

Hyaluronic Acid Caused of Wider Epithelialization Compare to Normal Saline in Severe Diabetic Ulcer

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Objectives: Diabetic ulcer, one of chronic complications of diabetes mellitus (DM), showed a high morbidity and mortality rate. The main treatment modality for diabetic ulcer was debridement, followed by wound treatment as local control to promote wound healing. This study aims to compare efficacy of hyaluronic acid (HA) and 0.9% sodium chloride (NaCl) in severe diabetic ulcer two after debridement.

Method: This study was a randomized clinical study to compare the efficacy of HA and NaCl 0.9% in severe diabetic ulcer two weeks post debridement. Thirty six severe diabetic ulcer (Wagner ≥ 3) samples were collected using consecutive sampling method and divided into 2 treatment groups: standard wound treatment using NaCl 0.9% and using hyaluronic acid. T-independent test was applied for statistical analysis data and $p < 0.05$ was considered a statistically significant.

Results: The two treatment groups showed insignificant difference in characteristics and laboratory findings. The mean tissue epithelialization width after two weeks of wound treatment using NaCl 0.9% was $17,22 \pm 3,25$ and using HA was $27,33 \pm 2,43$. Statistical analysis using t-independent test showed $t = 10.59$, $p = 0.001$ for both treatment groups.

Conclusions: HA improves wound healing rate in severe diabetic ulcer 2 weeks post debridement compared to 0.9% NaCl.

Keywords: Diabetic ulcer, Hyaluronic acid, epithelialization.

INTRODUCTION

Diabetic ulcer is one of diabetes mellitus (DM) complication which leads to the occurrence of tissue damage from skin to bone. Until today, diabetic ulcer still becoming general problem, in either medical, social, and economic worldwide and estimated around 15% experiencing all DM patients for a long of their life. This leads to morbidity and mortality.^{1,2,3} Standar treatment for diabetic ulcer includes optimal blood glucose levels control, debridement, off-loading/pressure reduction, antibiotic infection controlled, ischemic correction, and wound maintenance.^{1,3,4,5}

Curing process of chronic wound, such as diabetic ulcer is more difficult and need much more complex treatment. Local therapy or wound protection should protect wound from contamination and produce optimal wound environment that can faster wound improvement. A humid wound environment increases rate of epithelialization and wound improvement. This was introduced for the first time at the year of 1960 and since then many researchs were carried out by applying gel on treatment of chronic wound.^{4,6} Hyaluronic acid (HA) is an extracellular matrix component of connective tissue that can help wound healing process, creating

good condition for tissue regeneration. HA has long been used and results in a good improvement in ophthalmology and connective tissue disease, joint inflammation and rheumatoid arthritis. Penggunaan Topical HA has also an effective improvement on treatment of chronic wound.^{6,7}

Some researchs for using HA to treat wound results in significant wound healing.^{6,7,8,9} Researchs regardless the use of HA to treat diabetic ulcer has not been publish widely.¹⁰ Therefore, the aims of this study is to evaluate the effectivity of HA for treating diabetic ulcer after debridement.

METHODS AND PATIENTS

This is an open label randomized clinical trial of 36 severe diabetic ulcer (Wagner III-V) patients at Surgery Department Faculty of Medicine Udayana University/General Sanglah Hospital Bali-Indonesia from Desember 2010 until April 2011. Patients age between 40-60 years with blood glucose levels < 200 g/dL were included in this study. Patients with chronic liver, renal failure, taking chemotherapy and corticosteroid, albumin $< 2,5$ g/dL and Hb < 10 g/dL were excluded in this study. Sample were recruited consecutively and permuted block to two groups. The first group was treated 0.9% NaCl and the second group treated with HA. Debridement was carried out daily and applied for all group.

Effect of treatment was evaluated in two weeks after treatment by measuring the width of tissue

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epithelialization. Measurement was carried out by measuring average epithelialization distance, a distance from old wound to the wound edges in every cm and presented in mm.

Independent t-test was applied to determine the different between groups and $p < 0.05$ was considered to be indicative of statistically significant difference. For categorical data were analyzed using chi-square.

RESULTS

A number of 36 patients included in this study compromise of 15 (41.7%) male and 21 (58.3%) female. Subject characteristic data for all groups were presented in Table 1.

Table 1
Data of Subject Characteristic

Parameters	Hyaluronic acid (HA)	0.9% NaCl	<i>p</i>
Age (Years)	49.78±4.75	50.50±6.36	0.702
Sexes			
Male	8	7	0.735
Female	10	11	
Grade			
Wagner III	9	10	0.738
Wagner IV	9	8	
Wagner V	0	0	

Table 1 indicates that average age of the HA group was 49.78±4.75 years and for 0.9% NaCl group was 50.50±6.36 years. Significant test using independent-T test indicates value of $p > 0.05$. In addition, sexes and Wagner grade different between group were analyzed using *Chi-Square* and indicates no significant difference of these two parameter ($p > 0.05$).

Besides subject characteristic data, in this study other parameters obtained from laboratory test were also presented, as can be seen on Table 2.

Table 2
Supporting Data of all Groups

Parameters	Hyaluronic acid	0.9% NaCl	<i>p</i>
WBC	15.91±4.10	15.72±3.12	0.876
Albumin	2.93±0.34	2.91±0.33	0.913
Blood glucose	183.33±19.89	182.22±17.9	0.719
Hb	11.35±0.95	11.55±1.57	0.815
Culture			
Pseudomonas	6	8	0.708
Enterobacter	4	3	
Streptococcus	3	2	
Staphylococcus	4	3	
Proteus	0	1	
Klebsiella	1	0	
Acinobacter	0	1	

WBC = white blood cells

Summary of independent-t test for evaluating the different of treatment was presented in Table 3.

Table 3

Average Epithelialization Width between Groups					
Groups	N	Epithelialization (mm)	SD	<i>t</i>	<i>p</i>
HA	18	27.33	2.43	10.59	0.001
NaCl	18	17.22	3.25		

HA= Hyaluronic acid

DISCUSSION

Until today, diabetic ulcer still becoming general problem, in either medical, social, and economic worldwide. In the US, it was estimated around 16 millions people suffering of diabetes mellitus (DM), and 15% of them experiencing diabetic ulcer, 12% of them underwent amputation and 45% was a major amputation. Diabetic ulcer is the major cause of non traumatic amputation on inferior extremity. Healing process of chronic wound such as diabetic ulcer are more difficult and need a complex treatment. Local therapy or wound dressing of diabetic ulcer should protect the wound from contamination and creating a good environment, so that results in faster improvement.^{1,3,4,9}

In this study, 36 patients were recruited, compromise of 15 (41,7%) male and 21 (58.3%) female. Based on statistical analyzed, it was obtained that average age of HA group was 49.78±4.75 years, and for NaCl group was 50.50±6.36 years. Independent-t test indicates that there was no significant age different between the two groups. Likewise, sexes and Wagner grade of DM which were analyzed using chi-square were not significantly differ of the two groups ($p > 0.05$). That's means characteristic of the subject was not influence to the treatment. Furthermore, other parameters, such as blood glucose levels, albumin, white blood cells, Hb, and culture were not significantly different ($p > 0.05$), as indicates on Table 2.

Tissue epithelialization width occurs, based on data analyzed, it was obtained that for HA group was 27.33±2.43 mm and for NaCl was around 17.22±3.25 mm. Significant test by applying independent-t test results in there was a significant different between the two groups ($p = 0.001$ or $p < 0.05$).

HA is an extracellular matrix component of connective tissue that has a role to help wound healing, in which could create a humid environment, a good please for tissue regeneration. HA has been used since a long time and resulted in a good results in ophthalmology field and connective tissue disease, joint inflammation, and arthritis rheumatoid.

Application of HA locally shows an effective results in treatment of chronic wound.^{6,7,13,14}

This study was also supported by Barrois, et al.⁶ finding, they obtain that HA is an effective material for treating untreated diabetic ulcer with another methods. In addition, Ortone (1996),¹¹ reported that HA was effective in treatment of varicosum ulcer. He found that there was a significant decrease of 48% of ulcer size from 20.8 cm² to 10.8 cm² on the day 21st ($p < 0.001$). Ortone's results was in contrast to Dextranomer group in which he found that there was insignificant ulcer size decrease observed. Moreover, in a clinical trial of comparing the use of combination of HA with silver sulfadiazine (SS) to a group with SS only treatment on grade II burn wound, it was obtained a faster improvement rate for combine treatment.⁸

Furthermore, Vasquez (2003),¹⁰ in a research to find out improvement of diabetic ulcer treated using HA after debridement, he gain that in an ulcer diabetic with average size of 3.2 ± 2.2 cm², requiring 20 weeks for complete heal or average time needed is 10 ± 4.8 weeks). Based on above explanations, it can be concluded that HA can be used as an effective additional therapy to treat diabetic ulcer. Ballard and Cantor (2009),⁷ reported that some diabetic ulcer cases were difficult to heal due to physiologic and pathologic problems, such as fluctuated blood glucose levels, the present of comorbide factor, such as HIV, hypertension, and obesity. For these cases, treatment by applying topical HA resulted in a significant improvement.

Based on this study and other previous studies, therefore it can be proven that HA therapy is related to improvement tissue process. HA researches regardless of biological process, i.e. morphogenesis and oncology give also input of HA function in tissue improvement. Some HA functions probably related to its role as an integral part of extracellular matrix. These are related to the HA properties which are hygroscopic, rheologic, and viscoelastic that can affect cells by influencing of macro and micro environment surround the cells through a complex interaction to other component of extracellular matrix. HA and its oligosaccharides are probably directly influence cells function through receptor binding that straightly changes the specific gene expression.^{13,14}

One of initial response of traumatic tissue is formation of temporary matrix which were rich of HA and fibrin that help the entry of fibroblast and endothelial cell to wound and forming granular tissue. Both HA on cell and extracellular matrix, their natural hygroscopic property creates an environment to support cell migration to the new place, meanwhile, its ability to destroy free radicals creating

protection of cells and extracellular matrix molecule from proteolytic destruction. This is lead to improvement of in either acute or chronic wound.¹ HA's oligosaccharide has been proved to induce angiogenesis, an advantage potential property on healing of acute or chronic wound. The mechanism how the oligosaccharide healing the wound has not clearly understood yet. However, dissolved HA's fragment has been proved increase some cytokine expressions, such as interleukin-1 β (IL-1 β), TNF- α and insulin-like growth factor-1, and induce some inflammation gene expression on macrophage through CD44 receptor-mediated mechanism, also increase of collagen production by endothelial cells. These properties concistence to the view of HA directly influence cell activity through receptor-mediated mechanism.¹⁴

HA has an important function on normal epidermis. The function are part of extracellular matrix, free radical and its role on proliferation and keratinocyte migration. All of these indicate the important of HA in reepithelization process. On normal skin, HA was found in high number on epidermis basal layer in which CD44 was also located in the same site. Major function of HA in epidermis is maintaining extracellular space and producing hydrate structure as a nutrition lane. Increase of HA in skin leads to increase of tissue hydration.^{13,14}

Epidermic HA has also an important role to control keratinocyte proliferation in reepithelialization process. On wound healing, HA presents on wound gap on connective tissue matrix together with CD44 within migrated keratinocyte. Suppression of CD44 by epidermis antisense transgene that is specific in animal within low dermis superficial HA leads to keratinocyte basal morphology changes and keratinocyte proliferative defect as a response of myogene and growth factor. Besides that, decrease of skin elasticity, local inflammation process and tissue repair were also take place. This observation indicates the important role of HA in skin physiology and tissue repair.^{12,13,14}

CONCLUSION

HA causes of wider tissue epithelialization compare to 0.9% NaCl on severe diabetic ulcer treatment two weeks after debridement.

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