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Cataracts Risk Factors and Comparison of Blood Glucose Levels in Diabetic and Non-Diabetic Patients towards the Occurrence of Cataracts

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Keywords: Risk factors; Hyperglycemia; Cataracts

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Abstract

BACKGROUND: Cataracts are a multifactorial systemic disease that causes opacity of the optical lens. One aetiology of cataracts is chronic hyperglycemia, usually caused by uncontrolled diabetes mellitus.

AIM: The objective of this study is to identify risk factors of cataracts and to analyse if there is a significant difference in blood glucose values between diabetic patients with cataracts and non-diabetic patients with cataracts.

METHODS: This was an analytical case-control study with a sample size of 140 patients that were obtained via consecutive sampling of medical records.

RESULTS: This study found that age, high body mass index and hypertension were the dominant risk factors of cataracts. The mean value of blood glucose levels in diabetic patients with cataracts is 195.58 ± 63.9 and 109.7 ± 26.4 in non-diabetic patients with cataracts. There was a significant difference between the blood glucose values of diabetic patients with cataracts and non-diabetic patients with cataracts ($p < 0.001$). The dominant risk factors of cataracts were old age, high body mass index and hypertension. The majority of hyperglycemic patients belong in the group of diabetic patients with cataracts.

CONCLUSION: Chronic hyperglycemia can increase a patient's risk of cataracts.

Introduction

The Global Data of Visual Impairment: 2010 states that 33% of visual impairment and 51% of blindness worldwide is due to cataract [1]. Cataracts represent the world's largest cause of visual impairment and blindness (33%) after refractive problems like myopia, hyperopia and astigmatism (43%) [2]. Research on worldwide visual loss statistics shows that as many as 42% of blindness in Southeast Asia is caused by cataracts [3]. According to the *RisetKesehatanDasar* (RISKESDAS) of the Republic of Indonesia, 1.8% of the Indonesian population have cataracts, with almost 1000 new cases occurring annually. The RISKESDAS also states that 3.7% of the people of North Sumatera have cataracts [4].

Cataracts have multiple etiologies, one of which is chronic hyperglycemia due to uncontrolled diabetes mellitus. A study was done by Mohammad-Ali Javadiet al., (2008) showed that people with diabetes mellitus are 2-5 times more at risk of cataracts at a younger age [5]. While the prevalence of cataracts varies with ethnic population and geographical location, consensually, the incidence of cataracts is higher in patients with diabetes mellitus than that of those without diabetes mellitus.

Therefore, this study aims to identify the risk factors of cataracts and to compare the levels of blood glucose in diabetic patients with cataracts and non-diabetic patients without cataracts.

Material and Methods

This study was conducted analytically with a case-control design. The population of this study were all cataract patients of Haji Adam Malik General Hospital. The sample population were 140 patients who were obtained via consecutive sampling of the patients' medical records.

Seventy patients were categorised as the case group, and another 70 patients were the control group. The control group were patients with cataracts but without diabetes mellitus, while the cases were cataract patients with diabetes mellitus diagnosed before cataracts. Diabetes mellitus in patients were confirmed with a doctor's diagnosis and high HbA1c value. A high HbA1c value is a criterion to diagnose diabetes mellitus because it denotes chronic hyperglycemia for the past three months. Then the patients' ad random blood glucose level was checked to see if they were hyperglycaemic or not.

On the other hand, the control group were non-diabetic patients with cataracts. This is because blood glucose values were not provided in the medical records for other ophthalmic diseases.

Blood glucose was observed through a patient's ad random blood glucose levels taken at least 3 months before the diagnosis of cataracts.

Results

Description of Age in Diabetic Patients with Cataracts and Non-Diabetic Patients with Cataracts

Various studies have shown that there is a significant relationship between advanced age and the incidence of cataracts. The table below shows the frequencies of patients aged > 50 years and ≤ 50 years old.

Table 1: Description of age in diabetic patients with cataracts and non-diabetic patients with cataracts

	Age	n	%
Diabetic Patients with Cataracts	> 50 years	57	81.4
	≤ 50 years	13	18.6
Total		70	100
Non-Diabetic Patients with Cataracts	> 50 years	59	84.3
	≤ 50 years	11	15.7
Total		70	100

According to Table 1, there are 57 (81.4%) diabetic patients with cataracts aged > 50 years and 13 diabetic patients with cataracts aged ≤ 50 years old. Table 1 also shows that in the control group, there are 59 (84.3%) patients aged > 50 years and 11 (15.7%) patients aged ≤ 50 years old.

The majority of patients in both case and control groups were aged > 50 years old. This is supported by a study done in the University of Airlangga, Surabaya, Jawa Timur, Indonesia; where 66.7% of patients who required cataract surgery were aged > 60 years old [6].

Description of Sex in Diabetic Patients with Cataracts and Non-Diabetic Patients with Cataracts

A person's sex can influence their risk towards cataracts. Epidemiologically, females are more susceptible to cataracts. Table 2 shows the frequencies of male and female patients that were studied.

Table 2: Description of sex in diabetic patients with cataracts and non-diabetic patients with cataracts.

	Sex	n	%
Diabetic Patients with Cataracts	Male	40	57.1
	Female	30	42.9
Total		70	100
Non-Diabetic Patients with Cataracts	Male	37	52.9
	Female	33	47.1
Total		70	100

Table 2 shows that in the group of diabetic patients with cataracts, there are 40 (57.1%) male patients and 30 (42.9%) female patients. In the control group, there are 37 (52.9%) male patients and 33 (47.1%) female patients. Overall, there are more male than female patients.

In both the case and control groups, the majority of patients were male. However, this is contradicted by studies that have shown that female patients are more likely to get cataracts due to a decrease in estrogen levels due to menopause [7]. The higher number of male patients with cataracts in this study may be due to habitual smoking that is prevalent in Indonesian men, where studies by RISKESDAS have found that 67% of men in Indonesia are smokers [8]. Smoking tobacco can increase one's risk of getting cataracts [9].

Description of Body Mass Index in Diabetic Patients with Cataracts and Non-Diabetic Patients with Cataracts

The table below shows frequencies of body mass indices of the patients in this study.

Table 3: Description of body mass index in diabetic patients with cataracts and non-diabetic patients with cataracts

	Body Mass Index	n	%
Diabetic Patients with Cataracts	Underweight	1	1.4
	Normal	22	31.4
	Overweight	18	25.4
	Pre-obese	24	34.3
	Obese	5	7.1
Total	70	100	Total
Non-Diabetic Patients with Cataracts	Underweight	7	10.0
	Normal	36	51.4
	Overweight	9	12.9
	Pre-obese	17	24.3
	Obese	1	1.4
Total	70	100	Total

Table 3 shows that in the group of diabetic patients with cataracts, the majority of patients have higher than normal body mass indexes, with 18 (25.4%) overweight, 24 (34.3%) pre-obese and 5 (7.1%) obese patients. Only 22 (31.4%) of these patients had normal body mass indexes, and 1 (1.4%) patient was underweight.

On the other hand, the majority of patients in the control group have normal body mass indexes, with 36 (51.4%) patients. Nine (12.9%) of the patients were overweight, 17 (24.3%) were pre-obese, and 1 (1.4%) patient was obese. There were 7 (10.0%) patients who were underweight.

Obesity has an inconsistent relationship with the occurrence of cataracts; therefore, the causality of cataracts due to obesity cannot be proven as of now [10]. However, a cohort study by Ye et al., (2014) showed that an increase of 1 kg/m^2 in body mass index could increase the risk of senile cataracts by as much as 2% [11].

Description of Blood Pressure in Diabetic Patients with Cataracts and Non-Diabetic Patients with Cataracts

The pathophysiology of cataracts is influenced by severe systemic inflammation; therefore, hypertension can influence the pathogenesis of cataract formation via inflammatory mechanisms. Some theories state that hypertension can cause a permanent change in lens protein. However, epidemiologic studies have found inconsistent results for the mechanism of hypertension in cataract formation [12].

Table 4: Description of blood pressure in diabetic patients with cataracts and non-diabetic patients with cataracts

	Blood Pressure	N	%
Diabetic Patients with Cataracts	Normal	14	20,0
	Pre-hypertension	23	32,9
	Hypertension 1	19	27.1
	Hypertension 2	14	20.0
Total		70	100
Non-Diabetic Patients with Cataracts	Normal	18	25.7
	Pre-hypertension	42	60.0
	Hypertension 1	6	8.6
	Hypertension 2	4	5.7
Total		70	100

Table 4 shows that in the group of diabetic patients with cataracts, the majority of patients had higher than normal blood pressure, where 23 (32.9%) 0f patients were pre-hypertensive, 19 (27.1%) had type 1 hypertension and 14 (20.0%) had type 2 hypertension. Only 14 (20.0%) of patients in this group had normal blood pressure.

Forty-two (60.0%) of patients in the control group were pre-hypertensive, 6 (8.6%) had type 1 hypertension, and 4 (5.7%) had type 2 hypertension. There were 18 (25.7%) patients who had normal blood pressure in this group.

A meta-analysis by Yu et al., involving 25 other studies shows that the risk of cataracts is higher in populations with hypertension in cohort studies (RR 1.08: 95% CI: 1.05 – 1.12), and case-control or cross-sectional studies (RR 1.28: 85% CI: 1.12 – 1.45) [12].

Description of Ad Random Blood Glucose in Diabetic Patients with Cataracts and Non-Diabetic Patients with Cataracts

The following table shows the frequencies of ad random blood glucose levels of the patents in the study.

Table 5: Description of ad random blood glucose in diabetic patients with cataracts and non-diabetic patients with cataracts

	Blood Glucose Status	n	%
Diabetic Patients with Cataracts	Normal	41	58.6
	Hyperglycemia	29	41.4
Total		70	100
Non-Diabetic Patients with Cataracts	Normal	69	98.6
	Hyperglycemia	1	1.4
Total		70	100

Table 5 shows that in the group of diabetic patients with cataracts, 41 (58.6%) of patients had normal ad random blood glucose, while 29 (41.4%) of the patients were hyperglycemic. The table also shows that in the control group, 69 (98.6%) of the patients had normal ad random blood glucose while only 1 (1.4%) patient was hyperglycaemic.

The minority of diabetic patients with diabetes mellitus have normal blood glucose levels. This is most probably due to the medication taken by diabetic patients to control their blood glucose levels. However, studies have shown that the incidence rate of cataracts in diabetic patients is 20.4 per 1000 person-years but is 10.8 per 1000 person-years in non-diabetic patients [13]. Another study by Raman et al., (2010) showed that 2 / 3 of 1283 patients with type 2 diabetes mellitus had cataracts [14].

Comparison of Ad Random Blood Glucose in Diabetic Patients with Cataracts and Non-Diabetic Patients with Cataracts

The pathophysiology of cataracts due to uncontrolled diabetes mellitus is due to the chronic hyperglycemia that occurs. The following table shows the results of the Mann-Whitney-U test

Table 6: Comparison of ad random blood glucose in diabetic patients with cataracts and non-diabetic patients with cataracts

	Mean ± Standard Deviation	Mean Difference	p-value
Diabetic Patients with Cataracts	195.58 ± 63.9	85.88	0.001
Non-Diabetic Patients with Cataracts	109.7 ± 26.4		

Table 6 shows the results of the Mann-Whitney U test, where the p-value obtained was p = 0.001. Therefore, there is a significant difference in blood glucose levels between diabetic patients with cataracts and non-diabetic patients with cataracts. Since the mean value of blood glucose levels is higher in diabetic patients with cataracts as compared to non-diabetic patients with cataracts, there is a

significant relationship between blood glucose levels and cataract occurrence in diabetic patients. This is due to the pathophysiology of cataracts due to uncontrolled diabetes mellitus, where chronic hyperglycemia occurs and increases the risk of cataract incidence.

Discussion

The results of this study are supported by a study done by Kahloun et al., where significant visual impairment can occur to patients who have had diabetes for more than 10 years ($p < 0.001$) [15]. This study also showed that fasting blood glucose levels is related to 10-year incidence rates of cortical cataracts. Another study by Jingi et al., states that a patient who has had diabetes mellitus for more than 10 years is 1.42 times more likely to experience cataracts than patients who have had diabetes for less than 10 years [16].

This study found that cataracts have multiple risk factors which are old age, high body mass index and hypertension. Chronic hyperglycemia can increase a patient's risk of cataracts. The majority of hyperglycemic patients belong in the group of diabetic patients with cataracts. Healthcare providers and patients should be more aware of the risk factors of cataracts and work together to handle those risks appropriately.

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The Difference in Effect of Arabica Coffee Gayo Beans and Leaf (*Coffea Arabica Gayo*) Extract on Decreasing Blood Sugar Levels in Healthy Mice

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Abstract

BACKGROUND: High incidence rate of diabetes mellitus (DM) and increased incidence of complications from DM as well as the use of less effective antidiabetic drugs and high financing to treat demands alternative therapy.

AIM: This study was conducted to determine the difference in the effect of Arabica coffee gayo bean and leaf extract on blood sugar levels in healthy mice before and after the intervention of extract and fasting and postprandial blood glucose level after consumption of glucose.

METHODS: This research is an experimental research study. The study used experimental animals which were divided into 8 groups which are the control group (Aquadest), group given Acarbose, the group given the Arabica coffee gayo bean extract with dosage of 50 mg, 100 mg and 200 mg in healthy mice, the group given the Arabica coffee gayo leaf extract with dosage of 30 mg, 60 mg and 120 mg in healthy mice**.**

RESULTS: The results of the study obtained are before and after the intervention of extract showed the difference of p= 0.523 and after consumption of glucose the fasting and postprandial result showed the difference of p = 0.005.

CONCLUSION: The use of Arabica coffee gayo bean and leaf extract showed a high decrease in blood glucose levels (BGL) compared to the control group.

Introduction

Diabetes mellitus (DM) is a clinical symptom that arises due to an increase in chronic blood glucose levels due to lack of insulin, both relative and absolute [1]. DM is also a chronic disease that requires long-term management [2].

Type 1 diabetes patients and type 2 diabetes who prescribe flexible insulin therapy programs, education on how to use carbohydrate calculations and in some cases estimation of grams and protein grams to determine the time-consuming insulin dose is recommended to increase glycemic control [3].

Studies that examined the ideal number of carbohydrate intake for people with diabetes were inconclusive, despite monitoring carbohydrate intake and considering blood glucose responses to carbohydrate diets to improve postprandial glucose control. The literature on the glycemic index and the glycemic load in individuals with diabetes is complex often producing mixed results, although in some studies reducing the glycemic load of carbohydrates consumed has been shown to decrease A1C-0.2% to -0.5%. Study reports of more than 12 weeks have no significant effect on glycemic index or independent glycemic load weight loss on A1C; however, mixed results have been reported for fasting glucose levels and endogenous insulin levels [3].

Globally, an estimated 422 million adults lived with diabetes in 2014, compared with 108 million in 1980. The global prevalence (standard age) of diabetes has nearly doubled since 1980, increasing from 4.7% to 8.5% in the adult population. This shows an increase in related risk factors such as being overweight or obese. Over the past decade, the prevalence of diabetes has increased more rapidly in low- and middle-income countries than in high-income countries. Diabetes caused 1.5 million deaths in 2012. Higher than optimal blood sugar caused an additional 2.2 million deaths, increasing the risk of cardiovascular disease and others. Forty-three percent of the 3.7 million deaths occur before the age of 70 years. The percentage of deaths caused by high blood glucose or diabetes that occur before age 70 is higher in low and middle-income countries than in high-income countries [4].

A decrease in blood sugar levels can be reduced by taking acarbose treatment which is a treatment of artificial oligosaccharides which is competitive inhibitors and can inhibit the action of the α-glucosidase enzyme, which can slow digestion in carbohydrates [5].

Also, a decrease in blood sugar levels can be reduced by using Arabica coffee gayo bean extract as an alternative treatment. Consuming coffee can help reduce the risk of type 2 diabetes, while caffeine promotes lipolysis in adipocytes. Chlorogenic acid, another major constituent of coffee beans, has recently been reported to selectively inhibit hepatic glucose-6-phosphatase which is a limitation of the rate of enzymes involved in gluconeogenesis. However, roasting coffee beans has been shown to reduce the chlorogenic acid content in coffee. Arabica coffee gayo beans are rich in chlorogenic acid and their related compounds have a hypotensive effect [6].

Therefore, this study was conducted to determine the effect of Arabica coffee gayo bean extract on blood sugar levels in healthy mice after loading glucose.

Material and Methods

This research is an experimental study. The study used 25 animals as mice which were divided into 5 groups, namely the control group (-) (Aquadest), control group (+) (Acarbose) with a dose of 6.5 mg/KgBB, groups of Arabica coffee gayo beans with dosage of 50 mg, 100 mg and 200 mg and Arabica coffee gayo leaf with dosage of 30 mg, 60 mg and 120 mg. This research was conducted at the Pharmacology Laboratory at the University of North Sumatra (USU). In this study male mice (Mus musculus) will be used, Double Distsch Webster strain (DDW) age: 2-3 months (adults), weight 20-30 grams, healthy, never used in other studies. Mice were obtained from the FMIPA Biology USU Medan Laboratory. Arabica coffee gayo bean and leaf extract will be provided in the pharmacy faculty. The raw material for Arabica coffee gayo beans and leaves is obtained from a doctor.

The selection of samples and groupings was carried out using simple random sampling, in which every 40 samples that met the predetermined inclusion criteria will be numbered, then divided into 8 groups.

Arabica coffee gayo bean and leaf extract are done by drying. After that, the coffee beans and leaves are stretched to become smooth, and ethanol is 96% and mixed with Aquadest so it becomes 50% ethanol and left for 5 days for masturbation. Arabica coffee gayo bean and leaf extract are stirred every day. After 5 days, coffee bean and leaf extract are evaporated and left for 3 days to become thick, after 3 days coffee bean and leaf extract can be used for experimental research. Experimental research was carried out by administering aqua destilata, acarbose, Arabica coffee gayo bean extract with a dose of 50 mg, 100 mg, and 200 mg and Arabica coffee gayo leaf extract with dosage of 30 mg, 60 mg and 120 mg given to mice. Treatment and extract interventions were given for 3 days. On the third day, the blood glucose level (BGL) of mice were examined after the intervention and were fasted so that day 4. On day 4, BGL was examined for mice fasting, then given starch at a dose of 5 g/Kg to increase the BGL of mice and examined BGL postprandial mice. This research was conducted for 4 days. BGL mice are measured by the EasyTouch brand glucometer in mmol/L units and mice are given food every day while conducting research in the form of corn pellets, as much as 5 grams/day and also given as much as 30 ml water drinks. The thing that needs to be considered in this study is if the group of Arabica coffee gayo beans and leaves are effective to reduce BGL mice.

Results

The results of the study obtained are as followed below:

Table 1: Results Comparison of BGL of mice before and after the intervention of the green coffee bean group with the green coffee leaf group

Group	Comparison Group	N	Average	Standard Deviation	P
Arabica Coffee Gayo Bean	Arabica Coffee Gayo Leaf	14	-1.20	6.83	0.523

Description: Paired T-test = significance; $p < 0.05$.

Based on the results of Table 1, Paired T-test results of comparison of green coffee bean groups

with a dose of 50 mg, 100 mg and 200 mg with a group of green coffee leaves with a dose of 30 mg, 60 mg and 120 mg showed a p-value = 0.523 was not significant because the p-value < 0.05.

Table 2: Results Comparison of BGL fasting mice and Post Prandial groups of green coffee beans with groups of green coffee leaves

Group	Comparison Group	N	Average	Standard Deviation	P
Arabica Coffee Gayo Bean	Arabica Coffee Gayo Leaf	14	0.84	0.93	0.005

Description: Paired T-test = significance; p < 0.05.

Based on the results of Table 2, Paired T-test results of comparison of green coffee bean groups with a dose of 50 mg, 100 mg and 20 0mg with a group of green coffee leaves with a dose of 30 mg, 60 mg and 120 mg showed a p-value = 0.005 was significant due to a p-value < 0.05.

Discussion

This study showed that from 38 mice grouped into 8 groups with 5 mice per group, two mice were excluded due to the exclusion criteria of the study. In clinical trials, the results showed before and after the intervention of extract showed the difference of p = 0.523 and after consumption of glucose, the fasting and postprandial result showed the difference of p = 0.005.

The above results can occur because Arabica coffee gayo beans have chlorogenic acid compounds, consumption of chlorogenic acid in coffee can reduce the risk of type 2 diabetes mellitus. These compounds can stimulate glucose uptake in skeletal muscles through activation of adenosine monophosphate-activated protein kinase (AMPK). AMPK can show a positive impact that can direct the results of metabolites of useful substances such as decreased glucose production in the liver and fat synthesis [7].

There are epidemiological evidence and other significant evidence that coffee consumption reduces the risk of type 2 diabetes. One large study indicated a 50% risk reduction for people who drank seven cups of coffee a day compared to those who only drank two cups a day. Evidence shows chlorogenic acid as the active ingredient in coffee that prevents diabetes and improves glucose control in normal, prediabetic and diabetic patients [8].

Besides that, the Arabica coffee gayo leaves have mangiferin compounds which are useful for reducing the risk of heart disease and diabetes mellitus. Besides, coffee leaves also contain high amounts of antioxidants compared to green tea and black tea. The findings indicate that it promises that coffee leaves can be beneficial to be processed as a standardized herbal product that can be consumed daily and has efficacy as an anti-diabetes mellitus [9].

From the results of this study, it can be concluded as follows the use of Arabica coffee gayo bean and leaf extract showed before and after the intervention of extract showed the difference of p = 0.523 and after consumption of glucose the fasting and postprandial result showed the difference of p = 0.005

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The Influence of Health Promotion on Female Students' Behaviour in SMA An - Nizam Medan about Endometriosis

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Keywords: Endometriosis; Behaviour; Health Promotion

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Abstract

Endometriosis is a gynaecological disorder that is often characterised by dysmenorrhea, dyspareunia and or infertility. These symptoms are very common and often found as a symptom of other diseases so that endometriosis is more difficult to diagnose or often diagnosed late. Therefore, diseases like this would be better if we prevent them before they suffer. Although endometriosis is caused by multifactor, we can still do simple prevention and be positive. However, several other factors cause this prevention not to be applied in daily life such as lack of knowledge, inadequate facilities or an environment that does not support. Therefore, health promotion as a medium of information and the establishment of community self-reliance is expected to shift bad habits or form new good habits to be more vigilant and prevent endometriosis. The study was conducted using a quasi-experimental design with the pretest-posttest method and was processed using the Wilcoxon signed-rank test. Based on the results of data analysis, there is a significant increase in the behaviour of female students before and after health promotion.

Introduction

Endometriosis is the presence of endometrial glands and stroma growth in an aberrant and heterotopic location outside the uterus [1], [2], [3], [4]. The aetiology of endometriosis is still unclear but is thought to be caused by many factors including the involvement of the hormone estrogen. The incidence of endometriosis in the world reaches 10%. This number shows that the incidence of endometriosis is quite high and requires more attention. Clinical symptoms that are often displayed by endometriosis are not typical such as dysmenorrhea, dyspareunia and or infertility. These symptoms are very common and are often found as a symptom of other diseases. Variations in other symptoms of endometriosis are very diverse depending on the location of the lesion. Therefore, the selection of endometriosis therapy must be on target according to the symptoms caused. However, a definite aetiology has not been found so treatment is usually symptomatic and patients can experience recurrence after a period of treatment.

Because of the relationship between endometriosis and estrogen, the menstrual cycle and age of miners also contribute. Very few women remember exactly when their first menstruation or remember when the first day of their period every month. Similarly, pelvic pain when menstruation is often overlooked. This shows that ordinary people still feel unfamiliar with endometriosis.

Knowledge about endometriosis is still very low so that they do not intervene in any way to

prevent this situation. However, several studies have shown that high knowledge does not guarantee harmony in their practices and actions. Knowledge alone is not enough to make someone change their behaviour to prevent endometriosis, but also needs encouragement from the surrounding environment that supports infrastructure.

Health promotion is an approach to behavioral health factors; then their activities cannot be separated from the factors that determine the behaviour. According to Lawrence Green in the book [5], the behavior is determined by three main factors and the health promotion approach leads to these factors. First, the predisposing factors are factors that facilitate the occurrence of the behaviour in a person or society, in the form of knowledge and an overview of attitudes towards what will be done. Thus, health promotion implemented in the form of providing information about endometriosis or health messages and health education.

The purpose of this activity is to improve knowledge and attitudes about endometriosis, straighten traditions, beliefs, or values that are not conducive to healthy behaviours. Secondly, enabling factors such as the availability of facilities, equipment or infrastructures that support the behavior. So, health promotion is carried out in the form of community empowerment. The purpose of this activity is to facilitate themselves to behave well. This activity does not mean giving health facilities or infrastructure as a whole but provides the ability or expertise to support the provision of the necessary facilities. Third, reinforcing factors. Knowledge, attitudes and facilities that sometimes have not produced the expected behaviour in a society that needed reinforcing factors such as community leaders who set a good example in the prevention of endometriosis, such as greater scale forming official regulations, laws, and so forth.

The Jakarta Declaration states that the priority of health promotion is to improve community capacity and empower individuals and ensure health promotion infrastructure. Based on the above definition, health promotion needs to be socialised and is a cross-sector responsibility. Therefore, this study was conducted to observe the effect of health promotion on the actions of female students in SMA An-Nizam Medan on the topic of endometriosis. The aim of this study to see the effect of health promotion carried out by researchers, in the form of new good actions or habits.

Material and Methods

The type of research used was quasi-experimental design with the pretest-posttest method. This study was conducted for two months starting in September 2018 until November 2018 at the High School An-Nizam Medan. The school environment was chosen as the location of the study aimed at facilitating follow up and equalising the educational background of the research subjects to reduce external determinants that could influence the results of the study. The population is all female students of An-Nizam High School Medan. Determination of the number of samples using the total sampling method, that is, all population numbers were taken as a sample, 102 people.

This study uses a measurement tool in the form of a questionnaire that has been validated and tested for reliability. The intervention group came from the same group. Data collection is done twice, namely just before health promotion and one month after health promotion with the same instrument with the modification of the position number statement. The first measurement is that just before health promotion aims to collect preliminary data as a research control. Then, health promotion is carried out with an interactive (two-way) lecture method with the help of powerpoint media. The theme of health promotion is about endometriosis. The intervention that given is health promotion related to endometriosis. The second measurement, a month after health promotion, aims to see the effect of health promotion carried out by researchers, in the form of new good actions or habits.

Before data collection from research, subjects are carried out; the research subject must have understood the research to be carried out and signed informed consent. After being approved by the Ethical Committee, the research subject was given a questionnaire and filled it in according to the circumstances of each individual. Then, the data was processed with the Wilcoxon signed-rank test ($\alpha = 0.05$).

Results

All research samples filled out the questionnaire items in full so that no research subjects were excluded.

Table 1: Dependent Variable Frequency Distribution in Respondents

Variable	Health Promotion			
	Pretest		*Pretest*	
	N	%	N	%
Action				
Positive	27	26,47	44	43,14
Neutral	67	65,69	54	52,94
Negative	8	7,84	4	3,92

Based on the data processing of the research results in Table 1 shows that there are differences in the proportion of actions at the pretest and posttest. When depicted in Figure 1, this change is a positive

change where positive numbers shift to the right and vice versa. The positive category, which was originally 26.47% increased to 43.14%.

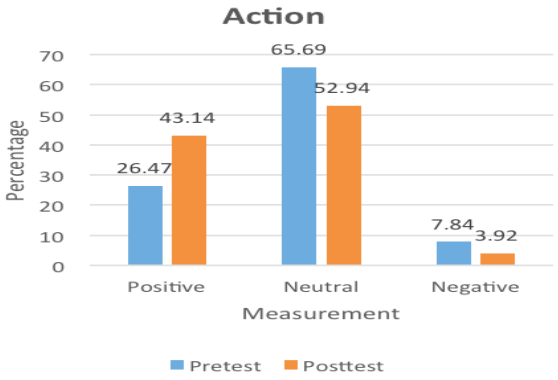

Figure 1: Percentage Frequency Distribution of Knowledge

The behaviour of respondents before and after health promotion is illustrated in Table 2. Tests on actions produce p = 0.001 or p < 0.05. Judging from the mean rank where positive rank (45.26%) is greater than the negative rank (44.34%) means that the differences that occur are parallel to the intervention. Health promotion causes an increase in the respondent's behaviour.

Table 2: Comparison of Respondents Action Before and After Health Promotion

Variable	Health Promotion			
	Pretest		*Pretest*	
	N	%	N	%
Action				
Positive	27	26,47	44	43,14
Neutral	67	65,69	54	52,94
Negative	8	7,84	4	3,92

Z-table score in the study with α = 0.05 is 1.645 (Z distribution table). The z-count value obtained in the measurement of the action is 3.678. When compared with z-table, the z-count score is greater. This means that H_0 is rejected or H_a is accepted; there is a difference or increase in the value of the action due to health promotion intervention. Testing through Z score strengthens the hypothesis test with p-value which also rejects H_0.

Discussion

The general objective of holding a health promotion, according to WHO is to change the behaviour of individuals or communities in the health sector. So, in this study observed changes in the actions of respondents who showed a positive difference. The proportion of the categories of positive actions before health promotion was 26.47%. After health promotion, the number in the positive action category increased to 43.14%.

The precautionary measures for endometriosis observed in this study include the act of maintaining hygiene during menstruation, calculating the menstrual cycle and regular checks to the doctor. According to [8] research, one of the efforts to reduce menstrual-related disorders is to get used to hygienic behaviour, especially the vulva hygiene. Similarly, the menstrual cycle must be calculated every month because there is an association with the hormone estrogen which is thought to be the main role in the incidence of endometriosis.

According to [6], there are three aspects of behaviour, namely knowledge, attitudes, and actions. These three elements influence each other. A person's behaviour is determined by knowledge, attitudes, beliefs, traditions and environment and many other aspects. If a person has a high knowledge of a thing, it is expected that the person also has a positive attitude and behaviour as well. However, these three aspects do not always go hand in hand as they may be influenced by many other factors, such as motivation, intentions, experience and so on. The effect of health promotion at a community also depends on other determinants that not all people will experience increased after the intervention [9], [10], [11].

It can be concluded that health promotion has a significant influence on the knowledge and attitudes of female students in An-Nizam High School Medan about endometriosis. Health promotion has the potential to increase positive knowledge and attitudes in the subject of research. Therefore, health promotion about endometriosis can be considered for more implementation and promotion in various regions as an effort to reduce the incidence of endometriosis. Medical and paramedics can also raise endometriosis material as an extension topic. Similarly, the government can take a role as a facilitator of implementation of various programs, including health promotion of endometriosis.

The little amount of knowledge absorbed through health promotion is the limitation of each individual, so health promotion methods and media must still be adjusted to the target community that will be addressed. Interactive and two-way methods are better choices so that the percentage of understanding goals is greater. Moreover, if health promotion is carried out repeatedly, it will produce more significant differences.

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Exercise Intensity Alter *Insulin Receptor* Gene Expression in Diabetic Type - 2 Rat Model

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Keywords: IR gene; Moderate continuous training; Severe continuous training; DM type-2

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Abstract

AIM: To analyse the differential expression of the *insulin receptor (IR)* gene between moderate continuous and severe continuous training in the T2DM rat model.

METHODS: This was an experimental study. Healthy male Wistar was used in this study, which divided into sedentary, moderate continuous training, and severe continuous training. Treated groups were assigned to run on the treadmill three times a week for eight weeks consequently.

RESULTS: The result shown that expression of mRNA *IR* gene in treated groups decline compared to control. There was a difference mRNA *IR* gene expression after eight weeks of exercise between MCT and control, SCT and control so are MCT and SCT. *IR* expression on skeletal muscle in treated groups was different compared with control. The distribution of IR on skeletal muscles in treatment groups was significantly increased compared control, but there was no significant difference distribution between MCT and SCT. HOMA-IR post-test in SCT was lower than MCT but FBG post-test lower in MCT than SCT.

CONCLUSION: The intensity of exercise makes a difference in *IR* gene expression between moderate continuous training and severe continuous training after eight weeks of assigned exercise in T2DM rat models.

Introduction

Nowadays, Indonesia becomes a global epidemic of Type-2 Diabetes Mellitus (T2DM). International Diabetes Federation (IDF) predicted the prevalence of T2DM patients in Indonesia is going to increase about 16,2 million by 2040 [1]. According to Indonesian Endocrinology Society (PERKENI), the increasing of T2DM prevalence is related to lifestyle changes, i.e. dietary and limited physical activity [2]. There were many studies related to exercise and insulin resistance but still limited yet to apply intensity altering insulin receptor gene expression.

Mechanism of insulin resistance in T2DM patient that caused by insulin signalling impairment is possible to happen at pre receptor, receptor and post-receptor level [3], [4], [5]. Chronic hyperglycemia caused an impact on insulin receptor such as *downregulation* receptor on the membrane surface of the skeletal muscle, thus causing impairment of insulin sensitivity [6]. Insulin receptors were found most at skeletal muscle, liver and adipose tissue [7]. Insulin receptor impairment in the liver will lead to blood glucose homeostasis disturbance at rest; while in skeletal muscle, glucose transporter-4 (GLUT-4) fails to exocytosis up to membrane surface when activity [8].

Insulin receptor expression on the skeletal muscle membrane was determined by mRNA receptor gene expression. The mRNA receptor gene expression will increase when our body requires more receptors. There was a relationship between gene expression and metabolism. Metabolic status and enzyme metabolites are inter-related, thus increasing one will affect others significantly [9].

Exercise can increase muscle contraction to full fill energy needs. Energy for muscle contraction is obtained from aerobic and anaerobic metabolism [10]. Power stroke produces ATP [11]. ATP and creatine ratio reduction activated enzyme AMP Kinase. This enzyme will then stimulate mitochondrial biogenesis through PGC-1α [12], [13]. Mitochondrial enzyme altered gene transcription [14]. Sympathetic activities during exercise suppressed insulin secretion from beta pancreas cell and at the same time, enhance gluconeogenesis in the liver, causing the blood glucose level to increase [10], [11].

Decreasing insulin levels stimulates an increase in receptor density on the surface of the cell membrane to facilitate blood sugar entry to the cell [15]. Rate of cortisol during exercise stimulates transcription of gene receptor insulin. The more vigorous intensity of exercises was carried out, the more accelerate the metabolism of the cell, drawing an effect of significant proportional in between the intensity of training and gene expression and the amount of insulin receptor involved [16]. This study aims to analyse the differential expression of the insulin receptor gene between moderate continuous and severe continuous training in T2DM rat models.

Material and Methods

Healthy male Wistar rats, 150-180 gram in weight, age eight weeks, were used in this study. These rats were acclimatised for seven days. Four rats were placed in the same cage, in a room with a temperature of 22-250°C with a dark light cycle 12 / 12 h. After acclimatisation, the animal experiment was given meals with a high-fat diet for five weeks consequently. They were then undergone fasting for one night before they were induced with Streptozotocin 30 mg/kg/intra-peritoneum diluted in 0.1 citrates, Buffer of pH 4.5. A repeated induction was given a week later with a dosage of 45 mg/kg [17]. Laboratory fasting blood glucose was tested on the next week for individual rats by taking the blood samples from the tail's veins. Rat determined as T2DM if fasting blood glucose was > 200 mg/dl and HOMA-IR > 6,5. Samples then classified into three groups, i.e. the *sedentary* group (control), *moderate continuous training* (MCT), and *severe continuous training* (SCT). There was no exercise assignment for the sedentary group. MCT group was running on the treadmill with a speed of 25 m/min and 30 m/min for the SCT group.

Fasting blood glucose level and HOMA-IR were recorded before and after exercise carried out, while the expression of the IR gene and muscle-skeletal insulin receptor were recorded after all exercise protocol was done. IR gene expression from gastrocnemius muscle was analysed using Real-Time PCR and immunohistochemistry used to assessed insulin receptor expression.

Exercise Protocol

The intervention carried out in this study is parallel with the protocol of Huang et 2016 [18], which the samples were placed on exercising on the treadmill. This intervention was held 3 times a week with 30 minutes/session for 8 weeks long. Before the protocol was given, rats are used to walk on the treadmill before the exercise protocol has begun. After exercise procedure was complete, rats were executed under sedation with ketamine 30 mg intramuscular.

The procedure of fasting blood glucose test and HOMA-IR

Blood glucose and HOMA-IR were measured before and after each session of assigned exercise. The samples fasted for 12 hours, and the blood samples were taken from the lateral vein of the tail, with a volume of 3 ccs. Anaesthesia with local Xylocaine spray was given before each blood samples were taken. Blood serum was extracted and tested for the blood glucose level with a spectrophotometer using GOD-PAP method (wavelength of 500 nm, Hg 546 nm, and temperature of 20-25°C / 37°C). Fasting insulin was tested with Insulin INS-Rat kit (Qayee-Bio, Shanghai, China) by ELISA. The resistance of insulin was determined by calculation of HOMA-IR, using the equation of:

Fasting Insulin (mIU/L) x fasting blood glucose level (mg/dl) 405

The procedure of expression Insr gene test

As much as 30-40 mg of gastrocnemius muscles were inserted in an Eppendorf tube filled with RNA fluid. Isolation of RNA gene carried out by using the standard procedure of the RNeasy Mini Kit (Qiagen-German). The result of isolation samples was kept under a temperature of -20°C for further analysis. RT-PCR test was carried out using One-Step RT-PCR with the SYBR® Green RT-PCR Reagents Kit (Biosystem).

Primer IR- Rat Forward (IDT): 5'- GGC CAG TGA GTG CTG CTC ATG C-3'

Primer IR-Rat Reverse (IDT): 5'- TGT GGT

GGC TGT CAC ATT CC-3'

Primer B actin Rat Forward (IDT): 5'- CAC CCG CGA GTA CAA CCT TC– 3'

Primer B actin Rat Reverse (IDT): 5'- CCC ATA CCC ACC ATC ACA CC – 3'

Thermocycler (rotor gene) were arranged according to the conditions of RT incubation 42°C (5 minutes), early denaturation 92°C (5 minutes), denaturation 45°C (10 seconds), annealing /extension 60°C (30 seconds) for 40 cycles.

Data Analysis

Normality of data obtained was testified using Shapiro–*Wilk* ($p < 0.05$). The t-dependent test was used to determine the difference between HOMA-IR and blood glucose level before and after assigned exercise in this study. Expression of *Insr* gene, HOMA-IR posttest among groups analysed by ANOVA test with the value of $p < 0.05$ and Post Hoc analysis with Bonferroni test. Meanwhile, the Kruskal Wallis test was used in this study to analyse the differences of skeletal muscle receptor in all three sample groups.

Results

Dismounts of HOMA-IR and blood glucose level were noted after eight weeks of assigned exercise were carried out. Reduction in blood glucose level in the treated groups was significant. FGB in MCT was lower than SCT and control. However, the reduction of HOMA-IR in MCT is not considered statically significant (table 1). According to the ANOVA test, there were different expressions on mRNA IR gene for MCT group samples and SCT group samples compared to control samples.

Table 1: Expression of gene *Inst*, insulin receptor, HOMA-IR and Fasting blood glucose level for control and independent sample

	Expression of gene *Insr* (ΔCt) Mean 2 ± SB	Insulin receptor muscles (%) Mean2 ± SB	HOMA-IR Mean2 ± SB	P value	FGB (mg/dl) Mean2 ± SB	P value
Control	1.0 ± 0.3	60 ± 20	Pre = 107.1 ± 14.4 Post = 49.1 ± 9.5		Pre = 474,8 ± 24.9 Post = 214.9 ± 5.7	
MCT	4.5 ± 2.2	86 ± 5.5	Pre = 90.5 ± 40.5 Post = 43.3 ± 8.6	0.066	Pre = 339 ± 103,7 Post= 191.6 ± 5.4	0.014*
SCT	2.8 ± 0.5	88 ± 4.5	Pre = 81.3 ± 6.1 Post = 18.5 ± 6.4	0.000*	Pre = 396.8 ± 25.7 Post = 198.2 ± 75.0	0.009*

Note: MCT = *moderate continuous training*; SCT = *severe continuous training;* ΔCt = *cycling threshold* target gene - *cycling threshold* refrence gene; SD = *standard deviasion*; HOMA-IR = insulince resistence; FGB = Fasting Blood Glucose; $p < 0.005$.

Table 1 showed that eight weeks of exercise has an effect on HOMA-IR and FGB. Both exercise in moderate continuous and severe continuous can reduce the average of HOMA-IR and FGB after 8 weeks of exercise in the T2DM rat model. Even 8 weeks of exercise has not quite enough to reduce HOMA-IR statistically significant in the MCT group. HOMA-IR post-test was found lower in SCT than MCT. But FGB was lower in MCT than SCT. mRNA IR gene cycling threshold and IR gene distribution on skeletal muscle increased both MCT and SCT.

Cycling threshold mRNA gene in MCT and SCT higher than control (Figure 1). Means that gene expression in MCT and SCT underwent eight weeks of exercise are found to have lower expression compared to control which are not given any exercise (MCT vs SCT vs Control = 4.5 ± 2.2 vs 2.8 ± 0.5 vs 1.0 ± 0.3; $p = 0.006$).

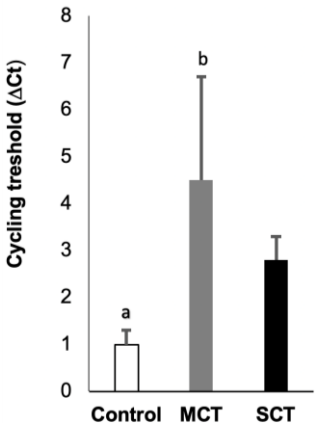

Figure 1: Insulin Receptor mRNA Gene Expression

Based on the Bonferroni post hoc test, there was a significant difference IR gene expression between the control group and MCT ($p = 0.002$), control and SCT ($p = 0.033$). IR gene expression in the MCT group also had a significant difference with IR gene expression in SCT ($p = 0.047$).

MCT gene *Insr* is found to have lower expression compared to SCT samples ($p = 0.047$) In the meanwhile, the amount of insulin receptor at skeletal muscle are found to rise at the group samples if compared to the control samples (MCT vs SCT vs Control = 86 ± 5.5 vs 88 ± 4.5 vs 60 ± 20; $p = 0.009$) (Figure 2) while group samples K3 having the most insulin receptor among all ($p=0.690$). A depression of expression *Insr* gene and rise in the amount of insulin receptor at the surface of skeletal muscle was noted, following by the reduction of HOMA-IR posttest within both group samples (control vs MCT vs SCT = 49.1 ± 9.5 vs 43.3 ± 8.7 vs 18.5 ± 6.4) (Figure 3). According to the ANOVA test, HOMA-IR, after eight weeks of assigned exercise, gave a significant reduction ($p = 0.001$) for the group SCT.

There was no significant difference in IR expression on the skeletal muscle between MCT and SCT, but both treated group were significantly differenced with control.

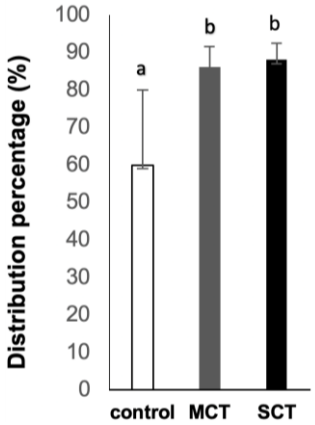

Figure 2: Insulin Receptor on Skeletal Muscle

HOMA-IR in MCT and SCT were reduced, but there was no statistical difference HOMA IR between MCT and control after 8 weeks of exercise. But in SCT group reduction of HOMA-IR after 8 weeks exercise was statistically different compared with control and MCT.

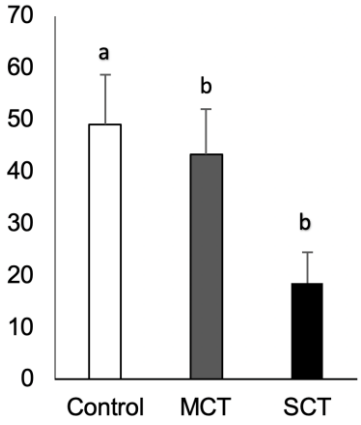

Figure 3: HOMA-IR; (**) note: groups with the same alphabet marked no statistically different, meanwhile group with different alphabet marked significantly different

Discussion

The purpose of this study was to analyse the difference *Insr* gene expression due to metabolic state in moderate and severe training in the T2DM rat model. We treated the group with the same condition between moderate and severe training group but the difference in intensity.

The result of this study shows that gene *Insr* expression, which underwent eight weeks of exercise, are found lower compared to the controlled samples, with a remarkable rose in cycling threshold (Δct) in the group samples that carried out the exercise assignment (Figure 1) Severe and moderate continuous aerobic exercise are found to have a significant effect on gene *Insr* expression (Figure 2). During exercise, ATP ratio and creatine will decrease, causing activation of enzyme AMP Kinase. The other metabolic enzyme that is mTOR also active. This enzyme plays a role as an integrated intracellular and extracellular signalling. Both AMPK and mTOR play together as a predominant enzyme in post-transcriptional gene and their activity can altered gene transcription [14]. Although the secretion of insulin have been depressed by sympathetic activity, by activating phosphorylation of AMPK — ACC, Ca2+ moduli skeletal muscle will stimulate translocation of GLUT-4 to the cell surface [15], [19].

State of metabolism highly affects the transcription of a gene [20]. A relationship was found among the state of metabolism, enzyme metabolic and transcription of a gene [9]. In the body of DM Type-2 patient, there is an interference of insulin signalling process causing the failure of translocation of GLUT-4 to the cell surface, resulting in an obstruction of glucose to diffuse into the cell [21]. Cellular stress is also one of the direct causes for the deficient of glucose in the cell by stimulating the secretion of cortisol in the adrenal cortex [11], [22]. Cortisol is essential in the gluconeogenesis process. Increase in plasma cortisol indicated that there would be stimulation to transcription of the gene insulin receptor, thus synthesising the insulin receptor [23]. Insulin synthesis will decelerate when insulin receptor is fully bonded, and the body is no longer in need of it.

Cortisol secreted during exercise will increase the rate of transcription of a gene. According to E.E. Hill et al., in a study, the frequency of cortisol in blood plasma increases significantly during exercise with the intensity of moderate and severe [24]. Metabolism among enzymes will stimulate biogenesis of mitochondria by activating PGC-1 of alpha mitochondria. High level of cortisol, enzyme metabolism rate and activation of PGC-1 alpha will stimulate the transcription of gene receptor insulin [9], [25]. The exercise that had been carried out eight weeks consecutively increase the amount of insulin receptor (Figure 2), increase in the sensitivity of insulin receptor, as well as decrease the insulin resistance (Figure 3), which results in the change of metabolic state. The difference in a metabolic state highly related to the decrease in gluconeogenesis in the liver causing a drop in gene transcription.

However, there were no such limitations observed in sedentary group samples, the limited amount of exercise carried out by sample, also causing a low glucose transportation rate in the cells. Increase in the rate of cortisol, produced by the compensated mechanism, will eventually stimulate the transcription of the gene to be continuously rising.

In this study, we found that there was a difference in the expression of gene *Insr* in rat models T2DM between moderate continuous exercise and severe continuous exercise. Samples which are given moderate continuous exercise have shown a higher

cycling threshold in mRNA gene *Insr* compared to the severe continuous exercise group. It is proven by there is a lower expression of gene expression in the moderate continuous exercise group compared to severe continuous, after eight weeks of exercise (Figure 1). Metabolisms of the cells in severe continuous exercise group samples are higher compared to the moderate continuous due to their intensity. According to E.E. Hill rate of cortisol in vigorous-intensity was higher compared to moderate one.

Reduction in insulin resistance and blood glucose level of the samples were found after eight weeks of exercise assignment. However, the decrease in insulin resistance in moderate continuous exercise group samples was not significant enough compared to the control groups, although there was a substantial reduction in blood glucose level compared to pretest.

In conclusion, exercise with high intensity will affect the expression of mRNA gene receptor and the synthesis of insulin receptor at the surface of muscle cells. Although a more drastic rise in the amount of receptor was recorded in severe continuous exercise group samples after eight weeks of exercise, a more precise consideration should be applied to the rate of cortisol and blood glucose level, appointed to T2DM patients. For those moderate continuous exercise individuals, it is recommended to carry out the more frequent exercise with the same consistency, in looking forward to a result of a significant decrease in insulin resistance.

Ethics

The study protocol was reviewed and approved by the Health Research Ethical Committee, Medical Faculty of Universitas Sumatera Utara, Medan, Indonesia (no. 263/KOMET/FK USU/2016).

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Curcumin's Effect on COX-2 and IL-10 Serum in Preeclampsia's Patient Undergo Sectio Caesarea with Spinal Anesthesia

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Abstract

BACKGROUND: Curcumin is a major component in curcuminoid which acts as an anti-inflammatory agent. Curcumin affects several biological markers that are thought to play a role in the pathogenesis of preeclampsia such as IL-10 and COX-2, resulting in an improvement in pregnant women with preeclampsia.

AIM: To see the effect of perioperative curcumin administration on IL-10 and COX-2 in preeclamptic patients undergoing caesarean section under spinal anaesthesia.

METHODS: This study was a double-blind, randomised clinical trial conducted at Pirngadi Hospital, USU Hospital and Sundari General Hospital Medan as a hospital network of Faculty of Medicine, North Sumatra University. Group 1 received a drug containing curcumin (as a treatment), and another group received a placebo (as a control).

RESULTS: There were no significant differences in the median values of COX-2 and IL-10 before and after treatment and also the p-values were greater than 0.05 in both groups (control and treatment).

CONCLUSION: There is no significant difference between the use of curcumin on serum COX-2 and IL-10 levels.

Introduction

Preeclampsia is a disorder in pregnant women with a gestational age of 20 weeks or more which characterised by hypertension and proteinuria that occurs in 5-10% of pregnancies. Preeclampsia also is known by a disorder of extensive endothelial function, starting with placental cytotrophoblast invasion, which can lead to clinical hypertension [1].

In pregnancy, there is a normal change in blood pressure due to hemodynamic changes to allow the exchange of blood and nutrients to the fetus. This circulation change usually occurs in the first trimester of pregnancy, during trophoblast implantation. The vascularisation changes of the decidua make the capacity of blood vessels that are initially high resistance with low capacity to be low resistance with high capacity, and this change will disrupt oxygen supply to the placenta. Increasing gestational age, the fetus will need a higher oxygen supply, but because vasoconstriction that occurs in the fetal blood vessels causes compensation to increase maternal blood pressure [2].

Curcumin which is the main component in curcuminoid theoretically acts as an anti-inflammatory agent by inhibiting NF-κβ activation, which is an important regulator of COX-2 expression [2]. Curcumin has been studied as a therapeutic agent for various types of diseases, the effect of curcumin as antiangiogenic, antioxidant and anti-inflammatory

affects several biological markers that are thought to play a role in the pathogenesis of preeclampsia such as IL-10 and COX-2 so that there is an improvement in women with preeclampsia.

This study was designed to prove that the administration of curcumin can change IL-10 and COX-2 levels and perioperative VAS in preeclamptic patients undergoing cesarean section under spinal anaesthesia.

Material and Methods

This is an experimental double-blind study with the type of pre and post-test control group design. Patients who diagnosed with preeclampsia and met the inclusion criteria, then divided into 2 groups which received drugs containing curcumin (as a treatment) and other group received placebo (as a control). The study was done at H Adam Malik Hospital, Medan, USU Hospital, RSUD dr. Pirngadi Medan, Hospital Putri Hijau Medan and Sundari General Hospital Medan, as a network Hospital for Medical Faculty of USU.

Pregnant preeclampsia women, with age range from 19-40 years, single pregnancy with a term of gestational age and planned for caesarean section with subarachnoid block regional anaesthesia techniques were included to inclusion criteria. Meanwhile, samples who had a systemic infection, chronic diseases (kidney disease & hypertension, diabetes mellitus) were the exclusion criteria for this study. Massive bleeding, patients who needed a hysterectomy, patients who had cardiac arrest and breathing problem during operation and prolong operation time were excluded from the study.

Fifty pregnant women were included in this study and had been informed about the study divided into two groups, one group received curcumin as a treatment group, and the other group received placebo as a control group. Three samples were excluded from this study because after blood samples were taken, the result was unreadable.

All samples who diagnosed with preeclampsia was being informed about the study after informed consent accepted blood were taken to examine all the biomarkers (T0), 90 minutes (T1) after the drugs given, second blood samples were taken for the second examination. Then sectio caesarean operation was done under spinal anaesthesia. 12 hours (T2) after the drugs are given another blood samples were taken to examine the biomarkers. All samples received Magnesium Sulphate based on preeclampsia's guidelines therapy.

Results

In this study, 47 samples were reported and divided into 2 groups, treatment groups (23 samples) and control groups (24 samples). The difference in serum COX-2 levels after being given curcumin is presented in Table 1.

Table 1: Differences in serum COX-2 levels after being given curcumin

Biomarker	Curcumin (-) (n=24)	Curcumin (+) (n=23)	P Value [a)]
COX-2 (T0)	54,95 (15,88 - 300,2)	55,71 (15,88 - 1074,92)	0,285
COX-2 (T1)	55,30 (10,94 - 91,88)	59,40 (33,34 - 489,86)	0,120
COX-2 (T2)	65,14 (23,84 - 91,06)	62,51 (16,4 - 504,96)	0,250
P Value [b)]	0,325	0,438	

Based on Table 1, comparison of COX-2 results in both groups at T0, T1 and T2 showed no significant differences indicated by the median values in the two groups that were not too far different and the p-values indicating values greater than 0.05. That is, from T0, T1 to T2 in both groups (control and treatment) no significant COX-2 levels were found.

Based on the value of COX-2 changes from T0 to T2 in each group indicated by the p-value in the Friedman test. The Friedman test results in the control group produced a p-value of 0.325, which was greater than 0.05, indicating that in the control group, there was no significant change in COX-2 levels from T0 to T2. Similarly, in the treatment group, a p-value of 0.438 which was greater than 0.05 showed that in the control group, there was no significant change in COX-2 levels from T0 to T2 (Figure 1).

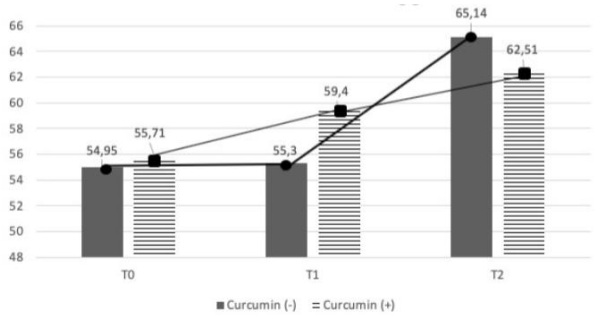

Figure 1: Differences in serum COX-2 levels after being given curcumin

The changes in serum IL-10 levels presented in Table 2, comparison of IL-10 results in both groups at T0, T1 and T2 showed no significant differences indicated by the median values in the two groups that were not too far different and the p-values indicating values greater than 0.05. That is, from T0, T1 to T2 in both groups (control and treatment) there was no significant IL-10 level found.

Table 2: Differences in serum IL-10 levels after being given curcumin

Biomarker	Curcumin (-) (n=24)	Curcumin (+) (n=23)	P Value [a)]
IL-10 (T0)	285,26 (8,32 - 636,4)	287,79 (6,37 - 6950,27)	0,158
IL-10 (T1)	309,8 (131,59 - 371,42)	289,63 (0,10 - 5759,2)	0,201
IL-10 (T2)	289,39 (9,70 - 412,12)	287,72 (1,53 - 6468,53)	0,170
P Value [b)]	0,582	0,296	

Based on the value of IL-10 changes from T0 to T2 in each group indicated by the p-value in the Friedman test. The Friedman test results in the control group produced a p-value of 0.582, which was greater than 0.05, indicating that in the control group, there was no significant change in IL-10 levels from T0 to T2. Similarly, in the treatment group, a p-value of 0.296 which was greater than 0.05 showed that in the control group, there was no significant change in IL-10 levels from T0 to T2 (Figure 2).

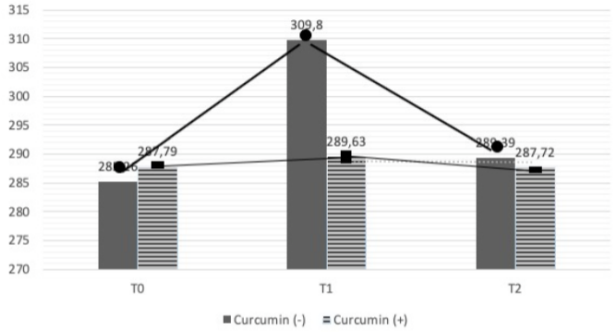

Figure 2: Differences in serum IL-10 levels after being given curcumin

Differences time by time can be seen in Table 3. There were no significant differences in IL-10 p-values (T0-T1 = 0.051, T0-T2 = 0.476 and T1-T2 = 0.317) and in COX-2 (T0-T1 = 0.437, T0-T2 = 0.890 and T1-T2 = 0.191) both in control and treatment groups.

Table 3: Differences in serum IL – 10 and COX-2 levels between observations

Biomarker	Curcumin - (n=24)	Curcumin + (n=23)	P Value
COX-2 T0-T1	-1,38 (-23,87 - 252,49)	-2,87 (-34,54 - 862,96)	0,437
COX-2 T0-T2	-3,83 (-49,67 - 233,45)	-5,36 (-222,48 - 1003,17)	0,890
COX-2 T1-T2	-11,86 (-48,68 - 32,35)	-1,00 (-232,78 - 140,21)	0,191
IL-10 T0-T1	-20,58 (-261,24 - 290,4)	38,34 (-299,42 - 1191,07)	0,051
IL-10 T0-T2	-10,59 (-252,86 - 273,72)	-1,25 (-623,07 - 481,74)	0,476
IL-10 T1-T2	-0,83 (-97,69 - 230,91)	-6,76 (-709,33 - 132,86)	0,317

Discussion

To our knowledge, our research was the first to assess the effect of curcumin in preeclampsia patients. So, the dosage that given was only once daily and only 100 mg of curcumin, although many studies used higher dosage in cancer or neurodegenerative patients.

IL-10 was first reported by Mosmann et al. as Cytokine Synthesis Inhibitory Factor (CSIF) as a protein with the ability to inhibit the activity of Th-1 type cells. CSIF is considered as the main factor that defines the difference between Th-1 and Th-2 type T cells because CSIF cuts T-cell activation. Although initially defined as Th-2 cell products, these cytokines have now been shown to be produced by various types of cells, including immune and non-immune cells. The report also shows that one way of regulating IL-10 is through a feedback loop that inhibits excessive inflammation [3].

IL-10 in humans is a homodimeric protein that is sensitive to monomeric acid with a molecular weight of 18.5 kDa which is encoded on chromosome 1 in mice and humans. IL-10 mice and human IL-10 are sufficiently conserved in their amino acid sequences to share 73% homology and are especially different in human N-glycosylation sites with IL-10 [4].

The serum and amount of placental IL-10 have been reported to increase in normal pregnancies. Sowmya et al., [5], in their study mentioned that variations in IL-10 production are largely genetically determined, which is mainly related to genetic variation in the study area that linked to the level of transcription. A study by Benian et al., [6], in the Istanbul population, have shown levels of IL-10 in plasma and IL-10 in the placenta. In contrast, Madazli et al., [7], reported an increase in IL-10 levels in maternal plasma of preeclamptic patients compared with normotensive women in the Istanbul population. Mansouri et al., [8], conducted a study on serum samples of preeclampsia patients and control subjects and revealed no significant changes in the examination. On the other hand, Makris et al., [9], showed that IL-10 promoter genotypes might not play an important role in circulating IL-10 levels but showed effects on IL-10 placental levels in the study population. This result suggests that IL-10 plays an important role [5].

There is increasing evidence that inflammation with COX-2 expression plays a key role in preeclampsia, so increasing the expression of neutrophils from COX-2 is an important finding regarding the pathophysiology of preeclampsia. Increased COX-2 expression seems to be widespread. COX-2 is increased in the placenta of women with preeclampsia where the expression is associated with an increase in thromboxane production. The placenta from preeclamptic women produces more thromboxane and less prostacyclin than the placenta obtained from normal pregnant women. Thromboxane is a strong vasoconstrictor, whereas prostacyclin is a strong vasodilator, so this imbalance contributes to reduced uteroplacental blood flow in preeclampsia [10].

Curcumin modulates the inflammatory response by reducing the activity of the cyclooxygenase-2 (COX-2) enzyme, lipoxygenase, and inducible nitric oxide synthase (iNOS); inhibits the production of inflammatory cytokines tumour necrosis factor-alpha (TNF-a), interleukin (IL) -1, -2, -6, -8, and -12, monocyte chemoattractant protein (MCP) and protein inhibitory migration also decreases mitogen-regulation activated and Janus kinases. (Julie, 2009). Curcumin is thought to be able to directly act on oxygenation of arachidonic acid or nitrogen-reactive species due to its antioxidant ability. Curcumin can modulate COX-2 via iNOS [11]. Inhibition of COX-2 and iNOS results from suppression of curcumin in NF-κ activation [12].

COX-2 expression regulation occurs not only at the transcriptional level but also at the post-transcriptional level. The main function of COX-2 is to mediate the conversion of arachidonic acid into prostaglandin H2, which is then converted to various PGs with various syntheses. The Study reported that COX-2 provides action on cell function, including modulation of cell proliferation and cell migration. Other researchers have reported the role of COX-2 in angiogenesis, seen by using a modified vascular endothelial growth factor (VEGF) as an endpoint. However, in the human placenta, the results are inconsistent, with several studies showing placental COX-2 levels not to differ between normal pregnancies and preeclamptic pregnancies. Others found that COX-2 levels in preeclamptic women were reduced as measured by immunoblotting, and this effect has been linked to oxidative stress [13].

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Tropical and Infectious Diseases Control and Management

Effectiveness Hand Washing and Hand Rub Method in Reducing Total Bacteria Colony from Nurses in Medan

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Keywords: Handwashing; Handrub; Total bacteria colony

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Abstract

BACKGROUND: Hospital-acquired infection (HAI) is a major problem for the patient's health care and may impact the duration of treatment. Hand hygiene is a simple procedure but giving good prevention usually done among nurses at the hospital.

AIM: Objective of the study is to determine the effectivity of handwashing method compared to hand rub to eliminate microorganisms on nurse's hands at Sumatera Utara University Hospital. This is an experimental analytic study using random sampling technique.

METHODS: There were 16 nurses enrolled in this study. There were 2 groups involved; the first group using handwashing with soap and the other one using hand rub. The swabs were taken from each hand from both groups before and after washing their hands. Moreover, the swabs directly sent to Microbiology Laboratory of Sumatera Utara University to identify bacteria which colonise the hand.

RESULTS: There were no significant differences between using handwashing method compared to hand rub in reducing total bacterial colony on hands ($p = 0.088$). The average of total colony decreased by using handwashing method is 59.5%, and by using a hand, rub is 47.2%.

CONCLUSION: Hand hygiene method using alcohol-based hand rub liquid has been recommended by WHO and can replace hand washing method in a particular situation.

Introduction

A hospital-acquired infection (HAI) is a major problem for patients health care and may impact the duration of treatment even increasing the risk of mortality. Prevalence of HAIs in low-income and medium countries ranged from 5.7-19.1%, while the prevalence in high-income countries ranged between 3.5 – 12%. In Indonesia which is part of the middle-income countries, HAI prevalence reached up to 7.1% [1]. Organisms that cause nosocomial infection are most commonly transmitted by the hands of physicians, nurses, physiotherapists, and other hospital personnel. Hand hygiene has often been singled out as the most important procedure in preventing nosocomial infection [2]. Hand hygiene is a simple procedure but giving good prevention usually done among nurses at the hospital. Sari study showed that using two types of hand rub from WHO which first formulation contains glycerol and ethanol, hydrogen peroxide.

In contrast, the second formulation consists of isopropyl alcohol, glycerol, and hydrogen peroxide, on the subject's hands before, after, and the average decline in the number of germs. Based on Total Plate Count showed bacterial colony difference before and after hand washing using hand rub WHO formula. The decline in several germs after using the hand rubs WHO formula is higher compared to the average decline in several germs after hand washing using a commercial hand rub [3].

Material and Methods

Study design

This is an experimental analytic study with pre-test – post-test design. Aim of the study is to measure the effectiveness of hand washing with soap and hand rub methods to eliminate microorganisms on nurse's hands at Universitas Sumatera Utara Hospital (USU-Hospital). This study has been done from October to November 2016. Moreover, Health Research Ethics Commission (KEPK) Faculty of Medicine, Universitas Sumatera Utara has approved the study protocol.

Subjects

These subjects were chosen by random sampling technique and who meets the inclusion criteria. The criteria include were the nurse's skin were intact and has not done hand-washing or using hand rub at least 4 hours before sample collection. There were 16 nurses enrolled in this study. Firstly, all of them using handwashing with soap for hand hygiene. After a week, all of them using hand rub for hand hygiene. The swabs were taken from each hand from both groups before and after washing their hands. Moreover, the swabs directly sent to the Microbiology Laboratory of Universitas Sumatera Utara.

Procedure

There were 16 nurses involved in this study with 2 different methods. Firstly, the nurses used handwashing with soap, and after a week, those nurses using hand rub. Both hands were swabbed before and after doing the method. After swabbing, the swabs directly sent to Microbiology Laboratory of Sumatera Utara University to identify bacteria which colonise the hand and to count the colony of the bacteria.

Statistical analysis

The effectiveness of the use of handwashing with soap and hand rub is defined as the reduction of the number of mean colonies on the samples before and after the action. The average obtained then compared with paired t-test.

Results

Hand wash with soap method

According to the results, before handwashing with soap, swab collected was obtained several bacteria named *Bacillus subtilis* 4 samples (20%), *Staphylococcus epidermidis* 9 samples (45%) and coagulase-negative Staphylococcus 7 samples (35%).

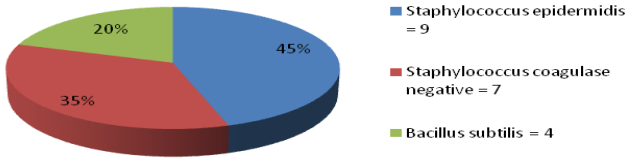

Figure 1: Bacteria found before handwashing with soap

After handwashing with soap Bacillus subtilis was not found moreover, bacteria still exist were Staphylococcus epidermidis 9 samples (56.25%) and coagulase-negative Staphylococcus 7 samples (43.75%) (Figure 1, and Figure 2).

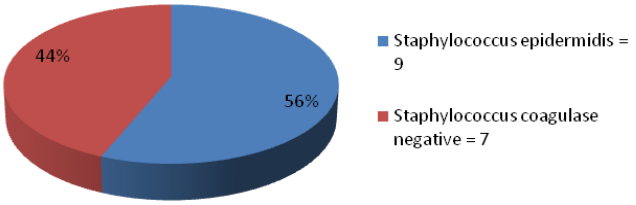

Figure 2: Bacteria found after handwashing with soap

Hand rub method

Based on the results of the study before using hand rub, bacteria found were *Staphylococcus epidermidis* 10 samples (47.62%), Staphylococcus coagulase-negative 6 samples (28.75%), *Bacillus subtilis* 2 samples (9.52%), *Klebsiella oxytoca* 2 samples (9.52%) and *Escherichia coli* 1 sample (4.76%). After using handrub, *Klebsiella oxytoca* and *E. coli* was not found but the other bacteria still exist.

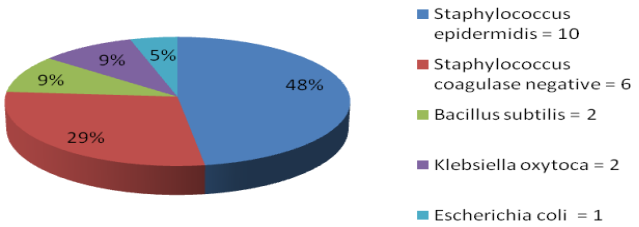

Figure 3. Bacteria found before using hand rub

There were *Staphylococcus epidermidis* on 10 samples (55.55%), Staphylococcus coagulase negative 6 samples (33.33%) and *Bacillus subtilis* 2 samples (11.12%) (Figure 3, and Figure 4).

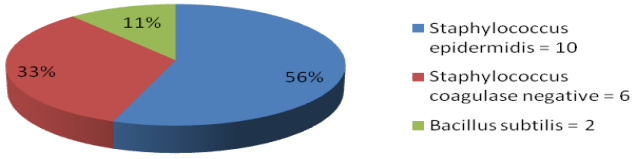

Figure 4: Bacteria found after using hand rub

Total plate count bacteria from hand wash with soap

It was found that the bacteria colony count reduced after handwashing with soap. Figure 6 described the total plate count (TPC) bacteria (CFU/ml) from each sample in a petri dish both before and after handwashing with soap. It was found that the average number of reduced colonies is 59.55%

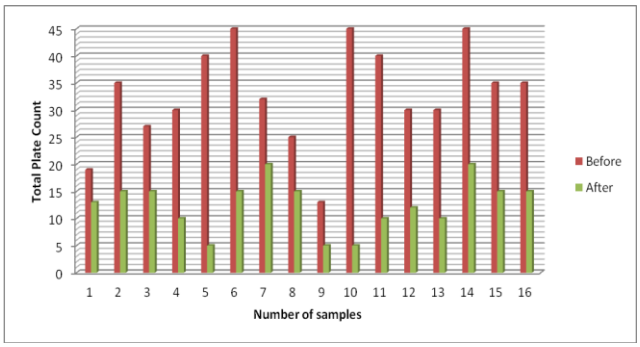

Figure 5: Reduced TPC after handwashing with soap

Total plate count bacteria from using hand rub

The same result showed when using hand rub. It was found that the bacteria colony count reduced after using hand rub. Figure 7 described the total plate count bacteria (CFU/ml) from each sample in a petri dish both before and after using hand rub. It was found that the average number of reduced colonies is 47.2%.

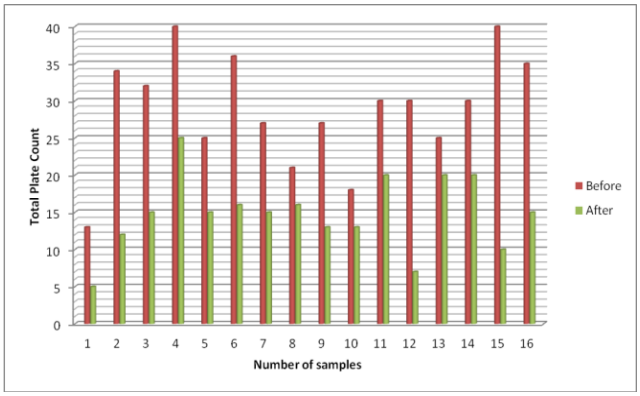

Figure 6: Reduced TPC after using hand rub

Effectiveness reduced TPC after hand wash with soap and using hand rub

The effectiveness of using soap or hand rub is defined as the reduction of the number of mean colonies on the samples before and after the method done. The value obtained when compared with the paired t-test. Paired t-test results showed that p = 0.088. However, the value of $p > 0.05$ indicates that there is no significant difference between handwashing with soap and hand rub usage in reducing the number of bacteria colonies on the hands of nurses. The average reduction number of bacteria was higher on washing hand with soap which is up to 12.35%.

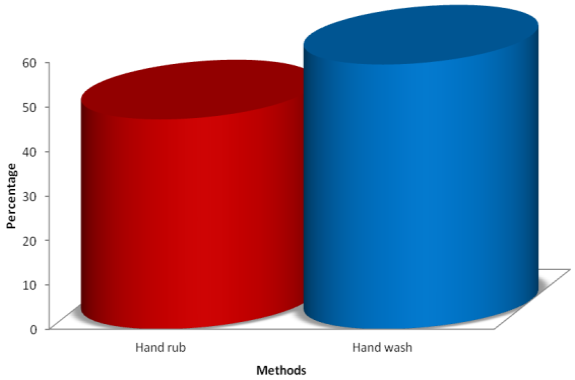

Figure 7: Comparison of reduced TPC from 2 methods

Discussion

The bacteria found on nurse's hands at USU hospital were *Staphylococcus epidermidis*, *Staphylococcus aureus* coagulase-negative, *Bacillus subtilis*, *Klebsiella oxytoca*, and *Escherichia coli*. This result was different from bacteria type obtained by Anwar Musadad and Agustina Lubis study which is 12.9% *Staphylococcus Albus*, 3.2% *Escherichia coli* and 6.4% *Pseudomonas sp*. [4]. The difference happened most probably due to the location of the medical officer and paramedic workplace are not the same as the previous study, so that the bacteria found may also different.

The hand is normally colonised by resident and transient bacterial flora, depending on the part of the skin where they colonised. Resident flora, the harmless bacteria is more difficult to destroy. (For example *Staphylococcus epidermidis* and *Staphylococcus aureus* coagulase-negative). Transient flora more often at risk of incurring diseases and more easily destroyed. (For example *Staphylococcus aureus*, *Bacillus subtillis*). Nurses' hands could be contaminated with both types of flora at the time of contact with patients. Moreover, it may be contaminated from the patient's environment. The amount of flora also increased along with the length of the duration of the activity [5].

Using hand rub recommended by WHO due to practical use and education promoted on the function of hygiene in replacing soap. The content on the hand rub used in this research is 70% ethyl alcohol and soap used is 0.175% chloroxylenol and 0.3% salicylic acid. The alcohol effect was bactericidal against gram-positive and gram-negative bacteria, *M. tuberculosis*, some types of fungus and non-enveloped viruses. Alcohol works on the bacteria by protein and cytoplasm membranes denaturation.

Finally, alcohol destroys the work by denaturation on the cell membrane [6].

The results showed that washing hands with soap (59.55%) or using hand rub (47.2%) had a significant difference between before and after doing hand hygiene (p = 0.001). These results are by research by Sari[3]. Whom found the significant difference after hand washing using soap (p = 0.002) and after using hand rub (p = 0.001). The same result also found by Mona [7]. Whom found the significant difference after washing 30 hands using soap (p = 0.01-0.03) and after using hand rub (p = 0.002).

However, in this study, washing hand with soap has an average reduction in total plate count of bacteria colony (59.55%) higher than using hand rub (47.2%). This result is contrary to Abaza et al., [5] who found the result of handwashing using alcohol-based hand rub is much more efficient than hand washing using soap. Abaza compared 4 products alcohol-based hand rubs with an efficiency of 99%, 97%, 77% and 87% while the soap only shows the efficiency of 30%. Similar to Abaza, Amy et al., [8]. Found where a total of 204 samples showed that hand rub is more efficient than handwashing with soap and have a meaningful difference (p = 0.01). Hand hygiene method using alcohol-based hand rub liquid has been recommended by WHO and can replace handwashing with soap method in a particular situation. It suggests that hand hygiene method using hand rub liquid could be chosen in hand hygiene method [6].

In conclusion, there was no significant difference between handwashing with soap and using hand rub in reducing bacteria which colonised the nurse's hand in this study (p = 0,088*)*. The average of total colony decreased by using handwashing with soap method is higher (59.5%) than using hand rub (47.2%).

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Correlation of Ki-67 Expression as Tumor cell Proliferation Activity Marker with Cutaneous Squamous Cell Carcinoma Grading

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Abstract

Cutaneous Squamous Cell Carcinoma (cSCC) is a malignant keratinocyte tumour that develops through the suprabasal epidermis. This malignant tumour is the second most common skin malignancy after Basal Cell Carcinoma (BCC). The increased incidence of cSCC is directly proportional to increasing age. Generally, the predisposing factor of cSCC is exposure to recurrent sunlight for a long time, so localisation of cSCC is a part of the body that often exposed to direct sunlight, such as the forehead, face, ears, scalp, neck, and back of the hand. The carcinogenesis process of cSCC is a cumulation of a series of events, one of which plays an important role is the proliferation index assessed by Ki-67. Forty-eight tissue paraffin blocks were diagnosed histopathologically as cutaneous squamous cell carcinoma from the Anatomical Pathology Laboratory of the Faculty of Medicine, Universitas Sumatera Utara and the Anatomical Pathology Unit of Haji Adam Malik General Hospital Medan, as the research sample. The results of protein expression from Ki-67 were assessed based on area. There was no significant correlation between cSCC grading and Ki-67 expression ($p > 0.05$). Ki-67 antigen tumour marker, widely used to determine the level of tumour cell proliferation.

Introduction

Cutaneous Squamous Cell Carcinoma (cSCC) is a malignant keratinocyte tumour that develops through the suprabasal epidermis [1]. This malignant tumour is the second most common skin malignancy after Basal Cell Carcinoma (BCC), with an amount of 20% of all malignancy in the skin. The American Cancer Society noted a comparison between cSCC and BCC of 1:3 [2]. It was in line with the data on the number of cases at the Polyclinic of Department of Dermatology and Venereology at the Dr Cipto Mangunkusumo, Central General Hospital, Jakarta, who reported the number of cases of BCC as many as 261 cases and followed by 69 cases of cSCC in 2000-2009. However, epidemiological data from the Dharmais Cancer Hospital during 2005-2007 noted that the most common skin cancer was cSCC followed by BCC [3]. The research conducted by Edi Kerina at the Haji Adam Malik General Hospital, Medan in 2012-2015 also placed cSCC as the most common type of skin malignancy above BCC with 59 cases of cSCC, while the number of BCC cases was only 29 cases [4]. The increasing incidence of cSCC is directly proportional to increasing age [2]. In general, a predisposing factor for cSCC is repeated sun exposure over a long period in the older age group. People with cSCC with a younger age usually have a brown skin colour. The impact of race on the incidence of cSCC appears to be at a less frequent incidence rate in black groups [5].

Sun exposure predisposes to cSCC, so localisation of cSCC is the part of the body most often exposed to direct sunlight, such as the forehead, face, ears, scalp, neck, and back of the hand. Other locations that also often experience malignant transformation from keratinocytes are the lower lip vermilion [5].

The process of carcinogenesis to become cutaneous squamous cell carcinoma is the cumulation of a series of events in a cell which then undergoes malignant transformation. Each stage of the event primarily influenced by various factors, both genetic, environmental, and food patterns [6].

One of a series of events in a cell that plays an important role in the carcinogenesis process is a cell proliferation index that can be assessed by Ki-67. Ki-67 expression is associated with the proliferative activity of intrinsic cell populations of malignant tumours, thus allowing the use of these markers in evaluating tumour aggressiveness [7].

This study aims to assess tumour cell proliferation in cutaneous squamous cell carcinoma through Ki-67 expression. Therefore, the researchers were interested in investigating the level of cell proliferation (Ki-67 proliferation index) in Squamous Cell Carcinoma using histopathological tissue preparations obtained from biopsy or excision that were fixed with formalin and planted in paraffin blocks.

Material and Methods

This study is a descriptive-analytic study with a cross-sectional design which aims to assess tumour cell proliferation in cutaneous Squamous Cell Carcinoma through Ki-67 expression.

This research was conducted at the Anatomical Pathology Laboratory of the Faculty of Medicine, Universitas of Sumatera Utara and the Anatomical Pathology Unit of Haji Adam Malik General Hospital Medan. This research is conducted for 16 months, starting from June 2017 to October 2018, which includes library studies, data collection, data processing and writing research reports.

Samples size in this study were calculated based on the sample formula for testing hypotheses in one population. Calculations are carried out using a confidence level of 95% and 80% power. Based on the formula, the samples size of at least 47 was found.

The sample in this study were 48 tissue paraffin blocks diagnosed histopathologically as cutaneous squamous cell carcinoma that met the inclusion criteria. Sampling is done using consecutive sampling technique.

Product data marker Ki-67 antibody biology option listed in the core with clone MIB-1; Monoclonal antibodies; Dako and dilution of 1:300. Ki-67 are proliferating cell nuclear antigen (PCNA) that indirectly may reflect the proliferation of tumour cells. Ki-67 expression assessment using the method semiquantitative.

The percentage of tumor cells at all stained: - Score 0 = ≤ 5% positive cells; - Score 1 = 6-25% positive cells; - Score 2 = 26-50% positive cells; - Score 3 = 51-75% positive cells; - Score 4 = > 76% positive cells.

We examine the complete clinical data needed. Re-reading of all slides from skin tissue was diagnosed as cSCC with Hematoxylin Eosin staining by two pathologists together with the researcher to determine the variance and histology grade. Then the serial slides of paraffin blocks were repeated in three slides to proceed with each examination with Ki-67 immunohistochemical staining.

Results

The expression of protein from Ki-67 was considered broad-based. There was no significant correlation between cSCC grading and Ki-67 expression ($p > 0.05$)

Table 1: Correlation Cutaneous Squamous Cell Carcinoma Grading with Ki-67

No	Grading	Ki-67			P
		Median	Mean	SD	
1	Well Differentiated	2	33.4	43.1	
2	Moderately Differentiated	85.5	61.8	42.4	0.167
3	Poorly Differentiated	50	53.8	37.6	

Discussion

Histopathological examination is still the primary support in establishing the diagnosis of cSCC in addition to holding on to the clinical picture. Histopathologically the cSCC found the irregular mass of epidermal cells that had proliferated and invaded the dermis layer. Cell proliferation rates at cSCC can be a consideration in determining cSCC differentiation so that it can establish grading from cSCC used to develop appropriate prognosis and management. It's also found in a study that states that cells from the outer layer of cSCCtumor mass will continue to proliferate. In contrast, cells in the layer of tumour mass will experience differentiation [8].

As a tumour marker, the Ki-67 antigen has been widely used to determine the level of

proliferation of tumor cells which were most commonly used in breast tumors. The Ki-67 antigen encodes two isoform proteins with molecular weights 345 and 395 kDa initially identified by Scholzer and Gerden in the early 1980s. This protein appears in all active phases of the cell cycle, namely G1, S, G2, and M, but does not appear in the G0 phase [9], [10]. Ki-67 is at a low level in the G1 and S phases and reaches a peak at the beginning of the mitotic phase, but in the next phase of mitosis (i.e., during anaphase and telophase), there is a decrease in Ki-67 levels. The amount of Ki-67 that appears in the cell cycle will be governed by the right balance between synthesis and degradation which is characterised by a short Ki-67 half-life, i.e., 1-1.5 hours. The Ki-67 expression is associated with the proliferative activity of intrinsic cell populations of malignant tumours, thus allowing the use of these markers in assessing tumour aggressiveness [7].

Marinescu et al., (2015) found that the highest expression of Ki-67 was found in low differentiated cSCC, after conducting p53, p16, and Ki-67 Immunoexpression studies on cutaneous squamous cell carcinoma and precursor lesions [11]. Referring to tumour grading currently used than conventionally, cSCC is divided subjectively into three histological degrees based on nucleus atypia and tumour-formed keratinisation [5], namely cSCC well-differentiated, cSCC moderately differentiated, and cSCCpoorly differentiated, so in this study, the highest Ki-67 expression was found in cSCC with poor differentiation. Accordance with Marinescu et al., (2015) who found that the highest Ki-67 expression was found cSCC with poorly differentiated [11].

In conclusion, the expression of tumour marker activity, Ki-67, found no significant correlation in establishing cSCC grading.

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Polymorphisms of *CYP2E1* rs2031920 is not Associated with Risk of Nasopharyngeal Carcinoma in Minangkabau Ethnic Group

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Keywords: CYP2E1 polymorphisms; Nasopharyngeal Carcinoma; Minangkabau

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Abstract

BACKGROUND: Various environmental factors have been suspected to be associated with the risk of developing Nasopharyngeal Carcinoma (NPC). Volatile nitrosamines found in salted fish are thought to be carcinogenic substances for NPC. Nitrosamines are activated by the CYP2E1 enzyme. Several studies investigated the relationship between polymorphism in the *CYP2E1* gene and susceptibility to NPC, but the results obtained were inconsistent.

AIM: This study was conducted to analyze the association of the *CYP2E1* rs2031920 polymorphisms with the incidence of NPC in the Minangkabau ethnic group.

METHODS: The subjects of this study were newly diagnosed NPC Minangkabau ethnic patients, while the controls were healthy people. A total of 23 cases of NPC and 23 aged (± 3 years) and sex-matched controls participated in the study. The method used to identify these polymorphisms is PCR sequencing.

RESULTS: On recent study we found *CYP2E1* rs2031920 gene polymorphism in both the NPC and control groups, in the NPC group there were 8.7% heterozygote mutants while in the control group there were 26.1% heterozygote mutants, and there were no homozygote mutants in the two groups, and statistically none a significant relationship between *CYP2E1* gene polymorphism and the incidence of NPC, with $p > 0.05$.

CONCLUSION: Our study reveals that there is no association of *CYP2E1* gene polymorphism (rs2031920) with the incidence of nasopharyngeal carcinoma in the Minangkabau ethnic group.

Introduction

Nasopharyngeal carcinoma (NPC) is cancer that has a unique distribution pattern both geographically and ethnically. The exact etiology of nasopharyngeal cancer is unknown, but it is thought to be a multifactorial interaction. Epstein-Barr virus (EBV) infection interacts with genetic susceptibility, and environmental factors are the main etiological factors [1].

Various environmental factors have been reported related to the incidence of Nasopharyngeal Carcinoma (NPC), including consumption of salted fish containing nitrosamines [2], [3]. Study on environmental factors as carcinogens has varied reports, but in endemic areas, nitrosamines from salted fish are a factor that is often associated with the incidence of NPC [3]. but clinical study at Dr. RSUP M. Djamil Padang did not get this association [4].

Nitrosamines are activated by the CYP2E1 enzyme, this enzyme also activates light molecules of nitrosamines such as N-nitrosodimethylamine (NDMA) and nitrosamines found in tobacco, N-nitrosonornicotine (NNN) [2]. Activation of nitrosamines can cause the growth of several malignancies. Several studies have found that CYP2E1 is also expressed on the nasal and

nasopharyngeal mucosa, CYP2E1 mutation can cause nasopharyngeal mucosa susceptible to the growth of NPC [2], [5], [6].

Study in Taiwan and Thailand populations found CYP2E1 RsaI (rs2031920) polymorphism in the promoter associated with an increased risk of NPC [7], [8]. while Guo X at al., who conducted studies in the Chinese population did not find an increased risk of NPC in mutant homozygote individuals variants of CYP2E1-RsaI (rs2031920) [9].

This study was conducted to analyze the association of the CYP2E1 rs2031920 polymorphisms with the incidence of NPC in the Minangkabau ethnic group.

Material and Methods

The subjects of this study were newly diagnosed NPC Minangkabau ethnic patients, while the controls were healthy people who were also Minangkabau ethnic. A total of 23 cases of NPC and 23 aged (± 3 years) and sex-matched controls participated in the study. Informed consent was obtained and blood samples were taken. The study was approved by the ethics committee of the Faculty of Medicine, Andalas University, Padang, Indonesia (No.422 / KEP / FK / 2018).

Primers for recognizing CYP2E1 gene polymorphism (rs2031920) are constructed using software Geneious 11.1.2. The method used to identify these polymorphisms is PCR sequencing. Primers used for CYP2E1 amplification are forward: 5 '- CAGTCGAGTCTACATTGTCAGT - 3' and reverse: 5 '-CTTGATGTCTGATGAGGAGGTTTG - 3', to amplify DNA with amplicon size 931bp. After DNA isolation and primer construction, PCR is then carried out.

All PCR product samples were sequenced at 1stBASE, Singapore. The results of sequencing data are then processed with Genious 11.1.2 software. In individuals who have CYP2E1 rs2031920 that do not experience polymorphism, no change in CC base is called a wild type, while those that change from CC to CT are called heterozygote mutants and those that change from CC to TT are called homozygote mutants. Sequencing results are then aligned with the reference genes (NG_055447.1).

Results

CYP2E1 rs2031920 gene polymorphism was found in both the NPC and control group, in the NPC group there were 8.7% heterozygote mutants while in the control group there were 26.1% heterozygote mutants, and there were no homozygote mutants in the two groups, and statistically none a significant relationship between CYP2E1 gene polymorphism and the incidence of NPC, with $p > 0.05$ (Table 1).

Table 1: Association of CYP2E1 Polymorphism (rs2031920) with NPC

Polymorphisms	Group		p
	NPC f (%)	Control f (%)	
Wild type (CC)	21 (91.3)	17 (73.9)	0.243
Mutant Heterozygote (CT)	2 (8.7)	6 (26.1)	
Mutant Homozigote (TT)	0 (0)	0 (0)	
Total	23(100	23(100)	

The presence of heterozygote mutant CYP2E1 rs2031920 gene polymorphism is indicated by the changes in CC base to CT. In the case of NPC, there were only heterozygote mutations in the two samples (K07 and K09), there were no homozygote mutations, whereas in the control group there were six samples also in the form of heterozygote mutants (S09, S10, S11, S17, S18, and S24) (Figure 1 and Figure 2).

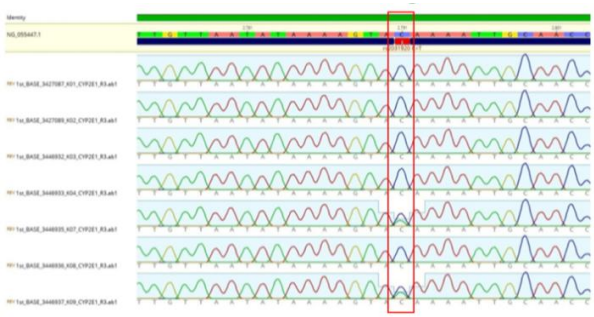

Figure 1: Alignment of sequencing results of NPC case group, samples; K01, K02, K03, K04, K07, K08 and K09 with reference genes CYP2E1

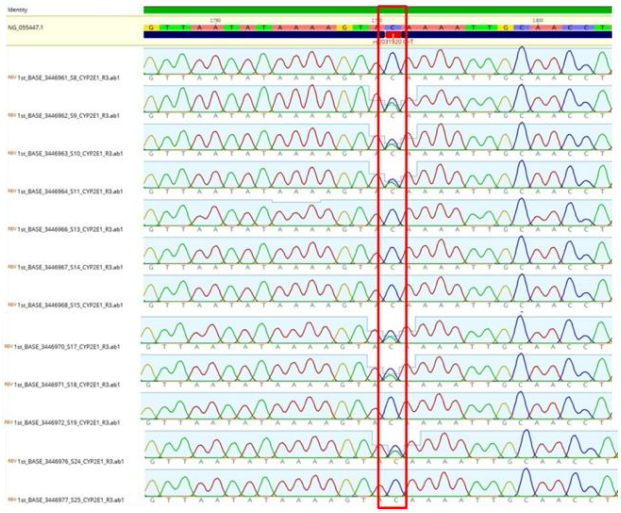

Figure 2: Alignment of sequencing results of several control group samples with the CYP2E1 reference gene

Discussion

This study shows that the CYP2E1 rs2031920 gene polymorphism was found in the NPC group and controls. The form of polymorphism found was only heterozygote (c1c2) mutants in both groups, and no homozygote mutants (c2c2) were found. Polymorphism is more common in the control group, but no significant differences were found between the two groups.

The CYP2E1 enzyme is involved in the metabolic activation of several lights and pro-carcinogenic molecules such as nitrosamines. Based on previous epidemiological studies, it was found that salted fish containing nitrosamines and nitrosamine precursors were associated with the incidence of NPC, so CYP2E1 gene polymorphisms were thought to cause the susceptible nasopharyngeal epithelium to NPC growth [6], [10].

Several studies have reported in several ethnic groups that have inconsistent results. The meta-analysis study that combined several studies in various ethnicities found a correlation between CYP2E1 rs2031920 gene polymorphism and the incidence of NPC, but also no association with the incidence of NPC if it was only heterozygote mutant polymorphisms [6].

In this study, Minangkabau ethnicity in both NPC and controls group found only heterozygote mutants (c1c2), and not related to the incidence of NPC. The same was reported by Lourembam et al. who conducted a study in India in areas with a high incidence of NPC also found only heterozygote polymorphisms and also did not get a significant relationship with the incidence of NPC [3]. Hildesheim et al., [2]. who conducted a study on NPC patients in Taiwan also, did not get an increase in the incidence of NPC in the heterozygote (c1c2) mutant CYP2E1 gene polymorphisms. Guo X et al., also found no increased risk of NPC in individuals both homozygote TT (c2c2) mutants and heterozygote CT (c1c2) CYP2E1-RsaI (rs2031920) in Han populations in South China [9].

The Kongruttanachok et al., [8]. report, which conducted a study on NPC patients in Thailand, only found an increase of incidence of NPC in homozygote variant polymorphisms both Thai and Chinese ethnic [8]. The same report in NPC in Tunisia has increased the incidence of NPC (OR = 8.39; CI95% [0.99 – 388.1]) in the c2c2 polymorphism and did not get in the c1c2 polymorphism.

Various reports concluding that there is a relationship between CYP2E1 gene polymorphism and the incidence of NPC generally combining homozygote and heterozygote mutants or calculating the frequency of c2 allele, whereas if only looking at heterozygote mutants, there are no reports that have a significant relationship with the incidence of NPC [6], [11], [13].

The RsaI position in the 5-Flanking region −1053 C > T is important in the difference in transcription activity. In vitro studies have shown that homozygote (mutant) c2c2 genotypes are associated with an increase of 10 times the CYP2E1 gene transcription. Further studies with liver sample microsomes showed that the level of mRNA subjects with c1c2 genotype was higher than those with c1c1 (wild type) genotype, thus also indicating that the transcription activity of c2 allele was stronger than that of c1. However, other in vitro studies did not find a significant increase in CYP2E1 activity in c2 carriers, compared to carrier c1 [14].

Increased expression in homozygote variants will cause more procarcinogens to turn into carcinogens, which will then cause DNA damage. The effect of differences in metabolic gene expression will be reduced if someone who has abnormal genotypes is not exposed to the substrate. So that mutations will not cause phenotype without interaction with environmental factors. The role of CYP2E1 polymorphisms is also likely to vary according to the amount of food consumed containing nitrosamines and precursors of nitrosamines (Kongruttanachok et al., 2001). Epidemiological studies in NPC patients in West Sumatra did not get a relationship between the consumption of salted fish and the increased incidence of NPC [4].

In conclusion, our study reveals that there is no association of CYP2E1 gene polymorphism (rs2031920) with nasopharyngeal carcinoma in the Minangkabau ethnic group.

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The Effectiveness of Acetone Breath Content Using Chitosan Based Sensor in Patients Diabetes Mellitus

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Keywords: Acetone breath; HbA1c; KGD; DM

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Abstract

BACKGROUND: The high incidence of diabetes mellitus in Indonesia to encourage researchers to continue to develop detection techniques are easy, inexpensive and minimally invasive. This study is expected to answer the challenge. Detection tool used in this research is the detection devices use traditional breath acetone levels of chitosan-based sensors. Acetone breath sensor works based on the chemical interaction between the breath of patients with diabetes and acetone sensor surface, which causes a change in the electrical response.

AIM: This study was cross-sectional using diabetes mellitus patients as research subjects by measuring breath acetone levels and HbA1c and KGD as a comparison.

METHODS: Research conducted at the primary health care facilities network services the University Hospital of North Sumatra. A total of 58 diabetic patients who meet the inclusion and exclusion criteria were included in this study. The relationship between the measurements analysed using Pearson correlation test.

RESULTS: Measurements showed that statistically there is a great relationship between breath acetone levels with or KGD HbA1c levels in the diabetic patient with each R = 0.05 and p = 0.708 for the relationship acetone breath and HbA1c and R = 0.1 and p = 0.445 for the relationship acetone breath and KGD. However, clinically there is a linear relationship between increased levels consistent acetone in the breath with increased HbA1c levels as well as levels of KGD.

CONCLUSION: Examination of breath acetone levels can be considered as an indicator of diabetes detection.

Introduction

Based on data from the World Health Organization (WHO), Indonesia is one of the contributor's patients with diabetes mellitus (DM), the largest by occupying the 4th position of the world and national causes of death in most cases 3 after heart disease and stroke (DG P3L 2010). Moreover, diabetes is not a disease that can be eliminated but can only be controlled so that the condition does not worsen. This fact is enough to conclude that prevention and early detection of diabetes is one step that absolutely must be done to curb the high prevalence of diabetes, especially in Indonesia.

Up to now has been a lot of testing done related to the early detection of diabetes. One of the testing using a blood sample*glucometer*. Although this method seems practical, testing of blood samples is considered invasive, painful; Accuracy remains low and relatively expensive. Likewise with HbA1c, although the DM detection method has high accuracy in measurement but is very expensive and not portable. Therefore, people mostly have to go to health institutions to know their blood sugar levels when not able to have a tool like Glucometer check blood sugar or HbA1c.

These deficiencies addressed with DM through the breath detection method that tends to *non-invasive*, Painless, more practical with a short

analysis time and is relatively inexpensive. Breathing method is a new method that does not exist conducted to analyse diabetes by analysing the blown gas released through the patient's mouth [1].

Health report showed that high levels of glucose encourage the formation of acetone. Acetone is one of the outputs produced by diabetics through the breath that has a higher concentration than healthy people. The concentration of acetone in the range of 0.3 ppm s / d 0.9 ppm was found in healthy human breath while in the breath of people with diabetes could reach 1.8 ppm [2]. Things need to be done in analysing the breathing is a detector that has very high sensitivity and presented online and in real-time.

Chitosan is *conductive biopolymer*. Very interesting to study as a new sensing material that is sensitive to acetone because it contains many amino groups (-NH2) and hydroxyl (OH) in the molecular structure. These groups provide sites for bonding active molecules of acetone to interact and cause changes to the electrical conductivity of chitosan. Besides, by maintaining the value of its pK, a ~ 6.5 Chitosan becomes soluble in acidic aqueous media that allows chitosan is deposited in the form of a thin film onto a substrate by electrodeposition method. Special advantages owned by chitosan is believed to lead to great success to produce acetone breath sensor that has high sensitivity, response time is faster and cheaper.

Based on the above matters, so in this study will be conducted using the breath acetone concentration detection sensor chitosan-based in DM patients were controlled and uncontrolled that is expected to replace the detection method using Glucometer blood sugar levels and HbA1c can match detection methods.

Material and Methods

This study is an observational study using a cross-sectional design (cross-sectional). The study was conducted in health care networks First Level University Hospital of North Sumatra. When the study is from March to October 2018. The target population was all patients with Diabetes Mellitus in Medan. The samples are all patients with Diabetes Mellitus who meet the inclusion and exclusion criteria.

All study subjects had requested her consent after an explanation of the purpose of the study, methods of sampling and research benefit from the researchers.

Approval of the study has been issued by the Health Ethics Committee of the Faculty of Medicine, University of North Sumatra.

Procedure

Factual testing procedures of chitosan-based sensors is done by testing patients with DM with acetone breath; they exhale into the sensor system. Furthermore, patients with DM will be tested her blood sugar levels using a glucometer and blood samples taken last them to be tested in the laboratory HbA1c levels.

Results

During the period from March to October 2018, to obtain samples such as blood sugar levels, HbA1c and acetone levels in the breath of people with diabetes in the Diabetes Clinic and Clinic Alifa Telkom in Medan. Total obtained 58 samples that meet the criteria for inclusion in this study (Table 1).

Table 1: Percentage KGD diabetic patient by Category Low, Borderline, High and Very High

Category	Total	Percentage
Low	6	10.3
Borderline	15	25.9
High	19	32.8
Very High	18	31.0
TOTAL	58	100

The table above shows that the percentage of people with diabetes are at KGD High category (32.8%). Average Blood Sugar Levels was 186.5 ± 83.8 (standard deviation).

Table 2 shows that the percentage of people with diabetes HbA1c values is in the High category (62.1%). Mean HbA1c was 9.33 ± 2.1 (standard deviation).

Table 2: Percentage of HbA1c in a diabetic patient by Category Normal, High and Very High

Category	Total	Percentage
Normal	5	8.6
High	36	62.1
Very high	17	29.3
TOTAL	58	100

Table 3 above shows that the percentage of DM patient's breath acetone levels is in the High category (89.7%). The mean Acetone (chitosan) is 368.24 ± 33.5 (standard deviation).

Table 3: Percentage of breath acetone levels in a diabetic patient by Category Normal, Low and High

Category	Total	Percentage
Normal	2	3.4
Low	4	6.9
High	52	89.7
TOTAL	58	100

Table 4 shows that the in DM patients breath acetone levels are found higher in line with higher

HbA1c levels (62.06%). Statistical test using the Spearman correlation test showed that acetone (chitosan) with HbA1c very weak with R = 0.05 and p = 0.708.

Table 4: Distribution Breath Acetone Levels of HbA1c levels compared with patients with DM

		Categories Acetone			Total
		Normal	Low	High	
Category KGD	borderline	1	1	13	15
	Low	0	0	6	6
	Very high	1	1	16	18
	High	0	2	17	19
Total		2	4	52	58

Table 5 shows that the in DM patients found higher breath acetone levels in line with the high levels of KGD (32.76%). Statistical test using the Spearman correlation test showed that acetone (chitosan) and KGD very weak with R = 0.1 and p = 0.445.

Table 5: Distribution of breath acetone levels compared to levels KGD DM patients

		Categories Acetone			Total
		Normal	Low	High	
Category	Normal	1	1	3	5
HbA1C	Very high	1	1	15	17
	High	0	2	34	36
Total		2	4	52	58

Table 6 shows that the in DM patients found that high levels of KGD not always followed by high HbA1c levels. Found there are 17% of patients with high HbA1c levels, but the levels of KGD borderline. Statistical test using the Spearman correlation test showed that HbA1c and KGD are strong with R = 0.7 and p = 0.01.

Table 6: Distribution of HbA1c levels compared with the levels of KGD DM patients

		Category KGD				Total
		Borderline	Low	Very high	High	
Category	Normal	3	1	0	1	5
hba1c	Very high	2	1	11	3	17
	High	10	4	7	15	36
Total		15	6	18	19	58

Discussion

Based on data from this study, it appears that the average blood sugar levels in the diabetic patient were 186.5 ± 83.8 (standard deviation). The mean Acetone (chitosan) is 368.24 ± 33.5 (standard deviation) and the mean HbA1c levels were 9.33 ± 2.1 (standard deviation). This is in line with the distribution of blood sugar levels, acetone breath and HbA1c showed that all of these parameters indicate a high category for each variable. It is clinically proven that we see that there is a linear relationship between the synergistic or three parameters. In Table 5 is shown that almost all patients with diabetes who have high levels of acetone breath then the patient HbA1c levels are also high. Although the levels are not always followed by an increase in blood sugar levels. This indicates that the clinical measurement of breath acetone levels following higher HbA1c levels than KGD. Therefore, according to researchers acetone breath can be used as a detector that can represent the levels of HbA1c. Still, before deciding whether the examination of acetone breath can be used in place of HbA1c, you should do more research to examine the levels of acetone in the blood for each subject were examined levels of acetone breath. In this research shows that there is a weak correlation between breath acetone levels with HbA1c or KGD. This is probably due to the number of samples that are still considered insufficient.

However, at least we see there is a connection even if the relationship is weak. For that, we need to do further research by increasing the number of samples. Therefore, according to researchers acetone breath can be used as a detector that can represent the levels of HbA1c. Still, before deciding whether the examination of acetone breath can be used in place of HbA1c, you should do more research to examine the levels of acetone in the blood for each subject were examined levels of acetone breath. In this research shows that there is a weak correlation between breath acetone levels with HbA1c or KGD. This is probably due to the number of samples that are still considered insufficient. However, at least we see there is a connection even if the relationship is weak.

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Further research should be done to check the levels of acetone in the blood for each subject examination of breath acetone levels. In this research shows that there is a weak correlation between breath acetone levels with HbA1c or KGD. This is probably due to the number of samples that are still considered insufficient. However, at least we see there is a connection even if the relationship is weak. For that, we need to do further research by increasing the number of samples.

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One interesting finding in this study is that although the statistically weak association between variables visible but clinically, we see that the relationship between variables is strong enough.

We can conclude:

1. Average Blood Sugar Levels DM patients were 186.5 ± 83.8 (standard deviation);

2. The mean HbA1c in the diabetic patient was 9.33 ± 2.1 (standard deviation);

3. The mean Acetone (chitosan) DM patients was 368.24 ± 33.5 (standard deviation);

4. Correlation acetone (chitosan) with HbA1c in diabetic patient is very weak with R = 0.05 and p = 0.708;

5. Correlation acetone (chitosan) and KGD diabetic patient is very weak with R = 0.1 and p = 0.445;

6. Correlation of HbA1c and strong enough KGD DM patients with R = 0.7 and p = 0.01;

7. Although statistically, the correlation test acetone breath on HbA1c and KGD showed a very weak but clinically shown that there is a consistent linear relationship between increased levels of acetone in the breath with increased HbA1c levels as well as levels of KGD.

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The Effectivity of Ethanolic Extract from Papaya Leaves (*Carica papaya* L.) as an Alternative Larvacide to *Aedes* spp

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Keywords: *Aedes* spp.; *Carica papaya* L; Dengue; Larvacide

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Abstract

BACKGROUND: Dengue haemorrhagic fever (DHF) is a disease found in most tropical and subtropical regions of the world, including Indonesia. One of the problems of vector control programs is insecticides resistance to Aedes spp.

AIM: The objective of this study is to determine the effectiveness of an alternative larvacide using papaya leaves (*Carica papaya* L).

METHODS: To obtain an ethanolic extract of *C. papaya* leaf (EECP), the dried of *C. papaya* leaf was macerated with ethanol 70%. Phytochemical compounds were screened qualitatively. Twenty-five larvae were entered into each cup that had been mixed with five concentrations of EECP i.e., EECPI (100-), EECPII (150-), EECPIII (200-), EECPIV (250), EECPV (300 ppm), 1% of Temephos (T), and water (A). Alkaloid carpain, saponin, flavonoid, tannin, glycosides and triterpenoid/steroid were traced in EECP. The mortality of larvae at 180, 360, 1440 and 2880 minutes were observed. The lethal concentration (LC50) and lethal time (LT50) were measured. Probit analysis was used to determine the concentration of killing larvae.

RESULTS: The mortality of larvae was found at 360 minutes only in EECPV. Then after 1440 minutes, all extracts shown the increasing of larvae mortality. LC50 and LT50 values were 215,96 ppm and 2,369 minutes of each.

CONCLUSION: EECP has larvicidal activity to *Aedes spp*.

Introduction

Dengue infection is a disease caused by the dengue virus found in most tropical and subtropical regions of the world. It is endemic especially in Southeast Asia, South Asia, Central America, South America and the Caribbean Islands [1]. The virus is transmitted to humans through the bites of female *Aedes aegypti* and *Aedes albopictus* [2]. The World Health Organization (WHO) estimates the incidence of dengue fever (DF) has increased over the past 50 years with attacks occurred in more than 100 million people each year and causes around 30,000 deaths, especially children [3]. The incidence of Dengue Hemorrhagic Fever (DHF) has also increased fourfold over the past three decades and there are now 2.5 billion people at risk for this disease [4]. Asia ranks first in the number of people with dengue fever annually. Between 1968 and 2009, WHO noted Indonesia as the country with the highest DHF cases in Southeast Asia with an incidence of 68.2% per 100,000 of the population [5]. According to the Indonesian Ministry of Health 2018, the incidence rate (IR) of dengue hemorrhagic fever in Indonesia in 2010 to 2017 was very fluctuating, in 2010 amounting to 65.70 per 100,000 population, 2011 down significantly to 27.67 per 100,000 population then in 2012 increase

to 37.27 per 100,000 population and 2013 it became 45.85 per 100,000 population, in 2014 it down to 39.80 per 100,000 population and 2015 increase again to 50.75 per 100,000 population and 2016 to 78.85 per 100,000 population until 2017 down significantly to 26.10 per 100.000 population. In 2017 there were 68,407 dengue cases with 493 deaths. This number down quite dramatically from the previous year 204,171 cases with 1,598 deaths [6].

In the North Sumatera region, DHF is still a public health problem. The Incidence Rate (IR) in North Sumatera showed an increase in the last 5 years: 18.5% per 100,000 population, 19.8% per 100,000 population, 21.2% per 100,000 population, 24.1% per 100,000 population, and 61.4% per 100,000 population in 2012, 2013, 2014, 2015 and 2016, respectively. The IR rate of DF in North Sumatera in 2016 was above the national indicator [7].

Dengue control program must be intensified, especially at the District/City level and at the Primary Health Centres [5]. With the evidence of a continuous increase in DHF cases in the last few years, it is necessary to consider other options for vector control of the vectors.

Since 1972, the government has been using malathion for fogging to prevent dengue transmission. However, the toxin residues produced by this insecticide may accumulate in humans and interfere with blood metabolism and acetylcholinesterase enzyme (AchE) disorder causing neurological manifestations like paresthesia, tremor, balance disorders to seizures [8]. Long-term effects include carcinogenic (formation of cancerous tissue in the body); mutagenic (genetic damage for future generations), teratogenic (birth of disabled children from poisoned mothers) and environmental pollution [9]. The resistance of *Aedes* spp. to temephos, 1% has also been widely reported in Latin America (Brazil, Cuba, Argentina, Peru, and Colombia), Thailand, Banjarmasin, and Indonesia [10], [13].

Due to the negative effects of the long-term use of chemical insecticides, it is important to study natural insecticides derived from plants as an alternative to larvacide [14]. Natural larvacide, including cyanide, saponins, tannins, flavonoids, alkaloids, steroids, and essential oils have been reported to have larvacidal effect [15]. The advantages of using these natural ingredients include lower risk for soil and water pollution due to rapid degradation or decomposition by sunlight, air, humidity and other natural components, and lower toxicity in mammals [16].

Young and fresh papaya leaves are rich in flavonoid compounds, alkaloids carpaines and other polyphenol ingredients [17]. These compounds can be potentially used as natural larvicides without harming the environment [18]. Hayatie et al. described the phytochemical constituents in *Carica papaya* L*,* such as alkaloid carpaines, tannins and flavonoids, have a lethal effect in larvae [19]. Alkaloid carpaines compounds have nervous toxins which kill *Aedes aegypti* larvae, however, are safe in humans [20]. Also, the saponin compounds reduce the digestive tract wall stress-causing larval death [21].

The effectiveness of plants as larvacides are generally assessed by the lethal concentration (LC) and lethal time (LT) [22]. WHO recommends the LC50 or LC90 values as the benchmarks in determining the effectiveness of a larvacide [23]. Kurniawan et al. showed that the ethanol extract of papaya leaves could kill the third instar larvae of *Aedes aegypti* with LC50 of 0.37% and LT50 at 1981 minutes [24]. When methanol extract of papaya leaves is combined with spinosad bacteria, the LC50 was 76.36 ppm on *A. aegypti* instar III and LC50 of 92.78 ppm on *A. aegypti* instar IV [25]. Others have also shown the effect of California papaya leaf extract on mortality of instar III and IV *Aedes aegypti* larvae with LC50 of 0.395% and LC90 of 0.625% [26]. These extracts have also been shown to be effective on other mosquitoes like *Anopheles* spp., and *Culex quinquefasciatus* larvae [27], [28].

Indonesia is the third-largest papaya producer in the world. Therefore, the use of papaya leaf as a source of natural insecticide is reasonable and affordable [29]. We aimed to evaluate the effectiveness of papaya leaves as natural larvicides on *Aedes* spp. instar III / IV larvae.

Material and Methods

This was an experimental study with a post-test control group design. Samples were 700 larvae of *Aedes* spp. mosquitoes, whereas each of 25 mosquito larvae was allocated in each tube with 200 ml of water in a 250 ml container according to the WHO 2005 provisions [30]. Samples in the study must meet the inclusion criteria that larvae of living *Aedes* spp. mosquitoes are at stages of instar III / IV, and *Aedes* spp. mosquito larvae are actively moving at observation.

Tools for producing papaya leaf extract include scales, tin cans for papaya leaf bases during the drying process, freeze dryer, macerator tool to form liquid extract, cotton and filter paper as filters placed in a macerator, bottles for the papaya leaf extract storage containers, and a rotavapor.

Tools to evaluate the effectiveness include 250 ml plastic cup containing 200 ml of water, pipette larvae to pick up the larvae, nylon gauze to close the glass of larval growth, glass cups as a container to measure the volume of water, timer to measure the length of time of research and a loop to observe the

movement of larvae.

The 250 ml plastic cups were divided into 7 groups to evaluate the effectiveness of 70% ethanol extract in *Aedes* spp. larvae. Of which five cups was for papaya leaf extract with concentrations of 100 ppm; 150 ppm; 200 ppm; 250 ppm; 300 ppm; one plastic cup for negative control containing distilled water (placebo, no treatment), and one plastic cup was for positive control containing temephos 1%.

Carica papaya leaf extract

The method for collecting young papaya leaves was done purposively without comparing with the same plants from other regions. Papaya leaves from the researchers' home garden, Medan Tembung, Medan City were collected, then cleaned from the dirt, washed with water until clean, afterwards dried in a freeze dryer at temperature of 50°C. The dried papaya leaves are called simplicia. Simplicia is considered dry if the material could be broken by bare hands. Simplicia was then soaked in 200 ml of 70% ethanol for 2 days then carefully placed into the macerator. The results of maceration were obtained from filtering the maceration process which was accommodated with a one-liter plastic bottle. The maceration process was repeated by adding 100 ml of 70% ethanol to the pulp then stirring and leave stand for 24 hours while continue to do stirring occasionally. The results of the second maceration was combined with the first one then stirred until evenly distributed and evaporated with a rotary evaporator so that dense extracts were obtained [31].

Phytochemical screening

The phytochemical of papaya leaf extract was screened to determine the content of secondary metabolites or active compounds in papaya leaf extract qualitatively. Compounds assessed including alkaloid group compounds such as glycosides, saponins, tannins, and flavonoids.

Larvicidal effect

Twenty-five larvaes were added into each cup that has been mixed with various concentration of papaya leaf extract, negative control and positive control. The numbers of dead larvaes were then counted at 180, 360, 1440, and 2880 minutes.

Data analysis

Data were not normally distributed. Kruskal-Wallis test was conducted to see the difference in the mortality of *Aedes* spp. larvae between the test groups and followed by post hoc analysis using the Mann-Whitney test to determine the correlation value of each pair and toxicity test with probit analysis for assessing the effectiveness of papaya leaf extract against *Aedes* spp. larvae.

This study was approved by the Ethical Committee of Faculty of Medicine University of Sumatera Utara (No.617/TGL/KEPK FK USU-RSUP HAM/2018).

Results

Phytochemical of papaya leaves used in this study is described in (Table 1).

Table 1: Phytochemical screening of ethanolic extract of *C. papaya* leaf (EECP)

Phytochemical Test	Result	Indicator
Alkaloid Carpaine	+	Formed orange deposits (Dragendorff's reagent) Formed white sediment (Mayer's reagent) Formed yellow deposits (Hager's reagent)
Flavonoid	+	Yellow fluorescence
Tannin	+	Formed in dark blue or greenish black
Saponin	+	There is foam that lasts for 10 minutes
Glicosides	+	Formed blue or green
Steroid/Triterpenoid	+	A brownish or violet ring is formed A greenish blue ring is formed
Essential Oil	-	It does not smell distinctive and there are no stains on filter paper

The average percentage of larval mortality from various observation times shows that found a significant difference in each concentration at the time observation (Table 2).

Table 2: The average percentage of larvae mortality *Aedes* spp. larvae between the test groups

Group	The Percentage of Larvae Mortality (Mean ± SD)				
	180'	360'	1440'	2880'	*p* value
Consentration 150 ppm	0 ± 0	0 ± 0	13 ± 8,2	19 ± 5,0	0.004
Consentration 150 ppm	0 ± 0	0 ± 0	19 ± 5,0	32 ± 5,6	0.002
Consentration 150 ppm	0 ± 0	0 ± 0	22 ± 2,3	52 ± 5,6	0.002
Consentration 150 ppm	0 ± 0	0 ± 0	65 ± 4,0	70 ± 4	0.003
Consentration 150 ppm	0 ± 0	1,0 ± 2,0	76 ± 6,5	85 ± 2,0	0.003
Temephos 1%	100 ± 0	100 ± 0	100 ± 0	100 ± 0	1
Aqua	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1
p value	0.00	0.01	0.00	0.00	

The probit analysis in this study showed LC50 of 215.96 ppm and LT50 of 2369.64 minutes (Table 3).

Table 3: Lethal concentration 50 (LC50) and lethal time 50 (LT50)

	LC_{50} Concentration (ppm)	LT_{50} Time (minute)
Value	215.96	2369.64

Discussion

This study determined the effectiveness of papaya leaf extract from papaya leaves of Bangkok type as an alternative larvacide [16]. Qualitative

phytochemical screening was carried out to determine the content of secondary metabolites from papaya leaf extract, which showed alkaloid carpaines compounds, flavonoids, saponins, tannins, glycosides and triterpenoids / steroids [32]. This finding is similar with those reported by Begum et al., Malathi et al., and Choundary et al., [29], [33], [34].

The alkaloids found in papaya leaves were alkaloids carpaines, that are compounds act by inhibiting the activity of the AChE. AChE affects the transmission of nerve impulses which causes disruption of muscle coordination, convulsions, respiratory failure and death [35]. Flavonoids work as a respiratory inhibitor or as a respiratory toxin. Flavonoids have a mechanism of action by entering the body of the larvae through the respiratory system which then causes wilting on the nerves and damage to the spinal and consequently insects will be unable to breathe and eventually die [36].

Saponins are stomach poison for cold-blooded animals, including mosquitoes. Saponins decrease the surface permeability membrane of the larval digestive tract. Hence the wall of the larvae's digestive tract becomes corrosive. Saponins inhibit the action of enzymes resulting in decreased digestive activity and the use of protein for insects [37]. Tanin causes difficulty for insects to digest food so there will be a decrease in growth [38]. Glycoside is a secondary metabolite in plants that is poisonous to stomach. It works by restraining appetite from the mosquito larvae [39]. Triterpenoids/steroid binds to free sterols in food digestion where the decreasing number of free sterols will interfere with the skin turn over the process in insects. Triterpenoid can also cause a decrease in digestive enzyme activity and affect the food absorption process. In this study, we have shown that all concentrations of papaya leaf extracts have a mortality effect on *Aedes* spp. larvae starting at 360 minutes although the significant effect was only seen at minutes 1440 and 2880. Nevertheless, their effects are much slower compared to temephos 1% which has shown an effect as early as at 180 minutes. Temephos 1% caused 100% mortality on larvae and water gave survival to the larve. This lower effectiveness of papaya leaf extract has also been seen in other studies with different amount of concentration and dose [40].

Our *post hoc* analysis also showed no significant difference in the mortality of *Aedes* spp. larvae at concentrations of 100 ppm to 300 ppm in minutes 180 to 360 but significant differences in all concentrations except for concentrations 100 ppm and 250 ppm were seen at minutes 360 to 1440 and from minutes 1440 to 2880. This result explained that the concentration is related to the length of time of exposure and begin to appear effective at a concentration of 200 ppm, which starts at 360 minutes to 2880 minutes and increasing with the length of time of exposure. The toxic concentrations accumulated with the increased exposure time before giving an effect to the larvae.

Kurniawan et al. showed a higher LC50 level than of that found in this study but with a shorter LT50 [24]. This indicates that concentration is inversely proportional to the exposure time. Kadafi et al. used a California-type of papaya leaf had an LC50 higher than in this study [26]. This might be explained by the differences in the composition of the active ingredients which influence the larval mortality [41]. While Refai et al., used 96% ethanol as solvents and had an LC50 value 20 times fold higher than in this study [14]. This difference can be influenced by the selection of improper solvents that affect the extraction process of active compounds in this plant, thus affecting the numbers of larval mortality [31]. The use of suitable types of solvents affects the effectiveness of larvacides in papaya leaves used [42].

We have shown that papaya leaf extracts have a fatal effect on *Aedes* spp. larvae. However, further study is still needed to determine the accurate dose of these extract as a larvacide. The effect of these extracts on the larvae of other species still also needs to be studied. Differences in species factors may affect the larval mortality against the toxic insecticide power [43]. One study has shown the same fatal effect on *Anopheles* spp. although the LC50 was higher [27]. Biological factors such as the geographical location of the original plant, storing methods, and the age of plants may also give vary influence [41]. Furthermore, other factors including temperature, air humidity, water pH may affect the body resistance of each larva [44], [46].

In conclusion, lethal concentration 50 from the ethanol extract of papaya leaves valued at 215.964 ppm or 0.021% with LT50 of 2369,642 minutes are effective as *Aedes* spp. instar III and IV larvacides.

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Diabetic Neuropathy among Type 2 Diabetes Mellitus Patients at Amplas Primary Health Care in Medan City

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Abstract

BACKGROUND: Diabetic neuropathy is one of the most complicated complications of diabetes patients with Type 2 diabetes.

AIM: The purpose of this study was to determine the diagnosis of diabetic neuropathy based on Clinical Neurological Examination (CNE) and the factors that influence the occurrence of diabetic neuropathy in Type 2 DM patients at Amplas Primary Health Care (PHC) in Medan City.

METHODS: The research design was descriptive-analytic with the cross-sectional approach. The study population was all Type 2 DM patients who came to Amplas PHC with a total sample of 53 people using the consecutive sampling. The research data source is primary data, namely the assessment of diabetic neuropathy using the Clinical Neurological Examination (CNE) criteria. Data were processed using SPSS and analysis using the chi-square test.

RESULTS: The results showed the majority of Type 2 DM patients had mild neuropathy as many as 24 people (45.3%). The Chi-square test results showed there was a relationship between age and duration of diabetes with the incidence of diabetic neuropathy in Type 2 DM patients at Amplas Primary Health Care.

CONCLUSION: Education and early detection with proper management can prevent more severe complications so that the quality of life of patients can be maintained better.

Introduction

Diabetic neuropathy is one of the macrovascular complications in Type 2 DM patients; this can occur due to uncontrolled blood sugar in diabetic patients, the more uncontrolled, the faster the neuropathy complaints [1].

Diabetic neuropathy is one of the chronic diabetes complications. People with diabetes have 11 times riskier of developing neuropathy compared to those without diabetes [2]. Some study found the most cases of neuropathy above the age of 55 years. People with DM have peripheral neuropathy as much as 25% of all people with diabetes mellitus in the world [3]. In the United States, 60-70% of diabetic patients (T2DM) have complications of diabetic neuropathy [4]. In Indonesia, experienced neuropathy in 43% of 16.800 patients Types 2 DM who were proven to be at risk of developing neuropathy [5].

Symptoms of neuropathy in patients with Type 2 DM such as numbness, burning, pricking, and other problems are often complained of but rarely noticed. Patients rarely treat neuropathy because they do not know the symptoms, and this problem can interfere with the quality of life of patients. Some study reported that out of a total of 4097 patients, 90.6% had type 2 diabetes. Among that 90.6 %, those with complications of diabetic neuropathy were 19.4%, followed by other complications of DM such as erectile dysfunction by 17. 5% and diabetic retinopathy of 16.8% [2].

Neuropathy is a serious health problem in patients with Type 2 DM because it can cause disruption of function and can even cause disability. Peripheral neuropathy can impact on muscle weakness and loss of reflex, especially at the ankles.

This situation results in changes in the way of walking and changes in the shape of the legs, such as hammertoes. As a result of suppression or injury to areas that experience numbness, often ulcers arise in the legs of patients with peripheral diabetic neuropathy, if it is not handled correctly, an infection can occur that spreads to the bone so it must be amputated [6].

Prevention of worsening of neuropathy can be done by early prevention. One form of early prevention is by examining peripheral neuropathy. Early detection of neuropathy is essential in DM patients, and preventive interventions can be applied to reduce morbidity. Clinical Neurological Examination (CNE) is used to detect and diagnose diabetic neuropathy in daily clinical practice. CNE includes a study of sensory function, leg muscle strength, ankle reflexes, and a specific score for each examination [7].

The Clinical Neurological Examination (CNE) is a scoring system that is used to assess sensory disorders and reflexes in the lower limbs. Clinical assessment is done to determine the presence or absence of neurological disorders, including sensory tests, tot strength, and ankle reflexes. Recently CNE has been used to detect and diagnose diabetic neuropathy in daily clinical practice. CNE is one modification of NDS because of the Neuropathy Disability Score (NDS), considered to be more complicated and challenging to apply in practical clinical use. Clinical Neurological Examination includes studies of sensory function, leg muscle strength, ankle reflexes, and the giving of sure scores for each examination.

The purpose of this study was to determine the diagnosis of diabetic neuropathy based on Clinical Neurological Examination (CNE) and the factors that influence the occurrence of neuropathy in Type 2 DM patients at Amplas Primary Health Care in Medan City.

Material and Methods

The study was descriptive-analytic (cross-sectional approach). The population of the study was all Type 2 DM patients who came to the Amplas Primary Health Care from January to March 2019. The participation of patients was voluntary by signing informed consent. Sampling was carried out by consecutive sampling with inclusion and exclusion criteria. Inclusion criterion is Diabetes patients residing in the Amplas PHC area, patients who come independently to the PHC, while the inclusion criteria are patients with a history of previous neuropathy (e.g., heavy smokers, known blood vessel blockage diseases from anamneses), patients who have experienced heart attack, patients who have had heart surgery.

The source of study data is Primary data. Neuropathy assessment is based on CNE criteria, where sensory tests, muscle strength, and ankle reflexes are assessed on both right and left limbs. The results of the examination are determined by scoring if the measurement is in normal condition the score is 0, decreases are 1, and a negative score is 2. The overall score for CNE examination ranges from 0 to 37 points. The total score is then categorised. Score 0 = no neuropathy, score 1-9 = mild degree, score 10 - 18 = moderate degree, while score 19-33 = severe degree. The data is processed using computer SPPS to assess the factors that influence the occurrence of diabetic neuropathy by doing the chi-square test.

Results

Baseline Characteristics of Type 2 Diabetic Patients

The result of Table 1 shows that the majority of patients aged 51-60 years as many as 25 people (47.2%), DM patients at most are female as many as 36 people (67.9%), based on the duration of diabetes it is known that most DM patients have 3-5 years and more than 5 years 19 people (35.8%).

Table 1: Basic Characteristics of T2DM patients

Characteristics	Frequency (n)	Percentage (%)
Age (years)		
40-50	19	35.8
51-60	25	47.2
61-70	8	15.1
> 70	1	1.9
Gender		
Male	17	32.1
Female	36	67.9
Duration Suffering DM		
< 3 years	15	28.4
3-5 years	19	35.8
> 5 years	19	35.8
Family History		
Father	10	18.9
Mother	15	28.3
Father and Mother	2	3.8
No one	26	49.0
Neuropathy (based on CNE Score)		
Normal (score 0)	18	34
MildNeuropathy (score1-9)	24	45.3
Moderate Neuropathy (Score10-18)	8	15.0
Severe Neuropathy (Score 19-32)	3	5.7

The majority of patients did not have a family history of diabetes as many as 26 people (49.0%). Based on the assessment, it is known that most patients have mild neuropathy of 24 people (45.3%).

Factors influence the Prevalence of Diabetic Neuropathy

Based on Table 2 shows that severe neuropathy is found in the age group of 40-50 as many as 2 people (10.6%) while moderate neuropathy

is located in the age group 51-60 years as many as 12 people (48.0%), based on chi-square test result that age and duration of diabetes have relationship with peripheral neuropathy experienced by Type 2 DM patients ($p < 0.05$).

Table 2: Factors that influence the occurrence of Diabetic Neuropathy

Risk Factors	Normal	Mild	Moderate	Severe	P
Age (years)					
40-50	7 (36.8)	7 (36.8)	3 (15.8)	2 (10.6)	0.03
51-60	8 (32.0)	12 (48.0)	4 (16.0)	1 (4)	
61-70	2 (25.0)	5 (62.5)	1 (12.5)	0 (0)	
> 70	1 (100)	0 (0)	0 (0)	0 (0)	
Gender					
Male	4 (23.5)	9 (52.9)	2 (11.8)	2 (11.8)	0.90
Female	14 (38.9)	15 (41.7)	6 (16.7)	1 (2.7)	
Duration DM					
< 3 Years	8 (53.3)	7 (46.7)	0 (0)	0 (0)	0.04
3-5 Years	8 (42.1)	11 (57.9)	0 (0)	0 (0)	
> 5 Years	2 (10.6)	6 (31.6)	8 (42.0)	3 (15.8)	
Family History					
Father	3 (30)	3 (30)	3 (30)	1 (10)	0.15
Mother	6 (40)	5 (33.3)	3 (20)	1 (6.7)	
Both	1 (50)	0 (0)	0 (0)	1 (50)	
No One	8 (30.8)	16 (61.5)	2 (7.7)	0 (0)	

Discussion

The study showed that there was a significant relationship between age and the prevalence of diabetic neuropathy. The results of this study are in line with other studies who get the most age in patients with diabetic neuropathy ranging from 51-60 years [8], while another study states that diabetic neuropathy patients are more than 50 years old [9].

In theory, it can be explained that increasing age stimulates the degeneration process and causes nerve cell damage. Changes in both large nerve fibres and small nerve fibres give rise to vulnerability in the elderly to neuropathy [4]. The number of patients with diabetic neuropathy in the age range of 45-65 years is due to the age of tissue damage caused by free radicals such as increased levels of lipid peroxide and changes in enzyme activity. Diabetes is degenerative, a disease that appears slowly along with the increasing age of the patient for years so that patients experience complications of diabetic neuropathy [10]. So that rising age will increase the risk of complications of diabetic neuropathy in diabetic patients [11].

Neuropathy based on gender is known to be more severe in male neuropathy (11.8%), while mild neuropathy is more common in female DM patients 15 people (41.7%). The Chi-square statistical test showed that there was no correlation between the incidence of neuropathy and the gender of Type 2 DM patients ($p < 0.05$).

The results of the study found that the prevalence of diabetic neuropathy was more prevalent in women ranging from 50.8% [4], [12]. Hormone differences in men and women affect the onset of neuropathy. High levels of oestrogen in women can interfere with the absorption of iodine, which plays a role in the process of nerve myelin formation while testosterone levels in men protect the body from type 2 DM, but not in women [4].

In general, in this study it can be seen that more women experience diabetic neuropathy than men, more women participating in this study can cause this no difference in the prevalence of diabetic neuropathy based on age in Type 2 DM patients at Amplas PHC.

Majority duration of illness of diabetic patients > 5 years, the results of the Chi-square statistical test revealed that there was a relationship between the duration suffering of diabetes with neuropathy ($p < 0.05$).

This study is in line with by Tabatabaei-Malazy where diabetic neuropathy sufferers are more common in people who have suffered DM for > 5 years [13], while according to [4] the average age of diabetic neuropathy patients has experienced DM for 10 year

Diabetic neuropathy generally occurs after 5 years of suffering from type 2 diabetes mellitus. Low glycemic control and dyslipidemia will increase the occurrence of diabetic neuropathy [14]. The more extended Type 2 DM, the higher the incidence of complications experienced. The duration of type 2 diabetes mellitus with high blood sugar levels will affect changes in blood vessel walls [15]. Chronic high blood sugar levels cause a decrease in insulin secretion. Glucose will turn into sorbitol, which causes nerve cell damage. The longer a person suffers from DM, the process will last longer and worsen the occurrence of nerve cell damage [16].

The duration of suffering from diabetes causes chronic hyperglycemia in patients whose blood sugar levels are uncontrolled. Chronic hyperglycemia causes microangiopathy that underlies the onset of neuropathy. In patients newly diagnosed with DM, it was found that less than 10% had symptoms of clinical neuropathy [4].

It was found that there were 16 people (61.5%) who had neuropathy without having a family history of DM, the Chi-square statistical tests stated that $p = 0.15$ ($p > 0.05$) means that there was no association between family history with neuropathy in Type 2 DM patients.

This study is the same with another study where the higher prevalence of diabetic neuropathy was found in groups without a family history [12]. In theory, stated that family history relates to diabetes mellitus where if one parent suffers from DM, a person's risk of developing DM is 15% if both parents have diabetes, the risk of developing DM increases to 75% [17]. Still, this research is not in line with the theory, possibly caused by the more significant role of

lifestyle in creating respondents in this study to be affected by DM.

The genetic risk will increase two to six times if parents or siblings experience type 2 diabetes mellitus [18]. Someone who has a history of hereditary type 2 diabetes mellitus will increase the risk of type 2 diabetes mellitus by 2-fold compared to someone who does not have a history of genetic type 2 diabetes mellitus. The result another study said the majority of female type 2 diabetes mellitus has a history of type 2 diabetes mellitus [19], [20] In this study indicate that lifestyle is a significant risk of diabetes compared to genetic patients, because of all people with diabetes, it is known that 49% do not have a family history.

In conclusions, diabetes neuropathy is one of the most common complications of microangiopathy experienced by Type 2 DM patients, from various factors known as age and family history, affecting the occurrence of diabetic neuropathy. Education and early detection with proper management can prevent more severe complications so that the quality of life of patients can be maintained better.

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The Burden of Malaria Incidence in Subaim, East Halmahera, North Maluku, Indonesia in 2016

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Keywords: Malaria; *Plasmodium vivax; Plasmodium falciparum;* Severe malaria; Incidence; Indonesia

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Abstract

AIM: This study investigated the distribution, characteristics, clinical manifestation and severity of Malaria in East Halmahera, North Maluku.

METHODS: A retrospective and an observational method were used in this study. Data were obtained through analysing medical records of malaria patients from January to December 2016.

RESULTS: There were 89 malaria patients enrolled in the study. The cases infected by *P. vivax* only were 75 cases (84.3%), by *P. falciparum* only (7, 7.8%), and by both infections (7, 7.8%). The incidence of malaria was higher in July and August 2016 in Cemara Jaya district (18, 20.2%) and Baturaja district (17, 19.1%). While severe malaria was higher in children (4, 28.6%) and pregnant women 2 (100%) by *P. vivax* infection.

CONCLUSION: *Plasmodium vivax* infection was higher than *P. falciparum*. Severe *P. vivax* infection was higher than *P. falciparum,* and most of the cases were in children and pregnant women.

Introduction

WHO stated that there are 91 endemic countries for malaria with 212 millions of new cases and 429.000 death. Half of the world's population was at risk for malaria in 2016 included South East region is that still endemic for malaria [1].

Malaria is caused by blood protozoa which is *Plasmodium* spp., transmitted to human through *Anopheles* spp mosquitoes bite [2]. Malaria is an endemic disease, mainly in tropical and subtropical countries [1], [2], [3]. The majority of South-East Asian countries are endemic for malaria [2], [3]. However, there is a decline in incidence and mortality in those countries for 54% and 46%, respectively, between 2010 and 2015 [1], [3]. Indonesia is one of the South-East Asian countries with high endemicity of malaria. This high endemicity is concentrated in East Indonesia. The 5 provinces with high endemicity of malaria are Papua, West Papua, South East Nusa, Maluku and North Maluku [2], [3].

North Maluku was ranked number 5 for malaria burden in 2015 with medium cumulative incident API 1-5. Malaria incidence in an area with medium endemicity tend to increase in 2012-2015, and it reached its peak in 2016 [4]. This study aims to investigate the distribution and characteristics of malaria patients, the clinical manifestation of malaria, as well as its complications.

Material and Methods

The study was approved by the research and an ethical review committee of the Faculty of Medicine, Universitas Islam Indonesia. Inclusion criteria included inpatients who had been diagnosed with malaria that had been proved by laboratory test result in 2016. Exclusion criteria included inpatients who had been diagnosed with malaria without a laboratory test and those who had been diagnosed by RDT only.

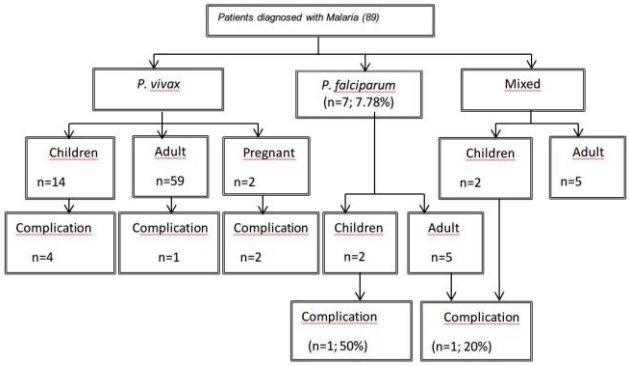

Figure 1: Flow chart of the study

This study assessed some parameters such as age, sex, address, admission month, occupation, clinical symptoms, blood smear examination results, malaria history, malaria treatment, and severe malaria event. Classification of malaria with complications was based on WHO criteria such as haemoglobin < 5 gr/dl, seizure, hyperpyrexia, and hemoglobinuria [5]. Patients were then categorised into age groups < 1 y.o; 1-5 y.o; 6-10 y.o; 11-15 y.o; 16-25 y.o; and > 25 y.o; sex groups, occupation groups, body temperature, symptoms, previous history, previous treatment, and haemoglobin levels. The data obtained such as clinical manifestations, demographic, and parasitology data were analysed using descriptive analysis method.

Results

Distribution, population characteristics & transmission of Plasmodium sp.

The data were taken from January to December 2016. There were 90 in patients who had been diagnosed with malaria, but 1 patient was excluded as s/he did not meet the inclusion criteria (Figure 1). The monthly incidence of malaria occurred throughout the year 2016 and reached its peak in July and August 2016 for 22 patients (24.72 %) (Figure 2).

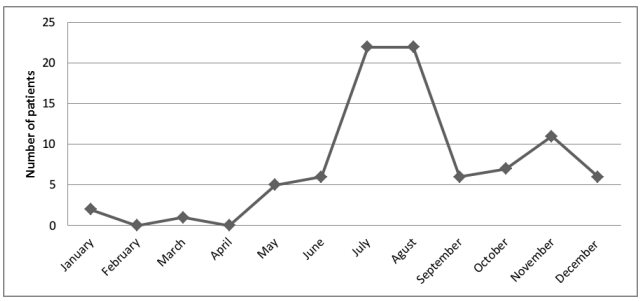

Figure 2: Malaria prevalence in Subaim Primary Health Care, East Halmahera in 2016

The increase of malaria incidence was associated with the transmission factors, including the presence of vectors and gametocyte stage within malaria patients. Gametocyte stage of *P. vivax* and *P. falciparum* in the Subaim region were found in 1% of the total population (Table 1).

Table 1: The distribution of Plasmodium sp. in Subaim Primary Health Care, East Halmahera (n = 89)

Location	Number of the patient, *n* (%)	Type of *Plasmodium*			
		P. f		*P. v*	
		T	G	T	G
Baturaja	17 (19.1)	+	-	+	-
Buli	1 (1.12)	-	-	+	-
Bumirestu	7 (7.78)	+	-	+	-
Cemara jaya	18(20.2)	+	-	+	-
Dakaino	2 (2.25)	-	-	+	-
Volly	1 (1.12)	+	-	-	-
Dodaga	1 (1.12)	+	-	+	-
Fayaul	5 (5.62)	-	-	+	-
Gulapapo	4 (4.49)	+	-	+	-
Mekarsari	10 (11.2)	+	-	+	-
Rawamangun	3 (3.37)	-	-	+	-
Subaim	11 (12.3)	+	+	+	+
Ternate	1 (1.12)	-	-	+	-
Toboino	1 (1.12)	-	-	+	-
Tutulingjaya	2 (2.25)	+	-	+	-
Waisuba	4 (4.49)	+	-	+	-
Wasile	1 (1.12)	-	-	+	-

P. f: Plasmodium falciparum; P. v: Plasmodium vivax; T: Trophozoit; G: Gametocyte.

Clinical manifestation & malaria complications

Amongst 89 total patients, there were 40 (44.9%) women and 49 (5.1%) men. Sociodemographic and clinical manifestation data of malaria patients are shown in Table 3.

Severe malaria symptoms amongst patients infected by Plasmodium sp.

There were 9 patients diagnosed with severe malaria. Table 3 describes severe malaria caused by *Plasmodium vivax* infection. Five patients (6.7%) had seizure, 1 patient (1.1%) with hemoglobinuria, 1 patient (1.1%) had hyperpyrexia, and 2 patients (2.25%) got severe anemia (Table 2). The seizure occurred in 4 of 5 patients, and all of them were children under 5 years old, while 1 patient was 17 years old. Severe malaria characterised by hemoglobinuria occurred on a 23-year-old patient, whereas hyperpyrexia (body temperature > 40°C) was found in a patient aged 7 months old.

Table 2: Clinical manifestation of malaria by different Plasmodium sp. in Subaim Primary Health Care, East Halmahera

Characteristic	*P. v* (n = 75) %	*P. f* (n = 7) %	*P. mix* (n = 7) %
Febrile	55 (73.3)	7 (100)	5 (71.4)
Chills	19 (23.3)	3 (42.9)	1 (14.3)
Nausea	31(41.3)	3 (42.9)	4 (57.1)
Vomiting	40 (53.3)	4 (57.1)	2 (28.6)
Diarrhea	9 (12.0)	0	1 (14.3)
Abdominal pain	17 (22.7)	1 (14.3)	2 (28.6)
Headache	37 (49.3)	2 (28.6)	4 (57.1)
Seizure	5 (6.7)	1 (14.3)	1 (14.3)
Hemoglobinuria	1 (1.3)	0	0
Temperature (^{o}C)	38.0 ± 1.1	38.4 ± 0.8	38.2 ± 0.9

P. f = Plasmodium falciparum; P. v = Plasmodium vivax; Mix = mixed infection.

It can be seen in Table 3; there was a 1-year-old malaria patient with complications caused by *P. falciparum* infection and characterised by a seizure. Similarly, there was a 4-month-old patient who suffered from a mixed infection and had a seizure.

Table 3: Sociodemographic and clinical manifestation of malaria in Subaim Primary Health Care, East Halmahera

Characteristics	Proportion
Age	
< 1 y	6 (6.7%)
1-5 y	4 (4.5%)
6-10 y	8 (8.9%)
11-15 y	7 (7.9%)
16-25 y	17 (19.1%)
> 25 y	46 (51.5%)
Sex	
Male	49 (55.1 %)
Female	40 (44.9 %)
Occupation	
Carpenter	20 (22.5%)
Employee	17 (19.1%)
Student	38 (42.7%)
Housewife	14 (15.7%)
Body temperature (°C)	38.09 ± 1.1
Febris	67 (75.3 %)
Chills	23 (25.8 %)
Nausea	38 (42.7 %)
Vomiting	46 (42.7 %)
Seizure	7 (7.9 %)
Diarrhoea	10 (11.2 %)
Abdominal pain	20 (22.5 %)
Headache	43 (48.3 %)
Currently on Malaria medication	6 (6.7 %)
Having malaria history	3 (3.4 %)
Mean Hb level (g/dl)	7.1 ± 2.3

Discussion

The Indonesia Government, through the Ministry of Health, has continuously tried to control the incidence of malaria [6]. The decreasing malaria incidence indicates this from year to year [4]. This study described the incidence of malaria at Subaim Primary Health Care, East Halmahera, North Maluku in the year 2016. Malaria remains an endemic disease in East Halmahera and needs to be controlled, especially in East Indonesia [3], [4].

The highest case of malaria in this study was caused by *P. vivax* infection, and this is similar to other studies [3], [7], [8]. The result of this study suggested that the incidence of malaria increased in July and August 2016 (Figure 2) with the highest incidence of malaria occurred in Cemara Jaya Village in 18 cases and Baturaja in 17 cases. The malaria transmission factor supports this in the presence of gametocytes in the patients' blood [9]. The presence of gametocytes in the bloodstream is also linked with seasonality prevalence at the local state, sub-microscopic and asymptomatic infection [10]. Other factors that also affect the emergence of malaria outbreaks are the climate factor from local regions such as La Nina, rainy season, and human population dynamic [8], [11], [12].

The presence of gametocyte phase in the patients' bloodstream is a key factor influencing the transmission of malaria disease. Further, this is also enhanced by several factors, such as the presence of *Anopheles* spp. as a vector, occupational factor, as well as outdoor activities [13]. This study discovered that malaria attacks mostly students and farmers. This might be due to both occupations are owned by the productive age group, which most of their activities are outdoor. Therefore, the possibility to be bitten by *Anopheles* spp. is higher than the other age group [2], [12].

Plasmodium vivax and *P. falciparum* are the most common malaria-causing species in Indonesia [2], [3], [14]. Likewise, *P. vivax* was the most common cause of malaria disease (82.3%) found in this study [3].

Malaria is one of the most common infectious diseases with high morbidity and mortality in Indonesia [4], [14]. In this study, the elements such as age, occupation, especially students and pregnant women are important to be noted as the predisposing factors for severe malaria [14], [15]. Based on this study, the incidence of malaria in Subaim Primary Health Care was mostly suffered by the working-age group and in children aged 15 years or less. This is in line with a previous study [13].

This study described that 25 people were children, likewise to other studies which clarified that malaria cases were found in young age groups [8], [16], [17]. In this study, 6 patients were diagnosed with malaria in the age under 1 year old and the youngest age was 4 months old (1 baby), this is similar to another study [8], [16]. The previous study reported out of 18 malaria-infected children, 7 of them were diagnosed with severe malaria, which characterized by seizures [16]. Therefore, all malaria patients < 1-year-old were diagnosed with severe malaria, whereas another severe malaria patient is 17 years-old. Additionally, a study in Ethiopia also showed similar results where children were at higher risk for severe malaria [15], [18], [16].

There are approximately 11.6-28.4% of infected pediatric patients that progress to severe malaria [15], [18] and most of them were infected by *P. vivax*. Our study also found that pediatric patients who were diagnosed with malaria accounted for 16%, and they were infants, preschoolers, and school-aged

children.

Further, this study also discovered pregnant malaria patients was accounted for 2.3%, which is similar to the previous study [19]. It should be noted that all pregnant women in this study were in their first trimester of pregnancy and were diagnosed with severe malaria as they suffered from severe malaria. This is important to note as it has also been reported in previous studies [19], [20]. The results of this study prove that *P. vivax* causes malaria with complications [15]. This suggests that a condition in the endemic area is associated with the disease's pathway.

Some studies suggest that pregnant women with malaria may have a complication with a Hb level < 9.3 g/dl [21], [22]. This complication was not affected by age, parity, gestational age as well as education level [20], [21]. The previous study stated that pregnant women are at high risk for *P. vivax* infection, which causes severe malaria conditions [23]. Immunity status is believed to be the underlying factor that causes severity in pregnant women [24]. In addition, malaria in pregnancy may pose a risk to abortion, prematurity, low birth weight, malaria congenital, intrauterine fetal death or stillbirth [25].

Most of the children with malaria had a complication [15], [18] as were seen in our study. In Africa, where the diseases have a higher prevalence, a study of 263 patients showed that 17.5% were diagnosed with severe malaria [15].

Mixed-infection of *Plasmodium spp.* was found for 7.8% patients in our study. This number was range from 1.2% to 22.5% worldwide [15], [26]. It is important to be understood that mixed infection can cause severe illness than a single infection. However, based on the current theory, *P. falciparum* infection is the most common cause of severe malaria [27]. Yet, this study discovered that the incidence of severe malaria is triggered by age, pregnancy and *P. vivax* infection. The incidence of malaria with complications occurred in infants and children that were characterized by anemia, and seizures [18], [28]. Severe malaria in children is commonly occurred due to the episode of *Plasmodium* spp. infection, parasitemia degree, parasitic virulence, and immune response [18], [28].

The result of this study supports the results of the previous study, where *P. vivax* is responsible to the incidence of malaria complications [28], [29]. Some metabolites products such as heme and lipid in the patients' blood can be used as a marker of severe malaria which is caused by *P. vivax* infection [29]. Another study proved that in *P. vivax* infection, there is phosphatidylserine, a cytoadherence factor that could be found on the surface of the Plasmodium-infected erythrocytes [30].

We conclude that *P. vivax* infection was higher than *P. falciparum* and was associated with severe malaria. Severe malaria was higher in children and pregnant women and was caused by *P. vivax.* Further study needs to find malaria risk factors in East Halmahera, East Maluku.

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The Effect of Isometric Exercise Plantar Flexor on Osteoblast Activity Measured by Bone Specific Alkaline Phosphatase and Callus Formation in a Patient Post Open Reduction Internal Fixation with Non-articular Tibia Fracture

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Keywords: Osteoblast; Isometric Exercise; Plantar flexor; Callus; Non-articular tibia fracture

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Abstract

BACKGROUND: Post-operative non-articular tibia fracture several problems that can occur include; pain, muscles atrophy, muscles weakness, joint stiffness, delayed union, and non-union that makes patients limited in their daily activities. Several factors that play a role in the process of fracture healing include osteoblast activity and exercise. Isometric exercises plantar flexor might effectively prevent the problem. Markers of osteoblast activity are bone-specific alkaline phosphatase (BSAP) levels and Hummer scale callus formation. Not yet known the effect isometric exercise of plantar flexor on osteoblast activity in the post open reduction internal fixation of non-articular tibia fractures, due to the lack of studies in this field.

AIM: This research was conducted to investigate the effect of isometric plantar flexor on osteoblast activity and callus formation in patients post open reduction internal fixation nonarticular tibial fractures.

HYPOTHESIS: There are differences in osteoblast activity and callus formation between groups that do isometric exercises of plantar flexor muscles with those that without isometric exercises plantar flexor.

ANALYSIS: Hypothesis test used a paired t-test with a value of α 0.05 and a confidence level of 95%.

METHODS: This clinical trial was true experimental with pre-post test control group design divided into two groups, group I obtained treatment of isometric exercises of the plantar flexor muscle, range of motion knee joint, and ankle while group II obtained the range of motion knee joints and ankle. Osteoblast activity measured with bone-specific alkaline phosphatase level and callus formation.

RESULTS: The result of the study found to increase mean bone-specific alkaline phosphatase group I; 15.6 and group II; 5.2. A paired t-test of independent samples with α of 0.05 and confidence interval 95% was obtained p-value = 0.000, there is a significant difference in increased levels of bone-specific alkaline phosphatase group I obtained isometric exercises plantar flexor with group II without isometric exercises plantar flexor in patients post open reduction internal fixation of a non-articular tibia fracture. Radiographic examination of Hummer scale callus in group I who carried out isometric exercises plantar flexor had an average value of 2.63, whereas group II without isometric exercises plantar flexor average of 3.06. Wilcoxon test with α 0.05 and 95% confidence interval obtained p-value = 0.000, there is a significant difference in callus image in both groups of patients post open reduction internal fixation non-articular tibia fracture. The linear regression calculated of callus assessment with bone-specific alkaline phosphatase obtained the value of R quadrat = 0.793, which showed that the formation of callus Hummer classification could predict the change in bone-specific alkaline phosphatase value by 79%.

CONCLUSION: The research found isometric exercise plantar flexor in patients post open reduction internal fixation non-articular tibia fracture enhances osteoblast activity and callus formation that will likely short the healing process time and prevent delayed union or non-union.

Introduction

The incidence of non-articular tibia fractures increases from year to year. Tibia is the bone most often broken because of the superficial structure and position. The increased number of tibial fractures over time results in increased morbidity and disability rates. Fracture is one of the problems that humans are faced with in a risky life because of the development of the industrialisation of society, the increasing number of vehicles, and increased activity [1]. The incidence of tibial fracture is recorded at 16.9/100,000 per year [2].

Treatment days averaged 7.2 days [2]. The most common causes of cases of tibia fractures are due to traffic accidents, falls from stairs, skateboarding, and sports. Tibial bone fractures often occur in men aged 15 to 29 years [3]. Fractures in the tibia are often accompanied by skin, muscles, fat and fascia [4].

The problem that usually occurs in patients who experience non-articular tibia fractures is the length of the healing process, muscle atrophy, reduced bone density, delayed union, and non-union. The process of fracture healing is influenced by several factors such as; osteoblast activity, fracture type, nutritional status, care, psychological conditions, time of medical intervention and rehabilitation as early as possible [5], [6]. Patients after open reduction internal fixation of the tibia fracture are long enough for several weeks without weight bearing which can cause complications in the form of calf muscle atrophy, calf muscle weakness, osteopenia, limited motion of the knee joint, and ankle [7].

Programs and types of muscle training must be designed accurately and so that complications do not occur and the healing time can be achieved quickly. One type of muscle training given is the isometric exercise of the plantar flexor muscle. Research on factors that influence the processof osteoprogenitor differentiation and osteoblast activity is very important in the process of healing tibial fractures. It is not yet known the effect of plantar flexor isometric exercises on osteoblast activity and callus formation in patients with non-articular tibia fractures.

Hypothesis: 1. There is a difference in the increase in bone-specific alkaline phosphatase and callus formation grade in the group of patients who are doing isometric exercises plantar flexor with groups that do not do isometric exercises of the plantar flexor; 2. There is a relationship between callus formation and bone-specific alkaline phosphatase levels in a group of patients who are doing isometric exercises plantar flexor with a group of patients who do not do isometric exercise plantar flexor.

Material and Methods

This research was conducted in the Department of Physical Medicine and Rehabilitation at Zainoel Abidin General Hospital Banda Aceh Indonesian. The study included 34 patients over a period of 1 month. The subject was recruited from Orthopaedic inpatient room post open reduction internal fixation non-articular tibial fracture after obtaining written informed consent and approval of the institutional ethical committee. Patients included in the study were those of age group 20-45 years, normal body mass index with isolated post open reduction internal fixation non-articular tibial fracture. Excluded patient with; loss of consciousness, diabetic, anemia, and multiple fractures. Experimental research with pre-post test control group design with subject selection used systematically random. Group I with the treatment of isometric exercises of plantar flexor muscles and standard exercises range of motion knee and ankle joints while group II is only with standard exercises the range of motion knee and ankle joints.

Methods

Group I

1. Range of motion exercise.

The patient is lying supine with the lower limbs straight and the ankle joint in a neutral position or 0°. The therapist helps the dorsiflexion ankle movement to the pain limit or the patient's ability then return to the starting position, this movement is repeated up to 10 times. Furthermore, the therapist helps move plantar flexion ankle to the extent of the pain or the patient's ability then return to the starting position, this movement is repeated up to 10 times. Knee at position 0°. Furthermore, the therapist helps to flexion the knee joint to the limit of pain or patients ability then returns the extension to the original position, this movement is repeated up to 10 times. Every day a training session is conducted, where each exercise session moves the knee and ankle joints 10 times.

2. Isometric exercise plantar flexor

Patient is lying on his back with the position of the leg straight and ankle neutral position or 0°, used modified ankle-foot orthoses (Figure 1). Purpose use ankle-foot orthoses to able fixation of ankle joint so that no occur joint movement and easy to use. Modification by make a window on the back side to be able palpate muscle plantar flexor contraction. Patient performs an isometric contraction of the plantar flexor muscle. Assessment of plantar muscle contraction is done by palpating the calf muscle belly. The long contraction in a matter of 10 seconds use stopwatch casio HS3. Every day three sessions were carried out, one training session carried out 10 repetitions with a break every 10 seconds. Every session rests for two minutes. Educational exercises are carried out first on healthy feet.

Group II

The only range of motion exercise without isometric exercise.

The patient is lying supine with the lower

limbs straight and the ankle joint in a neutral position or 0°.

Figure 1: Isometric Exercise Plantar Flexor

The therapist helps the dorsiflexion ankle movement to the pain limit or the patient's ability then return to starting position, this movement repeated up to 10 times. Furthermore, the therapist helps move ankle plantar flexion to the extent of the pain or the patient's ability then return to the starting position, this movement repeated up to 10 times. Knee at the straight position. Furthermore, the therapist helps to flex the knee joint to the limit of pain or patients ability then returns the extension to the original position, this movement is repeated up to 10 times. Every day a training session is conducted, where each exercise session moves the knee and ankle joints 10 times.

Bone Specific Alkaline Phosphataseanalysis

Bone-specific alkaline phosphatase (BSAP) is marker of activity osteoblast. This marker has value sensitivity 96% and specificity 80% and minimal diurnal variation. Measurement of isoenzymes BSAP by the calorimetry method using a spectrophotometer. The first blood collection from median cubital veins in both groups was examined for bone-specific alkaline phosphatase levels with the type of metra-sample stored that was performed on the second day after surgery. Second blood collection from median cubital veins in both groups for the examination of bone-specific alkaline phosphatase levels was carried out on thirty-first day after surgery.

Callus Formation Analysis

The first x-rays (AP/lateral position) of the tibia in both groups were examined on the first day after surgeryby the radiologist on the Cummer scale. The second x-rays (AP/lateral position) of the tibia in both groups were examined on the thirty-first day after surgery by a radiologist with Hummer scale. Reason chose Hummer because it has a valid level of measurement accuracy and Cohen's Kappa Hummer scale test results k = 0.65, CI 95% 0.59-0.75.

Statistical Analysis

Data analysis used: 1. Univariate which describing the distribution of the proportion of data; 2. Analysis of the homogeneity test against; bone-specific alkaline phosphatase, age, body mass index, and hemoglobin; 3. Analysis of hypothesis used a paired t-test with α of 0.05. and Confidence Interval 95%; 4. Analysis linear regression has used the relationship between the results of callus assessment with bone-specific alkaline phosphatase.

Results

The study population consisted group I of 13 (81%) males and 3 (19%) females with majority in the age group of 36-40 years 6 (37%) followed by 25-29 years 4 (25%), 41-45 years 3 (19%), 30-35 years 3 (19%) age groups. Group II (Control) of 12 (75%) males and 4 (25%) females with majority in the age group of 36-40 years 6 (38%) followed by 30-35 years 5 (25%), 25-29 years 4 (25%), 41-45 years 1 (6%) age groups (Table 1). Two samples drop out.

Table 1: Characteristics sample

Age (year)	GROUP I		GROUP II	
	Freq.	%	Freq.	%
25-29	4	25	4	25
30-35	3	19	5	31
36-40	6	37	6	38
41-45	3	19	1	6
Total	16	100 %	16	100 %
Gender				
Male	13	81	12	75
Female	3	19	4	25
Total	16	100 %	16	100 %

The level bone-specific alkaline phosphatase increased in both groups I and groups II (Table 2).

Table 2: Level Bone Specific Alkaline Phosphatase

GROUP I				GROUP II			
Sample number	BSAP 1	BSAP 2	Increase	Samplenumber	BSAP 1	BSAP 2	Increase
1	14.9	28.8	13.9	2	27.3	31.1	3.8
3	15.7	27.3	11.6	4	31.6	33.8	2.2
5	26.2	29.5	3.3	6	16.1	21.9	5.8
7	20.1	44.9	24.8	8	28.3	38.4	10.1
9	23.4	45.4	22.0	10	23.1	26.6	3.5
11	16.4	34.0	17.6	12	30.0	35.5	5.5
13	20.1	26.4	6.3	14	27.4	28.8	1.3
15	28.3	36.6	8.3	16	22.3	26.2	3.9
17	30.7	52.9	23.2	18	31.7	36.7	5.0
19	29.4	37.4	8.0	20	27.4	30.7	3.3
21	28.4	41.5	13.1	22	19.6	22.2	2.6
23	44.0	98.3	54.3	24	21.7	29.0	7.3
25	16.9	29,5	12.6	26	19.6	30.3	10.7
27	30.8	33.0	2.2	28	31.1	34.4	3.3
29	19.9	48.1	28.2	30	20.5	28.2	8.2
31	26.5	27.5	1.0	32	out	out	Out
33	out	out	out	34	20.1	26.4	6.3

Homogeneity test results; bone-specific alkaline phosphatase, age, body mass index,and haemoglobin with alpha 0.05 and 95% confidence intervals. Homogeneous test conducted on both groups were obtained;

1. The level of bone-specific alkaline phosphatase obtained a value of p = 0.869 which showed the levels of bone-specific alkaline

phosphatase in both groups were homogeneous.

2. Age in both groups obtained a value of p = 0.418 which showed the age in both groups is homogeneous.

3. Body mass index in both groups obtained p = 0.652 which showed that body mass index in both groups is homogeneous.

4. Hemoglobin levels in both groups obtained p = 0.158 also showed hemoglobin levels in both groups were homogeneous (Table 3).

Table 3: Homogeneity BSAP, Age, BMI, and Hb

No	Variable	Mean difference	Std.error	p
1.	Bone-specific alkaline phosphatase	0.869	2.29	0.869
2.	Age	0.418	2.06	0.418
3.	Bodymass index	0.652	0.38	0.652
4.	Hemoglobin	0.158	0.16	0.158

Because the distribution of bone-specific alkaline phosphatase levels in both groups are normal, then a paired t-test performed with α 0.05 and 95% confidence interval obtained the p-value = 0.000 (Table 4), which showed a significant difference in the increase in average bone specific levels alkaline phosphatase in the group that carried out isometric exercises plantar flexor with those who did not do isometric exercise plantar flexor in patients after open reduction internal fixation of non-articular tibia fractures.

The Paired t-test that was carried out on 2 groups was obtained bone-specific alkaline phosphatase levels in group I and group II with α 0.05 and CI 95% p-value = 0.000 which showed a significant difference in the increase in mean bone-specific alkaline phosphatase in the group that do isometric exercises plantar flexor with those who do not do isometric exercise plantar flexor in patients after internal open reduction fixation of non articular tibia fractures (Table 4).

Table 4: Paired t-test Bone Specific Alkaline Phosphatase Group I and group II

	Group I			Group II			p
	N	Mean	St.deviation	N	Mean	St.deviation	
Pct_BSAP	16	66.34	44.99	16	22.34	13.93	0.000

Analysis of callus assessment uses the Wilcoxon test on the group I who did the isometric exercise plantar flexor and group II did not do isometric exercises plantar flexor obtained the following results are obtained.

In the group, I the Hummer scale callus results obtained an average value of 2.63, the standard deviation of 0.72, and variance of 0.52, while in group II the results of the Hummer scale callus examination obtained an average value of 3.06, the standard deviation of 0.25, and a variant of 0.06. The Wilcoxon test obtained a p-value of 0.000, which showed a significant differences in callus formation between the two groups (Table 5).

Table 5: Wilcoxon test for Formation Callus of Group I and Group II

GROUP I			GROUP II			Wilcoxon
Mean	St.Deviasi	Variance	Mean	St.Deviasi	Variance	P value
2.63	0.72	0.52	3.06	0.25	0.06	0.000

Linear regression calculation results of callus examination with the results BSAP obtained by the value of R 2 quadrat = 0.793, which showed Hummer classification from callus formation able to predict a change BSAP in value 79%.

Discussion

The number of samples of patients with non-articular tibia fractures in this study we found 25 male (78%) and 7 female (22%). The highest age was 36-40 years old as many as 12 people (38%). Other researchers also reported more tibial fractures in male than female [8]. Chauhan et al., 2016 [9] reported that the results of epidemiological studies of tibia fracture were found in male as much as 78% (n = 156) and female 22% (n = 43) with the highest age in the range of 21-30 years. The study conducted by Amin MQ in Pakistan in 2015 out of 2120 cases of tibia fractures, 1980 male (93.4%) and 140 female (6.6%) with an average age of 33.28 ± 21.02 years. Clelland et al., 2016 [10] reported that the results of research on male incidents were almost four times higher than female. The incidence of the male is more dominant because of more work activities outside the home so that the risk of having an accident is greater. The incidence of non-articular tibia fractures is generally in the productive age or working age.

Stages of process osteoblast differentiation include; increase in cell number, matrix maturation, and matrix mineralization. The matrix maturation characterized by peak level of bone-specific alkaline phosphatase. Evaluation of bone healing processes is generally based on patient subjective statements in the form of complaints of pain and findings in the radiographic examination. Levels of bone-specific alkaline phosphatase as chemical marker in the early post-traumatic bone can predict fracture healing. Clinical measurement of isoenzymes bone-specific alkaline phosphatase is important to estimate the progress of bone healing after internal open reduction fixation of non-articular tibia fractures. Isoenzyme phosphatase is an isoenzymes group that plays a role in increasing the activity of serum or plasma bone-specific alkaline phosphatase. Clinical studies also show that serum levels of bone-specific alkaline phosphatase correlate with assessing bone formation processes [11].

Increase level bone-specific alkaline phosphatase in relation to trauma is a consequence of bone cell response to trauma, stress response, type of

injury and surgery. Bone-specific alkaline phosphatase is very important for bone mineralization and is considered a biochemical marker useful for bone formation, thus examining this enzyme in serum used in evaluating bone healing process from fractures. The level of bone-specific alkaline phosphatase in fracture depends on the stability of bone fragments, bone type, and the number of bone fractures. Studied increase level in bone-specific alkaline phosphatase in relation to the type of trauma and concluded that the initial decrease in bone-specific alkaline phosphatase level was not only a consequence of bone response to trauma but also the total stress response associated with injury and surgery [12]. Another study noted significant bone-specific alkaline phosphatase levels decreased until several days after injury, on the eighth day the enzyme returned to the values of the first day and then continued to increase until the twelfth week in tibial fractures [13]. In a group, I who received isometric exercises plantar flexor for 4 weeks, knee and ankle joint motion exercises had an increase in the average value of bone-specific alkaline phosphatase levels that exceeded that of group II who only gained a range of motion in knee and ankle joints. These data illustrate that there was an increase in osteoblast activity that was higher in group I than in group II.

Exercise can increase cytokine reactions, it is based on research that after a while physical exercise increases IL-1 and IL-6. Other researchersfound that IL-1 also increased at the same time in all high-intensity training groups and IL-6 levels had a tendency to rise immediately after exercise. Osteoblast activity is also affected by bone morphogenetic protein (BMP), Platelet Derived Growth factor (PDGF), as well as by physical loading that work through mechanical sensors. These biological and mechanical signals have been shown to stimulate proliferation, migration, and differentiation of mesenchymal stem cell (MSC) against osteoblasts. The cell response to mechanical load of exercise plays a very important role in MSC development process. Plasticyti of bone, supression, fixation, shear, and velocity fluid flow are main factors that act as stimuli for cell growth during bone repair. Mechanical stimulation with low magnitude has been shown to be anabolic to bone [14]. Osteoblasts have a pressure receptor. The receptor receives a mechanical load. Mechanical load stimulates some physical signals that induce the activation of osteoblasts. Bone is a porous network consisting of a liquid period, a solid matrix and cells.

Bone is very dependent on mechanical information that guides the population of cells to adaptation, maintenance, and improvement [15]. Mechanical transductive signals prove important for affect certain cell responses and the underlying mechanism. The pathway of mechanotransduction is very interesting to explain the burden of muscles contraction has an effect such as changes in bone mass, increased bone formation, and osteoblast activity [16]. Physical exercise affects the effects on physiological, hormonal, and immunological processes. Exercises involving skeletal muscle contraction will occur in the process of synthesis and release of interleukin-6 and interleukin-15. After muscle damage was induced by exercising/exercising the IL-6 ratio in myofibers immediately increased, with a peak in 12 hours. Simultaneously in the blood, there is an increase in the number of neutrophils that stimulate and trigger the release of macrophages and lymphocytes. Macrophages moving to the injury site perform phagocytic functions and chemotactic attract satellite cells. The interaction between satellites and macrophages is mediated by IL-6. These cytokines are also involved in increasing protein degradation, multiplying satellite cells that can support muscle regeneration. Satellite cells follow a series of proliferation, migration, and incorporation into adult myofibers which leads to the growth of muscle fibers or hypertrophy.

Apart from playing a role in muscle growth, interleukin affects the activity and differentiation of osteoblasts [17]. Changes in increase in mechanical forces stimulation the development of bone marrow mesenchymal stem cell against osteoblastogenesis [18]. These local mechanical exercises or stimuli produce mechanotransduction, which is the conversion of physical signals into a typical signal of intracellular biochemistry that produces a biological response to the bone. The mechanotransduction process also involves fluid movement (lamellar fluid flow) which stimulates detector cells, but the transduction of mechanical factors into cellular stimulation is not yet fully understood (Isaksson, 2012) [19].

The burden of muscle contraction on the surface of the bone will cause fluid flow through changes in intramedullary pressure [20]. This mechanical force will increase the expression of nitric oxide synthase in osteocyte so that the production of nitric oxide increases. Nitric oxide suppresses osteoclast activity and increases osteoblast activity which is very important in the process of fracture healing. Plantar flexor isometric exercises can also induce prostaglandin synthesis by increasing cyclooxygenase2 (COX2) activity, the level of COX2 levels depends on extracellular regulated kinase (ERK) phosphorylation. Prostaglandin stimulates osteoblast activity through the Insulin Growth Factor. Insulin hormone growth factor increases during mechanical stimulation [20].

Sclerostin signals decrease in the stimulation of muscle contraction exercises. Sclerostin is an osteocytic protein that can inhibit Wnt signals. This signal plays an important role in the proliferation and differentiation of osteoblasts. The ratio of RANKL and Osteoprotegerin in osteocyte decreases in strains. If mechanically stimulated, osteocyte will produce TGF

ß which plays a role in inhibiting osteoclastogenesis [21]. Transforming Growth Factor Beta (TFG-β) that regulate osteoblast activity including proliferation, differentiation, and tissue migration. After bone fracture will occur hematoma , expression of TFG-β increases and continues to be expressed throughout the remodeling phase. TFG-β plays a role in the process activation of mesenchymal stem cells (MSC).

Pressure from muscle contraction will be transferred through the muscle to the tendon that attaches the muscle to the bone, and therefore pressure is also applied to the bone specifically at the location of the adjacent bone surface [22]. The isometric contraction of the plantar flexor muscle will provide mechanical stimulation of pressure on the bone where mechanical stimulation is channeled through the extracellular matrix to osteoblasts, osteocytes, periosteal cells and osteoclasts. In the osteoblast cell wall where the pressure received at the receptor will trigger activity rather than osteoblasts. This type of mechanical stimulation can increase the proliferation and anabolism of osteoblasts to facilitate reconstruction of bone tissue, contributing to the process of homeostasis in bone tissue healing.

Effect isometric exercise plantar flexor and healing process of the tibia bone, activities osteoblast cells produce osteoid tissue and increase the amount of content alkaline phosphatase, which in the reaction of mineralization this enzyme prepares an alkaline atmosphere in the formed osteoid tissue. This enzyme in the bone increases phosphate concentration in the form of calcium-phosphate bonds in the form of hydroxyapatite which is based on mass law so that it plays an important role in depositing calcium and phosphate into the bone matrix.

The formation callus results were seen in group I which carried out plantar flexor isometric exercises had an average value of 2.63, whereas group II who did not do isometric plantar flexor exercises rated an average of 3.06. Bone-specific alkaline phosphatase levels in the blood of group I who obtained isometric plantar flexor exercise found a higher increase compared to group II who did not do isometric exercises of the plantar flexor muscle. This is because the activity of osteoblasts build bone by forming type I collagen and proteoglycans as a bone matrix or tissue osteoid through a process called ossification, and while actively producing osteoid tissue, osteoblasts increase the amounts of content alkaline phosphatase [23], [24].

In conclusion, isometric exercise plantar flexor 30 times a day for 4 weeks can increase osteoblast activity and callus formation in patients post open reduction internal fixation of non-articular tibia fractures. Thisexercise will prevent delayed union and non-union in non-articular tibia fractures.

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Antiplatelet Effectivity between Aspirin with Honey on Cardiovascular Disease Based on Bleeding Time Taken on Mice

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Abstract

BACKGROUND: The prevalence of cardiovascular disease (CVD) and its mortality continues to increase. Various studies have shown aspirin can reduce CVD mortality but has adverse side effects. Research on a comparison between aspirin and honey has not been done, but both have antiplatelet effects.

AIM: This study is aimed to prove the antiplatelet effects on honey and compare the antiplatelet effects of aspirin with honey based on the bleeding time in mice.

METHODS: This study is a true experimental design with a post-test only control group using 32 male mice, Double Ditsch Webster, ± 3 months old, the weight of 20-30 g, divided into 4 groups. Consisting of a negative control group (placebo), aspirin and honey. The suspension has given orally for 12 days using the probe. The research was conducted at the Laboratory of Pharmacology Department of Pharmacology and Therapeutics Faculty of Medicine, the University of North Sumatra in September until December 2015. The data collected was bleeding time in mice. Data analysed by Shapiro Wilk test, Kruskal Wallis and Mann Whitney.

RESULTS: The mean bleeding time was a placebo (102.88 seconds), aspirin (369.38 seconds) and honey (304.63 seconds). Mann Whitney test showed significant results in the aspirin and honey groups against the control group (placebo) with p = 0.001. There were no significant differences in the aspirin group against honey (p = 0.172). Honey has an antiplatelet effect in mice. The mean bleeding time in mice given honey is longer or closer to the mean bleeding time in the aspirin group.

CONCLUSION: The results could be used as a basis for further research to determine its use in humans with cardiovascular disease.

Introduction

Hemostasis is a body reaction that occurs sequentially to stop the bleeding. When blood vessels are damaged or broken, then the hemostasis process must occur quickly in areas that have been damaged and carefully controlled to be effective. Three major mechanisms that occur to reduce blood loss are vascular spasm, platelet plug formation, and coagulation (blood clotting) [1].

The process of hemostasis can occur without any additional injury to the blood vessels of patients with a history of heart disease and abnormalities in blood vessels [1]. In other words, thrombosis is the formation of a pathological hemostatic plugin blood vessels that do not bleed [2]. For example, in patients with transient ischemic attacks (TIAs), cerebrovascular disease (stroke), myocardial infarction or peripheral artery blockage are classified as cardiovascular disease (CVD) [3].

Cardiovascular disease (CVD) is the leading cause of disability and premature death worldwide. The underlying pathology of CVD is atherosclerosis [4]. According to the World Health Organization (WHO) in 2012 [5]. Deaths caused by noncommunicable diseases (NCDs) account for 38 million of the 56 million global deaths, which is about 68%. The main cause of NCD death in 2012 is cardiovascular disease (CVD) that is as many as 17.5 million deaths, or 46% of all deaths due to NCD.

In the Asia / Pacific region, the main cause of death is CVD, which is estimated at 9.3 million deaths

and accounts for about a third of all deaths in 2012 [3].

According to WHO-Noncommunicable Diseases (NCD), Country Profiles in 2014 [6]. Cardiovascular disease (CVD) has been the leading cause of death in Indonesia, which is about 37% of all deaths (1,551,000) in the country.

Administration of antiplatelet drugs such as aspirin can reduce mortality caused by CVD. This antiplatelet therapy is said to be effective in treating severe vascular disease with both short-term and long-term administration [7]. Aspirin (Acetylsalicylic acid-ASA) works by inhibiting the synthesis of thromboxane A2 (TXA2) in platelets and prostacyclin (PGI2) in blood vessels by irreversibly inhibiting cyclo-oxygenase enzymes [8]. However, the dose of aspirin used as an antiplatelet drug in clinical trials is not equivalent [9]. Excessive administration of single-dose aspirin, as well as long-term, can cause the risk of poisoning [10]. The severity of complications that occur with aspirin depends on the dose and duration of treatment. Complications can include bleeding and perforation of the gastrointestinal system [11]. It turns out that any pharmacological therapy has adverse side effects either directly or indirectly. Then safe, natural ingredients are needed as alternative pharmacological therapies or as supplements [12].

Honey is a sweet and thick liquid with a unique flavour generated by honeybees [13]. Honey is very efficacious in medical therapy because of the existence of various phenolic components that have many biological activities, including antioxidants and anti-inflammatory [14]. Research conducted by Ahmed et al., in 2011 [15], proved to have the effect of anti-platelet honey. Honey contains flavonoids, including hesperetin that serves as anti platelet aggregation [16].

With the effect of antiplatelet on honey, the researchers are interested in researching the comparison of the effectiveness of aspirin with honey as antiplatelet based on measurement of bleeding time on the tail of mice. This research is expected to be useful in preventing CVD both in primary and secondary.

Material and Methods

Types of Research

This research is a true experimental design because it performs randomisation (simple random sampling), control, and treatment. The study design is the posttest-only control group design.

P = Population (Mice);

R = Randomization;

K1 = Ex. Plasebo Control (-) without treatment;

K2 = Ex. Plasebo Control (+) with aspirin administration without treatment;

K3 = Ex. Treatment by giving oral Honey for 12 days.

Simple random sampling

1. Number labels are created on each animal that meets the inclusion criteria.

2. Then select as many as 32 mice from them to be sampled and divided into 4 groups at random.

Provision of intervention in experimental animals (mice) was made single-blind.

Time and Place of Study

This research has been conducted in Pharmacology Laboratory of Department of Pharmacology and Therapeutics Faculty of Medicine, University of Sumatera Utara. This research has been conducted from September to December 2015. Research has been conducted after obtaining approval from Ethical Clearance from the Ethics Commission of the Faculty of Medicine, University of North Sumatra.

Population and Sample Research

In this study have been used male mice (Mus musculus), Double Ditsch Webster strains age: ± 3 months (adult), weight 20-30 grams, healthy, has never been used for other studies. Mice obtained from Laboratorium FMIPA Biology University of North Sumatra Medan. The number of group animals is determined by the formula, according to Federer (1963), as follows:

$(t-1)(n-1) \geq 15$

Explanation:

n = sample size

t = number of groups of experimental animals

Then the required sample size is:

$(t-1)(n-1) \geq 15$

$(4-1)(n-1) \geq 15$

$(n-1) \geq 5$

$n \geq 6$

It takes a sample of six animals for each group based on Federer's formula. Added with an estimated drop out of 10%, then the minimum sample size required for each group are seven animals.

Based on the minimum number of samples

allowed statistically and not violating the 3 R (Reduction, Replacement, Refinement) principles in the experimental animal studies, the sample size is taken to eight for each group. So, the total number of experimental animals used is 32 mice.

Criteria for Inclusion, Exclusion and Drop Out

Inclusion Criteria: 1. Mice (Mus musculus) strain Double Ditsch Webster, male, age: 2-3 months, weight: 20-30 grams and 2. Mice healthy, active moves and come from the same group

Exclusion Criteria: Previous mice have received drug intervention.

Drop Out Criteria: Mice die within the study period.

Material

In this study, the materials used are 1. High Desert® 75 mg Honey and 2. Aspirin 80 mg.

Preparing and Maintaining Animals Try

Before the study, the adaptation of animals at the site with a light 12-hour cycle of dark schedule, a standard diet that is eating and drinking ad libitum. The food consumed comes from Charoen Pokhpand. Try animals kept at 25 ± 10°C, 60% relative humidity. Mice were preserved during the study period; weight was weighed before and after the trial.

Procedures

Male mice (Mus musculus), DDW strain, healthy, weight: 20-30 grams, divided into 4 groups: 1. Normal mice as control (-); 2. Mice with aspirin but not given intervention (positive control); 3. Mice with oral honey for 12 days; and 4. Admission was performed on the mice from the first day of the study, for 12 days at doses according to the Conversion Dosage Table 1.

Table 1: Table Dosing Calculation Calculations (Laurence & Bacharach, 1964) orally

	Mice 20 gram	Mouse 200 gr	Guinea Pig 400 gr	Rabbit 1,5 kg	Cat 2 kg	Monkey 4 kg	Dog 12 kg	Human 70 kg
Mice 20 gr	1.0	7.0	12.25	27.8	29.7	64.1	124.2	387.9
Mouse 200 gr	0.14	1.0	1.74	3.9	4.2	9.2	17.8	56.0
Guinea Pig 400 gr	0.08	0.57	1.0	2.25	2.4	5.2	10.2	31.5
Rabbit 1,5 kg	0.04	0.25	0.44	1.0	1.08	2.4	4.5	14.2
Cat 2 kg	0.03	0.23	0.41	0.92	1.0	2.2	4.1	13.0
Monkey 4 kg	0.016	0.11	0.19	0.42	0.45	1.0	1.9	6.1
Dog 12 kg	0.008	0.06	0.1	0.22	0.24	0.52	1.0	3.1
Human 70 kg	0.0026	0.018	0.031	0.07	0.076	0.16	0.32	1.0

Conversion Dosage Calculation: 1. Aspirin: 80 mg x 0.0026 x 1000: 20 = 10.40 mg / kg BW and 2. Honey: 75 mg x 0.0026 x 1000: 20 = 9.75 mg / kg BW;

After 12 days of intervention, all mice have been tested for measuring bleeding time by using Duke-Tail bleeding method as follows: 1. The area under investigation (tail of mice) has been cleaned with cotton alcohol; 2. First, the tail of the mice is cut (made wound) at 1 mm diameter using a scalpel and let the blood out freely, when the blood starts stopwatch is run; and 3. Exhausted blood is sucked with filter paper every 30 seconds until the blood stops flowing (do not let filter paper touch the wound), the stopwatch is stopped when the blood cannot be sucked again using filter paper, and the time is recorded.

Data Collection

Data collected in this study were primary data obtained from the measurement of bleeding time in mice tail after 12 days of intervention.

Data Analysis

The data obtained have been analysed using a computer program. The data have been tested for normality by using the Shapiro-Wilk test because of the small number of samples. Followed by a homogeneity test using the Levene test. The Kruskal-Wallis non-parametric statistical test is performed because the data distribution is normal but the data variant is not the same (not homogeneous), followed by post hoc analysis using the Mann Whitney test. A difference is significant when $p < 0.05$.

Results

Description of Research Location

This research was conducted in Pharmacology Laboratory of Department of Pharmacology and Therapeutics Faculty of Medicine, University of Sumatera Utara. The location is located on Jalan Dr T. Mansur, No. 5, Medan, North Sumatera.

Description of Research Sample

In this study used as many as 32 male mice (Mus musculus), Double Ditsch Webster strains age: ± 3 months (adult), healthy, weight 20-30 grams as a sample, which is divided into 4 groups each amounting to 8 mice, the control group (placebo), the aspirin group and the honey group.

Results of Data Analysis

The antiplatelet effect in mice counted from the time of bleeding was analysed using a computer program. From the research obtained data as follows:

Table 2: Bleeding Time Taken for Mice (sec)

Group	N	Mean	SD	Median	Minimum	Maximum
Control (Placebo)	8	102.88	15.93	101.50	74.00	125.00
Aspirin	8	369.38	120.97	402.00	201.00	507.00
Honey	8	304.63	141.29	380.50	248.00	536.00
				326.50	129.00	462.00

Data in Table 2 and Figure 1 showed the meantime of bleeding at the highest mouse tail was in group of mice with aspirin (mean = 369.38 sec) then followed by group of mice with honey (mean = 304.63 seconds), and the lowest was the control group (placebo) with a mean value of 102.88 seconds.

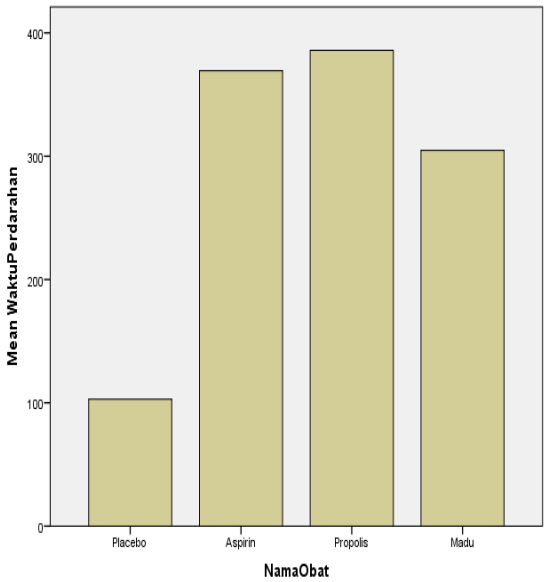

Figure 1: Mean Time Bleeding of Mice (sec)

Test Data Normality

Data on bleeding time in each group were tested for normality by using the Shapiro-Wilk test. The results show that the data is normally distributed ($p > 0.05$), presented in Table 3.

Table 3: Normality Test Result of Bleeding Time

Group	N	p	Explanation
Control (Placebo)	8	0.847	Normal
Aspirin	8	0.188	Normal
Honey	8	0.133	Normal

Data Homogeneity Test

Data on bleeding time on mouse tail were tested homogeneity by Levene's test. The results show a non-homogeneous data variant ($p < 0.05$), presented in Table 4.

Table 4: Homogeneous Test of Time Bleeding between Groups

Levene Statistic	df1	df2	Sig.	Explanation
9,760	3	28	0.0001	Not Homogeneous

Comparability Test

The comparability test aims to compare the meantime of bleeding in the placebo, aspirin and honey groups. Based on the result of the normality and homogeneity test, the data in this research are normally distributed but do not have the same variant (not homogeneous). The comparative analysis used is a non-parametric test that is Kruskal-Wallis. Non-parametric Kruskal-Wallis statistical tests showed significant differences in at least two treatment groups ($p = 0.0001$), thus followed by a post hoc analysis with Mann Whitney test.

Advanced Test (Post Hoc Test)

The follow-up test aims to see which groups have significant differences. In this study, the follow-up test used was the Mann Whitney test. The results of the test analysis are presented in Table 5.

Table 5: Data Analysis with Mann Whitney Test

Group	Control (Placebo)	Aspirin	Honey
Control (Placebo)	-	0.001*	0.001*
Aspirin	0.001*	-	0.172
Honey	0.001*	0.172	-

Data in Table 5 showed that there was a significant difference between aspirin and honey group to the control group (placebo), i.e., $p = 0.001$. There was no significant difference between aspirin group to honey ($p = 0.172$).

Discussion

This study is a true experimental design study to prove the effect of antiplatelet on honey and to know the comparison of effectiveness between aspirin with honey as antiplatelet based on the measurement of duration of bleeding in mice tail. The time of bleeding is the time to start bleeding on the tail of the mice that are cut until the blood stops. The presence of antiplatelet effect indicated by honey is characterised by the longer time of bleeding after the treatment of wound on the tail of mice.

The average bleeding time in the treatment group given honey was longer than the control group (placebo) and almost close to the average time of positive control group bleeding (aspirin). This is so because of flavonoids. One of the contents of honey has an antiplatelet activity that can be associated with the increased production of prostacyclin by endothelial cells. The prostacyclin inhibits the aggregation

process through cAMP synthesis which will inhibit the expression of GPIIb / IIIa platelet receptors [17]. Inhibition of the aggregation process leads to an average time of bleeding in the tail of the mouse with prolonged honey and propolis. From the statistical test, there were significant differences in bleeding time in the control group (placebo) against the treatment group given honey. In the treatment group treated with honey showed no significant difference to the positive control group (aspirin). This proves that honey has antiplatelet effects in mice as do aspirin.

The mechanism of inhibition of platelet aggregation by honey depends on several factors. A study has shown that exposure to hydrogen peroxide (one of honey content) can lead to platelet activity inhibition of platelet aggregation [18]. Also, honey may affect platelet function by inhibiting LDL oxidation that indirectly inhibits platelet aggregation [19]. This is because research has shown that activated platelets after aggregation will produce some cytokines that activate phagocytes resulting in increased production of oxygen free radicals that will eventually lead to the oxidation of LDL [18]. The active component of propolis CAPE has been shown to have an antiplatelet effect based on the following mechanism by increasing the formation of cGMP which will activate the cyclic phosphorylation of GMP-dependent VASP Ser157 and then inhibit the activity of PKC (protein kinase C). In the end, there is inhibition of phosphorylation of P47 and triggers the inhibition of platelet aggregation [20].

From the results of this study, it turns out the average time of bleeding in the tail of mice with the provision of honey longer or closer to the positive control group (aspirin). It can be considered as an alternative antiplatelet therapy or as a supplement in the prevention of heart disease and blood vessels. Therefore, further research needs to be done on humans for more effective results as well as doing this research using a large sample.

In conclusion, based on data analysis obtained in this study, the conclusions are: 1) this study proves that honey can provide an antiplatelet effect in mice with the occurrence of lengthening of bleeding time on the tail and 2) the mean bleeding time in mice tail (304.63 sec) was close to the meantime of bleeding with aspirin (369.38 sec) so that honey could be considered as a supplement in treating heart disease and blood vessels.

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The Influence of Nutritional Status and Ventilation on the Incidence of Pulmonary Tuberculosis at Langsa

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Abstract

BACKGROUND: Pulmonary Tuberculosis is still a global health threat and the cause of the average of 1.3 million deaths each year.

AIM: The objective of the research was to analyse the influence of ventilation and nutritional status on the incidence of pulmonary tuberculosis at Langsa, in 2018.

METHODS: The population was all pulmonary tuberculosis adult patients at Langsa, in 2018, with AFB+ of 315 people, and the samples were 116 patients in the case group and 116 patients in the control group. The data were analysed by using logistic regression with simple logistic regression test and multiple logistic regression tests.

RESULTS: The result of multiple logistic regression tests showed that there was the influence of nutritional status ($p \leq 0.001$; OR = 2.645 (95% CI 1.494-4.683) and ventilation ($p \leq 0.044$; OR = 1.816 (95% CI 1.015-3.250) on the incidence of pulmonary tuberculosis.

CONCLUSION: To break off the chain of pulmonary tuberculosis contagion, it is necessary to increase people's awareness of the importance of healthy life behaviour and healthful home by providing health counselling about the requirement for good nutritional status and ventilation.

Introduction

Southeast Asia has the highest incidence rate of Pulmonary Tuberculosis. It is estimated that there are about 44% of pulmonary tuberculosis incidence globally and about 4,440,000 new cases or 226 incidences per 100,000 people. 6% of them are found in Indonesia and 4% of them in the Philippines. Of the 30 countries throughout the world, Myanmar and Thailand had had the highest incidence rate of pulmonary tuberculosis until 2017 with 191,000 cases and 108,000 cases, respectively. The mortality rate of HIV- pulmonary tuberculosis is estimated about 638,000, and HIV+ pulmonary tuberculosis is 28,000. The mortality rate in this area ranks the second after Africa [1].

According to WHO, there had been 842,000 people who were infected by pulmonary tuberculosis in Indonesia until 2017, which ranked the second after India. New cases of HIV+ pulmonary tuberculosis is estimated about 36,000 people, and there are 107,000 people die because of tuberculosis, and about 10,000 people are infected by pulmonary tuberculosis with HIV+ die. According to Kemenkes RI [2], in its Health Profile, the highest rate of the incidence of pulmonary tuberculosis is found in Wes Java (78,698 cases), followed by Central Java (42,272 cases) and East Java (48,323 cases). Among them, there are 101,802 males (60.45%) and 66,610

females (39.55%).

The prevalence of pulmonary tuberculosis in Aceh is considered high since it ranks the 13th of the 34 provinces in Indonesia with 6,013 (0.71%) cases. This number has increased to 5,934 cases since 2016. This disease mostly affects 45-54-year-old people (640 cases or 10.78%), consisted of 422 males and 218 females [2].

Langsa is the town in Aceh Province which has a high rate of pulmonary tuberculosis which is increasing each year. According to the Health Agency of Langsa, new cases of pulmonary tuberculosis at Langsa have increased in the last five years. There were 155 cases in 2014, 177 cases in 2015, 178 cases in 2016, and 183 cases in 2018. This infectious disease had a significant increase in 2018, with 315 cases [3].

The increasing rate of pulmonary tuberculosis each year indicates that Eliminating and Eradicating Pulmonary Tuberculosis Program in public health can be achieved by maintaining the stability and the continuity of the Tuberculosis Control program. The sustainable implementation of this program needs multi-stakeholder involvement. Since 1990, this program has had its contribution of 25% to case finding, and 85% to the success in the treatment for pulmonary tuberculosis patients. The approach should be based on the basic health system (Puskesmas or Public Health Center) so that success can be continued in the long term [4].

The important role in the foremost segment of health care in supporting target fulfilment is seen from the rate of finding new cases of AFB+. The new finding of AFB+ in Aceh Province in 2017 was as follows: there were 1,857 cases (65.04%) of the 4,262 cases of pulmonary tuberculosis in males, and there were 998 cases (34.96%) of the 2,376 cases of pulmonary tuberculosis in females. This number indicates that there was the decrease in 2016 when the finding of a new case of AFB+ in males was 2.207 cases (64.14%) of the 3,683 cases of pulmonary tuberculosis, and AFB+ in females was 1,234 cases (35.86%) of the 2.150 cases of pulmonary tuberculosis.

There were 2,613 AFB+ patients (51.13%) who were treated and recovered, and 2,498 patients (48.7%) were under complete treatment. It was found that not all new AFB+ patients could be recovered, and this condition could be the source of contagion among the people that would eventually increase new cases of pulmonary tuberculosis each year [5], [2].

According to Permenkes RI No. 1077 on the Guideline for restoration to good air in rooms, healthful homes, viewed from physical factor, is influenced by some aspects such as minimal lighting is 60 lux, floors are waterproof and not moist, room temperature is 18°C-30°C, room moisture is 40%-60%, ventilation is the minimum of 10% of the floor area [6]. Resident's density calculated based on the need for space for each person is 9 $m^{2,}$ with the average height of the ceiling of 2.80 m [7].

Government Regulation No. 66/2014 states that environmental health is an effort to prevent sickness or health disorder from environmental risk factors to realise healthful environmental quality in the physical, chemical, biological, and social aspects. Health environment includes all physical, social, and biological factors of human beings and all other factors which can influence their behaviour. Condition and control from environmental health can potentially influence health [8].

The increase in the incidence of pulmonary tuberculosis at Langsa can be influenced by unhealthful home environment. A preliminary study conducted in five puskesmas and two hospitals at Langsa showed that in 2018 there were 315 AFB+ patients. All patients who were under complete treatment were recovered, while those who were under incomplete treatment had to take more medicines in the long term. It was found that not all patients who were under regular treatment, without intervals, within six months, on and off, had the risk for undergoing long term medication with the possibility of medicinal resistance.

It seems that pulmonary tuberculosis does not tend to decrease at Langsa. On the other hand, it has been increasing each year. There were 183 cases in 2017 and increased sharply to 315 cases in 2018. In the preliminary survey on visiting people's homes, it was found that, on the average, the houses of pulmonary tuberculosis patients were physically dingy and unhealthful.

The objective of the study was to analyse the influence of nutritional status and ventilation on the incidence of pulmonary tuberculosis at Langsa.

Material and Methods

Types of the Study

The study used the observational analytic method with a case-control design.

Source of Data

The population was all pulmonary tuberculosis AFB+ patients at each health facility of the public health facilities at Langsa. The samples were 116 patients in the control group and 116 patients in the case group (the ratio of 1:1) so that the total samples were 232 respondents, taken by using purposive sampling technique. It was used to determine the research samples according to the

criteria which had previously been determined.

Gathering of Data

The research variables consisted of natural lighting in rooms, floors, temperature, moisture, size of ventilation, resident's density, smoking habit, and nutritional status. The data were gathered by distributing questionnaires and conducting the direct observation. Statistical analysis was done by using multiple logistic regression tests.

Results

Description of Nutritional Status and Ventilation

Of the 116 cases of nutritional status variables, 55.2% of them (64/116) were BMI < 18.5, while of the 116 controls, 68.1% of them (79/116) were BMI ≥ 18.5.

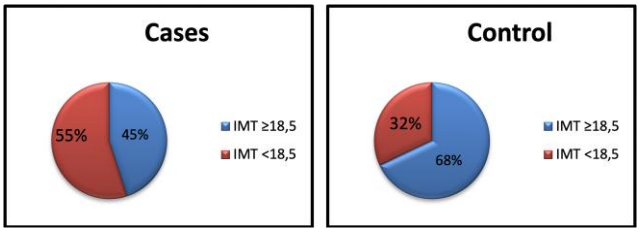

Figure 1: The percentage of nutritional status was indicated

Of the 116 cases of ventilation variables, 71.6% of them (83/116) were ineligible, while of 116 controls, 44.8% of them (52/116) were eligible (Figure 1 and Figure 2).

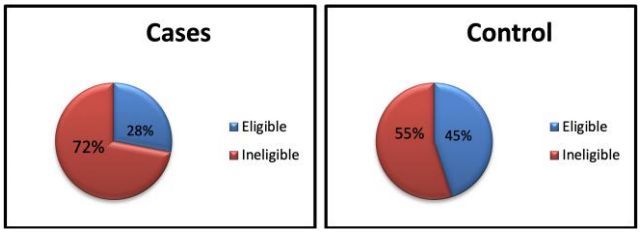

Figure 2: The percentage of ventilation was indicated

Correlation of Nutritional Status and Ventilation with the Incidence of Pulmonary Tuberculosis

The result of simple logistic regression test showed that there was the correlation of nutritional status p ≤ 0.001; OR = 2.628 (95%CI 1.539-4.487) and ventilation (p = 0.010; OR = 2.044 (95%CI 1.185-3.523) (with the incidence of pulmonary tuberculosis, as it was indicated in Table 1.

Table 1: Correlation of Nutritional Status and Ventilation with the Incidence of Pulmonary Tuberculosis

Variables	Pulmonary Tuberculosis		Non-Pulmonary Tuberculosis	
	n	%	n	%
Nutritional Status				
Good	52	44.8	79	68.1
Bad	64	55.2	37	31.9
P ≤ 0.001; OR = 2.628 (95% CI 1.539-4.487)				
Ventilation				
Eligible	33	28.4	52	44.8
Ineligible	83	71.6	64	55.2
P = 0.010; OR = 2.044 (95% CI 1.185-3.523)				

The Influence of Nutritional Status and Ventilation on the Incidence of Pulmonary Tuberculosis

The result of the study showed that the two variables (nutritional status and ventilation) had the influence on the incidence of pulmonary tuberculosis. The variable which had the most dominant influence on the incidence of pulmonary tuberculosis at Langsa was nutritional status at the value of OR = 2.645 (95%CI 1.494-4.683) which indicated that respondents who had BMI less than 18.5 had a risk of 2,645 times for pulmonary Tuberculosis exposure compared to respondents whose BMI was greater or equal to 18.5 (Table 2).

Table 2: The Influence of Nutritional Status and Ventilation on the Incidence of Pulmonary Tuberculosis

Variables	B	Sig.	OR	95% CI
Nutritional Status	0.973	0.001	2.645	1.494-4.683
Ventilation	0.597	0.044	1.816	1.015-3.250
Constanta	-.503	-	-	-

Discussion

The result of the research showed that most of the nutritional status of the majority had poor nutritional status. The statistical test results obtained that there was a significant effect between nutritional status on the incidence of pulmonary tuberculosis, and nutritional status was the most dominant variable affecting the incidence of pulmonary tuberculosis. The results of this study are in line with Oktavia's study [9]. That respondents with poor nutritional status increased the risk of 16.7 times exposed to pulmonary tuberculosis compared to respondents with normal/excessive nutritional status. This study was also in line with Ernawati [10]. That malnutrition was significantly associated with pulmonary tuberculosis with a value of p ≤ 0.001.

Nutritional status has a very close relationship with the risk of suffering from pulmonary TB. Individuals with poor nutritional status will be easier to switch status from Latent pulmonary tuberculosis to active pulmonary tuberculosis than individuals with good nutrition. Low nutritional status related to food intake that is not by the body's needs due to lack of knowledge (ignorance and low awareness) of the urgency to fulfil the needs of this aspect in society.

Poor nutrition in the poor also makes it easy to suffer from various diseases.

Tuberculosis and macroeconomics have a two-way relationship according to Aulia [11]. Tuberculosis has an impact on macroeconomic development and vice versa. Macroeconomic aspects have an impact on the increasing incidence and prevalence of pulmonary tuberculosis. The increase from the aspect of inflation has resulted in high prices that must be paid by the population to fulfil their daily needs. Inflation causes a large portion of the population to be unable to meet nutritious food needs, which affects the nutritional status of the community.

The poverty rate of the population of Langsa City in 2018 is high at 10.79 percent [12]. This is also influenced by the low capacity of the Langsa City government in carrying out development due to low Regional Original Income (PAD). Population density is also part of the macroeconomy which has led to an increase in cases. The uncontrolled population makes the availability of various aspects and facilities limited so that the limitation for healthy living occurs and the vulnerability to illness increases including pulmonary tuberculosis.

In conclusion, nutritional status and ventilation have the influence on the incidence of pulmonary tuberculosis. Nutritional status has the most dominant influence on the incidence of pulmonary tuberculosis. In order to break off the chain of pulmonary tuberculosis contagion, it is necessary to increase people's awareness of the importance of healthful houses and healthy life behavior by providing health counseling about the requirements for good ventilation and nutritional status.

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Co-Infection of Human Papillomavirus with Mycoplasma Hominis/Ureaplasma Urealyticum Among Female Sex Workers in Medan, Indonesia

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Abstract

Human papillomavirus (HPV) infection is one of the most prevalent sexually transmitted diseases among women aged < 35 years worldwide. Recent studies have suggested that the vaginal microenvironment influenced by bacterial infection poses for high-risk human papillomavirus (hrHPV) infection and cervical carcinogenesis. Female sex workers (FSWs) are a population susceptible to acquire Human Immunodeficiency Virus (HIV) and sexually transmitted infections (STIs), as well as transmitting the virus to others. The aim of this study is to evaluate the relationship between Mycoplasma/Ureaplasma infections and HPV infection among female sex workers. A total of 70 female sex workers of reproductive age were recruited from various location in Medan, Indonesia in 2018. Detection of Mycoplasma/Ureaplasma infections and HPV infection were obtained from PCR assessment. The results of this study showed that no correlation significant between Mycoplasma hominis/Ureaplasma urealyticum infection and HPV infection.

Introduction

Human papillomavirus (HPV) has been identified as an essential aetiology of warts, cervical intraepithelial neoplasia (CIN) and cervical cancer [1]. Among high-risk HPV, type 16 and 18 are the most carcinogenic for the progression of cervical disease and induce over 70% of cervical cancer, stated as the second most common cancer in women as reported in 2012 by the International Agency for Research on Cancer (IARC) [2]. The cervical cancer death currently accounted for about 57% of cases and 65% of cancer deaths worldwide [3], [4]. De Boer previously have stated that no information existed about the prevalence of HPV 18 or other HPV types in the Indonesian population and hypothesised that the high prevalence of HPV 18 in cervical cancer in Indonesia is caused by the high prevalence of HPV 18 in the Indonesian population [5]. The estimated incidence of cervical cancer in Indonesia is 17 per 100,000 women [6].

Beside persistent infection and an increase in viral load of HPV infection, other concomitant infections are also known risk factors causing rapid development of cervical cancer, while the negative contribution of smoking and contraceptive medication act as environmental risk factors [7]. Risk factors associated with cervical cancer include first intercourse at an early age, mutually change sexual partners, and suffering from chronic sexually transmitted infections (STIs) other than HPV and HIV [8], [9], [10].

Association between HPV infection with cervical cancer has been already known, even though several studies found Mycoplasma hominis and Ureaplasma urealyticum involvement contribute to the risk of HPV infection and the occurrence of abnormal cervical cytology [11], [12]. These bacterias were

found in 30 ± 80% women's urogenital tract as a commensal organism but had the pathogenic potential [13]. Several studies revealed that Mycoplasma hominis and Ureaplasma urealyticum played a role in developing abnormal cervical cytology associated with the development of precancerous cervical lesions [7]. Mycoplasma/Ureaplasma infections can cause chronic pelvic pain if the infection infiltrates the pelvis or genitourinary system. Persistent or untreated chronic infection can lead to cervical cancer in addition to pelvic pain, by causing persistent HPV infection or increased HPV levels [14].

Female sex workers (FSWs) are commonly known very vulnerable to exposure to HIV and sexually transmitted infections (STIs), as well as transmitting the virus or bacterias to others, due to behavioural risk factors as recurrent infections and repeated contact through high numbers of sexual partners [15]. This is compounded by the lack of knowledge and awareness of HPV infection, cervical cancer, and preventive efforts, as previously described in several studies [16], [17].

This study aims to evaluate the relationship between Mycoplasma/Ureaplasma infections and HPV infection among female sex workers in Medan, Indonesia.

Material and Methods

Study Population

This study is a descriptive study with a cross-sectional design to evaluate the relationship between Mycoplasma hominis and Ureaplasma urealyticum infection and HPV infection among female sex workers. A total of 70 female sex workers of reproductive age were recruited from various location in Medan, North Sumatera, Indonesia, between July and September 2018.

Subjects were women aged between 18 and above who were sexually active. Exclusion criteria were subjects recently diagnosed with the cervical disease within 6 months before the present study and currently pregnant. All participants were interviewed to collect information on socio-demographic background. The Ethics Committee of the University of Sumatera Utara approved the study, and informed consent forms were obtained from all subjects.

Detection of HPV and Mycoplasma/Ureaplasma

DNA samples were isolated from cervical swabs using Presto^{n} Buccal Swab gDNA extraction kit.DNA was then amplified using the established PCR method. PCR kit components used for HPV gene amplification were DNA template of 6 µl, *GoTaq*® *Green Master Mix (Promega)* of 12.5 µl, a primer of forwarding HPV. Mycoplasma hominis and Ureaplasma urealyticum used 2 primers: RNAH1 and RNAH2 that amplify the 16sRNA Mycoplasma hominis gene at 334 bp; UMS125 and UMA226 amplified Ureaplasma urealyticum serovar 3 which shows multiple bands at 403 bp for biovar 1 and 448 bp for biovar 2. The amplification mixture was carried out in 12.5 µl master mix PCR consisted of Taq polymerase enzyme, $MgSO_4$, and dNTP(Go Taq® PCR Core System, Promega).

Results

The data summarised in Table 1 showed the distribution of age according to the infection of Mycoplasma hominis/Ureaplasma urealyticum and HPV. This study showed that HPV 16 infection was higher in women aged 26-30 (12.5%), while that HPV 18 infection was found higher in women aged 18-25 (26.9%). The occurrence of Mycoplasma infections was more common in the age group 18-25 (31.4%), while Ureaplasma infections were more prevalent in the age group 31-45 (60.9%).

Table 1: Distribution of age by infection of Mycoplasma hominis/ Ureaplasma urealyticum and HPV

Infection	Total N = 70	Age in years 18-25 N = 26 (37.1%)	26-30 N = 16 (22.9%)	31-45 N = 23 (32.9%)	> 45 N = 5 (7.1%)
HPV16 (+)	3(4.3)	1(3.8)	2(12.5)	0(0)	0(0)
HPV16 (-)	67(95.7)	25(96.2)	14(87.5)	23(100)	5(100)
HPV18 (+)	13(18.6)	7(26.9)	3(18.8)	3(13.0)	0(0)
HPV18 (-)	57(81.4)	19(73.1)	13(81.3)	20(87.0)	5(100)
Mycoplasma (+)	22(31.4)	11(42.3)	5(31.3)	6(26.1)	0(0)
Mycoplasma (-)	48(68.6)	15(57.7)	11(68.8)	17(73.9)	5(100)
Ureaplasma (+)	32(45.7)	9(34.6)	6(37.5)	14(60.9)	3(60.0)
Ureaplasma (-)	38(54.3)	17(65.4)	10(62.5)	9(39.1)	2(40.0)

The previous study found that infections of *C. trachomatis*, *M. genitalicum*, and *U. parvum* were higher in the younger age group by comparisons based on age (< 50 years vs ≥ 50 years) [10]. Similar to the previous study, the respondents of this study mostly younger than 50 years, and bacterial infections more often to occur in the age group 18-25 years and 31-45 years.

Another study found that HPV infection peak in women aged 40-50 years [18]. Different from the results of the former study, HPV 16 infections were higher in women aged 26-30 years, while HPV 18 found high in women aged 18-25 years. Nevertheless, another study showed no correlation between HPV infection and the age of the respondent, and also found that age was not related to abnormal cytology or bacterial infection [19].

Table 2: Relationship between Mycoplasma hominis and Ureaplasma urealyticum infection and HPV infection

Bacterial infection	HPV 16 infection		OR	*P*-value	CI 95%	HPV 18 infection		OR	*P*-value	CI 95%
	Positive	Negative				Positive	Negative			
Mh										
Positive	0 (0,0%)	22 (32,8%)	-	0.547[a]	-	3 (23,1%)	19 (33,3%)	0.6	0.742[a]	0.148-2.440
Negative	3 (100,0%)	45 (67,2%)				10 (76,9%)	38 (66,7%)			
Uu										
Positive	2 (66,7%)	30 (44,8%)	2.426	0.589[a]	0.213-28.535	6 (46,2%)	26 (45,6%)	1.0	1.000[a]	0.305-3.422
Negative	1 (33,3%)	37 (55,2%)				7 (53,8%)	31 (54,4%)	22		

The results of this study as shown in Table 2, found that no correlation between Mycoplasma hominis infection with HPV 16 infection (p = 0.547) and neither with HPV 18 infection (p = 0.742). There were no correlation as well between Ureaplasma urealyticum infection with HPV 16 infection (p = 0.580), and also no correlation with HPV 18 infection (p = 0.639).

Discussion

The results were different from previous studies that found a significant association between HPV infection and the presence of Mycoplasma/Ureaplasma infection [11], [18]. Another study showed the correlation not only between bacterial infection and high-risk HPV infection but also between abnormal cytology with bacterial infection [10]. Another previous study from Korea from asymptomatic women of reproductive age with negative cytologic findings found that most HPV infections were not correlated with any specific STIs [20]. Another study from Estonia found the high risk-HPV was associated with STIs especially Chlamydia infection, and also with U. urealyticum infection in women over 41 years old. The same study found that U. urealyticum infection was not associated with HPV status [21]. A study from Nigeria found significant associations between persistent high-risk HPV infections and persistent M. Hominis in the vaginal microbiota, and the study suggested that M. Hominis may play a role in high-risk HPV induced cervical carcinogenesis [22].

Several studies showed that a high density of Ureaplasma might be associated with STIs and the differentiation of colonisation and infection contributed to co-infection, as reported by Kim et al., that showed only Ureaplasma colonization with greater than 104 CCU/mL was significantly associated with HPV infection [19]. Another study which in line showed patients with cervical lesions had higher U. parvum load colony-forming unit [23]. Unfortunately, in this study, we did not assess the bacterial load of Ureaplasma and Mycoplasma. The correlation between colonies number and infection may be explained by more pathogen colony could develop more cervical lesion as well due to chronic infections by activating the virulence factor. Few studies have been conducted to evaluate the association between ureaplasma load with cervical disorders especially with precursor cervical lesions of cervical cancer [5].

In conclusion, this study revealed that there is no significant correlation between the presence of Mycoplasma hominis/Ureaplasma urealyticum infection and HPV infection. Although among female sex workers, sexually transmitted infections like Mycoplasma spp, and Ureaplasma spp were frequently detected and as well as the HPV infection. However, this non-significant result might be due to the limitation of this study. We only recruited a small number of samples and therefore could not provide confirmed statistical significance for all of the tests performed.

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Viral and Host Factors are Related to the Progression of HIV Diseases in Mimika, Papua

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Abstract

BACKGROUND: Papua has a high cumulative number of HIV, which has expanded epidemic status with the most risk factors are heterosexuals.

AIM: This study aims to determine factors associated with HIV disease progression include host and viral factors.

METHODS: Eighty-four subjects recruited in Rumah Sakit Mitra Masyarakat (RSMM) VCT's laboratory, interviewed with questionnaires and also did laboratory examinations. HIV-1 subtypes were identified using RT-PCR, nested PCR and sequencing. Then, CD4+ data is checked using PIMA Analyzer. Demographic and clinical data obtained from the patient's medical record. After collected, data were analysed using Fisher's exact test.

RESULTS: The results showed two factors that influence the progression of HIV disease were HIV subtypes ($p = 0.002$) and Body Mass Index ($p = 0.033$). The HIV-1 subtype also correlated with CD4+ levels with a value of $p = 0.04$.

CONCLUSION: HIV-1 subtype correlates with HIV progression, so it is necessary to develop HIV/AIDS management strategies and clinical counselling.

Introduction

The report that provides by UNAIDS that the number of people living with HIV in the world reached 34 million people with 17 million (50%) are women, and 2.1 million are children less than 15 years [1]. In June 2018, Papua recorded with the cases of HIV were 14.315 inhabitants and 2.114 people are dead because of AIDS. Heterosexuals are the highest risk factors of HIV transmission in Papua with 13.888 cases, followed by mother to infant transmission by 208 cases [2].

Generally, it needs eight to ten years for HIV to develop into AIDS. Several factors were found to contribute to the progression of HIV infection is a factor immunological, virological, environmental and genetic factors hosts [3], [4], [5], [6]. Factors that may affect the host is the Human Leukocyte Antigen (HLA), CYP polymorphisms, gender, age, ethnicity, psychosocial and body mass index (BMI). Environmental factors that affect the progression of diseases such as transmission modes and socio-economic status [7]. Viral factors, including viral subtypes or mutations that destroy the virus [8].

A study in Thailand found that the subtypes of HIV-related manner and speed of transmission, where subtype B associated with the transmission of homosexuals and intravenous drug users (IDUs), while subtypes A, CRF01_AE, and C related to heterosexual transmission [9]. Studies conducted in Tanzania and Uganda found that subtype D correlated faster with a decrease in $CD4^+$ T cells and increased disease progression than other subtypes and recombinant forms [10], [11]. However, a retrospective cohort study conducted during 1996 and 2007 reveals that Africans infected with subtype B has the progression of HIV/AIDS faster than those infected with non-B subtypes [12].

Many studies in other countries have reported

correlations between various factors with the progression of HIV disease, but in Indonesia, the data is still limited or limited.

The purpose of this study is to determine what factors associated with the progression of HIV disease, including host factors, environmental and viral factors.

Material and Methods

Study and subject

The study was conducted for ten months, from January to October 2015. Blood sampling was taken at the VCT Laboratory of Rumah Sakit Mitra Masyarakat (RSMM) Mimika. Samples are HIV/AIDS patients were selected for continuous sampling and has received antiretroviral therapy who have met the inclusion criteria. Calculation of sample size for cross-sectional design uses the Lemeshow formula from the calculation results obtained eighty-four respondents. The results from interviews of demographic and clinical data with questionnaire collected for further processing. $CD4^+$ examination uses the PIMA Analyzer and haematology examination using Sysmex.

Subtyping HIV

The extraction process uses a standard kit from Qiagen with catalogue # 52906. Firstly RT-PCR (Reverse Transcriptase-Polymerase Chain Reaction) amplification using a specific primer. The primers used are obtained from the HXB2 reference journal access code Geneva K03455 (http://hiv.web.lanl.gov/NUM-HXB2.MAIN.html).
Second is electrophoresis, and this stage aims to see the results of amplification in the previous step. The PCR results were detected by electrophoresis of 5 ul PCR products plus 1 ul loading buffer on 2% agarose gel and 100 v voltage for 40 minutes. The PCR product was visualised by placing the gel on the doc-gel. DNA isolates which showed a band of 460 bp were affirmed to contain the target gene. The next stage is sequencing; Sequencing is done to find out the nucleotide sequence in several PCR gene envelope products. Sequencing using ABIPrism 3500 Genetic Analyzer (Applied Biosystem, USA). This sequencing process is carried out in 2 stages, namely, cycle sequencing reaction and purification of PCR products and sequencing. Sequencing results were analysed using Bioedit software. The last is BLAST (Basic Local Alignment Search Tool) process, the purpose of which is to get the HIV genotype and subtype. BLAST was conducted using the internet to two gene bank sites to confirm; the two sites are BLAST from NCBI (National Center for Biotechnology Information) at www.ncbi.nlm.nih.gov and the HIV sequence database at www.hiv.lanl.gov.

Statistical Analysis

Statistical analysis was performed using Fisher's exact test for categorical variables. Briefly, a 2 × 2 contingency table on the selected Data was constructed, and the two-tailed p-value. P values less than or equal to 0,05 were considered to be significant.

Results

Demographic Characteristics of Subjects

Demographic characteristics showed that HIV/AIDS patients in Rumah Sakit Mitra Masyarakat (RSMM) Mimika dominated by women as many as 61 people (72.6%), Papuans 60 people (71.4%), educated (81%), Working (83.3%), body mass index 18.5-25 kg/m2 69 people (82.1%), married 47 people (56%), heterosexual transmission routes 80 people (95.2%), $CD4^+$ levels as much as > 350 cells/mm^3 77 people (91.7%), and opportunistic infections of tuberculosis 69 people (82.1%).

The factors associated with the progression of HIV

A significant relationship between demographic variables clinical and clinical stage of HIV/AIDS is a subtype variable, BMI and Route transmission. The results of the analysis are shown in Table 1.

Table 1: Demographic Characteristic of study Subject

Characteristic	Clinical Stage (WHO)		∑	*p*
	I, II, III	IV		
Sex				
Male	17	6	23	0.356
Female	51	10	61	
Ethnic				
Papua	50	10	60	0.375
Non-papua	18	6	24	
Subtype				
CRF01_AE	30	8	38	0.002*
Non-CRF01_AE	20	26	46	
Opportunistic Infection				
TB	56	13	69	1.000
Non-TB	12	3	15	
Body Mass Index				
Other	9	6	15	0.033*
Normally	59	10	69	
$CD4^+$				
< 350 cell/mm3	6	1	7	1.000
> 350 cell/mm3	62	15	77	
Hemoglobin				
< 12 g/dl	22	14	36	0.826
12-15 g/dl	28	20	48	
Trombosit				
< 150.000 ul	5	6	11	0.340
150 – 400.000 ul	45	28	73	
CD4 Failure, < 50 cell/mm3/year				
Yes	37	7	44	0.580
No	31	9	40	

Table 2 shows the analysis of the relationship between the subtypes and clinical characteristics of the levels of $CD4^+$ HIV patients in Mimika. Results indicated exhibited significantly between subtypes of HIV-1 and $CD4^+$ cells of patients with p = 0:04 (α < 0,05). The results of the analysis are shown in table 2.

Table 2: The correlation between HIV-1 subtypes and the level of CD4

Characteristic	Cluster Differentiation-4		∑	*p*
	< 350	> 350		
Sex				
Male	2	21	23	0.62
Female	5	26	61	
Ethnic				
Papua	6	54	60	0.66
Non-papua	1	23	24	
Subtype				
CRF01_AE	6	33	39	0.04*
Non-CRF01_AE	1	44	45	
Opportunistic Infection				
TB	7	62	69	0.34
Non-TB	0	15	15	
Transmission Mode				0.70
Heterosexual	7	73	80	
Others	0	4	4	
Body Mass Index				
Other	3	12	15	0.10
Normally	4	65	69	
Hemoglobin				
< 12 g/dl	4	32	36	0.45
12-15 g/dl	3	45	48	

Discussion

Many factors affect the disease progression of HIV/AIDS, including host factors, environmental and viral factors [13]. Two factors that affect the progression in this study is a host factor is the body mass index and factor virus itself is a subtype of HIV-1. One element that has a relationship with the clinical stage is the body mass index (BMI). BMI is one of the WHO's clinical assessment parameters that weight loss in people with HIV/AIDS. However, the association of BMI with $CD4^+$ levels did not show significant results. In this study, the patients' BMI is normal for clinical stage I, II and III. A study in France showed that mortality was higher in patients with BMI between 16-18.4 kg/m^2 with HR 2.2 (CI: 1.6-3.0) and a BMI < 16 is 4.4 (CI: 3.1-6.3) and standard BMI 18.5 [14], [7]. These results are similar to studies in Surabaya which is one of the factors that affect the progression of HIV disease is the body mass index [7].

In addition to body mass index, HIV-1 subtype also has a significant relationship with clinical stage and $CD4^+$. Correlation between HIV-1 subtypes and the development of the disease is controversial. Several studies have reported a correlation between subtypes of HIV-1 and the progression of HIV/AIDS by relating the time needed by HIV to develop AIDS, the rate of change low $CD4^+$ counts, viral load high, and mortality associated with HIV/AIDS. CRF01_AE commonly identified in HIV/AIDS patients in Papua. This indicates that the recombinant form of the virus worldwide associated with faster disease progression, such as in Cuba and Brazil [15], [13].

This study shows that CRF01_AE associated with faster HIV/AIDS progression. Similarly, studies in China (Li, 2014; Ng, 2011). It found that CRF01_AE-infected seroconversion experienced a faster rate of decline in $CD4^+$ T cells, requiring earlier initiation of ART compared to non CRF01_AE patients [16].

The study held by Chu et al. reported that the level of low $CD4^+$ changes was related to the CRF01_AE subtype [3]. Research in Indonesia in 2013 reported that CRF01_AE subtype also has a higher prevalence than other subtypes and associated with the level of $CD4^+$ cell changes in patients who had received HAART [17]. Subtype connection with deaths related to HIV/AIDS is still contradictory. Subtype CRF01_AE estimated time of the death of people with an average of 7.8 (7.0 to 9.1) years [18].

It remains unclear why CRF01_AE associated with $CD4^+$ decline very quickly. However, several studies have shown that the high proportion of tropism X4 in the CRF01_AE subtype and also that X4 tropism is associated with an increased rate of $CD4^+$ decline and progression for advanced immunosuppression [19], [20], [21]. Also, a decrease in the immune system on the host after infection with HIV-1 can allow the virus to grow and replicate independently. It can explain the increase in the rate of disease progression in HIV-infected patients with subtype CRF01_AE [22].

Some literature suggests that the virus subtype may affect the pathogenesis and progression of the disease during HIV infection. Hu, has reported that in PWID (people with an injected drug) patients with subtype CRF01_AE have higher plasma viral load compared to patients with subtype B, but there was no difference in the number of $CD4^+$ T cells [23]. Recent research in Singapore reported a decline in $CD4^+$ T cells faster for a shorter time in patients with subtype CRF01_AE than other subtypes [16]. Besides, in Shanghai, it was also reported that HIV homosexual patients with the CRF01_AE subtype found that more patients had lower initial $CD4^+$ T cells. This subtype of HIV disease progression was faster to AIDS and CXCR4 tropism frequency greater than with other subtypes [24].

Direct comparison of HIV subtypes often complicated by uncertainty factors for instance: the way of transmission and timing of infection, host genetic diversity, effects of comorbid conditions, small sample size, identification methods that cannot distinguish between subtypes and recombinant strains. This analysis is limited to a relatively homogeneous population of incidences of cases with a clear infection time and a known mode of transmission (sexual exposure).

It should be noted that despite the historical existence of subepidemics separated by genotypes

and risk factors, CRF01_AE now appears to be dominant in all risk groups throughout Asia [25]. It remains to be determined whether this can be accounted for by genetic DRIFT, or if there are inherent differences between strains such as plasma viral load, transmission or other biological or epidemiological factors that might underlie this shift.

In conclusion, HIV-1 subtype CRF01_AE primarily associated with HIV disease progression, in this case, is a clinical-stage and $CD4^+$. Routine surveillance of the subtypes of HIV-1 and $CD4^+$ will be useful in monitoring the progression of HIV/AIDS and improving the management and clinical counselling. The further study combines subjects with different ethnic backgrounds, and functional evaluation can be used to examine the relationship between subtypes of HIV-1 and the progression of HIV/AIDS. The research may use more samples, and the factors involved include the use of viral load markers to monitor disease progression. Finally, the sequencing of the HIV genome as a whole is the ideal method for concluding the relationship of the subtype by conducting a co-receptor analysis.

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Neuromyelitis Optica Spectrum Disorder: A Case Report of Effective Combination Immunosuppressant, Corticosteroids, and Therapeutic Plasma Exchange

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Keywords: Neuromyelitis optica spectrum disorder; Aquaporin antibodies 4 immunoglobulin G; Therapeutic plasma exchange

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Abstract

BACKGROUND: Neuromyelitis optica spectrum disorder (NMOSD) is an autoimmune disease that causes severe demyelination, especially in the optic nerve and spinal cord with typical clinical manifestations of acute optic neuritis and transverse myelitis. The symptoms can occur simultaneously or separated by a variable period. NMOSD is associated with serum aquaporin antibodies 4 immunoglobulin G (AQP4-IgG).

CASE PRESENTATION: We report a case of a 22-year-old male with complaints of weakness of all four limbs, impaired vision, urinary incontinence, and dyspnea. The Expanded Disability Status Scale (EDSS) was nine. Spinal magnetic resonance imaging (MRI) showed longitudinal extensive transversal myelitis. The brain MRI showed a normal impression, whereas the brain magnetic resonance spectroscopy (MRS) examination showed a description of the mild demyelination process. The serum antibody AQP4 (AQP4-IgG) results were seronegative, the cerebrospinal fluid examination was normal, and the oligoclonal band was negative. The ophthalmoscopic examination found bilateral papillary atrophy but optical coherence tomography (OCT) was still normal. Somatosensory evoked potential and visual evoked potential examinations were abnormal. The patient was diagnosed with NMOSD and was given combination immunosuppressant therapy, corticosteroids, and therapeutic plasma exchange. The patient experienced significant improvement with EDSS decreased to six.

CONCLUSION: In the case of relapsing NMOSD patient, combination therapy of immunosuppressant's, corticosteroids, and TPE was used. There were significant improvements from EDSS nine to six.

Introduction

Neuromyelitis optica spectrum disorder (NMOSD), formerly known as neuromyelitis optica (NMO) or Devic's syndrome or Devic's disease, was initially considered as part of multiple sclerosis (MS) because the symptoms were considered overlapping. But now, it is known that the pathophysiology of these two diseases is different [1].

NMOSD is a central nervous system inflammatory syndrome that is different from MS, which is associated with serum aquaporin-4 immunoglobulin G (AQP4-IgG) antibodies [1], [2], [3].

NMOSD is an autoimmune disease that causes severe demyelination, especially in the optic nerve with typical clinical manifestations in the form of acute optic neuritis and transverse myelitis which can occur simultaneously or separated by a variable period [1], [2], [3], [4], [5], [6].

It is more common in the form of polyphasic (90%) such as optic neuritis or myelitis, or both occurring together. The monophasic form has only occurred in 10% of cases [1], [2].

Case report

We report a case of a 22-year-old male with complaints of weakness in all four limbs, impaired vision, urinary incontinence, and dyspnea. Previously the patient had experienced six similar attacks and the longer, the worse the symptoms got. A history of low back pain, muscle spasms, and numbness were found. Neurological examination found a weakness in all four limbs accompanied by increased physiological reflexes and the presence of pathological reflexes. Visual acuity examination on the right and left eyes showed a visual of 1/300 and 1/∞, respectively. Funduscopy examination revealed a picture of bilateral atrophic papillae (Figure 1). The optical coherence tomography (OCT) examination was normal. The presence of exteroceptive and proprioceptive disorders was accompanied by urinary incontinence. The score for the Expanded Disability Status Scale (EDSS) was nine.

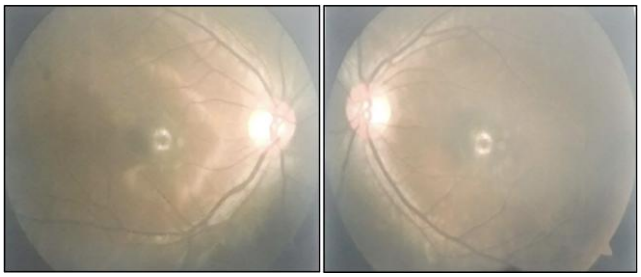

Figure 1: The ophthalmoscopic examination results of a 22-year-old male NMOSD patient with bilateral papillary atrophy

Blood tests results and analysis of brain fluid were within normal limits. Serology for the anti-herpes simplex virus, PCR analysis on herpes simplex virus and cytomegalovirus were negative results. Serum aquaporin 4 examination was negative. Autoimmune antinuclear antibodies (ANA) and anti-DSA analysis were normal. Electrophysiological examination of somatosensory evoked potential (SEP) found lesions between C2-7 and Th2-7 and visual evoked potential (VEP) found partial blocks of bilateral visual pathways. The spinal MRI examination showed a picture of myelitis involving C3-6 and Th2-6 (Figure 2). Brain magnetic resonance spectroscopy (MRS) showed a description of mild demyelination process. Brain magnetic resonance imaging (MRI) showed a normal impression.

Differential diagnosis at that time was NMOSD, MS, acute disseminated encephalomyelitis (ADEM), acute idiopathic myelitis transversalis (iATM) and systemic lupus erythematosus (SLE). Based on the results of clinical symptoms and other investigations, the patient was diagnosed with NMOSD.

Treatment to prevent relapse in this patient was azathioprine at a dose of 50 mg given twice a day. Nevertheless, the patient remained to experience recurrences. During an acute exacerbation, he was treated with intravenous methylprednisolone but no improvement was noted and his neurological symptoms worsened.

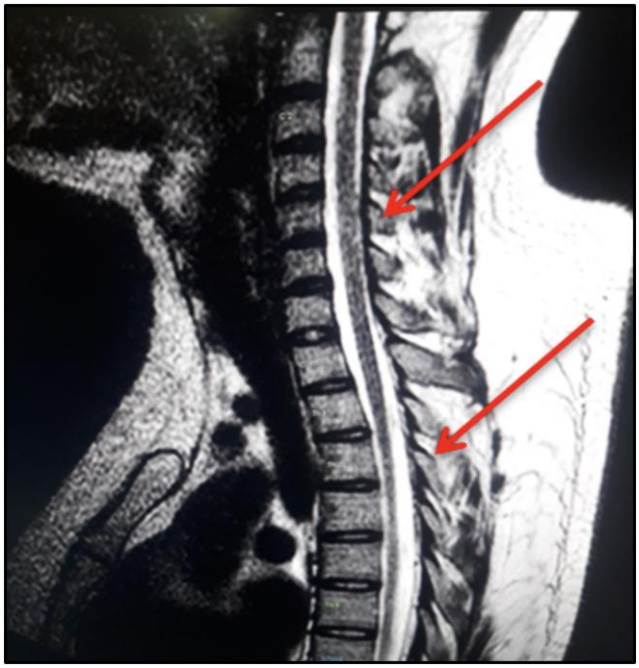

Figure 2: Spinal MRI result of NMOSD patient of a 22-year-old male with longitudinal extensive transversal myelitis involving C3-6 and Th2-6

The patient was then treated with combination therapy of intravenous dexamethasone 5 mg and 7 cycles of therapeutic plasma exchange (TPE) along with physiotherapy. He experienced significant improvement in his neurological symptoms with EDSS decreased to six.

Discussion

NMOSD is an autoimmune disease that causes severe demyelination, especially in the optic nerve and spinal cord with typical clinical manifestations of acute optic neuritis and transverse myelitis. It has been associated with serum AQP4-IgG [1], [2], [3], [4], [5], [6].

Eugene Devic (1858-1930) who first introduced the French term acute neuromyelitis optic "neuro-myélite optiquë aiugë" to show a new syndrome characterised by myelitis and acute optic neuritis. Lennon and Wingerchuck (2004) detected the presence of IgG-NMO or IgG-AQP4, the specific antibodies that distinguish NMOSD from MS [1], [7].

NMOSD is a rare syndrome with less than 1% demyelinating disease and the incidence varies in various countries. In general, the incidence of NMOSD ranges from 0.05-4.4 per 100,000 [1], [6]. It generally occurs in Asian, African and Hispanic

descendants [6]. It is more dominant to attack female than the man with a ratio of 3-9: 1, and in adults age between 30.5 and 55.2 years, but can also occur children and elderly [1], [6], [8]. NMOSD cases have been reported in a 3-year-old and a 90-years-old [7].

To date, the pathogenesis of NMOSD is still not fully understood [7], [9]. Antibodies to AQP4 play a key role in the pathogenesis of NMOSD. AQP4 is a water channel that is mostly expressed on podocytes of astrocytic cell membrane forming part of the blood-brain barrier [1], [7], [9].

The clinical features of NMOSD are severe recurrent attacks of myelitis and bilateral and unilateral optical neuritis that can occur simultaneously. It is more common to be found in the form of polyphasic (90%) than monophasic (10%) [1], [2]. In this patient, we found a weakness in all four limbs, accompanied by visual impairment, urinary incontinence, and dyspnea. Previously, he had experienced 6 attacks which at each time was increasingly worsened. Other clinical symptoms include brain stem symptom, posterior reversible encephalopathy syndrome (PRES), coma, hypothalamic dysfunction, depression, cognitive disorders, psychiatric symptoms, and abnormal endocrinopathy [1], [2], [10], [11], [12]. In this patient, no other clinical symptom was found.

Immunological and autoimmune examinations in NMOSD are generally norma[8]. In this patient, the immunological examination against the anti-herpes simplex virus, PCR analysis on herpes simplex virus and cytomegalovirus were negative. An examination of autoimmune antinuclear antibodies (ANA) and anti-DSA was normal.

Serum AQP4-Ig is detected in 60-90% of patients who met NMOSD clinical and radiological criteria [5]. In seronegative AQP4-IgG patients who met the clinical and radiological criteria of NMOSD, serum myelin oligodendrocyte glycoprotein (MOG) antibodies can be detected [2]. In this patient, AQP4-IgG examination was negative, but serum MOG antibody examination was not tested.

Cerebrospinal fluid examination in positive NMOSD patients with AQP4-Ab can be found moderate with normal pleocytosis in about 40% of cases during acute recurrence. Oligoclonal bands (OCB) are usually not found, the intrathecal polyspecific antiviral immune response against-Measles, Rubella and Varicella-Zoster viruses (MRZ reaction) are negative, and increased glial fibrillary acidic protein and neurofilament heavy chain (nfH) are commonly found [8]. In this patient, the cerebrospinal fluid examination gave a normal impression and OCB was negative.

The results of the ophthalmoscopic examination in NMOSD patients varied from optic neuritis, atrophic papillae, to normal features [10]. In this patient, the ophthalmic examination showed bilateral atrophy. A thinning nerve layer may also be seen in the OCT examination, but the changes take a long time after the development of optic neuritis[3]. In this patient, the OCT examination showed normal findings.

The electrophysiological examination on SEP and VEP often show changes in patients with NMOSD, with prolonged latency at around 40% of cases and decreased amplitude or potential loss in about 25% of patients [8]. In this patient, the SEP examination showed lesions between C2-7 and suspicious lesions between Th2-7. While VEP showed a partial block of bilateral visual pathways.

The MRI examination on the spinal cord in NMOSD have typical features of longitudinal extensive transversal myelitis (LETM), a lesion that extends over 3 or more segments of the adjacent spinal cord [1], [5], [13], [14]. MRI of the optic nerve can be seen as hyperintensity in optic neuritis and tends to have more posterior involvement of the optic nerve. Brain MRI features can vary, such as normal or periependimal lesions surrounding the ventricular system, dorsal brain stem lesions bordering the fourth ventricle, periependymal lesions that surround the lateral ventricles, white matter hemispheres, lesions involving the corticospinal tract, non-specific lesions and enhancing lesions [14], [15]. In this patient, an MRI examination of the spinal cord showed with the features of myelitis involving C3-6 and Th2-6 while a brain MRI examination was normal.

In general, the MRS examination for normal N-acetyl-aspartate (NAA), choline and Myo-inositol parameters are appropriate for normal axonal loss, inflammation, and gliosis [14], [15], [16], [17], [18]. In this patient, MRS examination found an increase in choline levels and the ratio of mild choline/creatine with the impression of a mild demyelination process.

The differential diagnosis of NMOSD is multiple sclerosis, acute disseminated encephalomyelitis, idiopathic acute transversal myelitis, and systemic lupus erythematosus [1], [5], [15], [16], [17], [19].

Therapy in NMOSD consists of acute exacerbation phase therapy to reduce the risk of relapse and long-term care [10], [20]. Treatment options for prevention of relapse include oral corticosteroids, immunosuppressant therapy, TPE, immunomodulatory therapy, and other new therapies. Azathioprine is the main treatment option for preventing relapse at a dose of 75-100 mg/day and is more effective when combined with oral prednisolone (1 mg/kg/day). Evaluation on hematology and long-term side effects including gastrointestinal complaints, leukopenia, infections, allergies, hematological general disorders, and congenital disorders are required [10].

Corticosteroids are the main choice in the acute phase. Intravenous methylprednisolone is

administered with a dose of 1-1.5 grams in 3-5 days [1], [2], [6], [7], [8]. Intravenous dexamethasone at a dose of 5 mg can be also a choice of corticosteroids [21]. Therapeutic plasma exchange (TPE) can be considered if the patient's condition does not improve or neurological symptoms worsen. Therapeutic plasma exchange dosage is carried out by giving 5-7 cycles in a period of 2 weeks with a dose of 1-1.5 plasma per time TPE [1], [6], [8], [9], [10], [20].

This patient has been treated with a dose of 50 mg azathioprine twice a day to prevent relapse, but the patient still had a recurrence. In acute exacerbations, initially, he was given intravenous methylprednisolone but there was no improvement in neurological disorders. Next, he was treated with the combination therapy of intravenous dexamethasone and 7 cycles of TPE. Azathioprine was also continued to be given with physiotherapy. He experienced significant neurological symptoms improvement from EDSS nine to six.

Long-term care is also needed such as medical rehabilitation, management of anxiety and depression, treatment of gastrointestinal problems and bladder, and pain management [20].

The probability of recurrence of disease activity is greater than 90% [21]. Attacks on NMOSD can be very severe, NMOSD can be life-threatening if the lesion extends to the cervical spinal cord and brain stem because it has the potential to cause respiratory failure [1], [7].

In conclusion, in the case of relapsing NMOSD patient, combination therapy of immunosuppressants, corticosteroids, and TPE was used. There were significant improvements from EDSS nine to six.

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Relationship between Chlamydia Trachomatis Infection with Patency Tubal and Non-Patency Tubal Occurrence in Infertile Women

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Abstract

AIM: To determine the relationship between *Chlamydia Trachomatis* infections with patency tubal and non-patency tubal occurrence in infertile women.

METHODS: This research was an observational analytic research with a cross-sectional study. This research was conducted at Haji Adam Malik General Hospital Medan, Pramita Laboratory Medan and Medical Faculty of Medicine Universitas Sumatera Utara. Laboratory on October 2017 until the number of samples was fulfilled. The samples were women who were referred to Pramita Laboratory Medan for hysterosalpingography procedure in accordance which corresponded to the inclusion criteria by using consecutive sampling technique.

RESULTS: From this research, it was found that the most infertile women were aged 31-35 years, with the most infertility type was primary infertility (83,8%) with the longest infertility duration was ≥ 3 years (44%). From all samples who were infertile, 26% among them were positive to chlamydia infection. Eight from twelve people who were infected by *Chlamydia Trachomatis* experienced non-patency tubal (66.7%) with p-value = 0.001 which showed that there was a relationship between *Chlamydia Trachomatis* infection with patency tubal and non-patency tubal occurrence in infertile women.

CONCLUSION: The proportion of *Chlamydia Trachomatis* infection in tubal abnormality in this study was 66.7%, whereas *Chlamydia Trachomatis* infection in the normal tube was 13.2%. It was obtained that there was a significant relationship between Chlamydia Trachomatis infection with tubal abnormality (non-patency tubal) with p-value < 0.005 (p = 0.001).

Introduction

Infertility is the absence of pregnancy after marriage for 12 months or more, even though the couple has had sexual intercourse regularly without any contraceptive use [1]. About 10-15% of couples worldwide experience infertility problems. The number of infertile couples in the world as well as in Indonesia is increasing, where there is an increase in the number of infertile couples of around 2% every 5 years [1]. In the United States, there are around 80 million reproductive age couples recorded [2].

According to the World Health Organization (WHO), the prevalence of women with infertility in Indonesia in 2004 at the age of 20-24 years was 21.3% and at the age of 25-29 years 16.8% [3]. Mashuri in 2006 against 93 infertile couples at dr. Pirngadi hospital Medan, found infertility originating from the wife's side as much as 49.46%, from the husband's side as much as 43.01% and 7.34% of both [4].

From the couple has had sexual intercourse regularly without using contraception, around 10-15% of couples worldwide experience infertility. Factors that cause infertility in women are divided into ovulation dysfunction factors (40%), pathological factors in the tube and peritoneal (40%), the presence of uterine problems (10%), and unexplained causes (10%). Genital infection by Chlamydia trachomatis is the main cause of tubal factor infertility [5], [6].

A case-control study conducted in Iran using

the Polymerase Chain Reaction (PCR) method showed that Chlamydia trachomatis was detected in 32% in infertile women and 8.7% infertile women [5]. Likewise in Nigeria, the prevalence of IgG Chlamydia trachomatis was 74% in infertile women [6]. In a study conducted in Egypt by Ahmed et al., IgG positive Chlamydia trachomatis was found in 30 of 78 infertile patients with tubal occlusion [7]. The study by Sharma et al., in Africa showed that tubal damage caused by Chlamydia trachomatis infection contributed more of 70 percent of infertility cases [8]. Various studies also found that the incidence of infertility due to tubal occlusion after infection with Chlamydia trachomatis ranged from 10-20% [9], [10], [11], [12]. From several studies it was also concluded that exposure to chronic infections from Chlamydia trachomatis is major predisposing factors resulting in chronic inflammation and causing damage to the tube resulting in infertility [13], [14].

Chlamydia trachomatis is an obligate intracellular microorganism that has the same cell wall as gram-negative bacteria and mainly infects the urethra, cervix, and tubal tract [15]. WHO reported the incidence of Chlamydia trachomatis infection is around 131 million worldwide with varying prevalence rates. in each country [16]. According to the 2014 Centers for Disease Control and Prevention (CDC), Chlamydia trachomatis infection is a sexually transmitted disease with the highest incidence in the United States of 1.4 million [17]. Chlamydia trachomatis is also the most common cause of sexually transmitted infections often reported in Europe, with a prevalence of 1.7-17% in European women and 4.1% in women in Spain [18], [19]. Chlamydia trachomatis infection as much as 80% in women is asymptomatic so screening is needed so infection control can be achieved [20], [21].

Chlamydia trachomatis infection can generally be diagnosed using culture examination, antigen detection test, antibody test, and Nucleic Acid Amplification Technique test (TAAN), such as PCR and Ligase Chain Reaction (LCR) [21]. TAAN has sensitivity 80-90% with specificity > 98% and can be used to check large quantities of samples [22]. PCR is one of the TAAN techniques by amplifying millions of DNA segments in just a few hours. This method is very promising for the initial detection of Chlamydia trachomatis infection with a sensitivity of 95% and specificity of 99.3% [23].

To date, there is no accurate data regarding the overall prevalence of Chlamydia trachomatis infection in Indonesia. Research on the prevalence of Chlamydia trachomatis infection in patients who experience differences caused by tubal patency with tubal non-patency in Indonesia is also not yet available. This is because Chlamydia trachomatis examination is not routinely done in infertility management in various education centers even though this has been recommended in the national consensus on handling infertility. At Haji Adam Malik Hospital itself, which is a national referral hospital, it has not yet made the examination and prophylactic therapy of Chlamydia trachomatis as a routine examination and therapy in temporary infertility management. most are caused by Chlamydia trachomatis infection.

So based on the above explanation, the author feels it is important to know the relationship between Chlamydia trachomatis infection and the incidence of tubal patency and non-patrol tubal infections in infertile women.

Material and Methods

This study aims to determine the relationship between Chlamydia trachomatis infection and the incidence of tubal patency and non-tubal patency in infertile women, an observational analytic study with a cross-sectional study approach. This research was conducted at Haji Adam Malik General Hospital Medan, starting in October 2017 until the sample was fulfilled then the sample was taken to the FK USU Laboratory for examination. The study population was all infertile women who were referred from SpOG private practice in the Medan region to Pramitha Laboratory who agreed to take hysterosalpingography (HSG).

The method of selecting samples in this study was done by consecutive sampling, where all infertile patients who met the inclusion and exclusion criteria and agreed to be examined after informed consent was included in the study until the required number of subjects was fulfilled.

The sample size is calculated statistically based on the formula:

Exp:

$$n = \frac{Z^2_{\alpha}\, p\, q}{d^2} = \frac{Z^2\, p\,(1-p)}{d^2}$$ (Snedecor GW & Cochran WG, 1967) (Lemeshowb dkk, 1997)

n = Minimum number of samples needed

Z = If α = 0.05 then Z score = 1.96

p = Proportion of Chlamydia trachomatis infection in tubal occlusion

q = 1- p = 0.15

α = Degree of trust = 5%

d = Precision = 10%

$$n = \frac{(1.96)^2 \; x \; 0.85 \; x \; 0.15}{(0.1)^2}$$

$$n = \frac{(1.96)^2 \; x \; 0.85 \; x \; 0.15}{(0.1)^2} = 48.98 \approx 50$$

Based on the above formula, the minimum number of samples obtained in this study were 50 subjects.

Inclusion Criteria: 1. Women aged 21-40 years; 2. Infertile women who are willing to do HSG examinations; 3. Infertile women who have never had abdominal and pelvic surgery, and have no myoma; 4. Willing to take part in the study and sign the consent form to take part in the study; and 5. Infertility occurs not due to male infertility.

Exclusion Criteria: Error results (illegible DNA on PCR).

Work Arrangement

Subjects were explained about the purpose of the study, the examination procedure, and the benefits of the research obtained from this study. If the subject is willing to take part in this study, the subject of the statement letter agrees to participate in the study Patients who meet the inclusion and exclusion criteria are interviewed about identity which includes: name, age, gender, type, and duration of infertility. How it works to take endocervical smears: 1. The subject is given an explanation in advance about the examination to be carried out so that the patient does not feel afraid; 2. Using personal protective equipment, in the form of laboratory suits, gloves, masks, and glasses; 3. The patient was previously asked to urinate and remove underwear, then the patient was positioned lithotomy; 4. If there are too many secretions in the vulva, clean them first with a NaCl solution; 5. Then the left hand opens the labia may then input the bottom sims and sims up inside the vagina; 6. With the help of floodlights. Look for the cervical portion; 7. Observe whether there are mucopurulent endocervical secretions, erythema, erosion, and edema; 8. The cervix: cleanses the endocervix area with sterile gauze, then smears with a sterile swab; and 9. The sample was put into a microcentrifugation tube which was filled with TE buffer liquid and then taken to the integrated laboratory of the Faculty of Medicine, University of North Sumatra.

Statistical Analysis

After all the data was collected, a descriptive analysis was conducted to determine the characteristics of the research subjects. Then bivariate analysis was performed to determine the relationship between Chlamydia trachomatis infection and the incidence of tubal patency and non-patency of the tube in women. To see the strength between the two variables, researchers used the Chi-square test in table 2 x 2 with a value of $p < 0.05$.

Results

Table 1 describes the characteristics of the study participants. In this study, it was found that the age most experienced Chlamydia Trachomatis infection was the age range in of 31-35 years, and the least was found in the age range of 21-25 years (Table 1).

Table 1: Characteristics of Infertile Patients by Age, Type of Infertility and Duration of Infertility

Characteristic	*Chlamydia trachomatis infection* (PCR)					
	Positive		Negative		Total	
	n	%	n	%	n	%
Age						
21-25	1	7.7%	0	.0%	1	2.0%
26 -30	2	15.4%	13	35.1%	15	30.0%
31-35	3	23.1%	20	54.1%	23	46.0%
36-40	7	43.9%	4	10.8%	11	22.0%
Total	13	100.0%	37	100.0%	50	100.0%
Infertility						
Primery	10	76.9%	31	83.8%	41	82.0%
Secondary	3	23.1%	6	16.2%	9	18.0%
Total	13	100.0%	37	100.0%	50	100.0%
Duration						
1-2	1	7.7%	6	16.2%	7	14.0%
2-3	7	53.8%	14	37.8%	21	42.0%
≥ 3	5	38.5%	17	45.9%	22	44.0%
Total	13	100.0%	37	100.0%	50	100.0%

Table 1 also illustrates the characteristics of the type of infertility. From 50 research subjects, there were 41 subjects with primary infertility and 9 subjects who experienced secondary infertility. Of the 41 subjects who experienced primary infertility, 10 people were infected with Chlamydia trachomatis 76.9% and 31 subjects who were not infected with Chlamydia Trachomatis. Of the 9 subjects who experienced secondary infertility, 3 of them were infected with Chlamydia Trachomatis, and 6 were not infected with Chlamydia Trachomatis.

Table 1 also illustrates the duration of infertility. From the table, it was found that the highest duration of infertility was > 3 years of parity, which was as many as 22 people (44%) and the least was with a duration of 1-2 years, as many as 7 people (14%). Of the 22 people who had > 3 years of infertility, 5 of them were infected with Chlamydia trachomatis, and 17 of them were not infected with Chlamydia trachomatis. Of the 7 people who had 1-2 years of infertility, 1 of them was infected with Chlamydia trachomatis, and 6 of them were not infected with Chlamydia trachomatis.

In Table 2 are shown the results obtained from 50 samples studied, and we can see that 13 people (26%) were infected by Chlamydia trachomatis, and 37 people (74%) were not infected.

Table 2: Frequency of Chlamydia trachomatis infection with PCR Method

	PCR		
	Chlamydia trachomatis (+)	*Chlamydia trachomatis* (-)	Total
Total	13 (26%)	37 (74%)	50 (100%)

In Table 3 we can see the prevalence of Chlamydia trachomatous infection in infertile women. As many as 8 people (66.7%) from 12 people with Non-Patent Tuba and as many as 5 people (13.2%) from 38 people with Tuba Patency have positive infection established with PCR. This is consistent with research by Sharma et al., in Africa showing that tubal damage caused by Chlamydia trachomatis infection accounts for more than 70% of cases of infertility.

Table 3: Prevalence of Chlamydia trachomatis infection with Tuba Patent and Non-Patent Tuba Events in Infertile Women

Chlamydia infection	Tubal Patency			
	Non-paten		Patent	
	n	%	n	%
Positive	8	66.7%	5	13.2%
Negative	4	33.3%	33	86.8%
Total	12	100%	38	100%

Relationship Between Chlamydia trachomatis infection and the incidence of tubal patency and non-patent of the tuba in infertile women

Chlamydia trachomatis infection is one of the causes of infertility experienced by mothers due to tubal patency.

Table 4 describes the proportion of Chlamydia trachomatis infection in tubal abnormalities. In this study, tubal abnormalities in the form of Non-Patent Tubes were found in 8 of 13 people infected with Chlamydia trachomatis (61.5%), higher than the proportion of Chlamydia trachomatis infection in normal tubal, i.e., 5 from 13 people (38.5%).

Table 4: Relationship Between Chlamydia Trachomatis Infection with Patency Tubal and Non-Patency Tubal

Tubal Patency	Chlamydia Infection						P Value
	Positive		Negative		Total		
	N	%	N	%	N	%	
Non patency	8	61.5%	4	10.8%	12	24.0%	
Patency	5	38.5%	33	89.2%	38	76.0%	0.001
Total	13	100.0%	37	100.0%	50	100.0%	

Discussion

Our results are not in accordance with several studies conducted by Ioannis which illustrate that the most Chlamydia trachomatis infections in the age range under 25 years in the USA. Due to this age, it is often associated with sexual experience, changing sexual partners and the number of new sexual partners. This may be different from the Indonesian state that does not adhere to free sex, so that hygiene and education factors are factors that influence the incidence of Chlamydia Trachomatis infection in the age range of 31-35 years, besides, it is said that the age at first sexual intercourse was also higher in the USA compared to developing countries [13]. The study conducted by Sayed on 150 infertile women in Iran, also illustrates that the most age for infection with Chlamydia Trachomatis is the age range of 25-29, which is as many as 80 people [8].

This result is consistent with a study conducted by Haifa in Saudi Arabia, of 456 who experienced infertility, 368 were primary infertility, and 89 were secondary infertility, according to a study conducted by Samiha, et al., conducted on 215 infertile women, of which 172 among them are primary infertility (80%), and 43 other women are secondary infertility (20%). This occurs because patients with primary infertility have greater concern than secondary infertility patients who already have children so that awareness of self-examination and seeking medical assistance in dealing with infertility problems is better than patients with secondary infertility. Women suffer from primary infertility because of several risk factors, such as menarche above 16 years, irregularities of the menstrual cycle, age at marriage, and infection.

This is in accordance with a study by Wafirotus in Indonesia in 42 infertile women who found that the duration of infertility was at most between 3 years and > 5 years, at 35.7%. Whereas in a study conducted by Gendarme et al., In Mongolia, of 430 infertile couples, the longest infertile duration was 4-8 years as many as 153 people (35.6%) and 2-4 years as many as 152 people (35.4%). The thing that causes the duration of infertility is because the average couple will seek treatment for infertile problems over 2 years [30].

Our results are consistent with a study conducted by Sayed et al., of the 150 samples of infertile women studied, where 48 (32%) subjects were infected with Chlamydia trachomatis established through PCR [8].

Similar results were also found in the Sami et al. From 100 samples of infertile women in the study group, 36 subjects (36%) were infected by Chlamydia trachomatis. The cause of infertility in Chlamydia trachomatis sufferers was due to non-patent tuba.

This is in line with the study by Sami et al., who examined the relationship between infertility and Chlamydia trachomatis infection in 200 women in Al-Sadar hospital, illustrating that 60 people had tubal disorders and 25 (41.6%) people with Chlamydia trachomatis infection with a value of $p < 0.005$, illustrates the close relationship between Chlamydia trachomatis infection with tubal disorders that cause infertility in women. The disorder is due to obstruction or occlusion of the tube due to untreated Chlamydia trachomatis infection. Another study conducted by Swapnil Singh on 200 infertile women, found 10 people suffering from Chlamydia trachomatis, and among those 10 people, all had tubal abnormalities, especially tubal occlusion. The most frequent occlusion site is in the fimbrial region as much as 34%, followed by the Cornual section 32%,

hydrosalpinx 20.6%, ampullary section 8.6% and the isthmus section 3.4%.

Another study conducted by Wafirotus et al., In 42 infertile women infected with Chlamydia Trachomatis, 76.1% had infertility due to tubal disorders, both those caused by perituba attachment, fimbriae phimosis, tubal occlusion, and hydrocephalic.

The study conducted by Gendarme et al., in Mongolia, performed in 280 women who experienced infertility, 55.6% were caused by infection, and 36% were due to tubal abnormalities, namely bilateral tubal occlusion and pelvic adhesion [30]. Another study conducted in Saudi Arabia by Alffarajj et al in 100 infertile women, found that 8% were infected by Chlamydia trachomatis, and 53.3% were due to tubal problems, this is because Chlamydia trachomatis that infects pelvic can spread to the uterus even to the fallopian tubes and cause local inflammation.

Also the study conducted by Ahmed et al., showed that of 80 infertile women, 30 women (37.5%) were infected with Chlamydia trachomatis, and 23 of them had tubal occlusion (76.6%), $p < 0.005$. Where 18 people experienced occlusion in the distal part, and 5 people experienced occlusion in the proximal part.

All tubal disorders caused by Chlamydia trachomatis infection causing infertility, have a complex mechanism. The mechanism by which Chlamydia trachomatis induces inflammation and tissue damage has not been fully understood. However, it is known that Chlamydia trachomatis target cells are squamocolumnar epithelial cells of the endocervix and upper genital tract in women. Apart from this place, the initial response to infection appears to be primarily a polymorphonuclear leukocyte response. Infected epithelial cells in vitro produce interleukin-8 (IL-8) and other proinflammatory cytokines, which stimulate the initial neutrophil response. Lipopolysaccharide may be the dominant Chlamydia trachomatis antigen capable of inducing proinflammatory cytokines.

Studies have explained the mechanism by which infected epithelial cells produce cytokines that direct the initial innate response and are obtained in Chlamydia trachomatis infection. Early neutrophil infiltration is followed by tissue infiltration with lymphocytes, macrophages, plasma cells, and eosinophils. In genital infections, plasma cells are generally present in large numbers. In genital diseases with biovar trachoma, lymphoid follicles (aggregate lymphocytes and macrophages in the submucosa) form when acute inflammation begins to subside. Thinning or loss of epithelium occurs around the follicle and can become necrotic when this disease occurs. When the infection begins to heal, fibrosis and scar tissue are formed.

The results of this study indicate that there is a significant relationship with a value of $p = 0.001$ where the value is smaller than 0.05, between Chlamydia trachomatis infection and the incidence of Non-Patent Tuba.

In conclusion**,** a significant relationship was obtained between Chlamydia Trachomatis infection and tubal abnormalities (non-patent tubal) with a value of $p = 0.001$ ($p < 0.05$), with the most research subjects being the age group 31-35 years and the primary infertility type being 41 subjects (82%). the highest duration of infertility is ≥ 3 years (44%). The frequency of Chlamydia trachomatis infection with the PCR method found that 13 subjects (26%) of the 50 subjects studied. The prevalence of Chlamydia trachomatis infection in tubal abnormalities in this study was 66.6%, while Chlamydia trachomatis infection in the normal tube was 13.2%.

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Prevalence and Knowledge of Soil-Transmitted Helminth Infections in Mandailing Natal, North Sumatera, Indonesia

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Abstract

BACKGROUND: Soil-transmitted helminth (STH) infection remains of public health importance, particularly in developing countries. North Sumatra province of Indonesia has reported high prevalences of STH in many of its districts.

AIM: In this study, we aimed to determine the prevalence of STH and evaluated STH knowledge and risk behaviours of school children in Mandailing Natal district.

METHODS: We conducted a cross-sectional study in two primary schools in Mandailing Natal district, North Sumatera province, Indonesia. Data were collected directly from the subjects using a questionnaire after receiving consent from their parents. A stool sample was also collected to determine the STH status of each subject. Samples were then transported to the parasitology laboratory at Universitas Sumatera Utara in Medan to be analysed by a trained analyst. Kato-Katz method was used to prepare the slides (World Health Organization, 2002). The intensity of infection is classified into light, moderate and heavy infection.

RESULTS: Prevalence of STH infections were 76.8% and 87.2% in Singkuand and Sikapas primary schools, respectively. The majority of infections were *Ascaris lumbricoides* and *Trichuris trichiura* at a light intensity. Only small numbers of children in the two schools used soap before eating, used soap after defecating, and wear sandal/shoes when walking outdoor. Less than 50% of children also knew the route of transmission of STH. Poorer knowledge and behaviours were shown in children from Sikapas than in Singkuang.

CONCLUSION: High prevalence and poor hygiene behaviours found in this study reinforce the need to identify the correct intervention to address this STH problem in the region.

Introduction

Soil-transmitted helminth (STH) infections (*Ascaris lumbricoides*, *Trichuris trichiura*, and hookworm) are widespread through tropical and subtropical regions with the highest prevalence in developing countries. According to the WHO, the burden of STH infection worldwide was estimated to be 1.5 billion people in February 2018, or have reached 24% of the world's population with the highest prevalence in sub-Saharan Africa, America, China and East Asia. This infection occurs in more than 267 million people in pre-school children, and more than 568 million in school-aged children [1].

In Indonesia, STH infection is one of the main public health problems with prevalences ranging from 45% to 65%. In areas with poor sanitation, the prevalence can reach as high as 80% [2]. In different districts in North Sumatra province, the prevalence of helminthiasis in suburban and rural areas have been reported from 87% in 2004, 84.6% in 2005, 64.3% in 2012 to 84.66% in 2015 [3], [4], [5], [6], [7], [8], [9]. Helminthiasis affects the nutritional status and cognitive function of the children, and in the long-term may interfere with their growth [10].

Factors associated with a high prevalence of helminthiasis in children including low personal hygiene such as handwashing behaviour before a meal and after going to the bathroom, nail cleanliness, defecation habit, availability of freshwater and soil contamination with helminth eggs. To prevent helminth infection and to reduce the prevalences, public education on family and personal hygiene, as

well as deworming programmes, are important [11].

This study aims to determine the prevalence and primary schoolchildren's knowledge of soil-transmitted helminth infection.

Material and Methods

We conducted a cross-sectional study in two primary schools in Mandailing Natal district, North Sumatera province, Indonesia. The study was done in March 2019. Subjects were children attending two primary schools in the district, namely Singkuang and Sikapas Primary School. Data were collected directly from the subjects using a questionnaire after receiving consent from their parents. The questions comprised of demographic data and knowledge on the potential STH risk factor and STH transmission. Teachers and research assistants assisted children in grade 1 and 2 to fill in the questionnaire. A stool sample was also collected to determine the STH status of each subject. Samples were then transported to the parasitology laboratory at Universitas Sumatera Utara in Medan to be analysed by a trained analyst. Kato-Katz method was used to prepare the slides [12]. The intensity of infection is classified into light, moderate and heavy infection (Table 1).

Table 1: Intensity of STH infection (World Health Organization, 2002)

Classification	*Ascaris lumbricoides*	*Trichuris trichiura*	Hookworm
Light	1-4,999 epg	1-999 epg	1-1,999 epg
Moderate	5,000-49,999 epg	1,000-9,999 epg	2,000-3,999 epg
Heavy	≥ 50,000 epg	≥ 10,000 epg	≥ 4,000 epg

Data were analysed using STATA SC/IC Version 15 (STATA Corporation, TX, USA). Chi-square analysis was used to determine significance. The level of significance was set at $P < 0.05$.

The study was approved by the Ethics Committee of Faculty of Medicine Universitas Sumatera Utara, Indonesia.

Results

During the study period, a total of 426 primary school children completed the questionnaires. Two hundred and twenty-nine (53.8%) were male, and 197 (46.2%) were female. Of those, only 54.9% have a private toilet inside their house, while the remaining used the public facility or defecate in the sea. The majority of children (71.3%) also admitted of helminth infection in the past (Table 2).

Table 2: Baseline characteristics

Characteristics	*n*	%
Grade		
1	81	19.0
2	102	23.9
3	81	19.0
4	89	20.9
5	73	17.1
Gender		
Male	229	53.8
Female	197	46.2
Sanitation facility		
Private toilet	234	54.9
Public toilet	41	9.6
Outdoor toilet	93	21.8
Not available	54	12.7
History of helminthiasis	305	71.3

Table 3 shows the hygiene behaviour of children in both schools. The use of soap for handwashing and after defecation was more likely among children in Singkuang primary school ($P < 0.001$), as well as the use of sandals or shoes when walking outdoor ($P = 0.04$). However, the proportion of children who used a toilet for defecating was similar in both schools ($P = 0.98$). During the study, we also inspected the cleanliness of the hand nails. Of 188 children at Singkuang primary school, 116 (61.7%) had dirty hand nails. Similar proportion ($n = 153$, 64.2%) at Sikapas primary school also had dirty nails.

Table 3: Hygiene behaviour among enrolled students

Behaviour	Singkuang (*n* = 188)	Sikapas (*n* = 238)
Use of soap for handwashing	77 (40.9)	40 (16.8)
Use of soap after defecation	63 (33.5)	44 (18.5)
Use of sandals or shoes when walking outside	40 (21.3)	33 (13.9)
Defecating in a toilet	121 (64.4)	169 (71.0)

We further collected data on the children's knowledge on STH transmission (Table 4), and only 4 (2.1%) of children in Singkuang did not know any route of STH transmission, while a more significant number (n = 84, 35.3%) of primary school children in Sikapas did not know about the route of transmission. As the route of transmission of each parasite is different, the most commonly known routes in Singkuang children were via the anus (93.1%) and skin (92.0%), as well as in Sikapas (62.6% and 63.9%).

Table 4: Knowledge of STH transmission

Knowledge on the cause of transmission	Singkuang (*n* = 188)	Sikapas (*n* = 238)
Dirty hand when eating (%)	86 (45.7)	135 (56.7)
Uncooked water (%)	129 (68.6)	133 (55.9)
Uncooked meal (%)	143 (76.1)	147 (61.8)
Via anus (%)	175 (93.1)	149 (62.6)
Via skin (%)	173 (92.0)	152 (63.9)
Via barefoot (%)	98 (52.1)	132 (55.5)

Of all enrolled children, only a third of children ($n = 56$, 29.7%) in Singkuang provided a stool sample for examination. While all children in Sikapas presented their stool. The prevalence of any STH infection (Table 5) were 76.8% and 87.2% for Singkuang and Sikapas, respectively. The most common infections were *A. lumbricoides* and *T. trichiura*. While hookworm infection only occurred in

one child in Singkuang, but more common in Sikapas (19.4%). The majority of infection intensity was light infection for both *A. lumbricoides* and *T. trichiura.* Although in Sikapas, more proportion of children had a moderate infection than in Singkuang ($P > 0.05$). Furthermore, the proportion of mixed infection was significantly more common in children in Sikapas than in Singkuang (72.0% vs 53.5%, $P = 0.01$).

Table 5: Prevalence and intensity of infection

Prevalence	Singkuang (*n* = 56)	Sikapas (*n* = 242)
Ascaris lumbricoide	33 (58.9)	169 (69.8)
Mean intensity	2011.4 (2426.4)	2780.8 (3270.6)
Light intensity	28 (84.9)	129 (76.3)
Moderate intensity	5 (15.2)	40 (23.7)
Trichuris trichiura	32 (57.1)	189 (78.1)
Mean intensity	514.1 (454.9)	633.4 (602.7)
Light intensity	29 (90.6)	144 (76.2)
Moderate intensity	3 (9.4)	45 (23.8)
Hookworm	1 (1.8)	47 (19.4)
Mean intensity	432 (0)	303.6 (191.7)
Light intensity	1 (100)	47 (100)
Any STH infection	43 (76.8)	211 (87.2)
Two mixed infections	23 (41.1)	110 (45.5)
Three mixed infecctions	0 (0)	42 (17.4)

Discussion

This present study reported the prevalence and basic knowledge of STH infection and transmission among school children in Mandailing Natal district, North Sumatra province. Further, we also assessed the hygiene behaviour in those children. Similar to our earlier studies in other districts in North Sumatra province, the prevalence of STH infections remains high [3], [4], [5], [6], [7], [8], [9], [13], despite existing health promotion and education to reduce STH burden.

Therefore, in this study, we complemented our data with the information regarding basic knowledge of STH infection, how they transmit and STH risk behaviours. This is part of a prospective study to evaluate the impact of hygiene education on children's knowledge and habit. In the two primary schools in the studied district, we revealed that the children had poor hygiene behaviour. However, children in Sikapas were less likely to use soap before eating or after defecating, and also less likely to wear sandals/shoes when walking outside to those in Singkuang. This is similar to a previous study [14]. When they were asked of how STH transmit, children in Singkuang also had better knowledge than children in Sikapas. This is by the higher prevalence of STH seen in Sikapas than in Singkuang. Although we did not perform analysis on individual risk, we are unable to determine a definite relationship between the risk behaviour and high prevalence.

This result also strengthens the importance of implementing other strategies to improve children's knowledge which lead to better health hygiene behaviour. The current deworming programme recommended by the government, as also recommended by the WHO [12], has not been successful in reducing the STH infections in this region, although efficacy studies in this province still demonstrated high efficacy of both albendazole and mebendazole for *A. lumbricoides* and *T. trichiura* infections [7], [15], [8], [13]. Therefore, anthelminthic resistance is not yet an issue. However, the routine deworming programme has been shown to have an impact on the infection intensity than in the prevalence which can be used as an indicator of improved health [16].

In this study, we have shown that the majority of children were infected with light intensity for either *A. lumbricoides* or *T. trichiura* infection. A WHO model evaluated the correlation between the species prevalence with the possibility of moderate to heavy infection, where prevalences of 58.9% and 69.8% as determined in this study correlated with a proportion of moderate infection of 17.8% and 29.3%, respectively. While *T. trichiura* prevalences at 57.1% and 78.1% correlated with the proportion of moderate infection at 10.8% and 21.4%, respectively. These estimations are by our results, and supports the continual use of periodic anthelminthic to reduce morbidity especially in individuals with moderate to heavy infections [16].

Our study has several limitations; first not all children returned their stool for STH examinations; therefore, the prevalence reported here may not reflect the true burden of STH in these communities. Second, we also did not correlate the STH results with individual data due to high numbers of missing STH status. Therefore, the association between high prevalence and children's behaviours and knowledge were only made based on assumption.

However, this study added more information on STH burden and risk behaviour in children. These data can be used to assess the changes in their knowledge and habits following our prospective health education intervention study.

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Comparison of Carcinoembryonic Antigen Levels Among Degree of Differentiation and Colorectal Cancer's Location in Medan

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Abstract

BACKGROUND: The most widely used tumour markers, especially in colorectal malignancy, is Carcinoembryonic antigen (CEA).

AIM: This study was aimed to investigate CEA value among the degree of differentiation and tumour location.

METHODS: A cross-sectional analytical study was used in this study on eighty consecutive patients with colorectal carcinoma (CRC) at Adam Malik General Hospital and Permata Bunda Hospital, Medan, Indonesia. All data were analysed using SPSS for Windows version 21.

RESULTS: They were rectal cancer 49.4%, left-sided colon cancer 43.2% and right-sided colon cancer 6.2%. Histopathology findings were well-differentiated 40.7%, moderate differentiated 32.1% and poorly differentiated 25.9%. There were no correlations between CEA level and haemoglobin level, white blood cells count, and platelet count. There was no significant difference between CEA and location of the tumour ($p = 0.70$), although CEA level was significantly differenced among histopathology findings ($p = 0.03$). CEA levels were and associated with the degree of differentiation.

CONCLUSION: CEA levels increased in well-differentiated colorectal carcinoma especially in rectal cancer.

Introduction

Cancer is a disease characterized by the unchecked division and survival of abnormal cells. When this abnormal growth occurs in colon or rectum, it is called colorectal cancer (CRC) [1]. CRC is one of the common tumour types in the world, which accounts for 400 000 deaths annually [2]. The incidence rates of CRC were 19.1 for men and 15.6 for women per 100.000 populations in Indonesia [3] with a major risk factor for CRC are smoking, alcoholism, physical inactivity and obesity [4].

In 1965 Gold and Freedman demonstrated that a CEA presents in extracts of tumours from the gastrointestinal tract and fetal gut tissues but not in extracts of adult intestinal tissues. CEA modulates intercellular adhesion of colon epithelial cell-collagen interactions. Since high concentrations of CEA presents in fetal tissues and tumours, it disrupts normal intercellular or cell-collagen adhesion forces allowing more cell movement and the development of less ordered tissues architecture and greater cell-cell interaction. CEA appears to have the greatest clinical use as evaluation of treatment efficacy and in follow-up for recurrent disease.

Carcinoembryonic antigen (CEA) is the most commonly used tumour marker for CRC diagnosis, prognosis evaluation or after treatment recurrence [5]. CEA is found in normal fetal gastrointestinal tissue and at very low concentrations in adult blood plasma. Its concentration increases in many tumours, such as CRC. Increased CEA concentrations were also reported in gastric, bronchial, uterine and ovarian cancers, and lymphomas as well [6].

American Society of Clinical Oncology (ASCO) defined right-sided colon cancer of cecum and ascending colon up to the hepatic flexure. Left-

sided colon cancer comprises of cancers in splenic flexure and regions distal to the splenic flexure, including the rectum. The transverse colon connects left and right-sides and on average is appreciably shorter than the right and left-sides. Colorectal adenocarcinoma can be divided into three distinct disease entities: right colon cancer, left colon cancer and rectal cancer [3].

High CEA production by tumours is associated with increased tumour growth and poorer prognosis [2], [3]. CEA can be detected and quantitatively measured in the serum and the tumour tissues of CRC patients although its role in the prognosis of CRC (Figure 1) remains controversial [7].

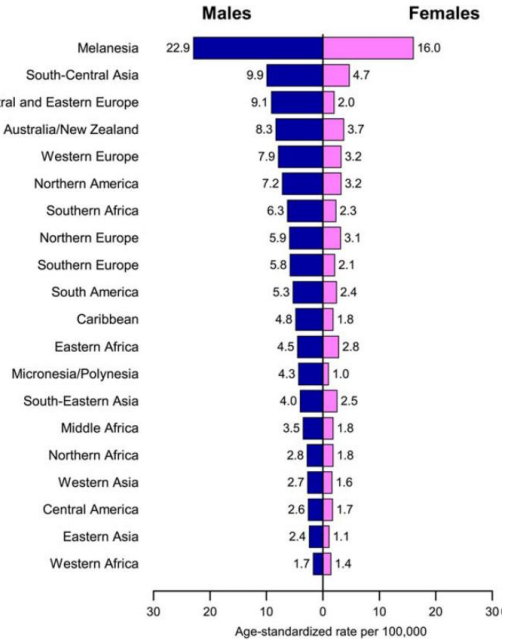

Figure 1. Colorectal Cancer Incidence, 2012

Current ASCO guidelines recommend that CEA examinations be routinely obtained at 3-month intervals during postoperative surveillance and at 1–3-month intervals during systemic treatment for metastatic CRC [2].

Normal value of CEA is 5 ng/mL in serum. Patients with appropriate symptoms, a highly increased concentration (e.g., 5 times the upper limit of normal reference value) should be considered strongly suggestive for the presence of cancer in that particular patient.

Factors affecting serum CEA levels in patients with CRC are tumour stage, tumour grade, liver status, tumour site within colon, presence or absence of bowel obstruction, smoking, ploidy status of the tumour. Well-differentiated CRC produces higher CEA level than poorly differentiated one. Certain benign liver diseases impair liver function and, thus, CEA clearance. Consequently, CEA increased in serum of patients with nonmalignant liver diseases. Smoking appears to double CEA serum concentration [6].

Material and Methods

Serum CEA levels in CRC patients were measured using CEA Elecsys analysers (Roche Diagnostics GmbH, United States) with a reference range of 5.0 ng/mL. CRC patients were then divided into two groups, those with normal serum CEA levels (e.g., ≤ 5 ng/mL) and those with elevated serum CEA levels (> 5 ng/mL)

A cross-sectional analytical study was used in this study on eighty consecutive patients with CRC at Adam Malik General Hospital and Permata Bunda Hospital, Medan, Indonesia. All data were analysed with SPSS for Windows version 21. Data were examined using the Kruskal Wallis test.

Results

Demographic characteristics of the patient are shown in Table 1. The recruited patients consisted of 48 males (60%), and 32 females (40%). The median age of these patients was 53 (25-80) years old. The majority of patients' education level was senior high school (43.2%), elementary school (25.9%), university (16%) and junior high school (13.6%).

The most common tumor location were rectal cancer (49.4%), left-sided colon cancer (43.2%) and right-sided colon cancer (6.2%). Histopathology examinations showed well-differentiated 41,3%, moderate differentiated 32.1% and poorly differentiated 25.9% CRCs.

Table 1. Demographic and clinical characteristics of the patients

Variable	N = 80
Gender	
Male	48 (60%)[a]
Female	32 (40%)[a]
Age	53 (25-80)[b]
education level	
Elementary School	21 (26.3%)[a]
Junior High School	11 (13.8%)[a]
Senior High School	35 (43.8%)[a]
University	13 (16.3%)[a]
Tumour Location	
Rectal cancer	40 (50%)[a]
Left-sided colon cancer	35 (43.8%)[a]
Right-sided colon cancer	5 (6.3%)[a]
Histopathology	
Well-differentiated	33 (41.3%)[a]
Moderately differentiated	26 (32.5%)[a]
Poorly differentiated	21 (26.3%)[a]
Haemoglobin	11 (5-19)[b]
White blood cells	8850 (1650-25 750)[b]
Platelet	32 5178 ± 135 551[c]
CEA	6.93(0.42-3340.08)[b]

[a] Categoric data: n (%); [b] Numeric data, abnormal distribution: median (min-max); [c] Numeric data, normal distribution: mean ± SD.

The correlation between routine blood count with CEA is shown in Table 2. Hb, WBC and platelet, showed no difference (p > 0.05).

Table 2. Correlation of routine blood test with CEA

Variable	CEA Levels	
	Correlation Coefficient	p
Hb	-0.111	0.32
WBC	0.002	0.98
Platelet	-0.109	0.33

Table 3 shows a comparison of CEA to tumor locations and histopathology grades. Mean of CEA level at rectal 223.90 ng/mL, left-sided cancer 156.79 ng/mL, and right-sided cancer 2.61 ng/mL ng/mL (p = 0.70). Histopathology examinations showed that there were well differentiated 387.66 ng/mL, moderately differentiated 36.62 ng/mL and poorly differentiated 33.90 ng/mL with significant differences among them (p = 0.03).

Table 3. Comparison of carcinoembryonic antigen (CEA) levels on histopathology classifications and tumour locations in colorectal cancer

	CEA Level Unit	F	p
Tumour location		0.37	0.70
Rectal cancer	223.90 ± 741.98		
Left-sided colon	156.79 ± 378.31		
Right-sided colon	2.61 ± 0.84		
Histopathology			
Well	387.66 ± 865.33	3.83	0.03
Moderate	36.62 ± 73.50		
Poorly	33.90 ± 66.22		

Discussion

Mostly sample is male (60%). This is according to the research of American cancer society 2012 obtained majority of gender is male than female. The localisation a large number of patients had colorectal cancer in rectal cancer 50% and Left-sided colon cancer 43.8% [10], [11] found that 44 cancers were in rectal region and 68 cancers were in other regions of the colon.

In this study differentiated histopathology findings the mostly well-differentiated 41.3%, this is similarly from the research of Aru W. Sudoyo et al. while the majority of colorectal carcinoma was well-differentiated [8].

CEA is the most widely used tumour marker worldwide and certainly the most frequently used marker in CRC [9]. In this study, there was a significant difference (p = 0.03) mean CEA among well differentiated 387.66 ng/ml, moderate 36.62 ng/ml and poorly 33.90 ng/ml. Well-differentiated CRC produces more CEA than poorly differentiated. Similarly, CEA tends to be higher in patients with well-differentiated compared to poorly differentiated [12], [13], [14], [15]. Thus, a lack of differentiation or poorly differentiated may explain why some patients with advanced CRC do not have increased CEA value (Park JW et al., 2013). Mean of CEA of location tumour obtained CEA increased to rectal cancer 223.90 and left-sided colon cancer 156.79. Some reports suggest that patients with tumours in the left-sided of colon cancer generally have a higher incidence of increased CEA than right-sided of colon cancer (Nicholson BD et al., 2015, Jeon BG et al., 2013) [16], [17].

In conclusion, CEA levels increased in well-differentiated colorectal carcinoma especially in rectal cancer.

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Comparison of Platelet to Lymphocyte Ratio between Degrees of the Barcelona Clinic Liver Cancer on Hepatocellular Carcinoma Patients at Haji Adam Malik General Hospital

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Keywords: Hepatocellular carcinoma; The Barcelona Clinical Liver Cancer System; Platelet to Lymphocyte Ratio

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Abstract

BACKGROUND: Hepatocellular carcinoma (HCC) is the fifth most common malignancy. The Barcelona Clinical Liver Cancer System (BCLC), guides the treatment of patients with HCC. Platelet to lymphocyte ratio (PLR) is an inflammatory marker used as a prognostic factor disease of HCC. An increase in PLR indicates higher host's inflammatory response and is associated with aggressive HCC behaviour, according to BCLC.

AIM: This study aims to determine the PLRs between among the degrees of BCLC (The Barcelona Clinic Liver Cancer) in HCC patients at Haji Adam Malik General Hospital in Medan during 2015-2016.

METHODS: This retrospective study involved 166 patients with HCC who were then classified by the BCLC guidelines. PLRs among the patient's degrees of BCLC were compared using Kruskal Wallis test.

RESULTS: A total of 166 HCC patients, 129 (77.7%) were men and 37 (22.3%) were women. The PLR value has a median value of 17841with the lowest value of 1776 and the highest value of 223684. There were differences in PLR levels with various BCLC stages in patients with HCC at Haji Adam Malik Hospital during 2015-2016 ($p = 0.026$).

CONCLUSION: There were differences in PLR levels with various BCLC stages in patients with HCC at Haji Adam Malik Hospital during 2015-2016.

Introduction

Hepatocellular carcinoma (HCC) is the fifth most common malignancy, with more than 30 cases per 100,000 people each year in Southeast and Central Asia. In the United States, the incidence has doubled over the past 20 years. The highest incidence of HCC was found in South East Asian Indonesia with a level of age standard (ASR) of 31.9 per 100,000 in men and 10.2 per 100,000 in women. HCC relates to some risk factors, including chronic hepatitis virus infection, Non-Alcoholic Fatty Liver Disease, alcoholism, and aflatoxin-contaminated food. The most common one is chronic Hepatitis B virus infection. Majority patients with chronic HBV infection have no typical symptoms, although the viruses are active and transmit into another person.

Guidelines for clinical practice is critical in directing and standardising the management of the disease. The Barcelona Clinical Liver Cancer System (BCLC), grades HCC and guides its. This staging system is complete and accurate since it does not only evaluate tumour characteristics, performance status and liver function but also connects disease staging to its treatment course [1]. Platelet and lymphocyte counts are basic haematological examinations that are very easy, fast to apply everyday and cheap. Platelet to lymphocyte ratio (PLR) is an inflammatory marker used as a prognostic

factor in various types of diseases. An increase in PLR indicates that the host's inflammatory response increased. This is associated with more aggressive tumour characteristics [2].

This study aims to determine the PLRs between among the degrees of BCLC (The Barcelona Clinic Liver Cancer) in HCC patients at Haji Adam Malik General Hospital in Medan during 2015-2016.

Material and Methods

Data collection

A retrospective study conducted at Internal Medicine Clinic Haji Adam Malik General Hospital in Medan from January 1, 2015, to December 31, 2016, in patients diagnosed with HCC. A total of 166 patients that had lymphocyte and platelet counts, liver function test, AFP, liver USG and 3-phase Liver CT Scans were recruited in this study and then grouped into BCLC A, B, C and D [2].

Statistics

All data were analysed using SPSS version 22. Demographic data were presented in numbers and percentages. Comparison of platelet to lymphocyte ratio between the degrees of BCLC of HCC patients was analysed using the Kruskal Wallis test.

Results

The demographic data of 166 HCC patients, consisted of 129 male patients (77.7%) and 37 female patients (22.3%). The median age of HCC patients was 52.8 years (20-83). Hepatitis B virus infection was found in 84.9% of patients, while hepatitis C virus infection and other causes were in 4.8% and 10.2% patients, respectively.

Table 1: Demographic characteristics of subjects

Variable	n = 166
Gender	
Male	129 (77.7 %)[a]
Female	37 (22.3%)
Age	52.8 (20-83)[c]
Aetiology	
Hepatitis B	141 (84.9 %)[a]
Hepatitis C	8 (4.8 %)
Other	17 (10.2 %)
LIM	15.1 (1.9 – 60.8)[c]
PLR	17841 (1776 – 223684)[c]
PLT	280819 ± 138268[b]
BCLC	
A	12 (7.2 %)[a]
B	54 (32.5 %)
C	76 (45.8 %)
D	24 (14.5 %)

Lymphocytes have a median value of 15.10 U/L with the lowest value of 1.90 U/L and the highest value of 60.80 U/L, and the PLR had a median value of 17841.57 with the lowest value of 1776.32 and the highest value of 223684.21. The average platelet value was 280819.28 ± 138268.57 then for the BCLC staging system; the most was BCLC class C (45.8%), followed by BCLC B (32.5%), BCLC D (14.5%) and BCLC A (7.2%) (Table 1).

Table 2: Comparison of PLR levels of HCC patients on BCLC staging

BCLC	PLR	P
A	18534 (2509 -61267)	0,026
B	14069 (1776 - 223684)	
C	18469 (2536 – 119117)	
D	30007(3724 – 117500)	

There were differences in PLR levels with various BCLC stages in patients with HCC.

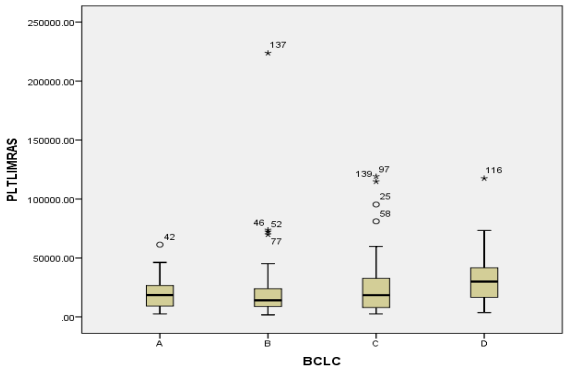

Figure 1: Comparison PLRs ratios between degrees of BCLC A, B, C and D

Discussion

In this study, it was found that the number of male HCC patient (77.7%) and female (22.3%) (Table 1). This is relevant with the research conducted by GLOBOCAN, cancer research (2012) which states that HCC patients are dominated with a male than a female with a ratio between 2: 1 to 4: 1. [3], the incidence of HCC in men was 40.0 and in women 15.3 per 100,000 population. Yuen et al., (2000) [4], and Cheung et al., (2006) [5], published that the percentage of male HCC patients compared to female 4: 1.

The median age of HCC patients in this study was 52.8 years (Table 1). Yuen et al., (2000) [4] and Cheung et al., (2006) [5] obtained that the median age of HCC patients in Hong Kong 61 and 63 years old. Park (2005) [6], studying HCC, with the highest incidence of HCC at the age of 55 years old. An analysis of subgroups in the United States examines Hispanic and African-American ethnicities, the highest incidence of HCC at the age of 45-65 years.

The most common aetiology of the incidence of HCC, according to this study, is hepatitis B infection (Table 1). This is also relevant to other studies. Chronic HBV infection has been known to be closely related to the incidence of HCC. HBV infection contributes greatly to the incidence of HCC and its mortality rate, around 63.9% of the incidence of HCC is caused by HBV infection [7], [8].

HBV carcinogenicity to the liver occurs through a chronic inflammatory process, increased hepatocyte proliferation, integration of HBV DNA into host cell DNA and HBV specific protein activity interacting with liver cells. Changes in hepatocytes from inactive conditions to replicate active cells determine the level of liver carcinogenesis. The coincidence of HBV infection with exposure to other oncogenic agents such as aflatoxin can cause HCC without going through liver cirrhosis condition. The amount of HBV and its genotypic factors affect the pathogenesis of HCC.

Other causes of HCC development are because HBV DNA can integrate with human genes, which can interfere with endogenous tumour suppressor and various other gene regulators or can also facilitate the emergence of proto-oncogenic activity. This imbalance between pro-oncogenic factors and tumour suppressor signals support the growth of HCC [10].

The effect of fat metabolic disorders, inflammation also contributes to insulin resistance. Proinflammatory cytokines and transcription factors are overexpressed in fatty tissue and liver. Obesity, which is mild chronic inflammation, is a risk factor for insulin resistance and NAFLD. There are induced by excessive intake, which is an early cause of decreasing insulin sensitivity. Obesity makes fat accumulation and activates the signalling flow of c-JunN-terminal kinase (JNK) and NF-κB) which results in increased production of proinflammatory cytokines such as TNF-α and IL-6. In addition to various proteins in fatty tissue such as adiponectin and leptin, it is considered the main link between obesity, insulin resistance and related inflammatory diseases. This series of inflammation is a risk factor for the development of HCC [11].

Inflammation is a protective immune response to dangerous stimuli such as pathogens and dead cells, which are regulated by the host. Inflammation is the stimulation of tumour progression. Tissue biomarkers reflect SIRS conditions that show prognostic value. Some haematological parameters include modified Glasgow Prognostic Score (mGPS), C-reactive protein (CRP), neutrophil to lymphocyte ratio (NLR), inflammation-based index (IBI) and platelet to lymphocyte ratio (PLR) [12].

Virchow suggested the relationship between inflammation and cancer through the theory of leukocyte infiltration in tumours, which generally characterise cancer cells. Since then, more evidence and research have suggested that the inflammatory response correlates with tumour progressions such as angiogenesis and tumour invasion. Invasion and migration of tumour cells are related to inflammatory cells, including lymphocytes, neutrophils and platelets. Platelets are important effector cells in hemostasis, recently have a role in the inflammatory response. Platelets recognise and kill pathogens and release various immune mediators and endothelial cell responses [13].

PLR is the result of calculating the ratio of platelet counts to lymphocytes. As an important component of the host's defence system, lymphocytes play an important role in various types of neoplasms. Tumour-infiltrating lymphocytes affect the outcome of patients with malignancy. High platelet counts indicate a poor prognosis, where lymphocyte infiltration around the tumour is associated with a better prognosis [14].

In this study, there were significant differences in PLRs to various BCLC stages in HCC patients (Table 2) and (Figure 1). Therefore PLR can be used to determine the progressiveness of HCC. The use of BCLC as a staging tool has been used universally. BCLC staging includes radiological assessment, while PLR is simpler, using routine blood tests [15]. Zheng et al. (2017) [16] stated that increasing NLR and PLR indicates a poor prognostic in HCC patients. NLR and PLR were reliable and cheap biomarkers in clinical decisions to the treatment of HCC.

Lee et al. (2015) [17], published that HCC patients with higher platelet counts had a greater risk of extrahepatic metastasis. With a large platelet count, it can secrete large amounts of VEGF (vascular endothelial growth factor) and PDGF (platelet-derived growth factor). This is an important factor for angiogenesis, cell proliferation and tumour metastatic. Ma W. et al. (2016) [15] found that increasing PLR indicated a poor prognostic in HCC patients. Wang H. et al., (2017) [18] stated that high PLR values are associated with the presence of HBV infection, tumour size, age over 60 years, and higher levels of BCLC. PLR indicates a poor prognostic in HCC patients. Likewise, in a similar study by Lin WF et al., (2018) [19], the same results were found where high PLR had worse prognostics.

In conclusion, there is a significant difference in PLR in BCLC degrees.

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The Influence of Albumin Level in Critically Ill Children to Length of Stay and Mortality in Paediatric Intensive Care Unit

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Keywords: Albumin level; Critically ill children; Hypoalbuminemia; Length of stay; Mortality

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Abstract

BACKGROUND: The use of albumin in the critical care setting is a very controversial issue. Low serum albumin concentration in critical illness is associated with a poor outcome.

AIM: We aimed to evaluate the influence of albumin level in critically ill children to the length of stay and mortality in the Pediatric Intensive Care Unit (PICU) Haji Adam Malik General Hospital, Medan, Indonesia.

METHODS: The study used an observational method with a cross-sectional design. The population of the study was all patients with major postoperative surgery and critically ill that admitted to the PICU at Haji Adam Malik Hospital from the period of June 2008 to September 2008. The albumin level of the subjects was determined on the first day admitted.

RESULTS: The group with hypoalbuminemia (< 3 g/dL) was given albumin supplementation according to a protocol in the PICU. The group with hypoalbuminemia have an average length of stay 7.6 days (9.7%) and mortality of 12 subjects (36.4%). The group with normal albumin level have 4.7 days (5.0%) and mortality 13 subjects (37.1%). There was no significant effect of albumin level to mortality.

CONCLUSION: Albumin level did not affect the length of stay and mortality in PICU.

Introduction

Albumin is a protein produced in the liver which plays a role in blood oncotic pressure. Besides, in colloid osmotic pressure, albumin also works as a transport molecule for bilirubin, fatty acids, and medications [1].

The role of albumin in critical illness is not fully understood. There are significant differences in the role of albumin between healthy and critically ill children. Low serum albumin concentrations in critically ill patients are associated with poor outcomes [2], [3], [4]. In healthy individuals, albumin act to maintain Colloid Osmotic Pressure (COP) but is less correlated in critically ill individuals [4]. Patient with a critical illness has a low COP. Among 200 patients, those with critical illness had a COP of 19.1 mmHg. Low colloid osmotic pressure correlates with high morbidity and mortality in critically ill patients. The COP of 15 mmHg is associated with a 50% life expectancy. Administration of albumin supplementation will increase COP and prevent fatal complications such as pulmonary oedema, which in turn, develop into respiratory failure [3], [4].

The osmotic pressure can be directly measured using oncometers or Van't Hoff equation: osmotic pressure = nx (c / M) x RT, where n = number of particles in the substrate (n = 1 for plasma proteins), c / M = molar concentration of the substrate, R = constant of 0.082, T = absolute temperature [5].

Based on a descriptive study of 134 children with critical illness in the pediatric intensive care unit (PICU), the incidence of hypoalbuminemia before

admission was 57% and may increase to 76% in the first 24 hours [6].

Hypoalbuminemia often occurs in critical illness. The causes of hypoalbuminemia are complex and affected by various mechanisms such as the imbalance between albumin production and destruction, increased capillary permeability, and altered intravascular and extravascular albumin distribution [2], [4].

The relationship between hypoalbuminemia and poor outcomes has motivated clinicians to provide exogenous albumin in patients with hypoalbuminemia. Although hypoalbuminemia directly leads to a poor outcome, there is still controversy about this [4].

Material and Methods

This study used an observational method with a cross-sectional design. The study was conducted at Haji Adam Malik General Hospital from June 2008 to September 2008.

The subjects of the study were all patients admitted to PICU at Haji Adam Malik General Hospital, Medan, Indonesia, from the period of June 2008 to September 2008. The subjects were taken with the purposive sampling method.

The inclusion criteria included major post-operative surgery and critically ill, while the exclusion criteria included multiple congenital anomalies, post-operative surgery patient with length of stay less than 24 hours, burn patient and those who refuse to take part in the study.

The variables being studied include 1) albumin level in the first day admitted; 2) length of stay; 3) mortality; and 4) type of cases, with classification surgery or non-surgery case.

Before the research was carried out, ethical clearance was sought and approved by the Ethics Committee of Universitas Sumatera Utara Medical Faculty.

Results

From this study, of 68 subjects in the PICU, there were 33 patients with hypoalbuminemia (48.5%) and 35 (51.4%) with normal albumin level. From the two groups, the mean albumin level was 3.05 g/dL (0.749), with the lowest levels of 1.5 g/dL and the highest at 4.7 g/dL. The mean albumin level was 2.33 g/dL in hypoalbuminemia group and 3.62 g/dL in normal albumin group with $P = 0.001$ (Table 1).

Table 1: The characteristics of subjects in both study groups

Parameter	Hypoalbuminemia (n = 33)	Normal albumin (n = 35)
Albumin level (g/dL), mean (SD)	2.33 (0.40)	3.61 (0.44)
Gender, n (%)		
Male	23 (69.7)	24 (68.6)
Female	10 (30.3)	11 (31.4)
Age (months) mean (SD)	53.3 (49.9)	53.1 (48.8)
Weight (Kg) mean (SD)	15.6 (13.13)	14.8 (9.55)
Eid index, n (%)		
< 70	9 (27.3)	11 (31.4)
70-80	3 (9.10)	7 (20.0)
80-90	6 (18.2)	7 (20.0)
90-110	10 (30.3)	7 (20.0)
110-120	3 (9.10)	2 (5.70)
> 120	2 (6.10)	1 (2.90)
Type of cases, n (%)		
Surgical	21 (63.6)	19 (54.3)
Non-surgical	12 (36.4)	16 (45.7)

This study showed that there was a higher proportion of male than female. In the hypoalbuminemia group, the proportion of male patients was 69.7%, and female patients were 30.3%, whereas in the normal albumin group, the number of male patients was 68.6% and female patients were 31.4%.

Furthermore, the mean age, weight, and nutritional status, in this case, were assessed by EID index and type of case (surgical or non-surgical) between the two groups did not show significant differences.

In this study (Table 2), we also found that the hypoalbuminemia group needs longer treatment (7.6 days) compared to the normal albumin group (4.7 days).

Table 2: The relationship between albumin levels and length of stay and mortality

Parameter	Hypo- albuminemia (n = 33)	Normal albumin (n = 35)	P
Length of stay (days); mean (SD)	7.6 (9.77)	4.7 (5.0)	0.134
Mortality, n (%)			
Yes	12 (36.4)	13 (37.1)	0.947
No	21 (63.6)	22 (62.9)	

This is different from the results of this study where the patients were not distinguished whether as surgical and non-surgical cases, and it was found that there was higher mortality in the normal albumin group (37.1%) than the hypoalbuminemia group (36.4%) although both were not significantly different.

Table 3: The results of multivariate analysis of length of stay

Variable	B	P	95% CI
Albumin level	-3.502	0.049	-6.984; -0.020
Type of cases	6.424	0.001	-2.838; 10.010
Nutritional status	-0.146	0.808	-1.339; 1.047

In this study (Table 3 and Table 4), nutritional status had no significant association with length of stay and mortality, but the types of cases, whether surgical or non-surgical had a significant association with length of stay and mortality ($P = 0.001$). From Table 3, the results of multivariate analysis showed that the variables with the greatest contribution to the length of stay were the types of cases.

Table 4: The results of the multivariate analysis of mortality

Variable	B	P	95% CI
Albumin level	0.371	0.540	0.442; 4.775
Type of cases	-2.093	0.001	0.038; 0.403
Nutritional status		0.816	
(1)	-6.73	0.621	0.035; 7.338
(2)	-2.38	0.758	0.174; 3.578
(3)	-0.378	0.654	0.110; 3.577
(4)	0.677	0.450	0.340; 11.40
(5)	0.734	0.430	0.205; 3.511
(6)	2.331	0.322	0.123; 4.712

Discussion

Albumin has several physiological functions and has been used extensively in the field of anaesthesia and intensive care as indicated. After more than 60 years of clinical research, administration of albumin is still questionable. In critically ill patients, several pathophysiological processes such as infection, trauma, or major surgery result in an inflammatory process that eventually releases mediators such as cytokines and leukocyte activation. This will result in disruption of endothelial function, increased microvascular permeability, and extravasation of fluid (including albumin) to the tissue. Acute-phase proteins, produced by the liver, are the sign of inflammation that is used to evaluate the relationship between hypoalbuminemia and poor outcomes [7], [8].

A prospective study of routine nutritional laboratory parameters examined less than 24 hours in 105 severely ill children in intensive care unit found a prevalence of hypomagnesaemia 20%, hypertriglyceridemia 25%, uremia 30%, and hypoalbuminemia 52% [9].

Hypoalbuminemia is a marker of morbidity and mortality in children with a critical illness. A retrospective study comparing groups of patients with hypoalbuminemia and groups with normal albumin levels in patients in the PICU showed that in the hypoalbuminemia group, the length of stay in PICU was longer (8.1 days) compared to those with normal albumin levels (4.4 days). The hypoalbuminemia group has a lower life expectancy and a higher rate of organ failure [10].

A study of cardiac and non-cardiac surgery patients and kidney impairment found that hypoalbuminemia was a predictor of poor outcome, where each 10 g/dL decrease in serum albumin would increase the mortality odds by 137%, morbidity by 89%, length of stay in the intensive care unit and hospital care by 28% and 71%, respectively [11].

Hypoalbuminemia is the result of a combination of inflammation and inadequate caloric input in patients with chronic renal failure. Inflammation and malnutrition will reduce the synthesis and increase protein catabolism which may reduce albumin concentration [12].

Transcapillary albumin excretion increased by 300% in patients with septic shock and by 100% after cardiac surgery. In septic patients, transcapillary changes will occur if appropriate treatment is administered. With increased albumin flow through the capillary membrane, there is an increase in lymphatic return to the intravascular space. Albumin movement during major surgery shows a decrease in lymphatic flow and albumin concentration in the lymphatic vessels. Measurements of total circulation and albumin exchange showed a 30% reduction with major surgery (Nicholson, 2000). In a prospective study, serum albumin was shown as a predictor of the outcome of postoperative patients [13].

A Cochrane Collaboration study concluded that there is no evidence which showed that albumin reduces mortality in patients with hypovolemia compared to cheaper alternatives such as normal saline and in critically ill patients with burns or hypoalbuminemia [14].

In this study, according to the intensive care unit protocol, all patients in the hypoalbuminemia group received albumin substitution as needed. Hypoalbuminemia is a frequent phenomenon in critical illness. Treatment focuses on the main causes of hypoalbuminemia rather than administering albumin. The results of several meta-analyses of albumin administration in hospitalised patients were inconsistent [15].

The role of albumin in critically ill patients is not supported by scientific evidence. Hypoalbuminemia correction does not have a significant advantage; treatment is aimed at basic diseases to treat hypoalbuminemia [13]. Albumin is recommended according to the appropriate indications for patients in the intensive care unit [8].

Intravenous albumin is appropriate for patients with cirrhosis with ascites, kidney failure, and hepatorenal syndrome awaiting liver transplantation. In patients with nephrotic syndrome who do not respond to standard therapy, severe symptomatic hypovolemia, administration of intravenous albumin and diuretics might be considered [16]. From an in-vitro study of sepsis patients, it was shown that albumin administration did not affect vascular permeability. The administration of 200 ml of 20% albumin was not significant in reducing microvascular protein leakage [17]. From a review of a randomised clinical study, administering low dose hyperoncotic albumin for hypovolemic resuscitation has several advantages such as reducing morbidity, renal impairment, and oedema [18].

Addition of albumin in parenteral nutrition solutions is also not recommended. It is said that fatal complications may occur compared to its benefits. Possible complications include infection, incompatibility, and chemical and physical instability

[19]. It was stated that the administration of albumin would cause a decrease in life expectancy in patients with critical illness [20]. This is in contrast to a study of a meta-analysis of randomised controlled trials that did not find the effect of albumin on mortality [21].

The study also found no difference between the hypoalbuminemia and normal albumin groups to mortality (P = 0.947). Based on previous studies and based on the results of our study, albumin levels do not affect the length of stay and mortality of patients in the intensive care unit.

In this study, there was no effect of albumin levels on the length of stay and mortality of patients in the pediatric intensive care unit. The type of cases has a significant association in the length of stay and mortality.

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The Association between CD-4 Level, Stress and Depression Symptoms among People Living with HIV/AIDS

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Abstract

BACKGROUND: The lives of individuals diagnosed with HIV and the subsequent illness, AIDS, were often chaotic because these individuals deal with the physical, emotional, and interpersonal sequelae of this illness. Depressive symptoms and stress were common and impact on functioning, quality of life, and health status, highlighting the importance of diagnosis and treatment of patients with HIV infection. Psychiatric clinical practice and rating scales have come to play an ever-increasing role both in determining specific symptoms and diagnosing an individual condition. Descriptive reports of psychiatric morbidity among those with HIV infection, that relied primarily on self-report rating scales, described high rates of symptomatic depression and stress.

AIM: This study aimed to determine the proportion of depression among people living with HIV/AIDS using the BDI-II and PSS to determine a relationship between symptoms of depression and stress with CD4 counts.

METHODS: This study was a numerical correlative analytic study with a cross-sectional study approach that assessed the correlation between stress, depression and CD4 level in people with HIV/AIDS that were receiving ARV therapy in the Voluntary Counselling Test (VCT) Polyclinic of General Hospital Haji Medan by recruiting 46 subjects. CD4 level was examined in the Pramita Medan laboratory.

RESULTS: When analysis of BDI-II level and CD4 scores were done, a significant correlation was found ($P < 0.05$). The strength of the relationship between the BDI score and the CD4 score was 0.548 revealing a positive correlation with moderate correlation strength

CONCLUSION: We have shown a significant relationship between depression, stress and CD4 level among people with HIV/AIDS in Medan, Indonesia. Psychological distress may affect the immunity in infected people, leading to the disease progressivity.

Introduction

HIV/AIDS was first launched in 1981 in California [1]. According to the United Nations Program on HIV/AIDS (UNAIDS) data, it is estimated that in 2016 the total number of people living with HIV was around 36.7 million people with new HIV infection rates reaching 1.8 million. The report on the highest number of HIV prevalence in the world comes from Africa, with an estimated 5.1 million people. UNAIDS data in 2016 also showed the prevalence of people living with this infection in Indonesia reaching 620,000 with a new infection rate of 48,000 [2].

Based on data in 2014, HIV infections people were most prevalent in the heterosexual group (61.5%), followed by Injection Drug Users (IDU, 15.2%) and homosexuals (2.4%), and unknown risk factors (17.1%) [3].

While report on the situation of the development of HIV/AIDS and Sexually Transmitted Infectious Diseases (STID) in Indonesia between January-March 2017, the highest number of HIV infections according to the risk factors was in men like men (MLM) (28%, *n* = 2,867), followed by heterosexual risk factors (24%, *n* = 2,448) [4].

HIV is a cytopathic virus classified in the Retroviridae family, subfamily Lentiviridae, Lentivirus genus. Based on its structure HIV belongs to the family of retroviruses, including the RNA virus with a molecular weight of 9.7 kb (kilobases) [5]. Human Immunodeficiency Virus (HIV) is a retrovirus that infects cells and the immune system [6].

Acquired Immune Deficiency Syndrome (AIDS) is a collection of symptoms or diseases caused by decreased immunity due to infection with HIV which belongs to the retroviridae family. AIDS is

the final stage of HIV infection. People with HIV and AIDS abbreviated as ODHA in Bahasa (Indonesian Language) are people who have been infected with HIV [7]. The course of HIV infection, CD4 T-lymphocyte count, number of viruses and clinical symptoms are classified into 3 phases: acute infection phase, latent phase of infection, chronic infection phase [5].

Psychosocial factors can influence the disease process with various biological parameters that reflect the progression of HIV infection, triggering a biological response through the limbic axis of the hypothalamic-pituitary-adrenal and sympathetic nervous system. Psychosocial factors with a high BDI-II score, high neurohormone factors such as cortisol and norepinephrine (NE) will significantly affect the progression of HIV infection to AIDS, resulting in CD level and an increase in the number of viruses [8], [9].

The Beck Depression Inventory is a self-assessment questionnaire for depression. BDI-II was developed as a concomitant level of validity and discriminant is acceptable among patients with HIV with an acceptable level of internal consistency. Analysis of the Main Components of BDI-II found three components with moderate correlation, one that assessed cognitive dimensions, the second assessed affective dimensions and the third with automatic dimensions of depression. While HIV and depression both have an automatic component, depression has affective, cognitive and automatic manifestations, with affective symptoms emerging as a disease while cognitive symptoms appear later, right for the assessment of the initial presentation of depression. Because of the symptoms of HIV infection and depression, it may be a symptom that is caused solely by depression [10].

In HIV infection, fatigue is formulated with the emergence of pathological, diffuse and extensive cell apoptosis which mainly attacks T lymphocytes so that the total and CD4 lymphocyte counts will gradually decrease. Psychological stress also triggers an increase in cortisol and catecholamine, which results in a decrease in the secretion of IL-1 and IL-2 by macrophages and a decrease in antibody formation/decrease in body resistance. The low IL-1 results in a decrease in T-helper monocytes and lymphocytes resulting in a decrease in antibody formation, and a decrease in phagocytic activity of inflammatory cells against germs that cause infection, thus individuals become susceptible to infection. While the increase in catecholamine results in suppression of T-helper cell activity and function. So, with the decrease in immunoglobulins, lymphocyte activity, and NK cells can be used as an indicator of psychological stress followed by suppressing the activity of the ability of macrophages and killer cells in the lysis of the virus [11].

Life stress can dramatically disturb people and their ability to function. Many studies have shown that the onset of episodes of depression is etiologically related to stressful life events. Stress can occur which causes induction of depressive symptoms in HIV-positive people. Roberts et al. conducted a cross-sectional study exploring the impact of stressful life events on emotional stress and life-threatening HIV-positive people. The relationship between acute life events and perceived stress affects the psychological state of dysfunctional attitudes, self-esteem, and neuroticism [12].

Based on the background mentioned above, the present study aimed to evaluate the relationship between CD4, stress, and depression in HIV/AIDS.

Material and Methods

Participants and Procedure

This study was a numerical correlative analytic study with a cross-sectional study approach that assessed the correlation between stress, depression and CD4 level in people with HIV/AIDS that were receiving ARV therapy in the Voluntary Counselling Test (VCT) Polyclinic of General Hospital Haji Medan by recruiting 46 subjects. CD4 level was examined in the Pramita Medan laboratory. The study period was from January to February 2019. Patients were recruited consecutively. Inclusion criteria included people with HIV/AIDS based on the WHO criteria, aged 20-50 years, had been diagnosed less than four years, received ARV therapy > 6 months, agreed to participate and could be interviewed, with last education at least Elementary School or equivalent. Exclusion criteria were people who had a history of mental disorders.

Beck's Depression Inventory-II

BDI-II is a 21-item scale measuring depression manifestations carried out by participants, with high internal consistency. It took about 10 minutes to complete. Each item consists of four statements (rated 0-3). Describes increasing the severity of the disorder in question. The interpretation of the results is as follows: a score of 5-9 shows normal ups and downs, 10-18 mild to moderate depression; 19-29 moderate to severe depression and 30-63 major depression [13].

Perceived Stress Scale

The Perceived Stress Scale (PSS) is used to measure psychological pressure. This instrument is a questionnaire consisting of 14 items that assess thoughts and feelings related to distress. Participants are asked on a 5-point scale to show how often they

have the same thoughts or feelings (0 never, 4 very often) [12].

CD4 T-lymphocytes

CD4 T lymphocytes are the main target for HIV infection due to viral affinity for CD4 surface markers. CD4 T-lymphocytes coordinate important immunological functions; loss of these functions shows a progressive decrease in the immune response. The results of CD4+ T-lymphocyte examination are used as the basis for clinical management and therapy of HIV-infected people. There are three categories of CD4 T-lymphocytes as follows: category 1 (500 cells/µl), category 2 (200-499 cells/µL), and category 3 (< 200 cells/µl) [13].

Infecting HIV T helper lymphocytes or via CD4 antigens. These infected lymphocytes then lysis when the new virion is released or removed by the cellular system immune. In progressive HIV infection, the T-lymphocyte CD4 is in number downhill. The absolute number of CD4 is an important measurement for predict, determine degrees, and monitor progression as well as response to treatment in HIV infection. Check the number of viruses' complete laboratory inspection for disease monitoring. The size inversely proportional to CD4, so the number of CD4 and the number of viruses immediately shows the patient's immune status. This is useful for determining a diagnosis, prognosis, and management of medication in patients who are infected with HIV [14].

Data Analysis

Subjects who fulfilled the criteria were asked for consent to take part in the study. They completed the demographic data and filled in the BDI-II and PSS questionnaires. Blood samples were taken to determine the CD4 level of the subjects.

Measurement of CD4 level was carried out in a private laboratory in Medan using flow cytometric.

Flow cytometry is a method that can be used for identifying the surface characteristics of each cell with ability separating cells that are in a suspension according to the characteristics each automatically through a gap penetrated by a beam of laser light. The method of flow cytometry continues to grow in line with electrical development of computers and reagents, including their use of a monoclonal antibody. Until now, measurements with flow cytometry use fluorescence labels, in addition to measuring cell size, too can detect cell wall markers, intracellular granules, infra-structure cytoplasm, and the cell nucleus.

All data were processed with SPSS. Before data analysis, normality test using the Shapiro-Wilk test was carried out because numbers of samples were less than 50. Then data were analysed to obtain a correlation value (r). If data were normally distributed, data was going to be analysed using the Pearson Correlation test. If data was not normally distributed, data analysis was performed using the Spearman correlation test. A value of $p < 0.05$ was considered significant.

Ethical Consideration

This study was approved by the Ethical Committee of Research, Faculty of Medicine Universitas Sumatera Utara (ID 215/KEPK FK USU-RSUP HAM/2018). Informed verbal consent was ensured before administration of the questionnaire. The beneficiaries were taken into consideration, and subjects were not coerced to participate in the study and confidentiality was maintained.

Results

We enrolled 46 patients in the study. The demographic description of patients was presented in Table 1. Variables with categorical scales were presented in frequency and proportion, and variables with numerical scales are presented in mean and standard deviation.

Table 1: Distribution of Demographic Characteristics of Participants

Variable	n	%
Gender		
Male	28	60.9
Female	18	39.1
Age (year)		
20-30	21	45.7
31-40	20	43.5
41-50	5	10.9
Education		
Junior High School	7	15.2
Senior High School	21	45.7
Undergraduate	18	39.1
Marital Status		
Single	27	58.7
Married	19	41.3
HIV stage		
I	19	41.3
II	22	47.8
III/IV	5	10.9
Transmission		
Sex	21	45.7
Drugs	25	54.3

Table 1 showed that the proportion of the sex of patients was male (60.9%) and female (39.1%). The age distribution was 20-30 years (45.7%), 31-40 years (43.5%), and 41-50 years (10.9%).

The proportion of patients with junior high school education, high school education, and undergraduate education were 15.2%, 45.7%, and 39.1%, respectively. The majority of patients also went into HIV stage I (41.3%) and stage II (47.8%), while the remaining were classified as stage III-IV (10.9%). HIV transmission occurred through sex (45.7%) and drugs (54.3%).

Table 2: Distribution of stress (PSS scores) and depression (BDI-II Scores) among HIV Patients

Variable	Average	SD	Min	Max
BDI-II	46.35	16.60	24	63
PSS	30.63	8.12	15	40

Table 2 showed that the PSS scores with a mean of 30.63, the standard deviation of 8.12, a minimum value of 15, and a maximum value of 40. The BDI-II score with a mean of 46.35 and a standard deviation of 16.60, a minimum value of 24, and a maximum value of 63.

Table 3: Distribution of CD4 among HIV Patients

Variable	Average	SD	Min	Max
CD4	345,30	104.67	158	564

Table 3 showed that CD4 counts with a mean of 345.00, the standard deviation of 104.46, the minimum value of 158, and the maximum value of 564.

The Shapiro Wilk Test showed that the CD4 score was normally distributed. Therefore a correlative test of baseline data used the Pearson Correlation test. When analysis of BDI-II level and CD4 scores were done, a significant correlation was found ($P < 0.05$). The strength of the relationship between the BDI score and the CD4 score was 0.548 revealing a positive correlation with moderate correlation strength ($r = 0.5 - < 0.6$).

The results of the Pearson Correlation test for PSS level and CD4 scores obtained a *P*-value of < 0.05, showing a significant correlation between PSS and CD4 score. The strength of the relationship between PSS scores and CD4 was 0.45 meaning a positive correlation with moderate correlation strength ($r = 0.5 - < 0.6$).

Discussion

Depression can affect behaviour and contribute to adherence to therapy so that HIV infection damages immune function. A study by Moosa (2005) recruiting forty-one people aged 18 years or over from an HIV outpatient clinic showed that about 56% of patients in this study had significant depressive symptoms (BDI > 10). However, this study did not find a significant correlation between BDI and CD4 scores ($r = 0.274$, $p > 0.05$) [13].

In this study, we also showed similar findings as described previously by Moosa et al., where BDI-II was an appropriate tool to determine depression in these patients. Nevertheless, our study was able to show a positive correlation between BDI-II scores and CD4 scores. This was in line with a longitudinal study conducted by Ironson et al., (2015) of 177 individuals with HIV/AIDS that underwent ARV treatment. The study showed that psychosocial and neurohormone influences the predictors of the progression of HIV/AIDS infection. Psychosocial factors determined by a high BDI score, high neurohormone factors such as cortisol and norepinephrine significantly affected the progression of HIV infection to AIDS by allowing a significant decrease in CD4 level and an increase in viral load [9].

In 2007, a study by Remor et al., at the Outpatient Clinic at the Madrid Teaching Hospital assessed psychological distress as measured by the PSS was associated with a decrease in CD4 level in 59 men and 41 women living with HIV/AIDS within six months. The study showed that the perceived stress was significantly ($p = 0,0001$) associated with a decrease in CD4 level in people living with HIV, i.e. every increase in 1 point PSS score (score between 3 and 43), is followed by a decrease in the score of 4.82 of CD4 counts after observation for six months [15]. We did not follow the patients in our study. Nevertheless, we have shown that the PSS score had a moderate correlation with the CD4 level among our patients.

In conclusion, we have shown a significant relationship between depression, stress and CD4 level among people with HIV/AIDS in Medan, Indonesia. Psychological distress may affect the immunity in infected people, leading to the disease progressivity. Routine screening of psychosocial status in these population is important to determine their mental health status and allow early management and prevent disease progression.

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The Relationship of Physical Activity and Obesity with the Incidence of Hypertension in Adults Aged 26-45 Years in Medan

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Abstract

BACKGROUND: The incidence rate of hypertension is increasing in Indonesia concerning unhealthy behaviours such as unhealthy physical activity and eating pattern which trigger obesity. In Indonesia, the prevalence of hypertension in > 18-year-old people was 34.1% in 2018.

AIM: The objective of the research was to find out the relationship between physical activity and obesity with the incidence of hypertension in adults (26-45 years old) in Medan.

METHODS: The research was done in Medan, using a case-control study design. The samples were 150 hypertension patients, taken by using proportional allocation: 75 of them were in the case group and the other 75 of them were in the control group. The data were gathered by conducting interviews, measurement, and questionnaires and analysed by using simple logistic regression test.

RESULTS: The result of the research showed that there was significant relationship of physical activity (p = 0.000; OR = 3.6; 95% CI, 1.802-7.270) and obesity (p = 0.000; OR = 4; 95% CI, 2.030-7.900) with the incidence of hypertension in 26-45-year-old respondents.

CONCLUSION: Make a habit of a healthy lifestyle in their daily life by doing physical activity regularly and good eating pattern to forestall hypertension.

Introduction

High blood pressure or hypertension is a global health problem. All countries in the world from high, middle and low income have the same burden in overcoming hypertension. World Health Organization statistics show the prevalence of hypertension in the world for those aged 18 years, or older is 22.1%, with a ratio of 24.1% in men and 20.1% in women [1]. This organisation reports that of the 56.9 million deaths worldwide, 54% of them are due to non-communicable diseases with the most common causes being ischemic heart disease and stroke [2]. Although most of this book addresses hypertension in the United States and another developed country, it should be noted that cardiovascular diseases are the leading cause of death worldwide, more so in the economically developed countries but also the developing world [3].

Hypertension is also referred to as "the silent killer" because most cases do not manifest any symptoms or signs even though blood pressure has far exceeded normal. This can last for years until eventually, the patient falls into an emergency such as a heart attack, stroke or kidney damage [4]. Hypertension is one of the important factors as a trigger for non-communicable diseases which is currently the number one cause of death in the world [5]. Based on data from Riset Kesehatan Dasar 2018, hypertension is the most non-communicable disease in Indonesia with the prevalence rate of 34.1 [6], which has increased from the prevalence rate reported by Riset Kesehatan Dasar 2013 (25.8%) [7].

The course of the disease (progressive hypertension) begins with high blood pressure starting from pre-hypertension in patients with asymptomatic age 10-30 years (marked increase in cardiac output), continuing to become early hypertension at the age of 20-40 years (increased resistance or peripheral

vascular power) then the age of 30-50 years develops into hypertension, in the final phase that is at the age of 40-60 years into hypertension with complications [3].

Regular physical activity is useful to regulate body weight and strengthen the heart and vascular system. Lack of physical activity can result in someone suffering from hypertension. Sedentary lifestyles are risky behaviours for diseases such as blockage of arteries, heart disease and affect life expectancy. Based on data Riset Kesehatan Dasar (2013), it appears that North Sumatra is one of the provinces with the obesity category above the national average both in men and women, while the proportion of physical activity that is lacking is 26.5% [7].

Physical activity is one of the risk factors for hypertension. The proportion of sedentary behaviour ≥ 6 hours is greater for women, people with low education, not working, living in urban areas, and residents with a quintile of ownership indices that are higher than those who are lower [7]. According to FAO / WHO / UNU (2001), the amount of physical activity carried out by a person for 24 hours is expressed in physical activity level (PAL). PAL is determined by the formula [8]:

$$\frac{\sum(PAR \times W)}{24}$$

PAL = Physical Activity Level

PAR = Physical Activity Ratio (energy cost of activity)

W = Time allocations (hours)

Physical Activity Level (PAL) is divided into three categories, namely mild, moderate and severe activity. Mild physical activity (sedentary lifestyle) has PAL values between 1.40-1.69. Someone with light activities uses a vehicle for transportation, does not exercise and tends to spend time only for activities carried out just by sitting and standing, with a little body movement. Moderate active activity (active or moderately active life) has a PAL value of 1.70-1.99. Someone with a moderate level of activity does not need large energy, but the energy needs of this activity are higher than light activities. Heavy physical activity (vigorously active lifestyle) has a PAL value of 2.00-2.40. Heavy activity is carried out by someone who works hard for a long time [8].

Obesity is a chronic condition that is the accumulation of fat in the body so that it exceeds the limit for good health. Obesity increases the risk of other diseases, such as diabetes mellitus and high blood pressure. Adult body weight is measured by calculating body mass index (BMI) with the formula of body weight in kilograms divided by height into units of meters squared [9]. The obesity criteria in this study used the criteria of BMI value of > 27 kg/m^2 [10].

Preliminary surveys are carried out at the Medan city health office by recapitulating hypertension cases according to age < 45 years in 2017, indicates that of the 39 Public Health Centers, a high number of hypertensive patients are in Community Health Centers of Mandala, Medan Deli, Amplas, Medan Johor and Teladan. In terms of the proportion of hypertension in the age < 45 years in the health office of Medan City in 2016, there was a figure of 11% compared to 12% in 2017 [11].

This study aimed to analyse the relationship of risk factors in the form of physical activity and obesity with the incidence of hypertension in adults aged 26-45 years in Medan in 2018.

Material and Methods

Types of the Study

The study used the observational analytic method with a case-control design.

Subjects

Determination of the selected location was carried out by stratified random sampling by dividing public health centres based on the high or a low number of managed cases. The study was conducted in Medan City by taking samples from the public health centres of Mandala, Amplas, Tuntungan and Padang Bulan Selayang dua. The consideration in the selection of study locations is that the public health centres represent high and the low numbers of the hypertensive case at aged ≤ 45 years in Medan City.

Population in this study where all hypertensive patients aged 26-45 years in four selected public health centres in Medan city. The sample in this study consisted of cases and controls. Cases are some patients with hypertension aged 26-45 years who were recorded in the registration book of the four selected Public Health Centers in Medan City and fulfilled the inclusion criteria, among others, namely not complicated by stroke, able to communicate well, and willing to participate in research. The controls were a portion of the population without hypertension aged 26-45 years and residing in the area of selected Public Health Centers in Medan City. This research has received approval from the Health Research Ethics Commission of the University of North Sumatera Nursing Faculty with numbers. 1778 / IV / SP / 2019.

The sample size was determined by the case-control study formula by calculating the sample size for hypothesis testing of the odds ratio for the difference of two proportions with a confidence level of 95% by which the sample consisted of 75 cases and 75 controls were obtained. The total sample in this study was 150 respondents [12].

Gathering of Data

Primary data were obtained through interviews in the form of filling out questionnaires of recalling 24-hour physical activity, and measurements of Body Mass Index (BMI). Secondary data were obtained from the register of public health centres. Data were analysed descriptively and analysed statistically using simple logistic regression.

Results

Characteristics of Respondents

Age distribution of respondents in the dominant case and control groups of 36-45 years, and by gender, the majority were female both in the case and control groups. Most respondents have high school level education and the majority of respondents by employment, are housewives. Respondents were dominantly ethnic Batak.

Table 1: Distribution Characteristics of Respondents in Cases and Controls at the Age of 26-45 years in Medan in 2019

Characteristic of Respondent	Hypertension (case)		Non-Hypertension (control)	
	n	%	n	%
Age				
26-35	9	12	24	32
36-45	66	88	51	68
Total	75	100	75	100
Sex				
Male	21	28	17	23
Female	54	72	58	77
Total	75	100	75	100
Employment				
Civil Servant / Army / Police	13	17	12	16
Private employed / Self-employed / etc	28	37	25	33
Housewife	34	38	38	51
Total	75	100	75	100
Level of Education				
Midle-High School (SMP-SMA)	53	71	34	47
Diploma (D1-D3)	13	17	26	35
Bachelor/Master (S1-S2)	9	12	15	23
Total	75	100	75	100
Ethnic				
Batak	43	57	55	73
Jawa	24	32	11	15
Minang, Melayu, dll	8	11	9	12
Total	75	100	75	100

Physical Activity

Based on the data in Table 2 showed that of the 75 people who suffer from hypertension 57 (76%) people have mild physical activity and 18 (24%) have moderate and severe physical activity. While from 75 control groups, it was seen that 35 (46.6%) people had mild physical activity and 40 people have moderate and severe physical activity. Respondents with mild activity were more commonly found in the case group, while those with moderate to severe activities were found more in the control group

Obesity

Based on Table 2 below, it is known that most patients with hypertension are obese (68%), whereas, in the control group, there are 65% of subjects with obesity. Respondents with obesity were found more in the case group compared to the control group.

Table 2: Frequency Distribution of Physical Activity and Obesity of Respondents of Adult Aged 26-45 years in Medan in 2019

Variable	Hypertension (case)		Non-Hypertension (control)	
	n	%	n	%
Physical activity				
Mild	57	76	35	46.6
Moderate	16	21,3	32	42.6
Severe	2	2,6	8	10.6
Total	75	100	75	100
Obesity				
Obesity	51	68	49	65
Non-Obesity	24	32	26	35
Total	75	100	75	100

Relationship between Physical Activity and Hypertension

Based on the simple logistic regression test, it is determined that there was a relationship between physical activity with the incidence of hypertension (P = 0.000 and OR = 3.619, 95% CI: 1.802-7.270).

Relationship between Obesity and Hypertension

The result of simple logistic regression test it was determined that there was a relationship between obesity with the incidence of hypertension in adults aged 26-45 years in Medan (P-value = 0.000 and OR= 4.005, 95% CI: 2.030-7.900) as it was indicated in table 3.

Table 3: Relationship of Physical Activity and Obesity with the Incidence of Hypertension in Adults aged 26-45 years in Medan in 2019

Variable	Hypertension (case)		Non-Hypertension (control)	
	N	%	n	%
Physical activity				
Mild	57	76	35	46,6
Moderate-Severe	18	23	40	42,6
P = 0.000*		OR = 3.619	95% CI. 1.802 - 7.270	
Obesity				
Obesity	51	68	49	65
Non-Obesity	24	32	26	35
P = 0.000*		OR = 4.005	95% CI. 2.030 - 7.900	

Discussion

Characteristics of respondents indicated that the age group of 36-45 years had more hypertension case, compared to the age group of 26-35 years. This is in line with the theory that as a person ages, the risk of developing hypertension is greater. This study is in line with Prasetyo Reseacrh (2017) in Sibella Surakarta health center at young adults (18-40 years) which shows that the highest age of the case group was distributed at the age of 36-40 years, namely the case of 19 people (45.2%) [13].

Respondents in this study were dominated by

high school educated women with the type of work as housewives. This study is in line with basic health research (2013), where hypertension sufferers were more prevalent in women and worked as housewives [7].

The results of the statistical test showed that there was a relationship of physical activity with the incidence of hypertension with a value of OR 3.619. It follows that people with mild physical activity have a risk three times more likely to suffer from hypertension compared to people with moderate and severe physical activity. This is in line with the study by Harahap (2017) which showed a significant relationship between physical activity and the incidence of hypertension [14]. Similarly, Rihiantoro's research at the Tulang Bawang community health centre Lampung, showed that there was a relationship between physical activity and the incidence of hypertension with an OR value of 2.255 (95% CI: 1.245-4.084). This means that respondents who did mild physical activity were 2.26 times more likely to suffer from hypertension than respondents who did moderate and severe physical activity [15]. This study is also in line with a longitudinal study by Daniela Schmidt and colleagues (2006) that American adults showed that there was a significant effect of moderate to high physical activity, after adjusting for age and sex, on the risk of death [16].

At the same time as the evidence for protection from cardiovascular diseases and all-cause mortality by regular physical activity has become incontrovertible, most people in all industrialised societies are becoming a less physical activity in their daily lives, spending more and more time in sedentary activities [3].

Theoretically, physical activity greatly influences the stability of blood pressure. Heart rate tends to be higher in people who are not active in activities. The harder the heart muscle pumps blood, the higher the blood pressure that burdens the arterial wall so that peripheral resistance causes blood pressure to rise. Lack of physical activity can also increase the risk of being overweight by thereby causing an increased risk for hypertension [17].

The results of statistical tests in this study indicated that there was a significant relationship between obesity with the incidence of hypertension with a value of OR = 4.005 (95% CI: 2.030-7.900). Thus, it can be said that the probability of occurrence of hypertension in adults aged 26-45 years in Medan which obesity, is four times greater than those who are not obese. This is in line with the study of Rohkuswara (2016) in the city of Bandung with AOR = 1.681 and P-value = 0.031 [18]. Obesity has become an epidemic today. According to the American Heart Association (AHA) and the American Physiological Society, about 70% of adults in the United States suffer from obesity. Estimated risk shows that as many as 65% to 70% of the incidence of essential hypertension is related to obesity, although population-based longitudinal studies show a somewhat lower number. Obesity is closely related to hypertension, with an increase in systolic blood pressure (SBP) of 6.5 mmHg for each 10% increase in body weight [20].

The nature of modern life, with more caloric intake, particularly from fast food and less physical activity, engenders more obesity, which is now a worldwide epidemic. Any degree of weight gain, even to a level that is not defined as overweight, is associated with an increased incidence of hypertension and, even more strikingly of type two diabetes. According to Willett et al., in Kaplan (2006) said in along-term follow up of 85,000 nurses, the incidence of hypertension increased threefold and the incidence of diabetes more than six-fold at an initial BMI of 26 as compared to at an initial BMI of 21 [3].

In conclusion, there is a relationship between Obesity and physical activity with the incidence of hypertension at the age of 26-45 years in Medan. To prevent early hypertension, it is recommended to make a habit of a healthy lifestyle in their daily life by doing physical activity regularly and good eating pattern to forestall hypertension.

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The Body Weights' Follow Up Before and After 6 Months Therapy of Oral Anti-Tuberculosis Therapy in Children in Medan, Sumatra Utara

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Abstract

BACKGROUND: Tuberculosis remains an important issue of children health, particularly in developing countries. Body Weight is one of the tuberculosis symptoms and used to identified children tuberculosis scoring in Indonesia.

AIM: The study aims to get an overview of body weight and body weight increment during oral anti-tuberculosis in Medan, Sumatra Utara.

METHODS: Medical records of children with tuberculosis in the Haji Hospital of Sumatra Utara located in Medan during January 2018 till July 2018 were compiled for the children characteristic, body weight before and after oral anti-tuberculosis treatment.

RESULTS: There were 99 children medical records included in the study, 42.4% children 1 to 5 years old treated as tuberculosis. At the early treatment, many children were in severe malnutrition (85%). However, after 6 months of tuberculosis treatment, there were many children (78%) got their body weight increment.

CONCLUSION: Body weight is an important sign and symptom of children with tuberculosis.

Introduction

Tuberculosis remains the global challenge in the health sector, particularly for children. Indonesia is the second-largest country (after India) which get burdens of Tuberculosis in the world. In Indonesia from 2013 to 2017, children under 14 years old who got tuberculosis were ranging from 8 to 10,1 percent [1]. Body Weight (BW) is one of the tuberculosis symptoms and one of the indicators as Tuberculosis scoring used to identified children tuberculosis. Tuberculosis scoring is widely applied by paediatricians since its inception in 2008, particularly in resource-limited facilities [2].

The study aims to get an overview of body weight and body weight increment during oral anti-tuberculosis in Medan, Sumatra Utara.

Material and Methods

This is a hospital-based retrospective study where medical records of children (< 18 years old) diagnosed and treated as tuberculosis during January 2018 till July 2018 were chosen and compiled. The Haji Hospital of Sumatra Utara was purposely selected as this is the type B hospital owned by the provincial government and serve as one of referring hospitals for the district and another municipalities hospital in Sumatra Utara. Medical records show tuberculosis and HIV, cancer, organ transplantation, diabetes or chronic diseases (heart and kidney) were excluded. The body weight in each medical record was noted at the beginning of the tuberculosis treatment and the end of 6 months of therapy. The applicable curve (WHO or CDC) was applied to bodyweight per age accordingly.

Results

During the study period, there were 128 children treated as tuberculosis; there are 99 medical records included in the study sample. There are 42.4% children age 1-5 years old treated as tuberculosis and 33.3% age 6 – 10 years old (Table 1).

Table 1: Characteristic of respondent

Age	N	%
< 1 year old	2	2
1-5 years old	42	42.4
6-10 years old	33	33.3
11-15 years old	17	17.2
16-17 years old	5	5.1
Total	99	100

Mainly the children treated as tuberculosis are male (53.5%) Table 2.

Table 2: Sex distribution

Sex	N	%
Male	53	53.5
Female	46	46.5
Total	99	100

At the early of treatment started, many respondents suffer to malnutrition. There are 84 children had severe malnutrition (85%) and 3 children (3%) had mild malnutrition (Table 3).

Table 3: Bodyweight per age of children at the early stage of OAT treatment

BW / Age	N	%
Normal	12	12.1
Mild	3	3
Severe	84	84.8
Total	99	100

At the end of OAT therapy, there were 77 children (78%) showed BW increment, 16 children (16%) keep normal and there were 6 children (6%) had lower BW than before (Table 4).

Table 4: BW comparison before and after OAT therapy

BW comparison	N	%
Similar	16	16.2
Increment	77	77.8
Lower	6	6.1
Total	99	100

Discussion

This study found that the age of children who got tuberculosis was from 1 to 5 years old and dominated by male. Children can present with TB disease at any age but most commonly, in TB-endemic countries, between 1-4 years. Pulmonary TB is the commonest type of TB in children [3]. In most countries, the adult male seems to be more susceptible to tuberculosis (TB) than adult female [4]. This study shows that boys are more frequent to have tuberculosis than girls but this result is too early to conclude. Sex difference of tuberculosis is so far contributed after puberty [4]. Malnutrition and tuberculosis are the two problems that interact with each other, particularly in the underdeveloped regions of the world [5]. Tuberculosis mortality rates are equal to their economic level. Similarly, nutritional status is significantly lower in patients with active tuberculosis compared to healthy controls [5].

For any infection, including tuberculosis infection, there is a complex interaction between the host response and the virulence of the organisms, which modulates the overall metabolic response and the degree and the pattern of tissue loss. Children with tuberculosis, a reduction in appetite, nutrient malabsorption, micronutrient malabsorption and altered metabolism leads to wasting [5]. Anorexia is also a contributing factor for wasting in tuberculosis [5]. This study found that more than 85% of respondents' body weight (BW) per age were under normal. This is mainly due to the children intake of food was insecure as part of the household food insecurity. A case-control study from India concluded that household food insecurity is part of the risk factors associated with tuberculosis [6].

However, how the child nutritional status is mainly back to normal after the tuberculosis treatment course is poorly understood. This study found that more than 75% of respondents got their body weight increment at the end of tuberculosis therapy, although there were 6% of respondents got lower body weight. It is assumed that the increment is due to the tuberculosis therapy which causes a rapid drop in bacillary load and improves nutritional status [7]. Unfortunately, this study was unable to find an in-depth assessment of food intake before and during tuberculosis treatment.

Leptin is thought to be a mediator in the complex process between TB, nutrition status and host immune response. Leptin level increment in the body is correlated to increment energy, protein and fat intake which is increased after administration of oral anti-tuberculosis therapy either in intensive phase or maintenance phase [8]. Thus, the more energy intake will increase body weight during and after tuberculosis therapy.

In conclusion, this study confirmed that the body weight and body increment is still an important sign and symptom of tuberculosis before and after treatment. However, how the bodyweight plays the role of tuberculosis infection and its connection vice versa, is poorly understood.

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Absolute Neutrophil Count Levels among Degree of Differentiation and Tumor Location in Colorectal Cancer Patients in Medan

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Keywords: Absolute Neutrophil Count (ANC); Colorectal carcinoma (CRC)

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Abstract

BACKGROUND: Absolute Neutrophil Count (ANC) recently a derived score composed of white blood cell and has been evaluated in a large number of the malignancy.

AIM: The aim of this study was to investigate ANC levels among degree of differentiation and tumor location in Colorectal Cancer (CRC) in Medan.

METHODS: This study was a cross-sectional analytical study on eighty consecutive patients with CRC. Data collection was obtained from the medical record of the patient at Adam Malik General Hospital, Medan, Indonesia. Inclusion criteria were included male or female aged ≥ 18 years old, the patient with CRC, blood test especially ANC, and the patient with histopathology test. The exclusion criteria were defined as a patient with chemotherapy, HIV, and the patient with sepsis. Data were analyzed with SPSS for window version 21.

RESULTS: here were Rectal cancer 49.4%, Left side colon cancer 43.2%, and Right-side colon cancer 6.2%. Histopathological findings well differentiated 40.7%, moderate differentiated 32.1%, and poorly differentiated 25.9%. This study showed that no significant differences between ANC and location of the tumor ($p = 0.951$), but ANC level was significant in histopathological findings ($p = 0.0001$).

CONCLUSION: ANC increased in poorly differentiated, and location of the tumor is left side colon cancer.

Introduction

Colorectal Carcinoma (CRC) is a malignant tumor from colon and rectum [1]. CRC is the human tumor which equally affects both men and women. It belongs to common, solid tumors and is the third most common in men. By mortality, it ranks as fourth after lung, stomach, and liver. The highest frequency is recorded in the highly industrialized countries, such as countries of North America, Australia, and New Zealand. The incidence of CRC is rapidly increased including in Indonesia, making it the second most common of malignancies [2].

The population in Indonesia is more than 235,000,000 and the age-incidence rates per 100,000 for CRC by gender was 19.1 for male and 15.6 for female [3]. As one of the leading causes of cancer death in developed countries, much interest has grown in research in the development of biomarkers to improve the diagnostic process.

The tumor microenvironment is very important about the preservation and promotion of tumor development and progression; inflammation has been identified as the seventh hallmark of cancer [4], [5]. It is widely accepted that inflammation has a critical role

in the pathogenesis and progression of cancer [6]. On the other hand, systemic inflammatory response to tumors causes changes in the hematological components like white blood cells, specifically the neutrophils. Human neutrophils, initially recognized as effectors in the first line host defense against invading pathogens, are the most abundant subpopulation of leucocyte [6]. In addition to direct bactericidal activities, neutrophils can regulate angiogenesis and tissue remodeling by releasing multiple proteases. Recently, a derived score composed of white cell and neutrophil counts has been evaluated in a large number of cancer patient. Increased level absolute neutrophil count (ANC) have been found in various human tumors, and tumor is infiltrating neutrophils are capable of being pro-tumor effect [4]. Thus, the examination of ANC could be used as an additional effective marker in identifying those CRC patients at increased risk of tumor metastasis and/or progression.

The aim of this study was to investigate ANC levels among degree of differentiation and tumor location in Colorectal Cancer (CRC) in Medan.

Material and Methods

Data collection

This study was a cross sectional analytical study on eighty consecutive patients with Colorectal Carsinoma (CRC). Data collection was obtained from medical record of patient at Adam Malik General Hospital and Permata Bunda Hospital, Medan, Indonesia from January to December 2016. Inclusion criteria are stated as followings: male or female aged ≥ 18 years old, patients with CRC, patient with blood test especially ANC, and patient with histopathology test. While the exclusion criteria were defined as patient with positive chemotherapy, patient with HIV, and patient with SEPSIS.

All data were analyzed with SPSS for window version 21. If data with normal distribution using ANOVA test, but data with abnormal distribution using KRUSKAL WALLIS test.

Results

Total of 80 patients, consisted of 48 males (60%), and 32 females (40%). Demographic characteristics of patient are shown in Table 1. The median age of these patients was 53 (25-80) years old. The majority of patient's education level was senior high school (43.2%), elementary school (25.9%), undergraduate (16%) and junior high school (13.6%).

The most common of tumor location were rectal cancer 49.4%, left side colon cancer 43.2%, and right-side colon cancer 6.2%. The data of histopathology from patient were well differentiated 40.7%, moderate differentiated 32.1%, and poorly differentiated 25.9%.

The majority of histopathology was well differentiated (41.3%)

Table 1. Demographic and clinical characteristics of patient

	N = 80 (100%)
Gender	
Male	48 (60%)[a]
Female	32 (40%)[a]
Age	53 (25-80)[b]
EducationLevel	
Elementary School	21 (26.3%)[a]
Junior High School	11 (13.8%)[a]
Senior High School	35 (43.8%)[a]
University	13 (16.3%)[a]
Tumor Location	
Rectal cancer	40 (50%)[a]
Left side colon cancer	35 (43.8%)[a]
Right side colon cancer	5 (6.3%)[a]
Histopathology	
Well differentiated	33 (41.3%)[a]
Moderately differentiated	26 (32.5%)[a]
Poorly differentiated	21 (26.3%)[a]
Hemoglobin	11 (5-19)[b]
White blood cells	8850 (1650-25750)[b]
Platelet	325178.75 ± 135551.146[c]
ANC	11.86 (6.38-27.55)[b]

The correlation between routine blood count with ANC is shown Table 2. Hb, WBC and Platelet, there was no significant difference between them (p > 0.05).

Table 2. Correlation routine blood test with ANC

Variables	ANC Correlation Coefficient	P
Hb	- 0.01	0.99
WBC	- 0.102	0.36
Platelet	0.093	0.41

Table 3 shows a comparison ANC to location of tumor and Histopathology. Mean of ANC to location of tumor Rectal cancer 12.83, Left side cancer 13.01, and right-side cancer 12.3, there was no significant difference (p = 0.951). Differentiated histopathology, well differentiated 9.48, moderate differentiated 11.89 and poorly differentiated 19.45, there was significant difference (p = 0.0001).

Table 3. Comparison of Absolute Neutrophil Count (ANC) levels among degree of differentiation and tumor location in Colorectal Cancer

ANC			
Tumor location	Mean ± SD	F	P
Rectal	12.83 ± 4.63	0.47	0.951
Left side colon	13.02 ± 5.98		
Right side colon	12.3 ± 4.24		
Histopathology	Mean ± SD	F	P
Well	9.48 ± 2.14	77.23	0.0001
Moderate	11.89 ± 2.92		
Poorly	19.45 ± 3.58		

Discussion

Total of 80 patients are mostly male gender 60% than female 40% this is according to research of American cancer society 2012 obtained majority of gender is male than female. The localization the large number of patients had colorectal cancer in Rectal cancer 50% and Left side colon cancer 43.8%. Study of Bin Jin et al., [7] show that they have found 44 cancers in the rectal region and 68 cancers in the other regions of the colon.

In our study differentiated histopathology findings the mostly well differentiated 41.3%, this is similarly from study of Sudoyo et al., [8] where most colorectal carcinoma was well differentiated [8].

In CRC patients, tumor infiltrating have been shown to be independent prognostic factors of survival in all clinical stages. Elevated neutrophil count, however, may reflect tumor progression by providing an adequate environment or its growth [6]. Previous study showed that infiltration of neutrophils was increased in colorectal adenomas compared to normal mucosa and correlated with size of adenoma, suggesting that the presence of neutrophils is involved in the early stage of colorectal tumor [9]. In our study show that mean of ANC increased in poorly differentiated 19.45, there were significant differences between them (p = 0.0001). Similarly, the study from Eileen and carlos show that ANC was increased in poorly differentiated compared to well differentiated [10]. Mean of ANC to location tumor, ANC was increased in left side colon cancer 13.01. Rao et al., show that ANC was increased in colon than rectum [4].

In conclusion, in our study found that Absolute Neutrophil Count (ANC) increased in poorly differentiated and location of tumor is left side colon cancer.

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Association between Fine-needle Aspiration Cytological Features and CD4 Level in Human Immunodeficiency Virus-Associated Tuberculous Lymphadenitis Patients Admitted to Haji Adam Malik Hospital in 2017

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Abstract

BACKGROUND: World Health Organization stated that one-third of the world's population has tuberculosis with one infected person in every second. In 2015 there were 330,910 tuberculosis cases in Indonesia. This number increased compared to 2014, which amounted to 324,539 cases. HIV-positive patients with extrapulmonary tuberculosis accounted for 50% of TB cases, of which 35% were lymphadenitis. Lymph node fine-needle aspiration (FNA) is considered effective in determining the early diagnosis of lymphadenopathy.

AIM: This study aims to evaluate the association between cytological features and CD4 level in HIV-associated tuberculous lymphadenitis patients in Haji Adam Malik Hospital in 2017.

METHODS: This is an analytical study with a cross-sectional approach involving 42 samples. Cytological features were obtained by slide reviewed and CD4 level were obtained from the medical record.

RESULTS: Analysis of the association of cytological features with CD4 level association revealed p-value of 0.353.

CONCLUSION: This indicates that there is no significant association between FNA cytological features and CD4 level in HIV-associated tuberculous lymphadenitis patients.

Introduction

Tuberculosis is an infectious disease caused by Mycobacterium tuberculosis and is contagious [1]. World Health Organization stated that one-third of the world's population has tuberculosis with one infected person in every second. In 2015 there were 330,910 tuberculosis cases in Indonesia [2]. This number increased compared to 2014, which amounted to 324,539 cases [3].

Although pulmonary tuberculosis is the most common tuberculous infection, extrapulmonary tuberculosis is also an important clinical problem. The term extrapulmonary tuberculosis refers to a tuberculous infection that occurs in organs other than the lungs. Extrapulmonary tuberculosis accounts for 15-20% of all tuberculosis cases in HIV-negative patients and 50% of all tuberculosis cases in HIV-positive patients, where tuberculous lymphadenitis is the most common form (35% of all extrapulmonary tuberculosis) [4].

According to the World Health Organization (WHO), TB is considered a global emergency with 8.8 million new cases and 3.9 million cases of them are accompanied by HIV infection in 2013. HIV aggravates TB infection by increasing the reactivation and accelerating the progressiveness of tuberculosis. With low CD4 levels in patients with HIV / AIDS, the ability of the immune system against tuberculosis will decrease so that the necrotic mass will be more dominant in the pathological examination. Therefore, the increasing number of HIV cases will increase the transmission and the proliferation of Mycobacterium

tuberculosis in patients who have been previously infected [2], [5].

At the same time, HIV / AIDS is one of the health problems in the world, especially in Indonesia. According to the Indonesian Ministry of Health Surveillance report, there were 184,929 HIV / AIDS cases in 2015. Majority of HIV / AIDS cases were in the young adult group, ranged 20-29 years, with 46.4 percent of the total cases [3]. The number of these cases continues to increase from year to year. HIV / AIDS causes severe immunodeficiency which is characterised by a reduced number of CD4 levels which increases the risk of opportunistic infections and malignancies.

Cytological examination through fine needle biopsy (FNA) in the lymph node is considered effective in determining the initial diagnosis of lymphadenopathy. FNA can help in distinguishing infections from metastasis, malignancy, or lymphoma. Besides, this method is well-known, inexpensive, fast, and has a low risk. Delyuzar (2017) reported that FNA in TB lymphadenitis showed the sensitivity of 93.65% and specificity of 70.99% with AFB examination as the gold standard. The sensitivity and specificity of this examination reached 98.95% and 96.97%, respectively, with PCR as the gold standard [6].

This study aims to evaluate the association between FNA cytological features and CD4 level in HIV-associated tuberculous lymphadenitis patients admitted to Haji Adam Malik Hospital in 2017.

Material and Methods

This analytical study with cross-sectional approach was conducted between March and November 2018. All HIV-associated tuberculous lymphadenitis patients admitted to Haji Adam Malik Hospital in 2017 were included in this study. This study was approved by the Health Research Ethical Committee, Universitas Sumatera Utara, Medan, Indonesia.

CD4 level was obtained from the medical record and categorised into below 200 cells/μL and 200 cell/μL or above. Cytological features of tuberculous lymphadenitis were obtained by FNA method and the FNA cytology slides were independently reviewed by two pathologists. Cytological features were categorised into necrosis, epithelioid with necrosis, epithelioid without necrosis, and absence of epithelioid and necrosis. Data were analysed using Microsoft Excel software and presented in tables.

Results

Forty-two HIV-associated tuberculous lymphadenitis patients were obtained from 232 lymphadenitis patients. Baseline characteristics of HIV-associated tuberculous lymphadenitis patients were summarised in Table 1.

Table 1: Baseline characteristics of HIV-associated tuberculous lymphadenitis patients

Characteristics		N = 42	Percentage (%)
Gender	Male	32	76.1
	Female	10	23.9
Age	0-10		
	11-20	1	
	21-30	17	2.6
	31-40	19	40.4
	41-50	2	45.2
	51-60	3	4.7
	61-70		7.1
	71-80		

Based on Table 1, most of the HIV-associated tuberculous lymphadenitis patients were men (76.1%) and were diagnosed in 31-40 years of age (45.2%).

The distribution of CD4 level in HIV-associated tuberculous lymphadenitis patients was summarised in Table 2.

Table 2: CD4 level in HIV-associated tuberculous lymphadenitis patients

CD4 level	N = 42	Percentage (%)
≥ 200 cell /μL	9	21.4
< 200 cell /μL	33	78.6

Based on Table 2, HIV-associated tuberculous lymphadenitis patients with CD4 level below 200 cell/μL were found in 33 patients (78.6%), and with CD4 level above 200, cell/μL were found in 9 patients (21.4%).

The distribution of FNAB cytological features in HIV-associated tuberculous lymphadenitis patients was summarised in Table 3.

Table 3: FNAB cytological features in HIV-associated tuberculous lymphadenitis patients

FNAB cytological features	N = 42	Percentage (%)
Necrosis	9	21.4
Epithelioid with necrosis	27	64.3
Epithelioid without necrosis	4	9.5
No epithelioid and necrosis	2	4.8

Based on Table 3, the most common FNA cytological feature of HIV-associated tuberculous lymphadenitis was epithelioid with necrosis (64.3%), followed by only necrosis (21.4%), epithelioid without necrosis (9.5%), and without epithelioid and necrosis (4.8%).

Association between FNA cytological features and CD4 level were presented in Table 4.

Table 4: Association between FNA cytological features and CD4 level

FNA cytological features	CD4 level			P
	≥ 200 cell/μL	< 200 cell/μL	Total	
Necrosis	1	8	9	0.353
Epithelioid	8	23	31	
Total	9	31	40	

Table 4 showed that the association between FNA cytological features and CD4 level is not significant.

Necrotic feature with CD4 level 200 cell/μL or above was found in only 1 patient. Epithelioid feature with CD4 level 200 cell/μL or above was found in 8 patients. Epithelioid feature with a CD4 level below 200 cell/μL was found in 23 patients.

Discussion

Of all the samples in this study, most TB lymphadenitis patients were men and were in the 31-40 age group. These results were by Singh, et al., (2016) and Jayshree, et al., (2015) [7], [8]. Most HIV-associated tuberculous lymphadenitis patients in Haji Adam Malik Hospital showed CD4 level below 200 cell/μL and this is in line with Lakshmi, et al., (2017) [9]. The most common cytological features found in HIV-associated tuberculous lymphadenitis patients were epithelioid features with necrosis, followed by only necrosis. This finding is in line with Singh, et al., (2016) and Gupta (1993) [7], [10].

The most typical feature found in cytology of tuberculous lymphadenitis patients is the presence of epithelioid granuloma. However, in cases of tuberculous lymphadenitis accompanied by immunodeficiency condition, such as HIV, epithelioid granuloma features are absence due to low immunity, so the only feature found is necrosis [11]. This is contrary to the current study, where 23 of 31 cases with CD4 levels below 200 showed epithelioid, which may be caused by many factors, including the development phase of tuberculous lesions in the tissue. This finding indicates that there is no significant relationship between FNA cytological features and CD4 levels in HIV-associated tuberculous lymphadenitis patients, which is also following Sutoyo (2011) [12].

In conclusion, there is no significant association between FNAB cytological features and CD4 level in patients with HIV-associated tuberculous lymphadenitis.

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The Difference of Educational Effectiveness Using Presentation Slide Method with Video About Prevention of Hypertension on Increasing Knowledge and Attitude in People with the Hypertension Risk in Amplas Health Center

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Abstract

BACKGROUND: The prevalence of prehypertension in the world reaches 20-25% with a figure that is still high in Indonesia (48.4%). The role of health promotion is substantial in an effort of prevention and treatment of hypertension.

AIM: This study aims to determine the difference of effect between health promotion using media slides presentation and with video in increasing knowledge and attitude regarding the prevention of hypertension in patients at risk hypertension at Puskesmas Amplas.

METHODS: his study uses a Quasi-Experimental design which from 48 samples that meet the criteria inclusion will be immediately given health promotion interventions with slides presentation and video. Respondents will be asked to fill out a questionnaire about knowledge and attitudes before and after the intervention. Data processing is done using the SPSS version 20 application.

RESULTS: Distribution level of the knowledge of respondents before the intervention (Pretest) and after the intervention (Posttest) is (9.8, 2.68 vs 13.2, 1.58). Distribution of the pretest vs respondent's attitude level. Post test is (29.7, 2.76 vs 33.2, 3.52). Based on the comparison of effectiveness between video and slide presentation, the significance value of knowledge was 0.072, and the significance value of attitude was 0.000.

CONCLUSION: There was a significant difference in effectiveness between video with a slide presentation on improving attitudes towards hypertension prevention, and there were no significant differences in effectiveness between videos with slides presentation on increasing knowledge about prevention of hypertension.

Introduction

According to The Joint National Committee (JNC-7) on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure, prehypertension defined as systolic blood pressure 120-139 mmHg with or without diastolic blood pressure 80-89 mmHg for those who aged over 18 years [1]. Prehypertension affects 25-50% of adult worldwide [2]. Prevalence of prehypertension between young Indonesian adult is as high as 48.4%. Prevalence of prehypertension in Teladan Health Center, Medan Kota district 2018 is 40.8% with the highest distribution according to individual characteristics is 17-25 years (46.4%) and female gender (69.6%) [3].

Individuals with prehypertension have higher risk causing hypertension and increased cardiovascular disease than those who have normal blood pressure [4]. According to the Framingham Heart Study, people with prehypertension have 3.5 times higher developing heart attack than people with normal blood pressure [5]. Any increase in systolic blood pressure (TDS) or diastolic blood pressure (TDD) (20/10 mmHg) is twice the risk for cardiovascular disease[1]. Assuming a 50% risk in 5 years, 10 adults with prehypertension will require intensive lifestyle changes and 4-6 people will need

antihypertensive drugs to prevent one case of incident hypertension [2].

Someone who has hypertension risk factors must be more vigilant and earlier in making prevention efforts. One of them is to control blood pressure and improve the understanding of hypertension through health promotion to know and reduce the behavioural risk of hypertension. According to research at the Andalas Health Center clinic in Padang, there was a significant relationship between knowledge and attitudes with efforts to prevent hypertension with p = 0.0005 [6].

However, until now the community's knowledge about hypertension management is still lacking [7]. Though good public knowledge about hypertension helps professional healthcare agent on efforts to prevent and cure this disease [8].

Material and Methods

This study used a Quasi-Experimental design where from 48 samples that met the inclusion criteria (age 40-60 years, did not suffer from hypertension, and were willing to become respondents and filled out questionnaires) will be immediately given health promotion interventions about hypertension prevention using counselling media in the form of slides presentation and videos. Respondents will be asked to fill out a questionnaire about knowledge and attitudes about prevention of hypertension before and after the intervention. The grouping of sample members in the experimental group in this study was a non-randomized control group pre-posttest design [9].

Processing data using data analysis with the Wilcoxon test to analyse the relationship between differences in knowledge and attitudes after the intervention was conducted using extension media in the form of powerpoint slides and videos. To see the effect between the independent and dependent variables, an independent t-test was carried out.

Results

Univariate Analysis

From Table 1, mean result and standard deviation (SD) of respondents age in this research are 46.54 ± 5.771 years (video) and 49.50 ± 8.103 years (slide presentation). In this research, the majority of gender, educational status, marital status and job are female (52.4% with video dan 66.7% with slide presentation), Senior High School (62.5% with video dan 37.5% with slide presentation), married (91.7% with video dan 83.3% with slide presentation), and unemployment (45.8% with video dan 54.2% with slide presentation).

Table 1: Distribution of respondent characteristics

Variable	Video (24)	Slides Presentation (24)
Age	46.54 ± 5.771	49.50 ± 8.103
Gender		
Male	11 (45.8)	8 (33.3)
Female	13 (54.2)	16 (66.7)
Marital Status		
Married	22 (91.7)	20 (83.3)
Unmarried	2 (8.3)	0 (0)
Widow	0	4 (16.7)
Educational Status		
Elementary	4 (16.7)	6 (25.0)
Junior High School	0 (0)	5 (20.8)
Senior High School	15 (62.5)	9 (37.5)
D3/S1	3 (11.1)	4 (16.7)
Job		
Enterpreneur	10 (41.7)	11 (45.8)
Worker	3 (12.5)	0 (0)
Not Working	11 (45.8)	13 (54.2)

From Table 2, the level of knowledge before the most intervention was the level of good knowledge of 20 people (41.7%), while the post-intervention increased to a good level of knowledge of 45 people (93.8%). The highest percentage of pre-intervention attitudes is the level of knowledge of 23 people (52.1%), while the post-intervention level of respondent attitudes increases, namely the level of good knowledge of 40 people (83.3%).

Table 2: Distribution between respondents according to knowledge and attitude

Variable	Pre Intervention, n (%)	Post Intervention, n (%)
Knowledge		
Mean ± SD	9.8 ± 2.68	13.2 ± 1.58
Below (B < 6)	5 (10.4)	0 (0)
Sufficient (S = 6-10)	23 (47.9)	3 (6.2)
Good (G = 11-15)	20 (41.7)	45 (93.8)
Attitude		
Mean ± SD	29.7 ± 2.76	33.2 ± 3.52
Below (B < 16)	0 (0)	0 (0)
Sufficient (S = 16-29)	23 (52.1)	8 (16.7)
Good (G = 30-40)	20 (47.9)	40 (83.3)

Bivariate Analysis

Based on Table 3, the Wilcoxon test analysis showed a comparison of the value of pre-test and post-test knowledge with video media related significantly, namely (p = 0.000). Data on respondents who experienced an increase in knowledge with a median difference value (4.5). Comparison of the value of the pre-test and post-test attitude with video media was significantly related (p = 0.000). Increased knowledge with a median difference (6.00).

Based on the Wilcoxon test analysis showed a comparison of the value of pre-test and post-test knowledge with slide media percentage associated significantly (p = 0.001). Data found that respondents experienced an increase in knowledge with a difference in the median value (2.00). Comparison of the value of the pre-test and post-test attitudes with slide media percentage was significantly related (p = 0.001). Data found that respondents experienced an increase in knowledge with a difference in the median value (2.00).

Table 3: Wilcoxon Test for knowledge and attitude video and slide

	Median	Min-Max	Sig. 2 tailed
Video			
Knowledge			
Pre-test	9.50	4-13	0.000*
Post-test	14.00	8-15	
Attitude			
Pre-test	29.00	25-37	0.000
Post-test	35.00	28-40	
Slide Presentation			
Knowledge			
Pre-test	29.00	25-37	0.000
Post-test	35.00	28-40	
Attitude			
Pre-test	30.00	25-35	0.001*
Post-test	32.00	26-39	

Based on the comparison of the results of video and slide presentation, the significance value of knowledge was 0.000 (there were significant differences in effectiveness between video and slide presentation on improving knowledge towards hypertension prevention in people at risk of hypertension) and attitude significance values of 0.072 (There was no significant difference in effectiveness between video and slide presentation on increasing knowledge about the prevention of hypertension in people at risk of hypertension).

Table 4: Comparisons between *pretest -post-test* results media video dan slide presentation

Intervention	Knowledge		Attitude	
	Median	Min-Max	Median	Min-Max
Video	4.00	-3-9	5.50	0-11
Slide Presentation	2.00	-3-9	2.50	-1-7
Sig. 2 tailed	0.072		0.000	

*P < 0.05.

Discussion

Most distribution of respondents according to marital status was married, as many as 22 people (91.7%) with video and 20 people (83.3%) with slide presentation. This result is not much different from the previous research explained that the highest proportion of respondents based on marital status was married (58.7%) [10]. Marital status has an indirect relationship with health status, including hypertension through behavioural risk factors (lifestyle) and stress. Besides, it is also directly related to the cardiovascular, endocrine, immune system, sensory nerves, and other physiological mechanisms. Hypertension is riskier for those who are widows or widowers because of the loss of a spouse or loved one is the most stressful life and can be accompanied by the possibility of illness and death [11].

The mean result and standard deviation (SD) of respondents age in this research is 46.54 ± 5.771 year (video) and 49.50 ± 8.103 years (slide presentation). In another study conducted in Zhengzhou, Central China, the mean results and standard deviation of patients with prehypertension were 45.5 ± 12.2 years [12]. Whereas in prehypertension studies and the factors associated with adult outpatients in Northeast Malaysia, prehypertensive patients from the respondents studied had a mean and standard deviation of 35.7 ± 12.91 years [13].

The highest sex prevalence was women (52.4% with video dan 66.7% with slide presentation). This is different from the study in Bangladesh, where the prevalence of prehypertension was higher in men (33.6%) compared to women (30.3%) [14]. Other studies in China showed that the prevalence of prehypertension was higher in men (41.1%) than women (33.2%) [5].

Most educational status of the respondents was senior high school (62.5% with video dan 37.5% with slide presentation). In studies conducted in China, the prevalence of prehypertension patients was higher in patients with secondary education (50%) compared to higher education levels (30.3%) [12]. In studies in China, the prevalence of prehypertension was mostly in senior secondary education (37.9%), university-level (36.2%) and primary or non-school education (33.3%) [5].

The highest employment status of respondents is not working (45.8% with video dan 54.2% with slide presentation). Jobs are related to income which is also one of the causes of hypertension. Low income is known to be a greater cause of the incidence of hypertension when compared to other risk factors. Work is also related to one's eating habits. Those who work generally have higher physical activity than those who do not work. High physical activity can reduce the risk of obesity. Conversely, if low physical activity can increase the risk of obesity, thus increasing the incidence of hypertension [11].

In this study, based on the Wilcoxon, test analysis showed a comparison of the value of the pre-test and post-test attitude with video media associated significantly (p = 0.000). Increased knowledge with differences in medical (10.00). This is supported by research on the effect of health education with audio-visual media on hypertensive care behaviours in the elderly, where there is a positive influence on older adults with hypertension [15].

Distribution of the level of knowledge of respondents observed based on the pre-intervention (video and slide slides) on prevention of hypertension increased, with a mean, standard increase (9.8, 2.68 vs 13.2, 1.58). This is following previous research studies that showed an increase in knowledge of hypertensive patients after being given health education with community-based interventions [15]. The results of this study are also supported by the theory that health education is an activity to help individuals, groups or communities to improve their behaviour to achieve optimal health. Health education can provide direct knowledge changes [16].

Distribution of respondent's attitude level was observed based on pre-intervention (video and slide slides) about prevention of hypertension increased, with a mean, standard deviation that increased pre-intervention compared to post-intervention (29.7, 2.76 vs 33.2, 3.52). This is consistent with previous studies which showed that health counselling had an important influence in improving knowledge, attitudes, and practices in patients with hypertension [17]. According to the theory of health education is a business or activity to help individuals, groups or communities to improve their abilities (behaviours), to achieve their health optimally. Health education is a learning process. Counselling is a method in health education that can change one's attitude for the better [18].

From the results of this study, it was found that improved knowledge of prevention of hypertension was obtained by respondents after getting intervention through video media. Data analysis also showed a significant improvement in attitudes towards the attitude of prevention of hypertension which was the best after the respondents received intervention through video media. This is supported by one systematic review study which states that often journals that showed significant differences, seven of them showed a significant change in attitude and knowledge of the video method [19]. This result is also supported by previous research which states that there is an effect of health education with audiovisual media on hypertensive care behaviours in the elderly, where there is a positive influence on older adults with hypertension [20]. The results of this study are also following previous research which states that there is an effect of health education using audiovisual media on hypertension control knowledge in the elderly in Tumut Village, Sari-Sari, Yogyakarta with p-value 0.014 ($p < 0.05$) [21]

Video can improve knowledge and attitude because respondents get the new experience they get in the video because the video relies on hearing and vision of the target, where the use of audiovisuals involves all sensory devices so that more and more sensory tools are involved in receiving and processing information. The possibility of the contents of the information can be understood and maintained in memory effectively moving images, and sound effects can facilitate the goal of understanding the content of information so that it can increase knowledge [22]. Computer Technology Research (CTR) states that humans are only able to remember 20% of what is seen, and 30% of those heard. But humans can remember 50% of what is seen and heard, and 80% of what is seen, heard and done all at once [23].

In this study, it was found that there were significant differences in the improvement of attitudes using video, and there were no significant differences in the increase in knowledge using video. This may be caused by social support factors that have influenced the attitude of respondents from before. This is by the theory which states that attitudes can be formed through various ways, including through imitation of others, and through suggestions that result from the influence of others or something that has authority in his view [24]. This may also be caused by the age factor in this study which is mature enough to affect one's perception and mindset. The more of the age increases, the more their capture power and mindset will develop. Also, the more of the age, a person will be more mature and easy to believe so that information is more easily accepted. Information is the first condition for an attitude. If based on the information arises positive or negative feelings towards the object and raises the tendency to behave in a certain manner, then there is an attitude [16].

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Adherence to Consuming Medication for Hypertension Patients at Primary Health Care in Medan City

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Abbreviations: ANOVA: Analysis of variance; BRS: brain relaxation scale; CVP: central venous pressure; MAP: mean arterial pressure; GDT: goal-directed fluid therapy; PPV: pulse pressure variation; SPSS: Statistical package for social science;

Abstract

BACKGROUND: Hypertension is one of the major health issues among senior citizens considering the increase in the population of a senior citizen's over the age of 60 in Indonesia. The use of antihypertensive drugs alone has proved to be inadequate to exhibit long-term effects of blood pressure control if it is not supported by adherence.

AIM: This study aims to investigate the relationship between knowledge, attitude, action, and doctor's communication to adherence of hypertensive patient in taking medication.

METHODS: This is an analytical study with a cross-sectional design. The population of this study consist of patients who have been diagnosed with hypertension and are consuming antihypertensive drugs. Eighty samples were randomly recruited. The research data were collected using a guided interview questionnaire and were an analysed using the chi-square test.

RESULTS: This study shows that the patient percentages with poor knowledge are 76.3%, 82.5% with a bad attitude, 82.5% for negative action, 56.3% for lack of doctor's communication, and 58% for poor levels of adherence. Chi-square test shows a significant relationship ($p < 0.05$).

CONCLUSION: There is a relationship between knowledge, attitude, action, and doctor's communication towards the adherence of hypertensive patient in taking medication.

Introduction

Hypertension is one of the major health issues among the increasing number of senior citizens over the age of 60 in Indonesia. Hypertension poses a high level of cardiovascular complications among the elderly such as heart failures and strokes. However, the blood pressure level among hypertensive patients is very low (38.4%) in Indonesia [1]. Hypertension or high blood pressure is defined as an increase in systolic blood pressure greater than 140 mmHg and diastolic blood pressure over 90 mmHg on two occasions with an interval of five minutes with enough rest/quiet. Increased blood pressure that takes place in a long time (persistent) can cause damage to the kidneys (renal failure), heart (coronary heart disease) and the brain (causing a stroke) when not detected early and given treatment [2].

According to the American Heart Association (AHA), the American population aged over 20 who suffer from hypertension has reached up to 74.5 million, but almost about 90-95% of cases are of unknown cause. Hypertension is a silent killer whereby symptoms can vary for each individual and is similar to the symptoms of other diseases [2]. The symptoms that are headaches/heaviness in the nape, vertigo, palpitations, tiredness, blurred vision, ringing in the ears (tinnitus), and epistaxis. Based on the Health Research in 2013, the prevalence of

hypertension in Indonesia obtained through questionnaires is 9.4%. This shows that in 2013, using individual analysis units, 25.8% of Indonesia's population suffers from hypertension [2].

The goal of treating hypertension is to reduce mortality and morbidity of cardiovascular diseases. The decrease in systolic pressure should be a major concern because in most cases, the diastolic pressure will be controlled simultaneously when systolic blood pressure is under control [3].

Medication adherence in hypertensive patients is important because hypertension is a disease that is not curable; therefore, it must always be controlled to avoid complications that can lead to death. Noncompliance issues are common in the treatment of a chronic disease that requires long-term treatment such as hypertension. Antihypertensive drugs that exist today have shown to control blood pressure among hypertensive patients, and also lowering the risk of developing cardiovascular complications. However, the use of antihypertensive medications alone is insufficient to produce the effect of long-term blood pressure control if not supported by compliance in the use of antihypertensive drugs [4].

Therefore, this study is conducted to investigate the relationship between knowledge, attitude, action and physician communication and the rate of adherence by patients with hypertension in the working area of Primary Health Care in Medan, Indonesia.

Methods

Design and Research Samples

This research is an analytic study with a cross-sectional design conducted in Puskesmas Medan Tuntungan. Eighty randomly selected patients who have been diagnosed with hypertension were chosen for this study. The purpose and methodology of the study were explained to respondents through a written explanation sheet for approval. This study was approved by the ethics committee Faculty of Medicine, Universitas Sumatera Utara.

Characteristics of Respondents

The characteristics of the respondents were obtained through interviews using a questionnaire. Characteristics of respondents consist of age, sex, years of treatment, drugs, knowledge, attitude, action, communication between doctor and levels of medication adherence. The level of medication adherence is determined based on the number of days the respondents forgot to take the medication in the last week (Bowing ≤ 3 days; Less Complying > 3 days).

Statistical Analysis

Characteristics of respondents are determined using descriptive statistics. The relationship between knowledge, attitude, action, communication between doctors with medication adherence levels were tested using chi-square tests of hypothesis with SPSS. A p-value of < 0.05 was used to determine the significance level in statistical analysis.

Results

The demographic characteristics of patients with hypertension in Puskesmas Tuntungan of 80 samples with the age sample in the range of 50-59 years are as many as 48 people (60.0%). Of this sampling, 43 patients (53.8%) are female patients. Patients who have been receiving treatment for more than 5 years amounted to 43 patients (53.8%). The type of drug prescribed to 55 (68.8%) patients and the most widely used is amlodipine. Through the study, it is found that as much as 61 (76.3%) of patients have less knowledge about the importance of consuming medication, 59 (73.8%) of patients have negative attitude towards consuming medication, 66 (82.5%) of patients have less action towards consuming medication and 45 (56.3%) patients adhere to consuming medication as a result of good communication with doctors (Table 1 and 2).

Table 1: Characteristics of Respondents

Characteristics of Respondents	Respondents n = 80
Age, n (%)	
30-39	3 (3.8)
40-49	24 (30.0)
50-59	48 (60.0)
60-65	5 (6.3)
Gender, n (%)	
Man	37 (46.3)
woman	43 (53.8)
Years of Treatment, n (%)	
<5 years	37 (46.3)
> 5 years	43 (53.8)
Medication type, n (%)	
Amlodipine	55 (68.8)
Captopril	25 (31.3)

From Table 2, it is found that 31 (93.9%) of patients with less knowledge and about medication and Hypertension had poor adherence in consuming medication. A group of 30 (90.9%) of patients have bad attitudes towards consuming medication but dutiful in consuming medication. A total of 35 (74.4 %) of patients have a bad action towards consuming medication for hypertension. 33 (70.2%) of patients have shown to have bad/less communication with doctors, but they are dutiful in consuming medication. The results of chi-square analysis showed a p-value

of < 0.05, which shows that there is a relationship between attitude, knowledge, action, and communication with a doctor to the level of adherence in consuming medication among hypertensive patients in Tuntungan Puskesmas, Medan.

Table 2: Knowledge about medication and Hypertension

Variable	Respondents (n = 80)
Knowledge, n (%)	
Well	21 (26.3)
Bad	59 (82.5)
Attitude, n (%)	
Well	19 (23.8)
Bad	61 (76.3)
Action, n (%)	
Well	35 (43.8)
Bad	45 (56.3)
Communication with Doctor, n (%)	
Well	14 (17.5)
Bad	66 (82.5)
Compliance level, n (%)	
Well	33 (41.3)
Bad	47 (58.7)

About 76.3% of patients have low knowledge compared to 23.8% of patients who have a high knowledge of hypertension. Based on values obtained by the chi-square test $p < 0.05$ ($p = 0.002$), it shows that there is a significant relationship between patients' knowledge about hypertension and adherence to antihypertensive medication.

Eighty-two-point five percent 82.5% of patients have shown to have bad attitude compared to 26.3% of patients who have a good attitude towards consuming medication dutifully. Based on the statistical Chi-Square test results, p-value = 0.003 ($p < 0.05$, was obtained from the 80 respondents in identifying the relationship between attitude and adherence to hypertension medication.

Table 3: Relationship between the levels of Knowledge, Attitudes, Actions, and Communications with Doctors Against the Adherence of Consuming Medication among hypertension patients

Factor	Dutiful n (%)	Less Dutiful n (%)	The p-value
Knowledge			
Good	17 (36.1)	2 (6:06)	0.002
Less	30 (63.8)	31 (93.9)	
Attitude			
Good	3 (9:09)	18 (38.2)	0.003
Bad	30 (90.9)	29 (61.7)	
Action			
Good	2 (6:06)	12 (25.5)	0.024
Bad			
Physician communication	31 (93.9)	35 (74.4)	
Good	14 (29.7)	21 (63.6)	0.003
Bad	33 (70.2)	12 (63.6)	

Around 82.5% of patients have shown to have bad action compared to 17.5% of patients who have shown good action. Based on the statistical test Chi-Square results obtained from the 80 respondents to determine the relationship of action with adherence of hypertension medication, a result of p-value = 0.024 ($p < 0.05$) was obtained.

Discussion

It was found that respondents with a high level of knowledge of hypertension have high adherence. The respondents with a low level of knowledge of hypertension had lower medication adherence. Another study shows that there is a relationship between knowledge and attitudes towards the patients' compliance in taking medication. The more knowledge the patient has about hypertension, the higher the level of consciousness of the patient in consuming medication. Another study has also concluded that the level of knowledge about the disease affects the patient's adherence to medication [5]. However, this is different from the study of Wahyuni et al., (2018) [6], which states that there was no association between knowledge and medication adherence, this might be because patients had received a lot of information from various sources not only from medical workers. It has also been proven that increasing patient knowledge, both about their disease and about the drugs consumed, will result in better compliance with the drug. Involving patients in their care by providing appropriate knowledge, often makes patients more concerned about their health, where this can be achieved through patient counselling and health care, as well as good interactions between doctors and patients [7].

Patients with hypertension have a poor attitude to adhere to hypertension medication. It shows that there is a relationship between attitude and medication adherence among patients with hypertension. A study has been carried out in the field that indicates a link between the levels of adherence to the attitude of patients in the treatment of chronic diseases [6]. Patients who understand about the disease will instil an attitude that will increase the level of patient compliance in taking medication [8]. Attitude is the tendency to act, perform and feel in the face, objects, ideas, situations or value. Attitude is not the behaviour, but it is a tendency to behave in certain ways to the object [9]. Attitude is a person's response to a stimulus or a closed object. It involves factors such as opinions, and emotions of either happy or sad, agree or disagree, good or bad, positive and negative aspects [10].

Similarly, a study by Wahyuni et al., (2018)[6] which states that the main factor linking patients to behavioral compliance is the patient's attitude to treatment ($p \leq 0.05$). Of the 55% of patients who adhere to treatment, 67% have a positive attitude (good). Patients with good attitudes will be 3.7 times more obedient than patients with less good attitudes. There was a strong relationship between adherence to taking good medication and the respondent's attitude towards antihypertensive treatment. A good attitude towards treating hypertension will prevent misunderstandings, which can lead to non-compliance in taking medicine. People who have good attitudes

can make the right choices and appropriate lifestyle modifications that will motivate respondent compliance [11].

Based on research conducted by Ginting (2008) [12] in the district of Medan, Belawan showed that patients with hypertension have less action in adhering to hypertension medication. Logically, a patient's action will be exhibited in the form of behaviour, but this does not mean that the attitudes and behaviours have a systematic relationship. Knowledge or good attitude is not necessarily manifested good action or behaviour (overt behaviour). To turn an attitude into action, a condition or motivation is needed to enable a person to exhibit their knowledge [13].

The Chi-Square test obtained a p-value of 0.003, which means there is a significant relationship between communication and adherence. This is consistent with previous studies, such as a research in PHC Kintap, Tanah Laut, South Kalimantan, which indicated that the correlation between the provision of drug information to adherence of drug consumption and there is a relationship between compliance with systolic blood pressure ($p = 0.003$, $r = 0.398$) and diastolic ($p = 0.045$, $r = 0.274$) [14]. Another study conducted at the Gatoel Hospital also shows that communication in the form of interviewing (motivational counselling) compared leaflets improve medication adherence, shown by the increase in MMS score 8 in the test group with the interviewing method [14]. Another study conducted in Northeastern State Ethiopia at Dessie Hospital shows that there is a relationship between communication and adherence of consuming hypertension medication among patients with the p-value of 0.023 [15]. The Antoinette et al., (2009) [16] study also showed that physician communication considered by patients to be more collaborative significantly gave a better value to better medication adherence ($p = .003$). Higher levels of symptoms and depression ($p < .001$) were significantly associated with poorer medication adherence and doctor communication assessed by patients was less collaborative. Other studies outside of hypertension cases by Wahyuni et al., (2018)[6] regarding patient compliance with taking asthma drugs, also shows that patients who are not serious in understanding disease and doing asthma treatment are caused by the influence of less established communication between doctors and patients.

In conclusion, there is a significant relationship between knowledge, attitude, patient, and physician communication actions towards the level of medication adherence among hypertensive patients in Puskesmas Tuntungan, Medan.

We suggest:

1. It is expected that hypertension patients look for information related to the importance of compliance in taking antihypertensive medication every day because there are still many patients who have a poor level of knowledge about consuming anti-hypertensive drugs.

2. It is expected that health officers further improve the socialisation and counselling about the importance of always adhering antihypertensive medication which may prevent complications due to uncontrolled high blood pressure.

3. It is expected that hypertensive patients always come back to the health centre or to Puskesmas to establish good communication with health care providers and adhere to medication and making sure their blood pressure is always in control.

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Risk Factors for Catheter-Associated Urinary Tract Infection and Uropathogen Bacterial Profile in the Intensive Care Unit in Hospitals in Medan, Indonesia

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Keywords: Catheter-associated Urinary Tract Infection (CAUTI); Catheter; Uropathogen; ICU

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Abbreviations: ANOVA: Analysis of variance; BRS: brain relaxation scale; CVP: central venous pressure; MAP: mean arterial pressure; GDT: goal-directed fluid therapy; PPV: pulse pressure variation; SPSS: Statistical package for social science;

Abstract

AIM: To evaluate the risk factors of CAUTI in ICU patients at Haji Adam Malik General Hospital and Universitas Sumatera Utara Hospital, Medan, Indonesia.

METHODS: This hospital-based observational research was an observational analytic research with a cross-sectional study. This research was conducted at Haji Adam Malik General Hospital Medan, Universitas Sumatera Utara Hospital, and Department of Microbiology Medical Faculty of Medicine Universitas Sumatera Utara, on July to August 2018 until the number of samples was fulfilled. The samples were adults aged ≥18 years, admitted to an ICU between July until August 2018 with an indwelling urinary catheter during their admission, admitted in ICU with different complaints and presentations and developed clinical evidence of infection that did not originate from patient's original admitting diagnosis, in accordance which corresponded to the inclusion criteria by using consecutive sampling technique were included in the study. Patients who were shifted out of the ICU within 48 h of admission were excluded from the study. These critical patients were referred for monitoring, observation, and management from different departments, e.g., medic, general surgery, neurosurgery, gynaecology/obstetrics, and accident/emergency departments.

RESULTS: From this research, it was found that fifty-four patients with catheter were screened for UTI infection. Of those, 24 patients (44.4%) were confirmed to have UTI by urine culture. Pseudomonas aeroginosa (16.7%) and Enterococcus faecalis (12.5%) were the most common pathogens. Patients aged > 50 years old ($P < 0.03$) and catheter use > 6 days ($P < 0.03$) were both significantly associated with increased risk of developing UTI.

CONCLUSION: There are eleven uropathogens identified in this study: *Pseudomonas aeruginosa, Enterococcus faecalis, Escherichia coli, Klebsiella pneumonia, MRSA, Salmonella enteric, Acinetobacter baumanii, Acinetobacter lwoffi, Acinetobacter haemoliticus, Burkholderia cepacia,* and *Staphylococcus sciur.*

Introduction

The definition of urinary tract infection (UTI) is an infection in any part of the urinary system, including kidney, ureter, bladder, or urethrae. Urinary tract infection is the presence of the microorganism in the urine [1]. *Hospitals in Europe Link for Infection Control through Surveillance (HELICS)* divided urinary tract infection into three classifications; UTI-A (symptomatic with microbiology confirmation urinary tract infection), UTI-B (symptomatic without microbiology confirmation urinary tract infection), and UTI-C (asymptomatic bacteriuria urinary tract infection). The *Centers for Disease Control and Prevention* (*CDC*) simplified these criteria based on the growth of mycobacteria in the urine culture. UTI is determined when the urine culture had ≥ 10^5 *colonies forming unit* (CFU)/ml urine with evidence of one or two species of microorganisms, and with or without clinical features. Hospitalised UTI developed in approximately 96.2% of patients with a history of catheter use (*HELICS*, 2005). Long term using catheter urine is the major risk factor to develop UTI as a nosocomial infection [2], [3]. *Catheter-Associated Urinary Tract Infection* (*CAUTI)* is defined as the infection in patients who use urine catheter for a minimal of three days [4]. Long term duration of urine

catheter use become a predisposition factor for *CAUTI* event [5]. The *National Healthcare Safety Network* (*NHSN*) showed that *CAUTI* cases in ICU patients were more common in critical illness patients, due to the use of invasive equipment, like urine catheter, vein and artery catheter, an endotracheal tube [6]. The use of urine catheter interrupts the innate immune defence mechanism system by affecting the mucous barrier, which has a function to prevent uropathogenic adhesion and its migration to vesica urinary [6], [7]. Catheter stimulates the inflammatory responses and causes trauma in the mucous of the urethra and bladder neck. Inflammatory and mechanical damages in the urinary tract epithelium do not only increase the risks for UTI but also influencing one's immune response to uropathogenic [5].

The duration of urine catheter use is the main risk factor for the development of *CAUTI* and bacteriuria [7], [8], [9]. The other potential risk factors, including female gender, pregnancy, and conditions like poor nutrition, faecal incontinence, illness severity, and immunocompromised status [2], [9].

Several uropathogens related to *CAUTI* have been described, including *Escherichia coli* (21.4%), *Candida spp*. (21.0%), *Enterococcus spp*. (14.9%), *Pseudomonas aeruginosa* (10.0%), *Klebsiella pneumonia* (7.7%), and *Enterobacter spp*. (4.1%) [2], [7]. The emergence of resistance to these microorganisms has been increased in the last decades, probably contributed by increased use of antimicrobial treatment, long term use of urine catheter, and treatment without indication [7], [10].

Material and Methods

This study aims to evaluate the risk factors of CAUTI in ICU patients at Haji Adam Malik General Hospital and Universitas Sumatera Utara Hospital, Medan, Indonesia, an observational analytic study with a cross-sectional study approach. This research was conducted at Haji Adam Malik General Hospital Medan, starting in July 2018 until the sample was fulfilled then the sample was taken to the Department of Microbiology, Medical College and Hospital of University Sumatera Utara and H. Adam Malik Medan Laboratory for examination. The study population was all adult patients, aged ≥ 18 years, admitted to an ICU between July until August 2018 with an indwelling urinary catheter during their admission, admitted in ICU with different complaints and who were infection that did not originate from patient's original admitting diagnosis, were included in the study who agreed to take urine culture examination.

The method of selecting samples in this study was done by consecutive sampling, where all ICU patients who met the inclusion and exclusion criteria and agreed to be examined after informed consent was included in the study until the required number of subjects was fulfilled.

The sample size is calculated statistically based on the formula:

$$n = \frac{Z^2_{1-\alpha/2} PQ}{d^2}$$

Exp:

n = Amount number of samples needed

Z = If α = 0.05 then Z score = 1.96

p = Proportion of significant bacteriuria infection in CAUTI confirmation by electrical and traditional finding = 0,438

q = 1- p = 0.526

d = Precision = 15%

$$n = \frac{1,96^2 \text{ x } 0,438 \text{ x } 0,526}{(0,15)^2} = \frac{3,8416 \text{ x } 0,438 \text{ x } 0,526}{0,0225} = 42,04$$

= 42.04≈ 42

Based on the above formula, the minimum number of samples obtained in this study were 42 subjects.

Inclusion Criteria

1. ICU patients aged ≥ 18 years old.
2. All ICU patients wore urine catheter at the time of the study (minimal for recent 48 hours).
3. Patients with pyuria.
4. Who are willing to do urine culture examinations.
5. Willing to take part in the study and sign the consent form to take part in the study.

Exclusion Criteria

1. Patients who used antibiotics for UTI for 2-4 weeks before the time of the study.
2. Cystitis.
3. Benign Prostat Hyperplasia patients.

Work Arrangement

The urine sample was aseptically collected from the sampling port of indwelling urinary catheter with sterile syringe and needle from suspected cases of *CAUTI*. The patient was labeled as a case of suspect *CAUTI* after 48 hours admission to ICU. Urine culture tests were performed on clean and sterile

urine samples from the study to observe bacterial growth than in microbiology laboratories have been checked for 30 minutes or been saved in the refrigerator in 4°C for about 24 hours. The present study was conducted in the Department of Microbiology, Medical College and Hospital of University Sumatera Utara and H. Adam Malik Medan (University of Sumatera Utara), Medan, Indonesia for a period of 2 months (July to August 2018).

The culture assessment and bacterial identification from urine samples were inoculated by calibrated loop (0.01 mL) technique onto blood agar, MacConkey's agar, *cysteine-lactose-electrolyte-deficient (CLED)* agar, and Sabouraud dextrose agar (SDA) supplemented with 100 µg/ml of chloramphenicol. The culture plates were incubated at 37°C for 24-48 h. The urine culture results have done after 48-72 hours. Colony count of > 10^4 colony form units (CFU)/mL was considered as significant for bacteriuria, colony count of 10^5 CFU/mL was considered as significant. Additionally, a Gram-stained smear was prepared from a centrifuged urine sample.

Analysis of Statistical

After all the data was collected, a descriptive analysis was conducted to determine the characteristics of the research subjects. Then bivariate analysis was performed to determine the relationship between patient's risk factors and CAUTI infection also the incidence or proportion of uropathogenic of patients in ICU Adam Malik General Hospital and University of Sumatera Utara Hospital, Medan from July until August 2018. To see the strength between the two variables, researchers used the Chi-square test in table 2 x 2 with a value of $p < 0.05$ and 95% confidence interval to assess the relationship of risk factors with positive CAUTI results.

Ethical Consideration

This study was approved by the Ethical Committee of Research, Faculty of Medicine Universitas Sumatera Utara (ID 165 / KEPK FK USU-RSUP HAM / 2018). Informed verbal consent was ensured before administration of the questionnaire. The beneficiaries were taken into consideration; subjects were not coerced to participate in the study and confidentiality was maintained.

Results

A total of 54 patients were admitted to the ICU of Haji Adam Malik General Hospital and Universitas Sumatera Utara Hospital. Of which, 24 (44.4%) were diagnosed with CAUTI. Baseline characteristics are described in Table 1. Male was found to be more common (*N* = 30, 55.6%), and catheter use between 3 and 6 days was the most prevalent (*N* = 40, 74.1%).

Table 1: Demographic Characteristic of Research Subjects in ICU

Demographic Characteristic	N = 54%
Gender	
Male	30 (55.6)
Female	24 (44.4)
Age	
21-30 Year	5 (9.3)
31-40 Year	6 (11.1)
41-50 Year	16 (29.6)
51-60 Year	11 (20.4)
> 60 Year	16 (29.6)
Duration of catheter	
3-6 days	40 (74.1)
7-10 days	7 (13)
> 10 days	7 (13)

CAUTI was also more common among patients aged ≤ 50 years old (16/27, 59.3%) compared to patients aged > 50 years (8/27, 29.6%), with younger patients had a higher risk to acquire infection (OR = 2, 95% CI 1.034-3.870, *P*-0.028) (Table 2). Higher risk to develop UTI was also seen in patients who used catheter longer than 6 days (11/14, 78.6%) than those with a shorter duration than 6 days (13/40, 32.5%). The prolonged use increased the risk to 2.418 (CI 95% 1.432-4.082, *P* = 0.003).

Table 2: Relationship between Risk Factor Gender, Age, Duration of Catheter with CAUTI Events in ICU

Characteristic Subject	CAUTI		P*	95% IK
	Positive n = 24 (%)	Negative n = 30 (%)		Lower-Upper
Gender				
Male	10 (33.3)	20 (66.7)	0,066	0.571
Female	14 (58.3)	10 (41.7)		(0.311-1.05)
Age				
≤ 50 Year	16 (59.3)	20 (66.7)	0,028	2
> 50 Year	8 (29.6)	10 (41.7)		(1.034-3.870)
Duration of catheter				
> 10 days				7 (13)
≤ 10 days				7 (13)

Of 24 confirmed CAUTI cases, we identified eleven microorganisms including *Pseudomonas aeruginosa, Enterococcus faecalis, Escherichia coli, Klebsiella pneumonia, MRSA, Salmonella enteric, Acinetobacter baumanii, Acinetobacter lwoffi, Acinobacter hemolytic, Burkholderia cepacia,* and *Staphylococcus scour* (Figure 1). The most bacteria found in this research was *Pseudomonas aeruginosa* in 4 subjects (16.7%), followed by *Enterococcus faecalis* in 3 subjects (12.5%) and *Escherichia coli ESBL (+),* then *MRSA* and *Salmonella enteric* each in 2 subjects (8.4%).

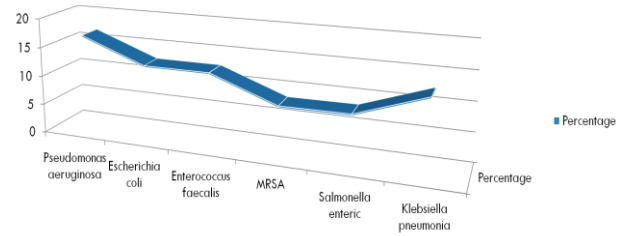

Figure 1: Uropathogen in CAUTI

Discussion

This study described the prevalence of CAUTI in Haji Adam Malik General Hospital and Universitas Sumatera Utara Hospital Medan, Indonesia. The prevalence in this study was relatively higher compared to findings in other published studies. In Indonesia, the prevalence rates of CAUTI ranged between 2.7% to 16% (REF).

This proportion is bigger than the prevalence rate of *CAUTI* infections in another country all over the world (2,4-35/1000 days of urine catheter using), data of *NHSN* in 2006-2007 (3,1/1000 days of urine catheter using), and study in other country such as at KNH hospital in East Africa (18%), other developing countries (9,9-35/1000 days of urine catheter using), developed countries (3,3-17,4/1000 days of urine catheter using), Europe (5,4/1000 days of urine catheter using), and United States of America (rate of *CAUTI* incidence 560.000/year).

The rate of *CAUTI* was expressed as the number of *CAUTI* per 1000 device days and was calculated using the following formula:

$$\frac{\text{Number of patients developing CAUTI}}{\text{Total number of catheter days}} \times 1000$$

In contrast to previous reports, younger age than 50 years old was found to be at higher risk for CAUTI in this study. While Nicole (2014) and Smeltzer & Bare (2008) described the opposite that elderly patients, having the age above 65 years old, and acute bacterial sepsis to increase the risk of the infection. Other factors, including poor environmental sanitation and health conditions, also contribute to the incidence of CAUTI. Patient's condition including nutritional status, history of previous antibiotic administration, immunological status, and the use of immunosuppressive drugs also enhance the risk for CAUTI [2], [10], [11]. In this study, we only evaluated the role of catheter duration in increasing the risk of CAUTI. We found a longer duration of 6 days had increased risk for infection, as also described in a study by Parida and Mishra (2013) and Xie (2011). In the latter studies, the authors explained the use of catheter longer than 5 and 7 days, respectively, allowed bacteria to multiply and cause infection. This duration of time is sufficient for the biofilm of the pathogen to form on the surface of the catheter and the drainage system leading to CAUTI [10].

There are a few limitations in this study. First, the numbers of samples enrolled in this study was relatively small, causing non-significant results in some of the analysis. Second, we only determined few risk factors to be evaluated the association with CAUTI. However, this study shows that CAUTI is prevalent in ICU patients in the two studied hospitals. Further study needs to be done to evaluate the cause of a higher prevalence of CAUTI in comparison to other hospitals in Medan, and more comprehensive risk factors need to be included in the data analysis.

In conclusion, a significant relationship was obtained between *CAUTI* infection with age as a risk factor, a value of $p = 0.028$ ($p < 0.05$), with the most research subjects being the age group 21 ≥ 60 years. The subject who aged ≤ 50 years has two times risk than a subject who aged > 50. A significant relationship was obtained between *CAUTI* infection with duration of the catheter as a risk factor, a value of $p = 0.003$ ($p < 0.05$), with the most research in 3-6 days of duration. The subject who uses a catheter for > 6 days has two times the possibility of *CAUTI* infected than a subject who uses a catheter for ≤ 6 days. The frequency of CAUTI infection with the urine culture examination found that 24 subjects (44.4%) of the 54 subjects studied.

There are eleven uropathogens identified in this study: *Pseudomonas aeruginosa, Enterococcus faecalis, Escherichia coli, Klebsiella pneumonia, MRSA, Salmonella enteric, Acinetobacter baumanii, Acinetobacter lwoffi, Acinobacter haemoliticus, Burkholderia cepacia,* and *Staphylococcus sciur.*

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Isolation of Surveillance Pathogenic Fungal Microbial Contaminant on Mobile Phone

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Abstract

AIM: Mobile phone has been used daily by almost everyone. This Research surveyed microbial contamination of mobile phones in the faculty of Medicine Universitas Swadaya Gunung Jati and identify the most influential fungal microbial species.

METHODS: A group of 15 samples was analysed to identify fungal isolates. The mobile phones were swabbed firmly passing its touch screen using sterile swabs then inoculated into media for fungi. Frequency distribution of isolates was calculated.

RESULTS: There were fungal isolates as follows: *Aspergillus Orchareus, Aspergillus flavus, Alternaria, Aspergillus niger, Penicillium sp., Cladosporium sp., Candida sp., Aspergillus Fumigatus*, and *Mucor sp.* at the rate of 19, 6, 1, 3, 2, 10, 2, 52, 2%, respectively.

CONCLUSION: The research indicates that all mobile phones were considerably having microbial infection, mostly from humans' natural flora and also from the air and soil. This determines that it is necessary to sterilize hands prior to a contact with mobile phones since it could lead into disease transmission.

Introduction

Data from the last 2 decades shows that Asian countries are increasing in mobile phone usage numbers in the world [1]. It was reported in China, that in the end of june 2017, there are 724 million mobile phone users, increasing from the previous data in 2016, that only 228.3 million users. Most of the users are aged 20 to 29, which are 29.7% of all the users [2]. With its affordable price, and ease of use of applications contained in the mobile phone, makes people interested in using the mobile phone. There are various features that make the mobile phone screen more comfortable to use, such as calculators, the internet, social media, games, cameras and more. In line with the advance of technology the mobile phone becomes an indispensable accessory in social life [3], [4].

In spite of that, mobile phones can cause electromagnetic radiation effects that can cause sleeplessness, headaches, reduce memory and reduce sperm quality [5]. Conveniences of mobile phones to be carried, allowing users to store them in various places such as pants, dining tables, kitchens or even toilets. This can be potentially dangerous to health and can be a carrier of a number of microorganisms that live in every inch of the mobile phone screen [6], [7], [8]. Microbes that live and thrive on mobile phones are due to daily contact with body parts such as the face, ears, and hands and can survive on the surface of the cellphone for weeks. These microbes can cause a risk of infection from contaminated mobile phone [9], [10], one of which make nosocomial infection [11], [12]. The application of personal hygiene with hand washing habits before

and after using a mobile phone is an effort to prevent disease transmission through a mobile phone [6], [13]. However, the results of the study show that the hands and instruments of health workers have the potential as carriers of microorganisms, although control and prevention efforts have been carried out such as hand hygiene, environmental decontamination, still have the potential for various objects such as laboratory equipment and mobile phones [12].

In this study, we focused on mobile phones used by laboratory officers and administrative officers in the education area, especially in the faculty of medicine.

Methods

The research subjects were 15 participants who worked in the UGJ faculty of Medicine consisting of laboratory staff, lecturers, academic staff and signed informed consent and disclosure of identification. Samples was taken using sterile cotton swab sticks, then rubbed over the surfaces of mobile phone and streaked immediately on Saburoud dextrose agar and incubated at a temperature of 35°C for 5 days and fungal growth was observed on the plate.

Results

Research is an effort to minimize the exposure of microbes that surround our lives. The habit of holding a mobile phone with an unclean hand makes the cellphone's surface greasy and sticky, allowing variations in the microfungi colonies. environmental factors where mobile phone users live and work can affect the diversity of pathogenic microfungi on mobile phones [12],[14], [15]. The results of the analysis show that the largest Colony forming unit (CFU) value is 0.2066 CFU, while the lowest CFU value is 0.036 which is isolated from the 2 subjects of mobile phone owners.

Table 1 show some microfungi that grow are those exposed to the environment, various microfungi environmental conditions allow a large diversity of microfungi [17]. Microfungi of the genus *Aspergillus* and *Candida* have pathogenic microorganism. So that microfungi of identified pathogens can indicate that mobile phones can be a vectors towards infectious transmissions for other individuals.

Fungal growth on the mobile phone can be caused by heat is delivered mobile phone, the heat temperature is the same as the temperature of the human skin [18], [19]. fungi can grow and thrive in the humid temperature; humidity Cleaner can spur fungus grows in a very optimum.

Table 1: Results of isolation and identification of pathogenic microfungi in mobile phones

No	Code	Mikrofungi Spesies	Colony Number	Total Colony Found Unit (CFU)
1	AI	*Aspergillus Orchareus*	2	0.0328
		Aspergillus flavus	2	
		Alternaria	2	
		Aspergillus niger	2	
		Penicillium sp.	1	
		Candida Sp	1	
2	AII	*Aspergillus Fumigatus*	1	0.0459
		Aspergillus Orchareus	2	
		Mucor sp.	1	
		Aspergillus Fumigatus	10	
3	AIII	*Aspergillus Orchareus*	3	0.2066
		Mucor sp.	1	
		Aspergillus Fumigatus	58	
		Penicillium sp.	1	
4	AIV	*Aspergillus Orchareus*	1	0.1082
		Aspergillus flavus	1	
		Cladosporium sp.	6	
		Aspergillus Fumigatus	25	
5	AV	*Aspergillus flavus*	10	0.0459
		Penicillium sp.	3	
		Candida Sp.	1	
6	BI	*Aspergillus Fumigatus*	26	0.0853
		Aspergillus Orchareus	1	
7	BII	*Mucor* sp.	1	0.036
		Cladosporium sp.	7	
		Syncephalastrum sp.	1	
		Candida Sp	1	
8	BIII	*Aspergillus Orchareus*	22	0.0885
		Cladosporium sp.	1	
		Penicillium sp.	2	
		Syncephalastrum sp.	2	
9	BIV	*Aspergillus Orchareus*	1	0.0098
		Aspergillus niger	2	
10	BV	*Mucor* sp.	1	0.0131
		Aspergillus niger	1	
		Aspergillus Fumigatus	2	
		Mucor sp.	1	
11	C1	*Aspergillus flavus*	1	0.0328
		Cladosporium sp.	7	
		Candida Sp	1	
12	C2	*Aspergillus Orchareus*	19	0.1279
		Aspergillus Fumigatus	15	
		Penicillium sp.	1	
		Aspergillus Versicolor	4	
13	C3	*Cladosporium* sp.	3	0.0098
14	C4	*Aspergillus niger*	1	0.0065
		Cladosporium sp.	1	
		Mucor sp.	2	
15	C5	*Aspergillus flavus*	2	0.036
		Aspergillus niger	3	
		Aspergillus Fumigatus	3	
		Candida Sp	1	

WHO 2009 states that fungal growth can be categorized as heavy (humidity > 90%), namely Aspergillus fumigates, Mucor plumbeus, Rhizopus spp, medium (humidity 80-90%), namely *Mucor circinelloides, Rhizopus oryzae, Aspergillus flavus* and mild (Humidity < 80%) namely *Sebi Wallemia, Aspergillus niger*. Some of these microfungi were found in this study. It is possible that the air also has an influence on the growth of fungi on a mobile phone, the fungal spores float in the air can be attached to the media on the mobile phone [20].

Other literature states that the colonies were found on the mobile phone can also lead to nosocomial infections [21], [22]. As in this study, we can find fungal species such as *Alternaria* sp., *Pencillium* sp., *Aspergillus* sp., Which are known to cause respiratory infections, allergic asthma, and irritation [23]. Long and Continuous exposure to these microfungi could leads into negative effectst towards humans, although the microfungi does not by itself clinically develops the disease. The infection occured

partly depending on microorganisms' characteristics, such as intrinsic virulence resistance, and also the infective material amount. Thus, exposure to recurrent moldy material can also cause respiratory or allergic irritation in some individuals [24].

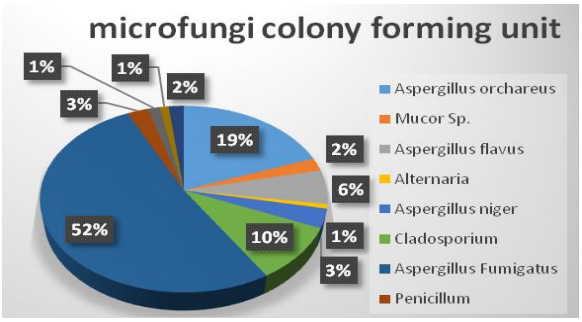

Figure 1: Presentage colony forming unit

Figure 2 shows that there is a variation of CFU on mobile phones for each mobile phone user, the diverse CFU shows that this fungal microbial can grow and develop through the skin on the hands or use handheld phones by exchanging hands with other users, allowing isolate populations.

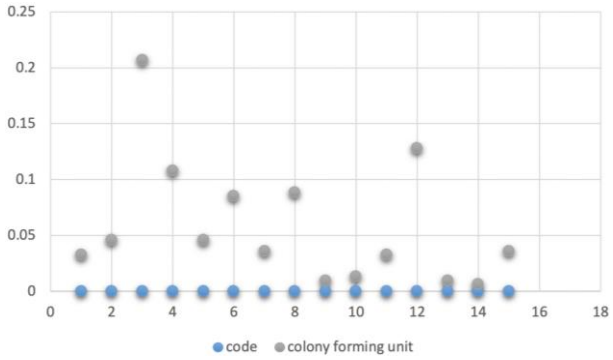

Figure 2: Variance colony forming unit in subject

Mobile phones really need sanitation procedures in order to suppress the growth of microfungi, especially developing dynamics such as Indonesia, especially in Cirebon, which is a tropical climate. Sanitation efforts on mobile phones could make microbial exposure minimal. Furthermore, tend to antibiotics usage reduction for infectious diseases [25]. It is necessary to suppress the number of infectious colonies and spores, eventhough the concentration is not high.

We can conclude:

1. There are 8 species in mobile phone; *Apergillus orchareus*, *Aspergillus flavus*, *Aspergillus niger*, *Alternaria*, *Mucor* sp, *Cladosporium, Penicillum*, *Apergillus fumigatus*

2. Analysis show that the largest Colony forming unit (CFU) value is 0.2066 CFU, while the lowest CFU value is 0.036

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The Relationship of Gender, School Sanitation and Personal Hygiene with Helminthiasis at Juhar Karo Regency in North Sumatera Province, Indonesia

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Keywords: Helminthiasis; Gender; School sanitation; Personal hygiene

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Abstract

BACKGROUND: Helminthiasis is a parasitic disease in human that causes a disturbance in food absorption and lead to malnutrition. The disease slowly impacts on the intelligence. The incidence of helminthiasis in Indonesia remains high, ranging from 2.5% to 62%. A preliminary study found that the proportion of helminthiasis in children is 31.25%.

AIM: The aim of the study to analyse the relationship between age, school sanitation and personal hygiene with helminthiasis elementary school children in Juhar Karo Regency in 2019.

METHODS: This was an observational study with a cross-sectional design. Subjects were children in grades IV, V and VI with and without helminthiasis. A total of 194 children selected by proportional random sampling were enrolled. Data were collected by interview, observation, and stool examination with the Kato-Katz method.

RESULTS: The proportion of helminthiasis in boys was 51.0%, the proportion of poor sanitation in school was 36.6%, and the proportion of poor personal hygiene was 67.5%. Personal hygiene was significantly associated with the incidence of helminthiasis (RP = 6.052; 95% CI = 3.029-12.902; P-value = 0.001). Improved personal hygiene may prevent the occurrence of helminthiasis.

CONCLUSION: The proportion of helminthiasis in elementary school students in this region was 50.0%. Personal hygiene has been shown to be related to helminthiasis. In this study, subjects with poor personal hygiene had a chance of 6.052 times greater to experience helminth infection compared to subjects who had good personal hygiene.

Introduction

Helminthiasis is one of the infectious diseases that remain a public health problem, particularly in Indonesia. This disease affects food absorption, nutritional status and reduces health condition, especially in elementary school children [1].

World Health Organization (WHO, 2017) estimated more than 1.5 billion people or 24% of the world population to be infected with soil-transmitted helminth (STH), and more than 880 million of children need treatment due to this parasitic disease [2]. The increased numbers of helminth infections in primary school children may be caused by behavioural factors including not washing hands before eating, or after defecating, not using sandals when leaving the house and not cutting the nails regularly. This situation can also be exacerbated by poor living environments such as no latrines and high humidity environment [3].

The Indonesian Ministry of Health (2017) proved that the prevalence of helminthiasis in Indonesia ranged from 2.5% to 62% [4]. The prevalence reported above is supported by the results of a survey conducted in several regencies in 2011,

where the prevalence of helminthiasis in Lebak and Pandeglang to be 62% and 43.3%, respectively; Sleman regency at 21.8%; Karangasem regency at 51.3%; West Lombok regency at 29.5%; Mataram City at 24.5%; and West Sumba at 29.6%[1].

A study by Martila et al., (2015) reported elementary school students in Pantai Jayapura, Papua had 50% prevalence of helminthiasis [5]. The numbers were dominated by infections by *Ascaris lumbricoides* (48.5%), followed by *Trichuris trichiura* (28.6%) and hookworm (14.3%). While other study reported prevalence as high as 59.3% in Wera district, Bima regency [6].

The initial survey conducted by the North Sumatra Health Office in 2014 found that in some North Sumatra regencies, the prevalence of helminthiasis was 29%; 25% was caused by *A. lumbricoides*, and 1% each was due to *T. trichiura* and hookworms. This prevalence decreased to 22.5% in 2016 [7].

In Karo regency, the prevalence was 41.1% in 2014 with *A. lumbricoides* as the predominant species. In 2017, this prevalence increased to 57.6% with *A. lumbricoides* at 41.4% and *T. trichiura* at 16.2% [7]. A repeat survey in 2018 in two elementary schools in Juhar District of Karo regency demonstrated a remaining high prevalence at 31.25% This may be due to several risk factors like human habits and environmental factors. Saeni and Arief (2016) have shown the relationship between helminthiasis and personal habits. The prevalence of helminthiasis was significantly higher in children who did not wash their hands before eating (64.0%) and after defecation (62.2%) [8].

Furthermore, environmental sanitation factors like sanitation, sewage, and waste facilities, and housing conditions like dirt floor, access to clean water, availability of trash bin and wastewater disposal facilities are associated with the helminthiasis incidence [9], [10].

The aim of this study was to analyse the association between gender, school sanitation and personal hygiene with the incidence helminthiasis on elementary school students in Juhar Village, Juhar District, Karo Regency of North Sumatra Province.

Methods

Study Design

This study was an analytic observational study with a cross-sectional design.

Subjects

Enrolled subjects in this study were all students of grades IV to VI at the Public Elementary School in Juhar Village in 2019. Inclusion criteria included respondents who had settled in Juhar village in a minimum of 3 months and respondents who agreed to be the subject of the study and followed all study protocols. Exclusion criteria included respondents who consumed anthelminthic in the last 3 months and respondents who had siblings in the grades IV, V or VI.

Data Collection

Data was collected directly through interviews using questionnaires. School personal hygiene and sanitation data were taken using a questionnaire in the form of tested and standardised questions. The stool was collected in a labelled pot that had been distributed previously to school children. The label included the student's name, date of birth, gender, and class. A stool sample, a size of a thumb, was taken using an ice cream stick, then put in a stool pot and closed tightly. Stool examination was done in the laboratory using the Kato Katz method.

The following data were identified: gender, school sanitation, personal hygiene of each respondent. Data analysis was done by stages, with univariate analysis, bivariate analysis with Chi-Square test at a significance level of 0.05.

Results

Table 1 showed the baseline characteristics of the subjects. The majority of subjects were male (99, 51.0%). Poor school sanitation was found in 36.6% of subjects, and poor personal hygiene was found in 131 people (67.5%). While the prevalence of helminth infections was 50%.

Table 1: Baseline characteristics (n = 194)

Characteristics	n	%
Gender		
Male	99	51.0
Female	95	49.0
School sanitation		
Poor	71	36.6
Good	123	63.4
Personal hygiene		
Poor	131	67.5
Good	63	32.5

Bivariate analysis showed a significant relationship between personal hygiene and helminthiasis (PR = 6.052), which means that the helminth infection was 6 times more likely to occur in children with poor personal hygiene than those with good personal hygiene. However, there was no significant relationship between school sanitation (*P* = 0.315), gender (*P* = 0.655) and helminthiasis.

Table 2: Gender relationship, school sanitation and personal hygiene with helminthiasis accidence in 2019

Variable	Helminthiasis (+)		Helminthiasis (-)		Total		p value	RP (CI=95%)
	n	%	n	%	n	%		
Gender								
Male	46	46.5	53	53.5	99	100	0.315	0.749
Female	51	52.7	44	46.3	95	100		(0.426 - 1.317)
School sanitation								
Poor	37	38.1	34	35.1	71	100	0.655	1.143
Good	60	61.9	63	64.9	123	100		(0.637-2.050)
Personal hygiene								
Poor	83	63.4	48	36.6	131	100	0.001	6.052
Good	14	22.2	49	77.8	63	100		(3.029 -12.092)

Discussions

Stool examination on 194 elementary school students in Juhar Village, Juhar District, Karo Regency showed 50% positivity for STH infection. This prevalence is considered high, which is similar to other findings in other parts of Indonesia [4].

There are 25 villages in Juhar District, Tanah Karo Regency with a population of 15,937 people and mainly works as farmers. Our previous study has shown that this area is disadvantaged areas as seen from the numbers of houses that still had not met the good environmental sanitation standards. During our observations, many houses still had the sewage run into their yard. School environmental sanitation facilities such as toilets were provided but not well maintained so they could not be used properly. Besides, there was also no sink for washing hands. As a result of inappropriate facilities, elementary school students can defecate anywhere near the school. This then leads to an increasingly uncontrolled spread of worms. This was also supported by the poor hygiene behaviour of elementary school children like lack of attention to nail hygiene, and not wearing footwear when playing on the schoolyard.

In this study, we found no significant relationship between gender and helminthiasis. This is in line with other studies by Ginting (2005) and Kartini (2014). These studies described that gender did not influence the incidence of helminth infection. In Karo region, the majority of occupation for both male and female is farming, therefore the same activities including washing, fetching water, farming and taking care of the house were done by both male and female [11], [12].

Similar to gender, school sanitation was also not associated with helminthiasis, as reported by Sumanto (2010) that school sanitation is not a risk factor for hookworm infection among school students [13]. In this study, school sanitation condition was assessed based on the condition of the bathroom, availability of public handwashing place in the canteen, bathroom tubs, and distance of garbage collection with a canteen < 20 meters. Despite poor condition found in all 4 studied schools, our study failed to show a significant association between poor sanitation with helminthiasis.

On the other hand, we found a significant relationship between personal hygiene and the incidence of helminthiasis. A similar association has been shown in other studies with children with poor personal hygiene had an increased risk to experience helminthiasis [14], [15], [16], [17].

Personal hygiene is very important in efforts to control the risk factors for helminthiasis. This can be done through efforts to improve personal hygiene or environmental cleanliness. Poor personal hygiene of a person may cause helminthiasis which is often influenced by the poor behavior of children such as not washing their hands after defecation, not using the soap when washing hands, not washing their feet and hands with soap after playing on the ground, not using footwear when playing and going out of the house, fail to maintain the cleanliness of nails, and consuming uncooked water [4].

Based on the results of the study, it can be concluded that helminth infection in this study can be influenced by various factors, one of which is personal hygiene. Poor personal hygiene will be at risk of contracting an infection, especially by STH. This has been shown in several other studies where respondents with poor personal hygiene experienced more infections than children who had good personal hygiene. Poor personal hygiene will aggravate the incidence of helminthiasis in elementary school students because at the age of the elementary school they are not able to independently manage their personal hygiene. And good personal hygiene is an important requirement in preventing and breaking the chain of the spread of infectious diseases such as helminthiasis.

The regulation by the Ministry of Health of Indonesia (2017) on helminthiasis prevention including reducing the prevalence of helminth infections by killing the worms through mass treatment to reduce the intensity of infections (number of worms per person), in order to improve health level. But the treatment of worms must be accompanied by the efforts to live clean and healthy, improved environmental sanitation, and nutritious food intake. For this reason, there is a need for cross-program cooperation and cross-sector collaboration between the government and the private sector. So continuous communication and understanding between these sectors will be achieved, and helminthiasis can be prevented in the short and long term [4].

In conclusion, the proportion of helminthiasis in elementary school students in this region was 50.0%. Personal hygiene has been shown to be related to helminthiasis. In this study, subjects with poor personal hygiene had a chance of 6.052 times greater to experience helminth infection compared to subjects who had good personal hygiene.

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Sepsis in Latent Autoimmune Diabetes in Adults with Diabetic Ketoacidosis: A Case Report

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Abbreviations: ANOVA: Analysis of variance; BRS: brain relaxation scale; CVP: central venous pressure; MAP: mean arterial pressure; GDT: goal-directed fluid therapy; PPV: pulse pressure variation; SPSS: Statistical package for social science;

Abstract

BACKGROUND: This case report intends to highlight the challenge in diagnosing type 1 diabetes on an adult patient. Latent Autoimmune Diabetes in Adult (LADA) types I diabetes Mellitus, which found in adulthood and characterised by progressive damage to pancreatic β cells that happened slowly. Incidence of LADA is around 2-12% of the total diabetes population. Sepsis in LADA patients will trigger diabetic ketoacidosis (DKA).

CASE REPORT: We report a case of a 33-year-old woman patient presents with decreased consciousness accompanied by rapid and deep breathlessness for 1 day. Before, the patient complains of fever and cough. Physical examination found soporous, blood pressure 120/80 mmHg, pulse 110 x/minute, temperature 38.8°C, breathing 32 x/minute Kussmaul. Bronchovesicular breath crackles in both lower lung fields. leukocytes were 22,100/mm^3, random blood glucose 638 mg/dL, urine ketone +++, HbA1C 17.2%, HOMA IR less than 2 units. C-peptide 0.3 ng/mL and GADAs 16.9 U/mL. Chest Xray indicated bronchopneumonia. Patients were diagnosed with diabetic ketoacidosis, LADA, and sepsis caused by bronchopneumonia. Patient treated with DKA management and sepsis. On the second day, the treatment of DKA was resolved and continued with the administration of short-acting insulin and regular long-acting.

CONCLUSION: Sepsis in LADA with DKA requires fast and appropriate management. Further search is needed to diagnose LADA.

Introduction

Latent Autoimmune Diabetes in Adult (LADA) types I diabetes mellitus found in adulthood which is characterised by progressive damage to β cells that lasts slowly [1]. Indonesia population was about 237.6 people in 2010, and make it become the world's fourth most populated country. Indonesia has the seventh-largest number of diabetic patients (7.6 million) [2], [3]. LADA occurs in about 2 - 12% among diabetic patients in the entire diabetes population. Symptoms of LADA are similar to Type 1 diabetes but are found in adulthood. The slow progression of pancreatic β cell damage in LADA is initially almost always diagnosed as type 2 diabetes mellitus [4], [5].

The Diagnosis of LADA is made from the clinical complaints of diabetes symptoms accompanied by high fasting plasma glucose, low *C-peptide*, without insulin resistance characterised by low HOMA IR [6]. Due to the slow progression of pancreatic β cell damage usually, in the first 6 months, LADA patients still respond with oral anti-diabetic therapy, but when plasma glucose levels can no longer be overcome with oral anti-diabetes drugs and lifestyle changes, daily insulin injections are needed [7].

It is estimated that more than 50% of patients diagnosed with type 2 diabetes without obesity are LADA, but not all LADA patients are underweight; some are overweight [6]. Glutamic Acid decarboxylase autoantibody (GADA), islet cell autoantibody (ICA), insulinoma-associated (IA-2) and zinc transporter autoantibodies (ZnT8) were found in

patients with LADA type DM [6], [7]. LADA patients usually have low C-peptide levels, although sometimes in moderate amounts according to the progression of the disease. While patients with insulin resistant or type 2 diabetes mellitus, usually have high C-peptide levels [8], [9].

Case Report

A 33-year-old woman comes with a major complaint is suddenly unconsciousness accompanied by shortness of breath that is fast and deep for 1 day. Before declining consciousness, the patient complains of fever, cough, and vomiting.

Figure 1: Chest Xray

On physical examination found soporous awareness, blood pressure 120/80 mmHg, pulse 110 x/minute, temperature 38.8°C, breathing 32 x/minute Kussmaul, BMI: 16.8 kg/m2. Bronchovesicular breath, fine wet cracks in both lower lung fields. On laboratory examination, leukocytes were obtained 22,100/mm3, blood sugar levels when 638 mg/dL, urine ketone +++, HbA1C 17.2%, HOMA IR was less than 2 units. C-peptide 0.3 ng/mL and GADAs 16.8 U/mL. Chest Xray indicated bronchopneumonia and plain radiograph of the pancreas, and no pancreatic calcification was found (Figure 1 and Figure 2).

Patients were diagnosed with diabetic ketoacidosis, LADA, and sepsis caused by bronchopneumonia. The management of DKA in these patients is given fluid resuscitation, intensive plasma glucose control with DKA, intravenous insulin protocol, and trigger factor control, namely infection with broad-spectrum cephalosporin class 3 antibiotics. On the second day, the DKA treatment is resolved, and insulin therapy is given for hyperglycemia. Short-acting and long-acting regularly.

Figure 2: Pancreatic Xray

Discussion

Infections are still the main cause of morbidity and mortality in diabetics patient. Diabetes could increase the risk of developing infections and sepsis to the patient [10]. The main reason for diabetes predisposes to infection appears to host response abnormalities, particularly in neutrophil chemotaxis, adhesion and intracellular killing, defects that have been attributed to the effect of hyperglycaemia [11]. There is also evidence for defects in humoral immunity, and this may play a larger role than previously. Diabetes is associated with elevations in C-reactive protein (CRP), tumour necrosis factor-alpha (TNF-α), interleukin (IL)-6 and IL-8, but no differences are seen in circulating cell surface markers or coagulation markers between patients with and without diabetes in the context of sepsis [11], [12].

Organ systems where bacterial infections predominate as well as fungal diseases were associated with substantial increases in magnitude among patients with both T1DM and T2DM, but risks were consistently higher for T1DM. Patients with T1DM are at approximately double the risk of patients with T2DM for infection-related to death. Bacterial eradication is needed to treat the infection. Antibiotic regiments are not different in a patient with or without diabetes [11].

Lung infections suffered by these patients trigger sepsis which then increases the risk of diabetic ketoacidosis. In stress situations, the body is thought to activate the central nervous system and neuroendocrine axes which release hormones such as catecholamines, glucagon and cortisol which are known to stimulate hepatic glucose production and lead to hyperglycemia [13], [14].

Ketoacidosis is a metabolic state associated with pathologically high serum and urine concentrations of ketone bodies, namely acetone, acetoacetate and beta-hydroxybutyrate. DKA can occur in patients with diabetes mellitus. This may be caused by precipitating physiologic stress or in some cases, maybe the initial clinical presentation in patients with previously undiagnosed diabetes [14], [15]. Some of the more common risk factors that can precipitate the development of extreme hyperglycemia and subsequent ketoacidosis are infection, non-adherence to insulin therapy, acute major illnesses like myocardial infarction, sepsis, pancreatitis, stress, trauma, and the use of certain medications DKA management must be carried out quickly and precisely given the high mortality rate [16].

DKA management protocols are replacement of lost fluids and salts, administration of insulin and management of infection. Considering that severe acidosis can interfere with the balance of homeostasis, it is reasonable to treat patients with pH < 7.0 using sodium bicarbonate. DKA in these patients can be resolved well because of the provision of therapy following the protocol, which are fluid resuscitation, blood sugar control and infection management with the administration of broad-spectrum antibiotics, which is the third generation of cephalosporin [11], [17].

A 33-year-old woman with a diagnosis of diabetic ketoacidosis, LADA, and sepsis caused by bronchopneumonia was treated. The problem with this patient is whether this patient is type 2 DM or another type of DM, given that clinically type 2 DM and other types of DM can be found in young adults [3], [4].

According to the literature where the percentage is 10%-20%. Usually, 85% are overweight or obese; urinary ketones are found in 33% of cases while 5%-25% with ketoacidosis [17]. DKA triggers in these patients are septic bronchopneumonia (Masharani, 2010). This is consistent with the literature that the most common trigger factor of DKA is an infection, and is thought to trigger more than 50% of DKA cases [15]. In infection, there will be an increase in cortisol and glucagon secretion so that there is a significant increase in blood sugar levels [12].

At the beginning of admission, this patient is diagnosed with type 2 DM, so to ensure diagnostic tests are carried out several tests including HOMA IR, C-peptide and GADAs. In these patients, there was a low HOMA IR; this was not my type 2 DM. Then examination was performed C-Peptide to prove whether this patient was pure type 1 DM or another type of DM. In these patients, C-Peptide is low, which is 0.3 ng/mL. The results of the examination C-peptide describe endogenous insulin secretion. Insulin and C-peptide are secreted equimolarly and released into the circulation through the portal vein. Low C-peptide indicates that endogenous insulin production in these patients is low and that high levels of GADAs are obtained, which is 16.9 U/mL, which indicates these patients have clear antibodies. In low HOMA IR conditions, low C-peptide and autoantibody abnormalities indicate that this patient does not type 2 DM but LADA type DM [18], [19], [20].

Diabetes is a much heterogeneous disease than the present subdivision into type 1, and 2 assumes. Both type 1 and type 2 diabetes seem to result from a collision between genes and environment [18]. Type 1 diabetes is believed to be an autoimmune disease characterised by genetic, immunological and metabolic features. The incidence is highest in children, but adults also get the disease. Data reported in LADA show that this is the most frequent form of adult-onset autoimmune diabetes and may account for 2% to 12% of all cases of diabetes in the adult population. Moreover, multicenter studies carried out in Europe, Asia and North America, reported that 4% to 14% of patients diagnosed with T2DM are positive for T1DM associated autoantibodies which are diagnostic for LADA [17]. In LADA, metabolic changes at diagnosis reflect a broad phenotype ranging from diabetic ketoacidosis to mild non-insulin-requiring diabetes [20].

Adult-onset autoimmune diabetes and childhood-onset type 1 diabetes are barely distinguishable immunologically. In LADA, the dominant autoantibody is GADA and lower C-peptide levels. At diagnosis, the clinical phenotype in patients with autoimmune diabetes is remarkably broad, ranging from diabetic ketoacidosis to diabetes that can be controlled with diet alone. Patient with LADA tend to have a lower age at diabetes onset, lower BMI and waist-to-hip ratio, but a more pronounced loss of C-peptide and an increased likelihood of insulin treatment [9].

To diagnose LADA, the Immunology of Diabetes Society has established three main criteria including (1) adult age of onset (> 30 years); (2) presence of any islet cell autoantibody; and (3) absence of insulin requirement for at least 6 months after diagnosis. However, the definition of LADA remains controversial, and open debate regarding these diagnostic criteria still exist [8].

A correct therapeutic strategy for LADA patients should aim to the preservation of residual β-cell function as well as improvement of neurometabolic control, to reduce the risk of long-term complications. Maintenance of β-cell function, as demonstrated by the Diabetes Control and Complication Trial, is indeed associated with a reduction of long teen diabetic complications [6].

In conclusion, sepsis in LADA with DKA requires fast and appropriate management. Further search is needed to diagnose LADA. LADA type DM is initially almost always diagnosed with type 2 DM or other types of DM because pancreatic β cell damage occurs progressively slowly (ADA, 2004). Diagnosis of

LADA patients are found clinically in diabetes, increased plasma glucose, HOMA IR less than 2 units, low C-peptide and the presence of autoantibodies.

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Vitamin A Supplementation in Pulmonary Tuberculosis Patients on Acceleration of Sputum Conversion in Medan City

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Abbreviations: ANOVA: Analysis of variance; BRS: brain relaxation scale; CVP: central venous pressure; MAP: mean arterial pressure; GDT: goal-directed fluid therapy; PPV: pulse pressure variation; SPSS: Statistical package for social science;

Abstract

BACKGROUND: Indonesia is in the second rank for the most TB (Tuberculosis) cases in the world after India. It is estimated that there are 1 million cases per year in Indonesia.

AIM: To find out the influence of Vitamin A supplementation in the medication of pulmonary TB on the acceleration of sputum conversion time at an intensive phase in the Working Area of Puskesmas in Medan Kota Subdistrict

METHODS: This research was Quasi Experiment method. With two groups. The samples were 30 newly diagnosed pulmonary TB patients consisting of of15 patients in the treatment group and 15 patients in the control group. The statistical data were analysed with t independent test, significance 95% ($p < 0.05$)

RESULTS: The treatment group discovered that they were mostly 18-27 years old 40.0%, male 73.3%, mostly university students 26.7%, had BTA conversion in the second week 80.0%; and the sputum conversion time was 2.4 weeks in average. The results of the research on the control group demonstrated that they were mostly 18-27 years old 33.3%, male 80.0%, mostly university students and entrepreneurs 20.0%, had BTA conversion in the second week 80%; and the sputum conversion time was 4.1 weeks in average. Vitamin A supplementation in the medication of pulmonary TB patients had some influence on the acceleration of sputum conversion at the intensive phase ($p \leq 0.001$).

CONCLUSION: We concluded that Vitamin A in the medication of pulmonary TB patients had some influence on the acceleration of sputum conversion at the intensive phase in the Medan Kota.

Introduction

Indonesia is in the second rank for the highest Tuberculosis (TB) cases in the world after India. It is estimated that there are 1 million new cases per year in Indonesia, twice more than the previous incidence forecast compared to that of in 2014 (WHO, 2015) [1].

It was reported in 2015 that North Sumatera Province was the province with the fourth-highest rate of New TB cases after West Java, East Java and Central Java. New cases of pulmonary TB patients (BTA) (+) who received medication in North Sumatera reached up to 16,946 people, with successful medication for 15.774 people (93.1%) (Kemenkes RI, 2016) [2].

According to the report on pulmonary TB in the last three years issued by the Health Agency of Medan, there were 1,586 BTA (+) cases in 2013 which were mostly contributed by Medan Kota Subdistrict with 200 patients; there were 1,650 BTA (+) cases in 2014 which were mostly contributed by

Medan Kota Subdistrict with 220 patients, and there were 1,576 BTA (+) cases which were mostly found in the working area of Medan Kota Subdistrict Puskesmas with 197 patients.

The results of the research by Pakkasi et al., in Indonesia in 2010 demonstrated that the group of patients who were supplemented vitamin A had an average of sputum conversion acceleration at 2.1 weeks, while the group of patients who were supplemented had an average of sputum conversion acceleration at 2.5 weeks [4].

One of the indicators used to monitor the medication of pulmonary TB is the conversion rate. Conversion Rate is the percentages of newly diagnosed pulmonary TB patients who are confirmed to bacteriology change into negative BTA (Acid Fast Bacteria) after going through preliminary medication period (Ministry of Health of Republic of Indonesia, 2014) [5].

Based on the above-explained background, the researcher is interested in studying the influence of vitamin A supplementation in the medication of pulmonary TB patients on the acceleration of sputum conversion at an intensive phase in the working is of Medan Kota sub-district.

Methods

Clinical testing research type is employed with an experimental approach and Quasi Experiment method. The research was done from May until October 2017 in the working are of Medan Kota Subdistrict Puskesmas.

This research took thirty (30) patients fulfilling inclusive criteria such as ≥ 18 years old, male and female, diagnosed by pulmonary TB with BTA (+) sputum, and medication using category I OAT, never had OAT medication before, willing to regularly take Category I OAT medicine during this research, and suffering from TB with BMI ≥ 18.5.

The data analysis was done using computerisation applying the software of SPSS 21. Mann Whitney testing was employed for the data analysis at a reliability rate of 95% and considered significant at $p < 0.05$.

Results

Age

The treatment group demonstrated that the findings were mostly 18-27 years old, i.e., 6 patients (40.0%) and those of the control group were also mostly 18-27 years old i.e. 5 patients (33.3%).

Sex

The findings in the treatment group were mostly male i.e. 11 patients (73.3%), and those of the control group were mostly male i.e. 12 patients (80.0%).

Occupation

The findings in the treatment group were mostly university students i.e. 4 patients (26.7%). The findings in the control group were mostly university students and entrepreneurs i.e. 3 patients each (20.0%).

BTA Examination and Mean Conversion

The BTA examination on the patients in the treatment group showed that most of their BTA were 3+ i.e. 9 patients (60.0%) with mean of conversion 2.7 weeks.

The BTA examination on the patients in the treatment group showed that most of their BTA were 3+ i.e. 9 patients (60.0%) with mean of conversion 4.4 weeks.

Patients' BMI (Body Mass Index)

Majority of the patients in the treatment group demonstrated an increase in the average of BMI resulted from comparing BMI from before medication and after conversion; the mean of BMI before the medication was 21.39, and the mean of BMI after conversion treatment was 21.62 and the increase was 0.23. The highest increase was demonstrated by respondent number 13 i.e. 0.67. The average of BMI resulted from comparing BMI from before medication and after conversion showed an increase; the mean of BMI before the medication was 21.75, the mean of BMI after conversion was 22.26 and the increase was 0.51. The highest increase in BMI was demonstrated by respondent number 4, namely 0.87.

BTA Conversion and Mean of Conversion

The treatment group demonstrated that most of patients' BTA conversion result was obtained in the second week i.e. 12 patients (80.0%) with mean of conversion 2.4 weeks. The control group demonstrated that most of patients' BTA conversion result was obtained in the fourth week i.e. 12 patients (80.0%) with mean of conversion 4.1 weeks.

Bivariate analysis

The results of the bivariate analysis using the Mann-Whitney test obtained a probability value of < 0.001 below the alpha value of 0.05. The meaning that, there was an effect of vitamin A supplementation on the treatment of pulmonary TB patients in accelerating sputum conversion in the intensive phase in the Medan Kota District Health Center Work Areas.

Discussions

The results of the research showed that most of the patients in the treatment group and control group were 18-27 years old i.e. 6 patients (40.0%) and 5 patients (33.3%) respectively. According to the report of the Minister of the Republic of Indonesia (2012) stated that approximately 75% of TB patients were from the most productive age group economically (15-50 years old). It is discovered that people in productive age are vulnerable to be infected by Pulmonary TB, as they have high mobility and frequently interact with other people around them. WHO (2013) [3] reported that 75% of all TB cases were found in the age group of 15-54 years old.

The results of the research on the treatment group also showed that most of the patients were male i.e. 11 patients (73.3%) and female i.e. 4 patients (26.7%), while those of the control group were mostly male i.e. 12 patients (80.0%) and females i.e. 3 patients (20.0%). It is in line with the report issued by WHO (2015) [1] stating that approximately 9.6 million of pulmonary TB cases consisting of more males (56.2%) than females (33.3%) so 3.2 million cases were found in females. Females contributed 480,000 cases out of 1.5 million of deaths caused by TB. The results of this research are in line with the report issued by the Minister of Health of the Republic of Indonesia (2013) stating that the prevalence of pulmonary TB is higher in males than in females (0.4: 0.3) [5]. It was found out that the males as the head of families and the financial supporter have more activities outside the house so that they are more vulnerable to be infected by pulmonary TB. As observed from sex, the number of treatments in males is 1.5 times higher than in the females.

As observed from occupation, the whole research subjects were dominated by university students i.e. 7 patients (23.3%). Four patients (26.7%) were from the treatment group and 3 patients (20.0%) from the control group. These results are in line with what was reported by the Minister of Health of the Republic of Indonesia (2013) that the prevalence of TB was mostly contributed by patients who did not work (11.7). An adult TB patient would lose 3 to 4 months of his working time on average that reduced 20-30% of his family income.

As observed from the BTA examination classifying into a group of 1+, 2+, 3+. The treatment group was dominated by +3 i.e. 9 patients (60.0%) with mean of conversion period of 2.7 weeks; the control group was dominated by 3+ i.e. 9 patients (60.0%) with mean of conversion period of 4.4 weeks. It is discovered that either the treatment or control group was dominated by pulmonary TB with BTA 3+. These results are following the research done by Tsani (2011) [6] stating that the results of BTA examination on 140 pulmonary TB patients showed that most of them (95 patients) had BTA 3+.

The results of the research on the treatment group (Vitamin A supplementation) demonstrated that the BTA conversion mostly took place in the second week i.e. 12 patients (80.0%), while that of the control group (without Vitamin A supplementation) mostly took place in the fourth week i.e. 12 patients (80.0%). The mean of conversion period obtained in the treatment and control groups were was 2.4 weeks and 4.1 weeks, respectively. These results were in line with the research done by Pakkasi et al. in Indonesia in 2010, stating that the patients supplemented by vitamin A had sputum conversion acceleration at 2.1 weeks in average [5]. These results were in line with the research done by Ahmad et al., [7] in India in 2012 stating that vitamin A supplementation best-accelerated sputum conversion i.e. 4.3 weeks. This research proves that pulmonary TB patients who have medicated with category I OAT and supplemented by vitamin A have BTA conversion earlier than those who are only medicated with category I OAT and not supplemented by vitamin A.

The statistical results using Mann-Whitney method obtained $p < 0.001$ which was still below the significance rate of alpha 0.05; which indicated that vitamin A supplementation had some influence on the acceleration of sputum conversion period at an intensive phase in the working are of Medan Kota Subdistrict Puskesmas. The earlier BTA conversion is, the faster the patients' recovery and the less the infection will be. This research is in line with the research done by Pakkasi in 2009, stating that there was a correlation between the low intake of vitamin A and the severity of TB with $P = 0.000$. The research was done by Karyadi et al., (2002) [8] discovered that vitamin A and zinc supplementation repaired the therapeutical effect of TB after 2 months.

There is an obvious causal correlation between two-malnutrition and active TB; nutrition assessment and management are integrated with medication and treatment of TB (WHO, 2013) [3]. TB reduces nutrition, and nutritional sufficiency weakens bodily immunity so that it increases the possibility of activating latent TB. Most individuals are catabolic and lose weight; some of them indicate malnutrition and mineral when being diagnosed. Weight loss in TP patients can be caused by some factors such as reducing food intake due to the declining appetite, nausea and stomachache, nutrition loss due to vomit

and diarrhoea. The low BMI (Bodily Mass Index), which is lower than 18.5 kg/m^2 is related to the increase if death risk, relapse and severity of TB. An effective TB therapy will repair nutritional status by improving patients' appetite and increasing metabolism efficiency (WHO, 2013) [3].

BMI below 18.5 increases 2-3 times higher risk of TB. Otherwise, weight gain will reduce this risk. BMI in average increases in cured patients by the end of the medication compared to the patients who are not cured by the same time (Nagpal et al., 2014) [9].

In conclusion, the mean of sputum conversion period in pulmonary TB patients who are ≥ 18 years old, medicated with category I OAT, BMI ≥ 18.5 in the group whose members are supplemented with vitamin A 6000IU + category I OAT is 2.4 weeks, whereas that of the group whose members are only medicated with category I OAT is 4.1 weeks. Vitamin A supplementation in the medication of pulmonary TB patients who are ≥ 18 years old, medicated with category I OAT and BMI ≥ 18.5 has an influence on the acceleration of sputum conversion at an intensive phase in the working area of Medan Kota Subdistrict Puskesmas.

Pulmonary TB patients who were ≥ 18 Tahun, medicated with category I OAT, whose BMI was ≥ 18.5 were mostly 18-27 years old, male, university students, whose BTA were 3+, in the treatment group had conversion in the second week and the control group had conversion in the fourth week.

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