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Research Article

Association of Sepsis-Related Mortality with Early Increase of MMP-9, TIMP-1, TIMP-1/MMP-9 Ratio

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Abstract

Background: Sepsis causes high morbidity. Matrix metalloproteinase 9 (MMP-9) and tissue inhibitor of matrix metalloproteinase 1 (TIMP-1) are involved in homeostasis, tissue regulation, physiological processes, angiogenesis, wound healing, and immunity.

Method: We conducted observational and prospective study from all sepsis patients who received sepsis therapy in the Emergency Department and Intensive Care Unit were recruited between April to September 2020 at the Haji Adam Malik General Hospital and Hospital Networks. We measured circulating levels of TIMP-1, MMP-9, TIMP-1/MMP-9 ratio, NLR and SOFA score as estimated mortality in critically ill patients.

Results: We found higher MMP-9, TIMP-1, and TIMP-1/MMP-9 ratio during 24 hours (n = 72). The levels of TIMP-1, MMP-9 differed significantly with the difference of sofa score or mortality rate in septic patients (p=0.001).

Conclusion: Septic patients showed persistently higher TIMP-1/MMP-9 ratio during 24 hours, which was associated with severity, and mortality, thus representing a new biomarker of sepsis outcome

Keywords

Sepsis; MMP-9; TIMP-1; SOFA score

Abbreviation

MMP-9: Matrix metalloproteinase 9; SOFA: Sequential Organ Failure Assessment; TIMP-1: Tissue inhibitor of matrix metalloproteinase-1

1. Introduction

The terminology of sepsis has been known since the Hippocrates. Sepsis borned from the Greek which means putrefaction. In 1989, Sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection [1]. Matrix metalloproteinases (MMPs) are implicated in degradation and remodel- ling of the extracellular matrix (ECM) and also in proteolysis of intracellular protein and are involved in innate immune defense and apoptosis. Previous clinical studies have shown higher circulating levels of MMP-9 and TIMP-1 in septic patients than in controls and higher levels of TIMP-1 at the time of severe sepsis diagnosis in non-surviving than in surviving patients [2].

Matrix metalloproteinase 9 (MMP-9) is involved in homeostasis, tissue regulation, physiological processes, angiogenesis, wound healing, and immunity. The MMP can change its nature depending on the transcription that occurs and the propeptides. Disruption of MMP regulation can lead to cancer and inflammation. MMP will be triggered whenever there is inflammation, angiogenesis, cellular and hormonal immunity, and stimulation of cytokines, chemokines, and growth factors. MMP-9 is released by inflammatory cell processes. In addition, MMP-9 is also tasked with activating cytokines, chemokines, growth factors, and cell adhesion which play a major role in the inflammatory process [3,4]. Inhibition of MMP-9 is regulated by tissue inhibitor of matrix metalloproteinase 1 (TIMP-1) [5]. In septic patients with AKI who examined levels of MMP-9, TIMP-1, and MMP-9 / TIMP-1 ratio, there was a significant increase in TIMP-1 and MMP-9/TIMP-1 ratio in cases of AKI with sepsis compared to non-septic AKI [6].

Matrix metalloproteinase appears as a response from body immunity where when cytokines are released, innate immunity such as polymorphonuclear cells (PMN) will be activated and the effectors (proteases and MMP) will be activated and will cause tissue damage [7]. The regulation of extracellular matrix degradation is regulated by MMP and TIMP. From the results of the study, it can be assumed that MMP-9 and TIMP-1 have the potential to be a biomarker for the prognosis of septic patients [8–10]. MMP and TIMP are markers for cell condition, if there is an imbalance between MMP and TIMP, it can be concluded that there has been damage to cells. MMP-9 is found mostly in polymorphonuclear leukocytes (PMN) which play an important role in the occurrence of sepsis [11].

2. Method

2.1 Participants

The study was conducted for 6 months, and was a randomized, and experimental controlled intervention study aimed to define association of sepsis-related mortality with early increase of MMP-9, TIMP-1, TIMP-1/MMP-9 ratio. All sepsis patients who received sepsis therapy in the Emergency Department and Intensive Care Unit were recruited between April to September 2020 at the Haji Adam Malik General Hospital and Hospital Networks. All patients included in the study were randomized using a computer-generated list of random numbers (randomizer.org). Patients were in the age range of 18-70 years. The patients/family of the patients agreed to participate in the study. Patients included in this study were those diagnosed with sepsis with qSOFA $\geq$ 2 or SOFA score $\geq$ 2 and received Hour One Bundle Sepsis therapy and other therapies that support sepsis therapy according to the procedure, and those with lactate levels $\geq$ 2 mmol/L.

Patients were tested for blood, and levels of MMP-9 and TIMP-1 (Elisa kit, Antibody-Sunlong Biotech Co. Ltd), This study protocol was approved by our Institutional Review Board of the Medical Faculty of the University of North Sumatra (No 54/KEP/USU/2020) and was performed in accordance with the ethical standards laid down in the Declaration of Helsinki. All data were analyzed by SPSS 25.0 package program. Friedman test was used for multivariate comparison of the non-parametric data that were distributed abnormally. The results were considered statistically significant if $p<0.05$.

2.2 Variables recorded

Each patient was followed and recorded for sex, age, comorbidity, history of operative procedure, microorganism responsible, antimicrobial treatment, pressure of arterial oxygen/fraction of inspired oxygen (PaO2/FIO2), international normalized ratio (INR), using furosemide, Sepsis-related Organ Failure Assessment (SOFA) score, MAP, NLR, albumin, and CVP. We assessed MMP-9 and TIMP-1 level from early admission ICU.

3. Results

3.1 Sample

Demographic and clinical parameters from septic patients (n=72) is shown in Table 1. This study showed that end-stage renal disease and cardiovascular disease as the most comorbids. Of the 72 sepsis patients enrolled in this study, 13.9% were found that Gram-negative bacteria cause most infections with cephalosporin as the most antibiotic used. Patients in this study had a median value of PaO_2/FiO_2 ratio 261.6 with moderate ARDS. In addition, the use of furosemide in ICU patients was reported to be 45.8% with a range for 24-hour urine volume is -406.7 to + 694 milliliters per day. Even though in this study the patient used furosemide, the CVP values were in the normal range of 6.3-10.1 cm H_2O. Higher NLR values from these patients indicated an ongoing infection process in patients with a median NLR value of 12.3. The MMP-9 and TIMP-1 values in this study showed very high results with median values 1300.6 ng/ml and 568.6 ng/ml respectively. Beside it, TIMP-1 / MMP-9 ratio was found in septic patients of 0.3-0.7. As shown in Table 2,

Table 1: Baseline clinical and biochemical characteristics of patients.

Characteristics	Description (n=72)
Age (years, mean ± SD)	52.4± 14.2
Gender	
Male, n (%)	36 (50)
Female, n (%)	36 (50)
Comorbidity	
Diabetes Mellitus, n (%)	7 (9.7)
Cardiovascular disease, n (%)	6 (8.3)
End-stage renal disease	9 (12.5)
Cancer	2 (2.7)
History of operative procedure	25 (34.7)
Microorganism responsible	
Unknown or not available, n (%)	62 (86.1)
Gram-negative, n (%)	10 (13.9)
Antimicrobial treatment	
Cephalosporins	43 (59.7)
Quinolones	23 (31,9)
Carbapenems	11 (15.2)
Aminoglycoside	4 (5.5)
Nitroimidazole	5 (6.9)
Tetracyclines	1 (1.3)
INR, Median (percentile 25-75)	1.1 (1.0-1.3)
Furosemide, n (%)	33 (45.8)
Fluid balance, L/day, median (percentile 25-75)	25.7 (-406.7 – 694)
MAP, mmHg (mean ± SD)	93.9 ± 20.7
Lactic Acid, median (percentile 25-75)	1.8 (1.2-4.0)
SOFA score, median (percentile 25-75)	6 (4-8)
NLR, median (percentile 25-75)	12.3 (7.0-24.8)
Albumin, median (percentile 25-75)	2.7 (2.2-3.3)
Central venous pressure (CVP), median (percentile 25-75)	8.2 (6.3-10.1)
MMP-9, median ng/ml (percentile 25–75)	1300.6 (720.7-1633.2)
TIMP-1, median ng/ml (percentile 25–75)	568.6 (473.2-676.4)
TIMP-1/MMP-9 ratio, median (percentile 25–75)	0.4 (0.3-0.7)

Table 2: TIMP-1, MMP-9 enzyme levels, and TIMP-1/MMP-9 ratio in septic patients.

SOFA Score (Mortality rate, %)	TIMP-1	MMP-9	TIMP-1/MMP-9 ratio
	(Mean±SD); n = 72	(Mean±SD); n = 72	(Mean±SD); n = 72
5.4	570.1±165.5	1088.3±603.4	1.1±1.3
20	542.7±142.8	1203.5±488.2	0.5±0.3
36.1	503.3±148.2	1043.4±412.0	0.5±0.3
73.1	582.9±136.1	1082.9±582.2	0.8±0.8
84.4	565.8±133.2	1229.7±529.2	0.6±0.6
p Value	0.001**	0.001**	0.001**

Note: Comparison of levels of TIMP-1, MMP-9 enzyme levels, and TIMP-1/MMP-9 ratio between mortality estimation of SOFA score, Friedman test); *Significant <0,05

the levels of TIMP-1, MMP-9 differed significantly with the difference of sofa score or mortality rate in septic patients, with a significant increasing (p = 0.001) with increasing mortality rate. While TIMP-1 / MMP-9 ratio decreased along with the increasing mortality rate.

4. Discussion

Several studies have shown that TIMP-1 levels increase significantly in septic patients compared to control patients even at the 0 point, which is the time when patients enter for treatment [6,12]. In the course of sepsis, the TIMP-1 level remains relatively high and is inversely proportional to the relatively lower MMP-9 with increasing time so that the TIMP-1 / MMP-9 ratio tends to increase. High TIMP-1 / MMP-9 ratios at day 1, 4, and 8 have been associated with mortality [12].

MMP is secreted as a soluble protein or expressed on the cell surface to regulate its interaction with the extracellular matrix. The extracellular matrix is a dynamic macromolecule such as collagen, fibronectin, laminin, and proteoglycans that represent an environment that influences behavior of cells. The cell will lose its differentiated phenotype and undergo anoikysis (death of apoptotic cells due to loss of cell adhesion). This can be seen from the activity of the TIMP cytokine which is independent of its role in inhibiting MMP. This will result in loss of MMP inhibition by interfering with the interaction of cysteine-1 in the catalytic of metalloproteinases [13].

MMP activity is regulated at different levels, namely proMMP synthesis and secretion, activation of inactivated MMP proenzymes by proteinase, inhibition of MMP enzyme activity by the role of TIMP [14,15]. MMP-9 is mainly produced by macrophages, but MMP-9 can also be found in neutrophil granules. The activity of MMP-9 is inhibited by the endogenous inhibitor TIMP-1 through a 1: 1 formulation and is usually released from the same cells that secrete this protease, so that regulation of the activity of this proteolytic enzyme is tight. An imbalance in protease and antiprotease levels will develop to cause a disease [16,17].

5. Conclusion

In conclusion, the novel findings of our study were that septic patients showed persistently higher TIMP-1/ MMP-9 ratio during 24 hours, which was associated with severity, thus representing a new biomarker of sepsis outcome.

6. Acknowledgement

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7. Conflict of Interest

The authors declare that there is no conflict of interest**.**

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