed any improvement. All patients of group C needed rescue analgesia; while, only 6 (13.3%) patients in group S asked for analgesic (Table 3).

Table 3: Neck spasm, Nausea, The need for rescue analgesia

	Group c (n = 45)	Group s (n = 45)	P value
Neck spasm	36/45 (80%)	36/45 (80%)	1.00
Nausea			
Before intervention	12/45 (26.7%)	12/45 (26.7%)	1.00
After intervention	12/45 (26.7%)	0/45 (0%)	0.00*
The need of rescue analgesia	45/45 (100%)	6/45 (13.3%)	0.00*

Data expressed as count and per cent; *Denotes significance between both groups.

Time to the first analgesic request was longer in group S compared to group C (10.17 ± 7.96 hours versus 1.00 ± 0.00 hours, P < 0.001) (Table 4).

Table 4: Time to first rescue analgesia

	Group c (n = 45)	Group s (n = 45)	P value
Time to first rescue analgesia (hours)	1.00 ± 0.00	10.17 ± 7.96	0.00*

Data expressed as mean ± (SD); *Denotes significance between both groups.

Discussion

We reported that suboccipital (neck) muscles injection successfully improved PDPH after cesarean delivery. To our knowledge, this is the first study to evaluate the efficacy of this intervention in PDPH.

PDPH is characteristically distributed over the frontal and the occipital region radiating to the neck and shoulders. The headache may be associated with other symptoms such as nausea, vomiting, hearing loss. Neck stiffness and muscle spasm are one of the most important characteristics of PDPH following spinal anaesthesia [12].

Although it remains speculative, traction on connective tissue link (myodural band) between the spinal dura mater and suboccipital muscles in the back of the neck may aggravate or even cause the headache [13].

Neck muscles injection with local anaesthetic has been used effectively for the treatment of various types of chronic headaches [14], [15], [16], [17], [18]; thus, we hypothesised that it could be effective in the management of PDPH.

The close characteristics of PDPH and cervicogenic headache could explain the similar response of both types of headache to neck muscle injection [17].

Myodural Bridge might have a role in headache production. This assumption was supported by the relief of chronic headache after surgical separation of the Myodural Bridge from suboccipital musculature [19]. Neck injection of local anaesthetic result in relaxation of suboccipital muscles; and thus, it could improve the tension on the dura by myodural

band.

Our findings provide new insights in the management of PDPH. Our intervention is simple, cost-effective, and less invasive compared to the traditional methods for management of PDPH such as epidural blood patch.

Other types of injections were used effectively for the treatment of PDPH such as greater occipital nerve block and sphenopalatine ganglion block [20], [21].

Future comparison between these blocks could be made and evaluation of suboccipital muscle injection using ultrasound might help select particular muscle for injection (rectus capitis posterior major, rectus capitis posterior minor and the capital inferior) where in all these muscles my-dural bands were identified.

Our study had some limitations: 1) It is a single centre study. 2) We evaluated the intervention in special population post-cesarean delivery. Future studies are needed for confirming our findings in PDPH after other operations. 3) We used a single dose of the local anaesthetic-steroid mixture; thus, dose-finding studies are warranted.

In conclusion, ultrasound-guided injection of the dexamethasone-lidocaine mixture in suboccipital muscles is effective management of PDPH after CS.

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Clinical Science

Instrumental Balinese Flute Music Therapy Improves Cognitive Function and Serum Dopamine Level in the Elderly Population of West Denpasar Primary Health Care Center

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Abstract

BACKGROUND: Musical artwork using Balinese flutes made from bamboo (timing buluh) by Agus Teja Sentosa, S.Sn is a combination of music played with flute as the main instrument which contains certain components resembling music therapy such as in western classical music by Antonio Lucio Vivaldi.

AIM: This study aims to determine the improvement of cognitive function and increase in serum dopamine in the elderly after listening to music with Balinese flute as the main instrument.

METHOD: The current study allocated 18 subjects in the control group listened to western classical music by Antonio Lucio Vivaldi, while 18 subjects in the intervention group listened to western classical music and music from Balinese flute as the main instrument by Agus Teja Sentosa, S.Sn. MoCA-Ina assessment and examination of serum dopamine levels were carried out initially and 21 days after listening to music intervention.

RESULTS: The mean increase in cognitive function score was higher in the intervention group (5.22; $p < 0.001$) than in the control group (4.67; $p < 0.001$), this increase was not statistically significant with a value of $p = 0.562$ ($p > 0.005$). The mean increase in dopamine levels in the control group (3.60) was greater than in the treatment group (3.56), but the mean increase was not statistically significant ($p = 0.085$).

CONCLUSION: There was a significant relationship between listening to the main instrumental Balinese flute music and the improvement of cognitive function, especially in the memory domain in all study subjects, but the mean increase in cognitive function and serum dopamine level did not reach statistical significance.

Introduction

The balance between brain, body, and soul is an integral part of human life. In Hindu religion, the philosophy of life is called *Tri Hita Karana*, which refers to a harmonious relationship that manifests happiness. The three major pearls of wisdom consist of *Parahyangan* or the harmony of life between human with God, *Pawongan* or the harmony of life between human with each other, and *Palemahan* or the harmony of life between humans and their natural surroundings.

Music can vibrate and resonate rhythm in our nature, in which as a metaphor, every cell in our body plays a role as a rhythmical sound resonator. Listening to music including in elderly associates with an improvement of brain plasticity, which is beneficial to stimulate cognitive function [1]. The process of brain neuroplasticity in its relationship with music is a part of enhancing cognitive ability or intelligence in the form of auditory intelligence. Listening to music induces powerful modulation activity on the mesolimbic pathway, impacts the nucleus accumbens and ventral tegmental area, as well as the hypothalamus and insula [2].

Musical listening experience necessitates complex auditory pattern-processing mechanisms, attention, memory storage and retrieval, and sensory-motor integration [3]. Music activates stored memory and stimulates cognitive function, also, recent brain imaging studies have shown that neural activity associated with listening to music extends well beyond the auditory cortex involving a wide-spread bilateral network of the frontal, temporal, parietal and

subarachnoid pathway [4]. Right cerebral hemisphere receives musical impulse and activates both hemispheres via the corpus callosum. Cognitive process is related to memory. Memory can be divided into 3 categories; it consists of sensory information storage, short-term memory, and long-term memory. Music impacts the encoding process of sensory memory. Positive emotion which people get from listening to music will encourage cognitive repair process [5]. Listening to pleasant music may increase cerebral blood flow or brain vascularisation in the mesocorticolimbic system, ventral striata (nucleus accumbens and mesencephalon), thalamic structure, cerebellum, insula, anterior cingulate cortex, and orbitofrontal cortex. The nucleus accumbens will be activated when a person is listening to pleasant music although it is never heard before [6].

Instrumental music is a type of music in which there are no vocals. The flute is a family of musical instruments in the woodwind group made from bamboo [7]. There are two principles in music reception which are equal loudness level (phone) and perceived level (sone). Phon is used to solve problems which cannot be fixed using only the decibel and hertz. In the human being, there is a natural human principle about surrounding things called senses [8]. The frequency range of human hearing which will travel to auditory cortex is 20-20.000 Hz [9].

Instrumental, low-pitched music with harmonious slow rhythm (60-80 beats per minute) are pleasing to the listeners, it may affect body physiology, slowing down both heart and respiratory rate, and might influence emotions through the limbic system [10], [11], [12]. This study utilised music from Balinese bamboo flute (*timing buluh*) as the main instrument, arranged together with modern music and played by Gus Teja (Agus Teja Sentosa, S.Sn). The song “Morning Happiness” has a tempo of 70-90 beats per minute and a frequency of 440 Hz.

Classical music is defined by Indonesian Language Dictionary (2008) as music composed and born from European culture and categorised according to certain periods. Listening to classical music will produce a positive effect, called a Mozart effect or Vivaldi effect. Western classical music used in such study is titled Spring by Antonio Lucio Vivaldi. Instrumental music with 60-80 beats per minute may affect brainstem neurons, activating neurotransmitters norepinephrine in conjunction with cholinergic and dopaminergic in the brainstem, mediating sensory and motor functions, with some influencing the cognitive function. Music directly activates the neuro-vegetative system (hypothalamus, hypophysis, suprarenal gland) to release neurotransmitters [2]. Cognitive function amelioration by listening to music is due to the relationship between orbitofrontal cortex (OFC) and a dopaminergic mesocorticolimbic circuit (nucleus accumbens/NAc and ventral tegmental area/VTA). Dopaminergic neurotransmitter in the neuronal pathway has a critical role in the brain’s ability to process heard music [6].

The brain will have difficulty in recalling (memory function) along with the ageing process, reduces its ability to make decisions and slower in carrying out activities which are known as cognitive function changes. MoCA (Montreal Cognitive Assessment) is a questionnaire to assess global cognitive function including executive function and memory [13], [14].

Methods

This research was an experimental study using a pretest-posttest control group design. This study was conducted between November 2017 and December 2018 over 21 days in a primary geriatric facility located in West Denpasar primary health centre. Pocock formula was used to calculate the sample size of this study. There were 32 healthy geriatrics aged 60-74 years old given their consent to be subjects in this study. These subjects were healthy and did not have any history of systemic illnesses including stroke, diabetes mellitus, dyslipidemia, hypertension, epilepsy, history of brain injury, brain tumour, brain infection, and hearing impairment. These subjects were divided into two groups, control, and intervention group. Subjects on control group listened to western classical music by Antonio Lucio Vivaldi, titled “Spring”, while intervention group listened to western classical music “Spring” with additional music piece of main Balinese flute “Morning Happiness” by Agus Teja Sentosa S.Sn. Each song was played for 20 minutes one time a day in the morning before subjects did their daily activity. All of the subjects used earphone to listen to the songs which were played from the recording tool provided by the researcher.

Cognitive function assessment in this study used the MoCA-Ina instrument, in which subjects were examined 2 times, before intervention and 21 days after the intervention of listening to music. Examination of serum dopamine levels also was carried out two times, before and 21 days after the intervention.

Descriptive analysis was carried out to see the characteristics of the research subjects. The Shapiro Wilk test was used to determine the numerical scale data normality, which was mean an increase in cognitive function scores and means an increase in serum dopamine levels. Comparative analysis of two mean increases in cognitive function scores and the mean increase in serum dopamine levels used unpaired T-test, significance level with p, and a 95% confidence interval.

Results

There were 36 subjects on this study, each group of a control group and intervention group consisted of 18 subjects. Male and female gender was distributed normally in each subject group consisting of 16 male and 16 female. Both groups had the same age interval, ranging from 60 to 74 years old. The subject baseline characteristics in this study including age, gender, education level, occupation, and mean score of the initial cognitive function are shown in Table 1.

Table 1: Baseline characteristics of research subjects

Variable	Group		p-value
	Control (n = 18)	Intervention (n = 18)	
Mean Age (years)	65.83 ± 4.27	69.44 ± 4.48	0.856
Gender			
Male	9 (50%)	9 (50%)	1.000
Female	9 (50%)	9 (50%)	
Education Level			
Junior High School	10 (55.6%)	8 (44.4%)	
Senior High School	2 (11.1%)	4 (22.2%)	
Academy/Diploma/Bachelor	6 (33.3%)	6 (33.3%)	
Occupation			
Retired Civil Servants	7 (38.9%)	8 (44.4%)	
Private Employees	5 (27.8%)	5 (27.8%)	
Entrepreneur	4 (22.2%)	1 (5.6%)	
Others	2 (11.1%)	4 (22.2%)	
Mean Initial MoCA-Ina Score	20.94 ± 3.45	21.33 ± 3.07	0.971
Mean Initial Dopamine Serum Level	36.50 ± 16.40	23.08 ± 8.63	0.002*

MoCA-Ina = Montreal Cognitive Assessment-Indonesia.

Table 2 shows the mean increase of cognitive function score on each subject group. The mean increase of cognitive function score on the intervention group (5.22 ± 2.02) was higher than in the control group (3.89 ± 1.55).

Table 2: Mean Increase of MoCA-Ina score before and after listening to music on the control and intervention group

Group	Mean Initial MoCA-Ina	Mean Final MoCA-Ina	Mean Increase MoCA-Ina	p-value
Control	20.94 ± 3.45	24.83 ± 3.54	3.89 ± 1.55	< 0.001*
Intervention	21.33 ± 3.07	26.56 ± 2.77	5.22 ± 2.02	< 0.001*

MoCA-Ina = Montreal Cognitive Assessment-Indonesia.

Statistical analysis using unpaired T-test to measure the effectiveness of MoCA-Ina score improvement between 2 subject groups showed that the increase was not statistically significant with a value of p = 0.562 (p > 0.05). The results of the analysis are presented in Table 3 below.

Table 3: Mean Increase of MoCA-Ina score between control and intervention group

Group	Mean Increase of MoCA-Ina score	p-value
Control	3.89 ± 1.55	0.562
Intervention	5.22 ± 2.02	

MoCA-Ina = Montreal Cognitive Assessment-Indonesia.

Independent t-test was performed to know the significance or effectiveness of each cognitive function domain between control and intervention group. The highest mean increase was found in memory domain on the intervention group (1.56 ± 0.78) compared to the control group (1.56 ± 0.78) with a value of p = 0.023 (p < 0.05). The mean increase of memory domain in the intervention group was statistically significant compared to in the control group. The analysis result for other cognitive function domains is presented in Table 4.

Table 4: Mean Increase of each cognitive function domain score (MoCA-Ina) on intervention and control group

Variable	Group		p-value
	Control	Intervention	
Visuospatial/Executive	0.89 ± 0.58	1.22 ± 1.06	0.093
Naming	0.17 ± 0.71	0.00 ± 0.34	0.377
Memory	1.56 ± 0.78	2.39 ± 1.24	0.023
Attention	1.06 ± 1.11	0.94 ± 1.35	0.453
Language	0.44 ± 0.62	0.33 ± 0.59	0.534
Abstract thinking	0.33 ± 0.49	0.28 ± 0.46	0.487
Orientation	0.06 ± 0.24	0.00 ± 0.00	0.331

MoCA-Ina = Montreal Cognitive Assessment-Indonesia.

The Shapiro Wilk test was used to determine the data normality since the sample size was less than 50 subjects. Mean increase of serum dopamine level data on both groups were being tested and showed that the data were not distributed normally with p = 0.000 (p < 0.05). Nonparametric study for the related sample, Wilcoxon test, was further conducted to test the mean difference of two groups that were not distributed normally. The analysis result is presented in Table 5.

Table 5: Mean concentration of serum dopamine before and after listening to music on the control and intervention group

Group	Mean initial dopamine concentration	Mean final dopamine concentration	Mean increase of dopamine	p-value
Control	36.50 ± 16.40	40.06 ± 72.50	3.60 (37.63-30.51)	0.085
Intervention	23.08 ± 8.63	26.65 ± 24.87	3.56 (16.84-9.71)	0.094

MoCA-Ina = Montreal Cognitive Assessment-Indonesia.

The mean increase in the control group was higher than in the intervention group, but this result was not statistically significant with a value of p = 0.085 (p > 0.05). The analysis result is showed in Table 6 below.

Table 6: Mean increase of serum dopamine concentration between control and intervention group

Group	Median (Minimum-Maximum)	p-value
Control	3.60 (37.63-30.51)	0.085
Intervention	3.56 (16.84-9.71)	

MoCA-Ina = Montreal Cognitive Assessment-Indonesia.

Discussion

All of the subjects in this study had the same range of age which was 60 to 74 years old. World Health Organization (WHO) in 1999 divided age range for geriatrics into 4 categories, consisting of middle age, elderly, old, and oldest-old. The range of age 64 to 74 years old in this study is classified as elderly [15]. A cross-sectional study found that the incidence

of hearing loss in the elderly occurred in 45% of people at the age of > 70 years [16]. Mean of age from the previous study was 74.1 years old [17]. The age range 60-74 years in this study is by the category of elderly by WHO and it was chosen to minimise the possibility of research subjects having hearing loss.

Baseline characteristic for mean serum dopamine level on both groups had a statistically significant difference ($p = 0.002$). There have been no similar studies before assessing serum dopamine levels in the elderly. The mean level of dopamine on both groups was below normal range, and the difference of initial serum dopamine level on control and intervention group was likely due to several factors, including the diversity of daily stressors experienced by the two groups, different feelings of comfort when listening to music and different daily habits such as smoking. The previous study in experimental animals found that psychological stress affects the level of dopamine release in mesolimbic, the assessment of dopamine level on this study was carried out by Positron Emission Tomography (PET) [18]. Nicotine consumption as in cigarette smoking is known for their effect in stimulating dopamine production [19]. The result from previous research using experimental animal found that as the increase of age, the basal ganglia structure changes and affects dopamine level [20]. The difference of mean initial cognitive function score between the two groups was not statistically significant with a value of $p = 0.856$ ($p > 0.05$). The result of the cognitive function examination on the elderly in this study is similar to the result from the previous study; it showed that the average cognitive function of the elderly aged 60-70 years was 21.48 [21]. The previous research about listening to Balinese flute music as a therapy showed the mean cognitive function score on the elderly was 20.75 [22].

The paired test found that both groups experienced a statistically significant increase in their mean cognitive function after listening to music ($p < 0.001$). The mean increase of cognitive function score in the intervention group was greater than in control group, the difference of the value based on the unpaired comparative test was not statistically significant ($p = 0.562$; $p > 0.05$). The intervention group listened to two types of music so that the possibility of improving their mean cognitive function was bigger than in the control group who only listened to one type of music, which was classical music. This condition may be caused by the weakness of this study which could not control the overall daily activities that can affect cognitive function. The subjects in this study listened to the music for 21 days (3 weeks) similar with the previously conducted study which concluded that experiencing music therapy for 2-3 times a week for 1-6 weeks period enhanced cognitive function [23]. Listening to western classical music for at least 10 minutes was able to improve cognitive function [24], [25].

The mean increase of cognitive function score after listening to classical music from the previous study was 3.17 which was less than the result from this current study that could happen because the subjects from the previous study listened to classical western music with a smaller frequency of 2 times a week for 7 weeks [26]. Both the control and intervention group were having improvement in their cognitive function score since the main Balinese flute instruments had similar characteristics with classical western music. Those two types of music have the appropriate component of music therapy. The mean increase of cognitive function score in the intervention group was higher than in the control group, that could be caused by the fact that all of the subjects on this study were Balinese people in which they were accustomed to listening to the Balinese flute. Balinese flute was earlier only played in the spiritual ceremony, but nowadays Balinese flute had developed and combined with modern music and played as recreational music. The intervention group had a higher increase in their cognitive function score compared to the control group since they were listening to two kinds of music that contained suitable components of therapeutic music. A prior research entitled The Effect of Exposure to Classical and Javanese Music on Cognitive Function in Patients with Acute Ischemic Stroke conducted at Dr. Sardjito Hospital Yogyakarta stated that the results of a cognitive function of subjects who received exposure to Javanese music and classical music were better than those who did not receive musical exposure [27]. The way people receive music is different from each other; it is influenced by history, place, culture, and taste of the listeners [6].

The previous research found that the mean scores of cognitive function in Balinese flute players were higher than in player of other types of musical instruments in the *Gong Kebyar* group in Bali. Metronome program was used to find out the components of music contained in the *Gong Kebyar* group. Music produced from the main flute instrument used in this study had some similar components to classical music, in terms of they did not have any lyrics, the frequency was 440 Hertz, and the tempo was 70-90 beat/minute, then the subjects listened with the volume of 40-70 decibel [28]. No similar research was found regarding the role of listening to the main Balinese flute instruments in improving cognitive function, especially in the elderly.

The comparison of the mean increase scores for each domain between the control and intervention group did not differ statistically ($p > 0.005$). Memory domain in both groups experienced the highest mean increase. The intervention group experienced a greater increase in their memory domain than in the control group, and the difference was statistically significant ($p = 0.023$). Primary auditory cortex (area 41) is located in the superior temporal gyri whereas the brain region responsible for memory function is

located in the temporal region. The result from the previous experimental study showed the dominant activity of the temporal region on functional Magnetic Resonance (fMR) imaging when subjects were asked to repeat words [29]. The outcome of the latter study parallel with the result from a previous experimental study which held in Italy, it was found that listening to western classical music increased mean memory function (recall memory) and visuospatial [24], [25]. Previous experimental studies yielded the same corresponding result where the increase of the memory domain was greater than of the other domains [30]. Improvement of cognitive function did not occur in all domains but there was an increase in memory function in elderly who listened to music therapy, the highest mean increase was found in the elderly listened to classical music titled Spring by Vivaldi then followed by the increase of memory domain in the elderly who listened to Mozart's White Noise music and the lowest score in the elderly who did not listen to music [31].

Attention and visuospatial/executive domains have increased higher than other domains after the memory domain. Previous research on the elderly showed that listening to music can improve the various domains of cognitive function, especially the domain of memory and attention [32], [33]. The domain of orientation and naming in the intervention group did not experience any increase in their average scores, because the initial and final scores were equally good. The fMRI scanning was done while subjects listened to music and the result showed activation of NAc and ventral tegmental area (VTA). The connection between NAc and VTA regulates the autonomic system, emotion, and cognitive function. Insula is activated since it is connected with NAc and play a role in addictive behaviour [6]. Functional Transcranial Doppler sonography (fTCD) was performed while subjects listened to music, the description of the results was music contains elements of harmony, and the music tempo increases cerebral blood flow velocity (CBFV) in the right hemisphere compared to the left hemisphere [34]. The music contains pitch elements which are processed in the auditory cortex of the right hemisphere and located in the temporal lobe, so the process of listening to music activates right hemisphere more than the left [35].

There was an increase in mean dopamine level of the control and intervention groups in this study, but this increase was not significant statistically. Similar research has never been done before. There was an improvement in cognitive function with music listening activities from the fMRI examination which done when subjects listened to music, it is due to the association between OFC and mesocorticolimbic dopaminergic circuits (NAc and VTA), but this study could not directly assess the serum dopamine levels [6].

The previous study in an experimental animal model with fMRI scanning produced an equal result as in this study, as auditory stimulation (music) increased the serum dopamine and serotonin levels, but no changes were found in the basolateral amygdala and NAcc dopamine level when listening to music. The difference between brain dopamine and the one in systemic circulation levels must be taken into account since dopamine metabolism may cause variation in measured levels [36]. The increase in mean serum dopamine level was not statistically significant. This might be caused by the ageing process itself. A study in a healthy animal model showed a decreased metabolism in the striatum and decreasing numbers of dopamine receptors D1 and D2 in older age. Other reasons not limited to other conditions and daily routine may also contribute to the insignificant result. The increase in dopamine levels may result from cocaine use and consuming preferred meals, elaborated in another study [20].

The weakness of this study was its inability to control daily activities of the research subjects which possibly capable of improving cognitive function; research subjects were not under monitored for 24 hours per day. Other obstacles were scattered sample location over several districts within the working area of West Denpasar primary health care and limited sample collector, making blood sample acquisition from each subject’s residences could not be done at the same time. All the limitations above may affect the mean serum dopamine level due to various timespan between the moment the study subjects finished listening to music and the blood sample collection from each subject. Not all the factors influencing serum dopamine levels such as medical condition, emotional stress, smoking and/or history of smoking, daily meal intake can be controlled. Study subjects listened to music individually at home with recording devices prepared by researchers, so supervisions were conducted indirectly through the music listening record.

The strength of this study lies in the homogenous control and intervention arms, strict eligibility criteria, and also have been randomized so possible bias or confounding variables have been reduced. There was no loss to follow up in this study.

In conclusion, this study proved that music listening intervention increased the cognitive function significantly in both the control (listening to classical music) and intervention group (listening to both classical music and main instrumental Balinese flute music). There was a substantial increase in the memory domain after listening to music. The differences from the cognitive function score increase and serum dopamine levels between both arms were not significant.

It is recommended to listen to classical and instrumental Balinese flute music regularly on a daily basis to maintain and/or increase the cognitive function in the elderly. Music can be utilised as one of

the non-pharmacological treatment modalities and raising the quality of geriatric home care. Further research might be necessary with different method and supervision.

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Clinical Science

The Evaluation of the Radiological and Functional Outcome of Distraction Osteogenesis in Patients with Infected Gap Nonunions of Tibia Treated by Bone Transport

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Abstract

AIM: The aims of this investigation were: 1) to study the Functional outcome of performing distraction osteogenesis in cases of infected non-union of tibia treated with Ilizarov and Limb Reconstruction System, and 2) to study the Radiological outcome of performing distraction osteogenesis in cases of infected non-union of tibia treated with Ilizarov and Limb Reconstruction System.

METHODS: The study was done with 27 patients of infected gap nonunions of the tibia at Sir JJ Hospitals, Mumbai from 2013-2016. After implant removal, if required radical resection of necrotic tissue and fractures were stabilised with Ilizarov or mono-lateral fixator depending on non-union site. Corticotomy was either done proximally or distally. Patients were followed up at monthly intervals for a minimum of 6 months.

RESULTS: The ASAMI-Bone healing score was Excellent or Good in 86% patients, and Functional score was Excellent or Good in 89% of patients. The commonest problems were of pin tract infection, wire loosening and angulation of the transported segment.

CONCLUSION: Elderly age, persistent infection, sensory loss in the foot, the stiffness of the knee, and above all the patient's reluctance to go any further given the protracted treatment besides, systemic disorders such as diabetes are all pointers for considering amputation as an alternative.

Introduction

Fractures of the tibia are one of the commonest injuries especially with the rise in vehicular accidents. The large subcutaneous antero-medial surface predisposes to open fractures and often leads to a bone gap devoid of soft tissue cover. Soft tissue damage and periosteal stripping are common in high velocity and open injuries, and this can compromise the vascularity of the tissues around the fracture. Infection of the wound, deformity, limb shortening and non-union are all known complications of fracture of the tibia. They often lead to a bone gap which may further increase on debridement of the infected or necrotic bone.

In the musculoskeletal system, the biomechanical environment plays a key role in repairing, maintaining, and remodelling of bone to meet its functional demands. Fracture non-union is a chronic condition associated with pain, functional and psychosocial disability. Stability, vascularisation and good rehabilitation are required for successful union of tibia fractures. Thus the management of tibia non-union revolves around attempts at satisfactorily restoring the above-mentioned factors to bring about an adequate union for physical and psychological rehabilitation of the patient. Different methods of treatment have been recommended for the management of infected gap non-union. One of these methods is the "Conventional" or classic method. This method focuses on eliminating the existing infection and drainage from the bone thereby facilitating the healing process. This is achieved by sequential debridement of all the infected and nonviable tissues. This line of management mandates the use of prolonged antibiotic therapy, bypass bone grafting and long-term orthotic support. The process of healing in these cases takes place by secondary intention. The

protracted time is taken in this procedure usually results in stiffness of adjacent joints. The second method is the “active” method in which attempts are made at obtaining early bony union and the period of convalescence is reduced to a minimum owing to which the motion in adjacent joints is preserved. In this method, the restoration of bony continuity gains priority over the treatment of underlying infection.

The requirements common to all successful techniques in the management of non-union are biomechanical stability, and biological vitality of bone obtained after all devitalized bone and soft tissue is removed, and infection is controlled aggressively. The distraction osteogenesis principle with bone transport is the mainstay of treatment in cases of infected non-union of the tibia with a bone gap.

The choice of the external fixator is now generally determined by the experience and preference of the surgeon, the complexity of the problem, and the number of sites that need treatment. As a general rule, monolateral fixators may not be as well suited as ring fixators for the mechanical correction of deformities with angulations or rotation or for those that need more than two sites of treatment. Each type of external fixator exhibits individual mechanical characteristics that may affect osteogenesis and healing. The stiffness and stability of a fixator system are dependent on many variables, including the diameter of the wires, the number of wires used, the tension on each wire, the diameter of the rings, the number of rings used, and the spacing between the rings. As the use of half-pins results in half the number of sites of soft-tissue transfixation, they can decrease the number of pain-related and soft-tissue complications and can potentially improve the comfort of the patient and the tolerance to treatment [1], [2], [3].

The conventional methods of treatment of infected gap non-union of tibia often require prolonged time to complete and are associated with many complications like the stiffness of joints, persistent deformity and limb length discrepancy. The distraction osteogenesis method of Ilizarov has the potential to correct the infection, deformity, limb length discrepancy, bone and soft tissue loss simultaneously. So we plan to evaluate the functional and radiologic outcome of distraction osteogenesis in patients with infected gap non-union of the tibia.

The aims of this investigation were: 1) to study the Functional outcome of performing distraction osteogenesis in cases of infected non-union of tibia treated with Ilizarov and Limb Reconstruction System, and 2) to study the Radiological outcome of performing distraction osteogenesis in cases of infected non-union of tibia treated with Ilizarov and Limb Reconstruction System.

Material and Methods

This prospective study was conducted at the Department of Orthopaedic Surgery, Grant Medical College and Sir J.J. Group of Hospitals, Mumbai from 2014-2016. A total number of twenty-seven patients of either sex of non-union tibia with an infected bone gap at fracture site were included in the study and treated by bone transport and distraction osteogenesis carried out by Ilizarov's ring fixator or mono-lateral fixator.

Inclusion criteria: Patients with infected gap non-union of the tibia with a minimum gap of 1 cm after debridement.

Exclusion criteria: Patients with pathological fractures and fracture associated with bone disorders, the presence of any debilitating systemic disease, hormonal disorders, mental disorders and those lost to follow up were excluded from the study.

Written Informed consent of all the patients was taken. A detailed history was taken regarding the mode of injury and treatment taken before admission. A detailed examination was done to assess the level of non-union, bone gap present, shortening of extremity, neurovascular deficit, deformity, the extent of infection, the condition of soft tissue in the leg especially the anteromedial aspect of tibia and function or stiffness of the knee and ankle joints. The non-union was classified according to the AO Classification of infected non-union [4], [5]. Standard anteroposterior and lateral skiagrams of patients were taken. After all pre-op investigations and obtaining pre-anaesthetic fitness, patients with obvious infection (AO Stages-Infected, draining and Active, non-draining) were debrided and stabilised with external fixator under spinal anaesthesia. Any implant, if present was removed during debridement. All non-viable bone was radically excised from the fracture site until punctate bleeding spots from the cortex were seen (Paprika Sign). During debridement, pus from the wound or sinus was sent for culture and sensitivity. The tibia defect was measured and noted after debridement, and the gap non-union was classified based on Paley’s classification [4], [5].

In some cases, the local antibiotic depot was employed after debridement for 3-6 weeks, in order to achieve control of infection at the non-union site. STIMULAN® was also used along with antibiotics as a strategy for infection management. The advantage of STIMULAN® is that it is biocompatible, sets at body temperature, non-pyrogenic and fully resorbable owing to which it does not act as a nidus for bacteria and also does not require removal. The commonly used antibiotics were vancomycin (for gram-positive organisms especially Methicillin-resistant Staphylococcus aureus), gentamycin and tobramycin (gram-negative organisms). After the deep culture sensitivity report was available, the patients were shifted to sensitive intravenous antibiotics if required.

When loss of soft tissue cover was to an extent wherein the wound was less likely to heal by secondary intention, plastic surgery intervention was called for, and a pedicled myocutaneous gastrocnemius flap or other local flap was done. After the restoration of the soft tissue cover, any antibiotic spacers (if present) were removed. The patients who were not previously stabilised with a bone transport mechanism were operated upon, and either Ilizarov or LRS was applied in such cases. The Ilizarov ring fixator was applied in 12 patients while the monolateral fixator was used in 15 patients.

Application of Ilizarov frame/LRS

Preoperative planning

The operative plan was formulated considering the fracture/non-union configuration and the preference of the patient. A few patients preferred the monolateral fixator due to its light frame. The ring fixator was preferred when there was deformity or angulation, or the proximal or distal tibia segments were short. Acute docking was done preferably when post debridement bone gap was less than 4 cm. The patients were counselled about the long duration of the treatment and the consequences of non-compliance with the treatment protocol. Informed consent was taken for surgery, and the complications were explained in the language they understood. A day before the surgery, appropriately sized rings were chosen, and the apparatus was pre-constructed. It was kept in mind that the internal diameter of the rings was at least 4 cm larger than the maximum diameter of the limb segment to be treated. It was made sure that all of the rings are of the same size. After this, the frame was sterilised for use during the surgical procedure. In the case of the monolateral fixator, the length of rail, type of clamp and configuration of the pin in each clamp were decided.

Surgical procedure

The patient was placed in supine position with the affected lower extremity supported by a bump under the greater trochanter to prevent external rotation of the limb.

With mono-lateral fixator three clamps were applied, one to the advancing or docked segment and one each to the proximal and distal tibia. The first tapered threaded pin was inserted over the anteromedial aspect of the tibia at the level of head of fibula parallel to the tibia joint line just piercing the second cortex. Care was taken not to withdraw the tapered threaded pin as it led to a loosening of the pin. The second pin was inserted over the anteromedial surface 2 cms above and parallel to the ankle joint. Once these two pins were secured, the rail was put in place along with the template. Rest of the pins were inserted using the template as the guide.

In most cases, the pins were placed in 1, 3, 5 configuration. Following this, the dummy clamps were removed and were replaced with central/end clamps. All the clamps were tightened to provide rigid stabilisation at the fracture site.

In case of ring fixator, 4 or 5 rings and four rods were applied along with 10-12 wires. One ring to the advancing or docked segment and 2 rings on either side. If one of the segments at the extreme was short enough only one ring was applied. The optimum diameter of the wire is 1.5 mm for children and thin bones, and 1.8 mm for adults and large bones [6], [7]. The first wire was inserted from lateral to medial just anterior to the head of fibula and parallel to the tibia joint line. Once the wire was inserted through the second cortex, it was not drilled but was gently tapped through the soft tissue to avoid wrapping of the neurovascular structures. The second wire was placed parallel to the first, 2 cms proximal to the ankle joint. These wires were then fixed to the ring frame. First, the proximal block of rings was centred on the leg. The distal block was positioned such that the anterior distance from the leg to the rings was the same distance as that of the proximal blocks, i.e. at least 2 cms on either side. These wires were then tensioned to 130 kgs using the dynamometer. When tensioning the olive wire, the side with the olive was tightened, and the tensioning was performed from the opposite side. Stability of the frame was created by inserting additional wires proceeding from proximal to distal avoiding the neurovascular structures. Multi-planar positioning of wires on each side of the rings or introduction of more wires, further apart with the posts further increased the stability of the assembly. If the length between the two rings of the component was more, it was preferred to minimise unsupported length by introducing drop wires.

After assembly of the rings or LRS, a small incision was made at the junction of middle and distal fibula for performing a fibular osteotomy if fibula was intact. Through a small incision preferably in the region of the metaphyseal-diaphyseal junction, a tibial osteotomy was performed with the help of an osteotome. The periosteum was elevated and preserved. The osteotomy began on the edge of the tibial crest and extended on to the medial and lateral sides of the tibia. The posterior-most fringe of bone was often broken by gentle external rotation of the distal rings/clamps.

The periosteum was repaired. After wound closure, the pin sites and the osteotomy sites were given external compression in the form of large dressing pads. It was ensured that all the connection bolts in the frame were tight and that the wires were cut and bent smoothly so as not to snag on clothing. Finally, the range of motion of the joints proximal and distal to the fixator was checked along with distal pulsations.

Post-operative care

Broad spectrum antibiotics were given three days of post operation. On the first post-op day, evaluation of pin and wire sites was done. Physical therapy was started. Graduated gait-training was attempted with the help of crutches or walker. A passive dorsiflexion device was given for the foot and ankle. In cases of acute docking or the patients having limb-length discrepancy were tackled with a shoe raise. The patient's bed was kept flat, and a pillow was placed under the most distal ring for a circular fixator or under the ankle for a unilateral fixator to force the knee into extension. Suture removal was done around 10-15 days post-operative, and pin-track dressings were done with normal saline if not infected. If redness or swelling was present, then betadine dressings were used. An adequate dose of NSAID was used consciously for pain management.

After the latency period which in our case was 7 days post-operatively, the frame was marked with adhesive tape, and the patient was taught distraction. The tape was placed in a position which will stop the patient from moving the nuts or the distraction elements in the wrong direction. The patient was taught to distract at the rate of 0.25 mm/6 hrs. Radiographs were taken every week during the initial period of distraction, and at monthly intervals after that The rate and frequency of distraction were altered relative to clinical circumstances and radiologic progression of the fibrous inter-zone. In the case of non-progression of the bony healing patient was taken up for bone grafting during treatment. The distraction was stopped when the desired bone transport or gain in length was achieved. The fixator continued to be in place to allow consolidation of callus.

Follow-up check-list

Patients were followed up at monthly intervals for a minimum of 6 months [8].

Clinical

- Distance moved on threaded rods compared to the previous visit.

- The range of motion of adjacent joints and physiotherapy.

- Neurological examination.

- Pin sites for signs of inflammation.

- Stability of frame and components.

- Ambulation.

- Assessment of complications like muscle contracture, joint subluxation.

Radiographic

- Distraction gap increasing as required

- Progress and correction of the deformity.

- Quality of regenerate

- Consolidation

Union was defined clinically by the absence of pain and motion at the fracture site. The radiological union was considered when at least three of four tibia cortices showed bridging callus with a sharp outline of the cortical bone. Finally, before actually removing the frame, the proximal and distal ends of bone were disconnected, and the patient was asked to bear weight. If the patient was able to do this, the fixator was removed. The patient was treated in patellar tendon bearing cast or crutches or brace for a minimum of 6 weeks. The patient was routinely followed up every 6 weeks for 6 months and thereafter every 3 months.

The final assessment for bone results and functional result was done using:

a) Healing time

b) External fixation index

c) Association for the Study and Application of the Method of Ilizarov (ASAMI) Scoring System

Results

Our study comprised of twenty-seven patients of infected gap non-union of tibia treated between September 2013 and November 2015. The treatment was done by an Ilizarov ring fixator in 12 (44%) patients and a monolateral fixator in 15 (55%) patients. Males (89%) outnumbered the fairer sex and were usually in the younger (25-35) age group (Figure 1, 2, and 3).

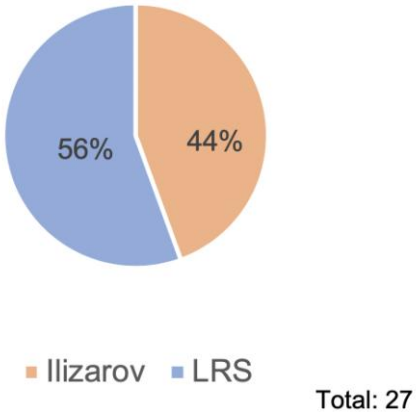

Figure 1: Definitive management of infected non-union

At the time of admission 18 patients were

discharged via an open infected wound or sinus and were categorised as Infected Active draining (IAD).

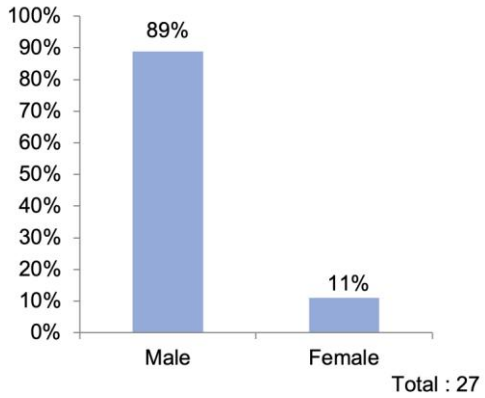

Figure 2: Sex distribution of patients with infected non-union

Five patients who were not actively draining at admission, but showed evidence of infection such as fever or abscess due to the injury to the leg were included in the Infected Active Not Discharging (IAND) group.

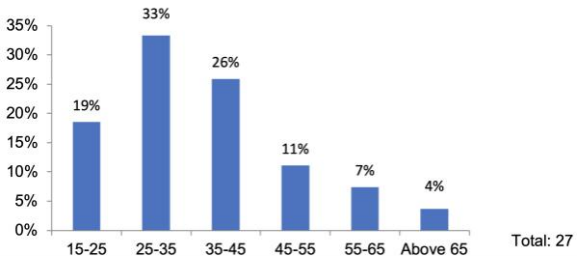

Figure 3: Age distribution of patients with infected non-union

There were 4 patients who conclusively showed evidence of infection of the bone or soft tissue in the form of a positive bacterial culture on exploration at the fracture site, a positive radioisotope scan, or imaging evidence including PET, but had not drained for the past three months and were not having fever or abscess. These patients were labelled as having Quiescent Infection (QI) (Figure 4).

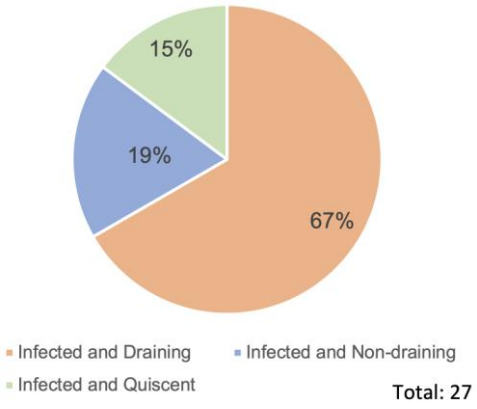

Figure 4: Type of Infected non-unions (AO Classification)

According to Paley's classification of non-union, 16 of the patient's post-debridement were in the B1 group having a bone gap > 1 cm with no bone shortening while rest 11 patients were in the B3 group with both bone defect and shortening. Though 20 of the patients did not present with an initial bone gap, the bone gap post radical debridement ranged between 1.3 cm to 9.3 cm with a mean of 4.38 cm (Figure 5).

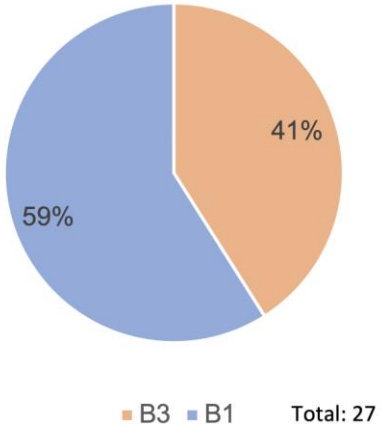

Figure 5: Paley's Classification of Gap non-union after Debridement

Most of the patients had multiple procedures before presentation as infected non-union. The main previous treatment that outlived the others was an external fixator in 17; an intramedullary nail in 5; plating in 4 and POP cast in 1. The average time between the fracture and presentation to us was 9.4 months with a range of 6.5 months to 16.5 months (Figure 6).

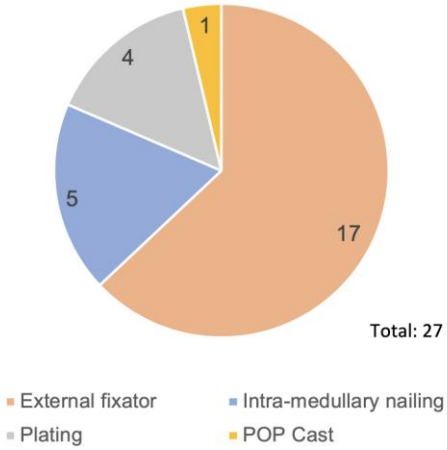

Figure 6: Predominant previous treatment modality

Acute Docking was done in 10 of our patients. The maximum post debridement bone gap in patients who underwent acute docking was 3.8 cm. Eight patients received a cancellous bone graft at the fracture site. We used Antibiotic-impregnated gradual release cement spacer in 6 of our patients and STIMULAN® pellets in 2 patients (Figure 7).

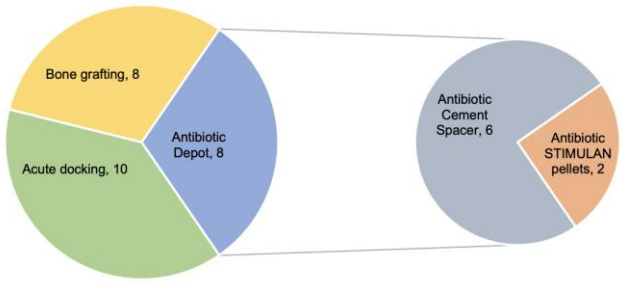

Figure 7: Patients who underwent Adjunctive Treatment

The healing time ranged between 4.1 to 10.1 months, and the average time was 6.5 months. The average bone healing index was calculated as 1.44 months/cm and fell in the range of 1.04 months/cm to 1.81 months/cm. The external fixator was applied on an average of 12.86 months within a range of 7.2 months to 19.2 months excluding the patients with plating and two amputations. The external fixator index average was 2.9 months/cm with a range from 1.7 months/cm to 3.69 months/cm.

In one of the patients after adequate bone lengthening, the transported segment tilt made it desirable to correct it by plating and bone grafting. The LRS was removed at the time of plating after the duration of 4.8 months.

Infection persisted despite radical debridement in four of the twenty-seven patients and played an important role in the decision for amputation. Two patients underwent a below-knee amputation, and both had persistent infection with non-union. Of these two patients who finally landed as failures, one of the patients had approximately 15-degree fixed flexion deformity of the knee with stiffness while the other had a partial sensory loss with decubitus ulcer on foot. The sensory loss along with persistent infection and non-union paved the way for amputation in this patient.

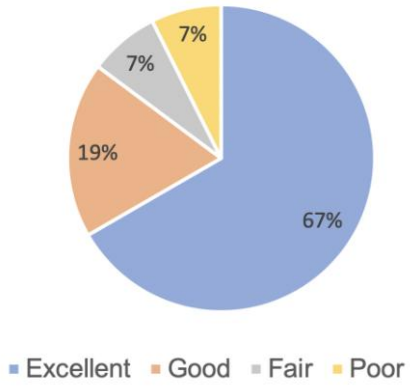

Figure 8: ASAMI-Bone

The Bony union was assessed by the ASAMI SCORING SYSTEM. It was found to be Excellent in 67% (18) patients; Good in 19% (5) patients; Fair and Poor in 7% (2) each. The poor score represented the two amputations. The functional ASAMI Score was Excellent in 63% (17) patients, Good in 26% (7) patients, Fair in 4% (1) and Failure in 7% (2) (Figure 8 and 9).

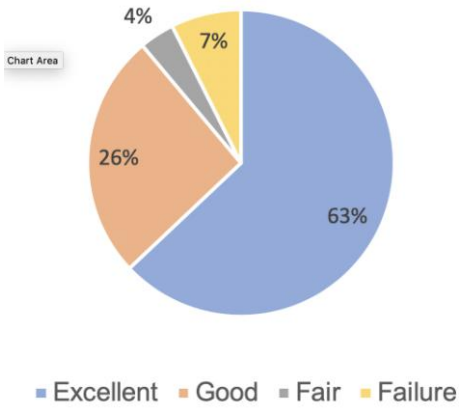

Figure 9: ASAMI-Functional

Pin tract infection was commonly encountered. Grade 0-1 DAHL was present in almost all the patients at some point of time, and in nine of our patients, DAHL 2-3 grade infection was present which responded to conservative treatment.

Equinus deformity occurred in 6 patients. Two had concomitant heel varus deformity. These were corrected by SUV application in few patients.

Discussion

With distraction osteogenesis at command, bone gap ceased to be the crucial deterrent it was at salvaging limbs a couple of decades back. Furthermore, the liberty with which the treating surgeon can radically debride bone without the fear of shortening to achieve good vascularised bone for the union has lessened the persistence of infection. Nevertheless, these strides in treatment have been more than matched by the challenges of shattered bones with a bone gap and extensive soft tissue injury commonly seen in the ever-rising high-speed vehicular accidents, and of late in the not too uncommon blast and firearm injuries.

The predominance of the younger age group (average age 36.3 years; range 19-69 yrs.) shows the same trend as in other studies. In a study done by Tang LIU et al., 2011 [9] (average 37.3 years, range 18-64 years); Kiran et al., 2012 [10] (average 34.54 years, range 21-54 years); Vignes et al., 2014 [11] (average 36 years, range 24-51 years). The higher incidence in the younger population corresponds closely with the increased rate of road traffic accidents at this age.

Conventionally many methods have been employed to overcome the problem of infected gap non-union in tibia, but all of them have several shortcomings and limitations. Multiple debridements, sensitivity guided antibiotics, irrigation of the wound with novel topical antibiotic delivery systems have all been used to eradicate infection with limited success. Autologous bone graft is good for small defects, but if the defect is large, the graft may have to be harvested from more than one site, adding to the morbidity of the patient. Also, there is a limit to the quantity of autologous bone graft that can be procured, which may not be enough for large defects [12].

In a study by Zile S Kundu et al., [2], it was seen that in adults the hypertrophy of the fibula usually took a long time compared to children. Therefore, they needed prolonged protective weight-bearing with the help of a brace. Some shortening of limb persisted in almost all cases. In spite of shortening, tibialization of the fibula provided an acceptable function to many patients and served as a good enough alternative to amputation, where there were adequate vascularity and intact sensation in the sole.

The bone transport and distraction osteogenesis pioneered by Ilizarov solves many of these problems in a much better way. It can produce bone of similar size and shape as lost; fearless radical debridement gives the best chance to eradicate infection; circumferential control minimizes deviation and deformity; weight bearing can be allowed early; early joint mobility minimises stiffness; soft tissue lengthening is simultaneously achieved obviating need for soft tissue reconstruction often and above all the procedure has stood the test of time and given good results consistently [3], [13], [14], [15], [16].

The monolateral LRS fixator is a clever offshoot of the Ilizarov osteogenic tree that brings in some positive features at the expenses of others. Because of its application to one side of the bone it is relatively easier to apply, has a lighter frame and thereby easy to carry around during ambulation, often cheaper versions are available, and it provides for greater access due to its openness for dressings and procedures which can be accomplished without removal of the frame. The price to pay is mainly the loss of circumferential manipulation, which increases deviations and deformities.

In our selection of the procedure, the choice of the frame was at times arbitrary but many a time guided by the patient's desire for a lighter frame or the requirements for dressings where an LRS was preferred. A small proximal tibia segment, need for deformity correction made a circular frame desirable. The average union time was 6.5 months and the average healing index 1.44 months/cm. Similarly, the average fixator application time was 12.86 months and the external fixator index 2.9 months/cm. Two of the twenty-seven patients did not achieve union. These results are not much different from other studies. In a study performed by Tranquilli et al., [17] in Italy on 20 patients with nonunion of the tibia, the mean time of union was 4.5 months. In a series of 25 cases studied by Kiran et al., [10], the mean time taken for union per cm of the bone gap was 1.7 months/cm.

We achieved an ASAMI Bone healing score of Excellent (67%) to Good (19%) in 86% of cases. The functional score was less in the excellent group (63%) but the Good group (26%) more than made up for it. The results compare favourably with other studies. Paley et al., [13] reported 60.87% excellent and 26.09% good result. His functional results were 64% and 28% respectively. Magadum et al., [14] had bony results of 76% (Excellent) and 20% (Good); and functional result of 60% (Excellent) and 32% (Good).

Two patients landed in non-union and had to undergo below knee amputation. Infection persisted in both. One of them had partial sensory loss in the sole and pressure ulceration along with ankle equinus deformity. The other had a stiff osteoarthritis knee and developed diabetes after the LRS system was in place. Although amputation is never a pleasant option either for the patient or surgeon but at times is the best option and saves lots of morbidities and should not be prolonged unnecessarily. They were aged 56 and 69 respectively. The ripe age, persistence of infection, neuropathy, and significant joint deformities all put their weight in favour of amputation.

Pin track infection [6] usually mild (Grade 0-1 DAHLS) often occurred at some time or the other in almost all patients and was managed conservatively. Grade 2-3 occurred in 33% of cases. Vignes et al., [11] reported Grade 0 Dahl in all patients and Grade 1 in 17 out of 20 patients. In a study by S.B Naique [18] significant pin-tract infections requiring a surgical procedure were seen in four of 45 fractures (7%) which had been stabilised with half pins and external fixators.

Equinus deformity occurred in five of the twenty-five healed patients, and two of them had varus deformity. Knee stiffness was present in one of the healed patients besides one amputee. Limb shortening of clinical significance producing limp was present in four cases and was corrected by the required rise in the shoe. Kiran et al., [10] reported equinus deformity and knee stiffness each in 4 of their series of 25 cases.

In conclusion, tibia fractures with or without bone gap are becoming increasingly common due to high-speed road traffic accidents and often present a challenge in reconstruction. The low vascularity of the leg in general and the accompanying soft tissue loss often with exposed and de-vascularised bone provides a fertile bed for infection. Even if the bone gap is not present initially significant bone gap results as radical debridement forms an essential pillar of successful treatment to eradicate the infection.

Distraction osteogenesis and bone transport can be considered to be the gold standard for infected gap non-union of the tibia as it simultaneously solves the problem of the bone gap, infection (radical debridement without fear of bone gap), deformity correction, early weight bearing and concomitant soft tissue expansion.

The classical ring fixator of Ilizarov is the principal device used for distraction osteogenesis and bone transport, but the monolateral fixator can be used in select cases where the lighter frame is preferred and is relatively easy to apply. LRS is not preferred when deformities need correction or are anticipated and if the fixation is too close to a joint. Intramedullary nailing or plating is best avoided till infection clears. General factors such as correction of anaemia, cessation of smoking, adequate nutrition or control of blood sugar in people with diabetes can never be overlooked. Besides counselling of the patient in preparation of the protracted treatment after an already long earlier attempt at the union is of crucial importance.

The present study comprised of twenty-seven patients of infected gap nonunion of the tibia {24 males: 3 females}. Fifteen {56 %} of these patients were treated with an LRS-mono-lateral fixator and the rest with Ilizarov ring fixator.

The overall ASAMI-Bone healing score was Excellent or Good in 86% patients {Excellent-67%; Good-19%}. ASAMI Functional score was Excellent or Good in 89% of patients {Excellent -63%; Good -26%}. The commonest problems were of pin tract infection and wire loosening, and angulation of the transported segment. Joint stiffness especially of the ankle in equinus was also encountered.

Elderly age, persistent infection, sensory loss in the foot, the stiffness of the knee, and above all the patient's reluctance to go any further given the protracted treatment besides, systemic disorders such as diabetes are all pointers for considering amputation as an alternative.

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The Impact of Upper Limb Training with Breathing Maneuver in Lung Function, Functional Capacity, Dyspnea Scale, and Quality of Life in Patient with Stable Chronic Obstructive of Lung Disease

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Keywords: COPD; Upper limb training; Breathing manoeuvre; Lung functions; Functional capacity; Dyspnea scale; Quality of life

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Abstract

BACKGROUND: Exercise tolerance is one of the main impacts of COPD. COPD patients often experience dyspnea and fatigue after doing daily activities using their limb parts, even in simple thing such as lifting or grooming. Nowadays, many pulmonologists concerned in pulmonary rehabilitation to modify some limb training with breathing manoeuvre to get positive impact in stable COPD patient.

AIM: The purpose of this study is to examine the impact of this modified upper limb training in lung function, functional capacity, dyspnea scale, and quality of life in patients with stable COPD.

METHOD: This was a quasi-experimental study held in 2017 on 22 stable COPD patients (based on GOLD 2018 criteria). Patients were given modified upper limb training with breathing manoeuvre that leads and monitored by a physiotherapist and physician in 10-20 minutes twice a week for 8 weeks. Before and after completed all sessions of training, we measured pulmonary functions test include FEV1 and FVC, functional capacity by 6 MWT, dyspnea scale by mMRC, and quality of life by CAT assessment. Statistical analysis was performed by Wilcoxon and paired t-test.

RESULTS: There was an improvement of lung function, both FEV1 (40.7 ± 13.8 to 47.3 ± 14.2; p-value 0.001) and FVC (50.7 ± 14.1 to 54.1 ± 14.7; p-value: 0.207) after training. There was a significant change of functional capacity in 6 MWT mean (277.3 ± 80.8 to 319.1 ± 78.3; p-value: 0.001). There was an improved quality of life after training, measured by decreasing in CAT score (23.9 ± 5.5 to 18.3 ± 5.2; p-value: 0.000). There was no significant change in the mMRC scale (p-value: 0.429)

CONCLUSION: There was an improvement of lung function, functional capacity, and quality of life in stable COPD after upper limb training with breathing manoeuvre in stable COPD patients.

Introduction

COPD (Chronic Obstructive of Lung Disease) is a common disease characterised by persistent respiratory symptoms and airflow limitations caused by airway and alveolar abnormalities due to significant exposure of noxious gases [1]. COPD is a preventable and treatable disease that challenge physicians to find new cases and treat patients with early respiratory symptoms.

Exercise tolerance is one of the main impacts of COPD. Many patients report the limitation of exercise that further impacts their quality of life. Some studies stated that COPD patients often experience dyspnea and fatigue after doing daily activities using lower and upper limb parts, even in simple things such as lifting or grooming [2], [3]. In people with COPD, there is an increase in metabolic and ventilatory, particularly in unsupported arm work. During arm

exercise, the accessory muscles of respiration will prioritise the arm task than breathing, and it leads to thoracoabdominal dyssynchrony then aggravate dyspnea. It also limits the tidal volume in results of increasing chest wall impedance to maintain the trunk and move the arms during arm activities [3], [4].

Many studies just concerned on lower body training to decrease impact in COPD patient, and there are just little studies that concerned mainly to upper limb training. However, upper limb training can improve lung function and functional capacity, then reduce symptoms and improve quality of life in patients with COPD [5], [6]. Supported and unsupported arm training have increased the endurance capacity and result in reduced breathlessness in daily life with a patient with COPD [7]. Upper limb training considered to decrease dyspnea scale, improve respiratory muscle coordination, and beneficial adaptations to the exercise [8]. During exercise, some patients often felt dyspnea or chest discomfort, particularly at the beginning of the training session. So, it is needed a few combinations of training include breathing pattern such as pursed lips breathing to minimalise the impact of training in patients symptom during a training session.

Exercise technique in this study is adopted in some pulmonary rehabilitation programs. However, there was no definite pattern of upper limb training with breathing manoeuvre pattern has been conducted. It gives a challenge to pulmonologist concerned in pulmonary rehabilitation to modify some upper limb training to get positive impact in stable COPD patient.

The purpose of this paper is to examine the impact of this modified upper limb training in lung function, functional capacity, dyspnea scale, and quality of life in patients with stable COPD.

Methods

This research was a quasi-experimental study held in the Department of Pulmonology and Respiratory Medicine, Faculty of Medicine, Universitas Sumatera Utara in 2017. This study protocol was approved by the Ethics Committee of Faculty of Medicine in Universitas Sumatera Utara.

Participants

Twenty-two participants enrolled in this study from a patient who came to the Department of Pulmonology and Respiratory Medicine in General Hospital of H. Adam Malik, Medan. Sampling was carried out by consecutive sampling with 22 patients who matched inclusion criteria and had no exclusion criteria. The inclusion criteria were stable COPD patients age 40-80 years and had not been involved in any exercise program for these two months. The diagnosis of COPD was established by history, physical examination, then confirmed by spirometry examination with GOLD 2017 criteria (FEV_1/FVC < 70). The exclusion criteria were patients in exacerbation state, did not want to follow or had an irregular exercise program, and had malignancy.

Protocol

After all, the participants had understood the contents of the study and filling the informed consent; they were scheduled for a training program. Before training, they had been given short-acting beta agonist (Salbutamol 2,5 mg) with a nebuliser, and they were confirmed in a clinically stable state when they came to the training program by a physician. First, they underwent warm up and muscle stretching for avoiding muscle injury for 10-15 minutes. Then, upper limb training for 10 minutes leads by physiotherapist and video prepared before. Upper limb training with breathing exercises consists of few manoeuvres such as:

1. Pursed lip breathing with exhaling while tilting your head towards your shoulder.
2. Bird-like pattern with inhaling while body straightening, exhale while bending forward to the bottom.
3. No-way pattern with pursed-lip breathing, seeing a movement to left and right alternately.
4. Shoulder shrug with pursed lip breathing.
5. Fan-like movement with pursed-lip breathing, hands are bent together, then turn right and left.
6. Chicken cuckoo like movements with rotating the shoulder with hands bent at the shoulder.
7. Vampire-like movement, hands straight forward while inhaling, then rotating the body to the right, left, and forwards while exhaling.
8. Calling movement, the hand is lifted, then touched it downwards, in the opposite direction.
9. Butterfly-like pattern, hands stretched straight forward then hands stretch.
10. It is cooling down.

This upper limb training held twice a week for 8 weeks. Before and after every session of training, vital sign measured and there were few physicians for leading and monitoring patients in the training program.

The followings were measured before and

after the training:

1. Lung function was measured by Forced Expiratory Volume in 1 second (FEV_1) and Forced Volume Capacity (FVC). The GOLD grade was made based on FEV_1 that divided into four categories. GOLD 1 for FEV_1 > 80%, GOLD 2 for FEV_1 50-79%, GOLD 3 for FEV_1 30-49%, and GOLD 4 for FEV_1 < 30%.

2. Oxygen saturation was measured by pulse oximetry. Oxygen saturation below 88 indicated hypoxia in stable COPD patient and needed for oxygen therapy while training session.

3. Functional capacity was measured by six minutes walking test (6 MWT). Patients were instructed to walk as fast as they can for 6 minutes on the hospital corridor. The patient may take a rest or decrease their speed if they experienced dyspnea or chest discomfort, but the timer was not stopped.

4. Dyspnea scale was measured by the modified Medical Research Council (mMRC) which score ≥ 2 indicates patients have more symptoms.

5. Quality of life measured by the COPD Assessment Test (CAT) questionnaire. The result ≥ 10 from CAT indicates patients' quality of life were impaired.

Statistical Analysis

All the collected data was entered and analysed by using SPSS (Statistical Package for the Social Science) for Windows version 16.0. Data was described in the distribution of frequencies then analysed using paired T-Test or Wilcoxon Test for bivariate analysis to know whether there is a significant change of lung function, functional capacity, dyspnea scale, and quality of life mean before and after the upper limb training program.

Results

Total twenty-two patients enrolled in this study consisted of women and men, age 40-80 years old, with a diagnosis of stable COPD and adequate adherence of completed all session of upper limb training rehabilitation program.

From Table 1, we can see that the majority of the subject was male in 60-69 years old with severe index Brinkmann (IB ≥ 600). Based on airway obstruction, most participants were in grade III with FEV_1 30-49%. All participants had more symptoms of COPD with low quality of life, based on CAT score ≥ 10. Most participants had high dyspnea scale from mMRC that interpret most participants had limitations in moderate to daily activities.

Table 1: General characteristics of participants in this study

Characteristics		N	%	Mean ± SD
Sex				
Female		3	13.6	-
Male		19	86.4	
Age				62.9 ± 8.56
(Years Old)	40-49	1	4.5	
	50-59	6	27.2	
	60-69	1	59.0	
	70-79	2	9.0	
Brinkman Index				1236 ± 954
	Mild (< 200)	3	13.6	
	Moderate (200-599)	3	13.6	
	Severe(≥ 600)	16	72.7	
GOLD severity (FEV_1)				40.7 ± 13.8
%	I (≥ 80)	0	0	
	II (50-79)	6	27.2	
	III (30-49)	1	45.4	
	IV (< 30)	6	27.2	
CAT score				23.9 ± 5.5
	< 10	0	0	
	≥10	22	100	
mMRC				
	0-1	6	27.3	
	≥ 2	16	72.7	

There was an increase of FEV_1 mean after 8 weeks of upper limb training compared to before training (40.7 ± 13.8 to 47.3 ± 14.2). It was in line with the statistical analysis using the Wilcoxon test that showed p < 0.05, interpret as there was a significant change of FEV_1 after 8 weeks of upper limb training. The same results also happen in FVC that showed an increased of mean after 8 weeks of training (50.7 ± 14.1 to 54.1 ± 14.7), but it was not significant in statistical analysis using paired T-test with p-value > 0.05.

Table 2: Mean and standard deviation of lung function, functional capacity, dyspnea scale, and quality of life before and after upper limb training

Characteristics	Mean ± SD Before training	Mean ± SD After Training	P value
FEV1	40.7 ± 13.8	47.3 ± 14.2	0.001**
FVC	50.7 ± 14.1	54.1 ± 14.7	0.207*
6MWT	277.3 ± 80.8	319.1 ± 78.3	0.001*
mMRC	2.36 ± 1.2	2.1 ± 0.9	0.429**
CAT	23.9 ± 5.5	18.3 ± 5.2	0.000*

*p-value from paired T-test, considered significant if p < 0.05; **p-value from Wilcoxon Test, considered significant if p < 0.05.

Functional capacity was measured by six meters walking test (6MWT) that showed significantly increased of mean after 8 weeks of training (277.3 ± 80.8 to 319.1 ± 78.3), and when analysed in statistical analysis using paired T-test, there was a significant change of functional capacity with p-value < 0.05.

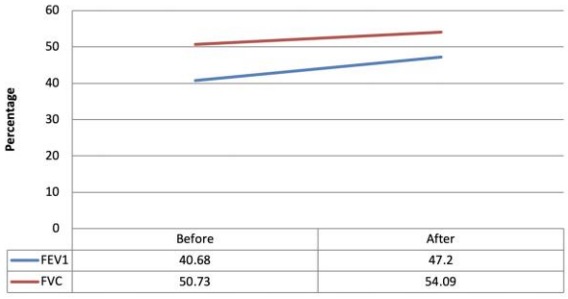

Figure 1: Comparison of lung functions between before and after training

Dyspnea scale could be measured by a few variables. In this study, we used the mMRC scale that

showed slight improvement after 8 weeks of training although it was not significantly changed in statistical analysis using the Wilcoxon test with p-value > 0.05.

Quality of life was measured by CAT questionnaire, a higher point in the CAT questionnaire showed the lower quality of life in a patient with COPD. In this study, there was a decreased mean of CAT after 8 weeks of training that interprets the increased quality of life in stable COPD patient.

Discussion

Some studies stated that COPD is a respiratory disease not only affecting the lungs, but also giving non-respiratory manifestations, including skeletal muscle dysfunction with atrophy and weakness, systemic inflammation, nutritional depletion and malnutrition, which can contribute to exercise limitation and affect patients' function and mobility [5], [9], [10], [11], [12]. Papaioannou et al. observed that higher systemic levels of oxidative stress in COPD patients may contribute to a reduction in the body mass and fat-free mass indexes, thereby contributing to impaired exercise capacity [13].

Muscles of the superior part of the thorax and scapular girdle, which serve for respiratory and postural functions, have thoracic and extrathoracic attachment points, such as the inferior/superior trapezius, latissimus dorsi, serratus anterior, subclavius and pectoralis major and minor. In COPD patients with pulmonary hyperinflation, which frequently occurs, the diaphragm lowers and loses its capacity to generate force, so that the ribcage muscles become more important to generate the inspiratory pressures [14].

Strategies have been used in COPD patients aiming to improve exercise performance. Pursed lip breathing is an intuitive technique that frequently COPD patients adapt to reduce dyspnea during exertion as a breathing retraining form. This strategy relieves the dyspnea sensation immediately after beginning to use the technique and encourages expiration time during the concentric phase of upper limb movements. Therefore it seems to be a form of physical exercise that could minimise the action of the accessory muscles [15].

COPD is a chronic disease that manifests in late age, after more than 10 years old insignificant noxious gases exposure. Long-term inflammation in airway and lung parenchyma due to free radical and oxidant in tobacco smoke and other environmental pollutions lead to subsequent airway and lung parenchyma damage [16]. Further, there was lung ageing theory that state lung function will decline along to increasing age because of structural and functional change. In elderly, there will be few changes in alveolar structure that sometimes called senile emphysema, changes in the chest wall caused by osteoporosis, stiffness in ribs, and reduced thickness in intervertebral discus [17], [18]. All these data related to our study that showed the majority of participants are in 60-69 years old.

Smoking is the main risk factor for COPD. The longer and the more of cigarette consumption, the longer and the greater inflammation will occur in airway and lung parenchyma. Cigarette smoke and nicotine can decrease the function of macrophages in lung, and specific enzymes that serve as energy for phagocytosis can be suppressed. It also inhibits mucociliary transport that made the most vulnerable to infection. Further, the materials in cigarette smoke can defect the alpha-1 antitrypsin that leads to the breakdown of elastin, component maintaining the elasticity of the lung, by neutrophil elastase [19], [20]. In this study the most Brinkman Index Values were severe (72.7%). This is similar to Ignatius et al., reported that COPD patients tend to have a Brinkman index medium to severe caused by high consumption of cigarette on patients [21].

From our study, we can see that there was an improved lung function, including FEV_1 and FVC, although FVC was not a significant change. This is line with Elmorsy study that stated improvement of FEV_1 and FVC after 8 weeks of upper limb training, but not significant in statistical analysis [22] (Elmorsy, 2013). Ries et al. found that there was a significant improvement of lung function include FVC, Residual Volume (RV), Functional Residual Capacity (FRC), Total Lung Capacity (TLC), but there was no significant change of FEV_1 after 6 weeks of upper limb training [23]. These inconsistent results caused by different type of upper limb training manoeuvre and clinical condition in every participant enrolled in each study.

In this data, there was no significant change in the dyspnea scale using the mMRC questionnaire. This is in line with McKeough study that stated there was no significant decrease in dyspnea in patients with stable COPD after upper limb training [6]. The same results also stated in Zanchet study that showed that there was no significant dyspnea improvement after pulmonary rehabilitation with upper limb endurance training after 6 weeks of training b session [24]. Sciriha study in South Europa also found there was no significant difference after 8 weeks of training program [25]. In contrast, Lacasse et al. showed a significant decrease in dyspnea means after 8 weeks of upper limb training [26]. In meta-analysis study in China, there was an improvement of dyspnea after more than 8 weeks of upper limb training, but it was not a significant change in statistical analysis. However, when upper limb training is given in shorter period, 3-8 weeks, there was a significant decrease of dyspnea, which suggest that short duration of upper

limb training can reduce dyspnea symptom in stable COPD patient [27].

Functional capacity is the ability to perform activities of daily life. An objective assessment of the functional capacity in this study was the 6 minutes walking test. There was a significant change of 6 minutes walking test mean after 8 weeks of intervention in this study ($p = 0.001$). There is an increase about of 41.81 ± 48.07 meters at the end of the training session. This is in line with few studies that showed improvement of functional capacity after limb training. Finnerty et al. reported an increase of about 59 meters after receiving 6 weeks of pulmonary rehabilitation compared to controls [28]. Bendstrup et al. reported an increase of 6 minutes walking distance of about 79.8 meters in the treatment group and 21.6 m in the control group ($p < 0.001$) [29]. Lacasse et al. conducted a meta-analysis of patients with COPD who received pulmonary rehabilitation and found the mean difference in the 6 minutes walking test is an increase of 55.7 meters and concluded that the minimum increased is 50 meters [30]. British Thoracic Society (BTS) recommends a minimum increase of 6 minutes walking test which considered clinically significant is 54 m [31].

Regular and intensive exercise in COPD will give effect in cardiopulmonary physiology, hormonal balance, and biochemical part in tissue. In general, regular exercise can induce oxidative capacity, decrease ventilation in submaximal workloads, and decreased oxygen consumption in submaximal workloads [32]. Exercise can increase the myoglobin amount in type 1 skeletal muscle fibre that helps the diffusion of oxygen from the cell membrane into the mitochondria [33]. All these processes impact the significant improvement of functional capacity in stable COPD patient.

Assessment of the quality of life in these patients also showed significant differences after the intervention. Participants had a decreased mean in CAT Questionnaire from 23.9 ± 5.5 to 18.3 ± 5.2 that interpret they had decreased symptoms in daily life. A multicenter study conducted by James W Dodd in 2011 correlate the CAT assessment before after 8 weeks of training in pulmonary rehabilitation and showed significant differences in which 162 patients feel better after pulmonary rehabilitation programs [31]. Lacasse et al. reported from a meta-analysis that pulmonary rehabilitation would reduce the symptoms of breathlessness and improve the activity of patients with COPD so that the functional capacity and quality of life also increase [30]. Subin et al., also conclude in their study that upper limb training can improve quality of life, although they used a different method to measure the quality of life, by using CRQ (Chronic Respiratory Questionnaire) [34]. Elmorsy et al., also stated that there is an improvement of the quality of life in patients who received upper limb training, and or without combination with lower limb training [22]. Berry et al., also explained that pulmonary rehabilitation would increase the maximum oxygen consumption and maximum work capacity thus increasing the functional capacity and quality life of patients with COPD [35]. Aerobic exercise can improve pulmonary symptoms by increasing maximum heart rate and anaerobic threshold in metabolic level, decreasing airway inflammation and increasing the usage of the ventilatory reserve [36], [37]. Besides, regular exercise has social, emotional and mental effect improvement. Patients can have more self-confident to control their symptoms in daily life [38] contribute to an improvement in the quality of life in patients with stable COPD after the upper limb training program.

There are some limitations of this study, include the number of participants and the methodological used. A small group of participants can make it was difficult to rule out the personal factor that could interfere with the result of this study. In the method, this study did not have a control group so we could not make the comparison between intervention study and control group. Dyspnea scale could be measured in some questionnaire, such as CRQ, mMRC, and SGRQ (St George Respiratory Questionnaire), but in this study, we use mMRC to measure the severity of dyspnea. Besides, we suggest measuring further parameters of lung function such as total lung capacity (TLC), residual volume (RV), maximal Peak Inspiratory Pressure (PImax), vital capacity (VC), and PEFR (Peak Expiratory Flow Rate).

From this study, we can conclude that upper limb training gives a positive impact on stable COPD. Improvement of lung function, functional capacity, and quality of life of stable COPD patient after 8 weeks of training showed significant change. So, upper limb training must be a part of a pulmonary rehabilitation program in the comprehensive treatment of COPD.

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Investigating Different Dimensions of Nomophobia among Medical Students: A Cross-Sectional Study

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Abstract

BACKGROUND: Today, mobile phones are recognised as an inseparable part of our daily lives, facilitating communication between users. Based on the studies, addiction to cell phones can lead to several complications including depression, anxiety, anger, and aggression.

AIM: This study aimed to investigate nomophobia (no mobile phone phobia) among medical students of Islamic Azad University, Tehran Branch.

METHODS: This descriptive cross-sectional study was conducted on 100 students studying in different majors of medical sciences in Islamic Azad University, Tehran Branch, from 2016 to 2017. Demographic data of all participants were recorded in a data sheet. In the next stage, a questionnaire was designed by the researcher to evaluate the effect of age, gender, education, and the duration of using cell phone variables on discomfort, anxiety, and insecurity due to lack of access to cell phone or other related issues. Raw data were analysed using SPSS statistical software version 21. The significance level was considered $P < 0.05$.

RESULTS: The results of the study showed that participants with lower mean age felt more discomfort, anger, anxiety, and insecurity due to lack of access to mobile phones and other related issues compared to other people. However, no variable was statistically significant (P-value > 0.05). Except anxiety, results showed that longer duration of mobile phone use might lead to a significant decrease in discomfort, anger, and insensibility variables among users (P-value > 0.05). The incidence of nomophobia (with its different aspects) was significantly lower in females (P-value > 0.05). Also, in participants with higher educational status, the nomophobia was recorded to be more frequent (P-Value > 0.05).

CONCLUSION: Understanding the pattern of nomophobia occurrence among cell phone users can facilitate our path to prevent its harms including discomfort, anger, anxiety, and feeling of insecurity among users of technology.

Introduction

Rapid advances in telecommunication technology and the efforts of researchers to transfer data and information as fast as possible have made today communities information dependent more than ever. In the recent years, technological advances have had a significant impact on economics, commerce, and financial markets, so that they have affected the trend of developing information technology and production processes, as well as increasing the productivity and standards of living in several countries [1]. Most thinkers and sociologists believe that techniques for utilising tools should be reconsidered based on the new advances in technology because they have a major impact on the lives of people [2]. The emergence of smartphones has progressed this trend. According to studies, the unlimited use of mobile phones affects the physical

health of users, especially children and teenagers [3], [4]. Researchers believe that the pattern of using cell phones depends on factors including gender, personality, marital status, socioeconomic status, age and educational status [5], [6], [7]. Using cell phones is common among people between the ages of 25 and 35. It is obvious that mobile phones are among the most utilised products in developed and developing societies, thereby, some researchers believe that mobile phones have facilitated societies to enter the age of information [8]. Considering that the goal of creating these products is the comfort of human, excessive use of them can lead to several physical and psychological harms, which eventually leads to the alternation of various aspects of people's lives. Today, the damage caused by technology has attracted the attention of many researchers. One of the damages related to communication is nomophobia. Nomophobia which is a combination of the phrases "No Mobile Phobia" was first reported in England [9], [10]. Based on the definition of Nomophobia, this phenomenon is a consequence of anxiety, stress, and fear due to lack of access to mobile phones and related issues [11]. This fear is followed by a feeling of frustration, insistence, expectation, persistence, and obsessive thoughts and imaginations. Researches have shown that the effects of nomophobia are more obvious in people with underlying diseases (such as depression, anxiety, fear, dependency, low self-confidence and social issues) [12]. Several people are involved with nomophobia globally. In a study conducted in 2005, it was shown that Nomophobia is more prevalent in teenagers and young adults [13]. Cognitive-behavioural therapy along with pharmacological interventions is recommended by the specialists to treat Nomophobia disorder [12]. Up today, some dimensions of this disorder are still not clear, which shows that this disorder is a suitable context for researchers to design studies. Therefore, in this study, we designed and conducted an evaluation of Nomophobia among students of medical sciences of Islamic Azad University, Tehran Branch.

Material and Methods

The population of this cross-sectional study included medical students of Islamic Azad University, Tehran Branch, between 2016 and 2017. Participants entered the study according to ethical principles of research and with complete knowledge about the research process. Exclusion criteria included individuals with no cell phone, disagreement with the principles of the study, studying in majors other than medicine, and not having smartphones. In this study, we investigated Nomophobia among medical students of Islamic Azad University, Tehran Branch. In the first step, the demographic data were recorded using a questionnaire. Then, data on nomophobia variables were extracted. The variables included 1. Discomfort, (Questions: 1) do I feel uncomfortable If I do not have access to my mobile data at any moment? 2) Do I feel very annoyed If I cannot access my mobile data during work? 3) Without my mobile, are all my connections to the network lost and do I get upset? 2. Anger, (Questions: 1) If I cannot get updates of the news via my phone, do I get nervous? 2) If I cannot use my phone or the battery dies, do I get angry and upset? 3) If my mobile phone is not with me, do I get nervous because of the taught that someone might try to contact me? 3. Anxiety, (Questions: 1) If my battery charge is running out, so I get worried? 2) If I use limited services on my phone, am I always nervous about the ending of them? 3) If I forget to bring my mobile phone, do I get worried, because of the possibility that I might be unable to contact my family or friends immediately? 4) If my mobile phone is not by my side, do I get nervous because of the possibility that I would not be able to access my messages and call logs? 5) If my mobile phone is not by my side, do I get worried about the status of my family? 6) If my cell phone is away, would all my relationships and access to my friends' numbers be limited so I might get anxious? 7) If my cell phone is far away from me, do I get nervous because I would not be able to access my emails? And 4. Insecurity, (Questions: 1) If my cell phone is away from me, do I feel insecure because someone might have accessed my data? 2) Do I feel insecure without my mobile phone and I don't know what to do? 3) If I forget to take my phone, do I feel nervous about leaving home?

Raw data were analysed using IBM SPSS Statistic software version 23. Descriptive and analytical statistics were analysed to reach the objective of the study which was evaluating factors affecting nomophobia including the prevalence of this phenomenon in males and females, the effect of age on the prevalence, and the occurrence of anger in nomophobia. After determination of the normality of data, K-S test was used for statistical analysis of ANOVA, as well as Kruskal-Wallis and nonparametric Chi-Square test. Also, the statistical significance (P-value) was considered less than 0.05 in this study.

Results

In this study, we investigated the effect of age, gender, education level, and duration of mobile phone using variables on the discomfort of individuals due to unavailability or other mobile-related issues. The results showed that discomfort variable decreases along with the increase in age. In other words, participants who were more uncomfortable with the unavailability of their phones or other mobile-related issues had a lower average age. However, this

level of difference was not statistically significant (P-value = 0.168). In this study, the relationship between the average duration of mobile use and discomfort due to unavailability of mobile phone or other related issues was studied. Although the difference between the results was not significant, the average duration of mobile use was less among users who were uncomfortable with the unavailability of mobile phone or other related issues. In other words, the increase in the duration of mobile phones use among users has led to a decrease in their discomfort due to unavailability of mobiles or other related issues (P-Value = 0.382) (Table 1).

Table 1: The effect of age variable and duration of mobile phone use on the discomfort status of individuals

Discomfort vs Age					
		N	Mean	Std. Deviation	P-Value
Age	No	44	24.4773	5.91210	0.168
	No comment	10	22.9000	4.79467	
	Yes	37	22.4595	3.14991	
Total		91	23.4835	4.89072	
Duration of mobile phone use vs Discomfort					
		N	Mean	Std. Deviation	P-Value
Duration of mobile phone use	No	63	8.0159	2.73857	0.382
	No comment	5	7.0000	2.73861	
	Yes	21	7.3810	3.62596	
Total		89	7.8090	2.95372	

Also, the descriptive statistics in this study showed that the frequency of discomfort variable in females (38%) is lower than males (46.4%). However, this difference was not statistically significant (P-value = 0.625). The results from the comparison between education level variable and discomfort indicate that there was no significant relationship between the level of education and discomfort variable in the participants. However, the descriptive statistics showed that participants with a bachelor's or higher degree have a higher relative frequency of discomfort due to nomophobia (P-Value = 0.793) (Table 2).

Table 2: The effect of gender and education level variables on discomfort status of individuals

Sex vs Discomfort						
		Discomfort			Total	P-Value
		No	No comment	Yes		
Sex	Male	13 (46.4%)	2 (7.14%)	13 (46.4%)	28	
	Female	35 (49.2%)	9 (12.6%)	27 (38%)	71	0.625
Total		48	11	40	99	
Level of Education vs Discomfort						
			Discomfort		Total	P-Value
		No	No comment	Yes		
Level of Education	Highschool graduate and Associate degree	6 (66.6%)	1 (11.11%)	2 (22.22%)	9	0.793
	Bachelor's degree	16 (47%)	3 (8.82%)	15 (44.1%)	34	
	Higher education	25 (45.4%)	7 (12.7%)	23 (41.8%)	55	
Total		47	11	40	98	

Investigating the effect of age, gender, level of education, and duration of mobile use variables on anger status due to unavailability of mobile phone or other related issues showed that participants with a higher mean age were less likely to show symptoms of neural nomophobia. In other words, the anger due to the unavailability of mobile phone or other related issues was higher in participants with lower mean age. However, this level of difference was not statistically significant (P-Value = 0.912). The results also showed that participants with lower duration of cellphone use were angrier with the unavailability of mobile phones or other related issues compared to others, though; the difference was not statically significant (P-Value = 0.247) (Table 3).

Table 3: The effect of age variable and duration of mobile phone use on anger status of participants

Anger vs Age					
		N	Mean	Std. Deviation	P-Value
Age	No	37	24.0811	6.12544	0.912
	No comment	15	22.8667	4.10342	
	Yes	40	23.1500	3.73171	
Total		92	23.4783	4.86404	
Duration of mobile phone use vs Anger					
		N	Mean	Std. Deviation	P-Value
Duration of mobile phone use	No	43	7.8140	2.71903	0.247
	No comment	9	9.1111	3.25747	
	Yes	38	7.4211	3.06357	
Total		90	7.7778	2.92904	

Statistical analysis indicates that the frequency of anger status in females (43%) was lower than males (44.4%). However, this difference was not statistically significant (P value = 0.829). Comparing the variables of education level and anger status showed that there is no significant relationship between the level of education and anger status in participants. Though, analysing the descriptive statistics showed that the percentage of diagnosed nomophobia is higher in participants with a higher level of education (P-value = 0.328) (Table 4).

Table 4: The effect of gender and education level variables on anger status

Sex vs Anger						
		Discomfort			Total	P-Value
		No	No comment	Yes		
Sex	Male	10 (37%)	5 (18.5%)	12 (44.4%)	27	
	Female	31 (43%)	10 (13.8%)	31 (43%)	72	0.829
Total		41	15	43	99	
Level of Education vs Anger						
			Discomfort		Total	P-Value
		No	No comment	Yes		
Level of Education	Highschool graduate and Associate degree	6 (66.6%)	1 (11.11%)	2 (22.22%)	9	0.328
	Bachelor's degree	16 (47%)	5 (14.7%)	13 (38.2%)	34	
	Higher education	18 (32.7%)	9 (16.3%)	28 (50%)	55	
Total		47	40	15	43	

The findings of the study showed that the increase in age led to a decrease in the feeling of insecurity due to the unavailability of mobile phone and other related issues. However, this relationship was not statically significant (P-Value = 0.172).

Table 5: The effect of age variable and duration of mobile phone use on the status of insecurity

Insecurity vs Age					
		N	Mean	Std. Deviation	P-Value
Age	No	63	23.8571	5.19881	0.172
	No comment	6	21.5000	5.31977	
	Yes	21	23.2857	3.67618	
Total		90	23.5667	4.88071	
Duration of mobile phone use vs Insecurity					
		N	Mean	Std. Deviation	P-Value
Duration of mobile phone use	No	47	7.7021	2.97756	0.663
	No comment	4	9.0000	3.46410	
	Yes	38	7.6158	2.87445	
Total		90	89	7.8090	

The results from Kruskal Wallis statistical test showed that participants who felt less comfortable with the unavailability of mobile phones or other related issues tend to spend less time with their cell phones (P-Value = 0.663) (Table 5).

According to statistical analyses, this disorder was seen less in females (39.43%) compared to males (50%). However, this difference was not statistically significant (P-value = 0.567). According to assumptions, there was no significant relationship between the level of education and insecurity variables due to unavailability of mobile phone or other related issues in participants (P-Value = 0.813) (Table 6).

Table 6: The effect of gender and education level variables on insecurity status

Sex vs Insecurity						
		Discomfort			Total	P-Value
		No	No comment	Yes		
Sex	Male	11 (42.3%)	2 (7.69%)	13 (50%)	26	0.567
	Female	39 (54.9%)	4 (5.63%)	28 (39.4%)	71	
Total		50	6	41	97	
Level of Education vs Insecurity						
		No	Discomfort No comment	Yes	Total	P-Value
Level of Education	Highschool graduate and Associate degree	7 (77.7%)	0	2 (22.22%)	9	0.813
	Bachelor's degree	21 (65.6%)	3 (9.37%)	8 (25%)	32	
	Higher education	42 (75%)	3 (5.35%)	11 (19.64%)	56	
Total		70	6	21	97	

It was found that the anxiety of participants reduced as their age increased (however it was not statistically significant) (P-value = 0.367). This means that participants with anxiety resulted from nomophobia tend to have lower mean ages, and as the age increases, the anxiety level decreases. The research shows that the anxiety of participants due to the unavailability of mobile phones or other related issues increased along with the increase in the duration of mobile phone use. (P-Value = 0.465) (Table 7).

Table 7: The effect of age variable and duration of mobile phone use on anxiety status

Anxiety vs Age					
		N	Mean	Std. Deviation	P-Value
Age	No	46	23.9130	6.01415	0.367
	No comment	6	21.1667	2.48328	
	Yes	38	23.1579	3.31684	
Total		90	23.4111	4.86883	
Duration of mobile phone use vs Anxiety					
		N	Mean	Std. Deviation	P-Value
Duration of mobile phone use	No	36	7.3333	2.98568	0.465
	No comment	14	8.2143	2.11873	
	Yes	41	8.0732	3.10153	
Total		91	7.8022	2.92202	

Based on descriptive data, anxiety resulted from nomophobia in females (20%) was lower than males (25%) (P-value = 0.864). Statistical data showed that a higher percentage of people with higher education experienced anxiety due to the unavailability of their cell phones or other related issues. Despite that, no significant relationship was seen between these statistics (P-Value = 0.672) (Table 8).

Table 8: The effect of gender and education level variables on anxiety status

Sex vs Anxiety						
		Discomfort			Total	P-Value
		No	No comment	Yes		
Sex	Male	19 (67.8%)	2 (7.14%)	7 (25%)	28	0.864
	Female	52 (74.2%)	4 (5.71%)	14 (20%)	70	
Total		71	6	21	98	
Level of Education vs Anxiety						
		No	Discomfort No comment	Yes	Total	P-Value
Level of Education	Highschool graduate and Associate degree	5 (55.5%)	1 (11.11%)	3 (33.33%)	9	0.672
	Bachelor's degree	20 (58.8%)	2 (5.8%)	12 (35.2%)	34	
	Higher education	24 (45.2%)	3 (5.66%)	26 (49%)	53	
Total		49	6	41	96	

Discussion

Today, cell phones have become an important part of life, especially among young people. According to studies, teenagers are more attached to their phones than adults. Also, the trend of using mobile phones is increasing among students [14]. Studies by experts on the negative physical and psychological complications of excessive use of cell phones indicate that it can lead to dependency syndrome [15]. According to researchers, excessive use of cell phones can lead to a wide range of complications including headaches and even microbial infections [16]. One of the most common new disorders in the world is nomophobia. Nomophobia is a modern phobia which is a consequence of interactions between a human being and mobile communications technology, especially smartphones [17]. Nomophobia is recognised as a behavioural addiction to cell phones which is characterised by psychological symptoms as well as physical dependence [18]. Most important characteristics of this disorder include discomfort, anxiety, anger, or stress due to lack of contact with the cell phone [19]. We tried to identify and describe the dimensions of nomophobia by creating a questionnaire for the students of medical sciences at the Islamic Azad University of Tehran. Up to date, several studies have addressed this issue. Dixit et al., (2010) designed and implemented a study entitled "investigating the level of mobile dependency among college students and medical students of the Indian Medical Center". Their results indicate that 73 per cent of students bring their mobile phones to bed at the time of sleep, and even do not let go of it all over the 24 hours of the day. Also, they found that 38.5% of the participants repeatedly checked their mobile phones for SMS and calls [20]. In another study by

Singh et al., (2013), it was found that the prevalence of nomophobia is increasing. They concluded that users are becoming more dependent on their mobile phones every day, which can lead to psychological and personality issues for them. They also stated that the psychological dependence to cell phones and the tendency to nomophobia is associated with several complications including hearing the fake sound of ringtone, and constant checking of pocket or bag to ensure the presence of the mobile phone [21].

Katharine et al. (2008) evaluated nomophobia in 2163 participants in England. The results of their study showed that 52 per cent of users experienced anxiety and stress in case of losing their mobile phones, dying battery charge, or being short on credit, so lacking access to their mobile phones. Also, their results showed that 58 per cent of males and 48 per cent of females had nomophobia. They concluded that the main reason for the stress of the participants was the lack of access to friends or family [22]. Prasad et al., (2017) evaluated the pattern of mobile phone usage among 554 dental students. The results of their study indicated that circa 39.5% of students believe that if they spend more time using their mobile phones, they will get lower grades in the professional exam.

Moreover, they stated that 24.12% of students are at risk of depression. Statistical analysis of the results in their study showed that there is a significant difference between education level and depression due to nomophobia in students. They concluded that the excessive use of mobile phone among dentistry students has led to an addiction to mobile phones and consequently depression, which has also affected the educational performance of them [23].

A comprehensive review of the results in the present study shows that nomophobia occurrence is increased with reduction in the duration of mobile phone use, and younger people, as well as people with higher education levels. Although different studies have addressed several aspects of nomophobia, the findings of the present study confirm the findings of previous researches and are in line with them. Considering the limited number of conducted studies in this area with a different methodology, all seeking to answer their questions, it is not possible to accurately compare the results at this time. However, the general concepts of the results are the same and consistent in all studies.

In conclusion, the advances in technology and the widespread use of it has emerged new disorders and dependencies. Addressing these issues can show us some vital but vague aspects of technical complications. By using the results of this study, we can conclude that the prevalence of homophobia is higher in lower ages, short-duration mobile phone users, as well as males and people with higher levels of education. Although the data of the study are not significant for any of the statistics, using the statistical descriptions would make us able to decide on the future studies and design them, and determine a target community with greater influence.

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Clinical Science

Uterine Fibroid Embolization via Transradial versus Transfemoral Arterial Access: Technical Results

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Abstract

AIM: This study was designed to compare the safety and feasibility of uterine fibroid embolisation (UFE) via transradial access (TRA) and transfemoral access (TFA).

MATERIAL AND METHODS: A retrospective analysis was conducted for 2 cohorts: 13 cases with already established TFA (from February 2016 to September 2018) and the first 11 procedures performed via TRA (from October 2017 to October 2018). Indications for embolization included: heavy menstrual bleedings (n = 18), lower urinary tract symptoms (n = 2), pelvic pain (n = 3) and abdominal pain (n = 1). One interventional radiologist and one fellow performed all procedures at one institution. Technical success, procedural time, access site complications as well as feedback from patients were assessed for analysis.

RESULTS: Technical success was achieved in 24/24 cases (100%). Unilateral uterine artery embolisation was performed in 7 cases (29.1%) and bilateral in 17 cases (70.8%). Mean procedure time was 72.4 minutes in TFA group, and 60.3 minutes in the TRA group. Mean fluoroscopy time was 25.3 minutes in the TFA group and 21.1 minutes in the TRA group. Access site-related and overall adverse events did not vary significantly among the study cohorts.

CONCLUSIONS: TRA represents a safe and feasible approach for UFE with a comparable safety profile to TFA.

Introduction

Uterine fibroids are the most common gynaecological benign tumours in women and can cause symptoms like severe menstrual bleedings with or without repercussion of blood account, pelvic or abdominal pain, swelling, urinary tract symptoms, bowel compression etc. Uterine fibroid embolisation (UFE) is a minimally invasive endovascular procedure which is performed for the treatment of fibroids. On the other hand, surgery is the standard treatment of choice for this pathology offered by gynaecologists. Hysterectomy and myomectomy, both in the classical or laparoscopic way are the most common surgical techniques. UFE is an established endovascular interventional technique which includes delivery of embolic agents through the uterine arteries directly in uterus and fibroids. This intervention is typically offered to women who refuse surgery.

Further it is commonly practiced in women who want to preserve their uterus by any means, who are contraindicated to surgery due to comorbidities, younger women etc. Traditionally, transfemoral access (TFA) has been the standard approach for performing this intervention as well as for other procedures in interventional radiology (IR), proven in a number of studies so far. Transradial access (TRA) is a relatively new approach in interventional radiology (IR) and so far has been used predominantly in interventional cardiology for the past few decades. Due to improved patient comfort, minor access site complications, earlier ambulation and reduced costs, TRA is becoming more popular also in some IR procedures such as: visceral embolizations with predominance of transarterial chemoembolization

(TACE) and radioembolization (TARE), embolization of gastrointestinal bleedings, carotid artery stenting and lately in some centers also for UFE. Reviewing the literature on this particular topic, there are not many papers connecting TRA and UFE. Those few small series published speak for its safety and good feedback from patients. In our hospital, UFE has been practiced from 2015, and from the end of 2017 we started using TRA for this procedure.

In this article, we present our initial experience using TRA for UFE compared to that of TFA regarding the safety profile and the procedural eligibility.

Material and Methods

Institutional review board approval was obtained for this retrospective study. Retrospective analysis was conducted for twenty-four women with UFE with 29 fibroids in the period from February 2016 to October 2018, reviewing our hospital information system. Thirteen women with TFA and eleven with TRA embolisation of uterine fibroid mean age 39 y. (range 29-47 y.) were analysed. Twenty-one of them (87.5%) have already completed reproduction, with at least one childbirth, with no particular desire for further reproduction. Two of them (8.3%) have no children (in these two myomas/fibroids were considered as one of the possible causes of infertility). One woman (4.1%) with one child, with a desire for another conception in future. All patients were symptomatic and indications for embolization included: heavy menstrual bleedings (n = 18) 75%, lower urinary tract symptoms (n = 2) 8.3%, pelvic pain (n = 3) 12.5% and abdominal pain (n = 1) 4.1%. Demographic patient characteristics between the two groups did not differ significantly (Table 1). Patients were divided into 2 groups: 13 cases with already established TFA (from February 2016 to September 2018) and the first 11 procedures performed via TRA (from October 2017 to October 2018). All procedures were performed by one interventional radiologist with experience of more than 400 visceral embolisations and one fellow in Radiology.

Table 1: Patient and procedural characteristics

	TFA group	TRA group
Number of women	13	11
Median age	31-45	29-47
Number of fibroids	16	13
Completed reproduction	10	11
Technical success	100%	100%
Total procedure time(minutes)	72.4	60.3
Fluoroscopy time(minutes)	25.3	21.1
Hospital days per procedure	2	0
Minor complications	1	0
Clinical symptoms improvement	13	9

In the TFA group, standard Seldinger technique was used with 5F introducer in the right common femoral artery (CFA). 5F Cobra 2 catheter was used for catheterisation of left internal iliac artery and Simmons 2 catheter for right internal iliac artery. In all femoral cases, 2,7F 130cm long microcatheter (Program, Terumo, Japan) was used for superselective catheterisation of uterine arteries and consequent embolisation.

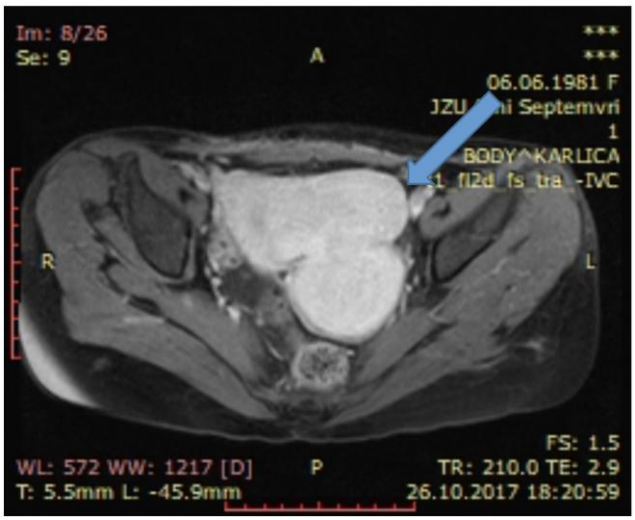

Figure 1: Pelvic MRI; T1 FS contrast-enhanced axial MRI of uterus pre embolisation shows 2 large intramural hypervascular fibroids (arrow) in the left side of uterus body with a displacement of cavum uteri

In the TRA group, the preprocedural US of the radial artery was performed together with Barbeau test for depicting patency of hand vessels. Radial arteries smaller than 2.5 mm in diameter were considered as too small for puncture, and these patients were excluded from radial puncture and converted to femoral access. Micropuncture set for transradial access (5F Slender Glidesheath, Terumo, Japan) was used in all radial cases with 110 cm MP or 125 angled catheter for cannulation of hypogastric artery.

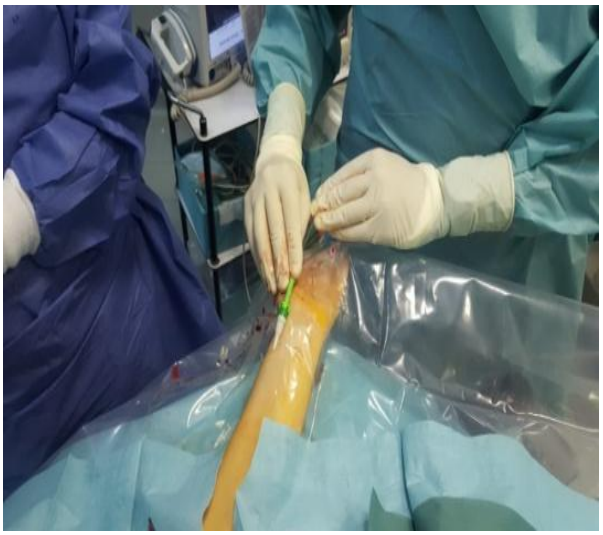

Figure 2: Left transradial access with the insertion of 5F Glidesheath, Slender, Terumo

One 150 cm long 2.8F microcatheter (Program, Terumo, Japan) in combination with GT microwire was chosen for uterine artery super selective catheterisation. Left radial artery was used for all of the eleven TRA cases.

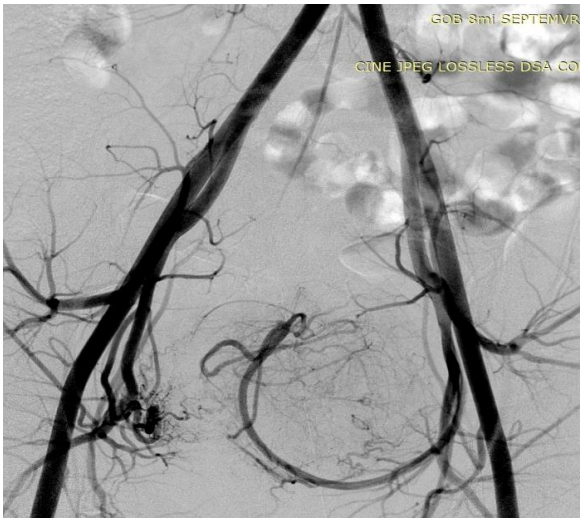

Figure 3: Digital subtraction angiography of pelvic arteries showed large and hypertrophied left uterine artery predominantly supplying the fibroids

Embolisation was performed by using PVA or PEG particles with size from 500-1000 microns. Type and size of particles were selected as per every case on the discretion of the operator.

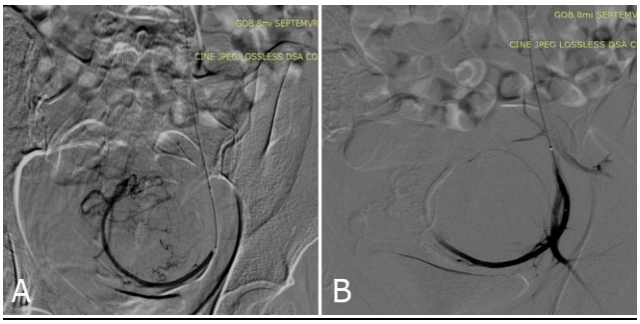

Figure 4: Angiographic findings pre and post-embolization; A) Transradial super selective angiography of left uterine artery pre embolization with 2.8F 150 cm long microcatheter shows large hypervascular fibroid; B) Postembolization angiography in the same patient shows complete embolization of left uterine artery with PVA particles and stasis of contrast at the tip of microcatheter while mail uterine artery is still patent

Haemostasis of the femoral artery was achieved with manual compression, and haemostasis of the radial artery was done with TR Band (Terumo Interventional Systems) in all cases.

Pelvic contrast-enhanced MRI and complete blood account tests were assessed before intervention in every case. The degree of achieved necrosis of the fibroids was assessed by control pelvic enhanced MRI 1-2 months after embolisation.

Conversation with the interventional radiologist about improving symptoms and quality of life, complete blood tests and radial artery ultrasound was also part of the postprocedural follow up at 1 month interval.

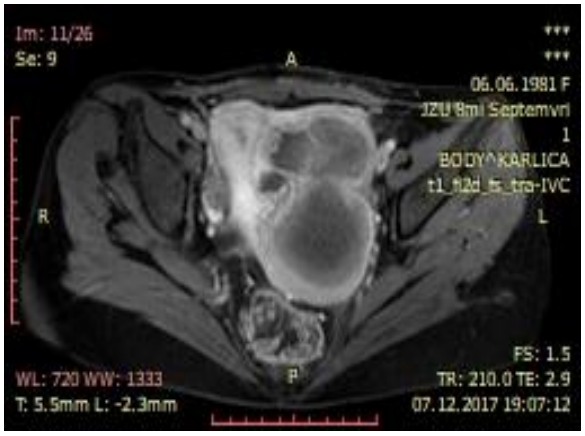

Figure 5: One and a half month post embolisation T1 FS contrast-enhanced axial MRI of the uterus in the same patient shows complete necrosis of both fibroids (arrow) and preservation of uterine body wall with normal enhancement

Results

Technical success of the procedure was achieved in 24/24 cases (100%). Unilateral uterine artery embolisation was performed in 7 cases (29.1%) and bilateral in 17 cases (70.8%). The decision for unilateral UFE was made by carefully reviewing of the preprocedural US and MRI, and in all these cases fibroids were predominantly vascularized by one uterine artery (> 80%). Mean procedural time was 72.4 minutes in TFA group, and 60.3 minutes in the TRA group. Mean fluoroscopy time was 25.3 minutes in the TFA group and 21.1 minutes in the TRA group. Access site-related and overall adverse events did not vary significantly among the study cohorts. One non-flow-limiting dissection of left internal iliac artery occurred in the TFA group during manipulation with hydrophilic wire which resolved spontaneously at the end of the procedure. In one patient in the TRA group, there was prolonged pain in the left forearm for 14 days which was managed conservatively with use of non-steroidal anti-inflammatory drugs.

Patients in the TRA group left the hospital the same day 3-4 hours after the procedure with a bandage at the left radial artery. In the TFA group patients stayed in the hospital for two nights, one day prior and one day after the procedure. There were no late major complications in both groups. In all TRA cases, radial artery remained patent without signs of thrombosis, which was confirmed with the US on one-month control examination.

Discussion

Transfemoral approach for uterine artery embolisation is an established technique for the treatment of uterine fibroids. Different catheters and manoeuvres have been described in the literature for cannulation of uterine arteries via transfemoral access. In recent years, transradial approach is gaining more and more popularity in the IR community, especially for some visceral artery interventions. It's proven safety and benefits in interventional cardiology interventions by a number of studies was followed by some small series in interventional radiology as well.

In this retrospective review of procedural safety and efficacy in a small cohort of patients treated with transradial approach, we did not observe any major complications during or after the procedure. Fluoroscopy time was less than that in TFA group without any statistical significance and we found that cannulation of the uterine artery is almost always easier when we used TRA. Main advantage so far is that the procedure can be performed as an outpatient one without patient hospitalisation. This was proven to be of great importance for patients when we were doing the postprocedural questionary. According to the results from this study, we can clearly say that TRA is a safe and effective alternative to TFA for UFE in carefully selected patients.

Every woman with the radial artery of 3 mm or greater diameter is a candidate for transradial UFE. Compared to TFA, there is shorter hospital stay and reduced total costs in the TRA procedure.

Limitations of the study: This is a small, retrospective study for gaining large and long-term conclusions. Also, it is a single centre so we cannot compare or share the results from other centres as well.

In conclusion, we think that TRA is potentially safe end effective approach for uterine fibroid embolisation. According to our experience so far it appears to be a highly promising treatment option for a woman with symptomatic fibroids. The main advantage of this procedure is early verticalization of the patients and same day discharge from the hospital.

Potential limitations so far are the lack of adequate materials (catheters and microcatheters) that can easily reach the ostium of every uterine artery via radial access. Also, the diameter of radial artery and total body height of the patient is a very important prognostic factor that can influence the success of the procedure.

Further studies with larger numbers of patients with follow-ups on longer intervals are necessary to assess the effectivness of TRA for uterine fibroid embolization better.

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Correlation between Serum Brain-Derived Neurotrophic Factor Level and Depression Severity in Psoriasis Vulgaris Patients

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Abstract

BACKGROUND: Psoriasis vulgaris is a chronic inflammatory skin disorder that can lead to depression. There is a similarity in neurotrophic substance in the pathogenesis of psoriasis and depression; it's called brain-derived neurotrophic factor (BDNF). BDNF level imbalance potentially affects the severity of psoriasis and depression.

AIM: This study aims to know the correlation between serum BDNF level and depression severity in psoriasis vulgaris patient and also the correlation between serum BDNF level and psoriasis vulgaris severity.

METHODS: This is an analytical cross-sectional study that 23 psoriasis vulgaris patients participated. All participants have performed serum BDNF level examination with enzyme-linked immunosorbent assay (ELISA). Depression severity assessed with Beck depression inventory-II (BDI-II) and psoriasis severity assessed with psoriasis area and severity index. Correlation between all variables was analysed with Spearman's correlation test.

RESULTS: Serum BDNF level and depression severity are a strongly negative correlation in psoriasis vulgaris patients (r = -0.667 with significant value p = 0.001). There is a moderate negative correlation between serum BDNF level with psoriasis vulgaris severity (r = -0.595 with significant value p = 0.003).

CONCLUSION: In psoriasis vulgaris patients, a low level of serum BDNF may increase depression severity and psoriasis vulgaris severity.

Introduction

Psoriasis is a chronic inflammatory skin disease with genetic factors may greatly affecting this disease [1]. Characteristics of psoriasis are a disturbance of epidermal proliferation and differentiation that involves biochemical, immunological, vascular and nervous system [1]. In general, it can be assumed that psoriasis, brain-derived neurotrophic factor (BDNF) and depression are related to each other. In psoriasis and depression, BDNF level is decreased. This condition may contribute to similar pathogenesis between psoriasis and depression. Psoriasis can be caused depression, as does depression can trigger psoriasis [2], [3].

There is a similarity between skin embryogenesis and the nervous system, its called ectodermal. Its allowed the role of growth factors in controlling skin homeostasis and remodelling, in this case, the role of neurotrophin [2]. The relationship between the nervous system and psoriasis was proved by the remission of psoriasis lesions after the cutaneous sensory nerve had removed [4], [5]. Some studies focused on the role of neurotropic in psoriasis, possibly due to activation of mast cells that continue into skin inflammation and then stimulate other neuroinflammatory cytokines [6].

In psoriasis, epidermal proliferation and apoptosis are influenced by neuropeptides and their receptors. Through its main receptor tyrosine kinase B (TrkB), BDNF plays a role in keratinocyte proliferation and apoptosis [2]. BDNF induces keratinocyte apoptosis but does not work on psoriatic transit-amplifying sub-population of basal keratinocytes [2].

In line with a low level of BDNF in psoriasis patients, research showed that BDNF level in depressed patients is also low. Psychological stress will reduce BDNF level through activation of the hypothalamic-pituitary-adrenal axis and sympathetic-adrenal-medullary axis which will increase cortisol and neuroinflammation cytokines and reduce BDNF level [7].

The problems of psoriasis are not limited to the skin. Psoriasis can result in psychological distress and a decrease in the quality of life [1]. Psychological stress, social stigma and embarrassment can lead to depression. The prevalence of depression in psoriasis patients is 10-62% [8]. A cross-sectional study found 32% of patients with psoriasis suffered from depression from a total of 265 psoriasis patients [9]. Psoriasis patients have a higher tendency to suffered from depression than leprosy, vitiligo and lichen planus [8]. Depression in psoriasis patients affects treatment adherence which remission is impossible to achieve. Based on these many studies, it is interesting to investigate the correlation between serum BDNF level and depression severity in psoriasis vulgaris patients.

Methods

This is an analytical cross-sectional study conducted from September 2016 to October 2017. This study was held at dermatology and venereology clinic Adam Malik General Hospital Medan Indonesia. The sample was taken using consecutive sampling method. This study was approved by the ethical commission of Faculty of Medicine Universitas Sumatra Utara Medan Indonesia.

Inclusion criteria in this study were patients diagnosed clinically as psoriasis vulgaris, age 20-65 years old, willing to take part in the study and sign an informed consent letter. Exclusion criteria were pregnant and breastfeeding patient, using topical drugs to treat psoriasis vulgaris such as a topical corticosteroid, calcipotriol, tazarotene and tar 2 weeks before this study was conducted. And patients who used systemic drugs such as methotrexate, acitretin, cyclosporine, corticosteroid 6 weeks before this study was conducted. Patients who suffered from bipolar disorder, schizophrenia and used antidepressant drugs are also excluded.

Blood sampling was taken at 8-9 am to avoid variations due to circadian rhythm. Measurement of BDNF serum level was done at the Clinical Pathology Laboratory of Adam Malik General Hospital Medan Indonesia using human brain-derived neurotrophic factor kit (R&D®, USA) and ELISA method. Measurement of depression severity was performed using Beck depression inventory-II (BDI-II). In this study, psoriasis severity was also evaluated using the psoriasis area and severity index (PASI).

The collected data was analysed to determine the relationship between variables using computer software. Correlation between serum BDNF level and depression severity and psoriasis severity was analysed with the Spearman correlation test. Significant correlation indicated by *p*-value ≤ 0.05.

Results

Psoriasis vulgaris patients characteristics

In this study 23 psoriasis vulgaris patients were participated and fulfil the inclusion and exclusion criteria. Characteristic of subjects were reported based on the gender, age, education, psoriasis and depression severity (Table 1).

Table 1: Psoriasis vulgaris patient's characteristic

No.	Characteristic	Amount (n)	(%)
1.	Gender		
	Male	12	52.2
	Female	11	47.8
2.	Age group		
	20-29	3	13
	30-39	7	30.4
	40-49	7	30.4
	50-59	5	21.7
	≥ 60	1	4.3
3.	Education		
	Elementary	0	0
	Junior high school	2	8.7
	High school	10	43.5
	Undergraduate	11	47.8
4.	Psoriasis severity		
	Mild	13	56,5
	Moderate	4	17,4
	Severe	6	26,1
5.	Depression severity		
	Minimal	7	30.4
	Mild	7	30.4
	Moderate	9	39.1
	Severe	0	0

The frequency difference between male and female patients was only 1 patient (4.4%). Based on the age group, the highest frequency are at the group 30-39 and 40-49 years old and have the same frequency of 7 patients (30.4%). The lowest frequency is in the age group ≥ 60 years old, who is only 1 patient (4.3%). Based on education, this study found the highest frequency is undergraduate patients that are 11 patients (47.8%). The lowest frequency is junior high school that is 2 patients (8.7%). It can be concluded that the subjects in this study were well-educated patients.

Based on the psoriasis severity, the highest frequency in this study was mild psoriasis, that is 13 patients (56.5%). While the lowest frequency is moderate psoriasis, that is 4 patients (17.4%).

The highest frequency of depression severity in this study was moderate depression that is 9 patients (39.1%).

Correlation between BDNF Levels and Depression Severity in Psoriasis Vulgaris Patients

This study found that the average serum BDNF level was 912.45 ± 180.94 pg/ml (Table 2).

Table 2: Correlation between serum brain-derived neurotrophic factor level and depression Severity in psoriasis vulgaris Patients and psoriasis severity

1. Serum BDNF level (Mean ± SD)	912,45 ± 180,94		
	p	r	r^2
2.Serum BDNF level and depression severity	0,001	-0,667	0,445
3. Serum BDNF level and psoriasis severity	0,003	-0,595	0,354

*Spearman's correlation test.

Serum BDNF level and depression severity were analysed with Spearman correlation, the value of the correlation coefficient (r) was -0.667 with a significance value (*p*) of 0.001 (Table 2). There is a strong negative correlation between serum BDNF level with depression severity. The lower serum BDNF level, the higher the severity of depression will be [14]. The coefficient of determination (r^2) in this analysis was found 45%, which indicate that 45% factor that influence severity of depression was serum BDNF level, and the remaining 55% are other factors (Table 2).

Discussion

Man and woman have the same opportunity to suffered from psoriasis [1], [10]. Psoriasis affects all age, but in children, the incidence is low (0.71%) [11]. Similar to the study by Kundacki et al., Who reported that psoriasis in childhood is rare, only 5.7% under the age of 10 years [12]. In this study, we have only recruited patients with psoriasis vulgaris at the age of more than 20 years old.

Fathy et al. reported that 90 psoriasis vulgaris patients had the average of PASI score was 20.8 ± 18.8, 70% of patients were categorised as moderate to severe psoriasis with a PASI score > 10 [13].

In this study, there were no psoriasis vulgaris patients with a severe degree of depression. Fatthy et al. reported that severe depression was higher in psoriasis vulgaris patients [13].

Fatthy et al. reported that BDNF level was lower in both groups of psoriasis (without depression 25.2 ± 6.5; with depression 16.9 ± 2.5) compared to the healthy control group (26.5 ± 3.6) [13]. BDNF level was significantly lower in psoriasis vulgaris patients with depression compared to psoriasis patients who did not suffer from depression (mean difference 8.3; $p < 0.001$) [13]. BDNF level was also significantly lower in psoriasis vulgaris patients with depression and depressed patients without psoriasis compared to healthy controls ($p < 0.0001$ and $p < 0.001$) [13]. The mean BDNF level was significantly lower ($p < 0.01$) in the group of psoriasis patients with depression (16.9 ± 2.5) compared to depressed patients without psoriasis vulgaris (21.5 ± 5.8) [13].

Duclot et al. reported that low BDNF level was known to play a role in depression pathophysiology, but can be increased by antidepressant [15]. However, BDNF level in serum does not correlate with depression severity. Therefore, utilisation of BDNF as a biomarker of depression is still unclear [15].

The role of BDNF in depression is proven by the presence of four things [16]. First, depression causes a decrease in BDNF level in the hippocampus and the prefrontal cortex. Second, depression triggers atrophy of the nerve dendrites in the hippocampus and the prefrontal cortex. Third, there is evidence of increased BDNF level in the hippocampus and the prefrontal cortex after administration of antidepressant. Fourth, the BDNF level increased in the amygdala and neural accumbent area which facilitate symptoms of depression. Therefore, Yu et al. concluded that depressive symptoms depend on BDNF level in the affected anatomic location [16].

BDNF level and psoriasis vulgaris severity were analysed with the Spearman correlation test; the correlation coefficient (r) was -0.595 with significant value (*p*) 0.003 (Table. 2). This result showed a moderate negative correlation between BDNF level and psoriasis vulgaris severity. The lower serum BDNF level, the higher psoriasis severity will be [14]. The coefficient of determination (r^2) in this analysis was found 35%, which indicate 35% factor that influences psoriasis severity was serum BDNF level and the remaining 65% were influenced by other factors.

Fatthy et al., reported that there was no correlation between BDNF level and PASI score ($r = 0.217$; $p = 0.250$) [13]. Similarly, Narbutt et al. reported that there was no correlation between BDNF level and PASI score [17]. The mean BDNF level in their study were 14.5 ng/ml and the mean of PASI score was 14.3 ($p > 0.05$) [17].

A study conducted by Brunoni et al. reported that there was no difference ($p = 0.59$) BDNF level in patients with mild psoriasis (3649 ± 3653 pg/ml) and severe psoriasis (3280 ± 2837 pg/ml) [2]. However, in their study psoriasis severity was not assessed with the PASI score, but was classified according to the presence of psoriatic arthritis and the used of

systemic therapy such as methotrexate, cyclosporine, mycophenolate mofetil, biological agents and phototherapy [2]. Raap et al. reported that there was no correlation between BDNF level and PASI score [18]. However, in their published article did not mention the mean of BDNF level in psoriasis patients, maybe because it was not the main purpose of their study.

It can be concluded from this study that the lower serum BDNF level, the higher the severity of depression and psoriasis will be. Serum BDNF level might be considered as a biomarker of depression severity as well as a biomarker of psoriasis severity in patients with psoriasis vulgaris. BDNF might be the new psoriasis treatment target. However, further investigations with better design are still needed to prove this result.

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Sero Conversion of Viral Hepatitis among End Stage Renal Disease Patients on Hemodialysis in Kashmir: Results of a Prospective Study

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Abstract

BACKGROUND: The seroconversion is a significant health concern in patients with end-stage renal disease undergoing hemodialysis particularly in high endemic zones of HBV and HCV.

PATIENTS AND METHODS: This prospective study was conducted from January 2009 to April 2018 at Sheri Kashmir Institute of Medical Sciences, Srinagar, Kashmir. A cohort of 459 end-stage renal disease patients on hemodialysis was enrolled from four dialysis centres and followed in a longitudinal manner. Their seroconversion rates, risk factors were studied. Positive patients were treated and followed up.

RESULTS: This study demonstrated HBV seroconversion rate of 7.4 % (n = 34) and HCV seroconversion rate of 10% (n = 46) in a cohort of 459 patients on hemodialysis attending four dialysis centres of Kashmir. Patients with diabetes mellitus outnumbered in seroconversion rates of (43.75%) followed by patients with glomerulonephritis (23.75%). Of 15 patients who had undergone renal transplantation 10 (66.67%), patients had seroconversion on hemodialysis which was statistically significant (P < 0.001). Patients who were dialysed at multiple HD centres had significant seroconversion than those who followed up at a single center. Seroconversion was associated with longer duration of dialysis (80.30 ± 30.92 vs 61 ± 9.41months, P < 0.000). HBV vaccination of the ESRD patient on hemodialysis was significantly protective against seroconversion (P = 0.000).

CONCLUSIONS: Hepatitis B vaccination, stringent precautions in all dialysis centres could help to reduce the high seroconversion rates which have a high financial burden on ESRD patients. Intense health education to both patients and medical staff will be beneficial to lower the seroconversion rates.

Introduction

End-stage renal disease (ESRD) is an immune compromised state. Disturbed cell-mediated immunity is the hallmark of advanced renal failure. Studies have shown that patients on maintenance hemodialysis have lymphopenia and their T4 & T8 lymphocyte are low. The uremic lymphocytes are shown to have lower proliferation rates compared to normal people and thus they become particularly susceptible to viral infections [1]. Over and above when on hemodialysis (HD) these patients are prone to contract various blood-borne infections like HBV, HCV, HIV etc. as HD requires access to the bloodstream and transmission can occur between patients and staff as well. Even ESRD patients receive multiple injections predisposing them to seroconversion.

In a study by Moloughney et al., [2] authors concluded that an untreated percutaneous exposure to an infected source carries a risk of seroconversion of up to 30% for HBV. The risks for HCV and HIV even though lesser than HBV are estimated to be at 1.8% and 0.31%, respectively after inadvertent percutaneous exposure. The seroconversion in ESRD patients on HD patients is particularly high. The Turkish multicentric trial has demonstrated the seroconversion rates to be higher among HD patients than on Continuous Ambulatory Peritoneal Dialysis (CAPD), authors advocated that CAPD compared to HD provided a potential advantage to the candidates

with the prospective renal transplant [3].

When patients with ESRD contract either HBV or HCV they invariably do not clear the virus and progress to chronic hepatitis. A meta-analysis of clinical studies based on 145,608 patients, anti-HCV seropositive status was a significant risk factor for death in patients on long-term dialysis [4]. Authors in the study mentioned above showed that ESRD patients with HCV positivity on dialysis are prone to have a higher cardiovascular risk making treatment of chronic HCV imperative among these patients.

Even though the treatment of HBV and HCV have become safer, better tolerated and more effective owing to the availability of direct-acting anti-virals for nearly all patients the cost factors continue to be high. Added to this chronic HBV and chronic HCV patients need regular follow up to assess the development of complications like decompensation and hepatocellular carcinoma. In a Canadian study, chronic HCV was shown to be highly burdensome to public health causing loss of productive years of life than any other infectious disease in that country [5]. Keeping in view a high prevalence of HBV and HCV in our region we were prompted to undertake this study, first of its kind, among hemodialysis patients. We estimated HBV and HCV seroconversion rates in patients undergoing maintenance HD at four dialysis centres as invariably maintenance hemodialysis is not being carried out at our tertiary care centre keeping in view limited resources and increased demand.

Patients and Methods

This study was carried out prospectively from January 2009 to April 2018. The enrolled patients gave written consent for the participation in the study and various laboratory tests were carried out at a bimonthly interval in each participant. The data were maintained on our dialysis register. Our study was conducted in full compliance with the guidelines for good clinical practice of the World Medical Assembly Declaration of Helsinki and the research guidelines of the Sheri Kashmir Institute of Medical science (SKIMS) Srinagar Kashmir, a tertiary care centre in the valley of Kashmir.

Study Population

From January 2009 to April 2018, 470, patients on hemodialysis were enrolled. Socio-demographic data were collected. These data included age, gender, duration of hemodialysis treatment, the frequency of dialysis, history of diabetes, blood transfusions, renal transplantation, surgical interventions and possible household acquirement of hepatitis infection. The medical records of the study participants were tabulated at the beginning regularly updated on follow up.

Definition of HBV and HCV Status

All patients had a baseline hemogram, liver function tests and HBsAg and anti HCV samples collected at the enrolment into the study. Then at a bimonthly interval, HBsAg detection was done by immunoassay and viral DNA (HBV DNA) Quantitative was estimated by real-time PCR (Roche Cobas Ampliprep) in HBV positive patients. Third generation ELISA test was used to detect anti HCV, and Hepatitis C viral RNA Quantitative was estimated by real-time PCR (Cobas TaqMan) in HCV positive patients.

The seroconversion was defined as a change from HCV antibody negative at the time of enrollment to HCV antibody positive status during the study period with high HCV RNA.

Exclusion criteria

1. Patients known to be positive for HBV and HCV at the time of enrollment were excluded.

2. Patients who had received a blood transfusion in the last three months at the time of enrolment.

3. Clinical Jaundice or high AST/ALT levels at the time of enrolment due to any reason.

Statistical Analysis

All the data were entered into Microsoft Excel. This included demographic profiles of participants, the cause of end-stage renal disease etc. We compared the two groups HCV and HBV positive with hepatitis negative patients. The analysis was focused on seroconversion, risk factors contributing to seroconversion during hemodialysis. Descriptive and analytical, methods were used throughout data analysis using SPSS version 21.

Data are presented as mean ± standard deviation or number (percentages). Chi-square test and Fisher's exact test were used, when appropriate. $P \leq 0.05$ was considered as statistically significant. Odds ratios (OR) were calculated considering the confidence interval of 95%.

Results

A total of 470 patients were enrolled during the study period. Eleven patients lost to follow up, and finally data on 459 patients on hemodialysis were evaluated. Of 459 participating subjects, the majority

275 (60%) were males. Mean age ± SD in the sero converted group was 63.09 ± 7.99 and age ranged 42-72 years. While the mean age in seronegative patients was 61.78 ± 9.411 and their age ranged 39-72 years. The duration of dialysis was 80.30 ± 30.92 (10-142 months) in sero converted patients and 61 ± 9.41(39-72) months in seronegative patients. The difference was statistically significant (p = 0.000) as shown in Table 1. Seroconversion was significantly higher in patients who had dialysis at multiple centres as shown in Table 1. Significant association of seroconversion was observed in patients with a renal transplant. Vaccination status was significantly protective in the prevention of seroconversion of viral hepatitis B (p = 0.00).

Table 1: Clinical data of seroconverted and seronegative patients on HD

Factors associated with seroconversion	Sero Positive N = 80	Sero negative N = 379	P value
Age in years (mean ± SD)	63.09 ± 7.99	61.78 ± 9.411	0.407
Males (No & %)	48 (60%)	242 (63.85%)	0.998
Mean duration of Dialysis in months	80.3 0± 30.92	61 ± 9.41	0.000
Previous blood transfusion			
a. No blood transfusion	17 (21.5%)	96 (25.3%)	
b. Less than 2 units	56 (70.9%)	245 (64.6%)	0.556
c. Three or > 3 units	6 (7.6%)	38 (10%)	
Previous renal transplant (Median & IQR)	10 (73.34%)	5 (14.7%)	0.034
Erythropoietin treatment %	55 (69.65%)	266 (70.2%)	0.920
Visit to Multiple HD centers	75 (94.9%)	173 (45.6%)	0.00
HBV vaccination status			
a. Fully vaccinated	6 (7.6%)	185 (48.8%)	
b. Partially vaccinated	35 (44.3%)	174 (45.9%)	0.000
c. Not vaccinated	39 (48.1%)	20 (5.3%)	

The study cohort had low haemoglobin levels, and there was no statistical difference between seroconverted or negative patients. The renal functions also showed no significant difference between the two groups. While AST and ALT levels were low in both groups but there was a significant difference between seroconverted and negative groups. Serum uric acid levels were significantly elevated in the seroconverted group compared to negative groups. There was no difference in the serum Albumin levels between the two groups Table 2.

Table 2: Laboratory data Investigations in the Cohort of ESRD on Hemodialysis

Investigation	HBV + ve	HCV + ve	Seronegative group	P valve
Hemoglobin (median (IQR))	8.5 (1.6)	8.6 (1.5)	8.5 (1.5)	0.575
Platelet (median (IQR))	180 (19)	188 (20)	180 (20)	0.361
WBC (median (IQR))	4.9 (0.62)	4.76 (0.74)	4.73 (1.0)	0.256
Urea (median (IQR))	182 (20)	180 (21)	187 (25)	0.166
Creatinine (median (IQR))	9.0 (2.4)	9.2 (2.1)	9.5 (1.0)	0.745
Uric acid	6.7 (0.5)	6.7 (0.5)	6.5 (1.0)	0.008
Calcium	9.8 (1.2)	9.4 (0.65)	9.5 (1.0)	0.098
Phosphorus	4.1 (0.4)	4.1 (0.4)	4.1 (0.4)	0.773
Bilirubin (mg/dl)	0.8 (0.5)	0.8 (0.4)	0.8 (0.3)	0.867
ALT IU	22 (8)	22 (8)	18 (0.5)	0.000
AST IU	23 (7)	22 (6)	18 (2)	0.000
ALP(KA)	80 (1.1)	80 (1.1)	80 (2.7)	0.403
T. Protein (g/dl)	7.6 (1)	7.7 (1.3)	7.6 (1.2)	0.888
Albumin (g/dl)	4.7 (.7)	4.7 (.7)	4.7 (.7)	0.942

Primary objective: Seroconversion of Hepatitis B and Hepatitis C

Of 459 patients on hemodialysis, 80 (17.42%) patients had seroconversion. HBV seroconversion was observed among 34 (7.4%) patients and HCV in 46 (10.2%) patients. The mean interval was 6 ± 1.2 months and ranged from 4 months to 11 months in our study. None of our study cohorts had HBV and HCV combined infection. Depending upon the cause of ESRD seroconversion rates varied as under as shown in shown in Figure 1.

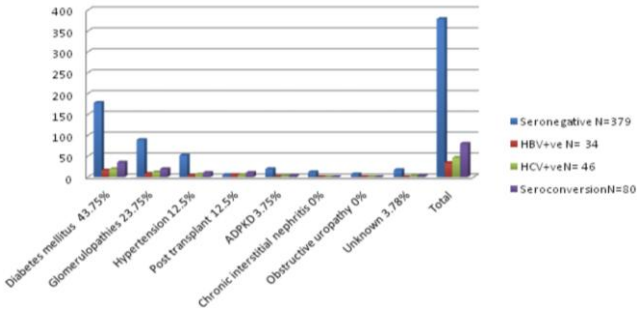

Figure 1: Seroconversion rates as per the aetiology of ESRD patients on HD

1. Diabetes mellitus: Diabetes mellitus was the most common cause of ESRD in this study. Of 213 diabetic patients on hemodialysis seroconversion was observed in 35 (16.43%) patients [HBV=16, HCV=19] corresponding to 35/80 (43.75%) of all seroconverted patients in our study cohort as shown in Figure 1.

2. Chronic glomerulonephritis: Of 108 patients with chronic glomerulopathies on hemodialysis seroconversion was found in 19 (17.59%) patients [HBV = 8, HCV = 11] corresponding to 19/80 (23.75%) as shown in Figure 1.

3. Hypertension: Of 62 patients with chronic hypertension on hemodialysis seroconversion was found in 10 (22.58%) patients [HBV = 4, HCV = 6] corresponding to 10/80 (12.5%) of our study cohort.

4. Post-Transplant: There were 15 patients post-transplant on hemodialysis in this study. Various reasons ascribed to their transplant failure were, chronic rejection 8 (53.34%) BK Virus 4 (26.67%) antibody rejection 3 (20%). Of 15 post renal transplant patients, seroconversion was observed in 10 (66.67%) patients [HBV = 5, HCV = 5], corresponding to 10/80 (12.5%) of our study cohort.

5. Adult polycystic kidney disease ADPKD: There were 22 patients with ADPKD on hemodialysis. The seroconversion was in 3 (13.63%) patients [HBV = 1, HCV = 2], 3/80 (3.75%) of our study cohort as shown in Figure 1.

6. Obstructive Uropathy: There were 7 patients with obstructive uropathy, and none of them underwent seroconversion.

7. Chronic interstitial nephritis CIN: Of 12 patients with CIN no patient underwent seroconversion.

8. Unknown cause of ESRD: Of 17 patients where the cause of ESRD remained unknown

seroconversion was observed in 3 (17.64%) patients [HBV = 0, HCV = 3] corresponding to 3/80 (3.75%) of our study cohort as shown in Figure 1.

Treatment of HCV, HBV and follow up:

All patients with HBV were treated with Tab. Entecavir 0.5mg once every 5 days. There was a progressive fall in HBV DNA levels in all patients. Patients with HCV were initially treated with Interferon-based therapy at the start of the study and later with oral drugs when oral drugs became available. Both HBV and HCV seroconverted patients were followed by ultrasound liver examination every six month, and AFP levels were monitored. There was no evidence of HCC in any of the study participants during the study period.

Discussion

This study demonstrated an HBV seroconversion rate of 7.2% and HCV seroconversion rate of 10% in a cohort of 459 ESRD patients on maintenance hemodialysis (HD)**.** The mean time from the start of HD to seroconversion was 6 ± 1.2 months, and it ranged from 6 months to 11 months. As summarized in Table 3 the prevalence of HBV and HCV in various HD units varies from country to country across the globe and the prevalence in the Indian HD centers continues to be high [6], [7], [8], [9], [10], [11], [12], [13], [14], [15], [16], [17].

Table 3: Global data of seroprevalence of HBV & HCV in HD centres

Virus	Place	Author	Year	Prevalence
HBV	Western Europe Japan &USA	Burdick et al [6]	2003	0-6.6%
	Middle East: Saudi Arabia & Bahrain	Almawi WY et al [7]	2004	11.8% & 3.7%
	Turkey	Yakaryilmaz et al [8]	2006	13.3%
	Brazil	Ferreira et al [9]	2006	2.4-10%
	Asia & Pacific countries	Johnson et al [10]	2009	1.3% & 14.6%
	North Indian data	Malhotra R et al [11]	2016	1.5-33.5%
HCV	United Kingdom	Wreghitt et al. [12].	1999	4%
	Saudi Arabia	Souqiyyeh et al [13]	2001	50%
	Germany	Hinrichsen H et al [14]	2002	6%
	Slovenia	Buturovic-Ponikvar et al [15]	2003	1.9%
	Casablanca	Boulaajaj K et al [16]	2005	76%
	Egypt	ZahranAM [17]	2014	49.6%

The risk of seroconversion in ESRD on HD is directly proportional to the prevalence of viral infection in a given society and the quality of dialysis centres. In this study, as shown in Figure 1 the patients with Diabetes mellitus had higher seroconversion rates than the non-diabetic population which has been demonstrated by other researchers as well [18], [19]. The seroconversion was proportional to duration of dialysis in years possibly due to increased nosocomial infection rates in HD population as demonstrated by Carneiro et al., [20]. Patients who were dialysed at multiple dialysis centres had more seropositivity rates compared to those who followed in a single centre as shown in Table 1 supported by Petrosillo et al., [21].

There was no marked elevation of AST and ALT levels in the seroconverted group. Nevertheless the difference between seroconverted and negative patients on HD was statistically significant (Table 2). Patients with the end-stage renal disease do not show a marked rise of AST and ALT levels following seroconversion warranting a high clinical suspicion as the actual liver damage may be profound irrespective of AST levels. As such, it has been suggested by Wong et al., [22] that in a dialysis patient with chronic HBV infection an unexplained elevation in serum ALT level persistently above 30 IU/L or just 0.75 times upper limit of normal (ULN), liver biopsy should be considered to rule out significant hepatic inflammation if the clinical evidence of progressive liver disease is high.

Our study demonstrated a significant elevation of serum uric acid (UA) levels in the seroconverted group compared to the seronegative group as shown in Table 1. In a study by Afzali A et al., [23] the data on 5518 participants during a mean follow of 12 years showed that the high serum uric acid levels strongly predicted the progression of liver disease. In their study serum UA level was associated with the development of cirrhosis. There was no significant association observed in hemogram, serum calcium, phosphorous, serum proteins, albumin levels between the two groups as shown in Table 2.

Prevention seems to be the cornerstone in declining seroconversion rates of HBV and HCV in HD patients. As per the guidelines of CDC endorsed by Kidney Disease: Improving Global Outcomes (KDIGO) [24], stringent precautions in dialysis units must be observed to decline seroconversion rates. These include wearing and changing of gloves after a clinical encounter with HD patient, isolation of positive patients, water-proof gowns between patients, systematic decontamination of the equipment circuit and surfaces after each patient's treatment. CDC also refrains sharing of various instruments like tourniquets, stethoscope, blood pressure cuff and use of multi-use vials of heparin between HD patients [25]. Apart from stringent precautions the use of recombinant erythropoietin (EPO) therapy seems to be the plausible solution in preventing the seroconversion among HD patients. However, blood transfusions cannot be avoided entirely in end-stage renal disease (ESRD). There are situations when ESRD patients need to be transfused exposing them to the risk of seroconversion especially when they develop EPO resistance due to suboptimal dialysis [26]. However, blood transfusion rates didn't affect sero positivity in our study as shown in Table 1.

The occult HBV remains another potential risk of seroconversion and its prevalence as demonstrated by Gutiérrez-García et al., [27] is parallel with the prevalence of apparent HBV infection prevalence.

This poses a significant risk of seroconversion not only during transfusion but also during the disease among ESRD patients on HD. Various studies have shown that the prevalence of occult hepatitis B in dialysis patients ranges between 0% and 58% [28], [29]. Thus whether the higher seroconversion rates observed in this study were due to activation of occult hepatitis B or due to some other reasons remained elusive. The enigma could have been solved had we checked HBV DNA in all patients undergoing hemodialysis but due to financial constraints, such practice may not be feasible everywhere. Having said this, keeping in view a high prevalence of HBV in our region whether testing for Anti-HBc antibody & HBV DNA levels both in a given blood donor and the ESRD patient before enrolment to hemodialysis will help to reduce further transmission needs to be studied. This has particular public health importance in our region as the number of dialysis units is limited, and the ESRD patient population on HD is on rise due to the shortage of renal transplantation facilities.

With the advent of Hepatitis B vaccination there has been a progressive decline in new conversion rates all over the globe. There was a significant association between vaccination status and seroconversion as shown in Table 1. Since there is no vaccine against HCV, aseptic precautions in various dialysis centres can decline the incidence of HCV seroconversion. Way back in 1977 when CDC guidelines [30] were laid Dinits-Pensy et al., [31] demonstrated a decline of new HBV among HD patients from 6.2 to 1% among US dialysis centres a few years later.

In another study, the prevalence of HBV infection in HD patients in the United States of America progressively fell from 7.8% to 1.0% between 1976 and 2002. Similarly, the prevalence of HCV infection fell from 10.4% to 7.8% from 1995 to 2002 [32]. Hemodialysis environment proves to be the vehicles of transmission of these viruses and renal transplant seems to be another modality in circumventing this vicious cycle. The Iranian data showed a significant fall of seroconversion after renal transplantation in ESRD patients [33].

There was a higher HCV seroconversion (10%) than HBV seroconversion rates (7.2%) possibly due to a higher prevalence of HCV in our region as demonstrated by Jasuja et al., [34]. We utilized utilised the third generation of enzyme-linked immunosorbent assay (ELISA) for the detection of anti HCV. Various studies have shown that the frequency of HCV RNA-positive among anti-HCV-negative patients undergoing HD varies from 0% to 12% [35], [36]. Nevertheless, routine screening of HCV RNA in our patient population with limited resources and no insurance coverage may not be feasible and serological testing**,** preferably by the third-generation enzyme-linked immunosorbent assay (ELISA) has been recommended for routine screening of HD patients [37]. Recently occult HCV has been demonstrated in the liver tissue and peripheral blood mononuclear cells of HCV RNA negative patients on HD connoting that despite negative HCV RNA and serology patients can have HCV infection and potentially transfer via dialysis units or get activated in the given patient [38].

The high percentage of seroconversion in this study could be partly attributed to the shortage of nursing staff, in an environment of a high prevalence of HBV and HCV positivity in our region. Further, invariably our HD units remain crowded units due to limited resources. Last but not least there are inadequate infection control policies and procedures in this part of the globe.

The drawback of our study, we believe, is that we did not calculate fibrosis scores in our cohort of seroconverted patients. However, all patients were Child Turcot Pugh class A and no patient had decompensation on follow up. The seroconverted patients were treated and regularly followed with Alfa-fetoprotein (AFP) levels and six-monthly ultrasound of the liver. None of our study participants developed Hepatocellular carcinoma during the study period. In a study by Cheng et al., [39], while comparing the outcome of hepatic resection in patients with CRF and normal kidney functions tests, it was observed that even though ESRD patients had high creatinine levels and low haemoglobin, a similar outcome in both ESRD & controls groups was observed by the authors. Their study highlights that patients with seroconversion must be followed up for HCC and operated as per the standard protocol.

We conclude that a high prevalence of viral hepatitis seroconversion among ESRD patients on hemodialysis was observed. All our efforts must be to prevent seroconversion of ESRD patients on HD by adequate HBV vaccination (40 µg HBV vaccine at 0, 1, 2 and six months) and unpermissive precautions in hemodialysis centres. Nevertheless, after an unfortunate seroconversion, ESRD patients must be treated with standard therapy which is safe and effective.

Our study emphasises enforcement of the quality control in various dialysis centres by healthcare authorities across the state, including periodic serological testing of dialysis staff. The practice guidelines must be laid, and rigorous infection control policies must be adopted by all the dialysis centres in the region to prevent the longterm consequences of seroconversion among ESRD patients on hemodialysis.

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Clinical Science

The Correlation between Hemoglobin Concentration during Pregnancy with the Maternal and Neonatal Outcome

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Abstract

BACKGROUND: The prevalence of anaemia is higher among women, including pregnant women. The estimation was about 24.8% of the population in the world suffering anaemia. Anaemia during pregnancy is a big problem because it can contribute morbidity and mortality, either in mother or newborn. The impacts of anaemia during pregnancy included post-partum haemorrhage, low birth weight (LBW), preterm delivery, and low Appearance, Pulse, Grimace, Activity, Respiration (APGAR) score.

AIM: This study aimed to determine the correlation between haemoglobin concentration during pregnancy and the outcome of mothers and newborns.

METHODS: It was a cohort study that included 200 pregnant women in second or third trimester at antenatal care of Sundari General Hospital Outpatient Clinic on February until September 2018. The participants were interviewed using a questionnaire, and their blood was checked to measure haemoglobin concentration using portable Easy Touch Hemoglobinometer. In the next three until six months, the following investigation was conducted to assess the maternal and neonatal outcome.

RESULTS: The result of this study showed among the maternal outcome, only antepartum haemoglobin concentration had a statistically significant correlation with the haemoglobin concentration during pregnancy ($p < 0.05$), meanwhile, among the neonatal outcome. LBW was the only factor that statistically significantly correlated to the haemoglobin concentration during pregnancy ($p < 0.05$).

CONCLUSION: We can conclude that once anaemia occurs in pregnant women, then the women kept suffering from anaemia with its correlation was statistically significant.

Introduction

In all over the world, anaemia has been a global health burden since it can affect anyone without considering age or gender group [1]. However, the prevalence of this nutritional disorder is higher among women, including pregnant women. About 24.8% of the population in the world suffers from anaemia [2], [3].

Anaemia during pregnancy has become a widespread nutritional disorder either in developing or even developed countries. According to the World Health Organization (WHO), the proportion of the population suffering from anaemia in pregnancy was 14% in developed countries and 51% in developing countries. WHO also estimated that among the population, the cases were the most frequently found in Africa and Southeast Asia. Indonesia is a part of Southeast Asia. Thus, anaemia in pregnant women is frequently found here. It is proven by the data from Basic Health Research Ministry of Health of the Republic of Indonesia in 2013 showing that the prevalence was about 37.1% [1], [2], [4].

Anaemia during pregnancy is defined as the haemoglobin criteria is less than 11 g/dL. Once pregnant women suffer from anaemia, iron deficiency becomes the most common cause that should be considered [3]. Other risk factors related to maternal anemia are unhealthy lifestyle, poor socio-economic

status, malnutrition, hemoglobinopathies, age (under 20 years or above 35 years), early marriage or teenage pregnancy, decreasing period of pregnancy interval, smoking or alcohol use, history of menstrual disorder or infection, and gemelli or multiple pregnancies [5].

Anaemia is a big problem especially when it occurs during pregnancy because it may contribute to morbidity and mortality, either in mothers or newborns. Anaemia (regardless the severity) accounts 12.8% maternal death which is the second leading cause of the death. In Indonesia, the national Maternal Mortality Rate (MMR) is still so high with 307/100.000 live births. Anaemia during pregnancy becomes an indirect cause of postpartum haemorrhage and results in maternal mortality in the latter [4], [5]. Meanwhile, the national Neonatal Mortality Rate is much higher with 987/100.000 live births. Moreover, the fetal or neonatal complication can include prematurity, low birth weight, and low APGAR Score. All complications were significantly ended with maternal and neonatal mortality [4], [6], [7].

Although anaemia during pregnancy may lead to many adverse effects, actually anaemia can be the most preventable cause of maternal and neonatal mortality. In the upcoming years, anaemia in pregnant women should be eradicated to improve maternal and neonatal health status. Hence, this study was aimed to investigate the correlation between haemoglobin concentration during pregnancy and the outcome of mothers and newborns. In the future, hopefully, this study can be the reference to counsel pregnant women, especially in Indonesia, to be more aware of how threatening anaemia is and to decrease the prevalence of anaemia itself.

Material and Methods

This study was conducted at antenatal care of Sundari General Hospital Outpatient Clinic. It was a prospective randomised study that included 200 pregnant women in the second or third trimester who met the inclusion and exclusion criteria.

This study took a period of 7 months — February until September 2018. After obtaining the consent, we interviewed the pregnant women who consumed Fe tablets, consist of ferrous sulfate 200 mg which contains 60 mg elemental iron for 90 days after the first trimester using a questionnaire to know their characteristics including age, gestational age, education background, and occupation. Then, the haemoglobin measurement was conducted by using Easy Touch portable hemoglobinometer. The data was collected during their antenatal care in the second or third trimester.

In the next three until six months, following investigation was conducted by asking the pregnant women to come again before giving birth. Then, we assessed the second haemoglobin measurement during their antepartum by the same portable measurement.

A few weeks later, the data about the outcome of maternal and neonatal was collected from the medical record if the subjects are giving birth in Sundari General Hospital. Unless they did not give birth in the same hospital, follow up was done by the phone. The maternal outcome includes the estimation of haemoglobin antepartum, bleeding volume while giving birth, and the initial breastfeeding. The neonatal outcome includes the data whether the newborn alive or not. Among the alive and healthy newborn, other data was collected, including preterm birth, low birth weight, and APGAR score. In this study, low birth weight defined as the infant birth weight which less than 2500 gram and preterm birth was considered as gestational age under 37 weeks. Because of our limitation, APGAR score was measured by only asking whether the newborn cried spontaneously or not. The newborn crying spontaneously was considered as good APGAR Score, but if they did not, it probably showed poor APGAR Score

Statistical Analysis

All data collected and recorded using the Statistic Product and Service Solution (SPSS) program 21st version. Values were expressed with the mean ± SD or percentage as appropriate. The analysis of the correlation between haemoglobin concentration during pregnancy and the outcome of mother and newborn were computed statistically using Chi-Square Test. A difference was considered significant at the p-value < 0.05.

Results

There were 200 pregnant women involved with their newborns that evaluated in this study. There was found no maternal mortality. In this study, the mean of haemoglobin (Hb) concentration was 10.73 ± 2 g/dl, the median was 10.6 g/dl, the maximum was 15.8 g/dl, and the minimum was 7.8 g/dl. The mean birth weight was 3015 ± 584 gram.

The subject characteristics were shown in Table 1. The mean age of all participants was 29 ± 5.5 years. For all characteristics, except birth method, either anaemia or normal group, had the similar result of majority variable. The most common age was 20 – 35 years. The majority of participants were in the third trimester of gestational age. Most of the participants were housewives. The majority education background

was senior high school. Most of the participants gave birth in a hospital. Otherwise, for the birth method, the anaemia group had different result compared to the normal group. The majority birth method in anaemia group was spontaneous birth. Meanwhile, cesarean birth was most commonly conducted in the normal group. By using Chi-Square Test, there were no characteristics which had a significant correlation with the hemoglobin during pregnancy (p-value > 0.05).

Table 1: Demographic of subject characteristics

Characteristics	Haemoglobin concentration during pregnancy		Total	P value
	Anemia (52.5%)	Normal (47.5%)		
Age	Mean 29 ± 5.5 years			0.218
< 20 years	0 (0%)	2 (2.1%)	2 (1%)	
20 – 35 years	88 (83.8%)	79 (83.2%)	167 (83.5%)	
> 35 years	17 (16.2%)	14 (14.7%)	31(15.5%)	
Gestational Age				0.267
2nd Trimester	28 (26.7%)	19 (20%)	47 (23.5%)	
3rd Trimester	77 (73.3%)	76 (80%)	153 (76.5%)	
Occupation				0.974
Housewife	77 (73.3%)	72 (75.8%)	149 (74.5%)	
Civil servant	9 (8.6%)	7 (7.4%)	16 (8%)	
Private servant	9 (8.6%)	7 (7.4%)	16 (8%)	
Entrepreneur	10 (9.5%)	9 (9.5%)	19 (9.5%)	
Education background				0.453
Elementary school	4 (3.8%)	2 (2.1%)	6 (3%)	
Junior high school	13 (12.4%)	14 (14.7%)	27 (13.5%)	
Senior high school	59 (56.2%)	57 (60%)	116 (58%)	
Diploma	6 (5.7%)	9 (9.5%)	15 (7.5%)	
Undergraduate	23 (21.9%)	13 (13.7%)	36 (18%)	
Mode of Delivery				0.121
Normal vaginal	76 (72.4%)	36 (37.9%)	112 (56%)	
Cesarean	29 (27.6%)	59 (62.1%)	88(44%)	
Birth location				0.210
Hospital	56 (53.3%)	59 (62.1%)	115 (57.5%)	
Midwifery unit	49 (46.7%)	36 (37.9%)	85 (42.5%)	

Chi-Square Test. p-value < 0.05 were considered as statistically significant.

The indication of cesarean birth can be seen in Table 2. The most common indication was the previous cesarean delivery. The other indications related to pregnancy problems, such as obstructed labour, placenta previa, abnormal fetal presentation, and cephalopelvic disproportion.

Table 2: The indications of cesarean birth

Factors	Anaemia	Non-anemia
Previous cesarean birth	21 (72.4%)	20 (58.8%)
Obstructed labor	3 (10.3%)	5 (14.7%)
Placenta previa	2 (6.9%)	1 (2.9%)
Cephalopelvic disproportion	1 (3.4%)	2 (5.8%)
Abnormal fetal presentation	2 (6.9%)	5 (14.7%)
Others	0 (0%)	1 (2.9%)

Table 3 showed how maternal outcome related to haemoglobin concentration during pregnancy. Among the three variables, only antepartum haemoglobin concentration that significantly associated with haemoglobin concentration during pregnancy (p < 0.05).

Table 3: The correlation between haemoglobin concentration during pregnancy and maternal outcome

Maternal outcome		Haemoglobin concentration during pregnancy		P value
		Anaemia	Normal	
Antepartum hemoglobin concentration	Anemia	89 (84.8%)	57 (60%)	0.000*
	Non-anemia	16 (15.2%)	38 (40%)	
Postpartum hemorrhage (PPH)	< 500 cc	85 (81%)	78 (82.1%)	0.834
	≥ 500 cc	20 (19%)	17 (17.9%)	
Initial breastfeeding	Yes	27 (25.7%)	27 (28.4%)	0.791
	No	74 (74.3%)	68 (71.6%)	

Chi-Square Test *p-value < 0.05 were considered as statistically significant.

It means that the pregnant women in either second or third trimester kept suffering from anaemia until the antepartum period.

Table 4 showed that among all participants, unfortunately, stillbirth (3.8%) was found. All stillbirth cases occurred in the anaemia group. There was no significant correlation between haemoglobin concentration during pregnancy and neonatal condition (p > 0.05).

Table 4: The correlation between hemoglobin concentration during pregnancy and neonatal condition

Neonatal condition	Hemoglobin concentration during pregnancy		P value
	Anemia	Normal	
Alive newborn	101 (96.2%)	95 (100%)	0.157
Stillbirth	4 (3.8%)	0 (0%)	

Chi Square Test.

To analyse the correlation between haemoglobin concentration during pregnancy and fetal outcome, participants with stillbirth were excluded. We found four cases of stillbirth. Thus, there were only 196 participants involved in this analysis. The result showed in Table 5. Among all variables of neonatal outcome, low birth weight was the only outcome that significantly related to the haemoglobin concentration during pregnancy (p < 0.05).

Table 5: The Correlation between haemoglobin concentration during pregnancy and fetal outcome

Neonatal outcome		Haemoglobin during pregnancy		P value
		Anaemia	Non-anemia	
Spontaneous cry	Yes	92(96.8%)	86 (90.5%)	0.892
	No	9(3.2%)	9 (9.5%)	
Preterm delivery	Yes	4 (4%)	10 (10.5%)	0.074
	No	97 (96%)	85 (89.5%)	
Low birth weight	Yes (< 2500 gram)	4 (81%)	11 (82.1%)	0.045*
	No (≥ 2500 gram)	97 (19%)	84 (17.9%)	

Chi-Square Test *p-value < 0.05 were considered as statistically significant.

Discussion

Out of 200 pregnant women in this study, more than half (52.5%) of them were suffering from anaemia. It is relevant to the previous study which stated that half population of pregnant women in the world affected by anemia. Meanwhile, the proportion of anemic pregnant women varies in each countries, such as 58% in China, 50% in Southeast Asia, and 40% in Istanbul [8].

Based on Chi-Square Test, this study showed that no significant correlation between hemoglobin concentration during pregnancy with any characteristic variables, included maternal age (p-value = 0.218), gestational age (p-value = 0.267), education background (p-value = 0.974), occupation (p-value = 0.453), mode of delivery (p-value = 0.121) and place of birth (p-value = 0.210). It was relevant to

the previous study conducted by Vural *et al*., showing that there was a statistically significant correlation between anaemia prevalence and mode of delivery. Otherwise, in Vural *et al.,* study, the correlation between maternal age and anaemia prevalence was statistically significant [8].

This study found that the mean of haemoglobin concentration was about 10.73 ± 2 g/dl. Based on both the World Health Organization (WHO) and the Center for Disease Controls and Preventions (CDC), the mean is defined as anaemia since the haemoglobin concentration less than 11 g/dl. [9]. Although it was categorised as anaemia according to criteria of WHO and CDC, the concentration of 10 g/dl in the mid-trimester of gestational age seems to reflect the adequate expansion of plasma volume [10].

Although more than half of the population were anaemic, there was found no maternal mortality in this study. It was suggested that the haemoglobin concentration still able to compensate for optimal plasma volume expansion. It was relevant to the previous study stated that the cutoff of extremely low haemoglobin concentration was less than 6.5 g/dl. This condition with other factors can contribute to maternal mortality. Even, the other cutoff with 8.9 g/dl associated with twice risk of maternal mortality [11].

In this study, out of 115 anaemic pregnant women, 20 participants (19%) had bleeding after birth ≥ 500 cc without significant correlation between haemoglobin level during pregnancy and the bleeding volume. The previous study of Frass had similar result showing 29.1% of anaemic pregnant women developed postpartum haemorrhage during cesarean delivery because of the uterine atony [12]. Even, the study of Kayle *et al.* showed that there was a strongly significant correlation between moderate-to-severe anaemia and blood loss severity [13]. Despite the widespread postpartum haemorrhage in all over the world, unfortunately, there is still a lack of data in the literature about contributing factors of it, especially in developing countries where many PPH and maternal death occur. David study stated that weak uterine muscular strength and lower resistance to infectious disease possibly occur due to severe anaemia. Meanwhile, the higher risk of PPH experience related to severity anaemia still needs further studies [13], [14].

This study investigated whether there is a correlation between initial breastfeeding and the haemoglobin concentration during pregnancy. Our study showed that most of the initial breastfeeding failure found in the anaemia group (74.3%). Despite the fact, the correlation was statistically not significant (p-value = 0.791). Another complication of anaemia during pregnancy that still not familiar is breastfeeding failure. However, an article review mentioned breastfeeding failure becoming the impact of anaemia during pregnancy, after puerperal sepsis and sub-involution [15].

Investigation of neonatal outcome was done by analysing three factors, including spontaneous crying to assess APGAR Score, preterm delivery, and low birth weight. Among the factors, only low birth weight had a significant correlation with the haemoglobin level during pregnancy (p = 0.045).

Birth weight is a good indicator to evaluate whether the mother supports the fetus adequately or not. Besides, it is the only determinant factor of newborn mortality in the first year of life. Birth weight less than 2500 gram is defined as low birth weight that is most commonly caused by anaemia during pregnancy. Previous meta-analysis literature showed that there was a significant correlation between anaemia during pregnancy and low birth weight in the 3rd trimester. Otherwise, the same study showed no significant correlation between both variables in 1st and 2nd trimester [9], [16]. Haemoglobin concentration of less than 10.5 g/dl was reported to increase the sevenfold risk of low birth weight [17]. Abnormally a previous literature stated that several studies reported low birth weight in anaemic pregnant women, but high haemoglobin concentration in 1st and 3r trimester also correlated with the risk of low birth weight due to similarly poor plasma volume expansion [10], [17].

Besides low birth weight, preterm delivery and low APGAR score were the other impacts of anaemia during pregnancy in neonatal outcome. This study showed that there is no significant correlation between haemoglobin concentration during pregnancy with any factor (p > 0.05). It seems relevant with the previous study conducted in Moshi Municipality showing a similar result about no correlation between anaemia and low birth weight and preterm delivery. However, a different study showed that maternal anaemia and preterm delivery had significant correlation statistically. Even, Hb < 10.5 g/dl can increase fivefold risk of preterm delivery [17], [18]. Our study can give more information about how the impact of haemoglobin concentration on the outcome of maternal and neonatal. However, our study had a limitation that did not bring any information about the dietary pattern of the pregnant woman, especially during pregnancy. This study also did not give any intervention for the anaemia correction before the childbirth.

In conclusion, from this study, we can conclude that once anaemia occurs in pregnant women, then the women kept suffering from anaemia with its correlation was statistically significant. Several studies showed that there was a significant correlation between anaemia during pregnancy and postpartum haemorrhage. However, this study found a different result with no significant correlation between haemoglobin concentration with PPH and initial breastfeeding. Therefore, further studies are needed to investigate more about the maternal and neonatal outcome due to anaemia during pregnancy. Moreover, this kind of study is still limited developed in Indonesia.

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Clinical Science

High Serum Lead Levels Increase the Incidence of Cognitive Impairment of Public Fueling Station Operators

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Abstract

BACKGROUND: Air pollution due to lead contained in motor vehicle fuel is inhaled for a long period causing cognitive impairment. Cognitive disorders in general fuel station operators are found in developing countries as a negative impact of environmental pollution.

AIM: This study aims to find out that high levels of lead in the blood increase the risk of cognitive impairment in operators of Public Fuel Filling Stations.

METHODS: This was a case-control study design to determine high lead levels in the blood increasing the risk of cognitive impairment in operators of General Fuel Filling Stations. There were 76 study subjects consisting of 38 case groups and 38 control groups.

RESULTS: Obtained lead levels of all research subjects in normal criteria (1.1-5.58 µg/dL). We used MoCA-Ina (a validated Indonesian version of MoCA questionnaire) to evaluate the cognitive function. High lead levels in the blood in the case group were 28 subjects (66.7%) and 14 subjects (33.7%) in the control group. Factors that significantly affected the occurrence of cognitive disorders are work periods of more than 3 years, which are 4 times higher risk of experiencing cognitive impairment (p = 0.021).

CONCLUSION: High lead levels in the blood have a 6 times greater risk of cognitive impairment than subjects with not high blood lead levels and work periods of more than 3 years have a risk of 6 times greater cognitive impairment.

Introduction

Some negative impacts on the environment and health are reported from the industrial sector. One effect of pollution is due to motorised vehicles. Cognitive impairment is a disorder of rational thought processes including the process of remembering, judging, orientation, perception and also paying attention. Disorders of cognitive function are often separated from our observations.

The Public Fuel Station is thought to be one of the places where air pollution occurs from vehicles using fuel which contains heavy metal components such as lead or Pb (plumbum) which are released as lead oxide, which is then inhaled by humans and causes the cognitive impairment. Symptoms of lead toxicity usually correlate with blood lead levels of 25–50 µg/dL in children and 40–60 µg/dL in adults [1]. The policy of standard quality specifications in the use of gasoline types does not fully eliminate the use of lead in 3 types of gasoline in developing countries. Gasoline type 88 (premium) consisting of 88 unleaded types of gasoline with a maximum lead level of 0.013 g/L and for 88 leaded gasoline types with a maximum lead level of 0.3 g/L. Gasoline type 92 (pertamax) with a maximum lead level of 0.013 g/L and gasoline type 95 (pertamax plus) with a maximum lead level of < 0.013 g/L [2].

This toxicity is a result of the ability of lead to replace Ca^{2+}, Mg^{2+}, $Fe^{2+,}$ and Na^{+} which subsequently affects the basic biological processes of the body, replacing calcium ions, so that it can cross the blood-brain barrier. Lead accumulates and damages immature astroglia cells, interferes with sodium ion concentration and increases the action potential

cause nerve cells damage and cognitive impairment [3], [4]. The lead that crosses the blood-brain barrier causes various neurological disorders such as behaviour changes, mental retardation, disorders in the prefrontal cortex, hippocampus and cerebellum [5], [6]. Cognitive impairment due to lead poisoning begins with a disruption of heme synthesis which inhibits the synthesis of aminolevulinic acid dehydratase (ALAD) in the cytoplasm and ferrochelatase (heme synthetase and protohemeferrolyase) in mitochondria. There is an increase in the initial aminolevulinic acid (ALA) urine, followed by an increase in erythrocyte protoporphyrin [7]. Inflammation results in disruption of the blood-brain barrier system and the nervous system as a whole. ALA enters and accumulates in the nerve tissue cause oxidative stress. The discovery of ALA in the nervous system is a neurotoxin and causes hydroelectrolyte changes and damage to nerve cells/apoptosis [8].

A cognitive function is an act of thinking, remembering, learning, and using language. Cognitive functions include attention, memory, consideration, and problem-solving abilities, and executive abilities such as planning, and evaluating [9]. There are several screening instruments for cognitive function disorders such as the Mini-Mental State Examination (MMSE), Clock Drawing Test (CDT), Montreal Cognitive Assessment (MoCA). We used the MoCA-Ina questionnaire (Indonesian version of Montreal Cognitive Assesment questionnaire) which evaluate the visuospatial, naming, memory, attention, language, abstraction, delayed recall and orientation. The MoCA-Ina Kappa value is 0.820 which shows that this questionnaire has a very good inter-rater agreement value [10].

This study aims to find out that high levels of lead in the blood increase the risk of cognitive impairment in operators of Public Fuel Filling Stations.

Methods

The study was conducted at the South Denpasar regional gas station from December 2017 to January 2018. All research subjects were gas station operators in the city of South Denpasar who fulfilled the inclusion and exclusion criteria, then performed cognitive function assessment using the MoCA-Ina questionnaire and blood sampling for lead level for each study subjects

Observational, analytical research method using a case-control design to determine high lead levels in the blood increases the risk of cognitive impairment in gas station operators. Measurements were made using the AAS method (Atomic Absorption Spectrophotometry) at Prodia Laboratory. Values of blood lead levels are grouped into high and not high levels of lead which are distinguished by the results of Receiver Operating Characteristic (ROC) procedure statistics and Area Under Curve (AUC). Data were presented on a nominal categorical scale, high lead levels when ≥ 2.45 µg/dL and not high lead levels < 2.45 µg/dL. Multivariate analysis was performed to determine the risk factors for cognitive function disorders such as working period which is categorised into a work period of 1-3 years and a work period of more than 3 years, and the use of masks as protective devices while working.

The data were analysed statistically by the comparative hypothesis test of 2 unpaired groups, namely bivariate analysis using Chi-square because the independent variables and dependent variables were nominal. If the observed/expected value is < 5, the Fisher exact test is used, and the multivariate analysis uses logistic regression analysis because the dependent variable is the nominal categorical variable. The level of significance is expressed as $p < 0.05$ with a 95% confidence interval.

Results

The research subjects were gas station operators in the city of South Denpasar that met the inclusion and exclusion criteria, a total of 76 subjects. The study subjects were divided into cognitive impairment (cases) and did not experience cognitive impairment (control). The results of examination of blood lead levels in all samples included in the normal criteria range from 1.1 to 5.58 µg/dL, with a mean ± SD of 2.56 ± 0.86 with 95% CI 2.35-2.749 (normal value less than 10 µg/dl). The characteristics of the research subjects consist of several variables summarised in Table 1 below.

Table 1: Characteristics of Subjects

Variable		Cognitive Impairment n (%)	No Cognitive Impairment n (%)	p-value
Age	Adult	20 (58.8)	14 (41.2)	0.166
	Adolesence	18 (42.9)	24 (57.1)	
Sex	Men	10 (45.5)	12 (54.5)	0.613
	Women	28 (51.9)	26 (48.1)	
Working period	≥ 3 year	16 (72.7)	6 (27.3)	0.011
	1 - 3 year	22 (40.7)	32 (59.3)	
Gloves wearing	Without going	38 (50)	38 (50)	
Masker wearing	Wearing masker not routinely	29 (52.7)	26 (47.3)	0.442
	Wearing masker routinely	9 (42.9)	12 (57.1)	
Blood lead levels	High	28 (66.7)	14 (33.3)	0.001
	Not High	10 (29.4)	24 (70.6)	

MoCA-Ina = Montreal Cognitive Assessment-Indonesia.

The overall lead blood level of the research subjects in the normal range, then performed the statistical method of the ROC and AUC procedure to determine the ability to examine blood lead levels that cause cognitive impairment, AUC value was 83% with 95% CI ranging from 73.5-92.6%. Statistically, this

AUC value of 74.9% shows the strength of a relatively high diagnostic value. The results of the ROC coordinates indicate that the cut off level of the lead of 2.45 µg/dL used in this study had a sensitivity value of 73.7% and a specificity of 63.2%. Figure 1 below shows the results of the ROC and AUC.

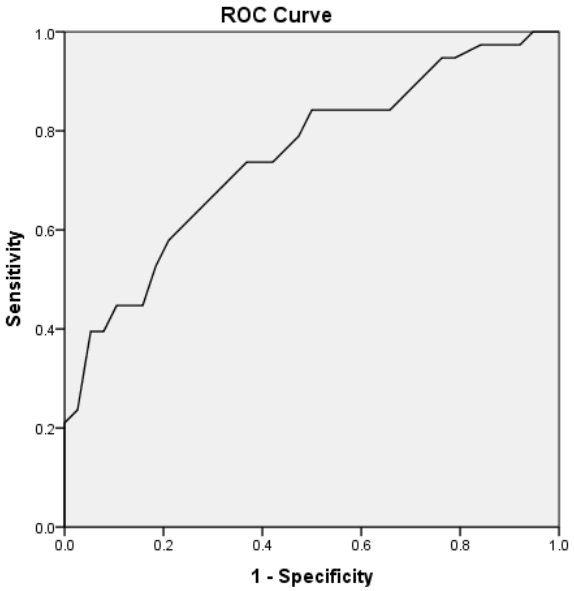

Figure 1: Results of the ROC procedure for lead levels against cognitive impairment

Research data on blood lead levels were grouped into two, namely high blood lead levels (≥ 2.45 µg/dL) and lead levels not high in the blood (< 2.45 µg/dL).

Bivariate analysis was performed to determine the relationship between high lead levels in the blood (independent variables) and cognitive function disorders (dependent variables), the hypothesis test used was risk estimate and Chi-square test. It was found that high blood lead levels had a higher risk of cognitive impairment than subjects with not high lead levels (OR 4.80; 95% CI 1.8-12.75; p < 0.001), shown in Table 2.

Table 2: Bivariate analysis of lead levels in the blood with cognitive impairment

Serum Lead Level	MoCA-Ina		Total n (%)	OR (95% CI)	p-value
	Cognitive Impairment n (%)	No Cognitive Impairment n (%)			
High	28 (66.7)	14 (33.3)	42 (100)	4.80	0.001
Not High	10 (29.4)	24 (70.6)	34 (100)	(1.80-12.75)	

*OR = odds ratio; CI = confidence interval; MoCA-Ina = Montreal Cognitive Assessment-Indonesia.

Bivariate analysis was performed to determine the relationship of age (independent variable) with cognitive impairment (dependent variable). Based on Pearson Chi-Square linear by linear data analysis for trends, p = 0.166, there is a significant relationship between age and cognitive impairment (OR 1.905; 95% CI 0.762-4.764), shown in Table 3 below.

Table 3: The relationship between adolescence and adulthood to cognitive impairment

Age	MoCA-Ina		Total n (%)	OR (95% CI)	p-value
	Cognitive Impairment n (%)	No Cognitive Impairment n (%)			
Adult	20 (58.8)	14 (41.2)	34 (100)	1.905	0.166
Adolesence	18 (42.9)	24 (57.1)	42 (100)	0.762-4.764	

*OR = odds ratio; CI = confidence interval; MoCA-Ina = Montreal Cognitive Assessment-Indonesia.

Gender did not affect the occurrence of cognitive impairment (p = 0.613), and cognitive impairment increased linearly with age (p = 0.011). The results of the analysis are shown in Table 4.

Table 4: Effect of sex and working period of gas station operators on the occurrence of cognitive disorders

Variable	MoCA-Ina		Total n (%)	OR (95% CI)	p-value
	Cognitive Impairment n (%)	No Cognitive Impairment n (%)			
Men	10 (45.5)	12 (54.4)	22 (100)	0.774	0.613
Women	28 (51.9)	26 (48.1)	54 (100)	(0.286-2.092)	
Working period > 3 years	16 (72.7)	6 (27.3)	22 (100)		
Working period 1-3 years	22 (40.7)	32 (59.3)	54 (100)	3,879 (1.312-11.467)	0.011

*OR = odds ratio; CI = confidence interval; MoCA-Ina = Montreal Cognitive Assessment-Indonesia.

It was found that the use of masks when working did not have a statistically significant effect on the incidence of cognitive disorders. This indicates that the use of personal protective equipment, especially masks, does not play a protective role in the incidence of cognitive disorders (p = 0.442). The results of the analysis are shown in Table 5 below.

Table 5: Effects of mask use when working on cognitive impairments

Masker Wearing	MoCA-Ina		Total n (%)	OR (95% CI)	p-value
	Cognitive Impairment n (%)	No Cognitive Impairment n (%)			
Wearing masker not routinely	29 (52.7)	26 (47.3)	55 (100)	1.487 (0.540-4.097)	0.442
Wearing masker routinely	9 (42.9)	12 (57.1)	21 (100)		

*OR = odds ratio; CI = confidence interval; MoCA-Ina = Montreal Cognitive Assessment-Indonesia.

The results of the bivariate analysis showed that the age, working period and blood lead levels were statistically significant for the incidence of cognitive impairment and the working period variable statistically with a value of < 0.25. The results of variable high blood lead levels (p = 0.001) and the working period (0.021) were statistically significant. Table 6 below shows the results of the analysis.

Table 6: Multinomial logistic regression on the variables of age, working period and lead levels in the blood with the incidence of cognitive disorders

	Coefficient	S.E.	Wald	DF	OR 95% CI	p-value
Constant	-1.962	0.632	9.639	1		
Adult	-0.151	0.645	0.055	1	0.860 (0.243–3.042)	0.814
Working period >3 years	1.742	0.756	5.309	1	5.707 (1.297-25.112)	0.021
High Lead Serum level	1.792	0.552	10.546	1	6.00 (2.035-17.695)	0.001

*OR = odds ratio; CI = confidence interval; S.E = standard error; DF = degree of freedom; MoCA-Ina = Montreal Cognitive Assessment-Indonesia.

Based on the equations above, high lead levels in the blood as a risk factor for cognitive impairment are 6 times greater than not high blood lead levels and work periods > 3 years as a risk factor for cognitive impairment 6 times greater than the working period of 1-3 year independently.

Discussion

The majority of the research subjects were in the teenage age category (55.3%), this was by the demographic characteristics of Indonesia, the majority of which were dominated by productive age. Female sex (71.1%) was obtained more than men because in this study many men were excluded because of active smoking. The working period is obtained for 1-3 years (71.1%) more than the other working period; this is due to several new gas stations standing around 1-5 years. In the case group (52.7%) and controls (47.3%) who were not routinely using masks while working for the past 1 month, the data shows that there is still a lack of discipline in the use of masks when working, caused by a lack of comfort when using masks.

This result is by previous studies which stated that the use of Personal Protective Equipment (PPE) was very influential and able to protect themselves from lead exposure (p = 0.038). The duration of working for 1-3 years was found to be the highest in the case group (71.1%); statistically, the duration of work affected the incidence of cognitive disorders.

The results of this study indicate that gender does not affect the occurrence of cognitive dysfunction, and there is a tendency for an increase in the incidence of cognitive impairment with age, so it was assumed that the incidence of cognitive impairment increases with age, regardless of factors in blood lead levels. This is probably because most of the age in this study sample tended to be in the age of adolescents so that statistically the apparent value was not significant with the opinions of previous studies. The results of this study found that the use of masks when working did not statistically affect the incidence of cognitive disorders; this indicates that the use of personal protective equipment, especially type 2 ply masks, does not play a protective role in the incidence of cognitive disorders.

Obtained subjects with high lead levels in the blood had a much greater risk of suffering cognitive impairment compared to subjects with not high blood lead levels. Lead can cause prominent abnormalities in the nervous system, in the form of slowness in action, decreased function of memory and concentration, depression, headaches, vertigo (dizziness), tremor (abnormal movement with rapid frequency), stupor (decreased consciousness), coma, convulsions, psychomotor disorders, mild intelligence disorders and personality changes. While the alkyl lead forms a special form of abnormalities in the central nervous system, with manifestations including insomnia, nightmares, and in severe cases can be schizophrenic. The strength of this study is that not many researchers have examined lead levels as a risk factor for cognitive impairment in gas station operators in Indonesia. This study cannot determine the length of exposure to lead that causes cognitive impairment and cannot determine the increase in minimum lead levels that cause cognitive impairment. Further studies are needed to determine these variables.

In conclusion, high lead levels in the blood have a 6 times greater risk of cognitive impairment compared to subjects with not high blood lead levels and work periods > 3 years have a6 times greater risk of experiencing cognitive disorders compared to 1-3 years of the work period. Blood lead levels and years of service are independent risk factors for the occurrence of cognitive impairments in oil refuelling station operators.

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Case Report

Single - Door Cervical Laminoplasty Using Basket Laminoplasty Device: A Case Report

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Keywords: OPLL; Single door cervical laminoplasty; Basket laminoplasty device

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Abstract

BACKGROUND: The management of the cervical canal stenosis as a result of ossification of the posterior longitudinal ligament (OPLL) is still evolving. Anterior and posterior approaches are still much in demand by the surgeons. In Japan, a posterior approach is more well-known to be used as the case OPLL is often on the populace. Single-door laminoplasty technique or “Hirabayashi” often used with either autograft or allograft, with or without an additional miniplate.

CASE PRESENTATION: In this case report, we would like to report the treatment of tetraparesis patients with “basket laminoplasty” using a special device with some advantages, not only providing stability of the lamina but also at the same time providing bone-graft container/basket for the benefit of the patient's bone fusion.

CONCLUSION: Basket laminoplasty device is an excellent choice for cervical OPLL. We believe the use of this device is very favourable for long-term patient outcome.

Introduction

Cervical root syndrome problems, such as cervical disc herniation and spondylosis including OPLL, often occur as one of the causes of cervical myelopathy. Because the result of conservative treatment in OPLL is unpromising, surgical treatment is selected in most cases [1]. The surgical management of OPLL is still in debate, whether it is better dealt with the anterior approach, posterior approach, or the combination of both.

It was agreed among experts that for a single-level spinal OPLL without canal stenosis, the anterior procedure is a better option, while in multi-level canal stenosis OPLL laminoplasty procedure can further facilitate the use of adequate decompression [2], [3], [4]. We reported a case of a patient who was successfully treated by open-door basket laminoplasty devices.

Case Presentation

A 65 years-old male presented with a history of weakness and numbness that were started from the legs. He also complained about stiffness and pain in his neck for two years. They were followed by arm weakness, especially at the left side. Sagittal and axial T2WI MRI revealed edema of the spinal cord and hyperintensity changes on C4-6 due to spinal canal compression by OPLL (Figure 1). On myelogram, there was a blockage of cerebrospinal fluid (CSF) flowed at the level C4-6 (Figure 2).

Further neurological findings were grade I-III tetraparesis and hypoesthesia of the arms and legs with increased deep reflexes. No history of trauma was documented. He received conservative management from a neurologist, but he considered himself not improved. He was then planned for a surgical decompression by the laminoplasty procedure.

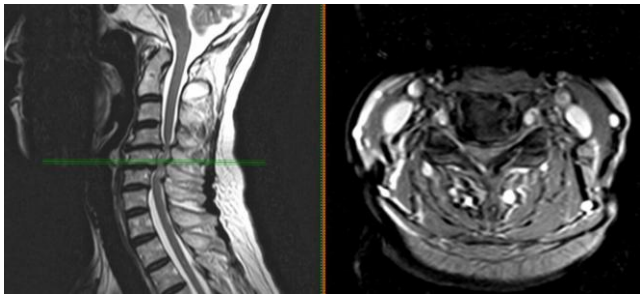

Figure 1: MRT2WI shows significant cord compression and intramedullary hyperintensity changes

A cervical midline skin incision was made from C2 spinous processes until C7. After the automatic retractor was placed, we discovered hypertrophic facet joints on C4-6, and C3-C7 lamina was exposed. The bone gutter of the medial border of the facets was made from C4-C6 both sides by using a high-speed drill.

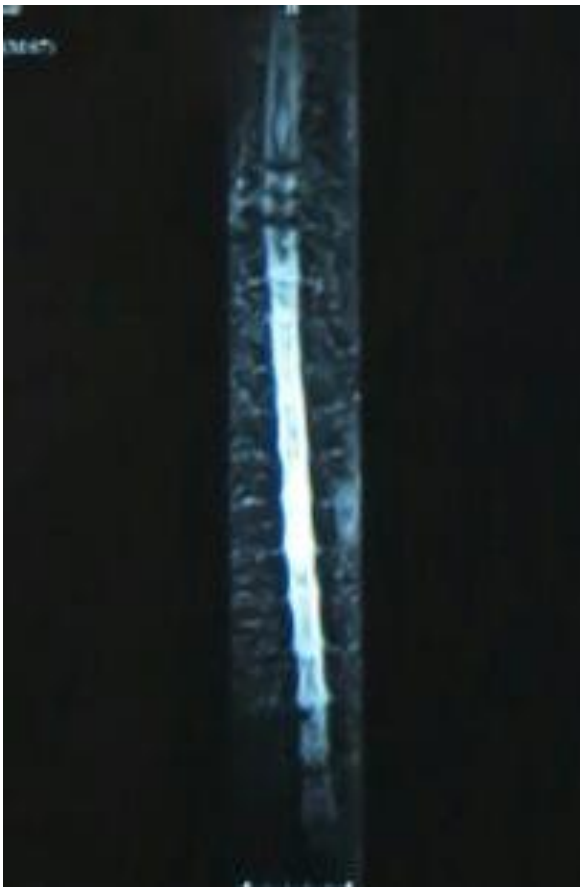

Figure 2: MR myelogram showed CSF blockage from C4-6 levels

By preserving the inner cortex of the lamina, we opened the lamina door gently from C4-C6 and basket laminoplasty devices were placed to maintain the door opening about 10 mm with titanium screws (1.5 mm in diameter, and 5 mm length) on the facet and lamina side (Figure 3). At this stage, the dural pulsation was observed, and after completing homeostasis a drainage tube size 10.0 was placed, an osteoligament reconstruction was done, and fascia-skin was closed by sutures. The patient was bed-rested for 3 days after the procedure, and with gradual ambulation, he was started mobilisation using a collar brace for 3 months.

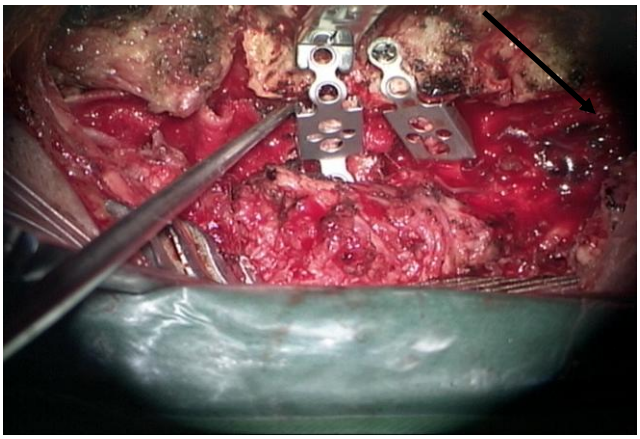

Figure 3: Intraoperative view of basket laminoplasty devices (arrow)

The patient made a good recovery. Motor paralysis improved remarkably, and gait disturbances were reduced. Upon discharge, the patient was able to sit using a wheel-chair and move all extremities against gravitational force. He continued the rehabilitation program for 6 months where he managed to get a partial resolution of his neurological deficits. A follow-up CT Scan was taken one month after the surgery, that revealed satisfactory implants position and sagittal balance of the neck segments (Figure 4).

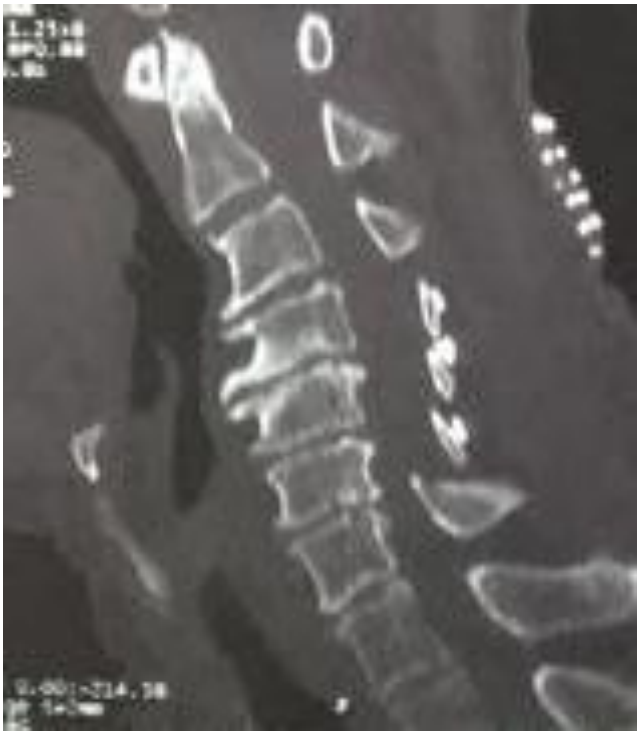

Figure 4: CT Scan revealed acceptable space for the cord and neck spine alignment

Discussion

Hirabayashi first described a technique of expansive open-door laminoplasty in 1991 [3]. In the beginning, a thread was used to hold the lamina of the spinous processes. But lately, various forms of spacers were available, although some surgeons still not confident to use them and some still use autograft [4], [5], [6], [8]. The tendency to use mini-plates or hydroxyapatite spacer to maintain the opened side have emerged, with 53.1% of the studies reported the use of mini-plates or hydroxyapatite spacer. The use of this hardware did not give a negative effect on the outcomes (p = 0.196) [5], [7]. Most of the previous studies failed to present sufficient data to conclude the pre and postoperative occurrence of post-laminoplasty kyphosis.[7-10] The use of spacers or miniplate does not affect significantly (p = 0.889) in spinal deformities or the neurological outcomes [5], [6].

Surgeons are still faced with some options regarding this procedure: using mini-plate without material for bone fusion, using materials such as hydroxyapatite bone-fusion, or using autograft with or without miniplate [6], [8]. Because of these choices, it is necessary to choose one device that is capable of carrying all the advantages stated above, easy to install, affordable, not adding foreign substances to the patient, ensuring bone fusion, sufficient to stabilise, not causing deformities, and does not cause neurologic deficits.

Basket system allows for the bone graft to be held in place, which maximises the speed of bone fusion. Screw placement is simpler than conventional miniplate because the basket is holding the lamina during the procedure. The results of pure titanium are faster osseointegration and lower artefacts. The unique shape of the laminoplasty basket is holding lamina properly and prevent itself from falling into the spinal canal.

In short, the device can provide all of the conditions stated above. Now the device has been used widely especially in Japan which does have many cases of cervical OPLL. To our best knowledge, our case was the first case outside Japan where this device was used. In terms of placing the device, we found it fairly easy in installation with no additional tools needed to place it.

In conclusion, basket laminoplasty device is an excellent choice for cervical OPLL. We believe the use of this device is very favourable for long-term patient outcome.

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Case Report

Giant Bowen's Disease on the Face: Case Report and Review of the Literature

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Keywords: Giant Bowen's disease; dermoscopy; histopathology

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Abstract

BACKGROUND: Giant Bowen's disease is a rare and unusual clinical manifestation. Presenting as extensive scaly erythematous patch or plaque, it gives rise to a wide spectrum of dermatological differential diagnoses.

CASE PRESENTATION: We report a patient with giant Bowen's disease on the face that was successfully treated with topical 5 % imiquimod. A review of the literature was made with the aim to analyse and compare the findings in it with our observation.

CONCLUSION: We present this case to draw attention to the importance of the self skin examination in the elderly population. Also, to prevent development to invasive squamous cell carcinoma from Bowen's disease, we recommend mandatory dermoscopic examination on every long-standing erythemosquamous lesion.

Introduction

Bowen's disease is considered an intraepidermal/*in situ* squamous cell carcinoma (SCC) as long as it has not spread beyond the basal membrane. Spreading into the dermis is a time-consuming process, and when it happens, it grows as an invasive SCC [1]. This precancerous nature of Bowen's disease was first recognised by John Templeton Bowen in 1912 [2]. The risk of progression to invasive SCC is estimated to be 3-5 % for extragenital lesions and 10% for genital lesions [3]. Referring to these assertions, its potential lateral spreading through the epidermis is expected. By this gradual progress, with time, Bowen's disease gets an unusual giant dimension.

Clinical presentation as a large erythemosqamous patch or plaque can be mistaken with various other dermatological differential diagnoses [4], [5].

Chronic UV irradiation exposure (solar, iatrogenic and sunbeds) is considered the most obvious trigger of Bowen's disease. Etiological causes also inspected are age, genetic factors, arsenic exposure and other carcinogens, human papilloma viruses (HPV), immunosuppression, trauma, x-ray irradiation etc. [4].

We present a case with giant Bowen's disease on the face, a sun-exposed area, an elderly patient. Our observation prompted us to consult the literature concerning this unusual presentation of Bowen's disease and compare the published findings with ours.

Case Report

An 85-year-old female was referred to our department with a history of slowly enlarging scaly erythematous lesion on the left cheek, developed over the last two years. Completely asymptomatic, it has

caused an only cosmetic disturbance. Patient has reported unprotected exposition to the sun 5-6 months per year, in the last 30 years. She declined previous cutaneous carcinomas of any kind. The attempts to treat it with topical corticosteroids and antimycotic creams did not reveal any results. After careful clinical and dermoscopic examination of the total skin, no other suspected lesions were noticed.

Dermatological status showed scaly, slightly elevated erythematous plaque with dimension 9 x 7.5 centimetres and well-demarcated borders from the surrounding healthy skin (Fig. 1).

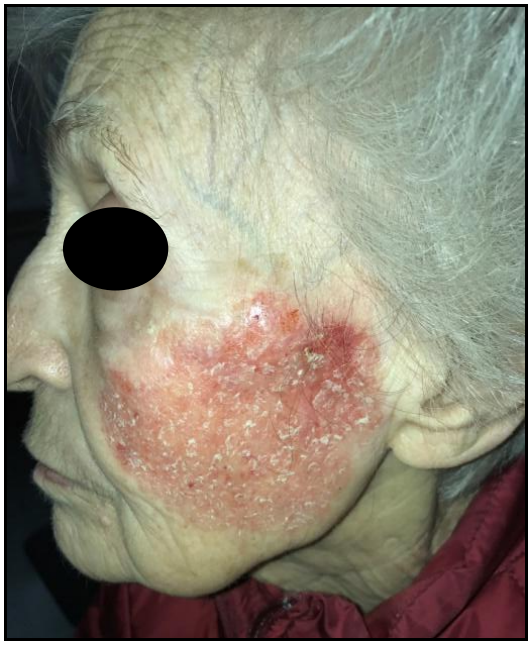

Figure 1: Large scaly erythematous plaque on the left cheek

Dermoscopic findings through the surface of the entire lesion showed glomerular or dotted vessels and yellow-white opaque scales on an erythematous background, suggestive for Bowen's disease (Fig. 2).

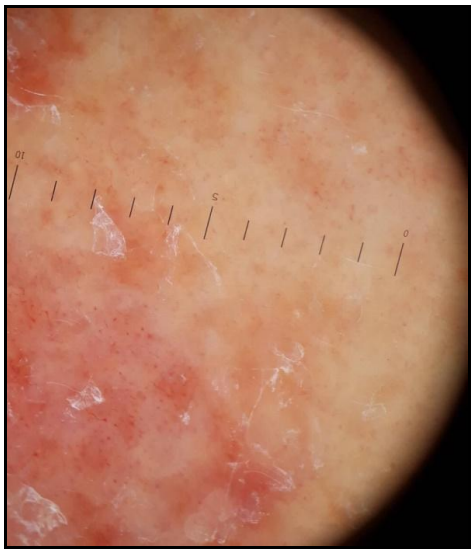

Figure 2: Dermoscopic findings: dotted vessels and yellow-white opaque scales on an erythematous background

Histopathological examination revealed parakeratosis in stratum corneum with atypia in cells throughout the epidermis. Individual dyskeratotic cells and increased mitotic figures were evident in the spinous layer. So, the diagnosis of Bowen's disease was confirmed (Fig. 3).

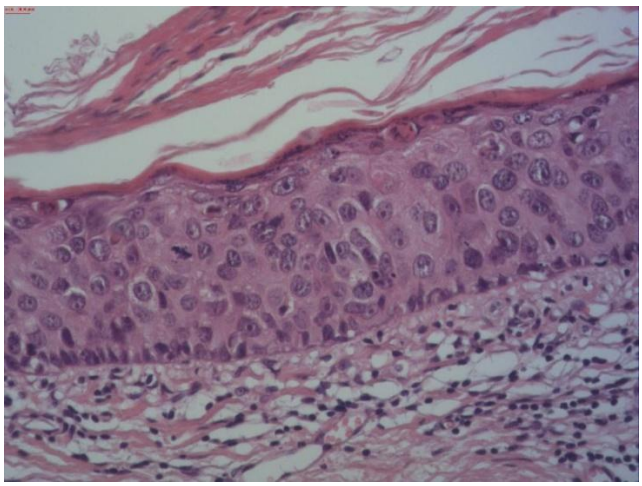

Figure 3: Histopathology, H&E: Parakeratosis in stratum corneum, atypia, pleomorphism, dyskeratotic cells and some mitotic figures in the epidermis

Treatment with 5% imiquimod cream was initiated 3 times weekly for 4 months. For the following 2 months, the application was reduced to 2 times per week. Impressive healing of the skin lesion was achieved [Fig.4]. Ten months follow-up did not show recurrence of the lesion.

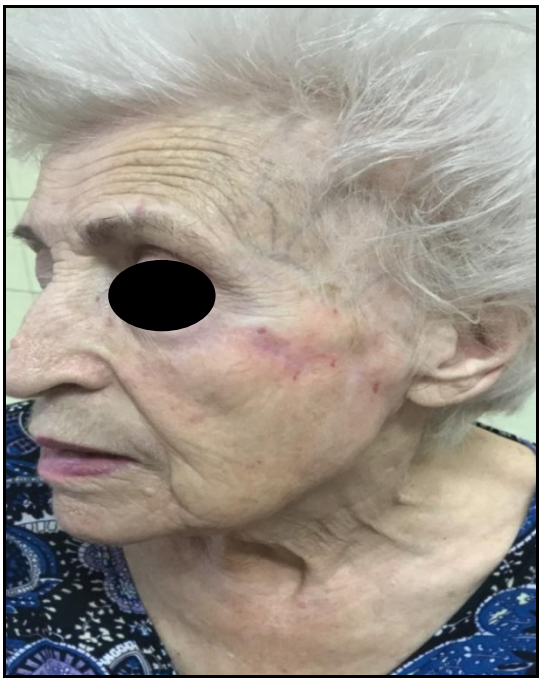

Figure 4: Complete healing of the Bowen's lesion after 6 months treatment with 5 % imiquimod

We aimed to review the literature and hence a Medline search was undertaken for the terms "Giant

Bowen's disease", "Huge Bowen's disease", "Large Bowen's disease" and "Extensive Bowen's disease". Additional articles were disclosed from the references given in the publications. All articles were methodically studied for the described clinical, dermoscopic and histopathological features. Clinical images and photomicrographs were reviewed and matched with those of our patient. All articles lacking any photomicrographs were excluded.

Table 1: Data from the literature concerning giant Bowen's disease, including our case

Reference	Sex	Age	Localisation	Dimension	Course
Sotiriou E, et al.2011 [6]	Male	79	Right fronto-temporal area	10 x 10 cm	2 years
Park JY, et al. 2013 [7]	Male Female	55 79	Left Flank Right breast	13 x 12 cm 21 x 14 cm	? 40 years
Bakardzhiev I, et al. 2015 [8]	Male	56	Above right flank	26 x 22 cm	More than 15 years
Shankar AA, et al.2015 [9]	Male	68	Abdomen	11 x 5 cm	25 years
Baykal C, et al. 2016 [10]	Male	67	Abdomen	15 x 15 cm	Since childhood
Akay BN, et al. 2016 [11]	Male	44	Abdomen	32 x 25 cm	10 years
Nagakeerthana S, et al. 2017 [12]	Male	73	Gluteal	15 x 16 cm	3 years
Ozlu E, et al. 2017 [13]	Male	62	Abdomen	13 x 13 cm	7 years
Caca-Biljanovska N, et al. 2019 [this paper]	**Female**	**85**	**Left cheek**	**9 x 7.5 cm**	**2 years**

Discussion

Giant Bowen's disease is rarely reported in the literature [8]. There is no consensus on an official definition of large, extensive or huge Bowen's disease. Morton et al. made efforts in their study to define "large Bowen's disease" as a lesion with a dimension more than 2 cm [14]. On the other hand, Lopez et al. used the term "extensive Bowen's disease" for those lesions larger than 3 cm in diameter [15]. Our patient with 9 x 7.5 cm sized Bowen's disease fulfilled the criteria proposed by Morton and Lopez [14], [15].

Although the clinical presentation can argue about plentiful of dermatological diseases, dermoscopic findings strongly suggestive for Bowen's disease were recently defined [16]. Anyway, histopathology remains the 'gold standard' for an accurate diagnosis of Bowen's disease [17].

The period for full expression of the Bowen's lesion is variable, from 2 years to maximum 40 years, a finding which is strongly in favour of the slow, lateral spreading of Bowen's disease.

Opposite to our patient, the most common position of giant Bowen's disease in other published cases is the abdomen, followed by flanks. These are covered parts of the body, non- sun exposed. We could assume that this observation and the fact that most of the patients with giant Bowen's disease are old males are the reasons for ignoring the lesion or delaying the dermatological consultation.

Only two of the patients from the literature, including our, have a lesion on the face, and both of them have the shortest history of 2 years duration of the disease. Upon that background, we can assume that cosmetically non-acceptance was the main motive to the patient to seek out dermatological help.

Asymptomatic as it is, the early lesions of Bowen's disease are very subtle and overlap with clinical features seen in many other dermatological conditions (seborrheic keratosis, superficial basal cell carcinoma, actinic keratosis, eczema, tinea, psoriasis vulgaris, seborrhoeic dermatitis etc.) [5].

Although early dermatological consultation can be accessed by the patient, these clinically not specific changes of Bowen's disease often mislead the dermatologist in making the correct diagnosis. Undiagnosed Bowen's disease ultimately advances to invasive SCC [11].

Treatment modalities for Bowen's disease are topical immunosuppressive/immunostimulating creams, cryotherapy, curettage, photodynamic therapy, radiotherapy, laser and finally surgical excision [18]. The therapeutic choice can be influenced by the age group, number, size and localisation of the lesion, preferable and comfortable solution by the patient and affordability of the therapeutic modalities [1].

In conclusion, the term "giant" is referred to clinical manifestation of the lesion, and it should not be used as a variant or distinctive form of Bowen's disease. We suppose that giant manifestation of Bowen's disease is due to neglecting the skin lesion by the patient, or not identifying it promptly by the dermatologist. Therefore, we strongly recommend self skin examination to be carried out, especially in older patients. Also, regular dermoscopy performed on every long-standing erythemosquamous skin lesion should be a rule. Early recognition of Bowen's disease and prevention of its undeniable progression into an invasive SCC is very important.

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Case Report

Chronic Osteomyelitis after Seven Years Neglected Bone Exposed in 12-Year-Old Boy: A Case Report

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Abstract

BACKGROUND: Access to modern medicine is still limited in some rural areas in Indonesia. This is mostly due to lack of people's knowledge and concern for their health, especially in orthopaedic cases. Osteomyelitis is generally described as infection and inflammation of the bone, which results in local bone destruction, necrosis, and apposition of new bone. Chronic post-traumatic osteomyelitis (CPTO) is a complex condition and one of the most challenging problems in orthopaedic surgery that cause considerable morbidity.

CASE PRESENTATION: We present a case of chronic post-traumatic osteomyelitis with radial nerve injury, in which radical surgical debridement and broad-spectrum antibiotic administration were done. A 12-year-old boy with a history of falling from the three-meter-high tree had swollen, deformed, and neglected humerus bone exposed. After stabilisation in the emergency room, surgical limb amputation was scheduled, yet the family refused this medical care and chose debridement instead.

CONCLUSION: This case is an important addition to the literature about chronic post-traumatic osteomyelitis with neglected bone exposed and the lacking of society's concern in regards to orthopaedic cases.

Introduction

Although government health programs, known as BPJS (*Badan Penyelenggara Jaminan Sosial*), has given access to health care, by the subsidised hospital cost and has become a way out for a poor urban's financial problem, the number of neglected fractures has not dwindled in recent years. Thus, fractures are often first treated by traditional bone-setters and healers whose method is not adapted to the management of open fractures. Most patients come to the hospital after a certain delay presenting with severe injuries or sequela that are difficult to cure with the diagnostic and therapeutic means available in traditional local facilities.

Chronic osteomyelitis occurs either after sequelae of acute osteomyelitis in children or secondary osteomyelitis due to trauma involving open fracture. The last entity defines our case which is post-traumatic osteomyelitis [1], [2], [3]. There are two most well-known classification systems for osteomyelitis, the Cierny-Mader, and the Waldvogel. Recently, new classification systems have been introduced and designed to be more specific to modern diagnostic and management approaches of osteomyelitis [3], [4]. Those important variables used in the new systems are bone involvement, antimicrobial resistance patterns of causative micro-organisms, the need for soft tissue coverage, and host status [4], [5]. Tenderness, effusion, increased warmth, pain with motion, and drainage on the area of the affected bone are signs and symptoms of chronic osteomyelitis [6].

Trauma is one of the risk factor for osteomyelitis [7]. Open fractures are at high risk of transcutaneous contamination of bacteria [8]. The pathophysiology of traumatic osteomyelitis depends on bones involved, initial injury's characteristic, and host condition [9]. Radial nerve injury (particularly radial neuropraxia) as the main presentation of

fracture middle third humerus (diaphyseal fracture), is one of the most aetiology for peripheral nerve palsy associated with humerus bone fracture. The incidence ranges from 1,8% to 22% [10], [11], [12], [13].

Lesions, caused by traumatic injury, can occur anywhere along the radial nerve. Therefore, the anatomy of it is clinically significant. The structures along its course also play an important role in determining the sites at which lesion might occur and in localising the origins of pathological lesions during diagnostic examinations [13], [14], [15], [16]. They are classified as lesions in the axilla (very high lesions), lesions in the arm in the radial groove (high lesions), lesions at the elbow, and lesions of the superficial branch of the radial nerve (low lesions). In our case, it is the high lesions type. The deficit of high lesions type presents brachioradialis weakness, wrist drop, finger drop, thumb drop, and sensory loss [16]. Diagnosis of osteomyelitis can be confirmed by bone scan or MR scan, along with needle aspiration, bone biopsy, elevated erythrocyte sedimentation rate (ESR), elevated C-reactive protein (CRP), elevated WBC count, and culture [6], [7]. Treatment of osteomyelitis is multiple surgical procedures, including surgical debridement of all necrotic bones, soft tissue coverage, the combination of appropriate antibiotics, and long-term follow-up, which are all still a problematic clinical challenge for osteomyelitis [1], [2], [3], [4], [8].

Identification of the pathogen in the bone, through a bone biopsy, is a definitive diagnosis of osteomyelitis. In the selection of antibiotic administration, most studies reported Staphylococcus aureus is the aetiology and responsible for 80% to 90% of its cases [7], [8]. Eighty-five to ninety percent of radial nerve lesion can recover spontaneously within three months. Thus, if nerve function doesn't return within three to four months, nerve surgical exploration should be performed [10], [11], [13], [15], [17]. The prognosis of radial nerve injury is influenced by the severity of humeral shaft fracture according to AO/OTA Classification [12].

Hereby we present a case report of a 12-year-old boy with chronic post-traumatic osteomyelitis (CPTO) after seven years of neglected bone exposed. He was successfully treated with radical surgical debridement, broad-spectrum antibiotics, analgesics, neuroprotective agents, and physiotherapy.

Case Report

A 12-year-old boy presented to the emergency department with a chief complaint of bone exposed and bleeding of the upper left arm every time he got minor trauma. It was caused by a past traumatic injury resulting from the impact of falling from a three-meter-high tree which happened seven years ago. He fell forward with left arm outstretched, struck the ground with shoulder flexed, elbow flexed, forearm pronated, wrist dorsiflexed, resulting in the bone of the upper left arm exposed. His mother admitted due to the fear of having surgery, the exposed bone was never consulted to medical care and was only treated by traditional medicine for seven years.

On clinical examination, of which consists of look, feel, move, the upper left arm was swollen with shortening deformity, contraction, angulation, exposed humerus bone with the size approximately 2,5 cm in diameter, and full thickness tissue loss, which was covered by dry, black eschar on the base of the ulcer on anterior and posterior aspect of the upper left arm. The eschar extended from the middle shaft into the proximal of the upper left arm. There is no slough and yellow-coloured pus seen on the wound base. Bleeding, tenderness and crepitus were presented on the middle shaft upper left arm (Figure 1). Sensation on first dorsal web space of his left hand was weakened. Extension of the left wrist and left fingers including the thumb was also impossible (wrist drop, finger drop, and thumb drop). Yet, radial artery pulsation was still poorly palpated. The left elbow's range of movement (ROM) including flexion and extension was moderately restricted because of deformity and pain. While the ROM of left wrist, particularly on extension movement, was severely restricted.

Figure 1: Open fracture of the middle one-third neglected upper left arm in a 12-year-old boy; a) lateral aspect; b) anterior aspect

Blood investigations showed severe anaemia (Hb: 7.3 mg/dL) and leukocytosis (20.200/µL). ESR and CRP values weren't investigated due to lack of facilities. Anteroposterior and lateral radiographs of the upper left arm showed both open, complete, transverse fracture on proximal one-third to middle humerus bone with osteomyelitis and a gap between

proximal and distal humerus. There was the irregular contour of the glenoid on the shoulder joint (Figure 2). The patient was managed and stabilised with the administration of normal intravenous saline, one unit of packed red cells (PRC), broad-spectrum antibiotics, analgesics, neuroprotective agents, and surgical debridement, as his family refused to be amputated. He then was followed up for seven days after debridement had been performed.

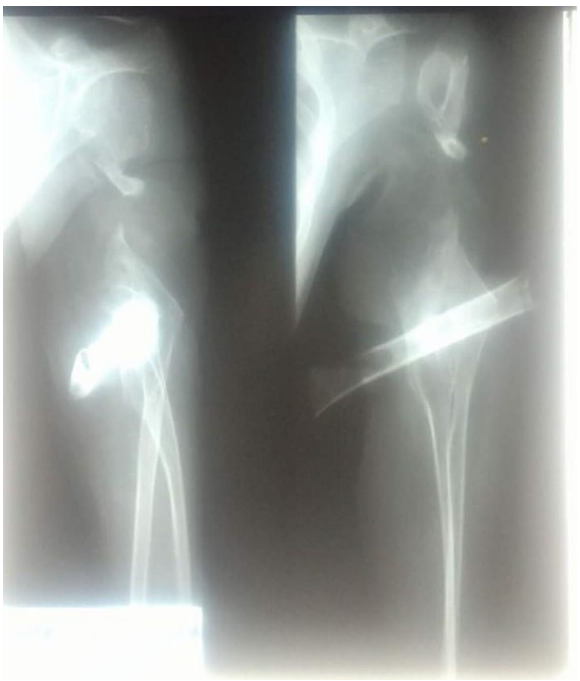

Figure 2: Anteroposterior and lateral X-ray films of the upper left arm. They showed a complete, transverse fragment fracture from proximal one-third to middle humerus bone which is displaced into anteroinferior with the lytic lesion and sclerotic on distal one-third humerus bone

Discussion

The incidence of post-traumatic osteomyelitis is rising recently because of the increased frequency of trauma, whereas the incidence of chronic and neglected cases has not dwindled due to lacking knowledge and concern of our society in regards to orthopaedic cases [10]. Physical examinations of osteomyelitis found on the patient were quite similar to the literature, such as tenderness, effusion, increased warmth, pain with motion, and drainage of the affected area [6].

Based on several classifications, our patient is type IIIB of Gustilo & Anderson's open fracture classification, contiguous type of Waldvogel's osteomyelitis classification, type IV class B host classification with unstable, segmental diaphyseal type of diffuse osteomyelitis defect on Cierny-Mader's osteomyelitis classification, and high lesions type of radial nerve injury's classification [1], [4], [18].

The patient has been diagnosed osteomyelitis based on history taking, physical examination, and has been confirmed by the elevated WBC count on laboratory findings and the appearance of the sclerotic and lytic lesion on proximal one-third humerus in x-ray [5].

His Mangled Extremity Severity Score (MESS) for limb salvage is more than seven, referring to score 4 for the massive energy of injury, score 2 for thready pulses, score 2 for a prolonged shock. His fracture meets the Apley's criteria for amputation, such as dangerous and damned nuisance criteria. We, therefore, suggest amputation to be performed, but his family refused it [3], [5], [8], [19], [20]. Consequently, we performed radical debridement and excision of all avascular scarred and infected granulation tissue, followed by debridement of the infected endosteum, reaming, and the insertion of closed suction drainage system [3], [20], [21].

Determining the appropriate antibiotics has been an issue due to the paucity of instruments (specimen culture). Therefore, we use a broad-spectrum antibiotic to prevent the increase of bacterial resistance cases [5]. Besides, early exploration of the radial nerve is not performed as well due to the lack of instruments and resources.

Although the infection control and satisfactory functional outcome of our patient can finally be achieved, this case proves knowledge and concern of our society in regards to orthopaedic cases is ironically superficial. Most of them are still taboo and afraid to have surgery, particularly amputation. Once the patient's fracture meets the MESS and Apley's criteria, amputation is essential to give the good prognosis to the patient.

In conclusion, this case is an important addition to the literature about chronic post-traumatic osteomyelitis with neglected bone exposed and the lacking of society's concern in regards to orthopaedic cases.

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Brief Communication

Weight Gain in Pregnancy and Weight Retention after Birth

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Abstract

AIM: Our study aims to determine the weight gain of pregnant women and their body weight one year after delivery. We compared these changes in body weight with education and place of residence (urban/rural).

METHODS: Secundigravidae women (N = 113) filled out the structured checklist regarding anthropological characteristics, such as body weight (the current and before and after the first pregnancy). Some sociodemographic characteristics were also obtained.

RESULTS: Average weight gain in pregnancy was 16.9 kg (Sd 6.1, median 16 kg; range 6-40 kg). Women with high school education only gained 2 kg more than women with college/university degree (F (1, 108) 4.11, $p \leq 0.05$). There was no significant difference in weight gain when the place of residence was compared (F (1, 111) 2.86, $p \geq 0.05$). The average weight difference one year after delivery was 3.3 kg (Sd 4.3, median 2 kg; spread -5 to 20 kg). There was no significant difference in weight difference one year after delivery in different educational groups. Women from rural area retained 2.5 kg more than women in an urban area (F (1, 109) 7.50, $p \leq 0.01$).

CONCLUSION: Our research has shown that women with higher education level gain less weight than women with lower degrees. They had more possibility to get access to information about health risks. The overall impression is that women do care about weight gain in pregnancy and actively work on getting back to desirable weight after delivery. This is even more important if we know that body weight before pregnancy, weight gain in pregnancy, pregnancy overweight and pregnancy obesity impact later life of mother and child. Therefore, the need for weight control in pregnancy and between pregnancies should be properly addressed.

Introduction

The increase in body weight is to some extent physiological process in pregnancy. At the beginning of pregnancy, women are often hypoglycemic due to the proinsulin's effect of hormones [1], [2]. Also, they often have hyperemesis, and there is usually a no sudden increase in body weight in the first trimester. Towards in the third trimester, more water accumulates in the body, and some women can get quite a bit of body weight.

Weight gain depends on hormonal changes, but the most important is the intake of nutrients.

High weight in pregnancy increases the risk of metabolic syndrome and obesity in later life of a child. Overweight and obesity before pregnancy have stronger correlations with metabolic syndrome and obesity in later life of a child than weight gain in pregnancy or postpartum weight retention [1], [2]. According to the World Health Organization classification obesity is defined as BMI ≥ 30 kg/m^2 and BMI 18.5-24.9 kg/m^2 is considered normal [3].

Weight retention after pregnancy can have serious consequences for women like postpartum depression, complications in later pregnancies and chronic conditions like diabetes and hypertension [4]. It is important to stress that overweight and obesity are risk factors for several gynaecological cancers [5].

Our study aimed to determine the weight gain of pregnant women and their body weight one year after delivery. We compared these changes in body weight with education and place of residence (urban/rural).

Material and Methods

The study was designed as a cross-sectional study conducted in the Department of Gynecology and Obstetrics, University Hospital Center Sestre milosrdnice, Zagreb, Croatia. Inclusion criteria were: all women were secundigravidae and time between pregnancies was more than a year. The study was approved by the Hospital Board of Ethics.

One-hundred and thirteen women participated in the study, of which there were 66 pregnant women (58.4%) in a second pregnancy and 47 parturient women (41.6%) that were admitted to the maternity ward for the delivery of their second child. Women anonymously filled out a structured checklist with anthropometric characteristics with an open-ended question on the current body weight, body weight before the current pregnancy, body weight before the first pregnancy, weight gain during the first pregnancy, and body weight after delivery of the first child.

Statistical analysis

Descriptive analyses were used to examine the average weight gain in pregnancy and one year after the delivery. ANOVA was used to test the differences in the weight gain and weight difference one year after delivery concerning education level, place of residence. P value was set to 0.05. All statistical analysis was performed using the SPSS version 20.0 for Windows.

Results

Average weight gain in pregnancy was 16.9kg (Sd 6.1, median 16 kg; spread 6-40 kg). Women with high school education only gained 2 kg more than women with college/university degrees. e (F (1, 108) 4.11, p ≤ 0.05). There was no significant difference in weight gain when the place of residence was compared (F (1, 111) 2,86, p ≥ 0.05), (Figure 1).

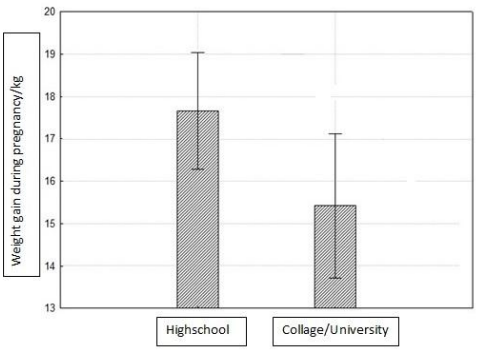

Figure 1: Weight gain in pregnancy in different education groups

The average weight difference one year after delivery was 3.3kg (Sd 4.3, median 2 kg; spread -5 to 20 kg). There was no significant difference in weight difference one year after delivery in different educational groups. Women from rural area retained 2.5 kg more than women in an urban area (F (1, 109) 7.50, p ≤ 0.01), (Figure 2).

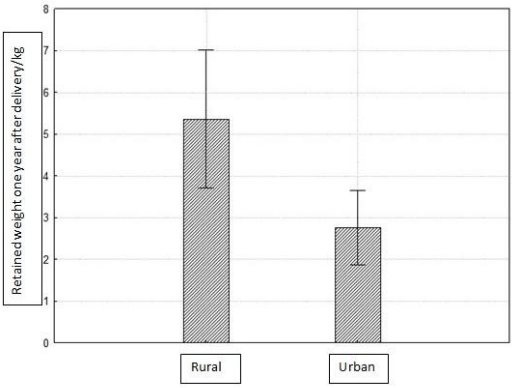

Figure 2: Retained weight one year after delivery in the rural and urban area

Discussion

Weight gain in pregnancy is getting a great deal of attention lately. Optimal weight gain ensures the better outcome of pregnancy and fewer complications in delivery, while high weight gain increases the risk of gestational diabetes, hypertension, macrosomic child, the incidence of Caesarean section and perineal injuries [5], [6]. Our research has shown that women with higher education level gain less weight than women with lower degrees, probably because they were educated about the dangers and the risks of getting too much weight in pregnancy. There was no significant difference in weight gain when the place of residence was compared what can be attributed to the proximity of rural areas to the city and therefore similar eating habits.

Women that gain more weight in pregnancy or fail to lose gained weight after delivery have increased the risk for complications in later pregnancies and health difficulties later in life [7]. In our study, the average weight difference one year after delivery was 3.3 kg. These results demonstrate a high level of silverness and discipline in postpartum women that influence overall health condition. We also found that women from rural area retained 2.5 kg more than women in the urban area. This can be attributed to social elements in the postpartum period like employment and professional activities.

The Institute of Medicine's report suggests that approximately half of women have the excess weight of 5 kg or more and one quarter 10 kg or more at 6 months postpartum [4], [8]. Postpartum weight retention (over 4.5 kg at 6-12 months after delivery) affects one million women in the United States each year. Particularly high risk of weight retention is in African American and Hispanic women. Lower income or certain geographical regions (South and Midwest) are also a risk for weight retention [9]. Parity is another known risk factor for weight retention [10]. All the women in this study are pregnant for the second time, and weight retention and weight gain of the first pregnancy was analysed. For weight retention prevention and general fitness after pregnancy exercise in and after pregnancy is recommended. In pregnancy, exercise is a novelty and should be moderate and adjusted for pregnant women. Outdoor activities can have a very important role in weight loss after delivery [6], [11].

In terms of socioeconomic parameters, the only the level of education seems to be related to weight gain during pregnancy. Low educations level is related to higher weight gain in pregnancy [12], [13]. This was confirmed in our results as well.

In conclusion, our research has shown that women with higher education level gain less weight than women with lower degrees. They had more possibility to get access to information about health risks. The overall impression is that women do care about weight gain in pregnancy and actively work on getting back to desirable weight after delivery. This is even more important if we know that body weight before pregnancy, weight gain in pregnancy, pregnancy overweight and pregnancy obesity impact later life of mother and child. Therefore, the need for weight control in pregnancy and between pregnancies should be properly addressed.

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Dental Science

Correlation of ABO Blood Groups and Rh Factor with The Severity of Generalized Chronic Periodontitis: Across Sectional Study in Riyadh, Saudi Arabia

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Keywords: Chronic periodontitis; Periodontal Diseases; ABO Blood Groups; Rh Factor; Severity

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Abstract

BACKGROUND: The development of periodontal diseases depends on the presence of causative microorganisms, host immunity and risk factors. Although variability present among the types of periodontal diseases, all are represented to a shared interaction between host and bacteria. ABO blood groups are the most investigated erythrocyte antigen system. However, limited investigations have been conducted to explore the alliance between ABO blood groups and periodontal diseases.

AIM: Our purpose was to explore any possible association between the severity of chronic periodontitis with ABO blood groups and Rh factor.

METHODS: A cross-sectional study was carried out on 205 patients out of 1126 generalised chronic periodontitis patients (GCP) who were referred to Al-Farabi Colleges, Riyadh, Saudi Arabia. They were categorized into; group I (mild), group II (moderate) and group III (sever).

RESULTS: The patients with blood group O were at a greater risk to develop GCP irrespective of its severity, followed by those with blood group A, B, and AB. The dispensation of the Rh factor in all groups exhibited a significantly greater distribution of Rh positive.

CONCLUSION: Genetic factors such as ABO blood group antigens may act as a risk influencer that affects the progression and severity of the chronic periodontitis.

Introduction

Periodontal diseases, including gingivitis and periodontitis, are known to be chronic immune inflammatory responses. The inflammatory reaction in periodontal diseases involves the triggering of leucocytes, neutrophils, T-lymphocytes and plasma cells, also the stimulation of antibodies and chemical mediators such as cytokines, chemokines and C-reactive protein. They are among the most common diseases in all communities and along with dental caries are considered the main causes of tooth loss [1]. The development of periodontal diseases depends on the existence of pathogenic micro-organisms, host immunity reaction and risk factors. These risk factors include oral hygiene, age, gender, immunity status, smoking, medications, drug abuse and socioeconomic status. Although variances that are present among the several types of periodontal diseases, all contribute a shared characteristic of complex host-microbial interactions. Disease development reflects the balance between homeostasis and the progression of the destruction of the periodontal tissues [2], [3].

ABO blood groups are the greatest reported erythrocyte antigen system where they have been utilised as haematological biomarkers in scientific studies and their associations with different diseases [4], [5]. The ABO blood type system implicates four blood categories: O, A, B and AB. Blood group O presents the erythrocytes without true antigen, but

create antibodies to A and B antigens while Type A and B blood groups present the erythrocytes that have the A and B antigens, respectively, and manufacture antibodies to the other blood type. In contrast, the Type AB blood group represents erythrocytes that do not make antibodies to others because they carry both A and B antigens [6].

The secretion of antigens of ABO blood groups in the saliva prohibits the ability of micro-organisms to adhere to the surface of a tooth; this is because many of these micro-organisms have surface lectins, which they use to adhere to surfaces of the body and are often ABO specific. Also, non-secretors tend to have minor levels of the immunoglobulin A (IgA) antibodies in the saliva, which may compromise their ability to keep bacterial counts low [7]. Therefore, the genetic factors may alter the oral ecology as genetic dissimilarities in the immune response and presentation of antigens may indicate the susceptibility to virulent and periodontal diseases. However, limited investigations have been revealed to explore the association between the ABO blood group and the diseases of periodontal tissues. The majority of the authors showed a positive correlation between periodontal diseases and ABO blood groups and claimed that the different ABO group could be a risk factor for periodontal diseases [8], [9], [10], [11], [12], [13], [14], [15]. In contrast, others did not find any associations between patients who have periodontal disease and ABO blood groups [16], [17].

Accordingly, this present study was done on generalised chronic periodontitis (GCP) patients and their corresponding blood types, to find out if there was any possible correlation between them. If any, whether of ABO blood types and the Rhesus (Rh) factor affect the severity of chronic periodontitis. Performing investigations in this field help us to understand more the risk factors of chronic periodontitis that will give more chances in prevention and successful treatment in the future.

Our purpose was to investigate any possible relationship between the severity of chronic periodontitis, ABO blood groups and Rh factor.

Patients and Methods

The study proposal was evaluated and accepted by the Ethical Committee of Al-Farabi colleges, Riyadh, KSA. Informed consent was received by participants after explaining all the details of the study.

Researchers investigated 1126 generalised chronic periodontitis (GCP) patients who were referred to the periodontology department, Al Farabi dental hospital, Riyadh, KSA. Patients were selected randomly according to the following criteria;

Inclusion criteria: -Healthy patients and former smokers with a clinically confirmed diagnosis of GCP according to AAP classification [18]; -Females and males ≥ 30 years old.; -Agreement (written informed consent) and compliance; -Clinical evidence of attachment loss in more than 30 % of the present teeth.

Excluded criteria: -Clinically diagnosed Gingivitis, Localized Chronic Periodontitis, Aggressive Periodontitis, non-inflammatory periodontal disease, acute periodontal diseases; -Current and former smoker patients; -Any systemic or mental diseases and systemic conditions like pregnancy; - Patients under any drugs, which affect periodontal health e.g. contraceptive pills; -Patients who had periodontal treatment 6 months prior to the examination; -Patients who had less than 20 teeth.

A group of 533 systemically fit patients were recruited, 319 medically compromised patients whether they smoked or not, and 9 systemically fit patients who had less than 20 teeth were excluded, only 205 patients (111 males and 94 females), aged 30-70 were enrolled in our cross-section study according to inclusion criteria (Figure 1). The study was based on the examination of the periodontal condition by two periodontal examiners, along with the detection of ABO blood groups.

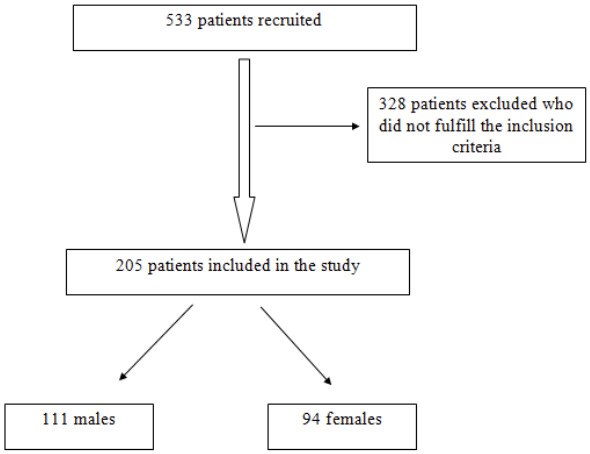

Figure 1: Flow chart

Periodontal Examination and diagnosis of chronic periodontitis

-Clinical examinations including documented patient age and gender, medical and dental history, oral care methods, extra and intra-oral examination were documented.

The Measurements of the Pocket depth (PD), Gingival marginal level (GML) and Clinical attachment loss (CAL) were done manually using periodontal probe (UNC-15), 3 readings were taken for each

surface Facially or Lingually/Palatally and the highest CAL reading was calculated and documented for each surface to assess the severity of the disease in periodontal tissues, The cementoenamel junction (CEJ) was used as reference point if not visible in case of fixed restoration; restoration margins were used for these measurements. The workshop of Australian dental association classified the severity of chronic periodontitis according to the clinical attachment loss of the tissues as follows: Mild = 1-2 mm CAL; Moderate = 3 to 4 mm CAL; and Severe = 5 mm CAL [19].

-*Horizontal furcation involvements* were evaluated and measured by Naber's probe, if present.

-*Panoramic radiographs* were used to evaluate bone loss all over the teeth.

-*Based on these clinical parameters, the subjects were segregated into 3 groups:*

Group I (Mild Generalized Chronic Periodontitis): Subjects displayed clinical attachment loss more than 30 % of sites, CAL between 1-2 mm.

Group II (Moderate Generalized Chronic Periodontitis): Subjects displayed clinical attachment loss more than 30 % of sites, CAL 3-4mm.

Group III (Severe Generalized Chronic Periodontitis): Subjects displayed clinical attachment loss more than 30 % of sites, CAL equal to or more than 5 mm.

Blood Investigations

Samples of blood were gathered using a sterile disposable lancet and finger prick methods. The blood grouping was done using the slide agglutination method (visual method) for the determination of the ABO blood group and Rh factor [20]. Three drops of the patient blood were mixed with anti-A, anti-B and anti-D separately on a glass slide. The blood agglutination pattern can be detected from which the ABO and rhesus D (Rh D) type of blood can be recognised. Patients were categorised regarding their blood groups (A, B, AB, O) and Rh status (+, −).

The participants were classified into three groups; Mild GCP (Group I), Moderate GCP (group II) and Severe GCP (group III). The participants and their ABO blood groups and Rh were analysed and tabulated. The percentages and distributions were estimated.

Statistical Analysis

To explore the relationship between the study groups, ABO blood groups and Rh factor, the percentage distribution was calculated and the tabulated data was statistically analyzed, the comparison of the distribution of blood groups was performed between the three groups using the Chi-square test using the SPSS version 19.00 program (SPSS Inc., Chicago, IL, USA).

Results

In this cross-sectional study, 205 patients (111 males and 94 females) with clinically and radiographically diagnosed GCP were divided into 3 groups regarding the severity of GCP. Table 1, and Figure 2 shows the patients' characterisation regarding gender and age disruption.

Table 1: Demographic data

Gender	30-39 years	%	40-49years	%	>50 years	%
Female (94) 45%	29	14.1	54	26.3	11	5.3
Male (111) 56%	35	17.1	64	31.2	12	5.8
Total (205) 100%	64	31.2	118	57.5	23	11.2

However, the ages of patients included in this study were above 30 years old where the noticed largest group ranged from 40-49 years which represents 57.5% of the total patients.

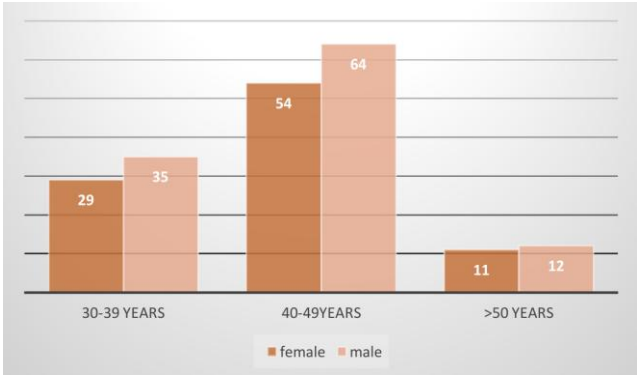

Figure 2: Demographic representation

Table 2, Figure 3 demonstrates the distribution of the ABO blood groups in 205patients with different grades of periodontitis, patients of mild chronic periodontitis (group I) had 6% of A blood group, 5.4% B blood group, 1.5% AB blood group and 10.7% O blood group.

Table 2: Distribution of periodontal status with the blood group of the study population

Blood group	Total no of patients	%	Group I	%	Group II	%	Group III	%
A	55	26.8	7 M+5 F	6	17 M+13 F	14	7 M +6 F	6.8
B	47	23	6 M+5 F	5.4	11 M +9 F	9.8	9 M +7 F	7.8
AB	19	9.3	2 M+1F	1.5	7 M + 2F	4.4	4M + 3F	3.4
O	84	40.9	11 M+ 13F	10.7	19 M + 17 F	18	11 M +13F	12.2

While moderate chronic periodontitis patients (group II) had 14% of A blood group, 9.8% B blood group, 4.4% AB blood group and 18% O blood group.

On the other hand, the third group which represented the severe form of chronic periodontitis (group III) had 6.8% of A blood group, 7.8% B blood group, 3.4% AB blood group and 12.2% O blood group.

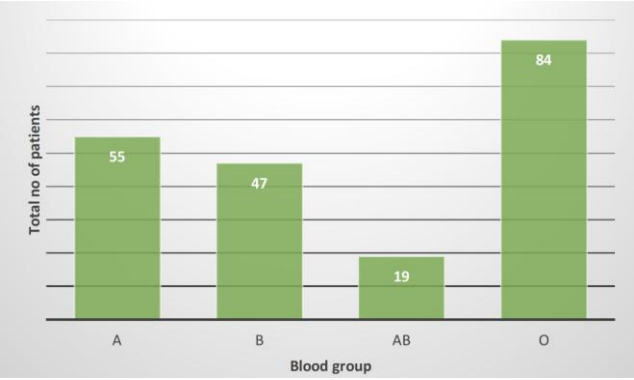

Figure 3: Distribution of periodontal status with the blood group of the study population

On comparison between the four blood groups, we found that all blood groups' highest percentage was related to the moderate form of GCP, and O blood group had the highest percentage of either mild (10.7%), moderate (18%) or severe form of GCP (12.2) in comparison with the other three blood groups. From this documented data, we find a higher frequency of GCP in patients with blood group O followed by A blood group, where among the surveyed 205 blood samples, O blood group represented 40.9%, and A blood group represented 26.8% of all three groups of chronic periodontitis.

Table 3: Frequency of Rh factor in subjects with periodontitis

Rh factor	%	Group I (Mild GCP)	Group II (Moderate GCP)	Group III (Severe GCP)
Rh+	90.2	41	83	60
Rh-	9.8	7	12	2

Table 3, and 4 exhibits the frequency of Rh factor of the total 205 participants who had chronic periodontitis, 184 (90.2%) participants were Rh Positive, and 21 (9.8%) participants were Rh negative, the variables of the equation showed statistical significance between the variables.

Table 4: Variables in the Equation for Rh Factor

	B	S.E.	Wald	Df	Sig.	Exp (B)
Step 0 Constant	.000	1.414	.000	1	.021	1.000

P Value = 0.021 < 0.05 Significant

Our results revealed that individuals with blood group O were at a greater risk to develop GCP irrespective of its severity, followed by those with blood group A, B, and AB respectively (Table 2). Thereby, the patients with the AB blood group had the least risk to develop GCP. The statistical analysis gave significant values when comparing the blood groups in different groups of Periodontitis (Table 5, and 6).

Table 5: Association between the between the blood groups

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	2.000[a]	1	.0137
Continuity Correction[b]	.000	1	1.000
Likelihood Ratio	2.773	1	.036
Fisher's Exact Test			
Linear-by-Linear Association	1.000	1	.317
N of Valid Cases	2		

The RH distributions in all estimated groups revealed significantly higher results of Rh Positive (Table 3).

Table 6: Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	12.000	9	.00
Likelihood Ratio	11.090	9	.90
N of Valid Cases	4		

Discussion

Plaque is a dynamic, well-organised microbial film that adheres tenaciously to teeth and other hard surfaces in the oral cavity. It is well known to be the main cause of periodontal diseases. Numerous factors may affect the qualitative and quantitative progress of the microorganisms responsible for periodontal diseases. Likewise, ABO blood groups were documented to be effective in bacterial colonisation and agglutination [21]. The progression of the disease can be related to host-based risk factors [13]. Genetic dissimilarities may act as risk or protective factors for chronic inflammatory reactions [10]. On the other hand, Offenbacher [22] stated that less than 20% of the variability of the severity of the periodontal diseases could be explained by the number of specific micro-organisms found in disease-associated plaques. At this moment, the role of genetic influences has been proposed.

Our study investigated the correlation between ABO blood group and the severity of the periodontal disease in systemically healthy patients with GCP above 30 years, as the chronic periodontitis don't explore significantly until the third decade [23].

A few early reports in the literature have considered the association between ABO blood group and periodontal diseases. Weber and Pastern were the pioneers in studying the relationship between ABO blood group and periodontal involvements in 1927. It was also noted that antigens of the ABO system could act as receptors for infectious agents [13]. Most of the previous studies have suggested that the ABO blood group is one of the genetic risk factors that may lead to periodontal diseases [3], [11], [12], [13]. While, Frias and Lopez [17], Barros and Witkop [24] and Pradhan et al., [25] stated that there is no correlation between ABO blood group and periodontal diseases.

In the present study, there was a positive and significant association between blood group and GCP. Also, the results revealed that blood group O is considered as a predictive factor for chronic periodontitis development and it increases the severity of this periodontal disease. While the patients with the AB blood group had the least risk to develop GCP. Similarly, Arati et al., in 2010 [14] reported that blood group O presented a greater percentage in the periodontitis patients and the blood group AB displayed the least percentage of periodontal involvements.

The results of our study also correspond to similar studies conducted by Gawrzewska [8], Demir et al., [9], Vivek S et al., [12], koregol et al., [15] and Anup et al., [16]. They conducted that individuals' blood group O and Rh positive had a superior predisposition for periodontitis. Instead, koregol et al., [15] and Gawrezewka [8] reported that patients with blood group A have more resistance to develop diseases of the periodontium.

Furthermore, Pai et al., [13] worked in 750 subjects with periodontal diseases. They determined that there was an existing relationship between periodontal disease and ABO blood group and there was a high prevalence of individuals with blood groups O and AB with healthy periodontal status, but individuals with blood groups B and A presented inclination toward unhealthy periodontal involvements. While, Aravind et al., [11] conducted that there was a relatively increased percentage of the B blood group in subjects with gingivitis and periodontitis while the subjects of O blood group had higher percentage healthy periodontium.

In contrast, some investigators demonstrated that patients with blood group B were found to be superior in the risk of developing periodontitis [3], [10], [13]. Also, Mortazavi et al., [26] found that periodontitis did not show any relationship with blood groups despite the most frequent blood group had periodontitis was O.

Some studies [27], [28] showed that O and B were the most frequent blood groups although blood group A and AB were detected at a lower incidence. Dental health providers in screening and prevention programs should consider this finding in addition to our results.

The Rh factor distributions in the present study exhibited a significantly greater percentage of Rh-positive than the Rh-negative factor. Similar conclusions were reported by numerous authors [3], [9], [13], [15]. This may be related to the difference in substitutes of cell membrane proteins, which is detected by a series of allelic genes at a single locus [15].

In conclusion, from the data of this study, it can be concluded that there was a clear relation between blood group ABO and Rh group with GCP and its severity. Genetic influences such as blood group antigens may act as a risk factor that affects the development and severity of the chronic periodontitis. Therefore, the information of the ABO blood group of patients in the periodontal clinics may be given an advantage in developing early treatment strategies in highly susceptible individuals. Because there is a conflict among results of studies, large, diverse population samples from different geographic regions with more extended investigations are recommended to create a more comprehensive evaluation of the influence of ABO blood group on the health of Periodontium.

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Assessments of Bone Height Loss in Telescopic Mandibular Implant-Retained Overdentures Retained by Two and Four End - Osseous Implants: A Randomized Clinical Trial

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Keywords: Bone loss; Bone height; Telescopic attachments; Over-dentures; Cad/cam

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Abstract

AIM: This randomized clinical study aimed to assess bone height loss when using mandibular implant overdentures retained by two and four endosseous implants using the telescopic attachments.

METHODS: Twelve completely edentulous patients were randomly allocated so that six patients were treated by telescopic implant overdenture retained by two implants (group A) and six patients were treated with overdentures retained by four implants (group B). Digital radiographic evaluation of bone height using Digora was made starting at the functional loading day followed by time intervals of 12 months.

RESULTS: No implant loss during the healing period or after functional loading. Radiographic evaluation revealed a statistically significant difference was found between (Group A) and (Group B) were ($p < 0.001$).

CONCLUSION: For bone loss, widely distributed four intraforaminal implants revealed more bone preservation than only two implants when using the telescopic attachments to support and retain an over dentures taking in consideration the type of the attachment will be used.

Introduction

Complete dentures wearer usually exhibit problems with their mandibular dentures due to lack of stability and retention as a result of the smaller denture bearing areas which affects the chewing ability [1]. The utilisation of dental implants, as a means for improving retention, has become a common and effective procedure in the last decades. Various authors have presented data on overdentures supported by 1–8 implants. Several attempts were made in recent years to find an answer to the question of how many implants should be used to retain/support an overdenture [2]. The advent of osseointegrated dental implants has offered additional treatment options for completely and partially edentulous patients. Implant prostheses can be classified as fixed or removable and as full-arch or partial-arch, similar to the principles of conventional prosthodontics [3]. The implant-supported prosthesis usually utilizes four or more implants to totally support an overdenture, obtaining its support totally from implants where the mucosa does not share in any load [4], While implant -tissue supported prosthesis depends on sharing the load between implants used and the mucosa of the distal extension part, utilizing fewer number of implants usually, two-interforaminal implants [3], [4]. Removable implant-retained restorations might be considered a better treatment option to fixed in patients with excessive ridge resorption which has led to the loss of facial support of the lips and soft tissues of the face as a result of severe residual ridge resorption and when inadequate accessibility to maintain good oral hygiene [5]. There are different attachment systems can be used, the most used connection systems between implants and overdentures are bars, balls with metal clips, locators, magnets, and telescopes depending in their selections on the anatomic, clinical situation, amount of retention needed, cost, implants position and parallelism [6]. Since 1989 non-rigid telescopic attachment have been

used to support a removable overdenture for the treatment of completely edentulous patients [7]. So, it has been over years of good clinical experience.

This study was conducted to evaluate the radiographic changes in bone height when using the telescopic attachment on two or four end-osseous implants supporting and retaining a mandibular over-denture.

Methods

Twelve patients with the eligibility criteria were recruited in the study from the Outpatient Clinic of the Prosthodontics Department, Faculty of Oral and Dental Medicine, Cairo University. The participants received oral and written information about the study and written informed consent was obtained before their recruitment. They were selected with completely edentulous maxillary and mandibular arches with class I maxilla-mandibular relationship, sufficient restorative space not less than 15 mm and adequate buccolingual width of keratinised mucosa equal to or greater than 5mm over the crest of the lower ridge. All Patient were selected with the good physical and psychological condition to tolerate conventional implant surgical protocol. A panoramic radiograph was taken for each patient to assess bone height and location of the nearby vital structure in the areas planned to receive implants. Complete maxillary and mandibular dentures were constructed with proper tissue fitness and ideal teeth setting to allow for prosthetic driven implant placement. When the patient already had a denture, it was checked for proper extension, mucosal fitness, esthetic and occlusion and then it was used to prepare the scan prosthesis. A preoperative CBCT scan was taken for the patient‘s mandibular arch with the scan appliance with PLANMECA Pro max 3D mid CBCT machine. After CBCT scan, the DICOM images were then imported in Blue Bio sky software (Blue sky Bio, LLC. planning software). For the patients receiving two implants the virtual planning was done to the area at the two canines, but for the patients receiving 4 implants, planning was made in the inter- foraminal area. Virtual implant models 3.5 × 10 mm and 3.5 × 11.5 mm were used for posterior and anterior implants respectively. Implants were placed inadequate bone locations guided by the radiolucent channels in the scan appliance at the place of canines and second premolars. The implants were planned parallel to each other as possible putting in consideration the nearby vital structure.

The surgical stent was inserted in the patient’s mouth; then bleeding points were made through the stent by using the periodontal probe opposing the proposed implants sites. The mid crestal incision was made slightly behind the location of implant placement with buccal realising incisions for easier releasing of the flap without laceration. Complete flap retraction was made by using mucoperiosteal elevator. Root form tapered implants (Neo Biotech Co. Ltd, Seoul, Korea) were placed using the submerged two-stage technique.

After the healing period of 3 months, the surgical stents were used again to relocate the position of the inserted implants. Infiltration anaesthesia was given to the patient, and crestal incision was made opposing to the site of each implant, healing abutments were screwed with collar height 5 mm to allow for proper gingival healing around the implants prior making the impression.

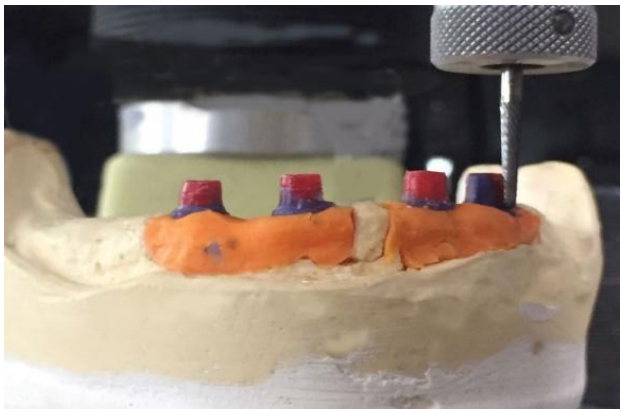

Figure 1: Milling surveyor

The impression was made with an open tray splinted implant level impression technique was used. Then, telescopic attachments were fabricated through the use of UCLA Ti-based plastic abutments (New Biotech ISUCH400, Korea) to fabricate the primary screw-retained abutments, adjusted the taper by the use of the milling surveyor (Figure 1), which was transferred from their laboratory position to the oral cavity through the use of an abutment jig (Figure 2).

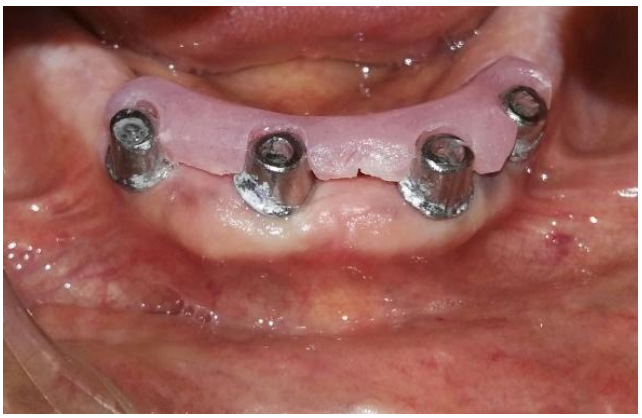

Figure 2: Acrylic jig

For the fabrication of the secondary coping, finished primary coping was scanned to design and fabricate the wax pattern of the secondary coping using the CAD/CAM (Shera Echo-scan 7 Dental

Wings Inc.2251,AvLetourneux Montreal H1V2N9 Canada), secondary coping was designed having two projected wings parallel to the ridge and properly fitted to the primary coping.

The casting of the resulted wax pattern was made, after finishing and polishing, secondary coping was checked on to the primary coping, together were placed on the cast and scanned to design and fabricate the framework using CAD/CAM) (Figure 3). Jaw relation registration was made, try in and then denture insertion and delivery to the patient with final occlusion adjustment.

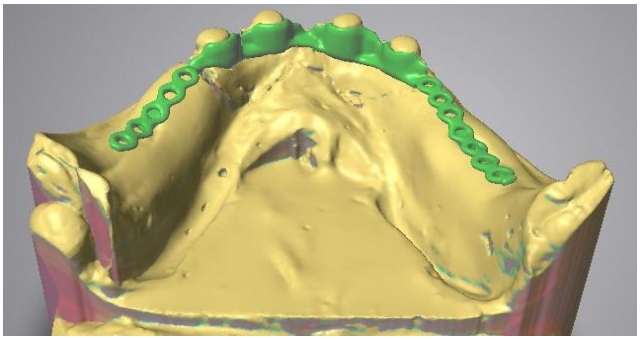

Figure: 3 Cad/cam designing of Framework

Radiographic assessment for the bone loss during the follow-up period:

Bone height was measured using Digora digital radiograph (Digora Computerized system, Helsinki, Finland) radiographs were taken at the following intervals at the day of loading of the final prosthesis and after twelve months of delivery.

The long cone parallel technique was used for making reproducible and standardised images during the follow-ups. At the time of the exposure, the lower denture was removed to allow for proper film alignment in front of the target abutments. Rubber base index (Figure 4) was made to allow for film stabilisation against the upper denture to stabilise the film during exposure. Then, the film was removed from film holder and placed inside Digora scanner opening. The images for each patient were saved in separate files with the patient's name until the end of the follow-up periods for interpretation.

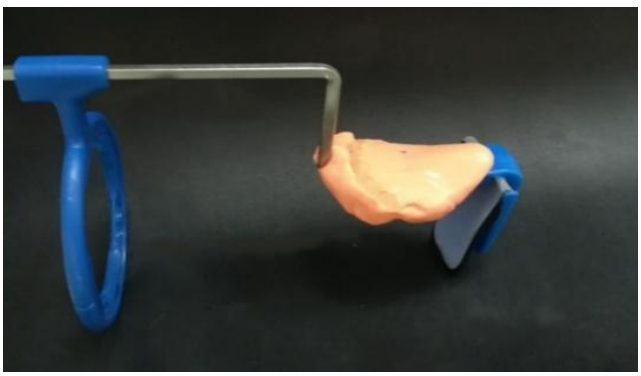

Figure 4: Putty Index for

Measurement of the amount of bone loss

The digital images saved were analysed to detect the amount of bone loss mesial and distal to the implant. To obtain actual images, the calibration option was used to detect the actual length of the implant by comparing the screen length of the implant to the known actual implant length. The reference point was taken at the junction between the implant platform and the abutment base, from which the loss of bone will be calculated by measuring the distance from the reference point to the first implant-bone contact. For each implant, bone height was measured at the mesial and distal surfaces in mm (Figure 5).

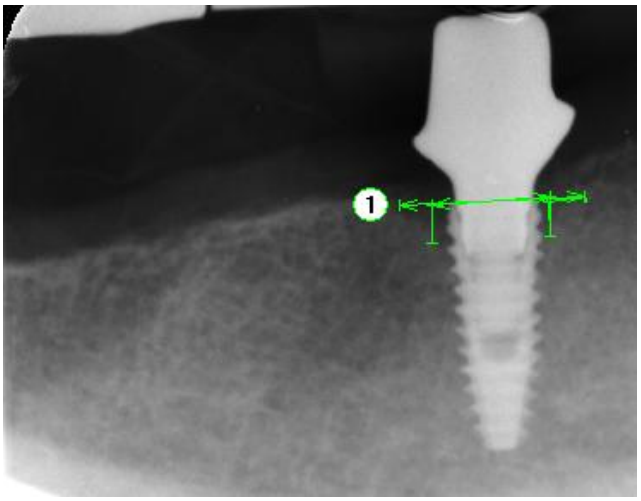

Figure 5: Digora measurements

Results

The mean and standard deviation values were calculated for each group in each test. Data were explored for normality using Kolmogorov-Smirnov and Shapiro-Wilk tests; data showed non-parametric (not-normal) distribution. Wilcoxon was used to comparing between two groups in related samples. Mann Whitney test was used to compare between two groups in non-related samples. The significance level was set at $P \leq 0.05$. Statistical analysis was performed with IBM® SPSS® Statistics Version 20 for Windows.

Table 1: The mean, standard deviation (SD) of bone height loss in different groups

Variables	Bone height loss				p-value
	At loading		After year		
	Mean	SD	Mean	SD	
Group A (2implants)	0.29	0.12	1.9	0.09	< 0.001*
Group B (4implants)	0.44	0.31	1.61	0.23	< 0.001*

*; significant ($p < 0.05$) ns; non-significant ($p > 0.05$).

Bone height loss results: Effect of time in each group as shown in Table 1 and Figure 6.

a) Group A (2 implants): A statistically significant difference was found between (At loading) and (After year) where ($p < 0.001$).

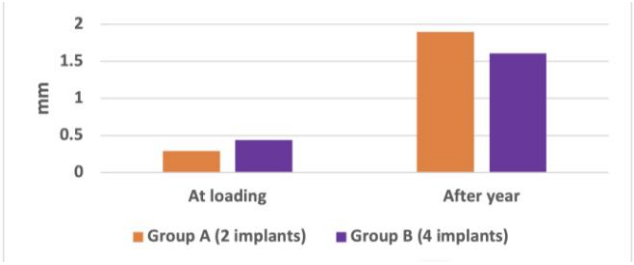

Figure 6: Bar chart representing a bone loss for each group

b) Group B (4 implants): A statistically significant difference was found between (At loading) and (After year) where ($p < 0.001$). Effect of time between groups: As shown in Table 2, and Figure 7.

Table 2: The mean, standard deviation (SD) of bone loss difference in different groups

Variables	Bone loss difference	
	Mean	SD
Group A (2implants)	1.51	0.23
Group B (4implants)	1.08	0.37
p-value	< 0.001*	

*; significant ($p < 0.05$) ns; non-significant ($p > 0.05$) .

At loading: No statistically significant difference was found between (Group A) and (Group B) where ($p = 0.051$). After a year: A statistically significant difference was found between (Group A) and (Group B) were ($p < 0.001$).

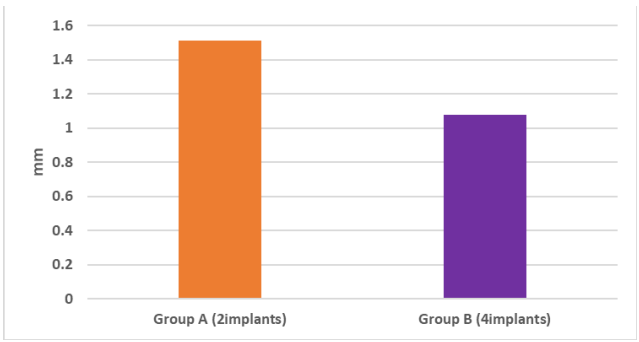

Figure 7: Bar chart representing bone loss difference for different groups

Discussion

Bone height measurement revealed a significant difference between the two groups where Group A showed a statistically significantly higher mean amount of bone loss than Group B.

Data was not much available due to the low number of studies comparing a different number of implants with telescopic attachments. There was a monthaversary within the studies addressing this topic; where some studies showed a non-significant difference between two and four implants, others showed a significant difference in the bone loss; where bone loss was more with the use of four implants. Other studies concluded that there is a need for more research for the long term effect of the use of different implants number. Although the results of this study were against the results of the following studies found in literature, those studies differed in study design, sample size, and also may be attributed to the different type of attachment used, where the studies comparing the bone loss between two and four implants were not addressing the telescopic attachments, where telescopic attachment should be fabricated with sufficient height to achieve the needed frictional retention; where this vertical height leads to more lateral forces than other attachments.

The height of the telescopic attachments in implant overdentures had a marked effect on the lateral force on implants and denture displacement. To protect implants supporting an overdenture and to prevent bone resorption, the height of the attachment should be carefully considered [8]. Wismeijer et al., 1997 evaluated over 100 patients with overdentures supported by two implants and four implants, no significant differences were found between the two modalities in 16 months; concluding the sufficiency of two implants to support an over-denture [9]. However; these results were against most of the literature which stated that there is a non-significant difference among two and four implants; this may be attributed to the differences in the attachment used and loading of the prosthesis.

Batenburg et al., 1998 studied 60 mandibular implant overdenture patients who were divided into 2 groups, one group treated with two endosteal implants and the other with four endosteal implants. They found no significant differences about peri-implant health. The authors suggested that additional study is necessary [10] in 2005; Visser presented 5-year results of a previous study made by Batenburget al., 1998, There was no difference in the clinical and radiographical state of patients treated with an Over-dentures on two or four implants [11].

Meijer et al., in 2009 reported a 10-year data of the previously published paper of Batenburg et al., (1998) concluding that there was no statistically significant difference between patients treated with a two or four implant mandibular Over-denture retained by bars concerning radiographic bone loss and prosthetic aftercare. For reasons of cost-effectiveness, a two-implant Over-dentures was advised [12].

Patients with two implants showed less marginal bone loss than those with four implants, suggesting that two implants seem to be preferable for mandibular implant-supported OD. The different

results of this study to be attributed to the loading and type of supra-structure that may influence the marginal bone loss, and that the more implants a patient has, the higher the probability of obtaining an implant with peri-implantitis affecting the bone level [13].

A randomized clinical trial, using a crossover design, by Burns et al., 2011, Thirty subjects received four implants in the anterior mandible, using three different Over-dentures attachment types were fabricated and/or fitted to the implants: 4-implant bar attachment, 2 implant bar attachment, and two ball attachments, the one-year data revealed that the risk of implant loss does not vary substantially by the number of implants [14].

In 2012, Roccuzzo et al., did a systematic review to assess the optimal number of implants for removable reconstructions. For the mandible, it cannot be concluded that bone loss, patient satisfaction, or a number of complications is significantly related to the number of implants supporting the overdenture. The author concluded that there is a need for a well-conducted research is needed to identify the prognostic factors for long-term success [15].

In conclusion, for bone loss, widely distributed four intraforaminal implants revealed more bone preservation than only two implants when using the telescopic attachments to support and retain an over dentures taking in consideration the type of the attachment will be used.

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Dental Science

Finite Element Study On Arthroscopic Anchor Design Aspects

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Keywords: Design; Finite element analysis; Endoscopy; Arthroscopic anchors; Suture eyelet; Internal drive mechanism

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Abstract

AIM: This research aims to study arthroscopic anchors design parameters. Prototypes were manufactured by new parameters values. The the performance of the prototypes was also tested.

METHODS: Five 3D arthroscopic anchor models were created to evaluate the role of some design aspects. Thread type, pitch and tip angle were tested as variable parameters. These models were produced on engineering CAD software then imported into ANSYS for finite element analysis. A tensile load of 300 N was applied to each model while the simplified bone base was fixed-in-place as a boundary condition. The finite element results were compared with prototypes tensile testing.

RESULTS: The finite element analyses showed stresses within physiological limits on the bone with all tested models. Thread type and pitch affected stresses on bone and anchor body. From stress point of view, two critical zones appeared on anchor body, anchor cortical bone connection and eyelet zone, while thread geometry (depth) affect the cortical bone response only. Laboratory tests matched finite element results and literature.

CONCLUSION: Increasing thread pitch of arthroscopic anchors decreases stress on the bone, while increases stress on anchor body. Arthroscopic anchors thread type has a negligible effect on bone, while it reduces stresses on anchor body if it placed more material around eyelet in internal drive mechanism and suture eyelet type of anchors. Anchor tip angle has a negligible effect on bone and anchor body.

Introduction

Suture Anchors are very useful fixation devices for fixing tendons and ligaments to bone. They are made up of: (1) the Anchor-which is inserted into the bone. This may be a screw mechanism or interference fit (like a raw bolt used in DIY) [1]. They may be made of metal or biodegradable material (which dissolves in the body over time) [2], [3]. The Eyelet-is a hole or a loop in the anchor to through which the suture passes. This links the anchor to the suture. (2) The Suture-is attached to the anchor by through the eyelet of the anchor. It also may be a non-absorbable material or a biodegradable material.

Suture Anchor is mostly self-tapping titanium implant that comes pre-loaded with HiFi high-strength sutures. It allows for more fixation points providing the ability to better distribute the load more evenly across the tendon. Also, it allows for versatile suture placement [4], [5.]

Arthroscopic Anchors' designs have a punch of parameters starting from diameter, length, angles (taper, cutting, ...etc.), ...etc. [6] where rare literature are seeking for the optimal design(s) for specific cases. Most of these researches' results are protected by patents [7], [8], [9], [10].

The modern kits of arthroscopic implants are single use, that it contains (1) hollow plastic handle (polyethene) with one internally threaded end, (2) Titanium tube (threaded end at the handle, and outer hexagon end), (3) one or two HiFi fibres for knitting between anchors each of one-meter length, (4) Arthroscopic anchor. Assembling the plastic handle and Titanium tube by thread resulted in anchor driver [11].

Arthroscopic anchors can be made of varied

materials, including stainless steel, pure titanium, titanium alloys and biocomposite materials. The three grades listed in standard specifications are austenitic types with specific compositions for these special applications. These materials are tested for biocompatibility and safety according to EN ISO 10993 and EN ISO 14971. The Chromium-Nickel-Molybdenum alloyed austenitic stainless steel used for BIOTEK implants complies with the international standards ISO 5832-1 and ASTM F138/ASTM F139. That production of such tools requires high-precision equipment including high-performance CNC machines, electropolishing facility, laser part identification, ultrasonic cleaning and passivation and state of the art inspection laboratories [12], [13], [14], [15], [16].

Recent studies reported PEEK (polyetheretherketone) as an alternative material to titanium implants. PEEK is biocompatible material with Young's modulus of 3.6 GPa. Additionally, the PEEK modulus of elasticity can be modified by reinforcing it with carbon fibres "CFR-PEEK (carbon fibre reinforced polyetheretherketone)" to reach 18 GPa, similar to that of cortical bone [2], [3], [11].

In this study, three major Suture Anchor parameter designs were investigated as; Thread type, pitch and tip angle via finite element analysis — laboratory testing for the prototypes to validate the theoretical study results against the in-vitro ones.

Material and Methods

Five 3D geometric models were prepared by "Autodesk Inventor" ver. 8.0 (Autodesk Inc. San Rafael, CA, USA) to investigate the three design parameters as:

a) Thread type (Models 1 and 3);

b) Pitch (Models 2 to 4);

c) Tip angle (Models 3 and 5).

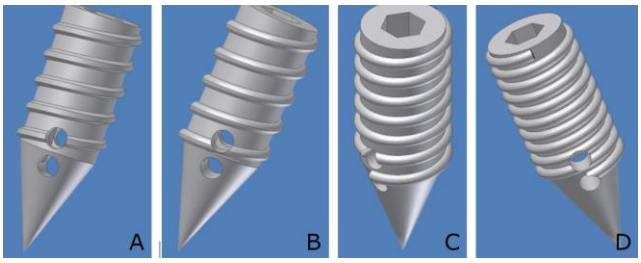

Figure 1: Sample of anchors with major modifications; a) Model 1, square thread type; b) Model 2, enlarged pitch; c) Model 3, regular thread pitch; d) Model 4, narrow pitch

The anchors 3D models were transferred to ANSYS Workbench Version 14 (ANSYS Inc., Canonsburg, PA, USA) as STEP files to be analysed. Where bone geometry was simplified and simulated as two co-axial cylinders. The inner one represents the spongy bone (diameter 14 mm & height 20 mm) which fills the internal space of the outer cylinder (shell of 2 mm thickness) that represents cortical bone (diameter 18 mm & height 24 mm). These models after assembly were subjected to 300 N [17], [18] tensile force located at eyelet (fibres resting). The base of the hollow cylinder representing the cortical bone was set to be fixed as a boundary condition. Linear static analysis was performed on a personal computer Intel Pentium Core 2 Duo, processor 3.0 GHz, 4.0 GB RAM. Figures 2, illustrate ANSYS screenshots show a sample of the analysis's models and meshed components before analysis.

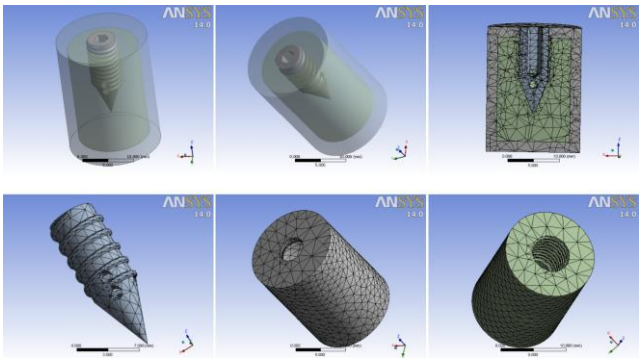

Figure 2: Sample of the analysed models and meshed parts from ANSYS workbench screen

Results

Comparing different thread types in models 1 and 3 showed the moderate effect on anchor body itself, which was not referring to the design rather than increasing material around the eyelet. Sharp-edged threads reduced stress on bone by more than 75% than blunt edged threads. Figures 3 and 4 illustrate Von Mises stress distributions in models 1 and 3 respectively.

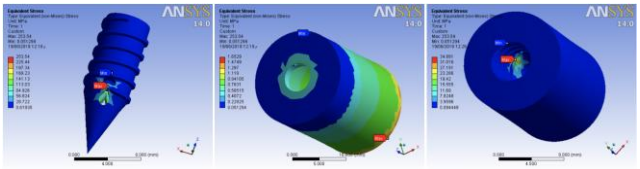

Figure 3: Model 1 (square thread design) Von Mises stress distributions

As presented in Figure 5, increasing pitch in model 4 showed negative effects on the anchor body itself by reducing material around the eyelet. On the other hand, increasing pitch reduces stresses dramatically on cortical bone by about 25%. Therefore, increased anchor pitch is very important for reducing bone stresses, which was verified by the results of model 2.

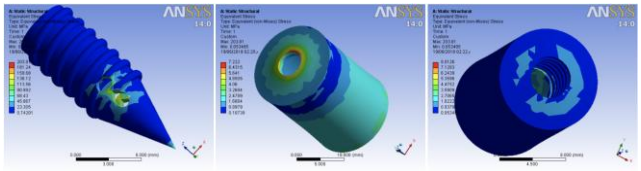

Figure 4: Model 3 (regular pitch design) Von Mises stress distributions

Changing the anchor tip angle as in model 5, and compare its results with model 3 results there will be no change in all values of stresses and deformation.

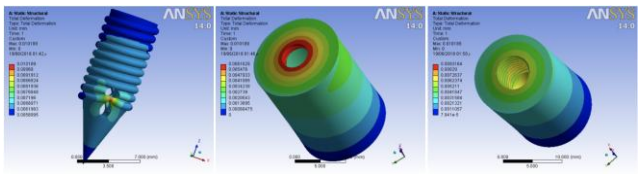

Figure 5: Results of model 4 (narrow pitch design) total deformation

Finally, set of 20 anchors (as model 3) were placed in transparent acrylic resin cube (dental laboratory prepared it) to make a trial for a tensile test of one Titanium anchor design as presented in Figure 6. Unfortunately, the HiFi and stainless-steel wires of 0.5 mm were cut inside the anchor at a tensile load lower than 280 N during a tensile test, and no failure was noticed on the anchor's body.

Figure 6: Test samples and one sample during testing on Universal Testing machine

Discussion

The internal drive mechanism and suture eyelet anchors performance are affected by; eyelet design, thread design, and material (metal, absorbable), the angle of suture pull, and insertion depth [5]. Failure can occur at the level of the suture, suture anchor, bone, and soft tissue. Anchors to be designed for suture pull along the axis of insertion, while the eyelet to be designed rounded or streamlined with channels that protect the suture [19], [20].

Reducing thread pitch decreases stresses dramatically on cortical bone. Therefore reducing implant pitch is very important for bone purchase. Reducing pitch also showed an improving effect on the implant itself by increasing material around the eyelet. On the other hand, a screw with a very small pitch may have a very high bearing area, but will not perform well because the threads are too close together to effectively engage the trabeculae [21]. Yakacki et al., [21]. The deeply inserted threads likely increased the pullout force past the predicted range based on smaller nominal insertion depth. The Bio-Corkscrew (Arthrex, Naples, Florida, USA) strength was consistently higher than the Opus Magnum, but this was simply due to the larger device size (5 vs 3 mm) and larger corresponding bearing area [21].

Thread type showed a moderate effect of implant body itself, which was not referring to the design rather than increasing material around the eyelet. Maximum Von Mises stress was recorded of order 250 MPa while the minimum was of order 190 MPa.

A screw of equal proportion but greater size will possess a higher strength than its smaller counterpart, comparing screws with different thread designs and sizes are difficult because of the different bearing area than the regular version [21]. The anchor has a short body with deep threads that secure it into the bone allowing decent holding strength. Finally, anchor tip angle has a negligible effect on anchor body, cortical and spongy bone.

According to in vitro tests, all sutures were failed at around 280N. That matches previous studies by Aktay et al., and Er et al., [5], [22], that find it of order 300 N. A common area of failure with metallic anchors is at the suture–anchor interface where the suture is serially abraded by the anchor's eyelet [19]. The eyelet design along with surface roughness and the arc of contact between the eyelet and suture all contribute to the frictional resistance created. A greater amount of friction leads to a lower maximal breaking strength of the suture. The failures occurred in most instances by rupture of the suture material. For the metal anchors, the threads almost always ruptured at the eyelets of the anchors [23].

In conclusion, titanium arthroscopic anchors design parameters investigations resulted in: 1) Increasing pitch increase stresses on implant itself, while decrease stresses on bone; 2) Thread type has a negligible effect on bone, while it may reduce stresses on implant body if it placed more material around eyelet; 3) Implant tip angle has a negligible effect on bone and implant body.

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Assessment of Osteoimmunological Changes Following Orthognathic Surgery

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Keywords: Orthognathic surgery; Immunosuppression; Stress response

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Abstract

BACKGROUND: There is a scarcity in the published literature which evaluates the postoperative inflammatory response and patients' immunity following orthognathic surgery.

AIM: The present prospective study aimed to evaluate the changes in two immunological callipers to measure the traumatic effect of orthognathic surgery

METHODS: In the present prospective cohort study, we included women (age range 16-30 years) with severe dentofacial deformities who were scheduled for bimaxillary osteotomy. Blood samples were collected for measurement of transforming growth factor beta one (TGF-β1) and osteoprotegrin (OPG) levels. The statistical analysis was carried with SPSS software.

RESULTS: In the present study, nine patients with severe dentofacial deformity were operated successfully under general anaesthesia. All patients reported decreased energy and fatigue in the early days after surgery and had difficulties with nutrition due to pain, oedema and paresthesia; however, no massive weight loss was reported. The levels of OPG started to increase immediately postoperatively (mean = 0.46 ± 0.08; p = 0.001). A significant increase in the concentration of OPG begun postoperatively and continued to rise significantly until the six weeks to reach 2.24 ± 0.30 ng/mL (p < 0.001). Similarly, the concentration of TGF-β1 increased at three days postoperatively and continued to rise until the six weeks to reach 1.28 ± 0.19 ng/mL (p <0 .001).

CONCLUSION: In conclusion, orthognathic surgery is associated with a significant rise in the pro-inflammatory cytokines until the six weeks postoperatively. These observed results may indicate a significant alteration in the immunity of the patients to undergoing orthognathic surgery.

Introduction

Orthognathic surgery is a common surgical procedure that aims to restore the normal anatomical and functional position in patients with severe dentofacial deformities [1]. It is usually indicated in the case of occlusal malfunction, improper aesthetic facial appearance, and temporomandibular joint dysfunction [2]. According to recent figures from England and Wales, more than 2600 orthognathic surgical procedures are performed annually [3]; a similar rate was reported from the United States (US) as well [4]. As patients with severe dentofacial deformities are more likely to suffer from poor psychological status related to their facial appearance, orthognathic surgery was reported to have a positive impact on patients' psychology and well-being [5]. However, the procedure is associated with some intra and postoperative complications such as severe haemorrhage, facial oedema, pain, and neurological injuries [6]. Intraoperative blood loss is inevitable as well which, in severe cases, may lead to a systemic inflammatory response with subsequent infection and end-organ failure due to lowered immunity [7].

On the other hand, the surgical stress response is a well-established consequence of surgical or accidental trauma; it is defined as the endocrine/metabolic changes resulted from injury-induced activation of hypothalamic-pituitary axis and

eventually led to the release of hormones such as the stress hormone, cortisol [8]. The response has significant associations with a wide range of postoperative complications, surgical stress response was reported to increase body demands and affect immune competence [9]; patients with surgical stress showed significant increase in the release of a number of key cytokines (prostaglandin (PG) E2 and transforming growth factor TGFβ) and suppression in the cellular immunity components until two weeks postoperatively [10], [12]. Thus, patients who undergo elective surgery are at increased risk of selective immunosuppressive effects during surgical stress and high rate of septic complications [13].

Few studies have evaluated the role of surgical stress in postoperative outcomes following oral and maxillofacial surgery; the level of IL-6 was reported to decrease following orthognathic surgery [7] markedly. To date, there is a lack in the published literature which evaluates the postoperative inflammatory response and patients' immunity following orthognathic surgery. Therefore, the present prospective study aimed to evaluate the changes in two immunological callipers to measure the traumatic effect of orthognathic surgery.

Material and Methods

Ethical approval

The study was conducted by the International and local ethical standards; the study was approved by the institutional review board of the Faculty of Oral and Dental Medicine, Cairo University, Egypt. Informed consent was obtained from all patients.

Sample size calculation

Prior data indicated that the difference in the response of matched pairs is normally distributed with standard deviation of 3661(pg/ml). If the true difference in the mean response of matched pairs is 7225 (pg/ml), 5 pairs of subjects at least needed to be studied to reject the null hypothesis that this response difference is zero with probability (power) 0.9. The Type I error probability associated with this test of this null hypothesis is 0.05.

Study design and Patients

The present study was a prospective cohort study; patients were randomly selected from those who were scheduled for bimaxillary osteotomy to correct severe dentofacial deformities. The deformities ranged between skeletal class III, vertical maxillary excess and mandibular retrognathia. Patients who had a history of previous orthognathic surgery, under steroids therapy, or suffered from bone disease were excluded from the study; smokers were excluded as well. The age ranges from 16 to 30 years old.

Preoperatively, patients underwent detailed clinical examinations and. Preoperative orthodontic treatment. Surgical procedures were performed at the Dental Educational Hospital, Cairo University. Mandibular and maxillary incisions were done through mucosa, muscles and periosteum using diathermy knife. Osteotomies were performed by surgical burs (Lindeman, fissure) and a reciprocating saw (Figure 1).

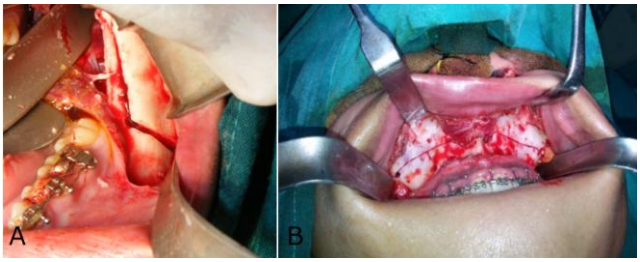

Figure 1: Bilateral sagittal split and Lefort 1 osteotomies; A) Osteotomy cuts through the cortical side on the medial side of the ramus and the lateral cortical plate; B) Osteotomy is done using a reciprocating saw through the buttress and lateral maxillary wall to the piriform rim followed by sectioning the lateral nasal wall by spatula osteotome

Postoperatively, cold compress, in the form of ice packs, were applied for 20 minutes every hour for 12 hours, fluid intake was carefully monitored, and antibiotics, analgesics, and intermediate-acting glucocorticoids were given for three days (IM 8 mg/2 ml of hydrocortisone was given intra-operatively and continued in the first operative day every 8hours, followed by half the dose in the second postoperative day followed by Methyl Prednisolone I.M. 40 mg/vial given once in the third day after surgery).

The patients spent an overnight hospital stay and discharged on the second day. Panorama, lateral cephalogram and posteroanterior views were taken one week postoperatively, the position of the jaw segments and condyle position were compared to pre-surgery films. Blenderized foods and high-calorie liquid dietary was consumed to prevent catabolism commonly associated with surgery.

Patients were instructed to maintain oral hygiene and returned for follow up visits during the first week, and then every two weeks. The pain was assessed using the visual analogue scale (VAS), swelling and paresthesia were evaluated subjectively by the patients marking yes or no. The patients returned to the orthodontist after two to three months postoperatively. Photographs were taken after three months.

Measurements of Osteoprotegerin (OPG) and Transforming growth factor beta one (TGF- β1)

Blood samples were collected preoperatively, immediately after the procedure, three days later, after one week, and then every two weeks till the six weeks postoperatively. Blood samples were allowed to clot for two hours at room temperature or overnight at 4°C in a serum separator tube. Centrifugation was done for 15 minutes at 1000 × g, and the aliquot was removed immediately and stored at -80°C. TGF- β1 level was measured by ELISA kit for TGF-β1 (catalogue no: ET3102-1), and OPG level was measured by Human ELISA Kit for OPG (catalogue noCSB-E04692h).

ELISA kit for TGF-β1

The Assay Max Human TGF-β1 ELISA kit was designed for the detection of TGF-β1 in cell culture supernatants. The assay employed a quantitative sandwich enzyme immunoassay technique that measures TGF-β1 in less than 5 hours. A murine monoclonal antibody specific for human TGF-β1 has been pre-coated onto a microplate. Samples were sandwiched by the immobilised antibody and a biotinylated polyclonal antibody specific for human TGF-β1, which were recognised by a streptavidin-peroxidase conjugate. All unbound material was then washed away, and a peroxidase enzyme substrate is added. The colour development is stopped, and the intensity of the colour was measured.

Human Osteoprotegerin (OPG) ELISA Kit

The assay employed the quantitative sandwich enzyme immunoassay technique. Antibody specific for OPG has been pre-coated onto a microplate. Samples were pipetted into the wells and OPG were bound by the immobilised antibody. After removing any unbound substances, a biotin-conjugated antibody specific for OPG was added to the wells. After washing, avidin conjugated Horseradish Peroxidase (HRP) was added to the wells. Following a wash to remove any unbound avidin-enzyme reagent, a substrate solution was added to the wells and colour develops in proportion to the amount of OPG bound in the initial step. The colour development was stopped, and the intensity of the colour was measured.

Statistical analysis

The statistical analysis was carried with SPSS software (Statistical Package for the Social Sciences, version 24, SSPS Inc, Chicago, IL, USA). Frequency tables with percentages were used for categorical variables, and descriptive statistics (mean and standard deviation) were used for numerical variables. The Kolmogorov-Smirnov test was used to verify the normality of distribution Quantitative data were described using range (minimum and maximum), mean, standard deviation and median. The significance of the obtained results was judged at the 5% level. ANOVA repeated measures test was used for normally distributed quantitative variables, to compare between more than two periods or stages. Post Hoc test (Bonferroni adjusted) was used to compare the values of the post-operatives to the pre-operative one and the immediate to the sixth week postoperative.

Results

In the present study, ten patients with severe dentofacial deformity were operated successfully under general anaesthesia, the operative time ranged from four to six hours. The second patient was not committed to the blood test appointments and dropped off from the study leading to decrease the sample size to nine patients. The estimated blood loss was about 650 cc to 850 cc, and none of the patients had a blood transfusion or experienced haemorrhage. Patients had phases of mild postoperative pain that declined progressively. Patients were encouraged to resume their normal activities as early as possible; they were discharged from the hospital on the second post operative day.

All patients reported decreased energy and fatigue in the early days after surgery and had difficulties with nutrition due to pain, oedema and paresthesia; however, no massive weight loss was reported. High caloric liquid diet was advised in the early postoperative period followed by a soft diet. Patients were instructed for oral health care at home and checked through the out-patient department.

Changes detected in Osteoprotegerin (OPG)

The levels of OPG started to increase immediately postoperatively (mean = 0.46 ± 0.08; p = 0.001). A significant increase in the concentration of OPG began at 3 days postoperatively and continued to raise significantly till the sixth week to reach 2.24 ± 0.30 ng/mL (p < 0.001) (Table 1 and Figure 2).

Table 1: Comparison between the different periods according to OPG and TGF-b ng/ml (n = 9)

	Pre	Immediate	3 days	1 Week	2 Weeks	4 Weeks	6 Weeks
OPG ng/ml							
Min. – Max.	0.24 – 0.46	0.35 – 0.54	0.45 – 0.68	0.54 – 0.98	0.97 – 1.80	1.20 – 2.40	1.80 – 2.90
Mean ± SD.	0.35 ± 0.07	0.46 ± 0.08*	0.59 ± 0.08*	0.84 ± 0.17*	1.24 ± 0.27*	1.73 ± 0.34*	2.24 ± 0.30*
Median	0.35	0.49	0.62	0.95	1.23	1.70	2.30
TGF-b ng/ml							
Min. – Max.	0.03 – 0.06	0.04 – 0.08	0.07 – 0.40	0.40 – 0.68	0.50 – 0.91	0.78 – 1.30	0.90 – 1.50
Mean ± SD.	0.04 ± 0.01	0.06 ± 0.01	0.21 ± 0.11	0.52 ± 0.09	0.77 ± 0.12	0.99 ± 0.16	1.28 ± 0.19
Median	0.04	0.05	0.20	0.51	0.80	0.93	1.30

*: Statistically significant at p ≤ 0.05. OPG, Osteoprotegerin; TGF-b, Transforming Growth Factor Beta.

Changes detected in Transforming Growth Factor Beta (TGF- β1)

There was an increase in the level of TGF-β1 level immediately post-operatively. However, the increase in TGF-β1 level was not statistically significant (mean = 0.06 ± 0.01 ng/mL; p = 0.135).

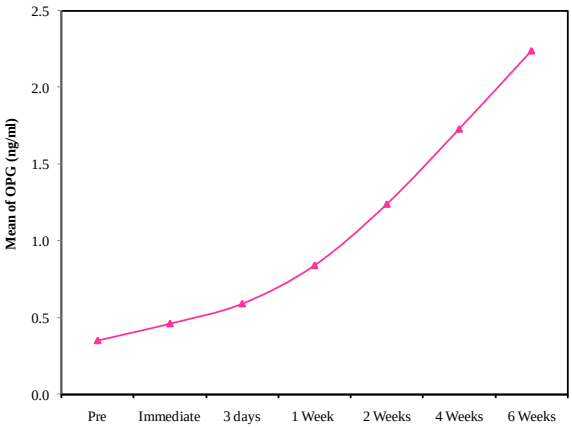

Figure 2: Comparison between the different periods according to OPG ng/ml

The concentration of TGF- β1 then significantly increased at three days postoperatively and continued to rise till the sixth week to reach 1.28 ± 0.19 ng/mL (p <0.001) (Table 1 and Figure 3).

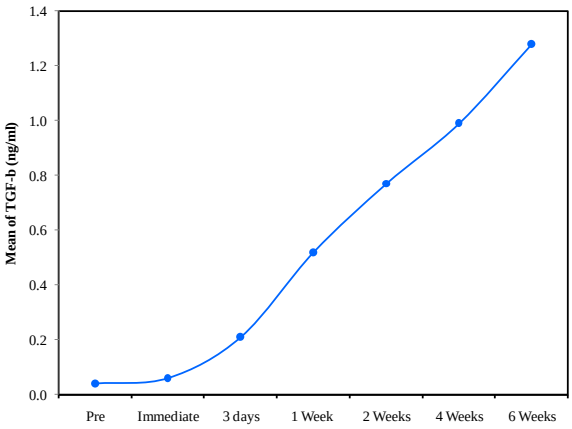

Figure 3: Comparison between the different periods according to TGF-b ng/ml

Discussion

The surgical stress response associated with orthognathic surgery may lead to significant immunosuppression and rise in the pro-inflammatory cytokines. Our results showed that both Osteoprotegerin (OPG) and Transforming Growth Factor Beta (TGF-β1) increased significantly postoperatively, the rise remained significant till the sixth week. These observed results may indicate a significant alteration in the immunity of the patients undergoing orthognathic surgery.

Orthognathic surgery is a type of elective surgery in which patients should be free from any associated co-morbidities, and the intra-operative complications are largely controlled [1]. Therefore, any changes in the postoperative immunological callipers can be attributed to surgery. In the present study, OPG and TGF-β1 levels were assessed as immunological callipers to measure the traumatic effect of orthognathic surgery. TGF-β1 plays an important role in suppressing the immune system and wound healing. It increases during fracture healing as it is known as a potent cytokine and growth factor that controls a wide range of cellular responses [14]. While OPG is an antiresorptive cytokine that controls bone homeostasis through regulation of osteoclasts formation and activities [15], it is also believed that OPG/RANKL/RANK system participates in the regulation of pro-inflammatory cytokines and immune response [16], [17]. Recently, a growing body of evidence showed a significant increase in OPG after major elective surgery [18], [19].

Our results showed that the levels of OPG and TGF-β1 steadily increased throughout the postoperative period. In concordance with our findings, Soliman and colleagues [20] reported a significant postoperative increase in OPG level among patients who underwent orthognathic surgery, the level of OPG remained high till the six weeks of follow-up. Similarly, Kunisada and colleagues [21] showed that OPG local serum levels significantly increased time-dependently after osteotomy ($P < 0.01$). Another report demonstrated an increase in TGF-β1 appeared at the later postoperative time and remained at higher levels compared with preoperative levels among patients who underwent orthognathic surgery [22].

The findings of these reports suggest the presence of significant inflammatory response following orthognathic surgery. The surgical stress response, which is known to be a spontaneous protective mechanism can be harmful and severe if prolonged during the perioperative settings [23]. Kasahara and colleagues [7] followed 46 patients for the occurrence of postoperative complications in patients who showed systemic inflammatory response syndrome (SIRS) following maxillary and mandibular orthognathic surgery, the incidence of SIRS was 50% and the rate of postoperative complications was significantly higher among the SIRS group compared to non-SIRS group (27.3% vs 0%; p < 0.01). On the one hand, the increased levels of the two immunological callipers prove and demonstrate the immunosuppressive effect of orthognathic surgery. OPG and TGF-β1 both are inhibitory cytokines as OPG inhibits the differentiation of osteoclast precursors and TGF-β1 inhibits the proliferation of T cells, the activation of macrophages and also inhibits the formation of osteoclast precursors. On the other hand, the inhibitory activity of both callipers induces

bone remodelling and tissue healing. The increased OPG expression following bone osteotomies induces bone healing by the increase in the OPG/RANKL and the inhibition of RANKL/RANK ratios.

The present study has some strength points. All of the included patients were females, unifying the gender should control for the hormonal, metabolic, and general condition influence in the immune system and body response. Moreover, we assessed the changes in the immune callipers following orthognathic surgery which is an elective surgery indicated for young, immunocompetent, and systemically free patients, so any change in the immune parameters are presumed to be a direct consequence for the surgery itself. However, we acknowledge that the present study has limitations. The sample size of the included studies was relatively small which may affect the generalizability of our findings. Also, the rate of postoperative complications was not systematically assessed.

In conclusion, orthognathic surgery is associated with a significant rise in the pro-inflammatory cytokines till the sixth week postoperatively. These observed results may indicate a significant alteration in the immunity of the patients undergoing orthognathic surgery. Alertness of the immunosuppressive effect which may occur because of the surgery should be highly taken into consideration and has the top priority to avoid it perioperatively.

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Factors Related to Breast Cancer Screening in Women in the Northern Part of Iran: A Cross-Sectional Study

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Abstract

BACKGROUND: Breast cancer is the most common type of cancer in women and affects many women around the world each year. Breast cancer screening is one of the best strategies that can be used to reduce the death rate from the disease. Different factors influence the breast cancer screening rate.

AIM: This study aimed to investigate the factors that affect the screening of women for breast cancer in the northern part of Iran.

MATERIAL AND METHODS: This cross-sectional study was conducted in the Mazandaran Province of Iran in 2016 on 1,165 women who participate in breast cancer screening programs, using a cluster-sampling method. A valid and reliable researcher-made questionnaire was used to collect the data. The collected data were analysed using descriptive and inferential statistics via SPSS 21.

RESULTS: In this study, 62% of the women had a history of breast self-examinations, 41.1% had breast examinations by healthcare staff, and 21.7% received mammography. The woman's age, age at first marriage, age at onset of sexual intercourse, occupation, spouse's occupation, household income, health status, history of infertility, smoking, and decision-maker on issues of sexual and reproductive health (SRH) were the best predictors of participation in screening for breast cancer ($P < 0.05$).

CONCLUSION: To encourage participation in breast cancer screening programs, women should be encouraged to seek preventive care. Also, factors that affect screening should be considered an appropriate educational method should be provided.

Introduction

Breast cancer is the most common type of cancer in women in developed and developing countries [1]. It affects 1.5 million women every year and has the highest death rate for all cancers in women [2]. According to the World Health Organization, 570,000 women died of breast cancer in 2015, which was equivalent to 15% of all cancer deaths in women (2 In Iran, the prevalence and death rate from breast cancer in 2014 was 9,795 and 14.2% respectively [3]. Globally, the incidence of breast cancer in developing countries is rising due to the increase in life expectancy, urban growth, and the acceptance of the Western lifestyle [4]. The goals of the Healthy People 2020 program by the US Office of Disease Prevention and Health Promotion include reducing the breast cancer death rate, reducing the number of people with late-stage cancer, and increasing the participation of women in breast cancer screening [5].

Breast cancer screening is the best strategy to reduce the death rate through early diagnosis, control, and treatment of the disease [1], [6], [7], [8], which improves patient survival and quality of life [4], [9]. Different methods, such as breast self-examination, physical examination by physicians or healthcare staffs, and mammography, are used to screen and diagnose breast cancer in early stages [2]. However, the detection of breast cancer in women in

developing countries, such as Iran, is often delayed until there is no hope of treating the disease [9]. The reasons for this delay include the lack of awareness of breast cancer screening methods, the lack of a widespread screening program in Iran, the lack of awareness of breast cancer symptoms at early stages, and the poor prognosis of the disease at the late stage. Promoting women's awareness of screening methods can play a major role in the early detection of breast cancer [4], [10].

Studies in various parts of Iran, such as Kerman [9], [11], Mazandaran [12], Gorgan [13], and Ilam [7], have shown that the breast cancer screening rate is not optimal. Different demographic, economic, social, and cultural factors, including age [1], [7], [13], [14], level of education [1], [7], [11], [14], status of occupation [7], [14], [15], and economic situation [11], can influence the participation of women in breast cancer screening programs.

Because of the high incidence of breast cancer in Iran and its occurrence in young Iranian women, an early diagnosis is important. Thus, this study aimed to investigate the participation rate of women in breast screening programs and its associated factors in women in the northern part of Iran.

Methods

This descriptive cross-sectional study was approved by the Research Council and Ethics Committee of the Mazandaran University of Medical Sciences, Sari, Iran. The study was conducted on 1,165 women in Mazandaran Province in 2016. The sample size was based on a study by Naghibi et al., in which 48.1% of women had a history of breast self-examination [1], and was calculated to be 1,200 women with a 95% confidence level and an error of 0.04 as follows: $n = [Z^2_{1-\alpha/2} * P(1-P)] / d^2 = 599*2$ (effect size) = 1198 $\cong$ 1200

Cluster sampling was used to organise the women into 60 clusters with 20 women in each cluster. The women had an equal chance to be selected from various urban and rural areas. The first household from each cluster was randomly selected based on a 10-digit postal code. The investigation began with the first household in each cluster and proceeded by moving from the right side. The remaining households were questioned by the investigation team until a sample population of 20 women was reached. Follow-ups were made if the eligible woman from the household in the sample was not present. If the researcher was not successful in reaching the woman after two visits, she was removed from the cluster and replaced by a woman from the next household. If more than one person in a household qualified for the study, the person whose birth date was closest to the date of the survey was included.

To collect the data about breast cancer screening and factors related to women's participation in breast cancer screening, in a deductive approach, an extensive literature review, opinions of 10 experts from various disciplines that included reproductive health PhD, sociologist, community nurse, gynecologists, midwives and surgeons (breast surgery fellowship) and 15 women referring to healthcare centers were used. A questionnaire with 33 questions (6 questions in breast cancer screening and 27 questions in related factors) was developed. Face and content validity and reliability of the instrument were assessed. The quantitative face validity of this questionnaire showed an impact source above 1.5 for all items. In the qualitative content validity process, 5 questions were revised and corrected. Also, 2 questions achieved a CVR less than 0.62, and 1 question had a CVI less than 0.79. The Cronbach's alpha and ICC of the questionnaire were 0.895 and 0.945, respectively. A dichotomous variable was used and a score of one was given for women who performed regular breast self-examinations, received breast examinations by a healthcare staffs, or were referred for mammography based on indications (every 1-2 years for women 40-74 years of age); a score of zero was given to women who don't participate in breast cancer screening. For the statistical analysis, descriptive and inferential statistics via SPSS version 21 (IBM Corp., Armonk, NY) were used. The absolute and relative frequency, mean and standard deviation (SD), tables and graphs, chi-square, analysis of variance (ANOVA), student's t-test, and multivariable logistic regression were used. The level of significance was set at $P < 0.05$.

Results

In this study, 1,165 women were investigated (35 questionnaires were not filled in completely). The mean age of the women was 37.15 ± 8.84 years. In terms of education, 519 women (44.5%) had not earned their high school diploma. In terms of breast examination, 722 women (62%) performed breast self-examinations, 479 (41.1%) received breast examinations by healthcare staffs, and 253 (21.7%) had received mammography. Of the women who performed breast self-examinations, 624 women (86.4%) had normal breasts, 88 women (12.2%) felt a mass, and 10 women (1.4%) observed nipple secretions. For women who received breast examinations by healthcare staffs, 421 women (87.9%) had normal breasts, and a mass was felt in 58 women (12.1%). The mammograms were normal in 204 women (80.6%), while 40 women (15.8%) had

a benign cystic mass, 8 (3.2%) had a benign solid mass, and 1 woman (0.4%) had calcification. The Student's t-test and chi-square test showed that women with a history of breast self-examination compared with women without a history of breast self-examination had a higher mean age at the onset of sexual intercourse (21.12 years and 20.23 years); a higher frequency of proper decision-maker on issues of SRH (63.8% and 56.2%); and a higher number of pregnancies (2.10 ± 1.16 and 1.93 ± 1.25). Women who received breast examinations by a healthcare staffs had a higher mean age (39.36 years), mean age of spouse (38.15 years), and frequency of infertility (49.6%) healthcare staffs than women who did not receive breast examinations by a healthcare staffs: 35.61 years, 36.52 years, and 40%, respectively. Women who had a history of mammography had a higher body mass index (BMI) (27.91), a mean number of pregnancies (2.36), and frequency of healthy health status (28.7%) than women without a history of mammography: 26.97, 1.95, and 18.9%, respectively.

The following variables were associated with a history of breast self-examination and had a P < 0.1 in the single-variable test and were entered into the multivariable logistic regression model: woman's age (P < 0.0001), age at onset of sexual intercourse (P < 0.001), age at first marriage (P < 0.008), occupation (P< 0 .004), health status (P < 0.062), household income (P < 0.030), the decision-maker on SRH (P < 0.021), number of pregnancies (P < 0.026) and having genital unclear or colored vaginal discharge (P < 0.010). Multivariable logistic regression showed that age, age at onset of sexual intercourse, occupation, and health status had significant statistical relationships with a history of breast self-examinations. The probability of performing breast self-examination increased 1.08 times for every year increase in age at the onset of sexual intercourse. The probability of a housekeeper performing breast self-examination was 32% less than the probability of employed women doing breast self-examination (Table 1).

Table 1: Multivariate logistic regression to determine the factors related to breast self-examination

Variable		Multivariate test		
		OR[1]	CI[2]	P-value
Age (year)		1.02	1.00-1.04	0.007*
Age (year) at onset of sexual intercourse		1.08	1.01-1.15	0.022*
Occupation	Housekeeper (unemployed)	0.68	0.46-0.99	0.04*
	Employed			
Health status	Healthy	0.71	0.54-093	0.015*
	Ill			
Ultimate decision-maker on issues of sexual and reproductive Health[3]	Desirable	1.14	0.85-1.52	0.36
	Undesirable			

*P< 0.05; [1]Odd's Ratio; [2]Confidence Interval; [3]The desirable decision-maker for issues of sexual and reproductive Health was the woman alone or with her husband; the undesirable decision-maker was the husband alone.

The following variables were associated variables with a history of receiving breast examinations by a healthcare staffs and had a P < 0.1 in the single-variable test and were entered into the multivariable logistic regression model: woman's age (P < 0.0001), husband's age (P < 0.009), BMI (P < 0.001), age at first marriage (P < 0.026), number of pregnancies (P < 0.0001), health status (P < 0.095), household income (P < 0.030), decision-maker for self-care (P < 0.022) and issues of SRH (P < 0.001), polygamy (P < 0.75), and a history of infertility (P < 0.030). According to the results of the model, age, age at first marriage, household income, decision-maker on issues related to SRH, and history of infertility had significant statistical relationships with a history of receiving breast examinations by healthcare staffs. The probability of having a breast examination by a healthcare staffs in a household with an income below 5 million Iranian Rials (120 US dollars) was 40% less than the probability of a woman from a household with an income greater than 15 million Rials (360 dollars) having a breast examination by a healthcare staffs. Furthermore, the probability of having a breast examination by a healthcare staffs in a household with an income of 5-10 million Rials (120-240 dollars) or 10-15 million Rials (240-360 dollars) was 35% less and 31% less, respectively, than the probability in households with incomes over 15 million Rials (360 dollars) (Table 2).

Table 2: Multivariate logistic regression to determine the factors related to breast examination by a medical team

Variable		Multivariate test		
		OR[1]	CI[2]	P-value
Age (year)		1.06	1.04-1.08	0.000*
Husband's age (year)		0.99	0.97-1.00	0.185
BMI[3]		1.02	0.99-1.05	0.199
Age (year) at first marriage		0.95	0.92-0.98	0.001*
Number of pregnancies		0.936	0.826-1.06	0.299
Health status	Healthy	1.04	0.789-1.37	0.766
	Ill			
Income (million Iranian Rials)	< 5	0.60	0.39-0.93	0.018*
	5-10	0.64	0.46-0.92	0.010*
	10-15	0.68	0.48-0.96	0.026*
	> 15			
Ultimate decision-maker in the level of self-care[4]	Desirable	1.07	0.81-1.41	0.62
	Undesirable			
Ultimate decision-maker on issues of sexual and reproductive health[4]	Desirable	1.67	1.22-2.29	0.001*
	Undesirable			
polygamy	Yes	0.62	0.29-1.32	0.22
	No			
Infertility	Yes	0.62	0.43-0.90	0.014*
	No			

*P < 0.05; [1]Odd's Ratio; [2]Confidence Interval; [3]Body Mass Index; [4]The desirable decision-maker for self-care, issues of sexual and reproductive Health was the woman alone or with her husband; the undesirable decision-maker was the husband alone.

The following variables were associated factors with a history of receiving mammography and had a P < 0.1 in the single-variable test and were entered into the multivariable logistic regression model: woman's age (P < 0.0001), husband's age (P < 0.0001), BMI (P < 0.003), number of pregnancies (P < 0.0001), education level (P < 0.018), husband's occupation (P < 0.0001), smoking (P < 0.068), health status (P < 0.0001), decision-maker for self-care (P < 0.001), buying home needs (P < 0.1), and SRH (P < 0.001), unclear or colored vaginal discharge (P < 0.002) and genital ulcers in the last 12 months (P < 0.041), and the use of a contraceptive method (P < 0.067). The Multivariate regression showed that age, husband's occupation, smoking, and decision-maker

on issues of SRH had statistically significant relationships with a history of receiving mammography. The probability of a nonsmoker receiving mammography was 83% less than the probability of a smoker receiving mammography. As each year of age increases, the probability of mammography increases by 7%. Furthermore, women with unemployed husbands received mammography 3.2 times more than those with self-employed husbands. If the decision maker is appropriate for SRH, 76% will increase the probability of mammography (Table 3).

Table 3: Multivariate logistic regression to determine the factor related to mammography

Variable		Multivariate test		
		OR[1]	CI[2]	P-value
Age (year)		1.07	1.05-1.10	0.000
Husband's age (year)		0.99	0.97-1.00	0.189
BMI[3]		1.01	0.97-1.05	0.429
Number of pregnancies		0.989	0.85-1.14	0.882
Educational level	Did not earn diploma	0.96	0.60-1.53	0.882
	Diploma and associate degree	0.92	0.59-1.44	0.735
	Bachelor degree and higher			
Husband's occupation	Unemployed	3.20	1.58-6.48	0.001
	Government employee	1.11	0.74-1.65	0.604
	Worker	1.12	0.75-1.67	0.561
	Self-employed			
Smoking	Yes	0.171	0.03-0.81	0.027
	No			
Health status	Healthy	1.36	0.98-1.89	0.059
	Ill			
Ultimate decision-maker for self-care[4]	Desirable	1.44	0.99-2.08	0.054
	Undesirable			
Ultimate decision-maker for buying home needs[4]	Desirable	0.94	0.68-1.29	0.717
	Undesirable			
Ultimate decision-maker on issues of sexual and reproductive Health[4]	Desirable	1.76	1.15-2.70	0.009
	Undesirable			
Vaginal discharge in the last 12 months	Yes	0.8	0.57-1.13	0.215
	No			
Genital ulcers in the last 12 months	Yes	0.53	0.27-1.02	0.061
	No			
Use of a contraceptive method	Yes	2.97	0.0	0.999
	No			

[1]OR: Odd's Ratio; [2]CI: (Confidence Interval); [3]BMI: Body Mass Index; [4]The desirable decision-maker for self-care, issues of sexual and Reproductive Health, and buying home needs was the woman alone or with her husband; the undesirable decision-maker was the husband alone.

Discussion

This study aimed to investigate the factors related to breast cancer screening in women from the northern part of Iran. According to the findings, 62% of women had a history of breast self-examinations, 41.1% had breast examinations by healthcare staffs, and 21.7% received mammography. The results showed that there was a higher rate of breast cancer screening than the rates reported by studies conducted in Nigeria [17] and in other places in Iran, such as Kermanshah, Kerman, Gorgan, Tabriz, and Mazandaran [1], [9], [11], [13], [14], [16]. However, Klug et al., in Germany showed that 82.8% of women had a history of breast examinations by healthcare staffs and 55.5% had a history of receiving mammography [18]. A comparison of the results of this study with the results by Kellogg et al. Indicating that Iran is not in good condition compared to the developed countries. Therefore, there is a need for more efforts to improve women's knowledge about breast screening methods and the importance of screening about an early diagnosis of breast cancer.

The results of this study showed that the predictors of participation in breast cancer screening were age, age at first marriage, age at onset of sexual intercourse, occupation, the occupation of spouse, household income, health status, history of infertility, smoking, and decision-maker on issues of SRH. Age was a predictor of women performing breast self-examinations, having a clinical examination by healthcare staff, and receiving mammography. For each year increase in age, breast screenings increased 1.02, 1.06, and 1.07 times, respectively. The results of this study were consistent with studies by Avci [19], Berdi-Ghourchaei et al. [13], and Nourizadeh et al. [16]. However, studies by Ahmadian et al., [15], Lee et al., [20], and Godazandeh [14] were inconsistent with the results of this study. Considering the direct relationship between age and onset of breast cancer, as women age they consider themselves to be more at risk of developing cancer. Therefore, the rate of screening increases with age.

Occupation was another predictor of breast cancer screening. The probability that a housekeeper would perform breast self-examination was 32% less than the probability that an employed woman would do breast self-examination. The results of studies by Soltanahmadi et al., [11], Bozorgi et al., [7], Godazandeh et al., [14], and Kim et al., [21] are consistent with the findings of this study. Employed women tend to have higher social relationships and higher levels of education than housekeepers and, thus, have an increased level of knowledge and increased participation rate in breast screening programs [11]. The relationship between job status and participate in a breast screening program indicates that, in addition to contributing to the household income, being employed presents more opportunities to use healthcare and social services [21].

The results of this study showed that household income was also related to breast cancer screening. Women with high household incomes had a high rate of screening. This finding was consistent with the findings by Soltanahmadi et al., [11]. One reason for this association could be the misconception by some women who think that a clinical breast examination must be performed by physicians, whereas clinical examinations are performed by midwives in healthcare centres for free [11]. However, a study by Lee et al., in Korea showed that household income did not influence the screening rate. The Korean government provides free screening services to low-income people, which might reduce the effect of household income on participation in screening programs [20].

Smoking was another factor that influenced breast cancer screening behaviours. In comparison with a nonsmoker, a smoker was 83% less likely to receive mammography. Rakowski et al. found that "the rate of mammography for current smokers was 16% lower than for never smokers for the past-year interval. Their interpretation was that "smoking is not a direct cause of reduced screening, but rather an indicator that a high-risk group of women receives less screening" [22]. One study reported that there was a significant difference between the psychological variables of smokers and nonsmokers, which influenced self-care behaviors and decision-making behavior about healthcare issues [20].

The decision-maker on issues of SRH was a predictor of participation in breast cancer screening. If the decision-maker was the woman alone or together with her husband, the probability of participating in screening by clinical examination or mammography was 1.67 times and 1.76 times, respectively, than when the decision-maker was the husband alone. Some reasons for this result could relate to having independence in making decisions about health and the financial dependence of 75% of the women in this study. Therefore, a lack of sense of control on resources and financial dependence on another person could influence a woman's decision-making about her health [23].

In women with a history of infertility, the rate of breast cancer screening by a healthcare staff was 38% less than in women without a history of infertility. The effect of infertility on the mental health of women can lead to high rates of depression, anxiety, and disappointment with life. Depression and other psychological problems in infertile women are barriers to seeking medical counselling, while disappointment with life can lead to reduced screening behaviours [24], [25].

There was a statistically significant relationship between the health status of women and breast self-examination screening. Women with underlying diseases, such as diabetes, high blood pressure, and thyroiditis, had a rate of performing breast self-examinations that were 29% lower than women who had no underlying diseases. Different studies have shown that the prevalence of depression and mood disorders was high in women with diabetes, high blood pressure, hypothyroidism, and hyperthyroidism [26], [27], [28], [29]. Given that depression and mood disorders are considered barriers to self-care behaviours [30], the results of this study were noticeable, and the rate of performing breast self-examination for women with a history of underlying diseases was lower than the rate for healthy women. It is recommended that breast examination and mammography be included in the routine care for women with underlying diseases. There was a significant statistical relationship between breast cancer screening and the age of onset of sexual intercourse and age at first marriage. Therefore, for each unit increase in age at onset of sexual intercourse and at first marriage, the probability of performing breast self-examination and receiving an examination by a healthcare staff increased 1.08 times and 0.95 times, respectively.

The husband's occupation was another predictor of participating in breast cancer screening. This study showed that the probability of receiving mammography in women whose husbands were unemployed was 3.2 times greater than in women whose husbands were self-employed. Women with unemployed husbands were probably the main income provider for their families. Therefore, women's financial independence from their husbands could be a factor in their decision-making about their health.

In conclusion, in this study, 62% of women had a history of breast self-examinations, 41.1% had a history of receiving breast examinations by healthcare staffs, and 21.7% of them had a history of receiving mammography. Also, it has been revealed that the woman's age, age at first marriage, age at onset of sexual intercourse, occupation, spouse's occupation, household income, health status, history of infertility, smoking, and decision-maker on issues of sexual and reproductive health were the best predictors of participation in screening for breast cancer. To reduce the total burden of disease of breast cancer, factors that are related to performing screening tests should be considered an appropriate educational method should be provided by healthcare providers.

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Survival Analysis of Cancer Patients in North Eastern Nigeria from 2004 – 2017 – A Kaplan - Meier Method

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Abstract

BACKGROUND: Cancer is a deadly malignant disease and is prevalent in Sub Saharan Africa. The North East part of Nigeria in particular and the country, in general, are struggling to cope with the increasing burden of cancer and other communicable and non-communicable diseases. The situation is worsened by the ongoing insurgency and terrorist activities in the area.

AIM: The aim of this paper is to present the research findings from a cohort study aimed at the analysis of the estimation of the survivorship time of the real data of cancer patients in the North-eastern part of Nigeria and to establish if the insurgency in the region has contributed negatively to the life expectancy of its inhabitants.

MATERIAL AND METHODS: The record of 1,090 patients from medical records departments of the University of Maiduguri Teaching Hospital (UMTH), located in Maiduguri, the capital city of Borno State in northeast Nigeria was obtained. The record showed patients that were diagnosed and died of one type of cancer or the other from 2004 to 2017. All the cancer cases included in the present study were grouped into sex, age, marital status, occupation, date admitted and date of death/discharge. Descriptive statistics and Kaplan-Meier method were used to analyse the data using SPSS version 23 while Microsoft EXCEL and Minitab 16.0 were used for data cleansing and organisation.

RESULTS: Of the 1,090 patients analysed, 920 (84.40%) experienced the event, i.e. death, while 170 (15.60%) patients were censored. The data were analysed based on the ages and sex of the patients. 50.20% of the patients were of ages 21-50 years. The proportions of patients in this age bracket surviving past 7 days are 75%, while those between ages 80 years and above is 12 days. Others are of survival time of 5 days (ages 0-20 years) and 7 days (51-79 years). Using sex, 75% of the patients' survival time is 7 days in the case of male and 6 days for females. It is safe to say that the survival time for cancer patients of the university the Maiduguri is 6 days and the result reflects the Northeastern part of Nigeria. This is because the hospital is one of few tertiary healthcare facilities in that area and consequently, cancer cases are often referred there.

CONCLUSION: Cancer incidence is high, and the probability of survival reduces as the survival time increases. This is a dire situation in need of urgent intervention from the government, groups and individuals to tackle the scourge of cancer, thereby improving on the life expectancy battered by the ongoing Boko Haram insurgency in that region.

Introduction

Sub Saharan Africa is one of the areas that plagued with the prevalence of non-communicable diseases, especially cancers which is on the increase. Cancer of any type is the most common and lethal malignancies in developing countries like Nigeria. Cancer is an unwelcome guest in every home, and it is seen as a death sentence in Nigeria. Northeast Nigeria is one of the least developed areas of the country, lagging dangerously behind in virtually every development index. The region is notoriously staggering under the weights of poverty [1], insurgency and terrorism [2], [3], [4], hunger and low life expectancy [5], polio, maternal and child mortality [6], [7] and so on.

Cancer is not only prevalent in the North East, Nigeria [8], [9], but to the entire country which are battling to cope with other health challenges such as mental health [10], [11], maternal and child health [12], [13] and HIV AIDS [14]. The high case fatality rate of cancer in Nigeria is due to low level of cancer awareness and screening, late discovery, unhealthy lifestyle, superstitious beliefs, limited or poorly funded healthcare facilities, the dearth of experts in oncology and others to mention but a few.

A patient's journey along a disease pathway can be highly complex and can be impacted by recurrences, co-morbidities, and interventions, to

name a few. Cancer as one of the world's best killer disease, for example, has killed many and left only a few to tell the tale. To disentangle the complexities, we need special kinds of survival models. These enable us to investigate diverse aspects of disease aetiology and explore the impact of risk factors at all stages. Crucially, we can then attempt to communicate risk profiles in many ways, understandable to both patient and clinician, through easily interpretable measures (such as the impact on life expectancy, postponable deaths, survival and transition probabilities). The study of survival of cancer patients will be an immense contribution to the fight against the dreaded disease.

Regard to sound statistical practice, in particular, the use of statistical approaches that provide clinically and peer-reviewed relevant information, will help maximise the potential of molecular markers for the care of cancer patients. Kaplan-Meier (K-M) method otherwise known as the product limit method is a statistical technique used to analyse cancer data. It is applied in analyzing the distribution of the patient's survival times following their recruitment into the study. The analysis expresses this in terms of the proportion of patients still alive up to a given time t, following the recruitment or entry into the study.

This paper aims to present the research findings from a cohort study focused at the analysis of the estimation of the survivorship time of the real data of cancer patients in the North-eastern part of Nigeria and to establish if the insurgency in the region has contributed negatively to the life expectancy of its inhabitants. Also, the result that established the observed relationship between survival time and survival probability is presented. Kaplan-Meier method was used in the survival data analysis of the cancer data, and the findings were discussed extensively.

Material and Methods

Data Collection

The record of 1,090 patients from medical records departments of the University of Maiduguri Teaching Hospital (UMTH), located in Maiduguri, the capital city of Borno State in northeast Nigeria was obtained.

The record showed patients that were diagnosed and died of one type of cancer or the other from 2004 to 2017. All the cancer cases included in the present study were grouped into sex, age, marital status, occupation, date admitted and date of death/discharge.

In survival analysis, follow up periods are calculated from when subjects were enrolled in the study (i.e. date admitted).

Research on cancer is often very interesting because of the high fatality rate of the disease is diagnosed later and or untreated. One of the methods used in the survival analysis in oncology and epidemiology studies is the Kaplan-Meier method (K-M) [15], [16], [17], [18], [19], [20], [21], [22], [23], [24]. K-M method is usually used in conjunction with Cox proportional hazard regression, immunohisto-chemistry, lognormal, hazard ratio, Chi-squared test, log-rank test and so on.

A summary of the use of the K-M method in conjunction with other statistical methods is given in Table 1.

Table 1: Kaplan-Meier and other statistical methods used in the survival analysis of different types of cancer

Type of Cancer	Statistical methods	Cases investigated	Author
Breast	KM, Chi-squared test, Cox regression	207	[25]
Breast	KM, Cox regression	300	[26]
Breast	KM, Cox regression	135	[27]
Gastric	KM	179	[28]
Breast	KM	308	[29]
Breast	KM, Cox regression	139	[30]
Leukemia	KM, Cox regression	527	[31]
Ovarian	KM	81	[32]
Rectal	KM, Cox regression	3786	[33]
Liver	KM, Cox regression	30,954	[34]
Thyroid	KM, Cox regression	12,128	[35]
Gastric	KM, Chi-squared test, Cox regression	4596	[36]
Breast	KM, Cox regression	1391	[37]
Breast	KM, Hazard ratio	10 226	[38]
Prostate	KM, Cox regression	579,608	[39]
kin	KM, Cox regression, Log-rank test	83	[40]

Data Preparation

In preparing the data for Kaplan-Meier survival analysis, each subject (patient) of the data component is mainly characterized by 3 variables: 1) their serial time (in days or years); 2) their status at the end of their serial time (event occurrence or censored); and 3) the study group or stage they are in.

For the computation of survival time curves and probabilities, the serial times for the patients are arranged from the shortest to the longest without regards to when they are recruited into the study as long as left censorship is not encountered. By this move, it can be ensured that all subjects within the group or stage begin the analysis at the same point and all are surviving independently until (event or censor) occurs.

Reasons for adopting the K-M method

The methodology is adopted for the following reasons. Firstly, the main target is to estimate a population survival curve from the sample obtained from the teaching hospital. Secondly, if every subject (patient) is followed until death, the curve may be estimated simply by computing the fraction surviving at each time t. Lastly, Kaplan-Meier curves have gravitated characteristics, which perhaps explains

their wide applicability in medical research as they provide a pictorial depiction of all the raw data, the failure times and the censoring times; yet they also provide a mathematical estimate of the given survival model.

It is glad to note that the data presented in this article did not violate the six assumptions of the K-M method. The assumptions are stated as follows: 1) The data is composed of two mutually exclusive and exhaustive states known as an event or censored; 2) The survival time was clearly defined and accurately measured; 3) The data is right censored; 4) The censoring and the event are independent. This is vital since the efficiency of the K-M method depends on the analysis of observed data; 5) No trend was observed in the data; 6) Right censoring is similar in all the groups (sex and age).

Data Processing

Raw data are stored in MS EXCEL format using actual calendar date and time. During analysis, serial time may be automatically calculated, and this is used in curve construction and data analysis. The first step in the preparation of K-M analysis involves the construction of a table using Minitab or Excel spreadsheet or in SPSS containing the three key elements required for input. These are: 1) serial time, 2) status at serial time (1 = Alive, 0 = Death) and 3) age group.

Results

These were performed using SPSS version 23.0. Data were reported by sex, age, marital status, occupation, date of admission and date of death/discharge. A descriptive analysis of the data was carried out, and each character was described by frequencies and percentages. Kaplan-Meier analyses were conducted to mainly estimate overall survival rates of the various types of cancer. The survival time of a patient is referred to the number of months or days (duration) from the date of diagnosis of cancer to the date of the patient died or last contact (censored) or the date of the end of the study for patients who were still alive or date of loss to follow-up (censored). The differences in survival between the stages were compared by the log-rank test. A two-tailed p-value of < 0.05 was considered as statistically significant.

Descriptive Statistics

Data on 1,090 cancer cases were gathered of which 478 (43.9%) were male, while 612 (56.10%) were female, shown in Table 2.

Table 2: The sex distribution of the patients

Sex	Frequency	Per cent	Cumulative Percent
Male	478	43.9	43.9
Female	612	56.1	100.0
Total	1090	100.0	

From Table 3, it can be seen that 50.20% of the patients were between the ages of 21-50 years old, 17.70% were of ages below 20 years old, 29.9% were between ages 51-79 years while 1.9% were 80 years and above.

Table 3: The age distribution of the patients

	Age	Frequency	Percent	Cumulative Percent
Valid	0-20	193	17.7	17.8
	21-50	547	50.2	68.1
	51-79	326	29.9	98.1
	80 & Above	21	1.9	100
	Total	1087	99.7	
Missing		3	0.3	
Total		1090	100	

A group of 227 (20.8%) of the patients are single, 832 (76.3%) are married, 12 (1.1%) are widowed, and 19 (1.7%) are missing values, shown in Table 4.

Table 4: Marital status of the patients

Marital status	Frequency	Per cent
Single	227	20.8
Married	832	76.3
Widow/Widower	12	1.1
Missing	19	1.7
Total	1090	100

Of the 478 females, 40.1% were housewives. 10.8% of the patients were children while 13.9% were civil servants. The details of the other occupation are given in Table 5.

Table 5: the Recorded occupation of the patients

	Occupation	Frequency	Percent	Cumulative Percent
Valid	Applicant	12	1.1	1.1
	Soldier	4	0.4	1.5
	Banker	1	0.1	1.6
	Bricklayer	1	0.1	1.7
	Business Man	71	6.5	8.4
	Butcher	1	0.1	8.5
	Civil Servant	151	13.9	22.7
	Caterer	1	0.1	22.7
	Cattle Rearer	5	0.5	23.2
	Child	118	10.8	34.3
	Clergy	1	0.1	34.4
	Contractor	1	0.1	34.5
	Police Officer	4	0.4	34.9
	Driver	11	1	35.9
	Politician	3	0.3	36.2
	Farmer	97	8.9	45.3
	Retired	19	1.7	47.1
	Fisherman	2	0.2	47.3
	Scholar	5	0.5	47.7
	House Wife	437	40.1	88.8
	Security Man	4	0.4	89.2
	Mechanic	2	0.2	89.4
	Student	85	7.8	97.4
	Tailor	5	0.5	97.8
	Teacher	8	0.7	98.6
	Technician	2	0.2	98.8
	Widow/Widower	13	1.2	100
	Total	1064	97.6	
Missing	System	26	2.4	
Total		1090	100	

The weird occupations seen in Table 5 can be attributed to high illiteracy of the patients and poor record keeping by the staff of the hospital. Finally, it

can be seen from Table 6, that 170 (15.6%) were alive and 920 (84.4%) were dead after admission in the hospital.

Table 6: End status of the patients

Status	Frequency	Per cent	Cumulative Percent
Dead	920	84.4	84.4
Alive	170	15.6	100
Total	1090	100	

Kaplan-Meier Results

The data presented in this subsection are from the K-M analysis. These are given as: case summary for age (Table 7), case summary for sex (Table 8), means and medians for survival time for age (Table 9), means and medians for survival time for sex (Table 10), overall comparison tests for the age (Table 11) and overall comparison tests for the age (Table 12).

Table 7: Case processing summary for age

Age	Total N	N of Events	Censored	
			N	Per cent
0-20	193	150	43	22.30%
21-50	547	473	74	13.50%
51-79	326	274	52	16.00%
80 & Above	21	20	1	4.80%
Overall	1087	917	170	15.60%

Table 7 displays the age of the cancer patients (categorised into age groups from 0-20 years, 21-50 years, 51-79 years and 80 years and above), the total number of patients in each age group, number of patients experienced events, and censored patients. Age 0-20 years has a total number of 193 patients with 150 been number of events and 43 (22.3%) as censored patients, age 21-50 has a total number of 547 patients with 473 patients that have experienced the event and 74 (13.5%) as censored patients, age 51-79 has a total number of 326 patients with 274 patients that have experienced the event and 52 (16.0%) as censored patients and age 80 and above has a total number of 21 patients with 20 total number of events and 1 (4.8%). It was noted that age group 21-50 has the highest number of events followed by 51-79 and age group 80 and above with the lowest number of events.

Table 8: Case processing summary for sex

Sex	Total N	N of Events	Censored	
			N	Per cent
Male	478	401	77	16.10%
Female	612	519	93	15.20%
Overall	1090	920	170	15.60%

Table 8 displays the sex of patients diagnosed with cancer, the total number of patients for each sex group, number of patients experienced events, and censored patients. Four hundred seventy-eight male patients were admitted with 401 been number of events and 77 (16.1%) as censored patients while there were a total of 612 females with 519 patients that have experienced the event and 93 (15.2%) as censored patients Clearly, the male patients have the lowest number of events.

Table 9: Means and medians for survival time for age

AGE	Mean				Median			
	Estimate	Std. Error	95% Confidence Interval		Estimate	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound			Lower Bound	Upper Bound
0-20	26.035	3.225	19.715	32.356	14	1.949	10.18	17.82
21-50	30.89	2.414	26.159	35.622	16	1.488	13.084	18.916
51-79	35.808	4.234	27.509	44.107	19	1.182	16.683	21.317
80 & Above	29.651	6.487	16.936	42.366	20	4.577	11.028	28.972
Overall	31.41	1.839	27.807	35.014	17	0.864	15.306	18.694

Table 9 gives a quick quantitative comparison of the typical survival times to effect for each of the age groups. The median survival time is calculated as the smallest amount of survival time for which the survivor function is less than or equal to 0.5.

The overall median survival time (i.e. the time at which the survival probability is 50% or 0.5) is 31 days. In other words, for cancer patients in the North, the chance of living beyond 31 days is 50%. The median survival time for ages 0-20 was 26 days, 30 days for ages 21-50, 35 days for age 51-79 and ages 80 and above was 29 days. These clearly show that the chance of living beyond 26 days, 30, 35 and 29 for ages 0-20, 21-50, 51-70 and above 80 years respectively after being admitted/diagnosed with the disease is 50%.

It can be seen that the higher the estimated mean time, the greater the chances of survival. This goes to show that ages 51-79 has the highest chance of survival while ages 0-20 years has the least chance of survival.

The log-rank test can be applied if the confidence limits do not overlap between the given levels. Clearly, from Table 9, there is no overlap between ages in the confidence intervals; hence differences in effect on time to an event can be inferred using the log-rank test.

Table 10: Means and medians for survival time for sex

Sex	Mean				Median			
	Estimate	Std. Error	95% Confidence Interval		Estimate	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound			Lower Bound	Upper Bound
Male	32.947	2.984	27.099	38.795	19	1.236	16.578	21.422
Female	30.184	2.286	25.703	34.665	16	1.33	13.394	18.606
Overall	31.355	1.833	27.762	34.948	17	0.852	15.331	18.669

From Table 10, it can be seen that the estimated mean time until death is 32 days for male, 30 days for females, which shows that females have a slightly increased chance of survival than males.

The median survival time for a male is 19 days while that of females is 16 days. These clearly show that the chance of living beyond 19 and 16 days for males and females, respectively for cancer patients in the Northeastern part of Nigeria is 0.5 after being diagnosed with the disease.

Clearly, from Table 10, there is a lot of overlap in the confidence intervals; hence it is unlikely that there is much difference in the average survival time.

Table 11: Overall Comparison Tests for the age

Test of equality	Chi-Square	Df	Sig.
Log Rank (Mantel-Cox)	3.812	3	0.283
Breslow (Generalized Wilcoxon)	6.845	3	0.077
Tarone-Ware	5.622	3	0.131

Table 11 is the test of equality of survival distributions for the different levels of age. The significance values of the tests (0.283, 0.077, 0.131) are all greater than 0.05. The interpretation of this is the acceptance of the null hypothesis means that there is no significant evidence of a difference in the observed survival times for the categories of age considered.

Table 12: Overall Comparison Tests for the sex

Test of equality	Chi-Square	Df	Sig.
Log Rank (Mantel-Cox)	1.440	1	0.230
Breslow (Generalized Wilcoxon)	3.573	1	0.059
Tarone-Ware	2.814	1	0.093

Table 12 is the test of equality of survival distributions for the different levels of sex. It shows that the significance values of the tests (0.230, 0.059 and 0.093) are all greater than 0.05. The interpretation of this is the acceptance of the null hypothesis implies that there is no significant evidence of a difference in the observed survival times for the categories of age considered.

Although, females have a slightly increased chance of survival even though, as inferred from the log-rank test, sex is not a barrier to death.

Plots of the Survival Functions and survival probability estimation

The survival curves (Figures 1 and 2) give a visual depiction of the life tables, the horizontal axis represents the time to the event, and the vertical axis shows the estimated probability of survival.

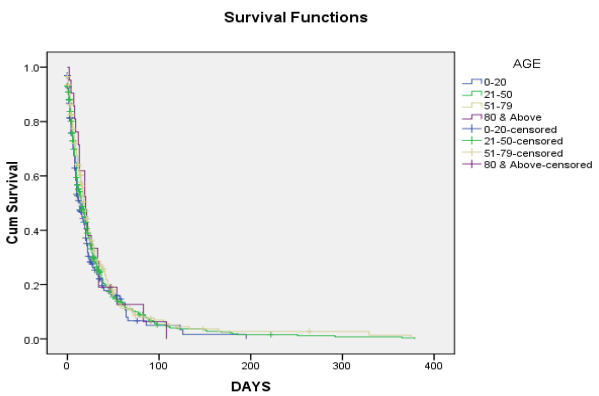

Figure 1: Kaplan-Meier survival plot for the ages

In this plot, drops in the survival curve occur whenever the patients experience the event

Also, the summary of survival probability estimate with the survival time for the age and sex are presented in Table 13 and 14 respectively.

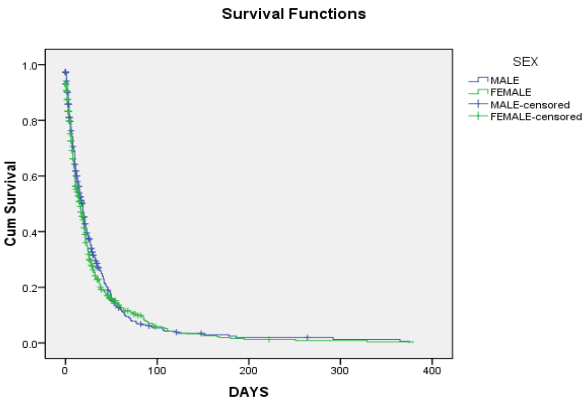

Figure 2: Kaplan-Meier survival plot for the sex

From Table 13, the proportion of subjects or patients surviving past 5 days is 75%, the proportion of subjects or patients surviving past 14 days is 50% and the proportion of subjects or patients surviving past 32 days is 25% for age 0-20 years.

Table 13: Survival probability estimate with the survival time for age

Age	25		50		75	
	Estimate	Std. Error	Estimate	Std. Error	Estimate	Std. Error
0-20	32	4.755	14	1.949	5	0.915
21-50	35	2.56	16	1.488	6	0.561
51-79	40	3.279	19	1.182	7	0.97
80 & Above	34	4.799	20	4.577	12	2.588
Overall	35	1.815	17	0.864	6	0.443

Note; Survival time is given in days (D). This can easily be seen in Figure 1.

The proportion of subjects or patients surviving past 6days is 75% and the proportion of subjects or patients surviving past 16 days (which is the median survival time) is 50% and the proportion of subjects or patients surviving past 35 days is 25% for age 21-50 years.

The proportion of subjects or patients surviving past 7 days is 75%, the proportion of subjects or patients' surviving past 19 days is 50% and the proportion of subjects or patients surviving past 40 days is 25% for age 51-79 years.

The proportion of subjects or patients surviving past 12 days is 75%, the proportion of subjects or patients surviving past 20 days is 50% and the proportion of subjects or patients surviving past 34 days is 25% for ages 80 years and above.

From this analysis, the probability of survival reduces as the survival time increases.

From Table 14, the proportion of subjects or patients surviving past 7 days is 75%, the proportion

of subjects or patients' surviving past 19 days is 50% and the proportion of subjects or patients surviving past 39 days is 25% for Males.

Table 14: Survival probability estimate with the survival time for sex

Sex	25% Estimate		50% Estimate		75% Estimate	
	Estimate (days)	Std. Error	Estimate (days)	Std. Error	Estimate (days)	Std. Error
Male	39	2.69	19	1.236	7	0.829
Female	32	2.048	16	1.33	6	0.52
Overall	35	1.816	17	0.852	6	0.44

Note; Survival time is given in days (D). This can easily be seen in Figure 2.

The proportion of subjects or patients surviving past 6days is 75% and the proportion of subjects or patients surviving past 16 days (which is the median survival time) is 50% and the proportion of subjects surviving past 32 days is 25% for Females.

Also, from this analysis, the probability of survival reduces as the survival time increases.

Discussion

A good glance at the results shows most patients died the same day or few days to date of admission. Hence, it was not a surprise that the analysis shows overall survival time of 6 days for 75% of patients with cancer at the University Teaching Hospital, Maiduguri. This is a far-cry to survival data obtained in another part of the country. The study shows that over 84% of the cancer cases died. We have more of the cases for women than for men. Over 40% of the cases for female were those who reported their occupation as housewives. Over 50% of the cases were age 21-50 years old. Only 1.9% of the cases were of ages 80years and above.

Following the result of the research, it is obvious that we have a major problem with the management of cancer in the region under review and Nigeria in general. The reasons for this might not be a difference of the below points.

The Northeast part of Nigeria is one of the poorest parts of Nigeria. Years of corruption and lack of investment in the health facilities have contributed to a near collapse of the health facilities of the region in particular and Nigeria in general. Poverty, illiteracy and superstition are contributory factors why diseases like cancer are endemic in the area. This is exacerbated by the ongoing Boko Haram insurgency and herdsman attacks. The insurgency has added to the already strain on the few available health facilities in the area and drastically reduction to access to health care. The effect is not limited to cancer, but other illness such as HIV AIDS epidemic, a cholera outbreak and others. It can be noted that the University of Maiduguri teaching is the only major tertiary health facility that covers Borno, Yobe, Gombe and Taraba states, an area and population that is greater than London, United Kingdom.

The precarious political and social factors are drivers to the culture of patients reporting of their cases very late. Most cancers have different stages, one, two, three and four. While stage one and two can achieve a cure, stage three and four are usually advanced where you no longer talk about radical treatment but palliative treatment to see how you can prolong the patient's life and improve the quality of life. The data for this research did not include the stage of cancer upon admission at the hospital, we suggest most cases to be at stage three and four going by the time to the event.

Most patients do not go for screening; they mostly consult roadside chemists, extreme cases among the people approach churches, Muslim clerics and herbalist.

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Assessment of Bottle-Feeding Practices in Kassala, Eastern Sudan: A Community-Based Study

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Keywords: Bottle-feeding; Urban residence; Breastfeeding education; Child hospitalisation; Sudan

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Abstract

BACKGROUND: The World Health Organization encourages exclusive breastfeeding up to six months and avoidance of bottle-feeding. There are few published research articles on the practice of bottle-feeding and associated factors in Sudan.

AIM: The study aimed to assess the usage and factors associated with bottle-feeding practices during the first six months of life among mothers with children aged between 6 and 24 months in Kassala, Eastern Sudan.

METHODS: A community-based cross-sectional study was conducted from July to September 2017. A structured questionnaire was used to collect relevant data from interviewed mothers.

RESULTS: A total of 242 mother-child pairs participated in the study. The mean (standard deviation) of maternal age and children's age was 27.13 (5.73) years and 12.2 (6.7) months, respectively. From the total, 96/242 (39.7%) used bottle-feeding for their children in the first six months of life. In multivariable analysis, urban residence (Adjusted Odds Ratio [AOR] 1.96, 95% Confidence Interval [CI] (1.06, 3.63), not receiving breastfeeding education (AOR 1.92, 95% CI 1.07, 3.45) and child hospitalization (AOR 1.83, 95% CI 1.02, 3.28) were significantly associated with bottle-feeding.

CONCLUSION: There was a high usage of bottle-feeding and it was found to be associated with child hospitalisation. To avoid bottle-feeding, urgent actions are required to support and educate mothers regarding breastfeeding with special attention to urban-residence ones.

Introduction

According to the United Nations Children's Fund (UNICEF) [1], the first 1000 days of a human being's life (nine months of pregnancy plus the first two years of life) are considered to be a crucial period. An inappropriately fed child is more vulnerable to malnutrition and its detrimental effects such as morbidity (diarrhoea and respiratory tract infections) and mortality [2], [3], [4].

Aiming to save children's lives, the World Health Organization (WHO) developed a set of recommendations, including exclusive breastfeeding up to six months and avoidance of bottle-feeding, safe complementary foods at six months and supporting mothers to practice this [5].

Various studies evidenced better cognitive development and intelligence quotients in breastfed infants compared to bottle-feed ones [6]. Previous studies have shown that bottle-feeding was a key factor for child morbidity and mortality in different settings [7], [8], [9]. For example, in the Philippines bottle-fed infants were found to be at high risk of hospitalisation due to infections [10].

The rate of bottle-feeding differs by country ranging from 15% in Nigeria [11] to 64% in Iraq [12]. Different reasons to practice bottle-feeding were mentioned by mothers such as mother's illness, breast-related health issues as well as perceived issues (i.e. perception of insufficiency of mother's milk) [13], [14]. Whatever the reason is for choosing

bottle-feeding, following the WHO recommendations, all mothers, even those who are HIV positive (the human immunodeficiency virus), can breastfeed their children [15]. In spite of the WHO adoption of the International Code of Marketing of breast-milk substitutes, still, poor adherence exists [16], [17], [18].

Breastfeeding education has been documented in many studies as an effective tool in promoting exclusive breastfeeding and avoidance of bottle-feeding in different settings [14], [19], [20]. Such breastfeeding education and support need to be directed to all mothers regardless of their residence and working status [21], [22]. Poor breastfeeding practices, such as low rates of exclusive breastfeeding, bottle-feeding and early weaning were documented in different regions of Sudan [23], [24], [25]. Early introduction of complementary feeding (i.e. before six months of age) was reported in Sudan [23], [26].

Our study aimed to examine bottle and breastfeeding practices amongst mothers in Kassala State, Eastern Sudan. Kassala State was selected to study breastfeeding patterns based on some factors. First is that most of the available data in Sudan about breastfeeding is derived from hospital-based studies [3], [26]. Also, the determinants of bottle-feeding are poorly understood, largely because this is an understudied area. Furthermore, the target area (Kassala State) is categorised as being amongst the most vulnerable regions with high rates of acute and chronic malnutrition, and most of the previous studies on breastfeeding were carried out in relatively more stable regions in the centre of Sudan [26], [27]. Kassala is more vulnerable to humanitarian crises as documented in many previous food and security reports [28], [29]. Also, the availability of data before the crisis is of paramount importance to build on them when a crisis occurred.

Therefore, the conduct of such a study at the community level, in an area characterised by both food insecurity and unstable security, is of great importance for the identification of the factors leading to bottle-feeding, which will ultimately provide the basis for future community-based interventions.

The study aimed to assess the usage and factors associated with bottle-feeding practices during the first six months of life among mothers with children aged between 6 to 24 months, at the community level in Kassala, Eastern Sudan.

Methods

A community-based cross-sectional study was conducted in Kassala, Eastern Sudan from July to September 2017. A two-stage random cluster study was used. Stage one, simple random sampling of the localities was performed to identify households randomly. Similarly, stage two involved random sampling of the household in identifying participants (any mother with a child aged between 6 to 24 months). Kassala has an estimated population of 453,159 inhabitants, of whom 55% live in urban areas, with 33,604 and 52,853 households in urban and rural areas, respectively [30]. Houses were mapped to select a representative sample. A structured questionnaire was used to collect relevant data from interviewed mothers. Two female medical officers were trained by the investigators to collect the data. The questionnaire was tested among 10 mothers (not included in the final sample), and the necessary corrections were completed before the field work. The inclusion criteria were as follows: willingness to participate in the study, having a child aged between 6 and 24 months (in case the mother had more than one child in this age group, she was interviewed about the youngest child) and availability at the time of data collection. The study excluded any mother who did not fulfil all of the inclusion mentioned above criteria.

The usage of bottle-feeding rate (%) was estimated based on the WHO definition for bottle-feeding: ‘any liquid (including breast milk) or semi-solid food from a bottle with nipple/teat’ [31]. In this study, the proportion of children aged between 6 and 24 months who were fed with a bottle during the first six months were considered as users of bottle-feeding, while others were excluded from this category. The first six months was specifically chosen because it is a period in which the infant should be exclusively breastfed [31].

A sample of 242 mother-child pairs was calculated based on the difference of the proportions of desired factors (education factor) which was assumed to be 61% vs 39% in the bottle user vs non-user. This sample has 80% power with a precision of 5% and assuming that 10% would not respond or have incomplete data.

Data were entered and analysed using the Statistical Package for Social Sciences (SPSS) version 20.0 for Windows (IBM Corp, New York, United States). The results were illustrated in tables and text by calculating the mean (M) and standard deviation (SD) for continuous variables, frequencies and percentages for categorical variables to describe the participants’ responses. T-test and Chi-square test were used to analyse continuous and categorical data, respectively. Bivariate analysis was applied with bottle-feeding practice as the dependent variable (user/non-user of bottle-feeding) and the other variables (e.g. child gender, age, birth order, education, residence (rural/urban), mode of delivery (vaginal/caesarean birth), breastfeeding education (received/not received), child hospitalization (yes/no)) as the independent variables. Furthermore, variables with a P-value of < 0.25 were entered in multivariable analysis to control confounding variables [32], [33].

Odds Ratio [OR], Adjusted Odds Ratio [AOR] (Backward LR) and 95% Confidence Interval [CI] were calculated and a variable with a P-value < 0.05 was considered as statistically significant.

Results

A total of 242 mother-child pairs participated in the study (Table 1). The M and SD of mothers' age and children's age was 27.13 (5.73) years and 12.2 (6.7) months, respectively. Maternal age ranged from 13 to 45 years, and 20/242 (8.3%) were ≤ 18 years. Child's order ranged from 1 to 9 (2.40 ± 1.42), and 70/242 (28.9%) mothers were primiparous. From the total, 96/242 (39.7%) used bottle-feeding during the first six months of their child's life, 99/242 (40.9%) lived in a rural area, 186/242 (76.9%) were housewives, and 164/242 (67.8%) had education less than secondary level.

Table 1: Socio-demographic characteristics of the studied participants in Kassala, Eastern Sudan (N = 242)

Variables		Total	Bottle feeding practice users (N=96)	Non-users (N=146)		
		Mean (SD)	Mean (SD)	Mean (SD)	Odds Ratio (95% Confidence Interval)	P-value
Maternal age, years		27.13 (5.73)	26.56 (5.63)	27.50(5.78)	0.97 (0.93, 1.02)	0.213
Birth order		2.40 (1.42)	2.41 (1.41)	2.40 (1.45)	1.01 (0.84, 1.20)	0.962
Number of children < 5 years		1.74 (0.73)	1.74 (0.73)	1.75 (0.73)	0.99 (0.69, 1.41)	0.942
Number of breastfeeding per day		7.31 (3.29)	7.59 (3.33)	7.12 (3.26)	1.04 (0.97, 1.13)	0.277
		N (%)	N (%)	N (%)	OR (95% CI)	*P*-value
Child gender	Male	131 (54.1)	51 (53.1)	80 (54.8)	1.07 (0.64, 1.79)	
	Female	111 (45.9)	45 (46.9)	66 (45.2)		0.799
Residence	Urban	143 (59.1)	61 (63.5)	82 (56.2)	1.36 (0.80, 2.31)	0.249
	Rural	99 (40.9)	35 (36.5)	64 (43.8)		
Living with extended family	Yes	125 (51.7)	51 (53.1)	74 (50.7)	0.91 (0.54, 1.52)	0.710
	No	117 (48.3)	45 (46.9)	72 (49.3)		
Mode of delivery	Caesarean delivery	42 (17.4)	20 (20.8)	22 (15.1)	1.48 (0.76, 2.90)	0.247
	Vaginal delivery	200 (82.6)	76 (79.2)	124 (84.9)		
Place of delivery	Institutional	132 (54.5)	52 (54.2)	80 (54.8)	1.03 (0.61, 1.72)	0.924
	Home	110 (45.5)	44 (45.8)	66 (45.2)		
Received breastfeeding education	No	99 (41.2)	49 (51.6)	50 (34.5)	2.02 (1.19, 3.43)	0.009
	Yes	141 (58.8)	46 (48.4)	95 (65.5)		
Faced breastfeeding difficulties	Yes	47 (19.8)	22 (23.9)	25 (17.2)	0.66 (0.35, 1.26)	0.291
	No	190 (80.2)	70 (76.1)	120 (82.8)		
Maternal education	< Secondary level	164 (67.8)	62 (64.6)	102 (69.9)	1.27 (0.74, 2.20)	0.390
	≥ Secondary level	78 (32.2)	34 (35.4)	44 (30.1)		
Paternal education	< Secondary level	138 (57.0)	56 (58.3)	82 (56.2)	0.92 (0.54, 1.54)	0.739
	≥ Secondary level	104 (43.0)	40 (41.7)	64 (43.8)		
Maternal medical disorders	Yes	20 (8.3)	8 (8.3)	12 (8.2)	0.99 (0.39, 2.51)	0.975
	No	222 (91.7)	88 (91.7)	134 (91.8)		
Maternal occupation	Housewives	186 (76.9)	71 (74.0)	115 (78.8)	1.31 (0.71, 2.39)	0.386
	Employed	56 (23.1)	25 (26.0)	31 (21.2)		
Paternal occupation	Governmental or private employed	121 (50.0)	51 (53.1)	70 (47.9)	0.81 (0.49, 1.36)	0.430
	Other than governmental or private employed	121 (50.0)	45 (46.9)	76 (52.1)		
Child hospitalization	Yes	79 33.3%	39 42.4%	40 27.6%	1.93 (1.11, 3.36)	0.018
	No	158 66.7%	53 57.6%	105 72.4%		
Weaned her child	Yes	54 (22.5)	20 (21.3)	34 (23.3)	1.12 (0.60, 2.10)	0.716
	No	186 (77.5)	74 (78.7)	112 (76.7)		

More than half of the mothers 132/242 (54.5%) of the children were institutional deliveries with a caesarean rate of 42/242 (17.4%), and 99/242 (41.2%) of the mothers did not receive breastfeeding education sessions.

Out of the 242 mothers, 54/242 (22.5%) had already weaned their children. The most common reasons mentioned by the mothers who had already weaned their children (N = 54), were pregnancy 11/54 (20.4%), appropriateness of the child's age for weaning 30/54 (55.5%), other reasons 13/54 (24.1%) such as child illness, mother illness, and return to work. Of those who did not wean yet (N = 188), 10/188 (5.4%) were planning to wean their children even before they reached the age of one year.

In multivariable analysis, urban residence (AOR 1.96, 95% CI 1.06, 3.63), not receiving breastfeeding education (AOR 1.92, 95% 1.07, 3.45) and child hospitalization (AOR 1.83, 95% CI 1.02, 3.28) were significantly associated with bottle-feeding during the first six months of the child's life (Table 2).

Table 2: Multivariable logistic regression analyses of factors associated with the use of bottle feeding among mothers with children aged between 6 to 24 months in Kassala, Eastern Sudan

Variables		Crude Odds Ratio (95% Confidence Interval (CI)	P-value	Adjusted Odds Ratio (95% CI)	P-value
Maternal age, years		0.96 (0.91, 1.01)	0.119	0.96 (0.91, 1.01)	0.111
Residence	Urban	2.03 (01.09, 3.79)	0.026	1.96 (1.06, 3.63)	0.032
	Rural (reference)				
Mode of delivery	Caesarean	1.80 (0.89, 3.64)	0.100	1.82 (0.90, 3.67)	0.095
	Vaginal (reference)				
Received breastfeeding education	No	1.83 (0.999, 3.33)	0.05	1.92 (1.07, 3.45)	0.029
	Yes (reference)				
Child hospitalization	Yes	1.84 (1.02, 3.29)	0.041	1.83 (1.02, 3.28)	0.042
	No (reference)				

Discussion

The usage of bottle-feeding in this study was 39.7% among all studied children. This is higher than the rates previously reported in central Sudan 20.5% [11], Nigeria 15% [19], Ethiopia 19.6% [26], Ghana 30.1% [34], and Namibia 35.7% [35]. Higher prevalence of bottle-feeding was reported in various studies, for example in Yemen 55% [36], and in Iraq 64% [12]. The high rates of bottle-feeding could be attributed to the degree of security instability in Eastern Sudan, or bottle-feeding experience gained in the past from donations (i.e. infant formula and other mother's milk substitutes) at the time of the previous humanitarian/refugee crisis in the area, and/or different methodologies as this is a community-based one. Therefore, in emergencies breastfeeding should be encouraged (i.e. psychosocial support) as much as possible and bottle-feeding should be avoided to save children's lives [37].

The current results showed that the risk of

bottle-feeding use amongst urban children was almost twice as much, compared to children in rural areas 1.96 (1.06, 3.63). In line with the current results, infants born to families residing in urban areas of Namibia [35], and Western Nepal [21], were at higher risk of bottle-feeding, 1.67 (1.26, 2.22) and 2.14 (1.37, 3.33), respectively. This could be attributed to the greater availability in urban areas of infant formulas at pharmacies as well as the promotion of these products by pharmaceutical companies through media, which is also abundant in urban areas. Therefore, the previous studies called for adoption and enforcement of the international code of marketing of breast-milk substitutes [17], [18]. Variations between rural and urban mothers regarding breastfeeding practices have been documented in many countries, including Sudan [38], [39]. Also, the work circumstances of mothers in urban areas are likely to motivate them to use bottle-feeding [22]. In particular, returning to work was documented as one of the weaning causes in the current study.

The results revealed that 99/242 (41.2%) of the mothers did not receive breastfeeding education sessions from healthcare personnel during pregnancy and/or after delivery, and these mothers had almost two times 1.92 (1.07, 3.45) the risk of bottle-feeding compared to mothers who received breastfeeding education. The prevalence of bottle-feeding among mothers who received and did not receive breastfeeding education were 46/141 (32%) and 49/99 (50%), respectively. This indicates that the prevalence of bottle-feeding practice is less likely to be among the breastfeed educated mothers by 18%. Previous studies have shown that breastfeeding education is effective in promoting exclusive breastfeeding and avoidance of bottle-feeding in different settings [14], [19]. Such education should be given to all mothers by healthcare workers to ensure reliability and most importantly, accuracy.

Furthermore, capacity-building regarding breastfeeding practices needs to be improved in Sudan, even among healthcare personnel [40]. Inadequate training of healthcare personnel was also reported in many other African countries including Ethiopia [41] and Nigeria [42]. Therefore, continuous breastfeeding education, ongoing support and encouragement from trusted family members or peers and healthcare personnel are essential for successful breastfeeding in future generations [43], [44].

In the present study, bottle-fed children were at higher risk of 1.83 (1.02, 3.28) of being hospitalised. Likewise, with the present results, previous studies [10], [26] documented the association between bottle-feeding and child morbidity. In Sudan, poor breastfeeding practice, including bottle-feeding has been associated with child morbidity and poor outcomes, i.e. deaths [2], [3], [4]. The risks of bottle-feeding for the children are as a result of contamination at any stage of food preparation, handling, storage and feeding [9], [24]. For example, among bottle-fed infants in Khartoum, 110 bacterial species including E. coli were isolated from bottle contents [45]. Even in certain circumstances where the bottle is used to deliver expressed mother's milk, there is still a risk from unsanitary methods of milk expression, with even worse consequences where fluid, other than expressed mother's milk, is delivered [9], [24]. Also, nipple confusion may happen when an infant has learned how to suck on the bottle and then struggles to adjust to sucking from the mother's breast [46]. Not only the contents of the bottle but also the material from which the bottle is made (e.g. plastic) can release toxic chemicals such as bisphenols, as it has been reported in recent studies including African countries (Cameroon and Nigeria) [47], [48]. Further research is required to overcome the limitations above and to investigate bottle content and composition (risk of exposure to bisphenols and other harmful substances).

The time at which bottle-feeding was introduced within the first six months and the reasons for bottle-feeding were reported to be addressed in the future intervention programs. Among the 96 mothers who introduced bottle-feeding in the first six months, it is clear that the first month 26/96 (27.1%), the fourth month 25/96 (26.1%), the fifth month 22/96 (22.9%), and other months 23/96 (23.9%) in descending order, were the most chosen times to introduce bottle-feeding, according to participant perception. Among the aforementioned 96 mothers, the most common reasons for bottle-feeding were insufficient breast milk 36/96 (37.5%), hot weather 20/96 (20.8%), maternal illness 14/96 (14.6%), work-related issues 12/96 (12.5%), child illness 9/96 (9.4%), and other reasons 5/96 (5.2%). The results of this study are in line with others in that the perception of insufficient mother's milk was reported by many authors as the main reason for bottle-feeding [13], [14]. Cultural reasons were also reported in the literature as mothers feel ashamed to breastfeed in front of strangers due to lack of privacy [14]. Identifying the reasons for bottle-feeding is of paramount importance for designing future breastfeeding education messages.

Unlike the current results, other factors such as maternal age [19], [26] mode of delivery [21], [49], parental education [11] and parents occupation [26] were reported to be significantly associated with bottle-feeding.

Our study tackled breastfeeding practices in an area which is characterised as a vulnerable area and provides valuable information which can be used to improve current breastfeeding practices. Our study has some limitations that need to be taken into consideration, including the possibility of recall bias. The study focused on one geographical area of Sudan (Kassala), so the results of this study cannot be generalised to the rest of the country. Moreover, the study failed to assess the feeding pattern of children

who were hospitalized and later died as the literature evidenced a strong correlation between bottle-feeding and child mortality.

In conclusion, the study showed high usage of bottle-feeding among mothers with children aged between 6 and 24 months in Kassala, Eastern Sudan. To avoid bottle-feeding and to improve child survival, urgent actions are required to support, promote, and educate all mothers regarding breastfeeding with special attention to those in urban residencies.

Ethics

The study was approved by the Research Board at the Faculty of Medicine, University of Gadarif, Sudan. Written informed consent was obtained from all the enrolled mothers.

Authors' contributions

AAH, ZT and IA designed the study and participated in the manuscript drafting. MAA, ZT and AAA collected the data. AAH, AAA and IA conducted the statistical analyses. All authors read and approved the final manuscript.

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Antisocial Personality Traits as a Risk Factor of Violence between Individuals with Mental Disorders

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Abstract

BACKGROUND: Mental disorder can increase the likelihood of taking violent acts of some individuals, but only a small percentage of violence in societies could be attributed to patients with mental health problems. For the past several years numerous studies related to forensic psychiatry has confirmed a close causal relationship between violent offenders and psychiatric comorbidity. Several studies have provided strong evidence that antisocial personality disorders (APD) represent a significant clinical risk for violence.

AIM: This study aims to show the relationship between antisocial personality disorder and antisocial personality traits with the other mental disorders and the manifestation of violence between the forensic populations of patients.

METHODS: The survey was conducted at the Psychiatric Hospitals and the Mental Health Centre. The research was carried out between two groups: one group of perpetrators of violence (PV) and a control group divided into two subgroups, a control group without violence (CG WV) and a group of respondents forcibly hospitalised CG FH. After obtaining consent for participation in the study, patients were interviewed, and questionnaires were applied. The research methodology included using measuring instrument-Psychopathy Checklist-revised (Hare's PCL-R).

RESULTS: The results show that in the group PV antisocial personality disorder is present in 45 patients, or 50% of the total sample. According to statistical research in between groups PV, CG WV and CG WV, there were determent significant differences in specifically listed items from Hare's PCL-R.

CONCLUSIONS: Psychopathological traits of mental disorders which are pathognomonic of committing violence are paranoid schizophrenia, as the most present and antisocial personality disorder in comorbidity, as the highest risk factor among the population with mental disorders that manifest violence.

Introduction

Mental disorder and violence

The myth that mental disorder by its very nature means the manifestation of violence persisted for centuries with the trend of intensifying of these beliefs even though in recent decades have made many campaigns to reduce fear in public [1]. There are no definitive answers to the questions of how the expression of violent behaviour is correlated with the nature of mental disorders under different circumstances or is associated with other developmental and life history variables. A mental disorder can increase the likelihood of taking violent acts of some individuals, but only a small percentage of violence in societies could be attributed to patients with mental health problems [2]. Ansis in his study found that 21 of 517 (4%) patients in the outpatient urban areas reported assassination attempts [3]. Psychiatric disorders that are associated with violence are ranging very widely and may include psychotic disorders, mood disorders, disorders of personality disorders and disorders associated with post-traumatic stress syndrome [4]. Elbogen & Johnson (2009) [5] used data from the National Epidemiological Survey on Alcohol and Related Conditions to prospectively identify risk factors for violent behaviour. They found that having a diagnosis of schizophrenia was not strongly associated with violent behaviour. In the Swanson's study, conducted

from 57 clinical sites across the United States, in the 6-month prevalence of any violence, the author found that, of 1,410 participants, 1,140 (81%) reported no violence, 219 (15%) reported only minor violence and 51 (4%) reported serious violence. Distinct, but overlapping, sets of risk factors were associated with minor and serious violence. Swanson reveals that the rate of violence linear increases with the number of diagnoses and concludes that mental disorder is a risk of violence among many others [6].

Comorbidity with personality disorder and violence

For the past several years numerous studies related to forensic psychiatry has confirmed a close causal relationship between violent offenders and psychiatric comorbidity [7]. Comorbidity in forensic psychiatry describes the co-occurrence of two or more conditions or psychiatric disorders known as dual diagnosis and defined by World Health Organization [8]. The majority of violent offenders have multiple psychiatric diagnoses. A high level of psychiatric comorbidity (50-90%) is associated with personality disorders [9], [10], [11], [12]. For the last few decades, forensic psychiatry is mainly concerned and focused on violent offenders with a history of psychiatric disorder, usually psychotic or personality disorder [13].

Several studies have provided strong evidence that antisocial personality disorders (APD) represent a significant clinical risk for violence. The relationship of greater risk for violence among persons with certain PD is in terms of four fundamental personality dimensions: 1) impulse control; 2) affect regulation; 3) threatened egotism or narcissism, and 4) paranoid cognitive personality style. Two of these dimensions-impulse control and affect regulation-are probably substantially affected by virtually all PDs linked to violence [14].

The main hypothesis of the study is that the manifestation of violence among people with mental disorders is not directly related to the diagnosis of severe mental disorder. The other hypothesis is that the violence caused by people with mental disorders is in direct correlation with comorbidity with an antisocial personality disorder or the presence of antisocial personality traits. This study aims to show the relationship between antisocial personality disorder and antisocial personality traits with the other mental disorders and the manifestation of violence between the forensic populations of patients.

Limitation of the study: The correlation between schizophrenia and schizophrenic disorders and antisocial personality disorders with criminal behaviour and manifestation of violence, was not followed in the continuum, but was confirmed. Research suggests that antisocial personality in adults and adolescents are the best to view as existing in a continuum. In our study, the selected participants were previously diagnosed as psychiatric patients in psychiatric hospitals. It opens up space for a deeper analysis of this connection, especially with some personal antisocial characteristics such as the most exposed

Subjects and Methods

It was a prospective study with a retrospective approach. The survey was conducted at the Psychiatric Hospital Demir Hisar, Psychiatric Hospital "Skopje" from Skopje and the Mental Health Centre in Prilep.

The timeframe in which the survey was conducted was between December 2016 to December 2017.

The study group consisted of 89 patients admitted to the Psychiatric Hospital Demir Hisar, most of the patients of the forensic psychiatric wards. These are patients who have committed crimes and who have been diagnosed by ICD 10. Based on this diagnosis and forensic expertise, the Court had determined the security measure "placement and treatment in a psychiatric institution." The survey excluded individuals admitted in the forensic psychiatric department with a diagnosis F 11 "drug addiction". After obtaining consent for participation in the study, patients were interviewed, and questionnaires were applied. The respondents of the study group were designated as perpetrators of violence (PV).

The control group consisted of 120 patients, most of the users of the Community Mental Health Center and some patients hospitalised at the Psychiatric Hospital Demir Hisar, which are not perpetrators of crime. The control group is divided into two subgroups. One control subgroup comprised 60 patients and in their history of illness, there were no records of violence. This group is referred to as a control group without violence CG WV. Another subgroup consists of 60 patients of the Psychiatric Hospital Demir Hisar who were forcibly hospitalised in the period from May 2016 to June 2017, which, according to sex and diagnosis of conditions responding to the survey. This subgroup is marked as control group involuntary hospitalised CG IH. The choice of respondents who are involuntarily hospitalized as a control group in this study was made, because that the act of involuntary hospitalization implies the existence of violence as one of the essential factors for the implementation of individuals with mental disorders to be admitted to hospital, as well as measures to control the threat of violence that could be forthcoming. This subgroup of patients is selected because it is an intention to show

whether there is a difference in the characteristics of patients who have already committed a crime and those in which there is a manifestation of violence in the form of aggressive behaviour, but they are not the perpetrator of the crime.

The research methodology included using the measuring instrument-Psychopathy Checklist-revised (Hare's PCL-R). The scale was created by Hare, RD in 1985 and formally published in 1991. It is a clinical assessment scale of psychopathy with 20 items. Each item refers to a different symptom or feature of a personality disorder. The closest equivalent to psychopathy in the APA guidebook is a condition called antisocial personality disorder. In a study published in 2013 in the Journal Assessment, a team of researchers from Florida State University compared the criteria for an antisocial personality disorder to the personality traits associated with psychopathy. These researchers concluded that the antisocial personality disorder definition captures many of the deviant or abnormal behaviours associated with psychopathy (Not 2013).

It is significant to note that this survey covered all forensic population placed on forensic departments in two psychiatric hospitals in Macedonia.

Results

To provide a detailed description, we used computations in which scores are presented as percentages, mean and medians. Determining the statistical significance of differences of continuous variables between the groups of patients was determined by the Pearson coefficient. We also combined ANOVA analysis. The level of statistical significance was ($p < 0.05$). Statistical analysis was conducted by software packages SPSS 15.0 and STATISTICA 8.0.

Table 1: Diagnostic structure of the patients

	PV		CG-WV		CG-IH	
ICD -10	N	%	N	%	N	%
F20.0	30	33.71	36	60.0	45	75.0
F21-25	14	15.73	18	30.0	8	13.33
F30	0	0	3	5.0	1	1.67
F31	0	0	0	0	0	0
F32	0	0	2	3.33	0	0
F32.3	0	0	1	1.67	0	0
F60.2	17	19.1	0	0	1	1.67
F60.2 F20.0	12	13.48	0	0	3	5.0
F60.2 F21	6	6.74	0	0	1	1.67
F60.2 F22	3	3.37	0	0	0	0
F60.2 F23	7	7.87	0	0	1	1.67
Total	89	100	60	100	60	100

Analysis of the structure of the patient's psychiatric diagnosis according to ICD 10, shows that in 19 (23%) patients in the study group (PV) were diagnosed with antisocial personality disorder (F60.2). In 28 (31%) patients were found double diagnosis, antisocial personality disorder in comorbidity with schizophrenia (F20) in 12 patients (13%), with transient acute psychotic disorder (F23) in 7 (7.9%) patients, with schizotypal disorder (F21) in 6 (6.7%), with delusional disorder (F22) in 3 (3%). These results show that in the study group – perpetrators of violence (PV)-antisocial personality disorder is present in 45 patients, or 50% of the total sample (Table 1).

Hare Psychopathy Checklist (PCL-R)

In the Hare Psychopathy Checklist (PCL-R) contains a group of items that are in direct correlation with the manifestation of violence It is evident that there is significant difference in the values of all variables that mark the disorder of personality (psychopathy) as a significantly higher in the perpetrators of the crime (PV) compared to two control groups CG WV and CG IH, except for the one variable "multiple, short-term marital relationships" (Table 2 and 3).

Table 2. Hare Psychopathy - 1 (PCL-R)

Hare Psychopathy Checklist	PV N (%)	CG WV N (%)	CG IH N (%)	P*
1. Glibness/superficial charm				
0	33 (37.08%)	47 (78.33%)	45 (75.0%)	PV/CG WV
1	33 (37.08%)	10 (16.67 %)	10 (16.67%)	P = 0.000003
2	23 (25.84%)	3 (5.0%)	5 (8.33%)	PV/CG FH
Total	89	60	60	P = 0.0000029
2. Grandiose Sense of Self Worth				
0	13 (14.61%)	32 (53.33%)	17 (28.33%)	PV/CG WV
1	46 (51.69%)	17 (28.33%)	20 (33.33%)	P = 0.0000029
2	30 (33.71%)	11 (18.33%)	23 (38.33%)	PV/CG FH
Total	89	60	60	P = 0.043
3. Need for Stimulation/Proneness to Boredom				
0	54 (60.67%)	14 (23.33%)	21 (35.0%)	PV/CG WV
1	23 (25.84%)	17 (28.33%)	33 (55.0%)	P = 0.0000015
2	12 (13.48%)	29 (48.33%)	6 (10.0%)	PV/CG FH
Total	89	60	60	P = 0.0014
4. Pathological lying				
0	31 (34.83%)	58 (96.67%)	48 (80.0%)	PV/CG WV
1	43 (48.31%)	2 (3.33%)	11 (18.33%)	P = 0.000000
2	15 (16.85%)	0	1 (1.67%)	PV/CG FH
Total	89	60	60	P = 0.0000025
5. Conning/Manipulative				
0	29 (32.58%)	55 (91.67%)	48 (80.0%)	PV/CG WV
1	37 (41.57%)	5 (8.33%)	9 (15.0%)	P = 0.000000
2	23 (25.84%)	0	3 (5.0%)	PV/CG FH
Total	89	60	60	P = 0.000000
6. Lack of remorse of Guilt				
0	5 (5. 62%)	40 (66.67%)	10 (16.67%)	PV/CG WV
1	14 (15.73%)	20 (33.33%)	36 (60.0%)	P = 0.000000
2	70 (78.65%)	0	14 (23.33%)	PV/CG FH
Total	89	60	60	P = 0.000000
7. Shallow affect				
0	1 (1.12%)	13 (21.67%)	4 (6.67%)	PV/CG WV
1	19 (21.35%)	43 (71.67%)	44 (73.33%)	P = 0.000000
2	69 (77.53%)	4 (6.67%)	12 (20.0%)	PV/CG FH
Total	89	60	60	P = 0.000000
8. Callous/Lack of empathy				
0	4 (4.49%)	36 (60.0%)	10 (16.67%)	PV/CG WV
1	26 (29.21%)	24 (40.0%)	44 (73.33%)	P = 0.000000
2	59 (66.29%)	0	6 (10.0%)	PV/CG FH
Total	89	60	60	P = 0.000000
9. Parasitic Lifestyle				
0	23 (25.84%)	48 (80.0%)	37 (61.67%)	PV/CG WV
1	31 (34.83%)	11 (18.33%)	21 (35.0%)	P = 0.000000
2	35 (39.33%)	1 (1.67%)	2 (3.33%)	PV/CG FH
Total	89	60	60	P = 0.000000
10. Poor behavioural control				
0	3 (3.37%)	37 (61.67%)	5 (8.33%)	PV/CG WV
1	20 (22.47%)	22 (36.67%)	45 (75.0%)	P = 0.000000
2	66 (74.16%)	1 (1.67%)	10 (16.67%)	PV/CG FH
Total	89	60	60	P = 0.000000

Participants from PV group with highly significant ($p < 0.001$) less compared to respondents from the two control groups need stimulation, or propensity to apathy The tested difference in the distribution of possible responses to the symptom

"lack of remorse or guilt" among groups PV and CG WV is highly statistically significant ($p < 0.001$), due to the significantly more common frequency of occurrence of this symptom of the disordered personality among respondents perpetrators of a crime (Table 2).

Cruelty and lack of empathy highly significantly more often ($p < 0.001$) were registered among respondents perpetrators of a crime. Also, respondents perpetrators of a crime are characterised by high significance ($p < 0.001$) as compared with the participants of the two control groups in terms of 10 item scale of analysis concerning the "weak control behaviour" (Table 2).

Tested differences in the distribution of possible responses to early behavioural problems among groups PV and CG WV is highly statistically significant, in the level of $p < 0.001$, due to the significantly more frequent early behavioural problems in the group of surveyed, perpetrators of criminal work (Table 3).

Table 3: Hare Psychopathy – 2 (PCL-R)

Hare Psychopathy Checklist	PV N (%)	CG WV N (%)	CG IH N (%)	P*
11. Promiscuous Sexual Behavior				
0	63 (70.79%)	55 (91.67%)	54 (90. 0%)	PV/CG WV
1	12 (13.48%)	1 (1.67%)	6 (10.0%)	P = 0.0063
2	14 (15.73%)	4 (6.67%)	0	PV/CG FH
Total	89	60	60	P = 0.0032
12. Early Behavioral Problems				
0	33 (37.08%)	52 (86.67%)	30 (50.0%)	PV/CG WV
1	23 (25.84%)	8 (13.33%)	25 (41.67%)	P = 0.000000
2	33 (37.08%)	0	5 (8.33%)	PV/CG FH
Total	89	60	60	P = 0.00036
13. Lack of Realistic Long-term Goals				
0	12 (13.48%)	24 (40.0%)	7 (11.67%)	PV/CG WV
1	23 (25.84%)	33 (55.0%)	46 (76.67%)	P = 0.000000
2	54 (60.67%)	3 (5.0%)	7 (11.67%)	PV/CG FH
Total	89	60	60	P = 0.000000
14. Impulsivity				
0	7 (7.87%)	44 (73.33%)	6 (10.0%)	PV/CG WV
1	25 (28.09%)	16 (26.67%)	46 (76.67%)	P = 0.000000
2	57 (64.04%)	0	8 (13.33%)	PV/CG FH
Total	89	60	60	P = 0.000000
15. Irresponsibility				
0	8 (8.99%)	39 (65.0%)	16 (26.67%)	PV/CG WV
1	29 (32.58%)	21 (35.0%)	37 (61.67%)	P = 0.000000
2	52 (58.43%)	0	7 (11.67%)	PV/CG FH
Total	89	60	60	P = 0.000000
16. Failure to Accept Responsibility				
0	4 (4.49%)	32 (53.33%)	8 (13.33%)	PV/CG WV
1	21 (23.6%)	28 (46.67%)	42 (70.0%)	P = 0.0063
2	64 (71.91%)	0	10 (16.67%)	PV/CG FH
Total	89	60	60	P = 0.0032
17. Marry Short Term Marital Relationships				
0	83 (93.26%)	59 (98.33%)	59 (98.33%)	PV/CG WV
1	2 (2.25%)	0	1 (1.67%)	P > 0.05
2	4 (4.49%)	1 (1.67%)	0	PV/CG FH
Total	89	60	60	P > 0.05
18. Juvenile Delinquency				
0	70 (78.65%)	60 (100%)	56 (93.33%)	PV/CG WV
1	4 (4.49%)	0	2 (3.33%)	P = 0.00065
2	15 (16.85%)	0	2 (3.33%)	PV/CG FH
Total	89	60	60	P = 0.034
19. Revocation of Conditional Release				
0	65 (73.03%)	60 (100%)	55 (91.67%)	PV/CG WV
1	8 (8.99%)	0	3 (5.0%)	P = 0.000065
2	16 (17.98%)	0	2 (3.33%)	PV/CG FH
Total	89	60	60	P = 0.013
20. Criminal Versatility				
0	0	55 (91. 67%)	2 (3.33%)	PV/CG WV
1	31 (34. 83%)	5 (8. 33%)	51 (85.0%)	P = 0.000000
2	58 (65. 17%)	0	7 (11.67%)	PV/CG FH
Total	89	60	60	P = 0.000000

Individuals with mental disorders who have committed crimes significantly more likely than respondents without violence and those involuntarily hospitalised, are characterised by impulsiveness in response (Table 2-part two). These high values of items that are in direct correlation+96/8 with the manifestation of violence confirm the connection between mental disorder and antisocial personality disorder as a mutual relationship which is the basis for violent acts. In PCL-Hare items, with statistical significance dominates the value of 2 (applies fully) in the subjects of the study group in a percentage much higher than the 31%, which is a representation of the entire sample of antisocial personality disorder. This frequency is even greater than 50%. Out of 21 items in PCL-Hare, in 14 (66%) item the rates is over 31% of representations of the traits that are characteristic of psychopaths, at the group of the perpetrators of violence (PV). According to our statistical research in between groups: PV (Perpetrators of violence), CG WV (control group-without violence) and CG WV (control group-without violence) we determent significant differences ($p < 0.05$) in a high rate (more than 50%) especially represented in the items listed in Table 4.

Table 4. **Database for different groups of items**

Items		PV N (%)		CG WV N (%)		CG IH N (%)	
Lack of remorse or guilt-		70 (78.65%)		0		14 (23.33%)	
Poor behavior control		66 (74.16%)		1 (1. 67%)		10 (16.67%)	
Failure to accept responsibility		64 (71.91%)		0		10 (16.67%)	
Callous lack empathy		59 (66.29%)		0		6 (10.0%)	
Criminal versatility		58 (65.17%)		0		7 (11.67%)	
Impulsivity		57 (64.04%)		0		8 (13.33%)	
Lack of realistic, long term plans		54 (60.67%)		3 (5.0%)		7 (11.67%)	
Groups	*Count*	*Sum*	*Average*	*Variance*			
70	6	292	48.66667	579.0667			
0	6	3	0.5	1.5			
14	6	31	5.166667	17.76667			
ANOVA							
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*	
Between Groups	8468.111	2	4234.056	21.22925	4.22E-05	3.68232	
Within Groups	2991.667	15	199.4444				
Total	11459.78	17					

Discussion

The results show that in the diagnostic structure in a violent forensic population that has committed a crime, located in psychiatric hospitals in Macedonia dominated comorbidity between schizophrenia and schizophrenic disorder and an antisocial personality disorder. Schizophrenia and antisocial personality, as autonomous disorder have been associated with a higher risk of violence. Despite a large number of studies examining the link between schizophrenia and its most prominent symptoms, the involvement of the manifestation of violent remains unclear [6], [15]. This raises the question of the impact of personal antisocial characteristics on a person who has schizophrenia and how this interdependence fosters violent behaviour. Numerous studies in the field of forensic psychiatry have confirmed a close causal relationship between the violent offender and comorbid psychiatric disorder [16], [17]. It confirms that the comorbidity has a significant influence in

clinical outcome, criminal relapse, on detention rate and length of detention [18], [9]. Schizophrenia and antisocial personality disorder are both characterised by impulsive, poorly planned behaviour. This behaviour may originate from a weak or poorly coordinated response inhibition system [19]. Comorbidity influences the assessment of criminal responsibility but may also affect the outcome of treatment and risk of relapse. The criminal activity of this comorbidity is interpreted as a result, among other things, of the fact that antisocial personality traits are regarded as being almost untreatable. Antisocial personality disorder presents a general pattern of disregard for and violation of the rights of others. Individuals with antisocial personality disorders lack insight into their disorder [20]. Psychotic who commit violent behaviours can be reincorporated into society once they are receiving medication and attended to since they immediately stop being dangerous. The same doesn't occur with psychopaths or antisocial personality disorders [21]. Although those individuals with antisocial personality disorders clearly could have been compulsorily treated very few were. Indeed, in Peay's study, compulsory admissions during the year 2007-8 there were 9995 admissions for those with mental disorders illness and only 147 for those with antisocial personality disorders [22].

The analysis of the results obtained by using the PCL HARE on forensic population placed in psychiatric hospitals in the country showed a significantly greater representation of the characteristics of antisocial personality disorder among individuals with mental disorders who are perpetrators of a crime, in terms of psychiatric patients who manifested violent behaviour. It confirmed that individuals with antisocial personality disorder in comorbidity with mental disorders are more criminally active than other perpetrators of violent acts [20], [23]. They often use psychological defence mechanisms like projection, denial, projective identification, and omnipotent ion, splitting, which are very early and primitive defence mechanisms that lead to disintegration [24]. The high values of the items "poor control behaviour" and "impulsivity" also suggest that the common denominator of APD – associated violence is anger. This is an emotion that is expressed with rage, resentment and irritability. Anger can be considered as a part of the neuropsychological response to a threat or perceived harm [14]. From the dimensional point of view, those antisocial personality traits having the greatest tendency towards violence are impulsiveness deficient affective regulation, narcissism and paranoid [25].

Identification and management of these psychological manifestations are extremely important in everyday clinical practice for the safety of the wider environment, but also the diagnosis and treatment planning [26]. That is confirmed by the findings in the survey. Analysing the items in the study, we can conclude that the most prominent items from the scale of PCL R in the perpetrators of crimes are those belonging to the emotional facet 2.

In conclusion, psychopathological traits of mental disorders which are pathognomonic of committing violence are paranoid schizophrenia, as the most present compared to other mental disorders and antisocial personality disorder, in comorbidity with paranoid schizophrenia is confirmed as the highest risk factor among the population with mental disorders that manifest violence. Personal traits of the individuals with mental disorders that correlate violent behavior are antisocial personality traits that are acknowledged as the highest risk factor among the population with mental disorders that manifest violence, sociopath orientation with inclination towards outsourcing of aggressive impulses through criminally behavior, defects in the moral sphere, with reduced feelings of guilt and remorse about past events and volatility in mutual relations. It confirms the conclusion that the diagnosis of schizophrenia itself does not constitute factors with risk of violence, but with statistically significant correlation with other factors is an important clinical indicator of violence [27].

This study opens the question of the relationship between mental disorders in violent behaviour. Many of the factors that are associated most with violent behaviour and people with a mental health condition, such as antisocial personality traits, antisocial behaviour or anger are predictors of significant violence among subjects without mental disorders so that the independent effect of the mental disease and violence is not clear [28].

The identification and management of antisocial personality characteristics as well as specialised treatments for specific clinical correlates (e.g. specialised treatment of impulsivity), in addition to the treatment of mental disorder are extremely important in everyday clinical practice, the safety of the wider environment, but also because of the management and planning of treatment.

Continuous medication, social support, the non-stress environment may, in significant part to control these symptoms.

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Review Article

A Review of Pancreatic Cancer: Epidemiology, Genetics, Screening, and Management

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Abstract

Pancreatic cancer ranks among the causes of cancer-related deaths. The average size of pancreatic cancer during diagnosis is about 31 mm and has not changed significantly over the past 30 years. Poor early diagnosis of a tumour has been attributed to the late-presenting symptoms. Over the years, improvement in the diagnosis of pancreatic cancer has been observed, and this can be linked to advancement in imaging techniques as well as the increasing knowledge of cancer history and genetics. Magnetic Resonance Imaging, Endoscopic Ultrasound, and Computer Topography are the approved imaging modalities utilised in the diagnosing of pancreatic cancer. Over the years, the management of patients with pancreatic cancer has seen remarkable improvement as reliable techniques can now be harnessed and implemented in determining the resectability of cancer. However, only about 10% of pancreatic adenocarcinomas are resectable at the time of diagnosis and will highly benefit from a microscopic margin-negative surgical resection. Overall, the failure of early tumour identification will result in considerable morbidity and mortality.

Introduction

Pancreatic cancer in recent years has been one of the deadliest with an increased mortality rate of about 3% of all cancers and about 7% of all cancer death in the United States and Europe with an estimated five-year survival rate [1].

The estimated number of people expected to be diagnosed with pancreatic cancer in 2018 is about 55,440 (29,200 men and 26,240 women), and about 44,330 (23,020 men and 21,310 women) will die of pancreatic cancer [2].

Several factors have contributed to an increased risk of pancreatic cancer. Such risk factors vary from; tobacco use, overweight, obesity, workplace exposure to certain chemicals (benzene, petrochemicals, dyes, and pesticides), age, gender, race, family history, inherited genetic syndromes, diabetes, chronic pancreatitis, cirrhosis of the liver, stomach problems, diets, physical in-activities, coffee and alcohol [2].

The signs and symptoms vary due to the location and the stage of a tumour. The tumours located at the head of the pancreas cause obstructive jaundice and weight loss, which occur as a result of steatorrhea and diarrhoea. While tumours of the body and tail usually lead to abdominal pain and weight loss. Pain is also frequently associated with pancreatic cancer. The pain usually presents as a dull, deep pain, coming from the upper abdomen, radiating to the back [3].

Different case studies have shown that patients in the early stages with tumour size less than 3 cm without lymphatic metastasis have a better prognosis with a 5-year survival rate of up to 25-30%

following surgical resection of a tumour. This result suggests that early detection is essential for the treatment and management of the tumour [4].

Also, advancement in diagnostic Imaging has paved the way in dictating underlining internal diseases which do not present with pain at the onset as observed in pancreatic cancers. Several imaging modalities have been used over the years for the diagnosis of different cancers [5]. This review looks at the epidemiology, genetics, screening and the management of Pancreatic cancer.

Epidemiology of Pancreatic Cancer

The epidemiological study of pancreatic cancer and the rate of its occurrence from 2005 to 2014 showed stable rates in women with a decline of approximately 2% annually in men. The cancer death rate from 2006 to 2015 had a 1.5% decrease in its annual report for both men and women. The combined cancer death rate fell continuously from 1991 to 2015 by a total of 26%, translating to approximately 2,378,600 fewer cancer deaths than would have been expected if death rates had remained at their peak [6].

Incidence

The incidence of pancreatic tumour varies from one geographical population to another. In every 10000, about 7.4 are affected by the tumor in both Western Europe and North America. Other developed countries such as New Zealand and Australia have about 6.5 per 100000 affected with the tumor. Lower incidence of approximately 1.0 in every 100000 is observed in developing countries in Africa and south-central Asia [2].

Pancreatic cancer also varies by gender in various geographical regions. The occurrence rate of pancreatic cancer among men in 2012 was 4.9 per 100000 and 3.6 per 100000 in women. The risk of developing pancreatic cancer in men was high in Armenia (11.9) and Czech Republic (11.8), Slovakia and Hungary (equally-11.5), then in Japan and Lithuania (equally-10.6). However, the risk of having pancreatic cancer in men was lowest in Guinea (0.4) and Pakistan (0.5). The incidence of pancreatic cancer in women is higher in developed countries. The risk of developing the tumour is lowest in Polynesia and central Africa (equaling-1.0), while the risk is higher in Hungary (5.9), Denmark (5.9), Finland (6.2) and Armenia (6.1) [7].

Europe and North America have 33% of the overall occurrence. This reflects on the accuracy of the diagnosis rather than the aetiology. The differences in incidence around the world have to do with quality in the data collected [6]. The incidence of pancreatic cancer around the world is summarised in Figure 1.

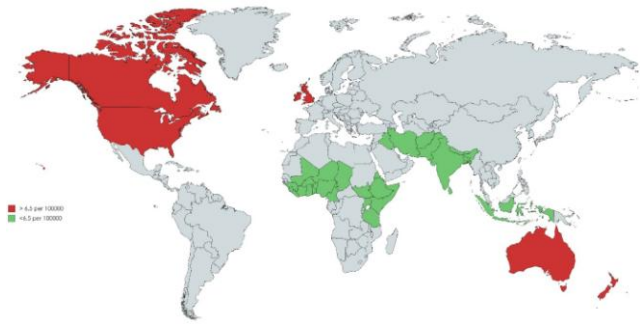

Figure 1: The figure showing the Incidence of pancreatic cancer around the world

The overall worldview of pancreatic cancer is showing the incidence rate. The incidence rate is more pronounced in developed countries than in the less developed countries.

The reduction in mortality incidence, when compared to past years, has been due to the improvement in medical care. Improved medical care showered significant positive effects on cancer treatment and management yet leaving some loopholes for some cancers such as pancreatic cancer [7]. Table 1 relates pancreatic cancer to other common cancers in the United States.

Table 1: Relation of pancreatic cancer to the common types of cancers that affect the American population with the estimated new cases and deaths for 2018

Rank	Common Types of Cancer	Estimated New Cases 2018	Estimated Deaths 2018
1.	Breast Cancer (Female)	266,120	40,920
2.	Lung and Bronchus Cancer	234,030	154,050
3.	Prostate Cancer	164,690	29,430
4.	Colorectal Cancer	140,250	50,630
5.	Melanoma of the Skin	91,270	9,320
6.	Bladder Cancer	81,190	17,240
7.	Non-Hodgkin Lymphoma	74,680	19,910
8.	Kidney and Renal Pelvis Cancer	65,340	14,970
9.	Uterine Cancer	63,230	11,350
10.	Leukemia	60,300	24,370
11.	Pancreatic Cancer	55,440	44,330

The table shows the relation of pancreatic cancer to the common types of cancers that affect the American population with the estimated new cases and deaths for 2018 [6].

Despite the improvement in the treatments of cancers in general, pancreatic cancer remains one of the deadliest cancers to date with high mortality as shown in Figure 2. For 2018 projection, new cases were estimated at 55,440 (3.2%) and 44,330 (7.3%) estimated deaths. The most predominant types of cancers are more common in comparison to pancreatic cancer. However, the late discovery of pancreatic cancer makes treatment and management challenging [6].

Survival Rate

Survival rate helps to generally estimate life expectancy after diagnosing cancer from the available data. It aids the comparison of patients diagnosed with cancer and the survival of people in the general

population within the same age, sex, and race who have not been diagnosed with cancer.

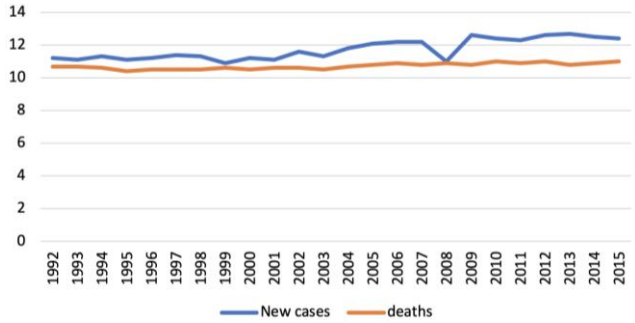

Figure 2: New incidence of pancreatic cancer was at 12.6 per 100,000 men and women annually. The number of mortality was 10.9 per 100,000 men and women annually. These rates are age-adjusted and based on 2011-2015 incidence and mortality

It's also important to note that survival statistics are based on large groups of people which obstruct the use of it to predict an individual status. No two patients are entirely alike, and treatment and responses to treatment can vary greatly [6]. The 5-year relative survival rate about the cancer staging is shown in Figure 3.

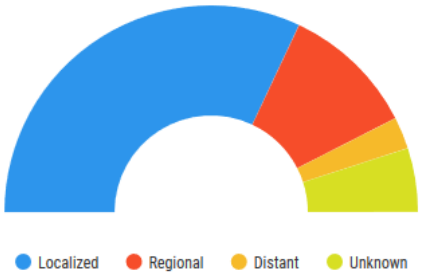

Figure 3: Five Years Relative Survival. The figure is showing a 5-year relative survival rate about the staging of cancer. The staging is broken down into Localized, Regional, Distant and Unknown.

Cases by Stage

Cancer staging during diagnosis refers to the extent of metastasis in the body. This helps to determine treatment options and life expectancy. When cancers do not undergo metastasis, it is known as localised cancer (stage 1). The spread of cancer to part of the body makes it regional or distant (stage II-IV).

The earlier a pancreatic cancer is identified, the higher the chance of survival rate for the five-year interval after being diagnosed. Only about 10.0% of pancreatic cancers are diagnosed at the local stage, and the 5-year survival rate for localised pancreatic cancer is 34.3% [6]. Figure 4 shows the staging of pancreatic cancers at the time of diagnosis.

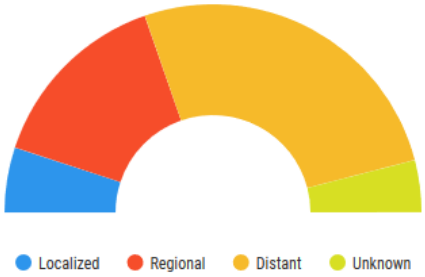

Figure 4: Percent of Case by Stage. The figure shows the staging of pancreatic cancer in the American population from 2008-2014 with all races, and both exes indicated

Prevalence in Gender, Race, and Ethnicity

The risk of Pancreatic cancer increases with age and more common in men than women. The number of new cases of pancreatic cancer was 12.6 per 100,000 for men and women annually based on 2011-2015 cases. Future predictions of pancreatic cancer at some point in some adults stands at 1.6 per cent according to the 2013-2015 data. In 2015, 68,615 people were estimated to be living with pancreatic cancer in the United States [6].

The period of 2014-2015 however, showed a decline in the occurrence of cancer in comparison to previous years. In 2015, the cancer mortality rate increased by 14% in non-Hispanic African Americans than non-Hispanic Caucasians overall. The mortality rate ratio was 1.14; with a 95% confidence interval. But the racial disparity was much larger for individuals aged < 65 years with the mortality rate ratio as 1.31 and the confidence interval at 95%, compared with those aged ≥ 65 years with mortality rate ratio as 1.07 and the confidence interval at 95%, 1.06-1.09 [6]. The comparison between different races and gender about new incidence and mortality has been summarised in Table 2 below.

Table 2: The table compares different race and gender about new incidence and mortality

	MALE		FEMALE	
	Incidence	Deaths	Incidence	Deaths
All Race	14.4	12.6	11.2	9.5
Caucasian	14.4	12.6	11.1	9.4
African descent	16.9	14.8	14.3	12.2
Asian/Pacific Islander	11.0	8.3	9.2	7.3
American Indian/Alaska Native	11.3	9.7	7.8	8.0
Hispanic	12.0	9.5	10.5	7.7
Non-Hispanic	14.7	12.9	11.3	9.7

This shows every new case and death per 100,000 persons in the population at the time of the census [6].

Genetics of Pancreatic Cancer

Genetics has become a vital aspect in the early detection of pancreatic cancer. The genetics of pancreatic cancer is classified into two major origins,

the exocrine pancreas, and the neuroendocrine pancreas. Among these two origins, 85% of cases seen is from the exocrine pancreatic origin which is the pancreatic ductal adenocarcinoma [3].

Genes like *Kras, CDKN2A, TP53, SMAD4* have been implicated in most cases of pancreatic cancer [3], the understanding of this main genes has given insight into the diagnosis and treatment of pancreatic cancer. However, the main driver genes for pancreatic tumour; *KRAS* (90%), *CDKN2A* (90%), *TP53* (70%), *SMAD4* (55%) undergo different mutations that give rise to carcinogenesis of a pancreatic tumour [1].

Furthermore, Roboslit pathway (5%), Notch signalling (5%), WNT (10%), chromatin (20%), DNA repair (17%), cell cycle processing (15%) are the minor pathways implicated in pancreatic cancer [8].

Kras gene is responsible for 90% of most pancreatic cancer cases. RAS protein is responsible for cell differentiation and proliferation by sending the signals for cell differentiation. The RAS protein binds to GTP in G coupled receptor and gives the signal for the hydrolyses of GTP to GDP resulting in other downstream signals for uncontrolled proliferation and growth. The mutation in *RAS* gene makes the gene bind to GTP simultaneously, and signals are given at the cellular level for uncontrolled proliferation [9].

TP53 is a tumour suppressor gene involved in cell cycle, the inactivation of this gene by point mutation causes several changes in the cell cycle. This causes several cell cycle check points to be bypassed, thus inducing gene mutations and hence cancer formation. P53 also plays important roles in apoptosis by mostly arresting cells in the G1-S phase [10].

The normal function of the p53 gene is to bind to other genes like miRNA34a which codes for p21 [4]. P21 is a protein that acts as a signal for the shutdown of DNA replication. Hence, mutation to p53 results in the inactivation of the p21 gene and results in uncontrolled growth and proliferation [1].

CDKN2A is a tumour suppressor gene for regulating G1-S phase of the cell cycle in a pancreatic tumour. When the *CDKN2A* gene undergoes inactivation, it leads to unregulated and uncontrolled growth and differentiation [1].

SMAD4 is a tumour suppressor gene which activates the attachments of TGFb immediately to cell surface receptors. This sends signals into the nucleus to turn on the *SMAD4* gene to attach themselves to other protein to regulate and control the growth and proliferation in specific areas of the DNA. Mutation in this gene causes uncontrolled proliferation and growth which gives rise to pancreatic cancer [1].

Studies have shown that targeting the *Kras* axis eliminates cancer cells and pancreatic tumour formation, so the *RAS* gene is the major contributing factor to pancreatic tumour formation [10].

A recent study has shown that *CCAT2* gene which is a long non-coding RNA is the oncogene in the development of pancreatic ductal adenocarcinoma (PDAC). A total of 80 human PDAC tissues and 3 PDAC cell lines were assayed, and it was shown that there was more *CCAT2* expression in the PDAC cell lines compared to the normal pancreatic tissues [8].

Studies have also shown that out of all the genes that are responsible for pancreatic cancer, the *SMAD4/DPC4* gene is a good marker of metastasis [11]. The study showed that 641 patients showed *DPC4/SMAD4* correlation with overall survival and recurrence patterns. The inactivation or loss of this gene has caused uncontrolled differentiation and metastatic development seen in pancreatic cancer [11].

Epigenetics

There are other genetic mechanisms different from the *Kras* mutation or the tumour suppressor deletion; these other mechanisms are also useful in the therapeutic management of PDAC [9].

DNA methylation is one of the mechanisms that inactivates suppressor genes. These genes do not undergo any mutation, but the cellular level methyl groups are added to carbon 5 of the pyrimidine ring which silences the gene [10]. Recent studies have shown that multiple genes are silenced or methylated in 45 pancreatic carcinomas. It was analysed that *RARb, p16, CACNAIG, TIMP-3, Ecad, THBSI, Hmlh1, DAPkinase, MINT31* are genes seen in pancreatic cancer [12]. Overexpression of EGF, EGF-R, HER-2/neu, and p185 has also been found to be common in pancreatic tumours of advanced stages [13].

It has been observed that some micro-RNAs are deregulated in some pancreatic ductal adenocarcinomas. MiR-21, for instance, is overexpressed in 20 pancreatic carcinoma tissues and cell lines compared to normal tissue or cell lines [14].

Most pancreatic neuroendocrine tumours show great phenotypic and genotypic heterogenicity, they also occur sporadically or as familial tumours in association with other familial diseases like multiple endocrine neoplasia types 1 (MEN1), Von Hippel Lindau disease (VHL) or tuberous sclerosis [15], [16].

In a research carried out by Yuchen Jiao *et al.*, in determining the genetic basis of PANnet resulted in the discovery that 44% of the tumours had somatic mutations in the *MEN1* gene, 43% had mutation in the *DAXX* (death domain-associated protein) and *ATRX* gene; they also found a 14% mutation in the *mTOR* gene [17].

PHLDA3b which is a tumour suppressor has also been implicated in the formation of PANnet, the

loss of heterozygosity at high frequency has been showed to lead to the development of PANnet, methylation of this gene has been implicated in the generation of PANnet [18].

Aberrant hypermethylation of 11 tumour suppressor genes were detected in PANnet, this gene includes *RASSFIA* (75%), *ink4a/p16* (40%), *OMGMT* (40%), *O-MGMT* (40%), *RAR-B* (25%), *hMLHI* (23%), *TIMP3, GSTπ, E-cadherin, P14ARF, APC*, the aberrant hypermethylation of this gene has been associated with advanced tumor stage of pancreatic neuroendocrine tumors [19].

Screening Modalities

Several types of modalities exist in Diagnostic Imaging. These modalities act as a benchmark in diagnosing pancreatic cancer. The different types of imaging vary from computed tomography (CT), magnetic resonance imaging (MRI), Positron-emission tomography (PET), Ultrasound and Nuclear scans [5].

Computed tomography is the combination of several x-ray pictures taking at the same time from different angles to produce a 3-dimensional image of the region been exposed to the CT machine. This creates an all-around view of the internal organs and structures for better diagnosis and analysis [20].

Based on available literature, CT has mostly been used in determining the staging of pancreatic cancer. However, it has been observed to underestimate the spread of cancer which results in the need for invasive surgeries or other imaging techniques to determine the extent of cancer. The combination of other imagines solutions have been proven to be more efficient when used together with the CT scan [21].

The most used combined imaging technique in the diagnosis of pancreatic cancer is the CT Scan and Positron Emission Tomography (PET Scan). The PET Scan utilises nuclear medicine in observing the metabolic processes in the body [22].

This system utilises gamma rays emitted indirectly by a positron-emitting radionuclide (tracer). The biologically active molecules used during PET scan examination aid in the visualisation of interesting areas in 3-dimension by reconstructing the image with computer analysis [23].

The molecule mostly used clinically is Fludeoxyglucose (FDG), an analogue of glucose. The concentration of this biomarker helps in showing the metabolic rate of tissues which in most cases indicates high metastasis in the presence of cancer [24].

Another imaging modality which is safer than the CT and PET scan relation to radiation emitted during the scanning process is Magnetic Resonance Imaging (MRI). It is used in conditions where the data for nonrigid motion characterised as tumour and organs will be at risk of radiation therapy. It also helps in identifying soft tissues such as blood vessels about tumour growth. The image produced is also in 4 dimensions like that of CT scan with some structural changes [25].

In an observation carried out in Karolinska University Hospital between 2010 and 2013, using an MRI procedure. All patients with the genetic risk associated with pancreatic mutations were checked [25]. This gave a clearer understanding of the MRI potential in identifying precancerous or early cancers in individuals at risk for pancreatic cancer. Based on the study it showed how effective the procedure and protocol used was in the early dictation of cancer [25].

Endoscopic Ultrasound (EUS) is the most accurate form of ultrasound useful in the diagnosis of pancreatic cancer. This imaging modality is achieved by a small ultrasound probe on the tip of an endoscope, which is a thin and flexible tube used in looking inside the digestive tract [26].

This procedure can be done in place of having a large opened incision to explore the extent of cancer. The procedure involves the probe being passed through the mouth down to the first part of the small intestine. It is then pointed towards the pancreas to view the extent of the tumour and take a biopsy for confirmation [26].

Factors that Affects Screening

Pancreatic cancer screening mainly focuses on people with an increased risk of developing the disease. Some of these risk factors include; smoking, diet, diabetes mellitus, obesity [2]. Individuals in the population with a family history of pancreatic cancer are also at risk; some genetic syndromes also pose risk factors of pancreatic cancer [2].

Screening is recommended for individuals considered to be at high risk of developing pancreatic cancer, individuals with > 5% lifetime risk [27]. The family history is the main tool used to determine pancreatic cancer risk; the number of affected family members and the relationships among the individuals (especially first-degree relatives) at risk is used as the basis for risk assessment [28]. The incidence of pancreatic cancer as relating to the number of affected first-degree relatives is summarised in Table 3.

Table 3: Table shows the incidence of pancreatic cancer by some affected first-degree relatives [33]

First degree relatives	Incidence ratio	Incidence per 10^4
1	4.5 x	41
2	6.4 x	58
> 3	32.0 x	288

The chances of occurrence of pancreatic cancer in an individual increase with the number of family members with pancreatic cancer. Familial

pancreatic cancer (FPC) is defined as having 2 or more of first-degree relatives with pancreatic cancer that does not meet the criteria of other hereditary cancer syndromes [29]. Familial pancreatic cancer accounts for at least 4-10% of pancreatic cancer. According to Matsubayashi H et al., European countries have been reported in FPC families, also seen in other hereditary syndromes; occurrence at a younger age and the worse prognosis are seen in the late years [30]. The resected pancreases of FPC relative often show multiple pancreatic intraepithelial neoplasia (PanIN) foci [31].

Individuals with mutations in the *BRCA2, PALB2, p16, STK11, ATM, PRSS1*, and *HNPCC* genes are associated with significantly increased risk for Pancreatic cancer and need to be screened [32]. These gene mutations are responsible for 10% of the familial susceptibility to pancreatic cancer [27]. Patients with Peutz Jegher syndrome also have an increased risk of pancreatic cancer [29].

Management of Pancreatic Cancer

Like various tumours or malignancies in the human body, it requires precise staging to determine if the tumor is respectable. The resectable nature of the tumour indicates the extent of its metastasis. This knowledge helps in choosing the best course of treatment and management [6].

The criteria for resectable pancreatic cancer are determined by the borderline of the tumour in contact with the superior mesenteric artery, a small segment of the celiac artery and the whole common hepatic artery [33]. However, the obstruction of the superior mesenteric artery and portal vein confluences caused by tumor growth is fixable by minimally inversive surgery [34].

Staging can only be very effective if the imaging of the malignancy is done with utmost accuracy. Most of the imaging techniques have one disadvantage or the other, but in cases where the advantage outweighs the disadvantage, they deemed fit for investigations [27].

However, there are some hurdles during the management which include;

1. Most patients are in the age group of the late sixties (70%) which undeniably predisposes them to multiple morbidities.

2. Some chemotherapeutic agents used during therapy present with other symptoms which affect the patient's functionality.

3. In addition to the reduced potency of chemotherapy; pancreatic cancer does not comply well with chemotherapy making treatment options restricted and cumbersome. The assessment of the effect of chemotherapy has been challenging due to the dense desmoplastic reaction (this refers to the growth of dense fibrous tissue around the tumour stimulated by various factors especially TGF-β) exhibited by pancreatic malignancies.

4. Obtaining biopsy samples from the tumor is difficult. However, chemotherapy has the capability of targeting specific cancerous cells [27].

Following the prognostic classification of pancreatic cancer, surgical intervention is only applicable to resectable tumours [10], [16]. Neoadjuvant therapy for resectable pancreatic cancer has shown varying results due to limited sample size and different patient responses to the therapies. The different means of classification has also led to the different types of therapy being used in the pre, peri and postoperatively state [16]. However, the data supporting its benefits justify its use [31].

Gemcitabine, a well-known medication has significantly increased the survival rate of patients with pancreatic cancer. Although, this comes with a heavy price in terms of negative side effects. Therefore, various combination therapies are being used to reduce side effects and be of more benefit than harm [31], [35].

Preoperatively Cisplatin in combination with gemcitabine significantly increases the resection rate by 70% which is almost doubled the rate of gemcitabine alone at 38% [33]. Studies were done by Adamska *et al.,* (2017) also shows an overall survival rate of 21% alive and pancreatic cancer free with this combination therapy [31]. Gemcitabine in combination with capecitabine, oxaliplatin and docetaxel improve resection rates in comparison with Gemcitabine alone [31], [33]. Borderline resectable patients treated with Xeloda, Taxotere, Gemzar, and radiation showed that 55 patients were able to have microscopically margin-negative resection out of 57 who were treated [1].

Neoadjuvant therapy for locally advanced and metastatic pancreatic cancer is more restricted due to the advanced state of cancer. The area concerned is more widespread and would involve lymph nodes in the case of metastasis. The effect of the drugs on other parts of the body can vary per individual. Nab-paclitaxel and Gemcitabine accompanied by Folfirinox therapy showed tumour regression and microscopically margin-negative resection of the tumour [16]. Other combinations which are also used for resectable and borderline resectable pancreatic cancer like Gemcitabine and cisplatin, gemcitabine and oxaliplatin, gemcitabine and capecitabine, PDXG (docetaxel, gemcitabine, capecitabine, and cisplatin) have also shown fairly good results encouraging the use of neoadjuvant in such patients [31].

The prognosis of resectable, borderline resectable and some locally advanced pancreatic cancer has a better probability for survival if managed properly. However, metastatic pancreatic cancer survival is solely based on increased therapies and palliative care [31].

Side Effects and Benefits of Therapy

The common side effects of some of the medications are neutropenia, thrombocytopenia. Examples of drugs which cause this side effect include 5-FU and gemcitabine, gemcitabine and erlotinib, gemcitabine and cisplatin, gemcitabine and nab-paclitaxel, FOLIFIRINOX which is a combination of fluorouracil, leucovorin, irinotecan, and oxaliplatin [31], [35].

The benefits of the monotherapy of gemcitabine include improved disease induced symptoms and increased survival response. However, it comes with several side effect except for haematological problems [35].

Benefits of novel agents include a reduction in pain, increased survival rate. Common side effects are haematological effects, musculoskeletal toxicities [35]. Examples include molecular targeting (olaparib), mitochondrial targeting (mFOLFIRINOX), microenvironment targeting tumor-associated-macrophages (CCR2 selective inhibitors), RAS inhibitors (Tipifarnib), metalloproteinase inhibitors (marimastat, BAY12-9566), epidermal growth factor receptor antagonist (erlotinib, trastuzumab, ZD1839), Antiangiogenics (thalidomide, paclitaxel, bevacizumab, combretastatin) [31].

Post-surgical patients are given adjuvant therapy, preferably gemcitabine or gemcitabine and capecitabine, although other regimens could be used. The use of other regimens is dependent on the response of the patients [35].

Discussion

Pancreatic cancer has been shown to have poor prognosis over the years. The disease falls within the most common causes of cancer-related deaths yet has a very low occurrence in comparison to the leading causes of cancer-related deaths. The availability of various diagnostic tools, treatment, and management have helped to contain the mortality of the tumour [1].

The study of pancreatic cancer from 2014 to 2015 showed a decline in mortality in men and a stable state in a woman. However, the decline in men is not significant enough to be considered clinically relevant [2]. The phenomenon seen between men and women is however not clear. Although, the decline in mortality for men can be correlated with the improvement in the health sector.

Research has shown that pancreatic cancers show good survival rates when diagnosed early [5]. Statistically, an increase of about 14% in mortality rate is seen in African Americans [7].

Genes like *Kras, CDKN2A, TP53, SMAD4* have been the point of interest in most cases of pancreatic cancer. The breakthrough in diagnostic techniques has paved the way for further examination to conclude early enough if an individual is at risk or has the tumour and guide early treatment or management.

Available research on pancreatic cancer indicates that genetics plays a vital role in detecting patients prone to developing the tumour [9]. Certain genes in the genetic line-up act as precursors for pancreatic cancer [16]. This understanding aids in further examination such as diagnostic imaging to confirm the state of the pancreas.

As shown by the literature, diagnostic imaging has over the years made some tremendous strides in aiding early detection of pancreatic cancer. Research has shown that MRI and EUS diagnostic technique is one of the best means of diagnosing pancreatic cancer without having to expose the patients to radioactive rays [25] further.

Various factors have been shown to cause pancreatic cancer. These factors mostly affect those who are genetically prone to having cancer. For instance, smoking acts as a carcinogen when used by genetically prone individuals [2]. The extreme exposure to some of the imagine radiations such as from x-rays and CT scans can escalate the dormant state [25].

The treatment and management of pancreatic cancer have improved between the 90s to date showing a decrease in mortality [35]. These improvements are all due to the advancement in technologies and medications.

The technological improvement includes the use of procedures such as interventional radiology. This improvement has aided in the precise removal of tumours with quick recovery time [5]. The medications Cisplatin and gemcitabine are helpful in tumour resectability. Radiation therapy with the combination of Taxotere and Gemzar also showed improvement in tumour resectability. Nab-paclitaxel and gemcitabine accompanied by folfirinox therapy significantly have been shown to help in the regression of the pancreatic tumour [16]. The medications also improve the disease-induced symptoms and increase survival response. However, the use of these medications is not devoid of side effects such as haematological and musculoskeletal toxicities [35].

Conclusion

Pancreatic cancer has had a history of poor prognosis because of its late detection. A family history of pancreatic cancer closely followed up with a

genetic screen has the potential to predict the likely incidence, early detection and possible management of pancreatic cancers. Also, further screening modalities and investigations using imaging techniques and interventional radiology have also helped to improve the early diagnosis and management of pancreatic cancer.

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Review Article

Templating Hip Arthroplasty

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Keywords: total hip arthroplasty; digital templating; prosthetic implant size

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Abstract

BACKGROUND: Different methods have been developed and employed with variable degrees of success in pre-operative templating for total hip arthroplasty. Preoperative templating, especially digital templating, has been claimed to have increased the effectiveness of total hip arthroplasty by improving the precision of prediction of prosthetic implant size.

AIMS: The overall aim of this systematic review is to identify whether the use of pre-operative templating in total hip arthroplasty procedures has resulted in increased accuracy, reliability and precision of the procedure. Various methods of templating, like traditional acetate overlay and digital method of templating that includes a single radiographic marker and double radiographic marker methods, have been compared to establish the most reliable method of templating.

METHODS: We searched the PubMed, Google Scholar Cochrane Central Register of Controlled Trials (CENTRAL), and MEDLINE (1966 to present), EMBASE (1980 to present), CINAHL (1982 to present), Psych INFO (1967 to present) and Clinical Trials Gov.

CONCLUSION: The results of this systemic review suggest that preoperative templating is resulting in an enormous increase in the accuracy of total hip arthroplasty and among various methods, King Mark is the most reliable method.

Introduction

Hip arthroplasty, also called hip replacement, is a surgical technique which involves the replacement of the diseased hip joint with a prosthetic implant. People with severe hip joint damage have gained a lot of benefit from this procedure. Hemiarthroplasty is another procedure that involves the replacement of only one half the joints with the prosthesis while the other half of the joint is left unaltered. However, researchers have shown that total hip arthroplasty shows better results than hemiarthroplasty [1]. The prosthetic implant used in this technique has 3 parts:

A femoral stem and head: This portion of the prosthesis is in one piece and is made of stainless steel

Acetabulum: This is made of polyethene.

Bone cement: Its major composition is acrylic.

The major aim while selecting for the composition of the implant is that it should be biocompatible and must not elicit an immune reaction in the body which would otherwise lead to rejection of the implant. Moreover, these implants must also resist corrosion and degradation so that they may last in the body for the longest period.

The most common indication of total hip replacement is osteoarthritis. A displaced fracture of the femoral neck that occurs in younger patients is also an important indication [2]. Other major indications include juvenile rheumatoid arthritis, ankylosing spondylitis, hip fractures, bone tumours (benign and malignant) and arthritis that is associated with Paget's disease. Total hip arthroplasty is also recommended for the management of unreduced traumatic dislocation of the hip joint that is very common in developing countries [3]. There are several techniques of carrying out arthroplasty; the major being posterior, lateral anterolateral and anterior approaches.

In modern times, hip arthroplasty has been

used extensively for managing severe hip damage. According to a review of peer-reviewed articles related to the experience of Rothman Institute, hip arthroplasty is a very safe, reliable and efficient procedure with a 10-year survival rate of the implant being greater than 99%. This remarkable rate of success has been attributed largely to the use of modern nonmetal implants [4]. Previously, metal on metal implants was used for hip joint replacement procedures, but their failure has been reported on numerous occasions [5]. So implants have now been replaced with a newer and more reliable prosthesis that has a higher success rate.

Like any other procedure, hip arthroplasty also has its complications. Dislocation is the most common complication, and it arises because the hip ball can get dislocated from its socket during the first 2 to 3 months after the operation. Venous thrombosis following hip replacement is a major problem that haunts the surgeons. However, this can be dealt with by the use of thromboprophylaxis in these patients [6]. Metal sensitivity is another complication that can lead to implant failure in patients receiving a metal prosthesis, and this should be suspected in patients who show the cutaneous signs of allergy after implantation of a metal device [7]. Sciatic nerve palsy, osteolysis and the difference between the lengths of the two legs are among other complications that are worth mentioning [8], [9].

Preoperative planning plays an important role in the success of any surgical procedure. Same is the case with hip arthroplasty in which preoperative assessment of various aspects of the procedure is of utmost importance. Researches have shown that unplanned hip replacement procedures have lower success rates than elective procedures. A prospective study was carried out at the University of Pennsylvania, and it depicted that unplanned and urgent hip arthroplasty resulted in a longer hospital stay of the patient and increase in the cost of the surgery with greater financial burden [10].

One of the important features in pre-operative planning of hip arthroplasty is the appropriate determination of the size of the prosthesis that has to be used in the procedure [11]. Preoperative templating ensures a greater degree of success of hip implant procedures. It has a profound effect on increasing the accuracy of the hip replacement procedure [12]. Accurately determining the size of the template also increases the precision of the procedure [13]. Preoperative templating also reduces the probability that the implanted prosthesis might loosen over time [14], [15]. Moreover, accurate templating before performing arthroplasty has also an important impact in decreasing the complications of the procedure among which leg length inequality and peri-prosthetic fractures are most notable [16], [17], [18], [19], [20], [21], [22], [23]. For this purpose, templates of both the acetabular and the femoral components are taken [24]. Many studies have shown that placement of the acetabular component of accurate size is of vital importance in determining how much successful the operation would be. But the major problem that the surgeons encounter in this regard is the accurate magnification of the radiograph to get the hard copy template of the radiograph for total hip arthroplasty. Accurately determining the magnification of hip radiograph and its correct application to find the exact size of the template has been the topic of interest for many years and this target, if achieved, can drastically increase the efficiency and accuracy of the hip replacement operation. For this purpose, several methods have been employed until now with variable degrees of success.

Previously, the process of templating was done by conventional methods which involved the use of drawings on transparencies of magnified implants [25]. Nowadays, digital radiography is used for this purpose.

In Digital radiography, computer programs are used to calculate the x-ray magnification and the templates are adjusted according to the magnification. The template and the radiograph are scaled in digital radiograph templating method to obtain the correct magnification. There are four basic steps in the process of templating from hip radiographs [26].

1. To identify the significant anatomical landmarks in the radiograph

2. To ascertain the quality of the radiograph.

3. The identification of various mechanical references, for example, femoral offset, acetabular offset, leg length discrepancy etc.

4. To optimise the position of the implant so that hip biomechanics are re-established.

In one study, one to one relationship was applied to assess the scaling to determine hip magnification. An object of known size, called the reference object, was placed adjacent to the hip to determine hip magnification. This technique was successful in establishing accurate templating for hip arthroplasty as the magnification of the reference object was equivalent to the magnification of the hip. However, it was found that for the method to be successful, it was compulsory that distance of the reference object and that of the centre of rotation of the hip should be at the same distance from the detector [27]. In another technique, a ten-penny coin was used as a marker in the process of scaling for magnification. Another method called two digital-line methods was also used for this purpose [28].

All the methods that are described above for digital templating have been designed in such a way that they use a single radiographic marker ball or disc whose diameter is known. However, for this method to be accurate, the marker has to be placed in the coronal plane of the hips so that the accurate

magnification of the hip may be obtained. If the marker is not properly positioned in the mentioned manner, the results of templating become inaccurate and the process becomes useless. For this purpose, the scientists felt a need to devise a new method of radiographic calibration that may provide more accurate results than the traditional single marker metal ball method of templating. Moreover, the condition that the radiographic marker should be positioned very precisely was also very annoying for the orthopaedic surgeons and in the course of developing a new device for templating; it was kept in mind that the new method must free the surgeons of this limitation. This lead to the new invention KING MARK by the University of Warwick and University Hospitals Coventry and Warwickshire (UHCW) which has revolutionised the whole procedure of digital templating and radiographic magnification for prosthetic implants. This technique has been claimed to be superior to the traditional metal ball method in many respects. King Mark is a double calibration device that is used for radiographic magnification. In King Mark, two markers are used for calibrating the template size. One marker is placed behind the pelvis, and the other marker is placed in front of the pelvis. The marker that is placed behind the pelvis is a pad that is radiolucent. Steel rods are embedded in it. The marker that is placed anteriorly is a strap that has radio-opaque balls secured in it. In this way, two markers are used for appropriate calibration. The King Mark is placed in the midline and if it is not placed in the midline, it appears on the radiographs that the markers are not properly positioned and this saves the surgeons from positioning mistakes that were encountered in the traditional single ball marker. One of the greatest advantages of this technique is that while single markers were not easy to use with very large patients, King Mark is easy to use in patients of all sizes. The method is non-intrusive and its accuracy and efficiency has been validated by a number of renowned institutions that are currently using it as part of the templating for total hip arthroplasty. Previously, in the UK, the sizes of the prosthesis for hip replacement were estimated correctly only in approximately 30% of the cases. With this new invention, the condition is expected to improve a lot and the inventors believe that it will go a long way in ensuring that the future total hip arthroplasty procedures are more successful at a much higher rate than they are currently. Professor of Trauma and Orthopaedic Surgery at Warwick Medical School, Professor Damian Griffin said:

"King Mark has removed all of the uncertainty about scaling digital radiographs for hip replacement. Our radiographers find it easy to use, and I can be confident that the measurements I make on scaled radiographs are correct. It is now unusual for my pre-operative templating not to be exactly right."

Using this technique, calibrations determined via digital templating will become more accurate and cost-effective. An orthopedic surgeon at UHCW Mr Steve Krikler said:

"Since changing to King Mark system, I have found the acetabular cup size to be very accurately predicted from the template, and the femoral size is also much more accurately predicted. I am now much more confident in choosing the stem offset and other parameters which are within my control, I always template my arthroplasties in TraumaCad, and I will only accept pre-operative images which include King Mark."

Given all this, it is expected that in the future, King Mark may also be used in other joint replacement procedures as well in addition to the hip arthroplasty.

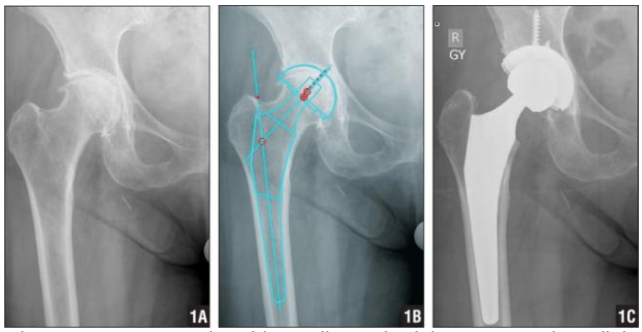

Figure 1: Preoperative hip radiograph (A), preoperative digital template using the traditional metal ball method of templating (B), and postoperative hip radiograph (C) of a patient who received a Right Total Hip Replacement

A small metal ball of known size can be seen at the left edge of the picture to help in identifying the magnification of x-ray

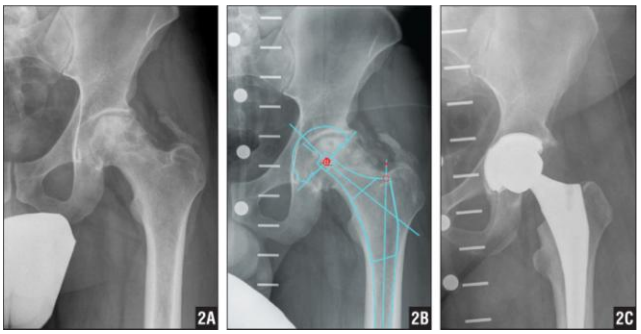

Figure 2: Preoperative hip radiograph (A), preoperative digital template using the newly designed KingMark (Double Markers) method of templating (B), and postoperative hip radiograph (C) of a patient who received a Left Total Hip Replacement

The overall aim of this systematic review is to identify whether the use of preoperative templating in total hip arthroplasty procedures has resulted in increased accuracy, reliability and precision of the procedure. The study also aims at assessing the effects of pre-operative templating on cost-effectiveness of total hip arthroplasty. Various methods of templating including traditional acetate overlay and digital method of templating that include a

single radiographic marker, and double radiographic marker methods have been compared to establish the most reliable method of templating. The aims also include assessment of the extent to which pre-operative templating has reduced post-operative complications of the procedure.

Methods

Criteria for considering studies for this review

Type of studies

Both prospective and retrospective studies have been selected for this review. Moreover, both conventional parallel designs and cross over type designs were included.

Type of participants

Studies included in this review only involved participants who had total hip arthroplasty, whether cemented or uncemented. Studies in which hemiarthroplasty procedures are performed were not considered.

Type of interventions

Study procedures that involve the following interventions will be considered.

- Total hip arthroplasty

- Digital templating for total hip arthroplasty

- Computed tomography-based templating for total hip arthroplasty

- Metal ball method of templating for total hip arthroplasty.

- Double radiographic method or King Mark method of templating for total hip arthroplasty

Primary outcomes

- Increased or decreased intra and inter observer reliability of templating procedure

- Increased or decreased accuracy or precision of total hip arthroplasty using the templating procedure.

- Decrease or increase in the cost-effectiveness of total hip arthroplasty after using templating

Secondary outcome

- Increased or decreased post-operative complications in patients with total hip arthroplasty after the use of templating technique.

Search strategy

We searched the PubMed, Google Scholar Cochrane Central Register of Controlled Trials (CENTRAL), and MEDLINE (1966 to present), EMBASE (1980 to present), CINAHL (1982 to present), Psych INFO (1967 to present) and Clinical Trials Gov exhaustively and comprehensively. In our search, we used descriptors like the accuracy of total hip arthroplasty following pre-operative templating, leg length discrepancy, leg length inequality, metal ball method of pre-operative templating, templating for total hip arthroplasty, THA, King Mark, a single radiographic marker for templating, a double radiographic marker for templating. We first searched for PubMed. Subsequent search strategies were derived from the MEDLINE strategy and Adapted for each database. A detailed description of the database outputs is shown in Tables 1 and 2, and Figure 1).

Table 1: Keywords used in the research strategy. Initial searches were performed by using keywords, alternate keywords combined with Boolean logic (OR). They were then combined using the Boolean logic (AND) to ensure that all the required terms for research will be included in the studies.

Search	Keywords
1	hip OR hip joint
2	hip prosthesis OR hip replacement OR hip arthroplasty OR total hip arthroplasty OR total hip replacement OR total joint replacement OR THA
4	King mark OR metal on metal implant OR non-metal implant OR metal ball method OR
5.	Preoperative templating OR leg length discrepancy
6.	1 AND 2 AND 3 AND 4 AND 5

As shown in Table 1, different databases were searched with the help of keywords and Boolean logic ‘AND’,’OR’. A group of 87 articles were retrieved in total out of which 25 were included in the systematic review. The flowchart below gives the process of article selection regarding the inclusion and exclusion criteria.

Reference list

Reference list of articles that the authors know has been searched. Other reviews that are found during the process of the search were not included.

Grey literature

We tried to contact authors of included studies to acquire other data that may either be unpublished, informally published or ongoing and is related to pre-operative templating for total hip arthroplasty.

Selection of studies

1. Review authors had independently screened and selected studies for possible inclusion in the study.

2. The titles and abstracts of trials identified from the search were independently reviewed and pooled for further screening.

3. Each review author independently examined the full text of all trials that were identified from the title and abstract screens.

4. Each reviewer compiled a list of studies that meet the inclusion criteria.

5. The contents of each review author's list were compared, and any disagreement was resolved by discussion and consensus between all of the review authors.

Table 2: The number of articles and databases

Databases	CINAHL FULLTEXT	MEDLINE	Pubmed	Cochrane	Embase	Psycho info.
Search 1	4524	1,314	13,847	3086	4930	5,277
Search 2	2458	854	5247	1014	2637	3,252
Search 3	852	524	325	626	1352	512
Search 4	354	303	101	352	462	405
Search 5	25	19	10	11	9	13

Data extraction and management

Two review authors had independently extracted data using specially developed data extraction forms. Information collection was based on:

1. Participant characteristics (age, sex, number of participants, indications for total hip arthroplasty, the type of method used in templating for total hip arthroplasty)

2. Intervention details (traditional method use of pre-operative templating, digital method, metal ball method, single radiographic method, King Mark method, double radiographic marker method)

3. Outcome measures (description of the measures used, continuous/dichotomous nature etc.)

Dealing with missing data

For missing data, the authors of the studies will be contacted. This would be done during the eligibility assessment and data abstraction. Moreover, the missing data will also be sought from secondary publications of the same study. However, if data are only available in the graphic format, we will impute approximations of the mean.

Publication bias

We assessed the risk of bias for each included study by an adapted Cochrane Collaboration "Risk of bias" assessment tool, including sequence generation, allocation concealment, blinding, incomplete outcome data/loss to follow-up, selective outcome reporting and other issues.

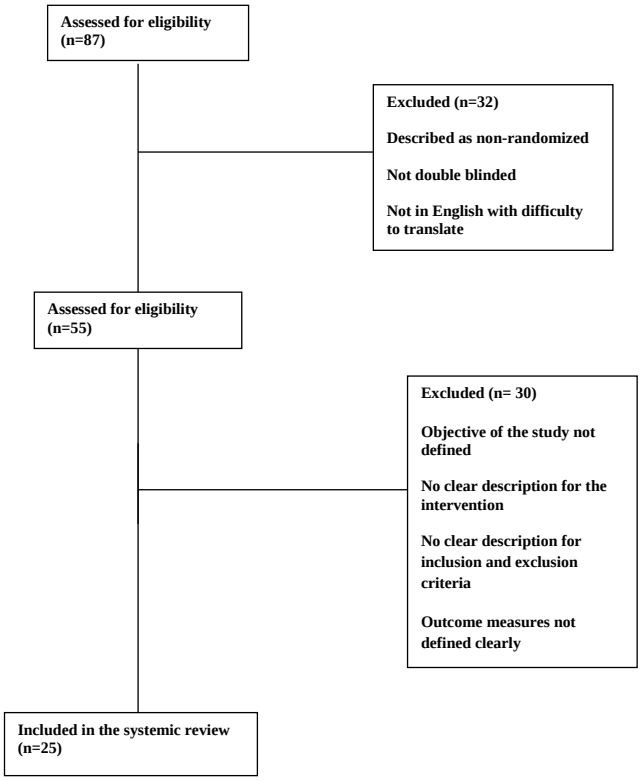

Figure 1: Consort diagram for the search strategy used

The response options for the quality assessment are defined as: yes (criteria applied and described appropriately or acknowledged in the study), no (criteria inappropriately applied) and unclear (criteria not described and impossible to obtain from the study). Each study has been classified into one of the categories below.

- High risk of bias: one or more criteria not applied/met.

- Moderate risk of bias: one or more criteria unclear.

- Low risk of bias: all criteria applied/met.

The review authors will discuss any disagreement in the assessment of the risk of bias to reach a consensus.

Assessment of quality of evidence across studies

We assessed the quality of evidence in this systematic review using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) tool with GRADE profiler (GRADEpro) version 3.6 software, defining the quality

of evidence for each outcome as the extent to which one can be confident that an estimate of effect or relation is close to the quantity of specific interest. The GRADE system rates the quality of evidence across studies as one of four levels: very low, low, moderate and high.

Sensitivity analysis

We hypothesize that pre-operative templating for total hip arthroplasty is less effective in increasing the reliability of templating hip arthroplasty in studies with a high risk of bias and in studies with short duration (that is, less than six months), thus we carried out sensitivity analyses by excluding studies classified as having high risk of bias and removing those having short duration. Also, the fixed-effects model was conducted for sensitivity analysis.

Assessment of reporting biases

We constructed a funnel plot to investigate the potential for publication bias for the primary outcomes relating to the accuracy and reliability of various methods of templating for hip replacement procedures.

Discussion

Several types of research have been carried out throughout the world to find out whether pre-operative templating for total hip replacement is effective or not, to determine what methods of templating are more reliable. Most of the studies that were done on the effectiveness of templating concluded that the role of pre-operative is certainly very important in estimating the success of the procedure. It was found that pre-operative is templating certainly increased the accuracy of total hip arthroplasty, increase in long term survival of the prosthetic implants in the body, a greater degree of patient satisfaction, decrease in post-operative complications of the procedure and better cost-effectiveness. Regarding the fact that what method of templating was more reliable, most of the studies revealed that the new digital method of pre-operative templating is clinically more significant than the traditional and conventional methods which had larger mean errors. Digital methods of templating comprise of single radiographic marker methods (metal ball methods) and the newly invented King Mark method which is a double marker technique. King Mark was found superior to the standard metal ball method.

Regarding the effectiveness of pre-operative templating research concluded that preoperative templating resulted in accurate planning of the size of prosthetic implants in 98% of the cases and hence it was recommended that templating must be done preoperatively in all cases of total hip replacement to make the process more reliable [29].

In a study, the digital method of templating was compared with a conventional method of templating for hip arthroplasty. Eighteen patients have included in the study, and all of them underwent uncemented total hip replacement procedures. The conventional templating was performed by using hard copy radiographic films and hard copy prosthetic overlays on a radiographic view box. The digital templating involved using computer-based software. Moreover, intra-observer and inter-observer variations were also assessed in all cases. The results of the study depicted that there was no significant difference between the conventional and the digital templating methods in determining the size of the actual prosthesis to be used in the procedure. Moreover, intra and interobserver variability were also found to be approximately the same for both conventional and digital methods. However, the basic limitation of this trial was that they included uncemented hip arthroplasties in their trial and so it is expected that carrying out the trial with the cemented templating hip arthroplasty might reveal some differences between the conventional and digital methods [30].

In another study, the standard acetate method was compared with the digital method of templating. It was found that while using the standard acetate method, the accuracy of the template for the acetabular component was 67% and was found to be 82% of the femoral component. In contrast, the accuracies for the acetabular and femoral components were found to be 78% and 90% respectively when the digital method was used thus reflecting the superiority of digital method of templating for the hip replacement procedure [31].

In another randomized control trial, it was found that the digital method was superior to the conventional methods both in accuracy and in long term survival of the implant [32].

Another study that focused on determining the reliability of digitally assessed magnification of hip radiographs for estimating the correct implant size stressed that the results of pro-operative templating must be verified once again intra-operatively to ensure the success of the procedure [33].

It has also been found that the accuracy of the templating procedure does not entirely depend upon the method of templating but is also determined by the efficiency and the experience of the physician. In a study, the implant sizes were determined using the same digital method of templating by two groups. One of the groups consisted if orthopaedic residents and the other group included experienced orthopedic surgeons. The results of the accuracy of the implants were compared afterwards. The group consisting of orthopedic residents accurately predicted the

acetabular component size in 63% of cases and the femoral component size in 87% of the cases. On the other hand, the group of experienced surgeons accurately predicted the acetabular component measurements in 88% cases and the femoral component in 97% cases. These figures certainly stressed that an experienced hand also has a significant role to play inaccurate pre-operative templating [34]. Similarly, it has been found training and experience level of the attending surgeon has no effect on the accuracy of acetabular component templates, but these factors do have a serious impact in determining the accuracy and precision of the femoral component template [35].

In a study, the templates developed by the same digital method of templating by a group of orthopaedic residents and by another group consisting of orthopaedic surgeons were compared. It was found that the accuracy of the templates developed by the orthopaedic residents was 63% of the acetabular implants and 89% of the femoral implants. Using the same method of templating, the orthopaedic surgeons correctly templates the acetabular component in 89% cases and the femoral component in 97% cases. This validated the statement that for the digital templating method to be accurate and reliable, it must be performed by an experienced hand rather than by untrained and unskilled personnel [36].

The conclusion of another study stated that digital templating predicts the size of the prosthetic implant with a high degree of precision and accuracy both for total hip arthroplasty and for short- stem hip arthroplasty and hence can cause a significant reduction in the postoperative complications and the failure of the surgical intervention [37]. Another study that assessed the accuracy of digital templating for uncemented hip arthroplasty revealed that digital templating predicted the size of the prosthetic implant accurately in 50% of the cases. However, when implants with two sizes of the template were used, the accuracy reached 100%. It was also found that the training level of the surgeon also affected the accuracy of the procedure. The Less experienced investigator had an accuracy rate of 82% while the more experienced ones had the accuracy rate of around 95% [38].

Another study stressed that although digital templating is a very reliable method for pre-operative detection of prosthetic implant size yet, it has been found that when implants are selected only from pre-operative templating, there is an increased risk that fractures may occur during the insertion of the implants [39]. Another study concluded that digital templating is very useful inaccurate correction of leg length in total hip arthroplasty [40].

Another study was done with the aim of assessing the reliability and accuracy of the digital method of templating. During the process, templates were made both for the acetabular component and the femoral component. The study also stressed as another one described previously, that all the implants that are selected by pre-operative digital templating must be verified intra-operatively to make the total hip arthroplasty procedure more efficient and reliable [41].

In other research, three methods of templating were compared with one another to find out which one of them was more reliable. One of the methods was analogue hard copy templating method, and the rest of the two were digital methods of templating. In addition to determining the accuracy of the method, the researchers were also interested in finding out whether these methods of templating were reproducible or not. In the study, both cemented, and uncemented implants were used to carry out total hip arthroplasty. The process of determining the best templating method was done using a retrospective study. The study included 33 patients out which 16 patients underwent cemented total hip arthroplasties, and 17 patients underwent cemented total hip arthroplasties. In the analogue hard copy method of templating, transparent sheets were taken, and the contours of the prosthesis were depicted on it. In this way, all the total hip arthroplasties were templated by analogue hard copy method. Digital Method 1 was performed with IMPAX™ ES Orthopaedic Application planning software, and the diameter of the reference object was used to determine template size. The digital method 2 was performed identically as the digital method 1. The main difference was that instead of using the diameter of the reference object as such, the corrected diameter of the reference object (that was determined by applying the linear relationship between magnifications of the reference objected the hip) was used. The results showed that digital method 2 was superior to the other 2 methods. Moreover, the reproducibility of all three methods was found to be moderate [42].

Another study was done with the aim of comparing the conventional method of templating with a new CT based method. The results of the study showed that the new CT based method of templating was much more accurate and easier than the conventional one. The new method was also found to be reproducible and easier to use even for the less experienced practitioners [43].

Another study compared the analogue and digital methods for total hip and knee arthroplasties. The results of the study, strikingly, were in favor of the analogue method of templating for hip arthroplasties. It was calculated by statistical analysis that analogue templates were accurate in 73% and 89% cases for cemented cups and stems and were 64% and 52% accurate for uncemented cups and stems respectively. Regarding the digital method, the accuracy of templates for cemented total hip arthroplasties was calculated to be 72% and 79% for cement cup and stem respectively and 52% and 66% for uncemented cups and stems respectively [44].

In another study, the accuracy and reliability of digital templating were assessed in uncemented hip replacement procedures. Orthoview software was used for this procedure. The accuracy in templating for femoral component was calculated to be 75% with 0.5 sizes and approximately 98% within 1 size. Head length templates were accurate in about 62% of the cases. The Acetabular component template was found to be accurate to 2 mm in 80% cases and with 4 mm in 98% cases. The results surely validated the efficiency of digital templating [45].

In a research procedure, validity, inter-observer reliability and intra-observer reproducibility of Mdesk, a digital method of pre-operative templating, were assessed. It was found that the validity of the system was good. The inter-observer reliability was found to be fair, and the intra-observer reproducibility was found to be excellent. By the observations, it was recommended that the pre-operative templating and the operation for the insertion of the implants must be done by the same surgeon to assure the success of total hip replacement [46].

Pre-operatively predicted prosthesis for total hip arthroplasty were compared in a study with the sizes of the original implants that were used in the surgical procedure afterwards. The mode of templating was digital. The researchers found that the pre-operatively predicted implants were of exact sizes as that of the original prosthesis used in hip replacement in 36% cases for femoral component and 33.7% cases for the acetabular component. The template size was with 1 size above or below of the originally used size in 77.5% cases for an acetabular cup and 82.3% cases for the femoral stem. This showed that digital preoperative templating was highly reliable and accurate. However, a difference of more than 2 sizes above or below the corrected size was found in a few cases, and so it was recommended that intraoperative X-rays must be used to verify the size of the prosthesis predicted by digital templating to ensure the successful hip arthroplasty [47].

A study in which metal ball method for templating was used to depict that the method accurately predicted the prosthesis size in 58.5% cases for total hip arthroplasty and approximately 93% of the templates were about +/- 1 size of the original implants. Moreover, none of the templates taken by digital metal ball method of templating was beyond 2 sizes of the originally implanted prosthesis, i.e. approximately all of the cases of templating hip arthroplasty were sized correctly. Metal sphere also correctly estimated the femoral head size in 100 % cases [48].

A study for the assessment of the reliability of digital templating in carrying out total hip arthroplasty in patients with Crowe type 2 and 3 dysplastic hips was done. Two groups of patients were assessed. One of the groups had Crowe type 2, and 3 dysplastic hips and the other group had other primary hip diseases. Total hip arthroplasty was to be done in both the groups and hence templates of the prostheses were prepared using digital templating. In patients with Crowe type 1 and 2 dysplastic hips, the accuracy of digital templating was 48.8% for acetabular component and 73.2% of the femoral component. On the other hand, in patients with other primary diseases, the results of digital templating to carry out hip arthroplasty were quite encouraging, and the accurate prediction of the acetabular component was achieved in 70.8% cases and 79.2% cases for the femoral component. Nevertheless, digital templating is still useful in patients with Crowe type 1 and 2 dysplastic hips [49].

Striking evidence in favour of manual methods of templating and against the digital method was provided in a study. It showed that the manual acetate method accurately predicted the femoral prosthetic component in 75% cases and that of the acetabular component in 83.3% cases. The digital method, on the other hand, was accurate in 41.6% cases about femoral component and in 75% cases for the acetabular component. The manual method of templating was much cheaper than the digital method of templating. Moreover, manual templating was also proved to be faster than the digital templating technique [50].

Four different methods of digital templating were compared in research. In two methods, metal balls were used for calibration. In method 1, the ball was placed laterally, and in method 2, the ball was placed medially. In method 3, the fixed magnification of 121% was applied and in method 4, object-film distance was applied. The results of templating were then compared and revealed that method 1 and 2 were associated with mean errors of 2.55% and 2.04% respectively. Mean error for method 3 was 1.42% and for method 4 it was 1.57%. The greater degree of errors in methods 1 and 2 was mostly due to the high degree of precision with which the balls have to be placed in order to get correct magnification for templating. Methods 3 and 4 proved to be more clinical significance [51].

In another similar study, computer based digital templating in uncemented total hip arthroplasty was compared with standard templating techniques. Digital templating again proved to be of better significance and reliability than standard templating technique. Good intra observer and inter observer reliability was found with the use of digital method of hip magnification for total hip arthroplasty. The Infraclass correlation coefficient was calculated to 0.7. There were significant differences in the accuracy of these two procedures. Using the digital templating technique, the size of femoral stem was predicted accurately to within 1 size in 85% cases and that of acetabular component in 80% cases. The only standard technique, however, was much less accurate especially in the prediction of acetabular component size. The accuracy was only 60% to within 1 sizes.

This validated the superiority of digital computer-based method of templating over the standard only templating [52].

The usual method of performing digital templating is by the use of radiographic markers. However, hip magnification can also be calculated without the use of a radiographic marker. This can be done by measuring the distance from the x-ray focal spot to the object and the distance from the x-ray focal spot to the radiological cassette or image receptor. These two methods of digital templating were compared in research. Medical records were taken, and the original size of the femoral head was obtained that was used in the surgical intervention. The methods mentioned above were then used to find out hip magnification and predict the size of the femoral head prosthesis. The accuracy of these methods was compared with each other afterwards. It was observed that both the methods had an almost equal accuracy. The radiographic marker method had a mean error of 2.6%, and non-marker methods had a mean error of 2.8%. However, the distance method was expected to be more acceptable to the patients and the technician. The distance method was also less complicated as there was no compulsion of the accurate placement of the radiographic marker for obtaining an accurate calibration of the prosthetic implant [53].

Another study investigated the utility of computer software based digital templating and also compared it with a traditional method in which magnification of 115-120% was assumed in the development of templates. The results of the study suggested that if magnification is assumed to be 115%, then the prosthesis size is not accurately determined and we get an over-sized magnification by 6 mm. So the use of digital templating was recommended [54].

To calculate magnification for templating, the object that is being used for templating must be placed at its centre and lack of fulfilment of this condition frequently leads to an error in determining accurate template for hip replacement. In a study, a new method was devised to alleviate this compulsion, and a planar disc was introduced. This disc was placed in the radiographic cassette and predicted magnification was obtained afterwards. The researchers concluded that the use of planar disc was associated with a greater degree of accuracy in predicting the correct magnification than the sphere. It was also noted that the technique was cost-effective and hence the use of planar disc instead of the sphere was recommended [55].

It has been declared by research work that to minimise the complaints of leg length discrepancy in patients who undergo total hip arthroplasty, pre-operative digital templating fooled by an Intraoperative x-ray is much helpful. By using this method of pre-operative and intraoperative assessment, the mean postoperative leg length inequality was reduced to 0.33 mm in contrast to greater than 6 mm that is usually encountered with the use of digital templating [56].

A prospective trial was done to identify accurate calibration methods of the digital radiograph to be used in pre-operative templating for total hip arthroplasty. One method involved positioning a coin between the patient's thighs and another method involved the use of callipers to measure the width of the pelvis. Their accuracy was then determined by comparing predicted sizes of the head of implants with the size of originally used implants. Coin method was found to more accurate clinically than calliper method [57].

Another prospective study compared analogue and digital techniques of pre-operative templating. Digital templating was found to be less accurate than analogue templating. For uncemented acetabular and femoral components, accuracy for the digital method was 52% and 66% respectively, and for cemented components, accuracy was 72% and 79% respectively. The accuracy of the analogue method for cemented acetabular and femoral component was 73% and 79& and for uncemented components was 64% and 52%. However, limitations of the study were that digital templates were not developed by experienced surgeons and analogue templates were planned by skilled surgeons and that might have resulted in bias [58].

A study was done to analyse the reliability and efficiency of the computed tomography-based method of pre-operative templating and compared it the traditionally used methods. Patients selected for this study were those who underwent uncemented total hip arthroplasty. It was suggested that except for a few cases in which combined femoral neck anteversion and external rotational contracture of the hip is less than 15 degrees, computed tomography-based computer software generated templates were more reliable, accurate and precise in determining prosthesis size both for a femoral and acetabular component in total hip arthroplasty [59].

Accuracy of pre-operative templating for uncemented total hip arthroplasty. The results of the trial depicted that the template size of femoral stem was exactly accurate only in 50% cases. However, the accuracy of templating was increased to greater than 85% when the femoral component implant was used with +/- 1 size of the template size. Accuracy rose to almost 100% when the femoral prosthesis within two sizes above or below the template size was used in operation. The experience level of the attending surgeon also greatly affected the precision of templating. Cases involving acute femoral neck fractures and proximal bone deformity were particularly difficult to template accurately [60].

To analyze whether the advent of templating techniques by digital methods has resulted in any positive impact on the success of total hip arthroplasty

in orthopaedic procedures, a study was done and concluded that as digital methods have resulted in mean magnification of 97%, therefore, templating by digital methods might result in improper selection of prosthetic implants for total hip arthroplasty as most of the manufacturing industries assume magnification to be 115-120% [61].

Assessment of precision, accuracy, reliability and utility of digital and analogue pre-operative templating procedures in determining the success of total hip arthroplasty was done in a prospective study. The accuracy of the analogue method in predicting the acetabular component size was 97%, and that of the digital method was 81%. In the case of the femoral component, the accuracy of the analogue method was found to be 98%, and that of the digital method was found to be 81%. Analogue method of templating was found to be more accurate than digital methods especially in estimating the size of the acetabular component. It was concluded that a large error in digitally predicted prosthesis template sizes was due to inaccurate positioning of the radiographic marker because for digital templating to be accurate, it is necessary that the radiographic marker should be appropriately positioned [62].

In other research, the accuracy of pre-operative templating in cementless total hip arthroplasties was calculated. A total of 109 surgical cases was included in the study. For an acetabular component of the prosthesis, accuracy of the predicted template was only 42.2%. In the case of the femoral stem, the accuracy of the predicted template was found to be 68.8%. However, when implants within 1 or 2 sizes of the template implants were used, the accuracy of templating rose to greater than 90% both for femoral and acetabular components. It was also noticed that if the patient had undergone total hip arthroplasty in the contralateral hip and it was used as an aid in predicting the size of the prosthesis, accuracy of templating increased even further but only for the femoral stem [63].

As mentioned earlier, leg length discrepancy is a common complaint among patients who undergo total hip arthroplasty. Preoperative templating has been noticed to play a major role in alleviating this complaint a great deal. A study was done with the aim of verifying this and concluded that in patients in whom the size of acetabular and femoral implants was determined preoperatively by templating, incidence of leg length inequality was reduced to almost nil and hence it was concluded that pre-operative templating is a reliable method for achieving leg length equality in patients who undergo total hip arthroplasty [64].

A study was carried out with the aim of assessing the degree of accuracy and reliability of the digital method of templating by comparing it with the traditional acetate method. Templating was done by using both methods one by one and the sizes of these templates were then compared with the size of original implants that were used in surgical procedures. About safety, digital templating was noted to be safer than traditional templating. About accuracy, the value of absolute error indicated that digital method was less accurate than acetate-based methods because the digital method underestimated the size of the femoral component and overestimated the size of the acetabular component [65].

Researchers tried to establish the most reliable position for placing the radiographic marker so that accurate template may be obtained for total hip arthroplasty. After carefully evaluating 106 patients, they concluded that the most reliable position for placing a radiographic marker to increase the accuracy and reliability of templating is at greater trochanter without skin overlap [66].

Similarly, it has been found that digital templating is very useful in total hip arthroplasties and is much more reliable than traditionally used acetate overlays that are prone to errors in magnification [67].

The studies mentioned above represent the analysis of digital templating methods using single radiographic markers. As mentioned previously, the single radiographic marker usage was associated with some complications in the procedure, and so, the King Mark method which uses two separate radiographic markers for hip magnification was devised. Although the method has been claimed to remove the major difficulties that were encountered using single marker templating methods and has been praised by the orthopaedic surgeons throughout the world, a new series of research and analysis has now begun to unfold various aspects of this new invention. Several researchers have compared the new method with the previous single radiographic marker method.

In a study, researchers investigated the double radiographic marker method of hip magnification for pre-operative templating and compared it with the normally used single marker method. In both cases, the calculation of magnification of the radiograph and its calibration was done by using Traumacad. The statistical analysis of the study revealed that the median error using double marker method of radiographic magnification was only 1.14% while using the single radiographic marker, the median error was estimated to be approximately 6%. The study concluded that the double marker method is certainly more accurate and reliable than the single marker method to template for hip arthroplasty procedure [68].

University Hospitals Coventry and Warwickshire NHS Trust in the UK also carried out a similar study in which they compared the single radiographic marker method with the recently invented double radiographic marker method. The predicted magnification of the hip radiograph was calculated by both the methods for the acetabular and femoral components of the prosthetic implant, and the true magnification of these components was also

determined. The researchers then compared the single and double marker methods. It was found that for the single marker method, the correlation between the true and predicted magnification was moderate for a single marker. The values were calculated to be r = 0.5 and n = 63. For the double radiographic marker, the values were r = 0.90 and n = 74, and so the correlation was good. Median error for the single marker method was 4.8%, and for the double marker, method was 1.1%. The intraclass correlation coefficient was 0.89 for the double marker method and 0.32 for the single marker procedure. So the validity of the double marker method was excellent. All these results proved that the double marker method was better than the single marker method [69].

Similarly, another study for the assessment of the accuracy and reliability of King Mark double radiographic marker and to compare it with the standard metal ball method was done in The Centre for Hip Surgery, Wrightington Hospital, UK. During the study, radiographic magnification was used by both methods to predict the size of the femoral head prosthesis and was then compared separately to the actual sizes of the prosthetic femoral head that were used in the surgical procedure. It was estimated that the median error was 1.02 mm for King Mark and 2.05 mm for the standard metal ball method. The intra-class Correlation coefficient was 0.83 for King Mark method and 0.76 for the metal ball method. So the ICC was better for King Mark. However, the analysis showed that the interobserver agreement was not very encouraging for both methods. The research concluded that King Mark was much more accurate and efficient than the standard metal ball method in templating for the size of the femoral head while on the other hand, the metal ball method might overestimate the template size [70].

Conclusion

Total hip arthroplasty is a very useful procedure that has played a really important role in the rehabilitation of patients suffering from various debilitating diseases of the hip and has saved a large number of patients from getting crippled for the lifetime. Thousands of total hip replacements are carried out throughout the world. Given this, it is very important that the prosthetic implants that have to be used in arthroplasty must be chosen precisely to avoid a large number of complications that may occur as a result of improper selection of the implant size. By discussion mentioned above, we conclude that preoperative templating for the process of hip arthroplasty has increased the success rates of templating hip arthroplasty throughout the world.

It has been observed that pre-operative templating has increased the success of total hip arthroplasty to almost 98%. By comparison between the accuracy of various methods of templating, it has been found that the digital method of templating is superior to all the other conventional and traditional methods in all respects. The mean error in predicting the size of the femoral and acetabular components of the implants is much lesser for the digital method as compared to the traditional methods. The incidence of postoperative failure of total hip arthroplasty has greatly reduced since the advent of digital techniques. Moreover, the risk of postoperative complications has also been minimised by the accurate use of digital methods. The problem of leg length discrepancy that previously was much more prevalent in patients who underwent hip replacement has been solved to a large extent using digital methods. The intra and interobserver reliability is also much better for digital methods than for the traditional methods. The ICC coefficient is also significantly greater for digital methods, and this provides the evidence of the reliability of digital methods. However, it has been found that the precision and accuracy of templating is not solely dependent upon the method of templating used but also depends on the skills and experience of the performing surgeon. The results of templating in predicting the size of the prosthesis accurately to be used in the original procedure also depend upon the level of training and experience of the personnel and the process of templating if performed by an adept surgeon increases the probability of accurate prediction of the implant size. It is also recommended that pre-operative templating and total hip arthroplasty should be performed by the same surgeon as it has been proved by several research procedures that this plays an important role in improving the success rate of hip replacement procedures.

Furthermore, the intra operative verification of the prosthesis is also recommended as the implants that have been inserted solely on the basis of results of templating have resulted in subsequent failure much earlier than those that were carefully verified intra operatively. However, the use of single radiographic marker had its problems with that high degree of precision was required in positioning the marker. To get rid of this, inventors have developed a novel method of templating for hip arthroplasty which has proved to be much more accurate, reliable and easy to use than the single radiographic marker technique. However, several aspects of King Mark, the double radiographic marker method, are yet to be revealed and with the passage of time, researchers will be done to assess the further credibility of this method.

Limitations: Although great care has been taken, but this review may have some limitations. This is because many types of research and trials that have been selected for this systemic review might be associated with bias. Moreover, studies that were published in non-English language could not be translated and then included in the review procedure.

If these studies were thoughtful and necessary for incorporation into our systematic review, then our review might be subjected to selection bias.

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Review Article

Low Protein Diets for Pregnant Women and Its Association with Insulin Secretion and Resistance

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Abstract

Gestational diabetes mellitus (GDM) complicates 3.5% of pregnancies in England and Wales and continues to show an increase in incidence each year. GDM can lead to diabetes postpartum, it is associated with an increased perinatal risk, and an increase in neonatal mortality. This review article looks at different studies regarding protein diets and their potential effects on GDM. We aimed to determine if a certain protein diet could potentially help protect against GDM using. We found that while a few studies have shown that increasing proteins in the diet of pregnant women, specifically that from poultry, whey, fish, nuts and legumes, may reduce the risk of GDM, there is certainly room for further research on the topic.

Introduction

Gestational diabetes mellitus (GDM) is defined as an impaired glucose tolerance within the onset and duration of pregnancy [1]. Around 3.5% of pregnancies in England and Wales are complicated by GDM [2] and after delivery; a cumulative incidence of diabetes from 2.6% to over 70% in several studies that followed women from 6 weeks to 28 years postpartum was reported, adding to the existing burden of diabetes on the NHS [3]. Furthermore, a pregnancy complicated with GDM is still associated with high perinatal risk and increased neonatal mortality and morbidity [4]. Much research has been undertaken to find potential ways of preventing the insulin resistance that can occur during pregnancy and our review looks at the evidence of low protein diets and its relationship with GDM.

The relationship between pregnancy and hyperglycaemia

GDM, much like the other types of hyperglycemia, is characterised by a pancreatic β-cell function that is insufficient to meet the pregnant body's insulin needs [5], [6], [7]. During a normal pregnancy, the growth of the fetus and placenta causes increases in growth hormone, cortisol, estrogen, progesterone, prolactin and human placental lactogen which all result in hyperinsulinemia, insulin resistance, fasting hypoglycaemia and postprandial hyperglycaemia [6], [7], [8], [9]. Subsequently, pancreatic beta cell function adapts to compensate for the decreased insulin sensitivity and the increased requirement [6], [7], [10]. To compensate for the increased insulin levels, peripheral muscle glucose is utilised, however, as gestation advances, these responses become inadequate to meet the demands of the fetus, and consequently, insulin resistance occurs [6], [7].

Adverse Maternal and Fetal Effects

Women with GDM have an increased risk for pregnancy-related morbidity and high risk of developing type 2 diabetes in the years following the pregnancy [3], [13], Further to this, their offspring have a higher risk of perinatal morbidity and an increased risk of childhood obesity and early onset type 2 diabetes mellitus [13], [22]. Studies have also shown that the risk of spontaneous preterm birth increased with increasing levels of glycemia during pregnancy [16], [17], [18], [19], [21]. Moreover, GDM has been shown to increase the risk of gestational hypertension and preeclampsia [20], [23].

Interestingly, it has been hypothesised that women with decreased insulin sensitivity may increase nutrient availability to the fetus, thus accounting for the possible fetal overgrowth and adiposity, otherwise known as macrosomia, a frequent result of GDM [14], [15], [18]. Another frequent complication for the infant is neonatal hypoglycaemia.

Methods

We searched for articles published in English through PubMed and Embase using the following search phrases: "GDM and protein diets", "GDM and insulin sensitivity" and "insulin sensitivity and protein diets". We included only published articles, from no more than 20 years ago, that have reported protein diets that influenced insulin sensitivity, insulin resistance and insulin levels in both animal and human models. We also included large cohort studies that looked at the incidence of GDM following specific dietary patterns.

Studies on the Effect of High Protein Diets and GDM

One group hypothesised that feeding insulin resistant rats with a high whey protein diet (32%) (HWP) containing whey protein concentrate (WPC) would increase insulin sensitivity compared to a diet containing red meat (RM). They fed rats a high-fat diet (300 g fat/kg diet) for 9 weeks, then changed to a diet containing either 80 or 320 g protein/kg diet, provided by either WPC or RM, for 6 weeks. They found that dietary WPC reduced plasma insulin concentration by 40% ($P < 0.05$) and increased insulin sensitivity, compared to RM ($P < 0.05$). Thus, their findings support the idea that an HWP diet is more effective than red meat in increasing insulin sensitivity [24].

Another study tested 57 overweight volunteers with fasting insulin concentrations > 12 mU/L. The participants were fed either a high-protein diet of meat, poultry, and dairy foods (HP diet) or a standard-protein diet low in those foods (SP diet) during 12 weeks. Interestingly, they found that among the volunteers on HP diet, there was significantly ($P < 0.03$) lowered postprandial glycemic response at weeks 0 and 16 compared to those on the SP diet. They concluded that replacing carbohydrate with protein from meat, poultry, and dairy foods has beneficial effects on glycemic response [25].

One group looked at pregnant patients with polycystic ovarian syndrome on a 1500-calorie/d, high-protein, diet, with 30% of calories as fat and metformin therapy, they found that women taking metformin along with the low-carbohydrate diet may have contributed to reduced development of gestational diabetes [26]. In another study, which included 21,411 pregnancies, they looked at prepregnancy low-carbohydrate dietary patterns in these women, and the subsequent incidence of GDM. They documented 867 GDM pregnancies, and their results showed that a prepregnancy low-carbohydrate dietary pattern with high protein and fat from animal sources is associated with an increased GDM risk, whereas a prepregnancy low-carbohydrate dietary pattern with high protein and fat from vegetable sources does not show a risk [27]. Interestingly, another paper also reported that, after adjustment for age, parity, nondietary and dietary factors, and body mass index (BMI), they found that the substitution of red meat with poultry, fish, nuts, or legumes showed a significantly lower risk of GDM [28].

One study examined the associations between dietary patterns and the risk of GDM in 3063 pregnant Chinese women. Their findings suggested that a vegetable-rich diet was associated with a decreased risk of GDM, while the sweets and seafood pattern was associated with an increased risk of GDM. They concluded that a high protein diet did not provide statistically significant findings on preventing GDM [29].

The Australian Longitudinal Study on Women's Health included 3,853 women without pre-existing diabetes who were followed-up between 2003 and 2012. They studied pre-pregnancy dietary patterns with the incidence of GDM. They suggested from their results general dietary recommendations for women of reproductive age, including consumption of a diet rich in vegetables, whole grains, nuts and fish, and low in red and processed meats and snacks [30].

Conclusion

Only a few published papers have studied the effects of a low protein diet on GDM, suggesting the need for further research in this, particularly topic. However, from the studies published, it is clear that selecting the right type of protein i.e. that from

poultry, whey, fish, nuts and legumes contributes to a reduced risk of GDM and it is important in maintaining a healthy lifestyle during pregnancy. It is also important to note that whilst the majority of studies here did show the benefits of increased protein from sources other than red meat, one study did show that a high protein diet had no significant effects on the risk of GDM.

Furthermore, it is important to discuss that the reasons for the protective effect against GDM may not necessarily be a direct result of increasing the right types of protein in the diet, but may be indirect, by decreasing a woman's consumption of other foods associated with an increased risk of GDM such as carbohydrates and fats [31], [32], [33], [34], [35].

To conclude, while a few studies have shown that increasing proteins in the diet of pregnant women, specifically that from poultry, whey, fish, nuts and legumes, may reduce the risk of GDM, there is certainly room for further research on the topic. Future studies should aim to determine the exact type of protein and their specific quantities using a large sample group of pregnant women that will eventually lead to a recommended diet plan to reduce the risk of GDM.

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Review Article

Prognostic Factors of Local-Regional Recurrence in Patients with Operable Breast Cancer in Asia: A Meta-Analysis

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Keywords: Prognostic factors; Recurrence; Operable breast cancer; Asian Population

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Abstract

BACKGROUND: Cause of recurrence of breast cancer is multifactorial. Also, the occurrence of breast cancer in Asian patients has some different factors from the recurrence of breast cancer in western countries.

AIM: This study aims to determine the prognostic factors of local-regional recurrence in patients with operable breast cancer in Asia.

METHODS: The authors conducted a meta-analysis of published research articles published in an online database of PubMed, ProQuest and EBSCO between January 2000 and July 2018. Pooled risk ratios (RR) were calculated using fixed and random-effect models. Data were processed by using Review Manager 5.3 (RevMan 5.3).

RESULTS: This study reviewed 879 articles. There were 11 studies conducted a systematic review then continued by meta-analysis of relevant data with total patients involved were 5,213 patients. The prognostic factors found of local-regional recurrence in patients with operable breast cancer were Nodal (N) stage with the highest risk ratio (RR = 6.35 [95% CI 3.78-10.67]) followed by HER2 positive (RR = 2.14 [95% CI 1.16-3.97]), stage of cancer (RR = 1.82 [95% CI 1.44-2.31]), tumor size (RR = 1.55 [95% CI 1.04-2.31]), tumor grade (RR = 1.43 [95% CI 1.23-1.65]), PR status (RR = 0.65 [95% CI 0.48-0.88]) and the least was ER status (RR = 0.60 [95% CI 0.39-0.91]). Homogeneity of variance was found in N stage, tumor size and tumor grade for recurrence of operable breast cancer.

CONCLUSION: This meta-analysis confirmed the correlation of N stage, HER2, stage of cancer, tumour size, tumour grade, ER and PR status with recurrence in patients with operable breast cancer in Asia.

Introduction

Breast cancer, the most common cancer in women, is highly heterogeneous with various clinical courses and outcomes [1], [2]. The disease recurrence and prolong survival have been reduced by several proven adjuvant systemic therapies, including chemotherapy, hormonal treatment, and anti-HER2 (human epidermal receptor). Breast cancers are classified into genomically defined subgroups, including subtypes: luminal A, luminal B, HER2+, and triple-negative (TN) tumours. Clinical courses, patterns of metastasis, and prognosis of these subgroups may be various. Most relapses happen during the first 5 years after diagnosis, even though the late recurrence of luminal breast cancer has been reported.

Breast cancer may recur 5-10 years after first treatment. High-bulk disease, high proliferative index, and HER2-positive malignancies correlated to recurrence earlier than 10 years, whereas progesterone receptor-positive (PR+) group was associated with relapse later than 10 years [3], [4]. The term of local-regional recurrence refers to recurrence either in ipsilateral breast structure after lumpectomy, in chest wall recurrence after mastectomy or recurrence in axillary or supraclavicular lymph nodes (less common infraclavicular and/or internal mammary nodes)

Approximately, local recurrence develops in 10-15% of stage I-II of breast cancer after breast-conserving surgery and 10-20% chest wall recurrence in the cancer stage I-IIIA after mastectomy [4].

Annual follow-up visits are usually scheduled for patients who have five disease-free years or completed hormonal treatment. Some of those patients develop rapid and extensive metastasis during the follow-up intervals; with few of these patients can not undergo chemotherapy due to organ dysfunction or unwell performance status as a result of systemic metastasis. In spite of the decrease of recurrence risk by adjuvant chemotherapy in the first 5 years, the effect of the therapy beyond 5 years is still unknown. Patients with estrogen receptor-positive (ER+) breast cancer have benefited from adjuvant tamoxifen, with the greatest benefit is in the first 4 years and an additional decline of recurrence risk carryover for more than 5 years [5]. Tamoxifen use extended to 10 years in women with early-stage breast cancer reportedly reduces the risk of late recurrence [6].

The cause of recurrence of breast cancer is multifactorial. Nowadays, several risk factors for breast cancer recurrence have been reported. The risk factors known are age, menopausal status, clinical T (tumour), clinical N (nodal involvement), LN (lymph-node), lymphovascular invasion, margin status, histologic grade, nuclear grade, hormonal status, HER2 status, chemotherapy, and antihormonal treatment [6], [7]. Also, the occurrence of breast cancer in Asian patients has some different factors from the recurrence of breast cancer in western countries.

This study determined prognostic factors of local-regional recurrence in patients with operable breast cancer in Asia by performing a meta-analysis study in which the conclusion had drawn have better accuracy. The result in this study will be useful and assist physicians in determining prognostic factor of recurrence in Asian patients with operable breast cancer.

Material and Methods

Study design and research sample

This research was a quantitative study performed by using meta-analysis study design. The meta-analysis followed PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analysis) Statement [8]. Meta-analysis was used to find prognostic factors of local-regional recurrence in patients with operable breast cancer in Asia. The research samples consisted of published research articles published in online article databases of PubMed, ProQuest and EBSCO between January 2000 and July 2018.

Operational definitions

The variables of this study included several independent variables of prognostic factors, i.e: age, menopausal status, clinical T (tumor), clinical N (nodal involvement), LN (lymph-node), lymphovascular invasion, margin status, histologic grade, nuclear grade, hormonal status, HER2 status, chemotherapy, and antihormonal treatment; and a dependent variable, i.e: local-regional recurrence of operable breast cancer.

Research procedure

This study was conducted by collecting data through the identification of published research articles on prognostic factors of recurrence in patients with operable breast cancer in Asia in online article databases of PubMed, ProQuest and EBSCO (Figure 1).

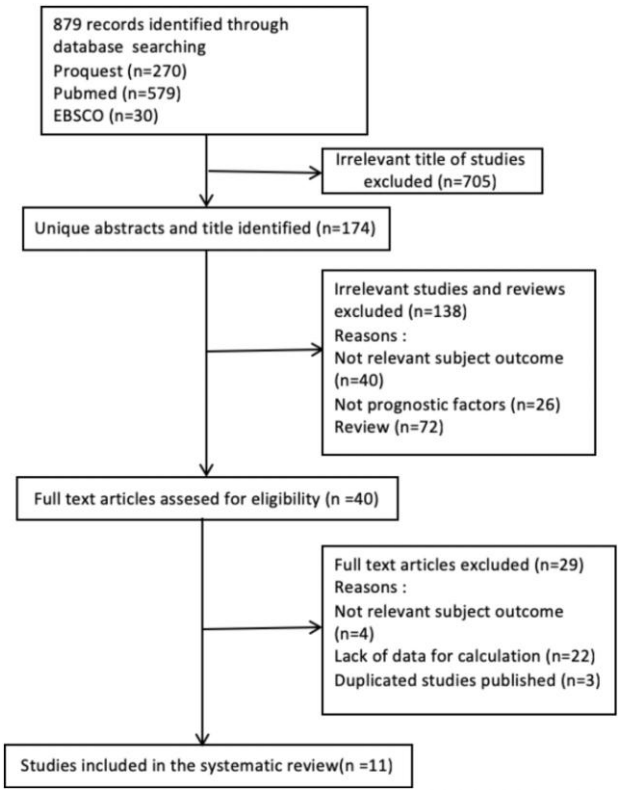

Figure 1: Flow diagram research procedure

Identification process of 913 articles was held by identifying the title of the articles, continued by abstract and full-text review of the articles. The article excluded if: (a) unrelevant to subject outcome, (b) the methods was neither case-control nor cohort study (c) the data provided in the results section of the article was insufficient for extraction and (d) duplicate studies.

Data collection technique

The search was limited to English language articles. The article type was limited to research articles. The research subject was limited to research involved human subject. The time of publication was limited for January 2000 to July 2018 period. The abstract of articles with relevant title continued to review process, and the articles with the irrelevant title were excluded. After that, articles with relevant abstract were continued to be reviewed in full-text, while the others were excluded. The inclusion criteria of the sample included published research on prognostic factors of recurrence in patients with operable breast cancer in Asia. The exclusion criteria were either the research was not available in the full-text form, or the criteria were not satisfied or if the information provided was insufficient for data extraction. These data were obtained from the articles: name of the first author and publication date, study location, study type, total samples and risk factors identified.

The information fulfilled criteria of inclusion from the studies obtained were extracted carefully by two independent investigators by a standardised protocol. Three other investigators resolved the disagreements. Quality assessment was performed by using the Newcastle – Ottawa Quality Assessment Scale (NOS) and studies with a NOS score ≥ 7 were considered as high quality [9].

Table 1: Systematic review of prognostic factors for local-regional recurrence in operable breast cancer patients in Asia

First Author, Year	Region	Type of Study	Number of Samples	Risk Factors	NOS
Elkum et al [10]	Saudi Arabia	Prospective	867	Grade	8
Son et al. [11]	South Korea	Prospective	523	Stage	7
Tanioka et al. [12]	Japan	Prospective	88	HER2, axillary lymph nodes	7
Chen et al [13]	China	Retrospective	540	Positive nodes	7
Akbari et al [14]	Iran	Prospective	258	LVI, stage	7
Song et al. [15]	South Korea	Prospective	95	Stage, grade, p53, Ki67	7
Ahn et al. [16]	South Korea	Prospective	677	N stage, grade,	7
Wei et al [17]	China	Prospective	1498	Stage, tumor size, ER, PR, HER-2	8
Wangchinda and Ithimakin [18]	Thailand	Retrospective	300	Tumour size, N stage, Grade, ER, HER2	7
Ditsatham et al. [7]	Thailand	Retrospective	185	ER status, PR status	7
Ahmadi et al [19]	Iran	Retrospective	182	Grade, ER, PR status	7

NOS, Newcastle-Ottawa Quality Assessment Scale.

Data analysis

The analysis was conducted to obtain the value of pooled risk ratio as the combined risk ratio value from the collected research. Data analysis was held by using the Mantel-Haenszel method with a fixed effect model and the DerSimonian-Laird random-effect model. Meta-analysis was carried out by using *Review Manager* 5.3.

Results

The selection of studies was conducted to identify 11 studies related to prognostic factors of recurrence in patients with operable breast cancer in Asia with total sample of 5,213 patients [7], [10], [11], [12], [13], [14], [15], [16], [17], [18], [19] (Table 1).

We found 11 studies through the systematic review (7 cohort study and 4 case-control) which then analysed by meta-analysis. The research variables analysis was based on the systematic review that has been done included stage of cancer, grade of the tumour, HER2, tumour size, N stage, ER and PR status (Figure 2).

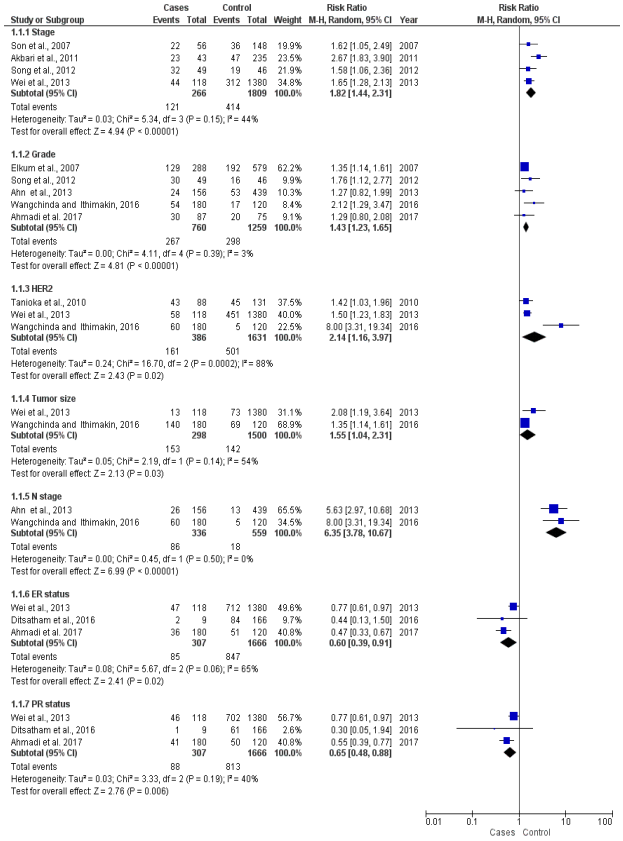

Figure 2: Forest plots prognostic factors of local-regional recurrence in patients with operable breast cancer in Asia

It was shown in Figure 2, based on prognostic factors known, N stage has the highest risk ratio (RR = 6.35 [95% CI 3.78-10.67]) followed by HER2 (RR = 2.14 [95% CI 1.16-3.97]), stage of cancer (RR = 1.82 [95% CI 1.44-2.31]), tumor size (RR = 1.55 [95% CI 1.04-2.31]), tumor grade (RR = 1.43 [95% CI 1.23-1.65]), PR status (RR = 0.65 [95% CI 0.48-0.88]) and ER status (RR = 0.60 [95% CI 0.39-0.91]). Funnel plots was performed to identify publication bias among publication on recurrence in patients with operable breast cancer in Asia (Figure 3).

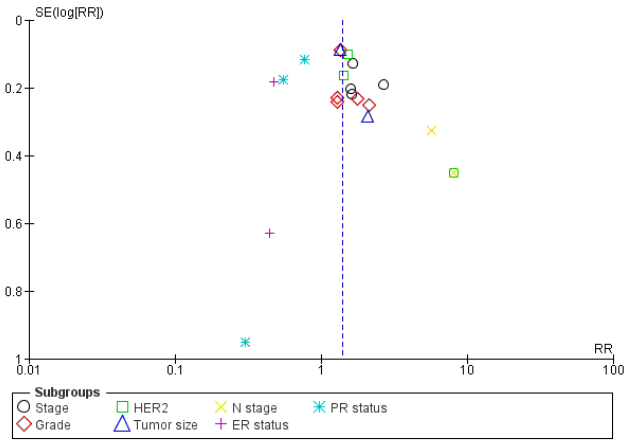

Figure 3: Funnel plots prognostic factors of local-regional recurrence in patients with operable breast cancer in Asia

Figure 3 shows that N stage, stage of cancer, tumour size and tumour grade have homogeneity of variance for recurrence of operable breast cancer resulted by the symmetrical plot based on the vertical line which means if the analysis conduct on a different population, time, place, and conditions, the results will be consistent. This is different from HER2, ER and PR status.

Discussion

Local recurrence is the reemergence of tumour in the ipsilateral breast chest wall or overlying skin. The incidence of local-regional recurrence after mastectomy is 9%-28% in early breast cancer. Approximately 40% of isolated local-regional recurrences are detected during routine examinations in symptomatic or asymptomatic patients. Identifying and management of local recurrence is important because 30-40% of local-regional recurrence will develop distant metastasis. The peak of annual hazard for recurrence occurs in 1 to 2 years after treatment and then declines slowly after 12 years. Therefore, the factors correlated to local-regional recurrence are essential to identify to provide adequate therapy so that metastasis can be prevented. Breast cancer in Asia seems to be different from western countries since it occurs at a younger age with an average age of 40 to 50 years and with smaller breast sizes leading to more difficult conservative surgery [20].

In this study, we compiled a total of 5,213 patients from 11 studies (7 cohort study and 4 case-control) that are appropriate for this systematic review. The races included in this study are Arabic, Japan, China, Korea and Malay. The basis of prognostic factors of recurrence in patients with operable breast cancer in Asia, consecutively, are N stage has the highest risk ratio (RR = 6.35 [95% CI 3.78-10.67]) followed by HER2 (RR = 2.14 [95% CI 1.16-3.97]), stage of cancer (RR = 1.82 [95% CI 1.44-2.31]), tumor size (RR = 1.55 [95% CI 1.04-2.31]), tumor grade (RR = 1.43 [95% CI 1.23-1.65]), PR status (RR = 0.65 [95% CI 0.48-0.88]) and ER status (RR = 0.60 [95% CI 0.39-0.91]). N stage, stage of cancer, tumour size and tumour grade have homogeneity of variance for recurrence of operable breast cancer.

The previous study known in Arab population has identified that young age (≤ 40) is an independent risk factor for relapse in operable Saudi breast cancer patients [10]. Another study in Japan found that HER2 status and axillary metastases are independent predictors of recurrence in breast cancer patients [12]. In China population, it has been reported that axillary lymph nodal status is the only risk factor of significant impact on 10-year [13]. In Thailand population, ER^+/PR^+ and $HER2^-$ patients have a higher risk of recurrence in later than 5 years, especially in patients with high ER titer and low nuclear grade. Tumour with larger and node-positive have a higher risk of early recurrence [18].

The first and second influential factor of recurrence in breast cancer patients is the involvement of axillary lymph nodes and HER-2 positive, respectively [12]. This systematic review also proves that the presence of lymph node metastasis plays a significant role as a predictive factor for local recurrence. By a report from the United States which Saphner et al., reported that the hazard for the recurrence is particularly high for those with 4 or more involved axillary lymph nodes during the first to 6 years of follow up as well as those with fewer nodes involved [21].

Following metastasis to the axillary lymph nodes, the HER2 type has the second highest hazard rate at local recurrence. The presence of HER2 positivity will be a major factor in recurrence if patients do not receive adjuvant trastuzumab therapy [22]. HER2 is transmembrane tyrosine kinase receptor regulating cell growth, proliferation. Amplification of this gene observed in 15 – 30 % of breast cancer patients and is a strong prognostic biomarker for aggressive disease. The prominence of HER2 positive factors as a second order risk factor in this study is interesting since this is different from the risk factor for local-regional recurrence of western countries. This phenomenon can be explained by the high percentage of young age breast cancer in Asia who have aggressive behaviour as well as a high percentage overexpression of HER2.

Stage of cancer is strongly correlated with tumour size. In this study, it was found that the stage and size of the tumour as a third and fourth risk factor for the occurrence of local-regional breast cancer recurrence in Asia. If the tumour size of more than 2 cm, positive lymph nodes axillary, histopathologic grading, the incidence of recurrence in stage III (25%)

is higher than stage I and II (5% and 12%) [23]. Many studies have demonstrated a linear correlation between the diameter of the primary tumour and both the presence of lymph node metastasis and clinical outcome. Among node-negative patients, tumour size is the only important prognostic factor [24].

The estrogen receptor impact in prognosis was significant in lymph node-positive patients [10]. In the first three years after diagnosis, estrogen receptor positivity affects prognosis significantly, but after three years this effect is not present. However, estrogen or progesterone receptor positive tumours will respond better to treatment with anti-estrogen drugs like Tamoxifen. A large study conducted in 37000 women in 1998 indicated that Tamoxifen treatment in estrogen receptor-positive patients would decrease recurrence and mortality rate for 47% and 26%, respectively [25]. In study patients with negative lymph nodes, estrogen receptor positivity has been associated with better prognosis [26].

Type of cancer and its grade, presence of tumour emboli, endolymphatic invasion, negative estrogen receptor, Increased expression of HER-2 and positive P53 are all variables that have been associated with risk of local recurrence [27]. A study reported that early recurrence associated with unregulated stress response signalling and certain clinical parameters, such as molecular subtypes, tumour size, and grade; while late recurrence correlated with mesenchymal characteristics of the tumour epithelium and gene expression alterations in the adjacent tumour stroma [28].

Local-regional recurrence may be associated with more aggressive tumour biology. Several factors have been associated with increased risk for local-regional recurrence in western. These factors are a lymphovascular invasion, young age, increasing tumour size, closed or involved margin status, positive nodal, high grade, extensive intraductal component, multifocal/centric disease, negative hormone receptors, lack of adjuvant systemic therapy [23], [27], [29]. The NSABP B-06 trial found a 3 times greater incidence of distant relapse in patients with local recurrence and 2.5 times higher increased risk of death. One-third of these patients have synchronous distance disease and another one-third subsequently develop metastatic disease, so re-staging of the patient with local-regional recurrence is important. Patients who have nodal recurrence has a higher risk than those with chest wall recurrence. Nodal recurrence may be a source of distant metastasis or a marker of systemic dissemination [30].

There were a few limitations in this meta-analysis. First, two studies seemed potentially eligible to be included in this meta-analysis, but the full texts were not accessible. This issue may raise the possibility of selection bias. Second, the number of cases sample in one study is relatively small (12), which can reduce statistical power.

Several studies have analysed and compared patients who died with the recurrent disease with those without recurrent disease. However, less attention has been paid to evaluating factors associated with the recurrence.

In conclusion, the development of recurrent breast cancer has been investigated as an effort to achieve successful breast cancer therapy and better clinical outcome as well as more number of breast cancer survivors. Loads of the number of follow up patients in this large population requires efficient, timesaving and cost-effective monitoring. Assessment for recurrence risk of the disease could be performed by the integration of the anticipated natural history of breast cancer based on its anatomic and biologic prognostic factors and the anticancer treatment administered.

This analysis confirmed the correlation of N stage, HER2, stage of cancer, tumour size, tumour grade, ER and PR status with recurrence in patients with operable breast cancer. We suggest that these patients should have proper treatment and be followed up frequently.

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Letter to the Editor

Cutaneous Bowen's Disease: an Analysis of 182 Cases according To Age, Sex, and Anatomical Site from an Italian Center

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Abstract

Bowen's disease (BD), also known as squamous cell carcinoma in situ, is a type of non-melanocytic intraepidermal malignancy characterised by a slowly enlarging erythematous to pink, scaly patch or plaque with irregular and well-demarcated borders. These lesions are usually persistent and progressive; it has been estimated that in general population around 3% to 5% of Bowen's disease transform into invasive squamous cell carcinoma. This report describes our experience with cutaneous BD and assesses the differences found about age, sex and anatomical site. Bowen's disease was seen more frequently in male patients rather than in female patients in contrast to what confirmed in literature - this difference is probably because being head-neck an exposed region, patients are more easily induced to autoexam and to consult the dermatologist.

Dear Editor,

Bowen's disease (BD), also known as squamous cell carcinoma in situ, is a type of non-melanocytic intraepidermal malignancy characterised by a slowly enlarging erythematous to pink, scaly patch or plaque with irregular and well-demarcated borders [1]. These lesions are usually persistent and progressive; it has been estimated that in general population around 3% to 5% of Bowen's disease transform into invasive squamous cell carcinoma [2], [3]. This report describes our experience with cutaneous BD and assesses the differences found about age, sex and anatomical site. One hundred eighty-two patients with cutaneous Bowen diseases were diagnosed between January 2010 and December 2017. All patients provided informed consent for our protocol conforming to the ethical guidelines of the 1975 Declaration of Helsinki. All patients had histopathologic-confirmed BD or squamous cell carcinoma in situ. This study was conducted at the Skin Cancer Unit of University of Federico II, Naples. Inclusion criteria were: (i) clinical (slowly enlarging, well-demarcated erythematous to pink patch or plaque with irregular borders and surface scale or crust) and dermoscopic (using pattern analysis) criteria for a diagnosis of Bowen's disease (e.g. multicomponent global pattern and a prominent vascular pattern of dotted and glomerular vessels together with a scaly surface); moreover, (ii) the presence of small brown globules or of grey to brown homogeneous pigmentation in the same dermoscopic structures were considered to be pigmented Bowen's disease. Superficial lesions such as Bowenoid papulosis were excluded. Lesions were recorded according to the age, sex of each patient, and by anatomical site (Table 1).

Table 1: Percentage of Bowen's disease found at each anatomical site according to the sex

	Males	Females
Head-neck	31%	17%
Upper limbs	15%	14%
Lower limbs	24%	39%
Trunk	21%	24%
Acral sites	9%	6%

A total of 182 Bowen's diseases were included. The mean age was 65 years (56-77). The χ^2 test of independence was used to compare sex-based differences. P-values < 0.05 were considered statistically significant. We found Bowen's disease to be more prevalent in male patients (94/182) rather than in female patients (88/182) (52% vs 48%).

Furthermore, head and neck region was the anatomical site most frequently observed in male patients (29/98, P = 0.3), whereas, lower limbs were the most represented location in female patients (34/88, P = 0.4). This result is in line with other studies in which head and neck are the anatomical sites most frequently involved followed by the lower limbs [4]. Our study has some limitations. Firstly, this is a retrospective observational study. Two individuals evaluated features to eliminate misclassification bias, with a third individual performing the decisive assessment in case of disagreement. Several studies report that Bowen's disease occurs more frequently in females and frequency varies between countries.

Moreover, our data suggest that there is a well-demarcated sex-difference statistically significant according to the anatomical site; in fact, head and neck are the most represented sites in male patients (31%), while women more commonly have Bowen's disease lesions on the lower limbs (39%). In conclusion, Bowen's disease was seen more frequently in male patients rather than in female patients in contrast to what confirmed in the literature [5]; this difference is probably because being head-neck an exposed region, patients are more easily induced to autoexam and to consult the dermatologist. Further studies are required to evaluate these results and to better compare sex-based differences of Bowen's disease.

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Letter to the Editor

Cutaneous Tumour of the Left Cricothyroid Area!?

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Keywords: Cutaneous tumour; Basal cell carcinoma; Cricothyroid area; Surgery; Lymph node; Antibiotic treatment

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Abstract

BACKGROUND: The cricothyroid area is an atypical localisation for placement of basal cell carcinomas. The main differential diagnosis for cutaneous tumours in this area is between BCC, spinocellular carcinoma and melanoma. The area is problematic about the choice of therapeutic approach, especially in the case of a vague clinical tumour type accompanied by enlarged lymph nodes in the immediate proximity.

CASE REPORT: We present an 84- year- old woman with a tumour formation located next to the left cricothyroid area. The lymph node ultrasonography performed during the hospitalisation revealed the presence of an enlarged lymph node in the upper third of m. Sternocleidomastoideus. The initial ultrasound data of the lymph nodes were in the direction of an inflammatory rather than a metastatic process. Therefore 5 days of therapy with Ceftriaxone x 2 g/day was conducted. The nodular tumour formation was surgically removed by radical elliptic excision. The subsequent histological study found that it was Stage II basal cell carcinoma (T2N0M0). A surgeon's consultation was conducted due to a patient's complaint about abdominal pain, and clinical evidence of a hernia inguinalis incarcerata was established for which the patient was urgently transferred to a surgical ward. Two weeks after the antibiotic treatment, a control echography of the enlarged lymph node in the area of m. Sternocleidomastoideus was performed, which showed complete involution of the lymph node.

CONCLUSION: Due to the specific anatomical features of the neck, such as a large number of lymph nodes and the resulting proximity between them and the primary tumours located in the area, it is often difficult to determine whether the lymph nodes are metastatically affected or inflammatory enlarged. In cases of missing ultrasound data for the metastatic process in the lymph nodes, surgical excision of the skin tumour with regular follow-up echographic control of the relevant lymph nodes represents an optimal therapeutic solution.

Dear Editor,

We present an 84-year-old woman with arterial hypertension and inguinal hernia. For arterial hypertension, the patient is on therapy with Bisoprolol fumarate 5 mg (1/2-0-0) and Simvastatin 20 mg (1-0-0). The patient was hospitalised for surgical removal of a tumorous formation in the left lateral part of the neck. The lesion dates from 5-6 years, during which it progressively increases in size, beginning to secrete and bleeds. During the dermatological examination, next to the left cricothyroid area, a nodular formation with a tight edge and an erosive bleeding surface was found (Figure 1a-c). According to the clinical data, the lesion was suspected for spinocellular carcinoma. In the differential diagnosis, it was also thought of Merkel cell carcinoma. A consultation was conducted with a vascular surgeon who identified the presence of an enlarged lymph node in the upper third of m. Sternocleidomastoideus, size 7.6 mm. Initial ultrasound of lymph nodes showed visual data rather for inflammatory than metastatic process. Five-day therapy with Ceftriaxone x 2 g/day was performed, and dressings with jodasept ointment were applied topically every day. Prophylactically, nadroparin calcium was applied at a dose of 0.4 ml/per day, subcutaneously. The nodular tumour formation was surgically removed by radical careful elliptical excision (Figure 2a, 2b). The resulting surgical defect was closed by a single interrupted sutures (Figure 2c).

The subsequent histological study found that it was basal cell carcinoma with extensive erosion, at places with the character of an adenoid-cystic variant; size 24/8 mm; free resection lines. The staging was performed according to which the data was for basal cell carcinoma stage II (T2N0M0). On the patient's complaint about abdominal pain, a surgeon was consulted, and clinical data for hernia inguinalis

incarcerated have been identified. For this reason, the patient was urgently transferred to a surgical department for surgical treatment. An ultrasound control of the enlarged lymph node in the area of m. Sternocleidomastoideus was planned after the antibiotic treatment, and the control echography after 2 weeks showed complete lymph node involution.

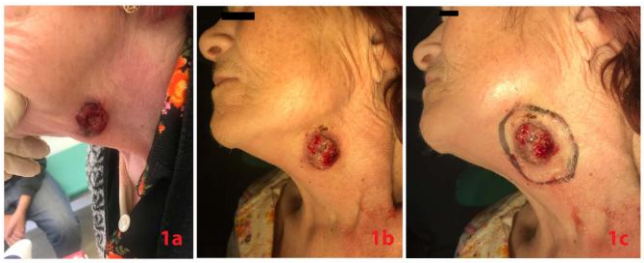

Figure 1: 1a- 1b, Clinical view of nodular formation with an erosive, bleeding surface, located next to the left cricothyroid area; 1c, Preoperative finding: outlining the surgical margins;

The presented case is interesting due to 1) atypical localization and clinical picture of basal cell carcinoma-adjacent to the cricothyroid region and 2) the choice of treatment approach in the case of a vaguely enlarged lymph node near the area of the primary cutaneous tumour. The most common localisations of basal cell carcinomas are nose and cheek area [1]. Unlike the clinical presentation described in our patient, BCC is usually presented as a nodule or plaque with pearly border and overlying telangiectasia and may be combined with a rodent ulcer [2].

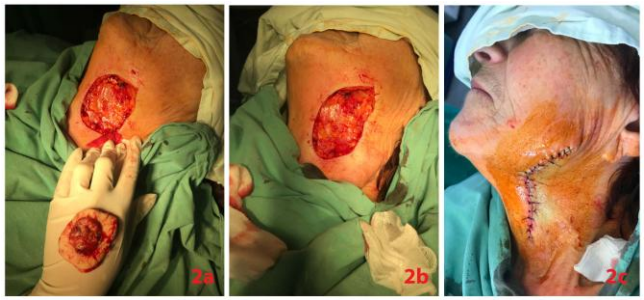

Figure 2: 2a-2b, Intraoperative view: elliptical excision of the nodular lesion; 2c, Postoperative finding: surgical defect closed by single interrupted sutures

The main differential diagnosis of skin tumours in the neck area is between BCC, spinocellular carcinoma (SCC) and melanoma [2].

In our case, based on clinical data, we considered SCC, an amelanotic variant of melanoma or Merkel cell carcinoma. It is believed that around 90% of the cases of skin tumours at the top of the aerodigestive tract are spinocellular carcinoma, which is characterized by being able to metastasize lymphogeneously [3]. Therefore, a surgical excision of the cutaneous lesion and a subsequent histological examination without an initial biopsy was selected for the described patient.

In the neck area, it is extremely difficult to determine the condition of the lymph nodes and whether they are metastatically affected by tumour cells due to 1) the anatomical characteristics of the area and a large number of lymph nodes and 2) due to the immediate spatial proximity between the primary tumour and the lymph nodes [3]. For this reason, our patient did not undergo lymph node dissection within surgical removal of the tumour formation, and a more gentle treatment approach was chosen with antibiotic treatment and observation of the therapeutic response from the lymph nodes.

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