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Research Article

Combination Therapy of Vitamin C and Thiamine on Matrix Metalloproteinases-9 (Mmp-9) for Septic in ICU

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Abstract

Introduction: Sepsis is a systemic and syndromic response to infection and is a common final pathway to death from many infectious diseases. Matrix metalloproteinase 9 (MMP-9) are potential sepsis biomarkers. Ascorbic acid and thiamine administration has been suggested to affect MMP-9 in several critical conditions, such as sepsis.

Methods: This study investigated effects of combination therapy of vitamin c and thiamine on MMP-9 in sepsis patients. A true experiment with pretest posttest control group and double blind design and randomized. This study conducted at Department of Anesthesiology and Intensive Therapy, including Intensive Care on April-December 2020.

Results: Based on the initial MMP-9 values, the levels of this enzyme increased in the NaCl 0.9% group, while in combination group, MMP-9 levels decreased after the intervention (17.9%). A significant decrease in MMP-9 was found in the ascorbic acid 231,5 □ 345,5 ng/ml (p=0.026).

Conclusion: In conclusion, combination therapy of vitamin c and thiamine as a adjuvant therapy in sepsis can reduced the MMP-9.

Keywords: Ascorbic acid; MMP-9; Sepsis; Adjuvant therapy

Introduction

epsis is a life threatening organ dysfunction caused by an imbalanced host response and infection [1]. This condition is the primary cause of mortality in the Intensive Care Unit (ICU). Although sepsis therapy has continued to develop in recent decades, the mortality rate of sepsis in Indonesia is still very high, around 61% [2]. Therefore, new approaches in managing sepsis need further upgrades and evaluations to reduce the high mortality rate.

The management of sepsis continues to evolve. In 2001, the early goal directed therapy (EGDT) developed by Rivers could reduce the mortality rate from 46.5% to 30.5% [3]. The first evidence based resuscitation approach in sepsis management was combined with the hour 6 bundle surviving sepsis campaign (SSC) 2004 guidelines [4]. After the publication of the 2016 SSC guidelines, this approach was revised and changed to the hour-1 sepsis bundle, combining the hour-3 and hour-6 bundle approaches. These guidelines recommend measuring lactate levels, drawing blood samples before giving antibiotics, administering broad spectrum antibiotics, administering crystalloid solutions of 30 ml/kg BW in hypotension condition immediately, or lactate levels>4 mmol in the first hour [5]. Although this revised sepsis bundle has successfully reduced the mortality rate, a recent study conducted in South Korea failed to exhibit deterioration of in-hospital mortality rate among subjects treated with this hour-1 bundle compared to the hour-3 and hour-6 bundles [6].

Several agents continue to be evaluated to reduce sepsis mortality. Ascorbic acid (vitamin C) and thiamine (vitamin B1) are potential agents that are currently being investigated for their function in the treatment of sepsis. The administration of thiamine in sepsis is based on the finding of this micronutrient depletion in critically ill patients, including sepsis. Thiamine depletion is associated with increased lactate levels which is a poor predictor of outcome in septic patients. On the other hand, multiple studies have shown that parenteral ascorbic acid can reduce the risk of multiple organ failure in sepsis and reduce levels of tissue injury biomarkers [7,8]. Controlled clinical trials showed lower 28-day mortality in surgical critically ill patients with septic shock receiving ascorbic acid than in the control group [9]. However, research on the benefits of these two micronutrients as therapeutic agents for sepsis is still limited,

especially the number of clinical trials and small samples.

Matrix metalloproteinase-9 (MMP-9) are one promising septic biomarkers to assess septic patients' therapeutic response and prognosis. MMP-9 is a protease enzyme of the metzincin class that degrades extracellular matrix (ECM) components [10]. This biomarker also directly describe physiological changes at the cellular and tissue levels.

Several agents continue to be evaluated to reduce sepsis mortality. Ascorbic acid (vitamin C) are potential agents that are currently being investigated for their function in the treatment of sepsis. On the other hand, multiple studies have shown that parenteral ascorbic acid can reduce the risk of multiple organ failure in sepsis and reduce levels of tissue injury biomarkers [7,8]. Controlled clinical trials showed lower 2 day mortality in surgical critically ill patients with septic shock receiving ascorbic acid than in the control group [9]. However, research on the benefits of this micronutrient as therapeutic agents for sepsis is still limited, especially the number of clinical trials and small samples.

Matrix metalloproteinase-9 (MMP-9) is as promising septic biomarkers to assess septic patients' therapeutic response and prognosis. MMP-9 is a protease enzyme of the metzincin class that degrades extracellular matrix (ECM) components [10]. The biomarker also directly describes physiological changes at the cellular and tissue levels. Therefore, research on the effect of combination therapy of vitamin c and thiamine, on MMP-9 in septic patients' needs to be done to reduce the mortality rate of sepsis remains a global health problem.

Methods

This study is a randomized controlled trial with a pretest-posttest control group design. Randomization was performed using randomizer.org. The study was conducted in two hospitals, Adam Malik General Hospital, Medan, and Grand Medistra Hospital Deli Serdang, between April 2020–May 2021. This study's inclusion criteria were adult sepsis patients admitted to ICU, with qSOFA or SOFA score ≥ 2, Lactate level ≥ 2 mmol/L, and received hour-1 sepsis bundle approach. Subjects who had an allergic reaction to thiamine and ascorbic acid were excluded from this study. The sampling method used in this study was non-probability sampling, which is consecutive sampling.

All patients with suspected sepsis were diagnosed with sepsis through the qSOFA criteria or SOFA score. Patients diagnosed with sepsis are subjected to a physical examination and supporting examination and then treated with an hour-1 sepsis bundle and other supporting sepsis therapies according to our standard operating procedures such as source control of infection. The 0.9% NaCl group got 0.9% NaCl 50 cc given by drip 60 minutes every 12 hours for three days. The combination group received both thiamine injection 200 mg and ascorbic acid injection 50 mg/kg BW in 0.9%NaCl 50 cc administered in 60 minutes every 12 hours for three days. Blood collection for checking the MMP-9 (ELISA kit, Antibody-Sunlong Biotech Co., Ltd) was carried out before giving the intervention at 0 hours. Second blood draw for checking the levels of MMP-9 after the intervention was carried out after 72 hours.

The data were tabulated into a master table using SPSS 25.0 software.

Results

In this study, a difference test was carried out using the ANOVA test for normally distributed data, and homogeneous results were obtained for the variables of age and MAP ($p>0.05$). Meanwhile, for the not normally distributed data, we carried out the test using the Kruskal-Wallis test and obtained homogeneous results for the variables sex, SOFA score, lactate, and NLR ($p>0.05$). In this study, the highest lactate levels were found in the 0.9% NaCl group. All the subjects in this study had MAP values>65 mmHg because of vasopressor support (Table 1).

Table 1: Baseline clinical and biochemical characteristics of patients

Characteristics	NaCl 0,9% (n=26)	Combination (n=24)
Age, years (mean ± SD)	48,8 ± 18,4	50,7 ± 11,1
Sex		
Male, n (%)	10 (38,5)	11 (68,8)
Female, n (%)	16 (61,5)	5 (31,3)
MAP, mm Hg (mean ± SD)	94.8 ± 17.0	92,4 ± 16.0
Lactate, mmol/L median (min-maks)	2,0 (1-10,0)	2,0 (1,0-4,0)
SOFA score median (min-max)	6 (2-18)	6 (2-12)
NLR median (min-max)	10,6 (2,5-30,3)	10,7 (1,4-64,6)

Table 2 shows that the levels of the MMP-9 enzyme before treatment in the two groups had homogeneous in value ($p=0.166$). Based on the initial MMP-9 values, the levels of this enzyme increased in the NaCl 0.9% group, while in combination group, MMP-9 levels decreased after the intervention (17.9%). A significant decrease in MMP-9 was found in the ascorbic acid 231,5 ± 345,5 ng/ml ($p=0.026$). However, the comparability test showed MMP-9 levels after treatment, and the difference was not significantly different between the treatment groups ($p>0.05$).

Table 2: Comparison of MMP-9 Levels in the combination and Control Groups

MMP-9 level (ng/ml)	NaCl 0,9% (n=26)	CCombination (n=24)	pa value
Before treatment			
Mean ± SD	983,8 ± 544,0	1289,3 ± 564,4	0,083
Median (min-max)	925,0 (141,1-2139,2)	1559,6 (129,2-1730,3)	
After treatment			

Mean ± SD	1236,6 ± 658,3	1057,8 ± 561,1	0,468
Median (min-max)	1267,5 (185,3-2585,1)	1198,6 (130,3-1741,9)	
Difference			
Mean ± SD	252,8 ± 646,8	-231,5 ± 345,5 (17,9%)	0,043*
Median (min-max)	29,2 (-609,7–1495,2)	-68,3 (-871,3–225,7)	
pb value	0,662	0,026*	

Note: pa (comparison of MMP-9 level between combination and control groups, independent t-test); pb (comparison of MMP-9 level before and after, dependent t-test); *Significance ± <0.05

Discussion

This study was conducted to explain the effect of thiamine and ascorbic acid on MMP-9 levels in septic patients. From the total of 50 subjects, the prevalence of men and women are almost equal. This finding is quite different from several previous studies where most septic patients were men [11]. A multicenter study in Italy showed that the prevalence ratio between men and women diagnosed with sepsis was about 3 to 2 [12]. Other studies have also reported that the incidence of sepsis is lower in women due to differences in reproductive hormones suggested being protective inflammatory factors in women [13]. This study shows the opposite result. This finding may be due to many samples in the study who dropped out because they didn't survive during observation. In addition, research in America shows that the incidence of sepsis is more influential on age, underlying disease, and source of infection [14].

In this study, the authors found a mortality rate of 36%. In addition, this study found a SOFA score in the range of 6-7, which predicts mortality of 10-20%. However, a study in Belgium showed this range of SOFA scores could lead to a higher risk of death if there were no significant reduction during therapy, namely 84.4% [15]. The high mortality rate in our subjects may be associated with a high number of dropouts. In a previous study, mortality in septic patients was found to be around 26.4%. Mortality can reach 30% in sepsis and 80% in septic shock [16]. In this study, the variables of age, MAP, gender, SOFA score, lactate, and NLR were evenly distributed in the four groups. However, this value does not show a significant effect on the outcomes in this study.

Although an increase in the MMP-9 and TIMP-1 levels indicates a severe inflammatory process in septic patients, it is expected that the MMP-9/TIMP-1 balance is the key to determine cell survival [17]. Elevated TIMP-1 levels, low MMP-9 levels, and low MMP-9/TIMP-1 ratios were found in non-surviving septic patients. Thus, the MMP-9/TIMP-1 ratio can be a good predictor of assessing sepsis's severity and mortality [18]. In the scatterplot graph, the MMP-9/TIMP-1 ratio and SOFA scores in sepsis patients who survived during the observation showed the same results—the more significant the decrease in the MMP-9/TIMP-1 ratio, the lower the SOFA score. In addition, this study shows exciting results when viewed from the correlation between MMP-9, TIMP-1, and MMP-9/TIMP-1 ratios in patients who didn't survive during observation.

A comparison of serum MMP-9 levels before intervention in the four groups showed that MMP-9 levels tend to increase only in the control group (0.9%NaCl). In the thiamine, ascorbic acid, and combination groups, MMP-9 levels decrease, although a statistical decrease was only found in the ascorbic acid and combination groups. These results proved that the intervention of thiamine, ascorbic acid, and the combination could suppress MMP-9 levels after 72 hours of intervention. This capacity can be seen in the control group, where MMP-9 increased after 72 hours of observation. The increase in MMP-9 levels is because MMP-9 plays a vital role in the cytokine storm, followed by the activation of the immune system. MMP-9 has been shown to aid in the migration of the immune system to sites of inflammation, has a vasoactive effect, and can induce vascular leakage in severe sepsis [19,20]. According to other studies, MMP-9 levels increased in the first few hours of systemic inflammation and were associated with organ injury severity [21,22].

The effect of ascorbic acid administration on MMP-9 in septic patients has not been studied before. However, the effect combination therapy consisting of hydrocortisone, ascorbic acid, and thiamine (HAT) in 140 septic patients is safe to use and capable of reducing the duration of shock and vasopressor use in septic patients. However, this study failed to prove that HAT administration reduced mortality and length of stay in ICU. Even patients given HAT had a 22.6% higher mortality than the placebo group of 20.4% [23].

In another study with a smaller sample than the previous study, it was reported that patients given HAT had lower mortality of 8.5% compared to 40.4% of controls. In addition, there was also a decrease in SOFA scores in patients given HAT. It was concluded that the initial intravenous use of ascorbic acid and corticosteroids, and thiamine effectively prevented the progression of organ dysfunction [24].

However, both studies used a combination consisting of glucocorticoids. Another study conducted in 2020 (The VITAMINS randomized clinical trial) in 216 patients reported that administration of the HAT combination did not show an increase in the duration of survival and freedom from vasopressor use during seven days of ICU treatment when compared to a group of patients given only hydrocortisone therapy [25].

The role of ascorbic acid in the therapy of sepsis can be summed up in three mechanisms. The first mechanism is the role of ascorbic acid as an antioxidant that acts directly as an electron donor so that it can fight free radicals, pre-

vent the formation of new free radicals by inhibiting the NADPH oxidase (NOX) pathway, and help to recycle other antioxidants. In addition, ascorbic acid also has an immune function. These micronutrients can regulate macrophages, suppress inflammatory mediators, and also have bacteriostatic effects. The last mechanism is the ability of ascorbic acid to increase vasopressor sensitivity to maintain hemodynamics [26].

Conclusion

In conclusion, combination therapy of vitamin c and thiamine as a adjuvant therapy in sepsis can reduce the MMP-9.

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Conflict of Interest

The authors declare that there is no conflict of interest

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