The Effect of Hyaluronic Acid on AcceleratingHealing of Diabetic Foot Ulcers and Decreasing Interleukin-6 Levels: A Literature Review

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Abstract

Because the process of healing chronic wounds is more challenging and requires more comprehensive treatment, patients with diabetes mellitus are at a higher risk of infection in their feet due to recurrent trauma and inadequate cleanliness. This can lead to a chronic problem. Hyaluronic acid (HA) is a component of the extracellular matrix in connective tissue that can help the wound healing process, thus providing the right conditions for the tissue regeneration process. Topical use of HA is considered effective in treating chronic wounds. The anti-inflammatory effect of HA works by inhibiting TNF-alpha, thereby preventing the increase in IL-6 and IL-8, which are pro-inflammatory cytokines. Patients with diabetic foot ulcers have higher plasma IL-6 levels compared to patients without diabetic foot ulcers. IL-6 is also an inflammatory marker that can differentiate infected diabetic foot ulcers (IDFU) from non-infected diabetic foot ulcers (NIDFU). Disturbance in the IL-6 pathway can cause delayed wound healing. Normally, IL-6 decreases significantly in the remodeling phase. This article was created to see how the effects of hyaluronic acid on reducing the time it takes for diabetic foot ulcers to heal and decreasing blood interleukin-6 levels from several studies that had been done previously.

INTRODUCTION

Patients with diabetes mellitus have a high risk of infection in their feet, lower legs, and upper limbs due to frequent trauma due to friction and poor hygiene[1]. Diabetic foot ulcers are one of the chronic complications of diabetes mellitus [2].

The process of healing chronic wounds, such as diabetic ulcers, is indeed more complicated and requires more complex treatment. Every surgeon wants the ideal wound dressing to accelerate the healing of chronic ulcers 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 [3].

Hyaluronic acid (HA) is a component of the extracellular matrix in connective tissue that can help the wound healing process, thus providing the right conditions for the tissue regeneration process in injured tissue. HA has been used for a long time and has developed good results in the fields of ophthalmology and connective tissue disease, arthritis, and rheumatoid arthritis. Topical use of HA is also effective in treating chronic wounds. [4]

On examination of exudate and plasma in chronic wounds when compared with acute wounds there is an increase in IL-1, IL6, and TNF alpha which is a pro-inflammatory cytokine. These cytokines will decrease in levels when the wound heals [5]. This article was created to see how the effects of Hyaluronic Acid on reducing the time it takes for diabetic foot ulcers to heal anddecreasing blood interleukin-6 levels from several studies that had been done previously.

OVERVIEW

Diabetic Foot Ulcer Etiology

Diabetic Foot Ulcer is a chronic complication of Diabetes Mellitus [2]. In general, diabetic foot ulcers are damage to the skin on the feet of a person with diabetes mellitus, which does not heal immediately, but no other abnormalities are found. Various causes lead to this skin breakdown, and once an ulcer has developed, many factors hinder the healing of the ulcer[6].

Recent studies have demonstrated that many risk factors are associated with the development of diabetic foot ulcers [7]. The risk factors were as follows: gender (male), having diabetes mellitus more than 10 years, old age, high body mass index, and other comorbidities such as retinopathy, peripheral diabetic neuropathy, peripheral arterial disorders, HbA1C levels, foot deformities, high plantar pedis pressure, infections, and inappropriate foot care habits [7].

Diabetic Foot Ulcer Pathophysiology

The artery's endothelial cell lining is a physiologically active organ. By managing the homeostatic balance between thrombosis and fibrinolysis, this organ governs the contact between the cellular constituents of the blood and the vascular wall. It also plays a key role in the interactions between leukocytes and the cell wall. The vascular system might be prone to atherosclerosis and other illnesses if endothelial function is abnormal. Endothelial and vascular function are abnormal in the majority of diabetic individuals, including those with peripheral arterial disease[8].

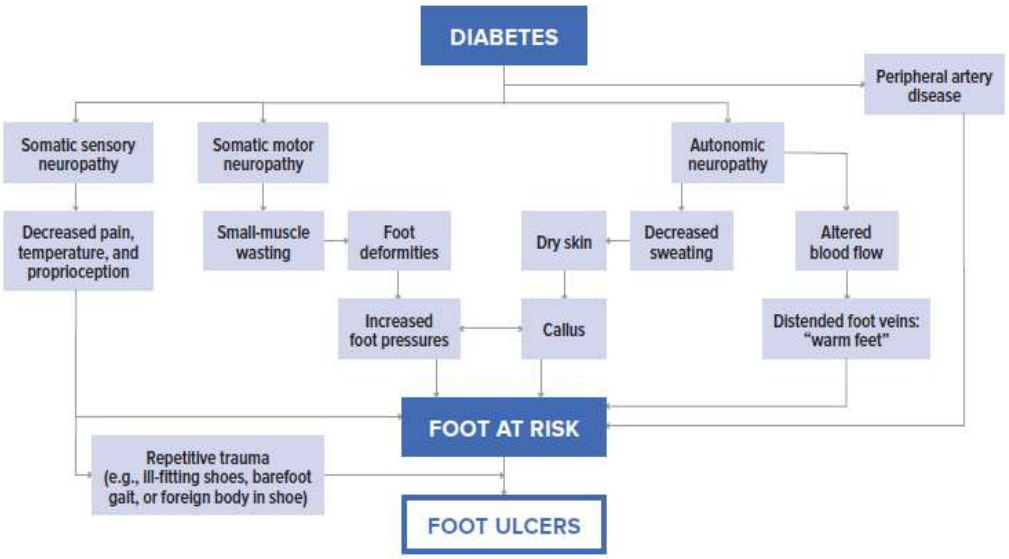

Figure 1. Pathophysiology of Diabetic Foot Ulcers [9]

Endothelial cell dysfunction in diabetics is caused by a variety of factors, the most significant of which is a drop in nitric oxide (NO) levels. Through interactions between leukocytes and the arterial wall, NO stimulates vasodilation and decreases inflammation. NO also inhibits the migration and proliferation of vascular smooth muscle cells (VSMCs) as well as platelet activation. As a result, a loss of NO homeostasis in the blood arteries might set off a chain reaction that leads to atherosclerosis and its problems. Endothelium dysfunction is caused by a number of factors, including hyperglycemia, the generation of free fatty acids (FFAs), and, most critically, insulin resistance. [8].

Hyperglycemia alters endothelial vasodilator homeostasis by inhibiting the activity of endothelial nitric oxide synthase (SNOe) production. Insulin resistance, in addition to hyperglycemia, contributes to the loss of NO homeostasis. Insulin resistance may cause impaired vasoreactivity, which can harm glucose metabolism by reducing nutrition input at the base of muscle capillaries[8].

Classification of Diabetic Foot Ulcers

There are several categorization methods available today for evaluating and determining the severity of diabetic foot ulcers (such as location, depth, presence or absence of neuropathic symptoms, infection, ischemia, etc.).Three main diabetic foot classification systems are commonly used as a reference for clinical diagnosis of diabetic foot ulcers are :[7]

1 *Wagner-Meggitt Classification*
2 *Depth-Ischemic classification*
3 *University of Texas classification*

The Wagner-Meggitt classification system is the most often used classification system (Table 2). This is an anatomical system with traits such as superficial ulcers, deep ulcers, osteitis abscesses,

forefoot gangrene, and complete foot gangrene. Infection is only discussed in third-degree circles. The severity of foot lesions is categorized into many categories in this system, ranging from grade 0 to grade 5. This technique, on the other hand, does not address ischemia or neuropathic symptoms, which is a flaw in the system.[7].

Table 1. Wagner-Megitt Classification

Degrees	Symptom
0	No open lesions
1	Superficial Ulcer
2	Deep ulcers that have extended to the tendons and joint capsules.
3	Deep ulcers are accompanied by abscesses, osteomyelitis.
4	Localized gangrene of the forefoot or heel.
5	Gangrene of the whole leg.

Diabetic Foot Ulcer Diagnosis

Patients suffering from diabetes should undergo examination including symptoms of arterial insufficiency and neuropathic disease in a scheduled and structured manner, based on existing risk factors. Check the patient's body temperature, respiration, heart rate, and blood pressure in the limbs, and document any irregularities [7].

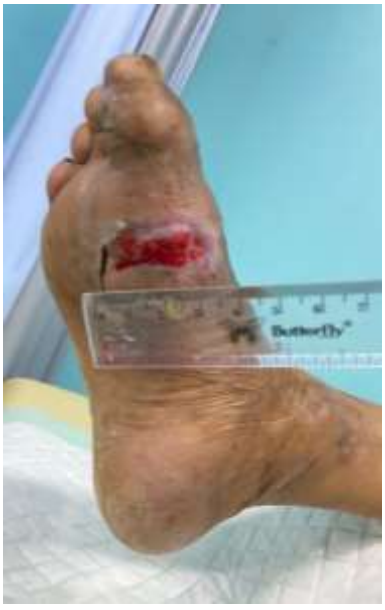

Figure 2. Photograph of Diabetic Foot Ulcer (Courtesy of RSUD Dr. Soetomo)

Wound measurements were carried out before and after treatment, using the PUSH Tool method. With the PUSH Tool method, the wound was observed by measuring the area of the wound, the amount of exudate, and the type of wound tissue present. Measurement of the area of the wound using a ruler, measured the length of the wound times the wound (cm2). Measurement of exudate by assessing no exudate, a little, moderate, and a lot. In tissue evaluation, a score of 4 is given if there is necrotic tissue, a value of 3 if there is slough without necrotic tissue, a value of 2 if the wound is clean and there is granulation tissue, a value of 1 if there is re-epithelialization, and a value of 0 if the wound has closed.

Table 2. PUSH Score Table

Pressure Ulcer Scale for Healing (PUSH)
PUSH Tool 3.0

Patient Name _____ Patient ID# _____
Ulcer Location _____ Date _____

Directions:
Observe and measure the pressure ulcer. Categorize the ulcer with respect to surface area, exudate, and type of wound tissue. Record a sub-score for each of these ulcer characteristics. Add the sub-scores to obtain the total score. A comparison of total scores measured over time provides an indication of the improvement or deterioration in pressure ulcer healing.

LENGTH X WIDTH (in cm²)	0 0	1 < 0.3	2 0.3 – 0.6	3 0.7 – 1.0	4 1.1 – 2.0	5 2.1 – 3.0	Sub-score
		6 3.1 – 4.0	7 4.1 – 8.0	8 8.1 – 12.0	9 12.1 – 24.0	10 > 24.0	
EXUDATE AMOUNT	0 None	1 Light	2 Moderate	3 Heavy			Sub-score
TISSUE TYPE	0 Closed	1 Epithelial Tissue	2 Granulation Tissue	3 Slough	4 Necrotic Tissue		Sub-score
							TOTAL SCORE

Diabetic Foot Ulcer Management

Wound healing of the skin is a physiological process consisting of the collaboration of cells. Efforts to restore a wound on the skin begin with local inflammatory cell aggregation in the inflammatory stage. Ultimately, this process results in the repair of the tissue structure consisting of collagen, cell regeneration and proliferation also occurs using differentiation from pre-existing cells [10].

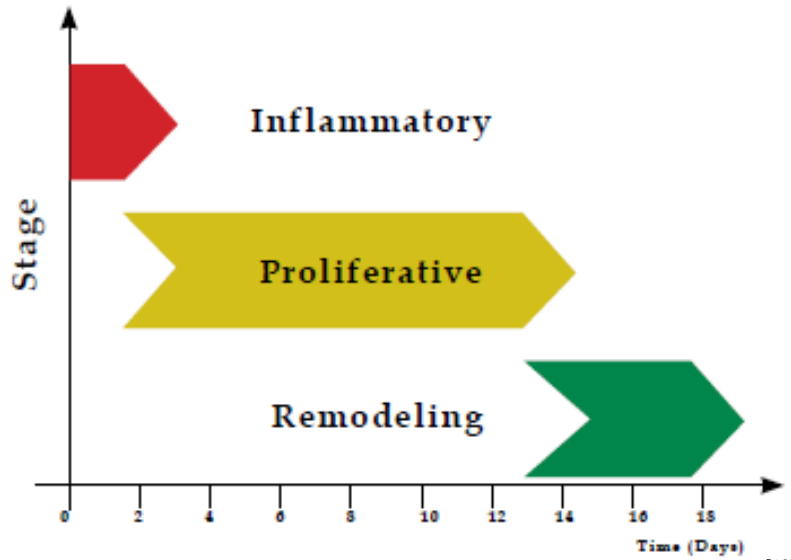

Figure 3. Illustration of the Length of the Wound Healing Process[10].

The body has a complex physiological response to injury which consists of three phases: hemostasis & inflammation, proliferation, and remodeling. This process is influenced by many factors, both internal and external. The main principle of wound management is to help the process occur effectively [11].

At the onset of injury, there is local vasoconstriction in the arteries and capillaries to help stop bleeding. This process is mediated by epinephrine, norepinephrine, and prostaglandins released by injured cells. After 10-15 minutes the blood vessels will experience vasodilation mediated by serotonin, histamine, kinins, prostaglandins, leukotrienes, and endothelial products. This causes the wound site to appear red and warm [12].

In the proliferative phase, there is a decrease in the number of inflammatory cells, reduced signs of inflammation, the emergence of proliferating fibroblast cells, the formation of new blood vessels, epithelialization, and wound contraction. A fibrin matrix filled with platelets and macrophages secretes growth factors that activate fibroblasts. Fibroblasts migrate to the wound area and begin to proliferate until their number is more dominant than the inflammatory cells in that area. This phase occurs from the third day to the fifth day [12]..

The formation of new blood vessels/angiogenesis is a process stimulated by high energy requirements for cell proliferation. In addition, angiogenesis is also needed to regulate vascularity damaged by injury and stimulated by high lactate conditions, acidic pH levels, and decreased oxygen tension in tissues [13].

The scar tissue remodeling phase is the longest phase of the healing process. This process starts around the 21st day to a year. Collagen formation will begin to decline and stabilize. Although the amount of collagen has been maximized, the wound resistance strength is only 15% of normal skin. [12]

Chronic Wounds and the Role of Cytokines

Many factors hinder the wound healing process. Systemic factors such as malnutrition, age, tissue hypoxia, and diabetes. In chronic wounds, there are changes in the wound healing process, one of which is an increase or decrease in the expression of several cytokines, growth factors, and proteins. On examination of exudate and plasma in chronic wounds when compared with acute wounds there is an increase in IL-1, IL6, and TNF alpha which is a pro-inflammatory cytokine. These cytokines will decrease in levels when the wound heals [5].

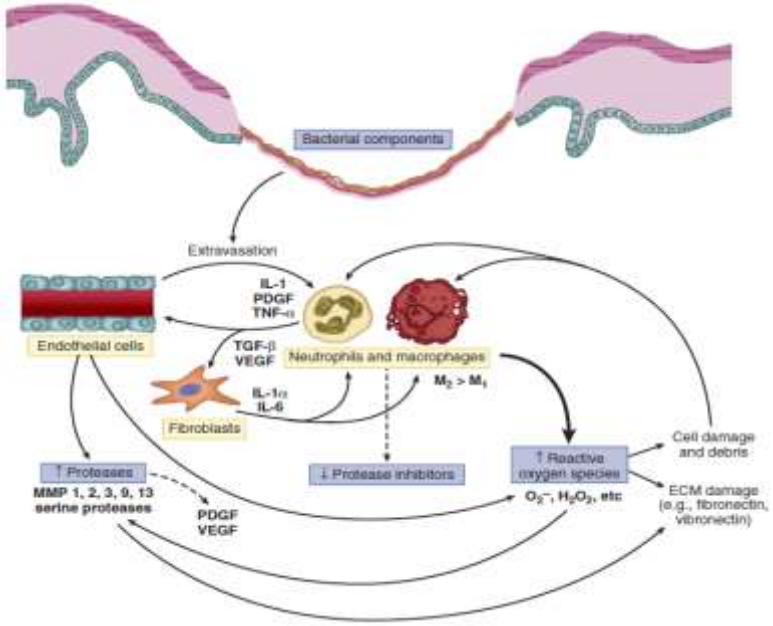

Figure 4. Mechanism of Chronic Wounds. [13]

The process of healing chronic wounds, such as diabetic ulcers, is indeed more complicated and requires more complex treatment. Local therapy or wound protection should prevent the wound from contamination and produce more optimal conditions to accelerate the wound healing process. Moist wound conditions can increase the epithelialization process and the wound healing process. [4].

Hyaluronic Acid

Hyaluronic acid (HA) is a component of the extracellular matrix in connective tissue that can help the wound healing process, thus providing the right conditions for the tissue regeneration process in injured tissue. HA has been used for a long time and has developed good results in the fields of ophthalmology and connective tissue disease, arthritis, and rheumatoid arthritis. Topical use of HA is also effective in treating chronic wounds[4] [14].

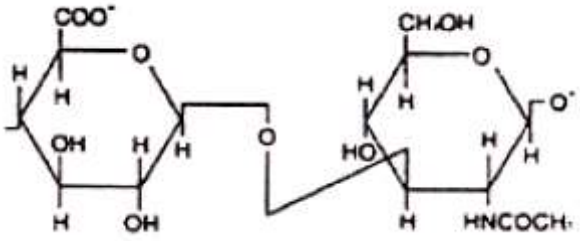

Figure 5.. Structure of Hyaluronic Acid [15]

Hyaluronic acid (HA) is a naturally occurring glycosaminoglycan that serves as a foundation for biomaterial production. HA, for example, is non-immunogenic, degradable by enzymes, and non-adhesive to cells and proteins. Angiogenesis, extracellular matrix, homeostasis, wound healing, and mediators of long-term inflammation are among processes in which HA plays a role physiologically. Hyaluronic acid is a glycosaminoglycan with repeating disaccharide units (1 – 4 – D – glycospiranosyluronic acid), (1 – 3) N acetyl 2 – amino – 2 deoxy – – D glycospiranosyl acid. These polysaccharides have a molecular weight of 104 to 107 Da and contain 500 to 50,000 monosaccharide residues per molecule. (Saranraj and Naidu, 2013).

HA has an important role in cell migration and proliferation, which are the two main processes required for wound healing. In addition, HA is also the main medium for tissue hydration with the ability to absorb water masses of up to 3 thousand times the mass of HA itself. According to the latest meta-analysis[16]. ten studies of HA produced good outcomes in healing burns, surgical wounds, and chronic ulcers [17].Hyaluronic acid also plays a role in increasing epithelialization, angiogenesis, lymphangiogenesis which supports the proliferation process in wound healing. In the maturation process, hyaluronic acid increases the remodeling of collagen [18]

A study by P. Rooney et al, who investigated the effect of HA on an inflammatory model of interstitial cystitis concluded that the anti-inflammatory effect of HA works by inhibiting TNF alpha thereby preventing the increase in IL-6 and IL-8 which are pro-inflammatory cytokines [19]. The fast and holistic healing process of diabetic foot ulcers is still a challenge, related to the condition and extent of the wound in unsupportive diabetic patients. As part of a multimodal treatment method for diabetic ulcers, the use of dressings with modern materials using Hyaluronic Acid is an effective method. However, several previous studies applied the use of HA in conjunction with additional biologic agents that could hinder the process of determining the true clinical effect of HA for Diabetic Foot Ulcers[20]

The Role of Cytokines in the Evaluation of the Wound Healing Process

A persistent inflammatory state and abnormal activation of macrophages are characteristic of chronic wounds in diabetic patients [21] This situation can be evaluated by looking at the expressions of inflammatory cells and cytokines. In chronic wounds, there is an increase in the expression of TNF alpha, IL-1, IL-6, and IL-8. IL-6 is a cytokine produced by monocytes and macrophages and has a role in the activation of B cells, T cells, and regulates hepatic acute-phase protein synthesis. IL-6 is detected 12 hours after injury and can persist for more than 1 week in certain cases [13]

Research conducted by Bekeschus et al in 2017 wherein that study compared the pattern of cytokines and *chemokine* in patients with diabetic foot ulcers and acute wounds explained that there was a significant increase in IL-1, IL-6, and IL-8 in patients with chronic wounds [4]. IL-1, IL-6, and IL-8 are pro-inflammatory cytokines, chronic inflammatory conditions, one of which is caused by these proinflammatory cytokines causing chronic wounds in diabetic patients [13] [21]

According to a study published in 2012 by Zubair et al, there is a link between diabetic foot ulcers and higher plasma IL-6 levels. When compared to individuals without diabetic foot ulcers, those with diabetic foot ulcers exhibited higher plasma IL-6 levels. Inflammatory marker IL-6 may distinguish between infected diabetic foot ulcers (IDFU) and non-infected diabetic foot ulcers (NIDFU) [5] [22]

At various stages of the wound healing process, IL-6 plays a critical function. Too much IL-6 can slow down the healing process because IL-6 will signal leukocytes to increase the inflammatory

process and eventually these cells will damage the ECM and the maturation process cannot occur. Therefore, in the remodeling phase, it is hoped that IL-6 levels will not be high [23].

The function of IL-6 in wound healing is not well known. Inflammation at the right time may help wounds heal faster, but IL-6 overexpression can slow down the healing process [13][23].However, the importance of IL-6 in wound healing should not be overlooked. In the remodeling phase, when a disruption in the IL-6 pathway might induce delayed wound healing, IL-6 generally drops considerably.[24]

CONCLUSION

Clinical aplication include the use of Hyaluronic Acid in treating diabetic foot ulcer has positive effect on accelerating healing of the ulcer. Then Hyaluronic Acid also has proven to decrease IL-6 which is a proinflammatory cytokine. Further research should be done with a larger sample and in a systematic way.

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