THE USAGE OF TIMP-1 / MMP-9 CUT-OFF POINT RATIO AS A PREDICTOR OF PRESSURE ULCER CLOSURE SUCCESS RATE

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ABSTRACT

Background: Pressure ulcers can be very painful and usually take a long time to heal. The right multi-modality treatments are needed to minimize this burden. However, pressure ulcers closure often fail. It is required to establish the right moment for wound closure. The degradation rate of the extracellular matrix as a result of matrix metalloproteinase (MMP) and tissue inhibitors of matrix metalloproteinase (TIMP) activity can be a good predictor of the success of surgical closure. Objectives: We propose a solution by setting the TIMP-1 / MMP-9 cut-off point ratio to determine wound closure success in pressure ulcers. Methods: This was a hospital-based nested case-control study at Hasan Sadikin General Hospital, Bandung. Data were collected from April 2012 to February 2014. Wound assessment was done every week until the third week to consider the successful and unsuccessful wound closure group. The relationship between variables was analyzed with bivariate analysis, while the contingency coefficient assessed its strength. The TIMP-1 / MMP-9 ratio cut-off point was determined using the Receiver Operating Characteristic (ROC) curve. Results: Twenty-four patients who underwent wound closure were enrolled as respondents. The relationship of TIMP-1 with the success rate of pressure ulcer closure did not show significant differences (P-value = 0.545). The MMP-9 in the group that succeeded and failed also did not show a significant difference (P-value = 0.367). However, the combination of TIMP-1 / MMP-9 ratio with cut-off point 0.732 was significantly different between the successful and unsuccessful group (P-value = 0.032; C = 0.610). Conclusion: TIMP-1 / MMP-9 ratio > 0.732 is related to the success in wound closure of pressure ulcers. Thus, it is important that surgeons measure the ratio of preoperative pressure ulcer patients due to their proven benefits in predicting the success rate of surgical closure.

KEYWORDS pressure ulcer; TIMP; MMP; wound healing
Introduction

Chronic wounds are substantial health problems which spend a lot of expenses on long-term management. More than 90% of chronic wounds are included in three main categories: venous ulcer, diabetic ulcer and pressure ulcer [1]. A pressure ulcer is a significant health problem with a high prevalence, especially among patients with immobilisation. Elderly patients contribute more than 65% of the incidence of pressure ulcers and increase the risk of death five times in pressure ulcer patients in hospital care [2]. Pressure ulcers are usually accompanied by severe complications, such as cellulitis, non-healing wounds, sepsis, and even death. These can be prevented if handled properly [3].

The prevalence of pressure ulcers in Scandinavian countries varies from 3 to 14.8%, in the United Kingdom from 5.3 to 19.7%, and in the United States showing prevalence rates from 4 to 23% [2]. The proper management of pressure ulcers includes microclimate control, nutritional improvement, repositioning and early mobilization, using support surfaces, treatment of infection and biofilms, wound dressing selection and good wound bed preparation, which is the act of cleaning the wound repeatedly to get a healthy wound before the surgical closure [3,4]. Although those actions are routinely done, failure rates remain high. A study conducted at Mansoura University Hospitals for three years stated that 5 out of 40 patients who underwent pressure ulcers closure with flaps were found wound dehiscence and seven others experienced recurrent wound [5,6]. 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.

One factor that connected with wound healing is the presence of matrix metalloproteinase (MMP). Based on how MMP works is divided into three main groups: collagenase, gelatinase and stromelysin. There are also smaller amounts of other types of MMP such as matrilysin, metalloelastase and membrane type MMP. Collagenase (MMP-1, -8, -13 and -18) main function is to degrade the interstitial structure of collagen, dominated by MMP-1. Gelatinase with the main components are MMP-2, and MMP-9 serves to degrade the collagen basement membrane and collagen denaturation structure. Stromelysin (MMP-3, -10, -11 and -19) degrades proteoglycan and glycoprotein matrix [7]. MMP-9 is the main protease responsible for matrix degradation in chronic wounds. High levels of MMP-9 during the inflammatory phase that cause changes and growth factors that cause wound healing fail [7-9]. One study revealed that serum activity of the pro-MMP-9 increased more in the patients with pressure ulcer than the healthy subjects. Their finding gave unprecedented evidence that the overexpression of MMP-9 protein is associated with pressure ulcer [10].

MMP inhibition is regulated by Tissue Inhibitors of Matrix Metalloproteinase (TIMP) [8]. TIMP makes MMP inactive by binding to the active part containing zinc from the enzyme. There are four types of TIMP: TIMP-1, TIMP-2, TIMP-3 and TIMP-4 [7]. However, among all of them, TIMP-1 has the highest affinity for MMP-9 [11].

In cases of chronic wounds that are refractory or difficult to predict whether or not a surgical closure is feasible, these immunohistochemistry tests are quite relevant. When compared with the operating costs incurred and the trauma experienced by the patient during the operation, these examination procedures benefit them. This study aims to find the TIMP-1 / MMP-9 cut-off point ratio and explore the relationship between TIMP-1 and MMP-9 to the success rate of pressure ulcer surgical closure.

Methods

This was a study at Hasan Sadikin General Hospital, Bandung, Indonesia. Data were collected from April 2012 to February 2014.

Inclusion criteria:

- Patients with pressure ulcer grade III or IV in the sacral area as big as 5-15 cm diameter.
- Age range 14 to 70 years old.
- Pre-operation hemoglobin >10 g/dl and leukocyte < 10,000 cell/mm³.
- Good wound bed preparation (healthy granulation tissue without pus and necrotic).

Exclusion criteria:

- Patients who have diabetes mellitus or other comorbid diseases.
- Patients with bad arterial perfusion including blood viscosity disorder, red blood cell deformities, and other artery diseases.
- Immunocompromised condition (history of radiation or cytotoxic drugs usage).
- Malnutrition (pre-operation albumin value < 2.5 gr/dl).
- History of corticosteroid use more than one week.

Drop out criteria:

- Patients who are unwilling to take surgery after informed consent about possible risks and complications.
- Patients who do not finish the complete follow-up procedure for any possible reasons.

Tissue biopsy was taken from the granulation tissue of the ulcers with a size of 0.5 x 1 x 1 cm3 by aseptic biopsy directly before surgical closure procedure. The samples were sent to the pathology anatomy department which assessed the expression of MMP-9 enzyme and TIMP-1 enzyme using immunohistochemistry method, semi-quantitatively by measuring the intensity and distribution of its density.

Wound closure was performed in a tertiary fashion. The wound was considered ready for closure if it had healthy granulation and no necrotic tissue. Surgical and mechanical debridement techniques were done for wound bed preparation. Surgeries were done by the first author, who has 20 years of average clinical experience, using fasciocutaneous flap without any tension. Antibiotic tulle, natrium chloride, and gauze were used as the dressing with daily changing. Oral broad-spectrum antibiotics were given according to the bacterial sensitivity test result. All patients have the same post-surgical handling. Patients were put in same prone position with 2-hourly repositioning in a clockwise direction on a standard mattress. Skin assessment was checked for additional damage at each turn or reposition. Supine position was avoided. Then the patients were assessed every week until the 3rd week. Patients were not put in supine position until all follow-up assessment has finished. Wound closure was considered successful if it was neither accompanied by the presence of pus nor wound dehiscence.

All data were analyzed using SPSS ver. 20.0 (IBM Corp., Armonk, NY, USA). The relationship between variables was analyzed with bivariate analysis, while the contingency coefficient assessed its strength. The Receiver Operating Characteristic (ROC) curve was used to determine the TIMP-1 / MMP-9 ratio value that can be used as a predictor of pressure ulcer closure.
### Table 1: General characteristics of the study subjects.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Wound closure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Successful</td>
</tr>
<tr>
<td>Gender: Male</td>
<td>9</td>
</tr>
<tr>
<td>Gender: Female</td>
<td>5</td>
</tr>
<tr>
<td>Underlying disease:</td>
<td></td>
</tr>
<tr>
<td>Trauma</td>
<td>8</td>
</tr>
<tr>
<td>Malignancy</td>
<td>3</td>
</tr>
<tr>
<td>Infection</td>
<td>2</td>
</tr>
<tr>
<td>Cerebrovascular disease</td>
<td>1</td>
</tr>
<tr>
<td>Age: Mean age + SD</td>
<td>37.86+14.7</td>
</tr>
<tr>
<td>Total</td>
<td>14</td>
</tr>
</tbody>
</table>

### Table 2: The immunohistochemistry results in the successful and unsuccessful group.

<table>
<thead>
<tr>
<th>Wound closure</th>
<th>P-value</th>
<th>C-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immuno-expression</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TIMP-1:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean + SD</td>
<td>4.21 + 1.42</td>
<td>3.80 + 1.68</td>
</tr>
<tr>
<td>MMP-9:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Ratio:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean + SD</td>
<td>0.86 + 0.26</td>
<td>0.72 + 0.31</td>
</tr>
<tr>
<td>C-value:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;0.732</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>&gt;0.732</td>
<td>4</td>
<td>12</td>
</tr>
</tbody>
</table>

*independent t-test; **Mann-Whitney test; ***chi-square

P-value: level of significance

C-value: contingency coefficient (C max: 0.707)

### Discussion

Most of the wounds that do not heal within three months are called chronic wounds [13]. No response to the normal regulation of the wound healing process is a predictive factor for wounds to be categorized as chronic wounds [14]. Pressure ulcers are defined as soft tissue injury due to persistent pressure on the bony prominence. A pressure that more than 32 mmHg in soft tissue results in ischemia and can become necrosis and ulceration if this continue, even in tissues that rich in vascularization [3,15,16]. Ninety-nine percent located below the umbilicus level, namely the sacral area (36-60%), heel (30%), and followed with ischium and trochanter as big as 6% [17].

From the total of 24 patients included in this study, trauma was found as the dominant cause of immobilization and pressure ulcer seems to happen more common in men. Fasciocutaneous flap was chosen because it provides the advantage of adequate oxygen supply, good wound closure, minimal potential for functional deformity and the donor site can be closed primary. Also, this type of flap does not preclude the use of other types of the flap in the reconstruction of recurrent ulcers, it provides simple techniques, flexible design, and proven to reduced hospitalization time [3,5].

From immunohistochemistry perspective, 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 [18]. TIMP is a physiological response to increased protease. The bacterial load has an indirect role in the increase of TIMP. TIMP increase is more affected by the growth factor stimulus on TIMP-producing cells (fibroblast, endothelial cell, and keratinocyte) proliferation [19].

Through debridement, the wound is expected to return to its acute phase and proceed from the prolonged inflammatory phase. The authors found a similar result in this study: wounds with successful surgical closure exhibited higher TIMP-1 and lower MMP-9 immunoeexpression. This study showed that the
examination of TIMP-1 or MMP-9 immunoexpression separately had no significant difference to the success of pressure ulcer surgical closure (P-value > 0.05). However, the TIMP-1 / MMP-9 cut-off ratio of 0.732 was related to the success of pressure ulcer wound closure with a sensitivity of 85.7% and a specificity of 60%.

Conclusion
In summary, the pressure ulcer surgical closure improvement can be improved using the measurement of immunohistochemistry, especially TIMP-1 and MMP-9. The TIMP-1 / MMP-9 ratio > 0.732 is related to the success in wound closure of pressure ulcers. Thus, it is essential that surgeons perform those tests on preoperative pressure ulcer patients due to their proven benefits in predicting the success rate of surgical closure.

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