Indonesia Journal of Biomedical Science (*IJBS*) 2019, Volume 13, Number 2: 57-62
P-ISSN.2085-4773, E-ISSN.2302-2906

DOAJ DIRECTORY OF OPEN ACCESS JOURNALS
DiscoverSys Whatever it takes...
Published by DiscoverSys

The effect of hyaluronic acid on the changes in Matrix Metalloproteinase-1 (MMP-1), leukocytes level, and bacteriocide effects among patients with Wagner 3 and 4 diabetic foot ulcer

Ida Bagus Anangga Kharisma[1*], I Nyoman Semadi[2], I Gede Raka Widiana[3]

ABSTRACT

Background: Diabetes Mellitus (DM) has become a major public health problem. A significant complication of Diabetes Mellitus is the emergence of diabetic foot ulcers that require special treatment in healing. Hyaluronic Acid plays a role in cell proliferation and cell movement and is needed in the process of wound healing. The main goal of treatment of diabetic ulcers in the foot is to achieve wound closure as quickly as possible, prevent recurrence and amputation.

Methods: A true experimental study with pre and post-test design has been conducted among 32 diabetic foot patients at Sanglah General Hospital which divided into 2 groups: Hyaluronic acid and NaCl 0.9% using consecutive techniques. The hyaluronic acid compared to NaCl 0.9% on MMP-1, leukocyte levels, and bacteriocide effects in patients with Wagner's diabetic foot 3 and 4 were assessed. Data were analyzed using SPSS version 20 software for Windows.

Results: The results showed that Hyaluronic Acid increased MMP-1 levels while NaCl 0.9% reduced MMP-1 levels significantly ($P<0.05$). Hyaluronic Acid was able to reduce leukocyte level significantly compared to NaCl 0.9% ($P=0.006$). A positive bacterial culture was also found markedly lesser in Hyaluronic Acid groups compared with NaCl 0.9% ($P<0.001$). Multivariate analysis using logistic and linear regression also showed significant differences in the MMP-1 and leukocyte level ($P<0.001$) and positive culture results using hyaluronic acids (OR: 0.020; 95% CI 0.003-0.166; $P<0.001$)

Conclusion: These results suggest that MMP-1 levels and leukocyte levels differed significantly between Hyaluronic Acid and NaCl 0.9% used. Hyaluronic Acid can increase MMP-1 levels in Wagner 3 and 4 diabetic care. The results of positive culture with Hyaluronic Acid used are less than NaCl 0.9%.

Keywords: Diabetic foot ulcer, Hyaluronic Acid, MMP-1 level, leukocyte level, bacteriocide effect

Cite this Article: Kharisma, I.B.A., Semadi, I.N., Widiana, I.G.R. 2019. The effect of hyaluronic acid on the changes in Matrix Metalloproteinase-1 (MMP-1), leukocytes level, and bacteriocide effects among patients with Wagner 3 and 4 diabetic foot ulcer. *IJBS* 13(2): 57-62. DOI:10.15562/ijbs.v13i2.195

[1]General Surgery Specialist Program, Faculty of Medicine, Udayana University, Sanglah General Hospital, Bali, Indonesia
[2]Department of General Surgery, Thorax Cardiovascular Division, Faculty of Medicine, Universitas Udayana, Sanglah General Hospital, Bali, Indonesia
[3]Department of Internal Medicine, Kidney-Hypertension Division, Faculty of Medicine, Universitas Udayana, Sanglah General Hospital, Bali, Indonesia

*Corresponding to:
Ida Bagus Anangga Kharisma; General Surgery Specialist Program, Faculty of Medicine, Universitas Udayana, Sanglah General Hospital, Bali, Indonesia; anangga.kharisma@yahoo.com

Received: 2019-05-20
Accepted: 2019-06-04
Published: 2019-12-01

INTRODUCTION

One of the complications in diabetes mellitus-type 2 (DM-2) is diabetic foot ulcer.[1] Diabetic foot ulcers cause increased amputation rates in diabetic foot patients. According to Edmonds ME and Katsilambros et al., showed that foot ulcer and amputation affect the quality of life and economy burden to the patient so that the prompt-identification of diabetic patients with a high risk of foot ulcer, prevention effort, and appropriate treatment is the main program of current health centers.[1,2] The incidence of amputation in diabetic patients varies worldwide, and the number is always increasing. This shows the failure of the health system management either by the government or by the private sector in dealing with diabetic foot problems.

The prevalence of foot ulcers in the overall diabetes patient population is around 4-10%, lower at a young age (1.5 - 3.5%), and increasing in old age (5 - 10%).[3] The main risk of diabetic foot complications is amputation. From the current study, approximately 85% of diabetic foot patients were amputated. A previous study stated that the prevalence of amputation in diabetic foot patients was 1.6% at age 18-44 years, 3.4% aged 45-64 years, and 3.6% at over 65 years of age.[3] While another study by Anggriani et al. stated that the mortality rate due to ulcers and gangrene reached 17.23%, where amputation complications result in approximately 14.8%.[4]

Failure of diabetic foot care is more dominant due to diabetes and education of patients regarding diabetes care. Besides, it is also caused by inadequate management by health workers. Thus there are many diabetic foot patients worldwide who do not get adequate treatment even though these patients have money to pay for medical expenses.[5] The main goal of diabetic foot ulcers management is a complete wound healing. The gold standards treatment of diabetic foot ulcers includes wound debridement, management of infections, revascularization

procedures for indications, and off-loading ulcers. Debridement must be performed on all chronic wounds to remove necrotic tissue and debris.[5,6]

An understanding of the wound pathophysiology leads to the implementation in the physiological approaches of diabetic foot syndrome management. One of the emerging technology that appears in the use of biomaterials for wound healing such as in the form of gels, membranes, mesh/nets was known containing hyaluronic acid.[7] Hyaluronic acid has several physiological and receptor-mediated functions (cell proliferation and metabolic modulation). Hyaluronic acid is involved in all phases of wound healing, from initial cell migration, through inflammatory and granulation phases to the last epithelialization.[7] In particular, hyaluronic acid has been reported to play an important role in controlling the level of tissue hydration by creating high osmotic pressure that appears earlier and is maintained during the formation of fetal skin as well as an inflammatory stage in the process of wound healing.[7] The binding properties of hyaluronic water maintain the ideal moisture environment needed for the cicatrization process.

The study conducted by Chen et al. showed that hyaluronic acid has an advantage in the formation of scar tissue for diabetic ulcers.[8] Diabetic ulcer healing rates after 12 weeks were significantly higher in patients using hyaluronic acid compared to those treated with standard therapy. Hyaluronic acid is an effective and safe as diabetic foot ulcer therapy with healing effects regardless of pharmacological preparations.[8]

However, a study conducted by Muller et al. reported that the effects of dermal scaffold hyaluronic acid (3D Hyalograft) and epidermal grafts were found no difference in the rate of cure of patients with diabetic foot ulcers compared to controls.[9] In vice versa, a study conducted by Uccioli et al. reported that the healing rates of the ulcer were faster in the 3D Hyalograft group (40 days vs. 50 days).[10]

Based on those mentioned above, a study regarding the effect of hyaluronic acid in the treatment of diabetic foot wounds is still controversial among several previous studies. So that, a study conducted on Wagner 3 and 4 diabetic foot ulcers was carried out to determine the MMP-1 levels, leukocyte levels, and bacteriocide effects from wound culture as an objective parameter in determining the wound healing process.

METHODS AND MATERIAL

A true experimental study with a pre and post test design among 32 patients diagnosed with Diabetics Foot Ulcers Wagner 3 and 4 at Sanglah General Hospital, Bali was conducted consecutively in February 2019. The exclusion criteria in this study include: 1) Patients who have received hyaluronic acid therapy; 2) Patients with incomplete medical records; 3) Patients who refuse to participate in the study. The independent variables in this study were hyaluronic acid and sodium chloride 0,9%, while the dependent variables were the number of leukocytes, MMP-1 levels, and bacteriocide effects. Wagner 3 and 4 diabetic feet in this study are assessed clinically and laboratory (blood glucose levels). Whereas the bacteriocide effect is the result of culture based on wound swabs before and after treatment expressed in the form (+) and (-) to indicate the presence of pathogens. Some other parameters assessed in this study included the duration of suffering from DM, levels of Hba1C, Albumin, Hemoglobin, and age.

Data were analyzed using descriptive, bivariate, and multivariate statistical analysis. The bivariate analysis included a normality test, variance homogeneity test, and Independent T test. While the multivariate test used linear regression analysis and logistic regression. The significance level was accepted if p-value less than 0.05, with a 95% confidence interval using SPSS version 20 for windows.

RESULTS

The characteristics of respondents based on age in the treatment group with hyaluronic acid were found 58±10.51 years, whereas in the NaCl 0.9% group, the mean age was 61±11.23 years (Table 1). According to gender, the male was 7 respondents (43.8%) in Hyaluronic Acid group, while in NaCl 0.9% found 6 respondents (37.5%). The random blood glucose level in the treatment group with Hyaluronic Acid was 440.87±84.62 mg/dL and blood glucose 2 hours post-prandial (PP) was 271.25±50.81 mg/dL. Similar results also found in the NaCl group where random blood glucose level was 0.9% 441.50±43.75 mg/dL and blood glucose 2 hours PP was 263.75±40.27 mg/dL (Table 1).

The onset of diabetes mellitus in Hyaluronic Acid group was 11.37±4.2 years and NaCl 0.9% about 13.9±4.9 years. Hba1C levels in the Hyaluronic Acid group was 6.49±1.00% while the NaCl 0.9% group was 6.69±1.09%. In addition, the albumin level in the hyaluronic acid group was 3.02±0.41 g/dL while in the NaCl 0.9% group was 2.9±0.50 g/dL. Similar results were also found in Hemoglobin assessment whereas 11.6±0.74 g/dL in hyaluronic acid and 11.67±1.05 g/dL in NaCl 0.9% group (Table 1).

Table 2 shows the MMP-1 levels before treatment in hyaluronic acid group was 2.7±0.93

Table 1. Characteristic of respondents

Variables	Intervention Groups (n=32)	
	Hyaluronic Acid n=16	NaCl 0,9% n=16
Age (mean, SD)	58±10.510	61±11,230
Sex (n,%)		
Male	7 (43.8%)	6 (37.5%)
Female	9 (56.3%)	10 (62.5%)
Random Blood Glucose (mg/dL) (mean, SD)	440.87±84.62	441.50±43.75
Post-Prandial Blood Glucose (mg/dL) (mean, SD)	271.25±50.81	263.75±40.27
Onset of DM-2 (years) (mean, SD)	11.37±4.2	13.9±4.9
Hba1C (%) (mean, SD)	6.49±1.00	6.69±1.09
Albumin (g/dL) (mean, SD)	3.02±0.41	2.9±0.50
Haemoglobin (g/dL) (mean, SD)	11.6±0.74	11.67±1.05

Table 2. The difference results of MMP-1 between Hyaluronic Acid (AH) dan NaCl 0,9% groups

Variables	Mean ±SD	Mean Difference	95% CI (Lower-Upper)	p-value
MMP-1 levels (pg/ml)				
(Pre-test)				
AH	2.7±0.93	0.11	(-0.84)-0.62	0.758
NaCl 0,9%	2.8±1.09			
(Post-Test)				
AH	2,9±0,83	0.66	0.09-1.22	0.025
NaCl 0,9%	2,3±0,72			
Leukocyte level (1x10^3)				
(Pre-test)				
AH	19.7±3.23	2.3	0.03-4.64	0.050
NaCl 0,9%	17.4±3.23			
(Post-test)				
AH	8.8±2.16	2.6	0.80-4.44	0.006
NaCl 0,9%	11.4±2.84			

Table 3. The difference of bacteriocide properties between Hyaluronic Acid and NaCl 0.9% groups

Groups	Bacterial Culture (n=32)		OR	p-value	95% CI
	Positive (n=16)	Negative (n=16)			
Hyaluronic Acid	2 (6.3%)	14 (43.8%)	0.020	<0.001	0.003-0.166
NaCl 0,9%	14 (43.8%)	2(6.3%)			

pg/ml and 2.8±1.09 pg/dL in NaCl 0.9% group with a no significant mean difference 0.11 (p=0.758; 95% CI: (-0.84)-0.62) between both groups. However, after treatment the hyaluronic acid was 2.9±0.83 pg/ml and 2.3±0.72 pg/ml in NaCl 0.9% group with a significant mean difference 0.66 (p=0.025; 95% CI:0.09-1.22) means that there is a significant difference between the treatment with hyaluronic acid compared with NaCl 0.9% (Table 2)

The results of this study also showed insignificant differences in the number of leukocytes before treatment between hyaluronic acid (19.7 ± 3.23) and NaCl 0.9% (17.4 ± 3.23) (MD: 2.3; 95% CI : 0.03-4.64; P = 0.053) (Table 2). However, the significant differences were found after treatment with hyaluronic acid (8.8 ± 2.16) compared with NaCl 0.9% (11.4 ± 2.84) (MD: 2.6; 95% CI: 0.80-4.44; P = 0.006) (Table 2)

Table 3 below shows the results of positive culture with the use of hyaluronic acid as many as 2 respondents (6.3%) and negative culture results of 14 respondents (43.8%), while positive culture results with 0.9% more NaCl use i.e. 14 respondents (43.8%) and negative culture results with the use of 0.9% NaCl as much as 2 respondents (6.3%). The p-value results show <0.001 (OR: 0.020; 95% CI: 0.003-0.166) which means that there was a significant difference in the results of bacterial culture between the use of hyaluronic acid and NaCl 0.9% (Table 3).

Determination of hyaluronic acid and 0.9% NaCl usage on the results of MMP-1 and leukocytes after controlling for age, DM duration, Hba1c, albumin and hemoglobin levels were carried out by linear regression test (Table 4). Description Table 4 shows that there was a significant difference in the average value of MMP-1 levels between hyaluronic acid and NaCl 0.9% group (P=0.025) (Figure 1A).

The results of the One-way ANOVA test in Table 4 show that there was a significant difference in the mean values of leukocytes between both groups (p=0.006) (Figure 1B). Logistic regression analysis in Table 5 shows that there was an effect of treatment with hyaluronic acid on the results bacterial culture which indicates a negative relationship (p<0.001; OR: 0.02; 95% CI: 0.003-0.166).

DISCUSSION

The results showed that MMP-1 levels increased with the use of hyaluronic acid compared to the use of NaCl 0.9% group. Hyaluronic acid is one of the basic compounds in the human body that has a function to bind water and hold cells so that it is always in a gel-like matrix.[8] Hyaluronic acid is a polysaccharide, which is present in connective tissue, epithelium, and nerve tissue in the body. Hyaluronic acid contributes to cell proliferation and migration, which is a key process needed in the wound healing process. 11 In addition, this material is considered to be a tissue hydrating agent, because the amount of water absorbed by hyaluronic acid molecules can exceed 3,000 times the size.[8] In recent years, an understanding of the pathophysiology of wound development with a physiological approach in the treatment of diabetic foot syndrome. one technology that arises in the use of biomaterials in the form of gels, membranes, mesh/nets containing hyaluronic benzyl ester (HYAFF-11), where grafts obtained from fibroblasts and autologous keratinocytes are placed there.[8] Also, the available

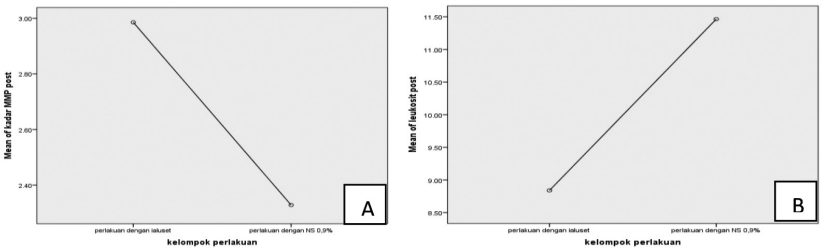

Figure 1. Scatter Plot difference in MMP-1 Level (A) and leukocyte level (B) between Hyaluronic Acid and NaCl 0.9% group

Table 4. The treatment of hyaluronic acid and NaCl 0.9% to the results of MMP-1

Variable	AH Δ±SD	NaCl 0.9% Δ±SD	p-value
MMP-1	2.98±0.83	2.32±0.72	0.025
Leukocyte levels	8.84±2.16	11.46±2.64	0.006

Table 5. Logistic regression test on the use of hyaluronic acid to the results of bacterial culture

Variable	B	SE	p-value	OR	95% CI
Hyaluronic Acid	-3.89	1.06	<0.001	0.020	0.003-0.166

treatment based on recombinant human platelet growth factor IV (becaplermin gel), two layers equivalent to living human skin, substituting allogenic skin.[7]

A study conducted by Ayuk SM showed that MMP-1 was a major collagenase that has an impact on wound healing, it has been shown that its specific type I proteolysis collagen (an essential component of the dermis) was indispensable for keratinocyte migration and thus epidermal re-formation.[12] The higher levels of MMP-1 was related to the good healing process and expected to provide a complete proliferation stage.[12] Furthermore; it has been suggested that an adaptation of MMP-1 regulation is essential for the proper development of the healing process.[9]

Several studies have been conducted regarding diabetic foot ulcers such as the study of Lobman et al. found that activated levels of MMP-1, MMP-8, MMP-9, and MMP-2 were significantly higher in diabetic foot ulcers and TIMP-2 levels were significantly lower than those of acute non-diabetic patients.[9] Only a few data were available regarding changes in MMP levels during the healing process of diabetic foot ulcer.[9]

The focus of diabetic wound healing is the ECM regulator which consists of growth factors and groups of enzymes that form the Metalloproteinase (MMP) matrix. The main function of growth factors is to regulate the initial inflammatory phase process until the final phase of granulation tissue formation. Injuries that do not heal often show a defect in growth factors.[13] Some cytokines such as TNF-β, IL-β and IL-6 increase in chronic venous ulcers and burns that do not heal.[13,14]

Leukocytes are a marker of infection in the body Infection often occurs in DM patients and is often found to be more severe than patients without DM and has a higher risk of osteomyelitis.[15] About 56% of diabetic foot ulcers become infected, and 20% of those infected undergo amputation lower extremity.[15] The results of other studies showed that there were differences in the number of leukocytes between the use of hyaluronic acid and NaCl 0.9%. Hyaluronic acid lowers leukocytes in the blood greater than NaCl 0.9% use in patients with diabetic foot ulcer Wagner 3 and 4. Hyaluronic acid is a polymer disaccharide of glucuronic acid and N-acetylglucosamine.[14] Hyaluronic acid is known to stimulate leukocyte destruction and phagocytosis from macrophage cells. This is still a debate in its use. Suzuki and Yamaguchi reported that hyaluronic acid suppresses the decrease of active oxygen and phagocytosis macrophage in molecular-dependent manner.[14]

Leukocytes themselves as cells that form components of blood that function to help the body fight various infectious diseases as part of the immune system.[16] Leukocytes or white blood cells are units that can move in the body's defense system (mobile), have functions to resist invasion of pathogens (disease-causing microorganisms) such as bacteria or viruses through phagocytosis, identify and destroy cancer cells that enter the body, function as cleansers that cleanse the body's waste by phagocytic debris from dead or injured cells, important in wound healing and tissue cleansing. Increased leukocytes in the blood indicate an infection or inflammation in the body.[16]

In diabetic foot ulcers, many pathogenic germs are found in both aerobic and anaerobic.[17] The presence of infection in the body causes an increase in leukocytes. Leukocytes or white blood cells function to help the body fight various infectious diseases as part of the immune system.[16] Data from the current research suggests that before being given treatment all respondents obtained positive culture results. After treatment with hyaluronic acid and NaCl 0.9%, there was a significant difference found in bacterial culture.[18] The results of positive culture with the use of hyaluronic acid were lesser compared with NaCl 0.9%. Hyaluronic acid is significantly more effective in treating local foot ulcers in terms of reducing wound size and reducing pain burden, with a good safety profile.[18]

Management of infections that are not optimal can cause wounds to become chronic and often

have polybacterial germs which are a mixture of gram-positive cocoa bacteria and negative gram-gram bacteria.[19] If this chronic infection continues, it results in deepening of the wound and wider necrotic tissue the presence of poor vascularization in DM patients causes low oxygen pressure in the injured area which results in easy anaerobic bacteria to multiply.[19]

Foot ulcers infected with pathogenic anaerobic bacteria showed a longer healing time than ulcers infected with aerobic pathogenic bacteria ($P < 0.001$). In anaerobic ulcer infections, Peptostreptococcus and Clostridium were found the amount to 69.4%, anaerobic Gram negatives such as bactericides and Fusobacterium were found approximately 30.6%.[17]

Hyaluronic acid is involved in the process of tissue repair as an important component in skin resurfacing and prevents scar formation. The osmotic ability of hyaluronic acid restores tissue hydration during the inflammatory process and viscosity prevents the passage of pericellular bacteria and viruses / around the cell.[20] Hyaluronic acid provides good long-term safety and has proven the ability to reduce bacterial adhesion and biofilm formation among various polymers tested.[21]

CONCLUSION

The leukocyte level in the blood patients with Diabetic Foot Ulcer Wagner 3 and 4 was significantly lower in the hyaluronic acid group. MMP-1 levels showed a significant increase in the hyaluronic acid group and the results of positive cultures with the use of hyaluronic acid showed significantly lesser colonies compared to NaCl 0.9%. In summary, it can be concluded that hyaluronic acid could reduce leucocyte level, increasing MMP-1 level, and provided lesser colonies of bacterial cultures. However, further studies using bigger sampler size and well-design are required to clarify the results to the population.

ETHICAL CLEARANCE

This study has been obtained the ethics approval from the Faculty of Medicine, Udayana University, Sanglah General Hospital, Bali, Indonesia with number 1340/UN14.2.2VII.14/LP/2019

CONFLICT OF INTEREST

The authors declare that there is no competing interest regarding the manuscript.

FUNDING

The authors are responsible for the study funding without the involvement of grant, scholarship, or any other resources of funding

AUTHOR CONTRIBUTION

Ida Bagus Anangga Kharisma is responsible for data gathering, data analysis, until reporting the results of the study. I Nyoman Semadi and I Gede Raka Widiana are responsible for study concept and supervised the study.

REFERENCES

1. Edmonds ME. A Practical Manual of Diabetik Footcare. USA; Blackwell Publishing. 2004.
2. Katsilambros N, Dounis E, Ten TP. Atlas of the Diabetik Foot. Inggris; John Wiley & Sons, Ltd. 2003.
3. Zhang P, Lu J, Jing Y, Tang S, Zhu D, Bi Y. Global epidemiology of diabetic foot ulceration: a systematic review and meta-analysis. Ann Med. 2017; 49(2):106-116.
4. Angriani Y, Restini M, Mitakda VC, Rochsismandoko, Kusumaeni T. Clinical Outcomes Penggunaan Antibiotik pada Pasien Infeksi Kaki Diabetik. Jurnal Sains Farmasi & Klinis. 2015; 1(2):111-121
5. Alexiadou K, Doupis J. Management of Diabetic Foot Ulcers. Diabetes Ther. 2012; 3(1):4
6. Golinko MS, Joffe R, Maggi J, Cox D, Chandrasekaran EB, Tomic-Canic RM, Brem H. Operative Debridement of Diabetic Foot Ulcers. J Am Coll Surg.2008; 207(6): e1-e6
7. Lee M, Han SH, Choi WJ, Chung KH, Lee JW. Hyaluronic acid dressing (Healoderm) in the treatment of diabetik foot ulcer: A prospective, randomized, placebo-controlled, single-center study. Wound Repair Regen. 2016 ;24(3):581-8
8. Chen CP, Hung W, Lin SH. Effectiveness of hyaluronic acid for treating diabetic foot: a systematic review and meta-analysis. Dermatol Ther. 2014; 27(6):331-6.
9. Muller M, Trocme C, Lardy B, Morel F, Halimi S, Benhamou PY. Matrix metalloproteinases and diabetic foot ulcers: the ratio of MMP-1 to TIMP-1 is a predictor of wound healing. Diabet Med. 2008; 25(4):419-26.
10. Uccioli L; TissueTech Autograph System Italian Study Group. A clinical investigation on the characteristics and outcomes of treating chronic lower extremity wounds using the tissuetech autograft system. Int J Low Extrem Wounds. 2003; 2(3):140-51.
11. Matsui Y, Inomata M, Izumi K, Sonoda K, Shiraishi N, Kitano S. Hyaluronic acid stimulates tumor-cell proliferation at wound sites. Gastrointest Endosc. 2004; 60(4):539-43.
12. Ayuk SM, Abrahamse H, Houreld NN. The Role of Matrix Metalloproteinases in Diabetic Wound Healing in relation to Photobiomodulation. J Diabetes Res. 2016; 2016: 2897656
13. McLennan S, Yue DK, Twigg SM. Molecular aspects of wound healing in diabetes. Primary Intention. 2006; 14(1):8-13
14. Lym HS, Suh Y, Park CK. Effects of hyaluronic acid on the polymorphonuclear leukocyte (PMN) release of active oxygen and protection of bovine corneal endothelial cells from activated PMNs. Korean J Ophthalmol. 2004; 18(1):23-8.
15. Everett E, Mathioudakis N. Update on management of diabetic foot ulcers. Ann N Y Acad Sci. 2018; 1411(1):153–165.
16. Alexander JS, Elrod JW, Park JH. Roles of leukocyte and immune cell junctional proteins. Microcirculation. 2001; 8(3):169-79.

17. Noor S, Zubair M, Ahmad J. Diabetic foot ulcer--A review on pathophysiology, classification and microbial etiology. Diabetes Metab Syndr. 2015 ;9(3):192-9
18. Dereure O, Czubek M, Combemale P. Efficacy and safety of hyaluronic acid in treatment of leg ulcers: a double-blind RCT. J Wound Care. 2012; 21(3):131-139.
19. Smith K, Collier A, Townsend EM, O'Donnell LE, Bal AM, Butcher J, et al. One step closer to understanding the role of bacteria in diabetic foot ulcers: characterising the microbiome of ulcers. BMC Microbiol. 2016; 16:54.
20. Triviño GC, Parra MR, González LV, Oyarzún JO, Solorzano G. Synthesis and Bactericidal Properties of Hyaluronic Acid Doped with Metal Nanoparticles. Journal of Nanomaterials. 2017; ID9573869.
21. Romanò CL, Vecchi ED, Bortolin M, Morelli I, Drago L. Review Hyaluronic Acid and Its Composites as a Local Antimicrobial/Antiadhesive Barrier. J Bone Jt Infect. 2017; 2(1): 63–72