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# Optimization of the Duration of the Administration of Mesenchymal Stem Cells Wharton's Jelly to the Level of Matrix Metalloproteinase-1 and Transforming Growth Factor-β in Osteoarthritis Rat Model

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## Abstract

**BACKGROUND:** Mesenchymal Stem Cell Wharton's Jelly (MSC-WJ) is promising candidates for osteoarthritis (OA) therapy since they have chondrogenic potential and the ability to form the extracellular matrix.

**AIM:** This study aimed to determine the effect of the time giving MSC-WJ on bioactive markers of osteoarthritis.

**METHODS:** The osteoarthritis rat model was treated by intra-articular injection with MSC-WJ and α MEM as a control. Four and 8 weeks later performed a histological analysis of cartilage and the determination of the levels of Matrix Metalloproteinase-1(MMP-1) and Transforming growth factor β1 (TGF-β1) in serum by ELISA.

**RESULTS:** The results showed that administration of MSC-WJ showed improvement in the histological picture of knee joints in experimental animals characterized by an increase in cartilage thickness on the joint surface. The administration of MSC-WJ showed a tendency to decrease MMP-1 serum levels of OA rats treated for 8 weeks, although statistically did not show a significant difference. Whereas, administration of MSC-WJ showed a decrease in serum levels of TGF-β1 OA rat treated for 8 weeks.

**CONCLUSION:** MSC-WJ can repair damaged knee OA cartilage tissue. The administration of MSC-WJ can reduce serum levels of TGF-β1 OA rats treated for 8 weeks.

## Introduction

Osteoarthritis (OA) is the most common chronic joint disease with age. OA is etiologically caused by various factors, including mechanical, biochemical, and genetic, all of which can contribute to OA [1], [2]. OA affects the knee, hand, hip, and spine joints most of all, which are the most important musculoskeletal joints. Symptoms are often associated with significant functional impairment, as well as signs and symptoms of inflammation, including pain, stiffness, and loss of mobility [3].

The causative factors for OA can alter the chondrocyte-matrix interaction in cartilage and stimulate chondrocytes to synthesize catabolic cytokines [4]. As a result of this event, there is an imbalance between degradation and synthesis in the articular joint tissue. Research has shown that chondrocytes produce several inflammatory mediators, such as interleukin-1-beta (IL-1β) and tumor necrosis factor-alpha (TNF-α) in tissue and joint fluid OA. Chondrocytes respond to pro-inflammatory cytokines by increasing the production of proteinases, prostaglandins, and nitric oxide (NO) [5] which degrade the matrix. At present, OA development is thought to be regulated in large part by excess matrix metalloproteinase (MMP), which contributes to the degradation of the extracellular matrix, such as MMP-1 and MMP-3, which play an important role in OA development by lowering the extracellular matrix [6]. Matrix metalloproteinase-1 is a member of MMP, which is highly responsible for the damage to articular joint tissue because it degrades native collagen, namely collagen types I, II, and III. Increased levels were not only seen in the synovial membrane, synovial fluid, and human cartilage diagnosed with OA [7] but also seen in the serum of OA mice significantly [6].

The degradation and synthesis of normal cartilage matrix molecules are constantly controlled by chondrocytes. Apart from catabolic factors, OA chondrocytes also express anabolic growth factors,

such as transforming growth factor (TGF)-β and insulin-like growth factor (IGF)-1 which stimulate extracellular matrix (ECM) production [8]. Transforming growth factor $\beta$ (TGF-$\beta$) is a multifunctional cytokine that modulates differentiation, proliferation, and extracellular matrix (ECM) production of various biological tissues [9]. However, the role of TGF-β is not only in regulating chondrocyte behavior and cartilage destruction but also in another feature of OA, namely, osteophyte formation. Osteophytes are growths on the margins of the joints that line the joint articulation surfaces and can cause clinical problems [10].

Mesenchymal stem cells (MSCs) are very promising candidates for articular cartilage regeneration and cell-based OA therapy because of their ability to differentiate into chondrogenic lineage cells and the ability to form a matrix [11], [12]. In addition, MSCs have immunomodulatory and trophic capacities by secreting anti-inflammatory and growth factors [13], which may improve the inflammatory and catabolic aspects of OA. MSC-based therapy is an action to replace cells that are damaged, lost or reduced by stem cells that can differentiate into the cells needed and MSC is easy to obtain and has minimal side effects. MSCs can differentiate into various specialized cells of mesoderm origin such as bone cells, cartilage, fat, cardiomyocytes, muscle fibers, and renal tubular cells and differentiate into cells from ectoderm origin, for example, neurons, and endoderm origin cells (endodermal origin), such as hepatocytes and pancreatic cells [14]. MSC can suppress the proliferation of inflammatory T cells, resulting in immunomodulatory and anti-inflammatory effects. These immunomodulatory mechanisms give rise to their potential use in autoimmune-mediated inflammatory conditions including inflammatory arthropathies [15]. In addition, the results of the study by Koh and Choi (2012) showed that intra-articular injection of MSC (derived from the infrapatellar fat pad) is effective in reducing pain and improving knee function in patients with knee OA [16].

Our previous study showed that the optimal time for MSC-WJ therapy has not been achieved to reduce MMP-1 levels, although histologically there has been the repair of cartilage tissue [17]. It is expected that from the results of this study, the optimal time will be obtained to decrease the level of MMP-1 which is one of the main proteases in degrading the cartilage matrix and the therapeutic effect of TGF-β which is one of the factors that functions to maintain cartilage homeostasis.

## Methods

### *Animals and experimental design*

Male white rats (*Rattus novergicus*) weighing between 250 and 300 g. The experimental animals were placed in clean cages, disinfected and free of pathogens and given standard food in the form of pellets and drinking ad libitum. The experimental animals were adapted for 1 week before treatment.

### *Osteoarthritis induction*

Osteoarthritis was induced by a single injection of intra-articular iodoacetate into the knee joints of anesthetized rats using intraperitoneal injection of 0.1 ml 10% xylazine and 0.2 ml 10% ketamine [18], [19], [20]. Prepare an MIA solution with a concentration of 20 mg/ml (sterile 0.9% NaCl solvent). After anesthetizing the rat's left leg was bent 90° at the knee. The patellar ligament is felt under the kneecap and is injected into this area. Each rat received 0.05 mL intra-articular injection into the left knee using a glass gas-tight syringe with a 27 gauge 0.5-in needle [18], [21]. Thirty osteoarthritis rats (3 weeks after induction of MIA, Janusz *et al*., 2001) were divided into five groups. The early OA group, the OA rat group after 4 weeks, the OA rat group after 8 weeks, the OA rat group was treated with MSC-WJ for 4 weeks, and the OA rat group was treated with MSC-WJ for 8 weeks. The treated group of OA rats was given 50 μL of MSC-WJ at a dose of $1 \times 10^6$ cells to the left knee, the control group and the untreated group were given complete medium after anesthesia.

### *Blood collection and knee joints*

Blood and Knee Joints were collected from the early OA group, the OA rat group after 4 weeks, the OA rat group after 8 weeks, the OA rat group was treated with MSC-WJ for 4 weeks and the OA rat group was treated with MSC-WJ for 8 weeks. In addition, the knee joint was also collected from healthy rats.

### *Flow cytometry analysis*

Mesenchymal stem cells used for the test were passage 3. MSC-WJ passage 1 originated from the human umbilical cord (UC-MSC) obtained from the UPT of Stem Cell Medicine Technology RSCM FKUI. Based on flow cytometry analysis, this MSC-WJ has expression of CD73-APC cell surface markers 99.8%, CD105-PerCP-Cys5.5 95%, and CD90-FITC 99.9%. Then MSC-WJ passage 1 is silenced (developed) and MSC passage 3 is obtained (Figure 1).

### *Histological analysis*

The rat knee joint was fixed with 10% formalin buffered phosphate, and then decalcified in 8% HCl for 1 week. Furthermore, it is processed into paraffin blocks and cut with a microtome with a thickness of 4 μm. The results of the cutting in the form of a ribbon are placed

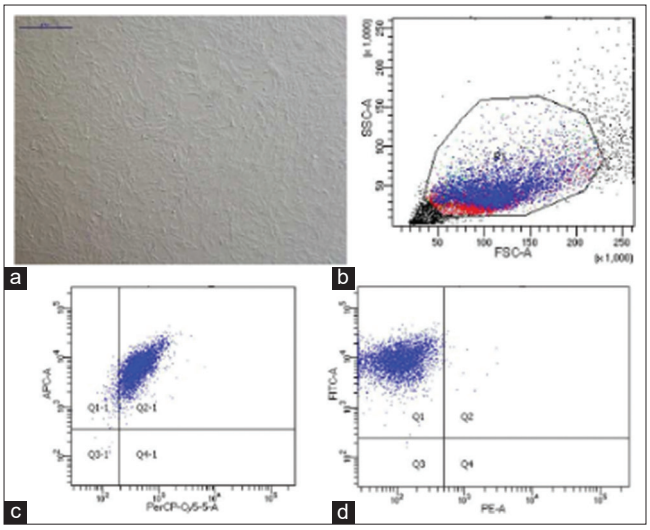


*Figure 1: Data on characteristics of mesenchymal stem cells Wharton Jelly. (a) Cells MSC-WJ reach confluence. Scale bar: 500 μM. Photographs of cells taken using a Nikon Ti-S microscope; (b) data flow cytometry. Forward scatter (FCS) plot & side scatter (SSC) plot. Population gated events (P1): 20,000; (c) cell surface markers expression: CD73-APC 99.8% and CD105-PerCP-Cy5.5 95%; (d) cell surface markers expression: CD90-FITC 99.9% and Lin (-) -PE 0.4%*

on the surface of warm water at a temperature of 45°C to eliminate folds in the ribbon due to cutting. Each ribbon (strip) was stained with Hematoxylin and Eosin.

### *Measurement of serum MMP-1 and TGF- β1 by ELISA*

Blood was drawn from the orbital sinuses of the rat eyes before being sacrificed and centrifuged at 3000 rpm for 15 min. The collected serum was stored at −80°C until the time of measurement. Serum MMP-1 and TGF-β1 levels were measured by an ELISA kit (Bioassay Technology Laboratory, China, and R&D Systems, Minneapolis, MN, USA). All samples were measured in duplicate.

### *Examination of MMP-1 Levels*

Prepare all reagents, standard solutions, and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature. Determine the number of strips required for the assay. Insert the strips in the framers for use. The unused strips should be stored at 2−8°C. Add 50 μL of the standard well. Add 40 μL sample to sample wells and then add 10 μL anti-MMP-1 antibodies to sample wells, then add 50 μL streptavidin-HRP to sample wells and standard wells (Not blank control well). Mix well. Cover the plate with a shaker. Incubate for 60 min at 37°C. Remove the sealer and wash the plate 5 times with wash buffer. Soak wells with at least 0.35 ml wash buffer for 30 s to a minute for each wash. For automated washing, aspirate all wells and wash 5 times with wash buffer, overfilling wells with wash buffer. Blot the plate onto paper towels or other absorbent material. Add 50 μL substrate solution A to each well and then add 50 μL substrate solution B to each well. Incubate plate covered with a new sealer for 10 min at 37°C in the dark. Add 50 μL stop solution to each well; the blue color will change into yellow immediately. Determine the optical density (OD value) of each well immediately using a microplate reader set a 450 nm within 30 min after adding the stop solution.

### *Examination of TGF-β1 Levels*

Prepare all reagents, standard solutions, control, and activated samples as directed in the previous sections. Remove excess microplate strips from the flate frame, return them to the foil pouch containing the desiccant pack, and reseal. Add 50 μL of Assay Diluent RD1-73 (for serum/plasma samples) to each well. Add 50 μL of Standard, Control, or activated sample per well. Tap the plate gently for 1 min. Cover with the adhesive strip provided. Incubate for 2 h at room temperature. A plate layout is provided to record standards and samples assayed. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (400 μL) using a squirt bottle, manifold dispenser, or auto washer. Complete removal of the liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels. Add 100 uL of TGF-β1 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 h at room temperature. Repeat the aspiration/wash (as in step 5) with Wash Buffer (400 μL). Add 100 μL of Substrate Solution to each well. Incubate for 30 min in the room (protect from light). Add 100 μL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing. Determine the optical density of each well within 30 min, using a microplate reader set a 450 nm.

### *Statistical analysis*

The Cartilage thickness data and MMP-1 were analyzed using the ANOVA test. Data of TGF-β1 were analyzed using a non-parametric procedure (Kruskal–Wallis). The data are expressed as the mean ± SD Test. $p < 0.05$ was said to be statistically significant.

## Results

Study of 30 male osteoarthritis rats induced by MIA for 3 weeks. The OA rats were divided into five groups, an early OA group, two groups of untreated OA rats and the other two groups treated with MSC-WJ for 4 weeks and 8 weeks.

### Histopathology

The results of the histopathological examination of the knee joint tissue showed changes in cartilage thickness (Figure 2).

### Cartilage thickness

Assessment of the thickness of the cartilage using the Betaview program, as well as the Beta 3.1MP Sony Exmor CMOS sensor. The results of the assessment showed a decrease in joint cartilage thickness between the control group and the 4 weeks OA group and the 8 weeks OA group. An increase in cartilage thickness occurred in the OA group treated with MSC-WJ.

**Table 1: Effect of MSC-WJ therapy on knee cartilage thickness in OA rats**

| Groups | Cartilage thickness (µm) | |
|---|---|---|
| | Mean ± SD | p |
| OA Control | 245.833 ± 61.8216 | 0.0001 |
| OA 4 weeks | 124.483 ± 37.2402 | |
| OA 8 weeks | 102.433 ± 21.0729 | |
| OA+MSC-WJ 4 weeks | 172.867 ± 53.8124 | |
| OA+MSC-WJ 8 weeks | 195.600 ± 17.3968 | |

Table 1 shows that the knee cartilage thickness of the rats was significantly different ($p < 0.05$). Knee cartilage thickness of the rats between the control group and the 4 weeks OA group and the 8 weeks OA rat group showed a significant decrease. Knee cartilage thickness increased after treatment with MSC-WJ. However, a significant increase in knee joint cartilage thickness occurred after the OA mice were treated with MSC-WJ for 8 weeks (Figure 3).

### ELISA examination

The blood obtained from the experimental animals was centrifuged and then serum was obtained. Serum before analysis was stored in the refrigerator at -80 $^{0}$C. The serum obtained was determined by the level of MMP-1 and TGF- β using the ELISA method. The implementation was carried out at the Biomedical Laboratory of the Faculty of Medicine, Andalas University.

### MMP-1

The results of measuring MMP-1 levels using the ELISA method showed a tendency to increase in serum MMP-1 levels of OA rats between the early OA rat group with the 4 weeks OA rat group and the 8 weeks OA rat group. Measurement data were analyzed using the ANOVA test.

Table 2 shows that the MMP-1 serum levels of OA rats were not significantly different ($p > 0.05$). MMP-1 serum levels of OA rat between the initial OA group and the 4 weeks OA rat group and the 8 weeks OA rat group showed an increasing trend, but this increase was not significantly different. Levels of MMP-1 between the 4 weeks OA rat group and the OA rat group treated with 4 weeks MSC-WJ showed an increasing trend but did not differ significantly. Meanwhile, MMP-1 levels between the 8 weeks OA rat group and the 8 weeks OA rat group treated with 8 weeks of MSC-WJ showed a decreasing trend but did not differ significantly (Figure 4).

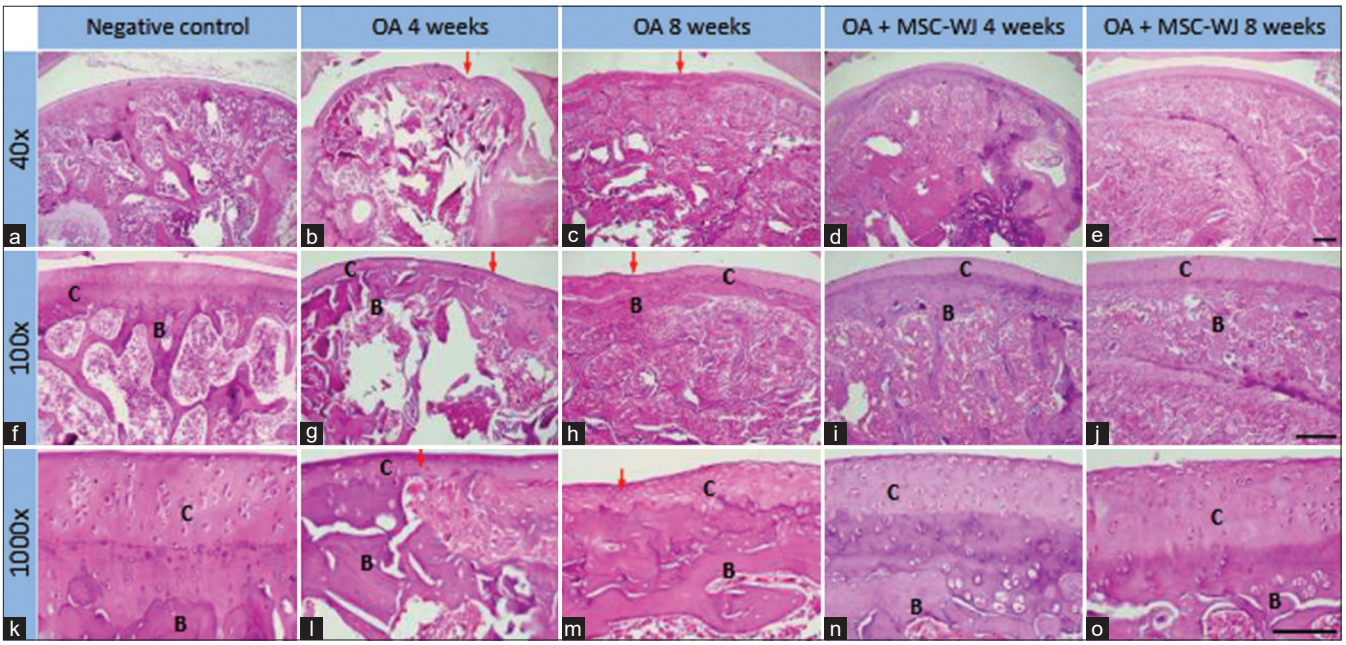


*Figure 2: Comparison of histology of genu joint tissue between experimental and treatment groups. Negative control (a, f, k), osteoarthritis positive control 4 weeks (b, g, l), osteoarthritis positive control 8 weeks (c, h, m), osteoarthritis MSC-WJ treatment 4 weeks (d, i, n) and osteoarthritis treated by MSC-WJ 8 weeks (e, j, o). The joint tissue of osteoarthritis animals showed a decrease in joint cartilage thickness with a thin cartilage area (arrow). On MSC-WJ administration, there was an increase in joint cartilage thickness compared to positive controls (OA 4 weeks and OA 8 weeks). Hematoxylin-eosin. 200 µm scale.*

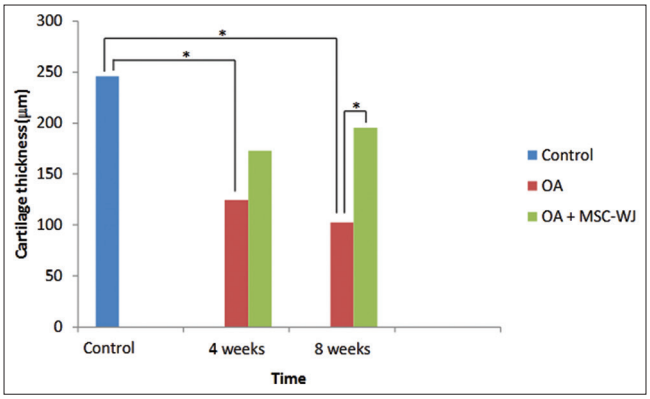


*Figure 3: Knee cartilage thickness in various treatment groups*

### *TGF-β1*

The results of measuring TGF- β1 levels using the ELISA method showed a tendency to increase serum TGF- β1 levels in OA rats between the initial OA rat group with the 4 weeks OA rat group and the 8 weeks OA rat group. Measurement data were analyzed using the ANOVA test.

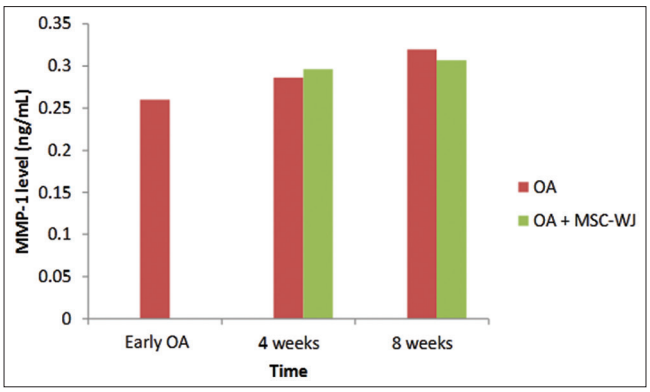


**Figure 4:** *MMP-1 levels in various treatment groups*

Table 3 shows that there is a significant difference in TGF-β1 levels between the initial OA rat group and the 8 weeks OA rat group and the OA rat group treated with 8 weeks MSC-WJ. Whereas,

**Table 2: Effect of MSC-WJ therapy on serum MMP-1 levels in OA rats**

| Groups | MMP-1 Levels (ng/mL) | |
|---|---|---|
| | Mean ± SD | p |
| Early OA | 0.26017 ± 0.05596 | 0.599 |
| OA 4 weeks | 0.28583 ± 0.07827 | |
| OA 8 weeks | 0.31950 ± 0.05222 | |
| OA+MSC-WJ 4 weeks | 0.29600 ± 0.08249 | |
| OA+MSC-WJ 8 weeks | 0.30650 ± 0.05356 | |

between groups of early OA rats with 4 weeks of OA rat and groups of OA rats treated with 4 weeks of MSC-WJ, there was no significant difference ($p > 0.05$). TGF-β1 levels between the 4 weeks OA rat group and the 8 weeks OA rat group treated with 8 weeks of MSC-WJ showed a significant difference ($p < 0.05$), whereas between the 4 weeks OA rat group with the 8 weeks OA rat group and the OA rat treated with MSC-WJ for 4 weeks had no significant difference ($p > 0.05$). The levels of TGF-β1 between the 8-week OA rat group and the 4 weeks MSC-WJ-treated OA rat group and the 8 weeks MSC-WJ-treated OA rat group were significant ($p < 0.05$). The levels of TGF-β1 between the 4 weeks MSC-WJ-treated OA group and the 8 weeks MSC-WJ-treated OA group were not found to be any significant difference ($p > 0.05$) (Figure 5).

**Table 3: Effect of MSC-WJ therapy on serum TGF- β 1 levels of OA rats using the Kruskal–Wallis test.**

| Groups | TGF-β level (ng/mL) | |
|---|---|---|
| | Mean ± SD | p |
| Early OA | 38.0433 ± 18.7827 | 0.004 |
| OA 4 weeks | 38.3167 ± 11.9387 | |
| OA 8 weeks | 64.9500 ± 27.9312 | |
| OA+MSC-WJ 4 weeks | 27.0283 ± 16.3462 | |
| OA+MSC-WJ 8 weeks | 19.8200 ± 3.6144 | |

## Discussion

### *Joint cartilage thickness*

In the induction of osteoarthritis, there is a decrease in cartilage thickness on the surface of the knee joint. With irregular cartilage thinning, there is a joint area with cartilage thinning and erosion. The thickness of the cartilage on the joint surface shows irregularity. In general, the thickness of the cartilage at week 8 is slightly lower than at week 4. Research conducted by Guzman *et al*. (2003) namely on the 28$^{th}$ day after the knee joint induced MIA an area of cartilage erosion occurred [21]. Januz *et al.* (2001) in their study found the occurrence of cartilage depletion on day 21 after MIA-induced mouse knee [18]. Research by Deng *et al.* (2015) showed an increase in surface irregularities, erosion, and loss of chondrocytes in the middle and deep layers of the OA joint [22].

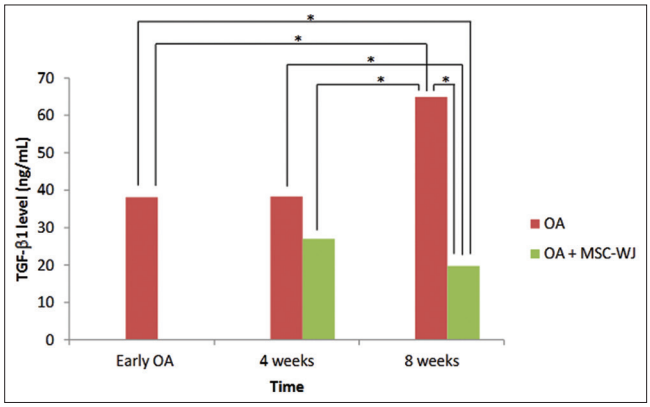


*Figure 5: TGF-β1 levels in various treatment groups*

Treatment with MSC-WJ showed an improvement in the histological features of the knee joint in experimental animals characterized by an increase in cartilage thickness on the joint surface in general. An increase in cartilage thickness was seen between the 8 weeks OA rat group and the 8 weeks OA rat group treated with MSC-WJ 8 weeks. The cartilage thickness that was treated for 8 weeks was slightly higher than

that for 4 weeks. The average cartilage thickness is still below the average bone thickness value of healthy animals. In some samples after MSC-WJ administration, areas with irregular cartilage thickness were found, but in general they gave the impression of improvement. This illustrates an improvement in cartilage which indicates an improvement in chondrocyte cells and an increase in protein synthesis that builds the matrix. Research by Deng *et al.* (2015) showed that OA joints treated with hyaluronic acid (HA) plus G-CSF-mobilized peripheral blood stem cells (gm-PBSCs) showed a significant reduction, while those treated with HA alone showed only a slight decrease in surface irregularity, erosion, and loss of chondrocytes in the middle and deep layers of the OA joint [22].

### *MMP-1*

Matrix metalloproteinase-1 (MMP-1) is one of the protease enzymes that act to degrade the main cartilage matrix components, such as collagen, aggrecan, protein links, and cartilage oligomeric proteins [23], [24]. This enzyme also serves to increase the proliferation and migration of MSCs [25]. MMP-1 increased its production in the synovial membrane, synovial fluid, and cartilage of humans with OA [7], as well as in the serum of OA rats [6]. The release of MMP-1 protein by human articular chondrocytes is stimulated by IL-1β [26]. According to Ponte *et al.* (2007) that MSC expresses MMP-1 constitutively [27]. Meanwhile, according to Chen *et al.* (2018) stated that MSC apart from expressing MMP-1 constitutive can also be increased by IL-1β [28].

This study showed that serum MMP-1 levels in OA rats treated with MSC-WJ had not shown a decrease at 4 weeks of therapy. MSC-WJ therapy for 8 weeks has shown a tendency to decrease MMP-1 levels, but this has not been significantly different. Research by Saulnier *et al.,* (2015) showed that administering MSC to OA rabbits had not been effective in reducing MMP-1 expression after 2 weeks of MSC injection, but was effective in reducing MMP-1 expression after 8 weeks of injection [29]. The results of research by Widowati *et al.* (2018) showed in vitro that MSC-WJ can reduce MMP-1 levels during the chondrogenesis process [30].

The results of this study indicate that MSC-WJ therapy has not been effective in reducing MMP-1 levels in <8 weeks after injection. This was seen at 4 weeks of MSC-WJ therapy which showed a tendency to increase MMP-1. Probably in this period, MSC-WJ synthesized and released MMP-1 to be needed in the migration and proliferation process of MSC [25], the process of MSC differentiation and the process of apoptosis [31]. According to Lejmi *et al.* (2015) that MSC migration depends on MMP-1 activity [32]. Research by Ho *et al.* (2009) demonstrated that MMP-1 plays an important role in the MSC migration function, via MMP1– PAR axis signaling [33]. Voronkina *et al.* (2017) showed the involvement of MMP-1 in the differentiation process of MSC, namely, the visible increase in MMP-1 activity during the differentiation process [34]. According to Zhao *et al.* (2016) stated that the apoptotic process induced by MMP1 is mediated by p38 MAPK [31].

The results showed a trend of decreasing MMP-1 at 8 weeks after MSC-WJ therapy. According to the research results of Saulnier *et al.* (2015), molecular analysis of rabbit synovial tissue showed a tendency to decrease MMP-1, MMP-3, and MMP-13 mRNA levels after 2 months of umbilical cord-mesenchymal stem cells (UC-MSCs) [29].

### *TGF-β1*

The transforming growth factor β (TGF-β) family plays a role plays a vital role in the development and homeostasis of various tissues. They regulate cell proliferation, differentiation, apoptosis and migration, as well as control ECM synthesis and degradation. In addition, these factors mediate the response of cells and tissues to injury and modulate immune function [35]. The induction of TGF-β1 is a response to mechanical stress in chondrocytes. Normal mechanical loading on damaged or overloaded joints in normal joints can stimulate chondrocytes to synthesize and release TGF-β1 in a mouse model OA [36].

This study showed that there was an increase in serum TGF-β1 levels of OA rats between the OA rat control group (Group I) with the 4 weeks OA mouse group (Group II) and the 8 weeks OA mouse group (Group III). Research by Yang *et al.* (2017) showed that there was an increase in TGF-β1 in OA model mice and lasted for a certain period [37]. The induction of TGF-β occurs in response to tissue damage, where TGF-β is an anti-inflammatory that plays an important role in various phases of inflammation [38]. In the early phase of OA, upregulation of TGF-β stimulates chondrocyte proliferation in an attempt to repair injured cartilage [39].

According to van der Kraan (2018) TGF-β in normal joints plays an important role as protective in maintaining differentiated chondrocyte phenotype, whereas in OA joints TGF-β will have a detrimental action due to its continued presence at high levels [40]. An increase in the concentration of TGF-β1 in subchondral bone triggers a cascade that leads to the development of osteoarthritis. This occurs because TGF-β plays a different role in the subchondral bone as opposed to its anabolic effect on articular cartilage [41]. Research by Zhen *et al.* (2013) showed that inhibition of TGF-β activity in subchondral bone may have a therapeutic effect [41]. Research by Chen *et al.* (2015) revealed that inhibition of the TGF-β1 signaling pathway could protect the knee joint from arthritis [42]. According to Chen *et al.* (2015) revealed that TGF-β1 is a potential pathogenic factor in the development of OA in adult joints [42]. Therefore, inhibition of TGF-β1 activity should be considered in the prevention and treatment

of OA.

The levels of TGF-β1 had not decreased significantly after 4 weeks of MSC-WJ therapy. However, the level of TGF-β1 decreased after being treated with MSC-WJ for 8 weeks. Mesenchymal stem cells can act as an anti-inflammatory by reducing the production of pro-inflammatory cytokines which directly inhibit T cell function and proliferation [43], prostaglandin E2 secretion, indolamine 2.3 dioxygenase, TGF-β1, and iNOS. In addition, research by Abou Elkhier *et al.* (2018) revealed that BMMSC therapy caused a decrease in TGF-β regulation in the OA group of rats compared to the untreated group of OA rats [44].

## Conclusion

Based on the results of the study, it can be concluded that the Mesenchymal Stem Cell Wharton Jelly can histologically repair damaged knee cartilage tissue in OA rats. Administration of Mesenchymal Stem Cell Wharton Jelly tended to reduce serum MMP-1 levels in knee osteoarthritis rats at 8 weeks of therapy, but the decrease in MMP-1 levels was not significantly different. However, giving Mesenchymal Stem Cell Wharton Jelly reduced serum TGF-β levels in knee osteoarthritis rats treated for 8 weeks.

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## Ethics of Study

This research has received ethical consideration and approval from the Research Ethics Committee Team of the Faculty of Medicine, Andalas University with registration number 349/KEP/FK/2019.

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