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The Effect of Topical Gel Secretome Hypoxic Mesenchymal Stem Cells (SH-MSCs) on IL-6 and TGF- β Gene Expression (Experimental Study in Male Wistar Alopecia-Like Rat Models Induced by Fluconazole)

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ABSTRACT: Alopecia induced by the accumulation of reactive oxygen species (ROS) stimulates changes in the immune and neuroendocrine systems. Hair follicles have dermal papillary cells (DPCs) that bind to corticotropin-releasing factor (CRF) receptors. Research shows that transforming growth factor (TGF-β) is one of the expressions that can be an indication of the influence of CRF. The effect of CRF itself is to cause hair loss. The purpose of this study was to determine the Secretome of Hypoxia Mesenchymal Stem Cells (SH-MSCs) against IL-6 expression and TGF-β expression. This study used experimentally in vivo with the Post Test Only Control Group Design method. The subjects of this study were male rats of Wistar strain which were divided into 4 treatments consisting of 1 healthy group, 1 negative control group, 1 treatment group with SH-MSCs dose 10% in a dose of 200 mg/rat/day gel, and 1 more treatment group with SH-MSCs dose 20% in a dose of 200 mg/rat/day. On the 21st day, IL-6 expression and TGF-β expression were examined. Data were analyzed using the One-Way ANOVA Test and the Kruskall-Wallis Test to determine the effect of each group. The One-Way ANOVA test showed the results of IL-6 expression ($p = 0.000$) showed a significant difference ($p < 0.05$) and the Kruskall-Wallis test of TGF-β expression ($p = 0.000$) with SH-MSCs dose 200 was a significant difference ($p < 0.05$). The administration of 10% and 20% doses of SH-MSCs in 200 mg gel may decrease IL-6 expression. Dosed SH-MSCs 20%increased TGF-β expression in alopecia-like rats. The development of the use of SH-MSCs is expected to be an alternative to alopecia therapy that is more beneficial and safe for the body.

KEYWORDS: Secretome Hypoxia Mesenchymal Stem Cells (SH-MSCs), IL-6x, TGF-β

I. INTRODUCTION

Alopecia is a disorder where there is loss of part or all of the hair on parts of the body or hair.[1] In alopecia, there are changes in several pro-inflammatory cytokines such as tumor necrosis factor-a (TNF-α) and interleukin-6 (IL-6) which causes changes in the morphology and growth cycle of hair which results in hair loss and baldness. Accumulation-induced alopecia reactive oxygen species (ROS) will stimulate changes in the immune and neuroendocrine systems. Hair follicles have dermal papillary cells (DPCs) that bind to receptors of corticotropin-releasing Factor (CRF). Research shows that transforming growth factor (TGF-β) is one of the expressions that can be an indication of the influence of CRF. The effect of CRF itself can cause hair loss.[1] Increasing levels of TGF-β as an anti-inflammatory mediator is expected to reduce CRF and reduce the rate of hair loss.Various therapies have been developed to treat alopecia, but conventional treatment is accepted Food Drug Administration (FDA) only Minoxidil and Finasteride. This treatment only provides temporary results as well has many side effects on long-term use.[1] Therefore, safe and effective therapeutic approaches are needed as a complement to existing conventional therapies. One of the therapies developed for the treatment of alopecia is using Secretome Hypoxia Mesenchymal Stem Cells (SH-MSCs).[1]Alopecia areata is a type of alopecia whose prevalence is known to occupy 2% of the total global population in the world. The prevalence has increased from 0.1% in 1970 to 2.11% in recent years. In the USA, the prevalence of alopecia areata is high0.57% to 3.8% of the population.[4] The prevalence of alopecia in the USA varies across different populations and studies.[5] Alopecia areata can develop into more severe forms, namely alopecia totalis (AT), which is the loss of all the hair on the scalp, and alopecia universalis (AU), which is the loss of

all the hair on the body.[5] Most alopecia patients come from the age group 30 to 59 years, namely the productive age group.[6] These various studies show that better therapy is needed to treat alopecia.SH-MSCs is an MSC culture medium that is conditioned in hypoxia and has gone through a filtration process using a technique of tangential flow filtration (TFF).[7] Preclinical studies show SH-MSCs can promote hair growth through nicotinamide adenine dinucleotide phosphate (NADPH) oxidase.[8] Preclinical studies show that SH-MSCs contain various anti-inflammatory cytokines, e.g vascular endothelial growth factor (VEGF), Insulin-like growth factor (IGF), hepatocyte growth factor (HGF), bone morphogenic proteins (BMPs), interleukin-6 (IL-6), and other cytokines that are closely related to hair regeneration1. SH-MSCs also contain TGF-β which is an anti-inflammatory cytokine and inhibits hair loss.[1,7] Other preclinical studies examined in vitro the effects of the secretome Adipose Stem Cells (ADSCs) in a mouse model of Androgenic Alopecia. This research found an immunomodulatory effect9. The secretome of ADSCs showed expression interferon-γ (IFN-γ), chemokine ligand 9 (CXCL9), chemokine ligand 10 (CXCL10), and T-cell infiltration. The effect of ASDCs on alopecia was also confirmed with preclinical studies. In this preclinical study, follicular genesis and hair growth are mediated by the secretory properties of white adipocytes. The results of this study pave the way for regenerative therapy of ADSCs for androgenic alopecia. There were thirty-five signaling proteins analyzed, VEGF levels, epidermal growth factor (EGF), IL-6, Eotaxin, monocyte chemoattractant protein-3 (MCP-3), IFNγ-inducible protein-10 and macrophage inflammatory protein (MIP-1α) which shows higher levels in the bald zone, conversely, MCP-1 is the lowest in the bald zone.[10] Clinical studies reported an increase in Wnt signaling in DPCs which is one of the main factors that increase hair growth. The Secretome of MSC and platelet-derived growth factor (PDGF) affects hair growth through cell proliferation to prolong the anagen phase fibroblast growth factor (FGF-7), induces cell growth (activation It extracellular signal-regulated kinase (ERK), stimulates hair follicle development (β-catenin), and suppresses apoptotic signals (Bcl-2 release and Akt activation).[16]Interleukin-6 is a cytokine that plays a role in the non-specific immune system and the specific immune system.[11] Clinical studies have found that IL-6 plays a role in the pathogenesis of one type of alopecia, namely alopecia areata, through the regulation of cell pathways T helper 1 (Th1) and T helper 2 (Th2). Th2-induced IL-6 will cause hair root collapse during the anagen phase through inhibition of the immune system and inadequate regulation of T cells. The clinical study found that disruption of the IL-6 signaling pathway can cause hair to grow back.[13]TGF-β is a functional regulatory polypeptide that controls many aspects of cellular function including independent cell renewal, differentiation, proliferation, migration, apoptosis, adhesion, angiogenesis, and survival. TGF-β as a pleiotropic cytokine has been shown to play a role in regulating stem cell differentiation through pathway-related cross-talk Smad and non-Smad.[12] TGF-β is also an anti-inflammatory mediator that is expected to reduce hair loss.Various studies report that SH-MSCs can suppress the secretion of pro-inflammatory cytokines. Administration of SH-MSCs can improve the condition of alopecia-like by inhibiting the inflammatory response through reducing levels of pro-inflammatory cytokines IL-6, IL-1β, TNF-α, and increasing anti-inflammatory cytokines IL-10 and TGF-β1. However, topical administration of SH-MSCs gel on the expression of IL 6 and TGF-β in Alopecia-like has never been done. Based on this background, it is necessary to carry out research to determine the effect of administering SH-MSCs-based gel on the expression of the IL-6 and TGF-β genes in the Wistar mice model of alopecia-like.

II. MATERIAL AND METHOD

This research is experimental research with a Post Test Only Control Group Design Group Design. The research subjects used 24 male Wistar rats 8-12 weeks old with a weight between 150-250 grams that meets the inclusion and exclusion criteria, adapted for 7 days. This study used 4 treatment groups, a control group of healthy mice (S), a negative control group of alopecia-like rat models with control 100 mg base gel Nacl (K), treatment group 1, namely model mice alopecia-like by administering topical 10% SH-MSCs gel at a dose of 200 mg/rat/day (P1), and treatment group 2, namely alopecia-like rats model by administering 10% SH-MSCs topical gel at a dose of 200mg/rat/day (P2), on the 28th day of male Wistar rats skin samples were taken to examine IL-6 expression and TGF-β expression

Research materials

The material for this research consists of culture material consisting of rat umbilical cord, fluconazole oral tablet, acetone-olive oil NaCl 0.9%, PBS, DMEM, FBS, fungizone, and penstrep. While the materials used for the treatment process are water-based gel, 70% alcohol, PBS, Ketamine, and Xylasine.

Research equipment

This research uses equipment in the form of cell culture equipment consisting of a Biosafety Cabinet (BSC), micropipette, CO incubator2, dissecting kit, 75T flask, microscope, and dissecting set to take skin samples after treatment. Hypoxic culture conditions are obtained using a hypoxic chamber. Oxygen meters are used to measure oxygen levels inside a hypoxic chamber.

Apart from that, this study also used sterile swabs to apply SH-MSC gel. The tool used for analysis of the cytokines Interleukin 6 and TGF-β is qRT-PCR.

Mesenchymal Stem Cell Isolation Procedure from Umbilical Cord

The entire process is done in-house biosafety cabinet class 2, using sterile equipment and working with high sterility techniques. (1) The umbilical cord separated from the rat fetus and the blood vessels were removed. (2) Using tweezers, the umbilical cord was placed in a Petri dish, and the umbilical cord was washed thoroughly using PBS. (3) Umbilical cord collected and placed in a sterile container containing 0.9% NaCl. (4) Umbilical cord chopped until fine and placed evenly in a 25T flask and let stand for 3 minutes until the tissue adheres to the surface of the flask. (5) A complete medium consisting of DMEM, fungizone, penstrep, and FBS) is added slowly until it covers the tissue. (6) The explants were incubated in an incubator at 37℃°C and 5% CO_2. (7) Cells will appear after approximately 14 days from the start of the culture process. (8) The medium was replaced every 3 days by removing half of the medium and replacing it with a new complete medium. (9) Cell maintenance was carried out until the cells reached 80% confluency.

Hypoxia Process

MSCs reaching 80% confluency were added with a complete medium of up to 10 mL. A flask that has been filled with MSC is then put inside a hypoxic chamber. Nitrogen gas is channeled through the inlet valve and an oxygen meter is placed in the sensor hole to measure the oxygen concentration inside the chamber. Nitrogen is added until the indicator needle shows a concentration of 5% oxygen. Chamber The containing flask was incubated for 24 hours at 370C. After 24 hours, the culture medium was taken and filtered using TFF to obtain SH-MSC which was then mixed with gel according to the dosage of P3 and P4.

RNA extraction and cDNA synthesis

Blood samples were taken and a multistage centrifugation process was carried out using Ficoll Paque so that PBMCs were obtained. Skin samples were fixed in 10% formalin and made into paraffin blocks. A 100 mg skin sample was then cut into small pieces and put into a tube filled with 50 mL of RNA Iso Plus. On the other hand, there are 10 PBMCs7 Each cell was transferred to a microtube and given 50 mL of RNA Iso Plus. The skin pieces were ground using a micropaste and 50 mL of RNAIso Plus was added and kept at room temperature for 5 minutes. Add 20 mL of chloroform and vortex until the solution becomes milky white. Incubate at room temperature for 2-3 minutes, and centrifuged at 15,000 rpm for 15 minutes at 400C until the solution in the tube appears to have 3 layers. The top layer is RNA (liquid phase), the second layer is DNA (semisolid phase) and the bottom layer contains cell debris. The top layer was transferred to a new centrifuge tube and the volume was measured, and a volume of isopropanol equal to the RNA taken from the top layer was added. The Eppendorf tube was shaken until white threads appeared, then centrifuged at 15,000 rpm for 10 minutes at 400C. The supernatant is discarded until a white pellet is visible at the bottom of the tube. After drying, 100 mL of 70% ethanol was added to the solution (Diethyl percarbonate) DEPC then turned back and forth repeatedly and centrifuged again at 15,000 rpm for 5 minutes at a temperature of 400C. The supernatant was discarded and 30-50 µm DEPC was added. The mixture was incubated at 550C for 10 minutes. Next, the total RNA solution was obtained and stored at -800C. RNA was quantified with Nanodrop. The quantification results were calculated to be 3000 ng. Synthesis of cDNA by making mixture A by mixing the calculated RNA samples, 1 µL of OligoDT, and PCR water until it reaches a volume of 10 µL, then incubating for 5 minutes at a temperature of 700C. Mixture A was added with mixture B consisting of 5X buffer 4 µL, DEPC-Treated H2O 5 µL, and ReverTraAce 1 µL. The mixture was incubated at 250C for 5 minutes, 420C for 50 minutes and 850C for 5 minutes.

Readings of Interleukin 6 and TGF B Expression with Time-Polymerase Chain Reaction (RT-PCR)

IL 6 and TGF-β expression readings were analyzed using reverse Transcription-Polymerase Chain Reaction (RT-PCR). A mixture of 3 µL cDNA samples, Taq master mix (dNTPs, Taq DNA polymerase, reaction buffer, and MgCl2) as much as 12.5 µL, specific primer for each target gene as much as 0.6 µL for forward and reverse primers and 8.3 µL Nuclease Free Water. PCR products were then analyzed using Illumine qRT-PCR. The GADPH primer sequences used were F: 5'-GCG ACA GTC AAG GCT GAG AATG -3' and R: 5'-TCT CGC TCC TGG AAG ATG GTGA -3'. The IL-6 primer sequences used were F: 5'-TCC TAC CCC AAC TTC CAA TGC TC-3' and R: 5'-TTG GAT GGT CTT GGT CCT TAG CC-3'. The TGF-β primer sequences used were F: 5'-TAC CAT GCC AAC TTC TGT CTG GGA-3' and R: 5'-ATG TTG GAC AAC TGC TCC ACC TTG-3'. The increase in gene expression was analyzed in the ratio of increase to housekeeping genes using the software EcoStudy.

III. RESULT

Validation of MSCs

SH-MSCs isolation was carried out in the laboratory of Stem Cell and Cancer Research (SCCR) Indonesia, Semarang, using the umbilical cord of 21-day-old pregnant mice. The isolation results were then cultured in T75 flasks with a complete medium. The results of the MSCs culture after the 5th passage showed an image of cells attached to the bottom of the flask with morphology spindle-like cells (Figure 1) using microscopic observation.

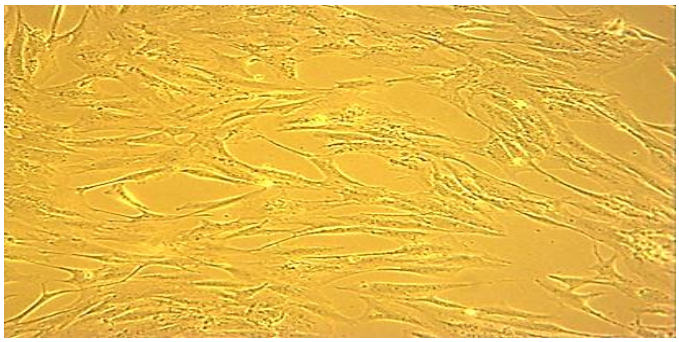

Figure 1. The morphology of MSCs resembles fibroblast cells at 200x magnification

The results of MSC isolation were validated using flow cytometry to show the ability of MSCs to express various surface marker special. Quantitative results are the percentage of positive expression of CD 90.1 (97.6%), CD 29 (97.7%), and negative expression of CD 45 (1.5%), and CD 31 (3.2%) (Figure 2).

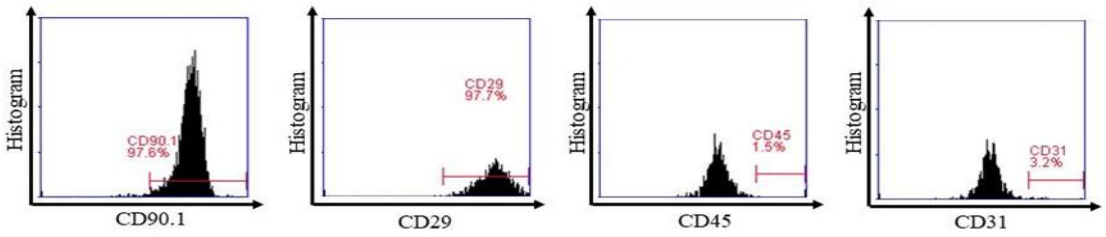

Figure 2. Expression of CD90, CD29, CD45, and CD31 on MSCs

MSC cells were cultured in osteogenic and adipogenic induction medium for 21 days to determine their differentiation ability into osteocytes and adipocytes. The image of osteogenic differentiation of MSCs is shown by arrows on cells containing calcium deposits with staining Alizarin Red Figure 3. Adipogenic differentiation of MSCs is indicated by arrows on cells containing accumulated lipid droplets with staining Oil Red O (Figure 4). Calcium and fat deposition resulting from the differentiation of MSCs into osteocytes and adipocytes are shown in red in each culture.

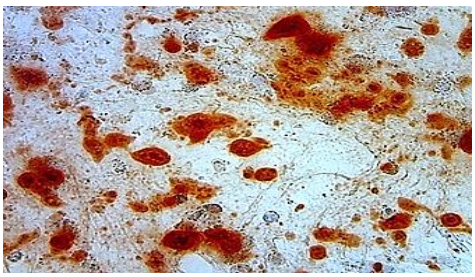

Figure 3. MSCs can differentiate into osteocytes

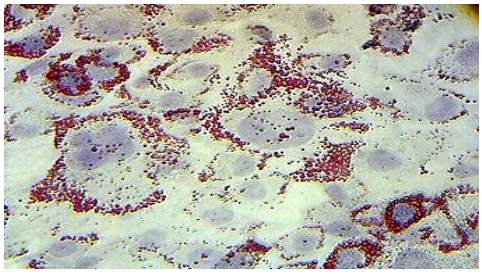

Figure 4. Adipocytes after staining Alizarin Red and Oil Red O at 200x magnification

Effect of Administration of SH-MSCs on Expression TGF-β and IL-6 expression

Study influence of hypoxic mesenchymal stem cell secretome on IL-6 expression and TGF-β expression Induced Wistar rat fluconazole was done for 21 days until the rat became an Alopecia-Like model. The results of the research are listed in Table 1.

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Table 1. Results of Mean Analysis, Normality Test, Homogeneity Test on IL-6 expression and TGF-β expression

Variable	Group S	K N=6	P1 N=6	P2 N=6	Sig.(p)
IL-6 (ng/L) Expression					
Mean	1.000	3.026	2.454	1.419	
Std. deviation	0.141	0.133	0.225	0.134	
Shapiro Wilk	0.960*	0.346*	0.584*	0.591*	
Levene Test					0.686**
One Way Anova					0.000***
TGF-β Expression					
Mean	1.000	0.683	1.302	3.176	
Std. deviation	0.141	0.183	0.131	0.394	
Shapiro Wilk	0.960*	0.434*	0.144*	0.007	
Levene Test					
Kruskall Wallis					0.000***

Information: *Normal p>0.05 **Homogeneous p>0.05 ***Significant p<0.05

Table 1 shows that the lowest mean IL-6 expression was in the healthy rat group (S), followed by the treatment group (P2) alopecia-like rat model by administering SH-MSCs gel at a dose of 200 µL/kgBW in 200 mg gel, and followed by the treatment group (P1) of Alopecia-like rat models by administering SH-MSCs gel at a dose of 100 µL/kgBW in 200 mg gel. The highest mean IL-6 expression was in the negative control (K-) mouse model group alopecia-like with control 200 mg NaCl base gel. All groups of IL-6 expression levels based on the Shapiro-Wilk test are normally distributed with a p-value >0.05 and the homogeneity test result shows a homogeneous p-value of 0.561 (p>0.05), so data analysis uses a parametric test One Way ANOVA. One-way ANOVA test result showed significant differences between all groups with a p-value of 0.000 (p<0.05), then continued with the test Pos Hoc LSD to find out which groups are the most influential.The highest mean TGF-β expression (Table 1) is in the treatment group (P2) of Alopecia-like rat models by administering SH-MSCs gel at a dose of 200 µL/kgBW in 200 mg gel, then followed by the treatment group (P1) of Alopecia-like rat models by administering SH-MSCs gel at a dose of 100 µL/kgBW in 200 mg gel, and followed by a group of healthy rats (S). The lowest mean TGF-β expression was in the negative control (K) alopecia-like rat model group with control 200 mg NaCl base gel. All groups expressed TGF-β expression based on the Shapiro Wilk test one of the groups, namely the treatment group (P2), did not normally distribute with a value of 0.007 p-value <0.05. Kruskal-Wallis test result shows significant differences between all groups with a p-value of 0.001 (p<0.05). Then continue using the Mann-Whitney test to find out which groups are the most influential.

Differences in IL-6 Expression Between Groups

The difference in IL-6 expression between the 2 groups was determined by the test Post Hoc LSD as presented in Table 2.

Table 2. Differences in IL-6 Expression between the 2 Groups

Group	p-Value
S vs K	0.000*
S vs P1	0.000*
S vs P2	0.000*
K vs P1	0.000*
K vs P2	0.000*
P1 vs P2	0.000*

*LSD Post Hoc Test with a significant value of p<0.05

Test Post Hoc LSD test Table 2 shows the expression of IL-6 in the healthy mouse group (S), there is a significant difference to the group (K) p-value 0.000 (p<0.05), the healthy mouse group (S) there is a significant difference to the group (P1) p-value -value 0.000 (p<0.05), and in the healthy mouse group (S) there was a significant difference to group (P2) p-value 0.000 (p<0.05). There is a significant difference in group (K) to group (P1) with a p-value of 0.000 (p<0.05) and there is a significant difference in group (K) to (P2) with a p-value of 0.000 (p<0.05). Then there is also a significant difference between groups (P1) and (P2) with a p-value of 0.000 (p<0.05). Based on the data above, it can be concluded that administration of SH-MSCs gel in doses of 10% and 20% in

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200 mg gel has a significant effect on reducing IL-6 expression in male Wistar rats with the alopecia-like model so the hypothesis statement is accepted.

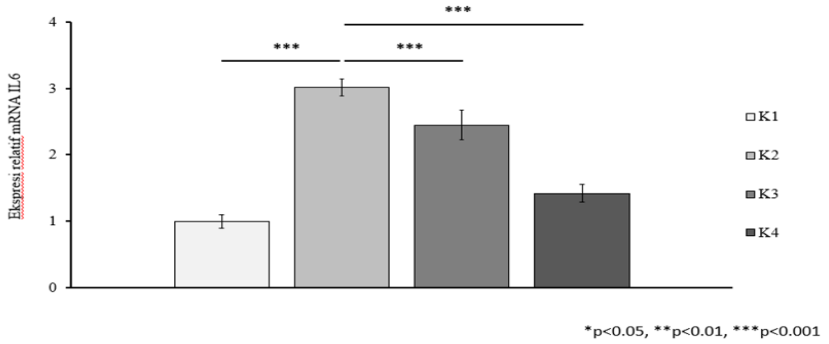

Figure 5. The expression of IL-6

The Different of TGF-β Expression between Groups

The difference in TGF-β expression between the 2 groups was determined by the Kruskal-Wallis test with the Mann-Whitney test as presented in Table 3.

Table 3. Differences in TGF-β Expression between the 2 Groups

Group	p-Value
S vs K	0.010*
S vs P1	0.010*
S vs P2	0.004*
K vs P1	0.056
K vs P2	0.000*
P1 vs P2	0.004*

*Mann-Whitney test with a significant value of p<0.05

The Mann-Whitney test result (Table 3), shows the expression of TGF-β in the healthy mouse group (S), there is a significant difference between the group (K) p-value 0.010 (p<0.05), the healthy mouse group (S) there is a significant difference compared to the group (P1) p-value -value 0.010 (p<0.05), and in the healthy mouse group (S) there was a significant difference to group (P2) p-value 0.004 (p<0.05). There is no significant difference in group (K) to group (P1) with a p-value of 0.056 (p<0.05) and there is a significant difference in group (K) to (P2) with a p-value of 0.000 (p<0.05). There is also a significant difference between groups (P1) and (P2) with a p-value of 0.004 (p<0.05). Based on the data above, it can be concluded that administration of SH-MSCs gel at a dose of 10% did not have a significant effect on increasing TGF-β expression so the hypothesis statement was rejected. Administration of 20% SH-MSCs gel in 200 mg gel had a significant effect on increasing TGF-β expression in male Wistar rats in the alopecia-like model so the hypothesis statement was accepted.

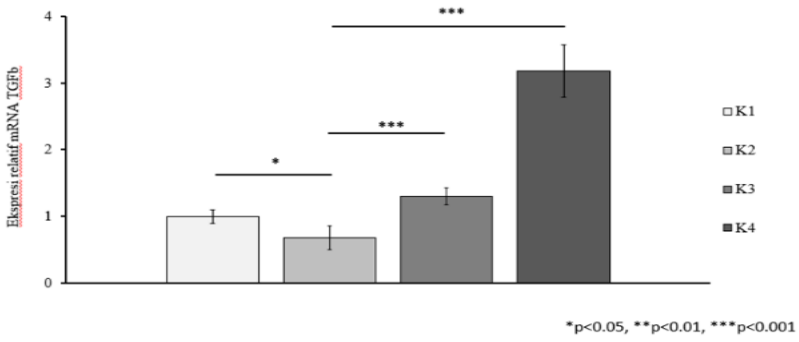

Figure 6. The expression of TGF-β mRNA

IV. DISCUSSION

Hair loss is determined by various factors: inherited (tricodystrophy and alopecia androgenetic), accompanying comorbid conditions, hormonal damage (thyroid organ disease, insulin resistance), immune system (alopecia and lupus erythematous), poor

nutrition, environmental elements (drugs, ultraviolet (UV) radiation), mental disorders (stress and trichotillomania), and aging. These factors are detrimental and affect the hair cycle, thereby reducing stem cell activity and requiring time for hair follicles to recover.[1]Perceived stress (internal or external) can stimulate changes in neuroendocrine immunity. It has been shown that prolonged stress can increase inflammatory cytokines (e.g. INF-ɣ) leading to inflammation and ending in apoptosis, cell senescence, and premature catagen transition. DPCs in hair follicles have receptors for corticotropin-releasing factor (CRF). CRF inhibits hair shaft elongation and DPC proliferation by arresting division in the G2/M phase. Additionally, it contributes to the accumulation of reactive oxygen species (ROS) which also stops the cell cycle. CRF decreases the expression levels of anagen-related cytokines, such as hepatocyte growth factor (HGF), Wnt5a, TGFβ, dan Vascular endothelial growth factor (VEGF). The stress of hair loss contributes to a negative feedback loop that increases the incidence of hair loss.[2] Hair Follicle (HF) are immunologically similar spots to the cerebrum, eyes, and gonads, and they are influenced by the neuroendocrine-immune system. In physiological conditions, this is influenced by (1) Low or no expression of the principal MHC I antigen, (2) the presence of non-functional Langerhans cells, and (3) local expression of immunosuppressive substances (TGF-β1 and α-melanocyte MSH). The conclusion from this is that HF can easily be used in transplantation.[16]MSCs actively participate in angiogenesis through direct differentiation, cell contact interactions with endothelial cell lineages, and the release of pro-angiogenic factors in a paracrine manner. Paracrine factors can increase the blood supply of damaged tissue through the activation and recruitment of stem and progenitor cells. MSCs possess several angiogenesis factors, interleukin-8 (IL-8), Insulin-Like Growth Factor 1 (IGF-1), and Vascular Endothelial Growth Factor (VEGF). These pro-angiogenic factors can form vascular networks and increase the migration of endothelial cell derivatives in vitro. In addition to the secretion of angiogenic factors by MSCs, it has been revealed that various factors present in the secretome can activate angiogenic properties in endothelial cells. For example, MSCs influence hair growth through cell proliferation to prolong the anagen phase (FGF-7), induce cell growth (ERK activation), stimulate hair follicle development (β-catenin), and suppress apoptotic cues (BCL-2 release and Akt activation).[1]This study showed that IL-6 expression decreased very significantly compared to the negative control group. Then, TGF-β expression increased significantly compared to the negative control group. This shows that SH-MSCs influence IL-6 expression and TGF-β expression. This is thought to be because another content of SH-MSCs is TGF-β, where TGF-β can activate SMAD2/3 phosphorylation for translocation to the nucleus and induce the expression of anti-inflammatory cytokines.[18]. Maintaining MSCs is critical for tissue homeostasis in repair. Their division does not occur frequently in mature organisms, and most of them are not in prime condition. Thus, it is very important to understand their activation and induction components, which will enable the use of multipotent cells in regenerative plastic surgery and hair regrowth. Their use is complicated by the fact that receptor expression on various growth factors and microenvironmental effects may vary.[16] TGF-β levels increased on the seventh day due to the proliferation phase. Higher than the healthy group because they are in the inflammatory phase. This is in line with previous studies which state that platelet aggregation and release of ADP, PDFG, tumor growth factor b (TGFb), and CXCL4 activate local cells (fibroblasts and keratinocytes) and the immune cascade to initiate inflammation. Keratinocytes, as the main cell type of the epidermis, release proinflammatory cytokines and antimicrobial peptides and proteins (AMPs). Together, these immune responses aim to decontaminate the wound bed by recruiting neutrophils and macrophages to the alopecia.[19]Each dose of SH-MSCs topical gel showed a different decrease in IL-6 expression and an increase in TGF-β expression. This indicates that each dose showed a different response to IL-6 expression and TGF-β expression, which means that each dose has different responses to inflammation. If compared between the two doses, the 10% and 20% doses both reduced IL-6 expression compared to the control group. This research is in line with previous research which stated that 23 weeks after the last treatment using MSC there was an increase in average hair thickness (29 ± 5.0%) above the initial value for the targeted area. 12 weeks after the last injection with A-PRP the average hair number and hair density (31 ± 2%) increased significantly[16]. Another study also said that among the thirty-five signaling proteins analyzed, the levels of VEGF, EGF, IL-6, Eotaxin, MCP-3, IFNγ-inducible protein-10, and MIP-1α were higher in the bald zone compared to the periumbilical zone[10]. 20% SH-MSCs topical gel showed increased TGF-β expression compared to the control group, while 10% SH-MSCs topical gel did not show increased TGF-β expression compared to the control group. This research is in line with previous research which compared MSC-CM-T doses of 10% and 20% for healing wounds. In this study, it was found that a 20% dose of MSC-CM-T was more effective in initiating an increase in wound closure rates[19].The weakness of this study is that histopathological examination of CD45 and CD68 was not carried out to determine the presence of inflammatory lymphocytes and macrophages (M1) after administration of SH-MSCs gel and in this study it is not known how long the application was carried out. It is necessary to carry out histopathological examination of CD45 and CD68 to determine the presence of inflammatory lymphocytes and macrophages (M1) after administration of SH-MSCs gel. Future researchers are expected to examine the duration of application of the SH-MSCs gel even longer.

V. CONCLUSION

The Administration of 10% and 20% SH-MSCs gel at a dose of 200 mg/rat/day can reduce IL-6 gene expression. The administration of 10% SH-MSCs gel at a dose of 200 mg/rat/day did not increase TGF-β gene expression, and the administration of SH-MSCs gel at a dose of 20% at a dose of 200 mg/rat/day can increase TGF-β gene expression.

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