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The effectiveness of topical hyaluronic acid on decreasing Interleukin-6 and acceleration of wound healing (Push Score) in Wagner II-III diabetic foot ulcer in Dr. Soetomo Hospital Surabaya

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ABSTRACT

Background: A diabetic foot ulcer is a complication that often occurs in patients with diabetes mellitus. The healing process of a chronic wound is more complicated and requires more complex treatment. There is an increase in IL-1, IL6, and TNF-α pro-inflammatory cytokines in a chronic wound. Hyaluronic acid (HA) is a component that has been used for a long time and is known to help the wound healing process. This study was at a time to determine the effectiveness of hyaluronic acid on the reduction of interleukin-6 in accelerating the healing of Wagner II-III diabetic foot ulcers.

Methods: This study is an experimental, randomized study of pre and post-test design on diabetic foot ulcer patients with the Wagner II-III classification who were controlled at the thoracic, cardiac, and vascular surgery polyclinic at Dr. Soetomo Hospital Surabaya. Data were analyzed using SPSS version 20 for Windows.

Result: About 39 samples are willing to participate in the study, divided into 20 samples in the treatment group and 19 in the control group. The mean concentrations of IL-6 in the treatment group before and after the intervention were 280.70±155.50 ng/ml and 126.60±145.60 ng/ml. There was a significant decrease in IL-6 levels ($p<0.05$) before and after the intervention. The mean concentrations of IL-6 in the control group pre and post were 315.20±127.40 ng/ml and 136.30±134.60 ($p<0.05$). The decrease in the control and treatment groups was no statistically significant difference ($p>0.05$). There was no significant difference in the PUSH Score ($p>0.05$) in the two groups before intervention/treatment. The decrease of wound area in the control and treatment groups were -4.80 ((-0.40)-(-24.50)) and -4.8 ((-0.70)-(-30.90)) ($p>0.05$).

Conclusion: Topical hyaluronic acid and wound care using 0.9% NaCl reduced interleukin-6 levels, PUSH scores, and wound area in Wagner II-III diabetic foot ulcers.

Keywords: hyaluronic acid, IL-6, PUSH score, diabetic foot ulcer.
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INTRODUCTION

Patients with diabetes mellitus have a high risk of infection in the feet, lower legs, and upper limbs due to frequent trauma due to friction and poor hygiene.[1] Compared to 2013, the prevalence of DM based on a doctor's diagnosis in residents aged 15 years from the 2018 Riskesdas increased to 2%. The prevalence of DM of all ages in Indonesia at Riskesdas 2018 is slightly lower than the prevalence of DM at the age of 15 years, which is 1.5%.[2]

Diabetic foot ulcers are a chronic complication of diabetes mellitus.[3] Approximately 85% of lower limb amputations are initiated by foot ulcers that do not heal.[4,5] Lower limb amputation rates are 15 times higher in diabetic patients compared to non-diabetic patients.[6]

The healing process of chronic wounds, such as diabetic ulcers, is more complicated and requires more complex treatment. Every surgeon wants an ideal wound dressing that accelerates the healing of chronic ulcer wounds without complications. A good wound dressing is a wound dressing that retains moisture and reduces adverse effects on the wound itself, such as infection, maceration, and allergies.[7,8] Since then, a previous study involving gels' application in treating chronic wounds has been carried out.[9]

Hyaluronic acid (AH) is a component of the extracellular matrix in connective tissue that can assist the wound healing process, thus providing the right conditions for the tissue regeneration process in injured tissue. AH has been used for a long time and has developed good results in ophthalmology and connective tissue disease, arthritis, and rheumatoid arthritis. The topical use of AH is also effective in treating chronic wounds.[9,10] According to the results of a previous meta-analysis, ten studies of AH produced good outcomes in healing burns, surgical wounds, and chronic ulcers.[11]

PUSH Score is the sum of scores by measuring the area of the wound, the amount of exudate and the type of wound tissue present. Measurement of wound area using a ruler, measured wound length times the wound (cm^2).[12]

IL-6 has a major role in the wound healing process at certain phases of the wound healing process. The amount of IL-6 that is too much can slow down the healing process because IL-6 will signal leukocytes to increase the inflammatory process.[13] Eventually, these cells will damage the ECM and the maturation process cannot occur. Therefore, it is hoped that IL-6 levels will not be high in the remodeling phase.

The role of IL-6 in the wound healing process is not fully known. Appropriate inflammation timing can accelerate the wound healing process, and overexpression of IL-6 can inhibit the wound healing process. However, the role of IL-6 in wound healing cannot be underestimated. Where interference with the IL-6 pathway can cause delayed wound healing, normally, IL-6 is significantly decreased in the remodeling phase.[13] We can make this a predictor of wound healing by evaluating the expression of pro-inflammatory cytokines in wounds. When IL-6 levels are always high in each phase, it will slow wound healing. It is expected that the use of Hyaluronic Acid is effective in decreasing levels of IL-6 in patients with chronic wounds and can accelerate the wound healing process.

On examination of exudate and plasma in chronic wounds compared to acute wounds, there is an increase in IL-1, IL6, and TNF-α, which are pro-inflammatory cytokines. These cytokines will decrease in levels when the wound heals.[13] Therefore, this study aims to determine the effectiveness of hyaluronic acid on the reduction of interleukin-6 in accelerating the healing of Wagner II-III diabetic foot ulcers at Dr. Soetomo Hospital Surabaya.

METHODS

This study is an experimental study of pre and post-test design on diabetic foot ulcer patients with the Wagner II-III classification who were controlled at the thoracic, cardiac, and vascular surgery polyclinic at Dr. Soetomo Hospital Surabaya. This research was conducted in August 2021 until the number of samples was met in April 2022 using a random sampling technique. The study population was diabetic foot ulcer patients with Wagner II-III classification with wound care using 0.9% NaCl and Hyaluronic Acid or 0.9% NaCl who were controlled to the thoracic, cardiac, and vascular surgery polyclinic at the Dr. Soetomo Hospital Surabaya. Inclusion criteria included patients with Wagner II-III diabetic foot ulcers, patients who agreed to participate in the study, and patients with complete medical records. Meanwhile, the study exclusion criteria were patients with liver cirrhosis, patients with stage V renal failure, and patients receiving chemotherapy treatment. Then randomization and group division was carried out. The first group received wound care with 0.9% NaCl+Tulle. Meanwhile, group 2 received ulcer treatment with 0.9% NaCl+Tulle+Hyaluronic Acid. Data were analyzed using SPSS version 20 for Windows.

RESULTS

There are 39 patients who are willing to take part in the study and meet the inclusion criteria and follow the study to completion. Each patient who came was randomly randomized by taking turns putting the patient into the treatment group and the control group. At the end of the study, there were 20 samples in the intervention group and 19 samples in the control group. The characteristic data of laboratory results before intervention in the treatment and control groups showed that the average hemoglobin level was 11.30±1.90 g/dL and 12.10±0.90 g/dL, respectively (Table 1).

IL-6 levels were taken at the beginning of the study before treatment and at the end of the study after treatment. The examination of IL-6 levels was carried out at the Clinical Pathology Laboratory of Dr. Soetomo Hospital. The mean concentrations of IL-6 in the treatment group before intervention were 280.70±155.50 ng/dL and 126.60±145.60 ng/dL, respectively. There was a significant decrease in IL-6 levels ($p<0.05$) before and after the intervention, as shown in Table 2. In addition, the mean concentrations

Table 1. Characteristics of laboratory results before intervention.

Variable	Intervention (n=20)	Control (n=19)	p
Hemoglobin (g/dL) (Mean±SD)	11.30±1.90	12.10±0.90	0.939
Leucocyte Count (Mean±SD)	10,675±3,488	12,850±6,819	0.612
Thrombocyte (ml)(Mean±SD)	458,600±195,996	367,157±139,504	0.216
Serum Ureum (mg/dL) (Mean±SD)	12.80±3.10	12.20±3.70	0.205
Serum Creatinine (Mean±SD)	1.10±0.40	1.30±0.60	0.259
SGOT (Mean±SD)	25.90±11.60	24.50±5.50	0.015*
SGPT (Mean±SD)	26.90±12.0	24.60±12.00	0.818
Albumin (Mean±SD)	3.70±0.10	3.70±0.10	0.585
Random Blood Glucose (Mean±SD)	138.00±14.90	150.00±13.90	0.009*

*Statistically significant if p-value less than 0.05

Table 2. The concentration of IL-6 Treatment Group before and after intervention.

IL-6 Concentration (ng/dL)	Pre	Post	p
Before Intervention	280.70±155.50	126.6±145.60	<0.001*
After Intervention	315.20±127.40	136.3±134.60	<0.001*

Wilcoxon signed rank test; *Statistically significant if p-value less than 0.05

Table 3. Decreased IL-6, PUSH Score, and wound area levels in the treatment and control groups before and after the intervention.

Variable	Control	Intervention	p
ΔIL-6 (ng/dL)	-195.60 (0.00- (-376.47))	-50.88 (0.00-(-379.99))	0.563
PUSH Score			
Pre-Intervention	13 (6-15)	13 (8-16)	0.348
Post-Intervention	9 (3-12)	8 (4-14)	0.692
ΔPUSH Score	1 ((-1)-3)	1 ((-4)-4)	0.282
Wound area (cm^2)			
Pre-Intervention	8.00 (1.70-52.80)	6.65 (0.90-38.60)	0.844
Post-Intervention	2.50 (0.20-28.30)	2.15 (0.10-24.80)	0.899
Δ Wound area (cm^2)	-4.80 ((-0.40)-(-24.50))	-4.80 ((-0.70)-(-30.90))	0.811

Mann-Whitney Test; *Statistically significant if p-value less than 0.05

of IL-6 in the control group pre and post-after interventions were 315.20±127.40 ng/dL and 136.30±134.60 ng/dL, respectively. There was a significant decrease in IL-6 levels ($p < 0.05$) from the pre-treatment control group to the post-treatment control group both before and after the intervention, as shown in Table 2.

The Mann-Whitney test was carried out the test to compare the decrease in IL-6 levels and the median values for the decrease in the control and treatment groups were -195.60 (0.00-(-376.47)) and -50.88 (0.00-(-379,99)), respectively. 99)]. There was no statistically significant difference ($p > 0.05$), as shown in Table 3.

PUSH Score data retrieval was carried out when the control patient was in the thoracic and vascular thoracic surgery clinic, which was calculated by the examiner and confirmed by one other examiner. The median PUSH Score before intervention/treatment in the control and treatment groups were 13 (6-15) and 13 (8-16, respectively). There was no significant difference in the PUSH Score ($p > 0.05$) in the two groups before the intervention, as shown in Table 3.

The median PUSH Score after intervention/treatment in the control and treatment groups were 9 (3-12) and 8 (4-14), respectively. There was no significant difference in the PUSH Score ($p > 0.05$) in the two groups after the intervention, as shown in Table 3. The median values in the control and treatment groups were respectively 1 ((-1)-3) and 1 ((-4)-4) and no statistically significant difference ($p > 0.05$), as shown in Table 3.

The data on the area of the wound was tested for normality of the data with the Saphiro Wilk test and the data was found to be not normally distributed. The test to compare the area of the wound was performed with the Mann-Whitney test. The median value of the wound area before intervention/treatment in the control and treatment groups were 8.00 (1.70-52.80) and 6.65 (0.90-38.60), respectively. There was no significant difference in wound area ($p>0.05$) in the two groups before intervention/treatment, as shown in Table 3.

The median value of wound area after intervention/treatment in the control and treatment groups was 2.50 (0.20-28.30) and 2.15 (0.10-24.80), respectively. There was no significant difference in wound area ($p>0.05$) in the two groups after intervention/treatment, as shown in Table 3. Data on the reduction in wound area was obtained by calculating the delta value of the wound area before and after intervention/treatment. The test to compare the decrease in wound area was carried out with the Mann-Whitney test and the median values of the decrease in the control and treatment groups were -4.80 ((-0.40)-(-24.50)) and -4.8 ((-0.70)-(-30.90)) and no statistically significant difference ($p > 0.05$) as shown in Table 3.

DISCUSSION

On examination of exudate and plasma in chronic wounds compared to acute wounds, there is an increase in IL-1, IL6, and TNF-α, which are pro-inflammatory cytokines. These cytokines will decrease in levels when the wound heals.[12,13] A study conducted by Bekeschus S et al. in 2017, which the study compared the pattern of cytokines and chemokines in diabetic foot ulcer patients and acute wounds, explained that there was a significant increase in IL-1, IL-6, and IL-8 in patients with chronic wounds.[14]

From the results of this study, there was a significant increase of IL-6 mean concentrations in the treatment group before and after the intervention ($p < 0.05$). In addition, in the control group, pre and post-IL-6 levels were also decreased significantly ($p < 0.05$). However, there was no significant decrease of IL-6 levels in the control and treatment groups ($p > 0.05$).

Kartika RW et al., in 2021 found that in Wagner II diabetic foot ulcer patients, there was a significant decrease in IL-6 compared to baseline before administration of platelet-rich fibrin and AH.[15] They also found a significant difference in the decrease in IL-6 levels on the 7th day of administration of platelet-rich fibrin and AH compared to the group that was given only platelet-rich fibrin. Meanwhile, in the control group who were not given either platelet-rich fibrin or AH, it was found that IL-6 levels increased on day 7.[15]

The data in this study showed the median PUSH Score before intervention/treatment in the control and treatment groups were 13 (6-15) and 13 (8-16), respectively. There was no significant difference ($p > 0.05$) in both groups. Meanwhile, the median PUSH Score after intervention/treatment in the control and treatment groups also did not find a significant difference in the PUSH Score ($p > 0.05$). By comparing the decrease in PUSH Score in the control and treatment groups, it was found that there was no difference in the median value of the decrease ($p > 0.05$) in the control and treatment groups,.

Ramos-Torrecillas J et al., in 2015 compared pressure ulcers with platelet-

rich growth factor administration without and with AH by observing the PUSH score from time to time; they found a reduction of up to 48%.[16] Their study found that 37.5% experienced complete wound healing within 36 days in the platelet-rich growth factor and AH group (p<0.004). Meanwhile, with the administration of platelet-rich growth factors alone, 32% experienced complete healing.[16]

A previous study found that a linear decrease in PUSH score observed weekly for 4 weeks in patients with Wagner diabetic foot 3 was a strong predictor of ulcer healing, while in ulcers that did not experience a decrease in PUSH score in 4 weeks, it was a sign of non-abdominal pain.[17] The use of the PUSH Score for monitoring wound healing is considered valid for diabetic foot ulcers, leg vein ulcers, and pressure ulcers.[17] A previous study also found that for an average of 31 days, a decrease in the PUSH Score of 3.9 indicates a healing ulcer, while a decrease in the PUSH Score of -0.6 indicates a non-healing ulcer.[17]

The components of wound area and depth are also measurements often used to observe wound healing. A previous study found that the administration of silver sulfadiazine and AH accelerated wound healing in 3 weeks by stimulating better epithelialization.[3] Meanwhile, Lee Y et al., in 2014, with a smaller number of samples, found that in 3 weeks, there was a 51.6% reduction in wound area in diabetic foot ulcers given HA dressing compared to a 29.7% reduction in wound area in the group without HA dressing, but statistically, it was not significant (p=0.184).[18] Meanwhile, Hwang et al., 2016, in a prospective study, found that diabetic wounds treated with AH experienced a faster and more significant reduction in size (p = 0.012), namely, a 50% reduction in size could be achieved in 21 days compared to 39 days in diabetic wounds without AH.[19]

The wound area in this study experienced a significant reduction in both the control and treatment groups. However, in comparing the decrease in wound area in the group without AH and with the administration of AH, there was no significant difference (p = 0.899). This study used an observation period of 2 weeks, while in previous studies, there were observations for more than 3 weeks.[17-19]

In conclusion, topical hyaluronic acid and wound care using 0.9% NaCl reduced interleukin-6 levels, PUSH scores, and wound area in Wagner II-III diabetic foot ulcers. However, there was no significant difference in the decrease in interleukin-6 levels, PUSH Score and wound area in the administration of topical hyaluronic acid compared to wound care using 0.9% NaCl in the 2-week observation period. Many factors affect IL-6 levels, so IL-6 cannot be used as the only marker for evaluating diabetic foot wounds.

This study also has some limitations. Firstly, we only observed patients for two weeks. We didn't observe patients until the wound closed. Secondly, the parameters we measure are blood IL-6, while blood IL-6 also describes the general inflammatory process in the body, for example, in diabetic patients or some patients with chronic inflammation.

ETHICAL CLEARANCE

Ethics approval was obtained from the local ethics committee.

CONFLICT OF INTEREST

All authors declare that they have no conflicts of interest.

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AUTHOR CONTRIBUTIONS

All authors have contributed equally from the conceptual framework, data acquisition, and data analysis until the study results are reported through publication.

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