



Journal Homepage: - www.journalijar.com
**INTERNATIONAL JOURNAL OF
 ADVANCED RESEARCH (IJAR)**

Article DOI: 10.21474/IJAR01/7662
 DOI URL: <http://dx.doi.org/10.21474/IJAR01/7662>



RESEARCH ARTICLE

RELATIONSHIP BETWEEN TIMP-1/MMP-9 RATIO, TYPE AND NUMBER OF BACTERIAL FOR SUCCESSFUL PRESSURE ULCER CLOSURE.

Hardisiswo Soedjana¹, Lisa Hasibuan², Dany Hilmanto³ and Theresia Risa Davita².

1. Head of Division of Plastic Reconstructive and Aesthetic Surgery, Department of Surgery, Padjajaran University - Hasan Sadikin General Hospital, Bandung, Indonesia.
2. Division of Plastic Reconstructive and Aesthetic Surgery, Department of Surgery, Padjajaran University - Hasan Sadikin General Hospital, Bandung, Indonesia.
3. Department of Paediatric, Padjajaran University - Hasan Sadikin General Hospital, Bandung, Indonesia.

Manuscript Info

Manuscript History

Received: 01 August 2018
 Final Accepted: 31 August 2018
 Published: 30 September 2018

Keywords:-

pressure ulcers, TIMP-1, MMP-9,
 bacteria, wound closure

Abstract

Background: Wound closure in pressure ulcers has a low rate of success. A tool is required to establish the right moment for wound closure. Matrix metalloproteinase-9 (MMP-9) is the main protease responsible for matrix degradation in chronic wounds. Its activity is regulated by Tissue Inhibitors of Metalloproteinase-1 (TIMP-1). The MMP-9/TIMP-1 ratio is inversely proportional to the success of wound healing in pressure ulcers. The type and number of certain bacteria also determine the success of wound closure. This study aims to explore the relationship between the bacterial load as well as the TIMP-1/MMP-9 ratio to determine wound closure success in pressure ulcers.

Methods: A hospital-based nested case-control was conducted in 24 pressure ulcer patients who underwent wound closure and were evaluated for 3 weeks. The relationship between variables was analyzed with bivariate analysis, while its strength was assessed by the contingency coefficient. We also performed a multivariate analysis with logistic regression to see which variables are more dominant in determining wound closure success.

Results: TIMP-1/MMP-9 ratio, as well as the bacterial load, was significantly associated with successful wound closure. The cut-off point of TIMP-1/MMP-9 ratio in the successful group is 0.72 ± 0.31 (P-value = 0.032). TIMP-1/MMP-9 ratio > 0.732 is related to the success of wound closure. Type and number of bacteria are significantly different between the successful and unsuccessful group (P-value = 0.034).

Conclusion: TIMP-1/MMP-9 ratio is more dominant than the bacterial load in determining the success of wound closure of pressure ulcers, with OR 11.45 vs. OR 0.63 compared to type and number of bacteria (P-value = 0.024).

Copy Right, IJAR, 2018,. All rights reserved.

Corresponding Author:- Theresia Risa Davita.

Address:- Division of Plastic Reconstructive and Aesthetic Surgery, Department of Surgery,
 Padjajaran University - Hasan Sadikin General Hospital, Bandung, Indonesia.

Introduction:-

Pressure ulcer is a major health problem with a high prevalence, especially among patients with immobilization.(1) Medical record in our hospital showed an increasing number of patients with pressure ulcers, with an average length of hospitalization of more than 3 months.(2) Complications of pressure ulcers include unhealing wound, cellulitis, sepsis, even death.(3)

Essentially, the management of pressure ulcer comprises of nutritional support, mobilization, and wound bed preparation. Preparing the wound bed can be done by repeated debridement to establish a healthy wound before wound closure is performed.(3) A wound is deemed ready to be closed if it is free from necrotic tissue and covered with healthy granulation tissue.(4)

Surgical measure is an important step in managing pressure ulcer. It includes debridement from necrotic tissue and wound closure by skin graft or flap.(5) However, the failure rate of wound closure remains high. This failure includes dehiscence, persistence of infection, and wound recurrence.(6-8) A diagnostic tool is required to determine the right moment to perform wound closure so that the incidence of failure can be reduced or prevented.

In chronic wounds, MMP-9 functions as the destroyer of extracellular matrix and growth factor, which in turn cause failure in wound healing. It also prevents cell migration and causes tissue destruction.(9,10) Its activity is regulated by Tissue Inhibitor of Metalloproteinase 1 (TIMP-1).(11) MMP-9 / TIMP-1 ratio is inversely proportional to the success of wound healing, as shown in diabetic ulcers.(12,13)

Another factor that interferes with wound healing process is bacterial contamination. The ability of wound to form granulation tissue depends on the bacterial load.(14) Schneider also found that the concentration and quantity of bacteria can be used to predict wound closure success.(15)

This study aims to explore the relationship between the type and number of bacteria as well as TIMP-1 / MMP-9 ratio and the success of pressure ulcer wound closure. This study also aims to determine the dominant factor.

Material And Method:-

This research was conducted as a hospital-based nested case control of 24 patients with pressure ulcer grade III and IV on their sacrum area. This study was performed in Hasan Sadikin Hospital, Bandung, Indonesia from April 2012 to February 2014.

Samples were biopsied from the granulation tissue of the ulcers from included patients. The samples were divided into two parts, each sent to histopathology and clinical pathology department. The histopathology department assessed TIMP-1 and MMP-9 enzyme expression through immunohistochemistry.

The clinical pathology department conducted culture, bacterial identification, and assessed the number of bacteria. Bacterial load was classified as the following score:

1. no bacterial growth
2. bacterial growth with low virulence (non-pathogenic) $<10^5$ CFU/gram tissue
3. bacterial growth with low virulence (non-pathogenic) $\geq 10^5$ CFU/gram tissue
4. bacterial growth with high virulence (pathogenic) $<10^5$ CFU/gram tissue
5. bacterial growth with high virulence (pathogenic) $\geq 10^5$ CFU/gram tissue

Wound closure was performed in a tertiary fashion. The wound was considered ready for closure if it had healthy granulation and no necrotic tissue. The author performed wound defect by a fasciocutaneous flap. After wound closure, the wound was assessed every week for three weeks to evaluate the success of wound closure. Wound closure was considered successful if it was neither accompanied by the presence of pus nor wound dehiscence.

The relationship between variables was analyzed with bivariate analysis. The authors assessed the strength and closeness of the relationship by looking at contingency coefficient. After that, we performed multivariate analysis with logistic regression to see which variables are more dominant in relation to the success of pressure ulcer wound closure.

Result:-

A total of 26 patients were included in the study. Two subjects dropped out from the study. One of them was deceased and the other refused further medical care. The ulcer closure was successful in 14 patients and failed in the remaining 10. The characteristics of study subjects are shown in Table 1.

Table 1:-General characteristics of study subjects.

Characteristics	Wound closure	
	Successful	Unsuccessful
Gender		
Male	9	7
Female	5	3
Underlying disease		
Trauma	8	6
Malignancy	3	1
Infection	2	1
Cerebrovascular disease	1	2
Age mean	37.86 ± 14.7	42.4 ± 18.3
Nutritional status		
Good	14	10
Inadequate	-	-
Poor	-	-
Total	14	10

The cutoff points of TIMP-1/MMP-9 ratio in the successful and unsuccessful group were 0.86 ± 0.26 and 0.72 ± 0.31 with P-value of 0.032. The successful group showed significantly greater ratio than the unsuccessful group. The type and quantity of bacteria were significantly different between the successful and unsuccessful group, with P-value of 0.034. TIMP-1/MMP-9 ratio was more dominant than the type and quantity of bacteria in successful wound closure of pressure ulcers, with OR 11.45 vs 0.63 and P-value 0.024.

To set a cut off point for TIMP-1/MMP-9 ratio we used Receiver Operating Characteristic (ROC) curve. A ratio > 0.732 is related to the wound closure success in pressure ulcers, with 85.7% sensitivity and 60% specificity. This ratio has a more dominant effect on wound closure success compared to the type and number of bacteria.

Table 2:-Immunoexpression in successful and unsuccessful group.

Immunoexpression	Wound closure		p	C (contingency coefficient)
	Successful	Unsuccessful		
TIMP-1	4.21 ± 1.42	3.8 ± 1.68	0.545	
MMP-9	5	5	0.367	
Ratio				
Mean	0.86 ± 0.26	0.72 ± 0.31	0.032	0.61
< 0.732	2	6		
≥ 0.732	12	4		

Regarding the bacterial load, no significant difference was found between bacterial type or number and the success of wound closure. However, when classified based on both bacterial type and number, the wound closure success showed significant difference.

Table 3:-Bacterial load in successful and unsuccessful group.

Culture result	Wound closure		p	C (contingency coefficient)
	Successful	Unsuccessful		
Bacterial type			0.921	
No growth	6	6		
Nonpathogenic bacteria	7	1		
Pathogenic bacteria	1	3		

Bacterial number			0.995	
No growth	6	6		
<10 ⁵ CFU/gram tissue	3	7		
≥10 ⁵ CFU/gram tissue	1	1		
Bacterial load score			0.034	0.653
0	6	6		
I	0	6		
II	1	1		
III	3	1		
IV	0	0		

To assess the relationship between TIMP-1/MMP-9 ratio as well as bacterial load and wound closure success, the author conducted multivariate analysis with double logistic regression as seen in Table 4.

Table 4:-Logistic regression analysis on the relationship between bacterial load as well as TIMP-1/MMP-9 ratio and wound closure success.

Variable	Coefficient β	SE	p	OR
Bacterial load	-0.46	0.442	0.298	0.63
TIMP-1/MMP-9 ratio	2.438	1.078	0.024	11.45
Constant	-0.817			

Discussion:-

Pressure ulcers are caused by continuous pressure on sites with bony prominence due to immobilization. From the total 24 patients included in this study, trauma was the dominant cause of immobilization as it was found in 14 patients. The other causes included malignancy (both primary and metastatic) in 4 patients, vertebral infection in 3 patients, and cerebrovascular disease in 3 patients.

Chronic wounds originate from acute wounds which are stuck in the inflammatory phase. Chronic wounds are associated with high MMP and low TIMP, resulting in a low TIMP/MMP ratio, inadequate extracellular matrix formation, and cellular migration and proliferation failure.(16) TIMP is a physiological response to increased protease. Bacterial load has an indirect role in the increase of TIMP. TIMP increase is more affected through growth factor stimulus on the proliferation of TIMP-producing cells (fibroblast, endothelial cell, keratinocyte).(17)

Through debridement, the wound is expected to return to its acute phase and proceed from the prolonged inflammatory phase. The author found similar result in this study: wounds with successful closure exhibited higher TIMP-1 and lower MMP-9 immunoexpression. A separate examination of TIMP-1 or MMP-9 immunoexpression did not show significant association with the success of pressure ulcer wound closure. However, the TIMP-1 / MMP-9 cutoff ratio of 0.732 was related to the success of pressure ulcer wound closure with sensitivity of 85.7% and specificity of 60%.

An open wound is bound to be contaminated by surrounding skin bacteria after 48 hours.(18) Bacterial contamination is associated with the persistence of chronic wounds. Wound healing depends on the presence of bacterial contamination, type and magnitude of bacterial burden, patients' immune state, and the wound bed preparation by the medical staff (debridement and wound care). Although wounds may show a healthy granulation, contamination may still be found on the wound. Therefore, bacterial culture should be done before attempting to close the defect.

This study showed that the examination of the type or number of bacteria individually had no significant difference to the success of pressure ulcer wound closure. However, when categorized by both criteria, significant difference was found.

Using double logistic regression analysis, TIMP-1/MMP-9 ratio showed a more dominant role in wound closure success compared to bacterial load. This analysis is in line with the findings in this study, in which successful wound closure is still found even on subjects with high bacterial burden and high virulence of the bacteria.

TIMP-1, MMP-9, and bacterial load are expensive tests to be done, but in cases of chronic wounds where the success of defect closure is unpredictable, these tests are relevant. This is especially true in chronic wound patients who had undergone repetitive surgical procedures but still suffer from refractory wound. Hence compared to the cost of surgery and trauma experienced due to surgery, these additional tests are considered beneficial to the patients.

Conclusion:-

TIMP-1/MMP-9 ratio as well as the type and number of bacteria significantly associated with successful wound closure of pressure ulcers. TIMP-1/MMP-9 ratio > 0.732 is related to the successful in wound closure of pressure ulcers. This ratio has a more dominant effect on the successful in wound closure of pressure ulcers compared with the type and number of bacteria.

Reference:-

1. Thoroddsen A. Pressure sore prevalence: a national survey. *J Clin Nurs*.1999; 8:170-179.
2. Medical Record Data at Division of Plastic Surgery Dr. Hasan Sadikin Hospital in 2007 – 2011.
3. Bauer JD, Mancoll JS, Phillips LG. Pressure sore. Dalam:Thorne CH, editor. *Grabb and Smith's Plastic Surgery*. Vol. 6. Philadelphia : Lippincott Williams & Wilkins;2007.page 722-729.
4. Porter A, Cooter R. Surgical Management of Pressure Ulcers. Primary Intention. 1999: 151-155.
5. Ferreira, Tuma Jr. P, Carvalho VF, Kamamoto F. Complex Wounds. *Clinics*. 2006; 61(6):571-578.
6. Shehab S. Local Fasciocutaneous Gluteal Flap (Dufourmentel) in Reconstruction of Parapelvic Pressure Sores.Egypt J Plast Reconstr Surg. 2003; 27(1):47-52.
7. Thomas D. Prevention and treatment of pressure ulcers : What works? What doesn't. *Clev Clin J Med*. 2001; 68(8):704-722.
8. Disa JJ, Carlton JM, Goldberg NH. Efficacy of Operative Cure in Pressure Sore Patients. *J Plas Reconstr Surg*. 1992; 89(2): 272-278.
9. Toy LW. Matrix metalloproteinases : Their function in tissue repair. *Journal of wound care*. 2005;14(1): 20-22.
10. Widgerow A. Wound fluid intervention: Influencing wound healing from the outside. *Wound Healing Southern Africa*. 2011;4(1): 12-15.
11. Phillips N,Auler S, Hugo R, Gonzalez S. Beneficial Regulation of Matrix metalloproteinases for Skin health. *Enzyme research*. 2010;2011: 1-4.
12. Liu Y, Wahl LM. Production of matrix metalloproteinase-9 by active human monocytesinvolves a phosphatidylinositol-3 kinase/Akt? IKK α /NF-kB pathway. *Journal of Leukocyte Biology*. 2005;78: 259-266.
13. Liu Y, Min D, Bolton D, Nub'e V, SW Twigg, Yue D, et al. Increased Matrix Metalloproteinase-9 Predicts Poor Wound Healing in Diabetic Foot Ulcers. *Diabetes Care*. 2009;32(1): 115-117.
14. Krizek TJ, Robson MC. Evolution of Quantitative Bacteriology in Wound Management. *Am J Surg*. 1975;30(5): 579-584.
15. Scheneider M, Vildozola CW, Brooks S. Quantitative Assesment of Bacterial Invasion of Chronic Ulcers. *Am J Surg*. 1983;15(2): 260-262.
16. Schultz G, Barillo D, Mozingo D, Chin G. Wound bed preparation and a brief history of TIME. *International Wound Journal*. 2004;1(1):19-32.
17. Gibson. D, Cullen. B, Legerstee. R, Harding. KG, Schultz. G. MMPs Made easy. *Wounds International*. 2009;1(1): 1-6.
18. Mustoe TA, O'Shaugney K, Kloeters O. Chronic Wound Pathogenesis and Current Treatment Strategies: A Unifying Hypothesis. *Plast Reconstr Surg*. 2006; 117(7): 35-40.