Infection and Drug Resistance

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ORIGINAL RESEARCH

The Effect of Thiamine, Ascorbic Acid, and the Combination of Them on the Levels of Matrix Metalloproteinase-9 (MMP-9) and Tissue Inhibitor of Matrix Metalloproteinase-1 (TIMP-1) in Sepsis Patients

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Background: Sepsis is a global health problem. Therapeutic agents continue to develop for the management of sepsis. Ascorbic acid and thiamine are currently potential agents intensively studied for their benefits in sepsis.

Methods: This study was a randomized controlled trial (RCT) with a pretest-posttest control group design. Randomization was using a computer. The research was conducted at Haji Adam Malik General Hospital Medan and Grand Medistra Hospital. Blood tests were at the Integrated Laboratory of the Faculty of Medicine, University of North Sumatra. The research was for 14 months. The number of patients in this study was 86 septic patients divided into four groups (NaCl, thiamine, ascorbic acid, and combination). The parameters measured were the enzyme MMP-9 and the enzyme TIMP-1.

Results: The number of subjects who met the inclusion criteria was 147 patients. Fifty-three patients passed away during the monitoring period, and eight blood samples were damaged. The final data analyzed consisted of 86 subjects. Of the 86 septic patients obtained Based on initial MMP-9 values, MMP-9 levels increased in the 0.9% NaCl group (25.6%), while MMP-9 levels decreased after intervention in the thiamine, ascorbic acid, and combination groups but a significant decrease in MMP-9 was found in the ascorbic acid group (17.2%; $p = 0.04$) and the combination group (17.9%; $p = 0.026$). For TIMP-1 levels, a decreasing trend was only in the ascorbic acid (5.1%) and combination (5.9%) but not significant ($p > 0.05$). The highest MMP-9/TIMP-1 ratio was in septic patients receiving thiamine with a significant linear correlation ($p < 0.05$) between levels of MMP-9 and the MMP-9/TIMP-1 ratio with a moderate correlation level and significant negative correlation ($p < 0.05$) between TIMP-1 and the MMP-9/TIMP-1 with a moderate level of correlation.

Conclusion: Administration of thiamine alone gives better advantages than ascorbic acid alone and their combination.

Keywords: sepsis, MMP-9, TIMP-1, MMP-9/TIMP-1 ratio, thiamine, ascorbic acid

Introduction

Sepsis is a global health problem in which one-third of patients admitted to the ICU contributed to 19.7% (18.2–21.4%) of all deaths globally in 2017.[1,2] The sepsis incidence in developed countries, such as in the United States, has increased from 82.7 to 240.4 patients per 100,000 population between 1979 and 2000, with an in-hospital mortality rate of around 30%.[3,4] In some centers, sepsis mortality can be greater than acute coronary syndrome or stroke, reaching 30% in sepsis and 80% in septic shock.[5] The past observational study of severe sepsis and septic shock patients at Dr. Cipto

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Mangunkusumo Hospital, Jakarta in 2012–2013, reported that the mortality rate of severe sepsis and septic shock was up to 61%.

Matrix metalloproteinase-9 (MMP-9) and tissue inhibitor of metalloproteinase-1 (TIMP-1) are two 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.[6] TIMP-1 plays a role in ECM remodeling by regulating specifically the activity of MMP-9.[7] These two biomarkers also directly describe physiological changes at the cellular and tissue levels.

The balance of MMP-9 and TIMP-1 can be assessed by their ratio and correlates with the outcome of septic patients. Although both MMP-9 and TIMP-1 levels were found equally elevated in sepsis, a decrease in the MMP-9/TIMP-1 ratio is significantly associated with severity and mortality.[8] Subsequent studies have indicated that an early increase in the TIMP-1/MMP-9 ratio was associated with mortality, especially measured on the first, fourth, and eighth days.[9] A study in Serbia also found the same results and concluded that the balance between MMP-9 and TIMP-1 is an essential biomarker of diagnosis and good prognosis in sepsis.[10,11]

The benefits of ascorbic acid and thiamine in sepsis management have been widely studied. The role of ascorbic acid in sepsis can be summed up into three main mechanisms. The first mechanism is the role of ascorbic acid as an antioxidant, capable of scavenging free radicals, preventing the formation of new free radicals, and assisting the recycling process of other antioxidants. In addition, ascorbic acid also has an immune function because of its macrophage-regulating ability, suppressing inflammatory mediators, and bacteriostatic effects. The last mechanism is the ascorbic acid's ability to increase vasopressor sensitivity to maintain hemodynamics.[12] Meanwhile, the thiamine administration can improve the condition of sepsis by promoting aerobic metabolism to prevent lactate formation, involved in the pentose phosphate pathway (PPP) reduce the production of glutathione to ward off free radicals/reactive oxygen species (ROS), and improve mitochondrial function.[13].

A previous study has reported that treating sepsis subjects with combination therapy consisting of thiamine, ascorbic acid, and hydrocortisone could reduce the mortality rate.[14] However, Fujii et al disputed this result. The therapy of thiamine, ascorbic acid, and hydrocortisone combination was not superior to intravenous hydrocortisone alone.[15] The Ascorbic Acid, Corticosteroids, and Thiamine in Sepsis (ACTS) trial, which measured sequential organ failure assessment (SOFA) score as the primary outcome, concluded that combination therapy should not be recommended as the routine therapy for sepsis.[12] This finding is interesting to be reviewed since many previous studies supported the combination therapy group in treating sepsis.[14] Several studies have shown the effect of ascorbic acid and thiamine on MMP-9 and TIMP-1. Thiamine and ascorbic acid administration have been linked to reducing TIMP-1 and MMP-9 levels in cancer cells.[16] The administration of thiamine and benfotiamine was associated with an increase in TIMP-1 concentration.[17] An experiment in mice also showed increased apoptosis and MMP-9 level in tumor cells deficient in ascorbic acid.[16] MMP-9 and TIMP-1 levels are easy to perform at any time and can depict changes at the tissue and cellular levels. Therefore, research on the effect of thiamine, ascorbic acid, and their combination on MMP-9, TIMP-1, and their ratios in septic patients needs to be done to reduce the mortality rate of sepsis remains a global health problem.

Methods

This study was a randomized controlled trial with a pretest-posttest control group design with randomization using a computer application (www.randomizer.org). Sample collection was at Adam Malik General Hospital Medan and Grand Medistra Hospital Deli Serdang from April 2020 – May 2021. The inclusion criteria of this study were adult sepsis patients admitted to ICU with SOFA score $\geq$ 2, Lactate level $\geq$ 2 mmol/L, and received management hour-1 sepsis bundle. The research excluded the allergic reactions to thiamine and ascorbic acid. The sampling of this study was non-probability sampling consecutive sampling.

Clinical Protocol and Participant

This research was conducted after obtaining informed consent and approval from the University of North Sumatra Medical Faculty Research Ethics Committee and permission from the Education and Training Division of Adam Malik Hospital, Medan. All patients were diagnosed with sepsis using qSOFA criteria or SOFA score according to the

Surviving Sepsis Guidelines. Patients, including the sample, were performed a physical examination and treated with an hour-1 sepsis bundle, then managed other supporting sepsis therapies according to our standard operating procedures such as source control of infection. Samples included in the inclusion criteria were randomized using a computer and divided into four groups: the normal saline group, thiamine, ascorbic acid, and both (a combination of thiamine and ascorbate). The normal saline group got 0.9% NaCl 50 cc (drip 60 minutes every 12 hours) for three days. The thiamine group received a 200 mg thiamine injection administered (drip 60 minutes every 12 hours) for three days. The ascorbic acid group received an ascorbic acid injection of 50 mg/kg BW administered (60 minutes every 12 hours) for three days. The combination group received a thiamine injection of 200 mg and an ascorbic acid injection of 50 mg/kg BW administered (60 minutes every 12 hours) for three days. Drugs were prepared and administered by the research assistant. The MMP-9 and TIMP-1 examination (ELISA kit, Antibody-Sunlong Biotech Co., Ltd) was carried out before intervention. Second blood draw for the levels of MMP-9 and TIMP-1 check after the intervention about 72 hours.

Statistical Analysis

The analyzing data were using SPSS 25.0 software. The normally distributed numerical data were presented in mean±SD (standard deviation), while the data that were not normally distributed were presented in median (minimum-maximum). Categorical data were displayed in numbers (percentages). The normality test was performed using Kolmogorov–Smirnov test. Statistical test for normally distributed data in 4 groups was analyzed using ANOVA, and not normally distributed data were analyzed using Kruskal Wallis. The relation between two variables of continuous data was analyzed using the Pearson correlation test. The effect of the one variable value in predicting the value of the other variable was analyzed using a linear regression model.

Results

The number of subjects who met the inclusion criteria was 147 patients. Fifty-three patients passed away during the monitoring period, and eight blood samples were damaged. The final data analyzed consisted of 86 subjects (Figure 1). A normality test was to assess whether the variables of the sample tested in this study were normally distributed or not. The normality test in each group in this study used the Kolmogorov–Smirnov test. The data were declared normally distributed if the p-value was >0.05 and not normally distributed if the p-value was <0.05. In this study, age and MAP were normally distributed, so it used the ANOVA test for the statistical test. Meanwhile, sex, lactate, SOFA score, NLR, MMP-9, and TIMP-1 were not normally distributed, so we used the Kruskal Wallis test (Table 1).

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 ($p = 0.574$) and MAP ($p = 0.824$). 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 ($p = 0.422$), SOFA score ($p = 0.577$), lactate ($p = 0.289$), and NLR ($p = 0.732$). In this study, the highest lactate levels were found in the 0.9% NaCl group and combination group. All the subjects in this study had MAP values >65 mmHg because of vasopressor support (Table 2). From 53 subjects who died during this study, the characteristics were presented in the Kaplan Meier survival curve. The curve shows that the survival of patients receiving thiamine and combination was higher in the first 10 hours than in the other two groups. However, after 24 hours of observation, the thiamine group had higher survival than the ascorbic acid, combination, and 0.9% NaCl group, with more than 60% of patients still alive. After 48 hours of observation, the combination group showed better survival, with more than 40% of patients alive. At 60 hours, all patients from the 0.9% NaCl, ascorbic acid, and combination groups died, while 10% from the thiamine group survived. Before 72 hours, a total of 53 patients from all groups died (Figure 2).

Figure 3 shows a moderately positive correlation between MMP-9 and TIMP-1 enzyme levels in septic patients who died during observation. The increase in MMP-9 enzyme levels was significantly correlated with the TIMP-1 enzyme levels increase ($r = 0.564$; $p = 0.001$).

Figure 4 shows a linear regression analysis that showed a strong positive correlation between MMP-9 enzyme levels and the MMP-9/TIMP-1 ratio in sepsis patients who died during the observation time ($R2 = 0.628$; $p < 0.001$; $r = 0.792$), meaning an increase in MMP-9 can affect the increase in MMP-9/TIMP-1 by 62.8%. TIMP-1 levels also have a positive correlation, but do not have a significant effect on the MMP-9/TIMP-1 ratio ($R2 = 0.001$; $p = 0.852$; $r = 0.031$). This

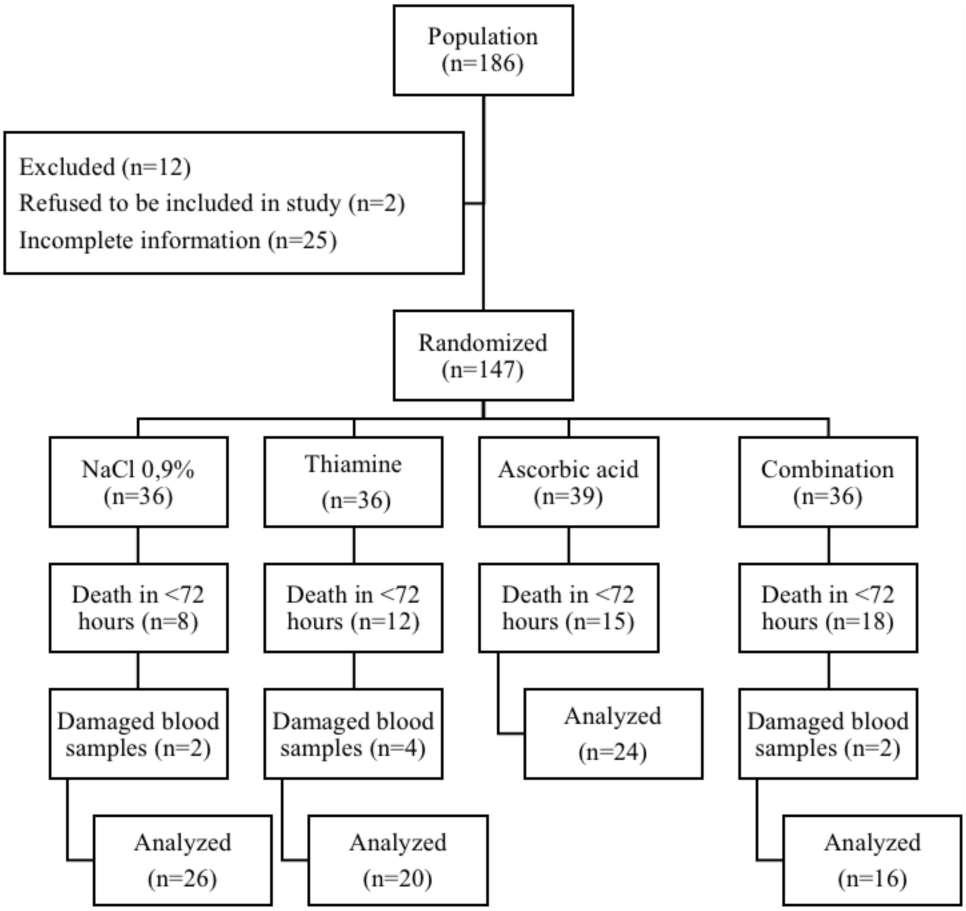

Figure 1 Flow of Patients Through the Ascorbic Acid, Thiamine and Combination of Them in Septic Patients.

finding shows that in non-survivor sepsis patients, there was an MMP-9/TIMP-1 ratio imbalance, indicated by the large effect of the MMP-9 enzyme on the MMP-9/TIMP-1 ratio offset by the influence of the TIMP-1 enzyme.

Linear regression analysis in survived sepsis patients showed an association between the MMP-9 enzyme and the MMP-9/TIMP-1 ratio, which had a moderate effect and a positive correlation (R2 = 0.235; p = 0.012; r = 0.485). This finding was offset by the moderate impact of the TIMP-1 enzyme on the MMP-9/TIMP-1 ratio with a negative

Table 1 Normality Test in 147 Subjects

Variables	*p*-value
Age	0.200*
Sex	0.001
Mean arterial pressure (MAP)	0.200*
Lactate	0.001
SOFA score	0.010
Neutrophil and lymphocyte ratio (NLR)	0.004
MMP-9 pre treatment	0.001
MMP-9 post treatment	0.031
TIMP-1 pre treatment	0.001
TIMP-1 post treatment	0.001

Note: *Kruskall Wallis test, normal distribution if p>0.05.
Abbreviations: MAP, Mean Arterial Pressure; SOFA, Sequential Organ Failure Assessment; MMP-9, Matrix metalloproteinase-9; TIMP-1, Tissue Inhibitor Of Matrix Metalloproteinase-1.

Table 2 Baseline Characteristics of the Study Population

Characteristics	NaCl 0.9%	Thiamine	Ascorbic Acid	Combination
Age, years (mean ± SD)	48.8 ± 18.4	52.3 ± 16.8	53.3 ± 11.7	50.7 ± 11.1
Sex				
Male, n (%)	10 (38.5)	10 (50.0)	12 (50.0)	11 (68.8)
Female, n (%)	16 (61.5)	10 (50.0)	12 (50.0)	5 (31.3)
MAP, mmHg (mean ± SD)	94.8 ± 17.0	97.6 ± 25.4	91.4 ± 16.0	92.4 ± 16.0
Lactate, mmol/L median (min-maks)	2.0 (1–10.0)	1.3 (1.0–5.5)	1.0 (1–10.0)	2.0 (1.0–4.0)
SOFA, median (min-max)	6 (2–18)	7 (3–14)	7 (2–18)	6 (2–12)
NLR, median (min-max)	10.6 (2.5–30.3)	14.4 (0.2–86.8)	13.9 (1.6–69.3)	10.7 (1.4–64.6)

Abbreviations: MAP, Mean Arterial Pressure; SOFA, Sequential Organ Failure Assessment.

correlation (R2 = 0.167; P = 0.038; r = −0.408) (Figure 5). This finding shows a balance in the MMP-9/TIMP-1 ratio in survived sepsis patients, indicated by the significant effect of the MMP-9 enzyme on the MMP-9/TIMP-1 ratio.

Table 3 shows that the levels of the MMP-9 enzyme before treatment in the four groups had significant or homogeneous differences in value (p = 0.166). Based on the initial MMP-9 values, the levels of this enzyme increased in the 0.9% NaCl group, while in the thiamine, ascorbic acid, and combination groups, MMP-9 levels decreased after the intervention. A significant decrease in MMP-9 was found in the ascorbic acid and combination groups (p = 0.04 and 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 4 shows the levels of TIMP-1 enzyme in each group. After administration of 0.9% NaCl and thiamine, there was no significant decrease and tended to increase, but a significant increase occurred in the 0.9% NaCl group. In contrast to the ascorbic acid group and the combination, there was a sharp but insignificant decrease in TIMP-1 (p > 0.05). Statistically, the change in TIMP-1 in the four groups was not significant (p = 0.165). A decreasing level of TIMP-1 was only found in the ascorbic acid and combination groups but was not statistically significant (p > 0.05).

Table 5 shows that thiamine administration could maintain the MMP-9 and TIMP-1 balance in sepsis patients, which means that the significant positive effect on the MMP-9/TIMP-1 ratio of the MMP-9 enzyme can be offset by the significant negative impact of the TIMP-1 level. Meanwhile, in the NaCl 0.9% and ascorbic acid groups, there was

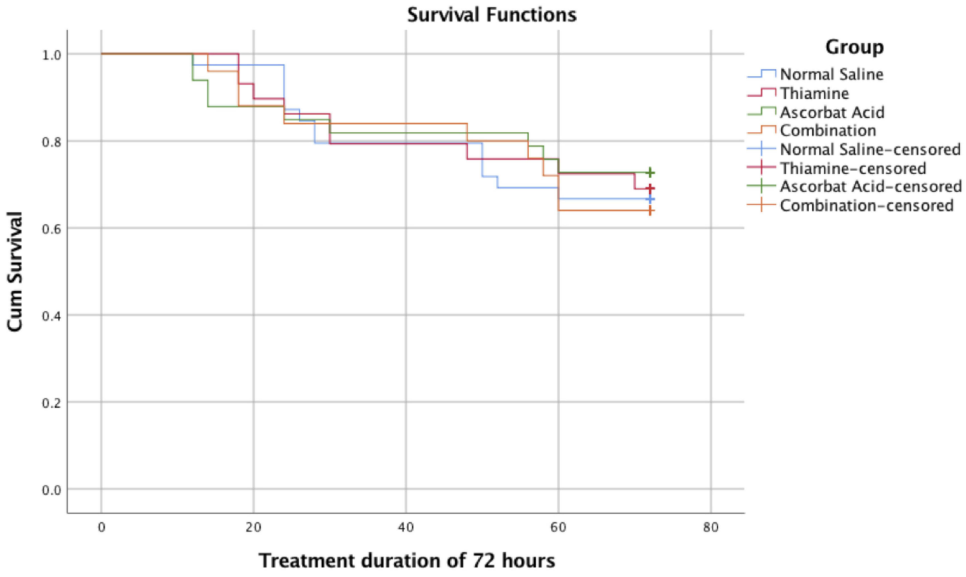

Figure 2 Kaplan Meier survival curve in non-survival subjects within 72 hours of observation.

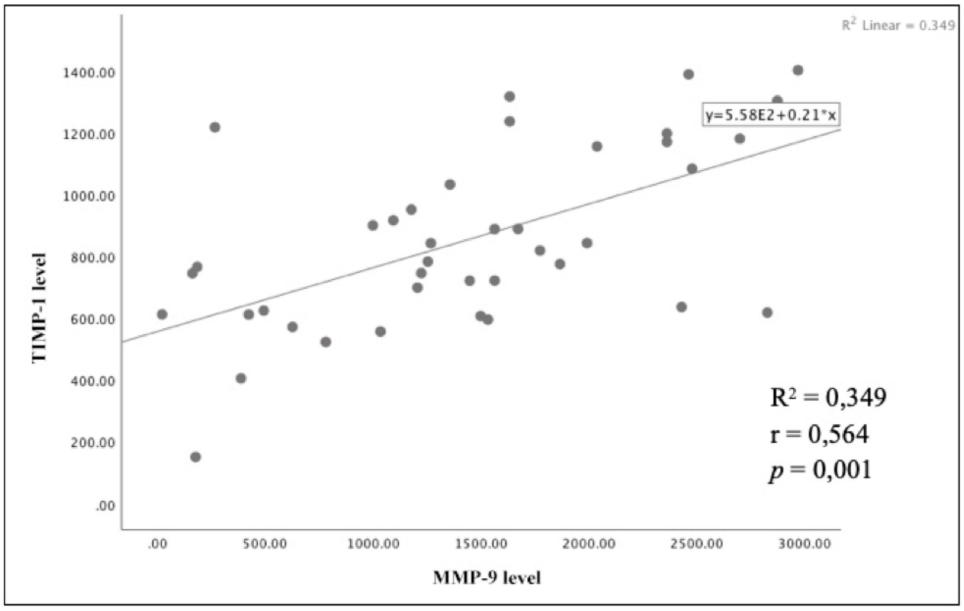

Figure 3 Scatterplot graph of MMP-9 and TIMP-1 levels in sepsis patients who did not survive during observation.

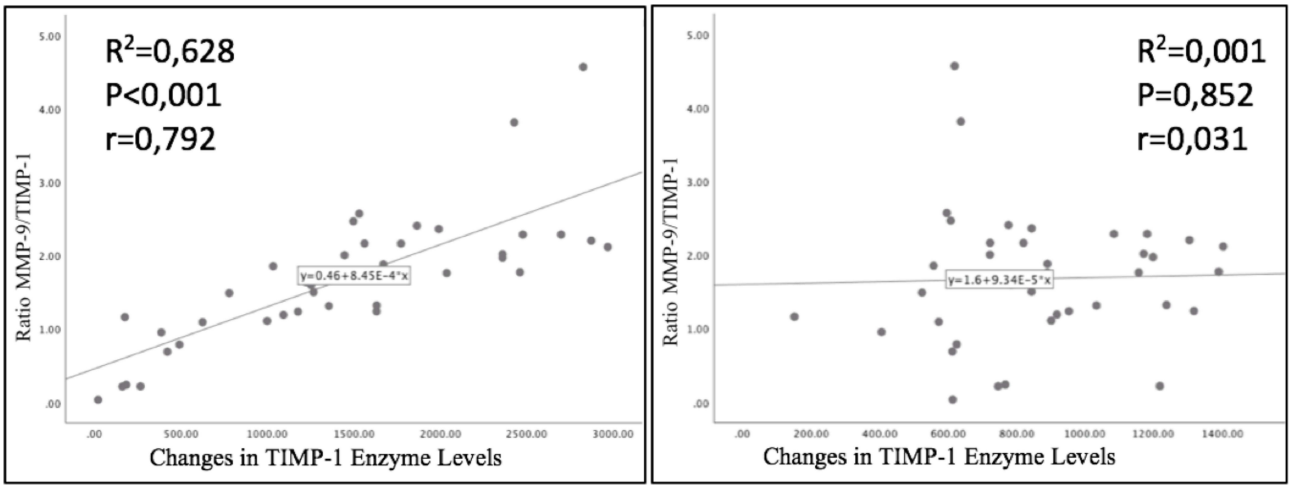

Figure 4 Scatterplot graph of MMP-9, TIMP-1 levels, and MMP-9/TIMP-1 ratio in sepsis patients who did not survive during observation.

a significant positive effect on the MMP-9/TIMP-1 ratio of the MMP-9 level, but the TIMP-1 level effect did not follow it. In the combination group, changes in MMP-9 and TIMP-1 enzyme levels did not affect the MMP-9/TIMP-1 balance.

Discussion

This study was conducted to explain the effect of thiamine and ascorbic acid on MMP-9 and TIMP-1 levels in septic patients. This study found that 53 patients (36%) died during the study before the observation finished or in less than 72 hours. On the Kaplan Meier survival curve, patients who died during 72 hours of observation showed that the thiamine group had a higher chance of survival than the 0.9% NaCl, ascorbic acid, and combination groups, and the thiamine group had a better and constant chance of survival after 30 hours of observation. There was a moderate and significant positive correlation between the MMP-9 and TIMP-1 levels of the 53 septic patients who died before completed observation or less than 72 hours. This result proves that an increase in MMP-9 levels followed by a more significant increase in TIMP-1 levels indicates a vital role for these two enzymes in the pathophysiology of sepsis.

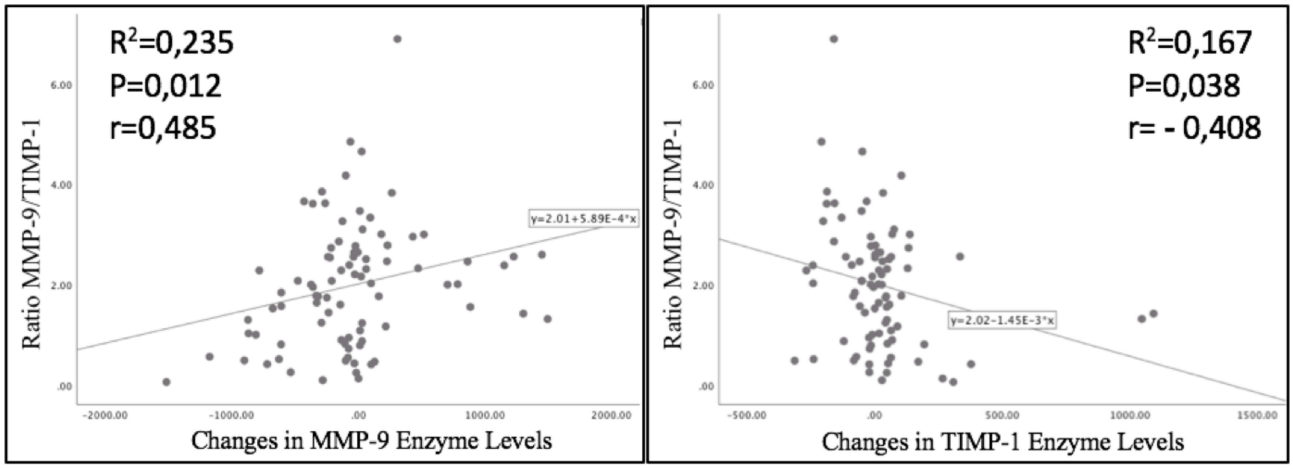

Figure 5 Scatterplot graph of changes in MMP-9, TIMP-1 level, and MMP-9/TIMP-1 ratio in sepsis patients who survived during observation.

MMP-9 and TIMP-1 are enzymes that are important in maintaining the balance of integrity and composition of the ECM in various tissues that play an important role in controlling signals generated from matrix molecules that regulate cell proliferation, differentiation, and cell death.[18] MMP-9 also helps lymphocytes and leukocytes to enter the blood and lymph circulation.[19] Another study showed that MMP-9 was elevated in septic shock patients and decreased after treatment with polymyxin B immobilized on fibers (PMX-F). Research on inhibiting the MMP-9 levels in septic rats using chemically modified tetracycline-3 (CMT-3) has also been carried out and found an increase in TIMP-1 levels, which lowers TGF and decreases caspase-3 signaling pathways, plays a role in cell life.[20] Many things can affect the MMP-9 and TIMP-1 levels increase. MMP-9 was correlated significantly positively with the number of circulating leukocytes, especially neutrophils.[21]

Table 3 Comparison of MMP-9 Level in 3 Groups

MMP-9 Level (ng/mL)	NaCl 0.9% (n=26)	Thiamine (n=20)	Ascorbic Acid (n=24)	Combination (n=16)	p-value
Before treatment					
Mean ± SD	983.8 ± 544.0	1312.8 ± 419.5	1133.1 ± 551.1	1289.3 ± 564.4	0.166
After treatment					
Mean ± SD	1236.6 ± 658.3	1168.9 ± 455.5	938.0 ± 587.7	1057.8 ± 561.1	0.455
Difference					
Mean ± SD	252.8 ± 646.8	−143.8 ± 436.3	−195.0 ± 458.4	−231.5 ± 345.5	0.113
p-value	0.662	0.135	0.04	0.026	

Abbreviations: MMP-9, Matrix metalloproteinase-9. Statistical significance if p<0.05, CI 95%.

Table 4 Comparison of TIMP-1 Level in 3 Groups

TIMP-1 Level (ng/mL)	NaCl 0.9% (n=26)	Thiamine (n=20)	Ascorbic Acid (n=24)	Combination (n=16)	*p*-value
Before treatment					
Mean ± SD	644.5 ± 371.1	581.8 ± 131.8	569.6 ± 161.2	587.1 ± 146.4	0.939
After treatment					
Mean ± SD	783.2 ± 330.7	584.8 ± 185.0	540.0 ± 167.7	552.3 ± 194.5	0.016
Difference					
Mean ± SD	138.7±320.5	2.9 ± 121.3	−29.6 ± 121.5	−34.7 ± 139.1	0.076
p-value	0.045	0.709	0.304	0.379	

Abbreviations: TIMP-1, Tissue Inhibitor Of Matrix Metalloproteinase-1. Statistical significance if p<0.05, CI 95%.

Table 5 Linear Regression Analysis Between the Balance of MMP-9/TIMP-1 with Levels of MMP-9 and TIMP-1 in Sepsis Patients

Variables	NaCl 0.9% (n=26)	Thiamine (n=20)	Ascorbic Acid (n=24)	Combination (n=16)
MMP-9				
Beta	0.415*	0.445*	0.457*	0.070
R^2	0.172	0.198	0.209	0.005
p-value	0.035	0.05	0.025	0.798
TIMP-1				
Beta	−0.120	−0.520*	−0.383	−0.173
R^2	0.014	0.270	0.147	0.030
p-value	0.558	0.019	0.064	0.522

Notes: *Pearson correlation test, independent-*t* test, Statistical significance if p<0.05, CI 95%.
Abbreviations: MMP-9, Matrix metalloproteinase-9; TIMP-1, Tissue Inhibitor Of Matrix Metalloproteinase-1.

In the scatterplot graph of MMP-9, TIMP-1 levels, and MMP-9/TIMP-1 ratio in septic patients who did not survive, MMP-9 levels were correlated positively with the MMP-9/TIMP-1 ratio with an effect of 62.8%, but TIMP-1 levels as an MMP-9 inhibitor could not affect the MMP-9/TIMP-1 ratio. Meanwhile, in the scatterplot graph, the levels of MMP-9, TIMP-1, and the MMP-9/TIMP-1 ratio in survived sepsis patients showed the opposite result. In survived patients, MMP-9 levels were correlated positively with the MMP-9/TIMP-1 ratio. Meanwhile, TIMP-1 can affect the MMP-9/TIMP-1 ratio inversely. This finding indicates that changes in MMP-9 level followed by changes in TIMP-1 level in maintaining the MMP-9/TIMP-1 balance are conditions that describe the ability of cells to survive.

Although studies of the therapeutic effect of thiamine, ascorbic acid, and their combination as adjuvant therapy in sepsis have not improved outcomes in septic patients, this study tried to examine the treatment effect by assessing the MMP-9 biomarker, which is a principal effector in acute inflammatory disease. MMP-9 stored in the tertiary granule of polymorphonuclear leukocytes can be released by inflammatory factors such as IL-1b, IL-8, and TNF.[22] This study proved that the administration of thiamine, ascorbic acid, and combination reduced MMP-9 levels as a biomarker of inflammation.

This study showed that the administration of 0.9% NaCl and thiamine did not decrease TIMP-1 levels but tended to increase them. A significant increase occurred in the 0.9% NaCl group. In contrast to the ascorbic acid group and the combination, there was an insignificant reduction in TIMP-1 levels. Compared with another similar study conducted in 2014, levels of TIMP-1 were higher in patients who did not survive than in those who survived. Elevated levels of TIMP-1 are associated with capillary thrombosis, multiple organ dysfunction worsening, and mortality in septic patients.[9] Another study found that TIMP-1 increased after 10–20 hours of sepsis, and TIMP-1 levels were higher in the healthy group. This study also recommends that elevated TIMP-1 is an excellent biomarker to predict poor clinical outcomes in severe sepsis patients.[23] When viewed from the results of our study, the control group showed worse condition because there was a very high and significant increase in TIMP-1 levels, which was 21.5% from the baseline TIMP-1 value. According to Serrano-Gomez et al (2017), TIMP-1 showed higher sensitivity, specificity, and negative predictive value, to predict the severity of sepsis and concluded that none of the biomarkers evaluated had a significant predictive value for mortality.[11] Meanwhile, there was only a slight increase in TIMP-1 levels in the thiamine group by 0.49%. The ascorbic acid and combination groups showed a decrease in TIMP-1 levels. The results of this study are consistent with the previous studies conducted by Lorente[8,9] that an increase in TIMP-1 was found in patients who did not survive occurred in patients receiving NaCl 0.9% therapy.

However, this study is interesting viewed from the correlation of MMP-9 and TIMP-1 levels in septic patients. The control and combination groups showed a positive correlation between changes in MMP-9 levels and TIMP-1 levels. Although the correlation is weak and insignificant, it shows the same correlation pattern. The higher the MMP-9 levels, the more substantial the increase of TIMP-1 levels. The balance of the MMP-9/TIMP-1 ratio in the control group and ascorbic acid group was only affected by MMP-9 levels when looking at the result of the linear regression test. While TIMP-1 levels could not affect the MMP-9/TIMP-1 significantly. As for the combination group, changes in MMP-9 and

TIMP-1 levels did not affect the MMP-9/TIMP-1 balance. This study proved that administration of thiamine cause changes in MMP-9 and TIMP-1 levels. These changes are helpful to maintain MMP-9/TIMP-1 balance in septic patients.

Although thiamine administration could not significantly reduce MMP-9 levels, it caused a minimal increase in TIMP-1 levels. Thiamine is the only group that maintained MMP-9/TIMP-1 balance in septic patients, which was not found in the other intervention groups. This balance becomes significant, especially in sepsis, where liver injury, vascular damage, multiple organ failure, immune system damage in controlling infection, and blood coagulation disorders occur.[24–28] Another study concluded a significant correlation between increased mortality and decreased MMP-9/TIMP-1 ratio on the first, fourth, and eighth days in septic patients. MMP-9 is a proteinase that plays a role in ECM degradation with specific activity regulating transcription and zymogen precursor activity, and high levels of MMP-9 in circulation can describe cytokine storm conditions. High MMP-9 activity contributes to cell death and accelerates apoptosis.[9,29,30] The role of TIMP-1 as a specific inhibitor of MMP-9 plays a role in controlling local activity in tissues, so it is expected to reach the balance in ECM.[31]

Finally, this study has a few advantages since it is a randomized controlled trial (RCT). The existence of randomization, a control group as a comparison, and assessment before and after treatment make this study more valid by minimizing the possibility of bias.

This study is also the first to evaluate sepsis therapy with thiamine, ascorbic acid, and a combination of the two by measuring the biomarkers MMP-9 and TIMP-1 levels. Previous studies assessed outcomes in terms of mortality, and none have utilized these enzyme biomarkers.[9,12] This study explains a biomolecular approach to the controversial role of thiamine, ascorbic acid, and their combination. That is still under debate by Marik et al (2017), ORANGES Trial by Iglesias et al (2020), VITAMINS Randomized Clinical Trial by Fujii et al (2020), and other investigators on the choice of thiamine, ascorbic acid and combination therapy for the management of sepsis. This study shows that the administration of thiamine alone provides better benefits than ascorbic acid alone and in combination.[14,15,32]

Conclusion

Administration of thiamine alone gives better advantages than ascorbic acid alone and the two combined.

Abbreviations

ASA, American Society of Anesthesiologists; BMI, body mass index; CI, confidence interval; HR, heart rate; MAP, mean arterial pressure; SD; standard deviation; SpO2, peripheral capillary oxygen saturation; MMP-9, Matrix metalloproteinase-9 (MMP-9); TIMP-1, Tissue Inhibitor Of Matrix Metalloproteinase-1.

Data Sharing Statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Ethics Approval and Informed Consent

Ethical approval (Ethical Committee No.54/KEP/USU/2020) was provided by the ethics committee of the Faculty of Medicine, Universitas Sumatera Utara, in 2020. All patients provided informed consent, and all procedures were conducted according to the Declaration of Helsinki.

Consent for Publication

The Authors agree to publication in the Journal of Infection and Drug Resistance.

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Author Contributions

All authors significantly contribute to the work reported, whether in the conception, study design, execution, acquisition of data, analysis, and interpretation, or in all these areas. Contribute to drafting, revising, or critically reviewing the article. Approved the final version to be published, agreed on the journal to be submitted, and agreed to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Marik PE, Taeb AM. SIRS, qSOFA and new sepsis definition. *J Thorac Dis*. 2017;9(4):943–945. doi:10.21037/jtd.2017.03.125
2. Rudd KE, Johnson SC, Agesa KM, et al. Global, regional, and national sepsis incidence and mortality, 1990–2017: analysis for the Global Burden of Disease Study. *Lancet*. 2020;395(10219):200–211. doi:10.1016/S0140-6736(19)32989-7
3. Neligan P. *Anaesthesia and Uncommon Disease*. 5th Infectious Disease and Bioterrorism ed. United States of America: Philadhelpia: Saunder Elsevier; 2006:377–411. doi:10.1016/B978-141602212-1.50015-9
4. Rhee C, Klompas M. Sepsis trends: increasing incidence and decreasing mortality, or changing denominator? *J Thorac Dis*. 2020;12(Suppl 1):S89. doi:10.21037/jtd.2019.12.51
5. Knoop S, Skrede S, Langeland N, Flaatten H, Brakenridge S. Epidemiology and impact on all-cause mortality of sepsis in Norwegian hospitals: a national retrospective study. *PLoS One*. 2017;12(11):e0187990. doi:10.1371/journal.pone.0187990
6. Vandooren J, Swinnen W, Ugarte-Berzal E, et al. Endotoxemia shifts neutrophils with TIMP-free gelatinase B/MMP-9 from bone marrow to the periphery and induces systematic upregulation of TIMP-1. *Haematologica*. 2017;102(10):1671–1682. doi:10.3324/haematol.2017.168799
7. Galliera E, Tacchini L, Corsi Romanelli MM. Matrix metalloproteinases as biomarkers of disease: updates and new insights. Clinical Chemistry and Laboratory Medicine. *Walter de Gruyter GmbH*. 2015;53:349–355. doi:10.1515/cclm-2014-0520
8. Lorente L, Martín MM, Labarta L, et al. Matrix metalloproteinase-9, −10, and tissue inhibitor of matrix metalloproteinases-1 blood levels as biomarkers of severity and mortality in sepsis. *Crit Care*. 2009;13(5):1–9. doi:10.1186/cc8115
9. Lorente L, Martín MM, Solé-VioĺnJ, et al. Association of sepsis-related mortality with early increase of TIMP-1/MMP-9 ratio. *PLoS One*. 2014;9(4):e94318. doi:10.1371/journal.pone.0094318
10. Bojic S, Kotur-Stevuljevic J, Kalezic N, et al. Diagnostic value of matrix metalloproteinase-9 and tissue inhibitor of matrix metalloproteinase-1 in sepsis-associated acute kidney injury. *Tohoku J Exp Med*. 2015;237(2):103–109. doi:10.1620/tjem.237.103
11. Serrano-Gomez S, Burgos-Angulo G, Niño-Vargas DC, et al. Predictive Value of Matrix Metalloproteinases and Their Inhibitors for Mortality in Septic Patients: a Cohort Study. *J Intensive Care Med*. 2020;35:95–103. doi:10.1177/0885066617732284
12. Moskowitz A, Andersen LW, Huang DT, et al. Ascorbic acid, corticosteroids, and thiamine in sepsis: a review of the biologic rationale and the present state of clinical evaluation. *Critical Care BioMed Central Ltd*. 2018;22:548. doi:10.1186/s13054-018-2217-4
13. Donnino MW, Carney E, Cocchi MN, et al. Thiamine deficiency in critically ill patients with sepsis. *J Crit Care*. 2010;25(4):576–581. doi:10.1016/j.jcrc.2010.03.003
14. Marik PE, Khangoora V, Rivera R, Hooper MH. Hydrocortisone, Vitamin C, and Thiamine for the Treatment of Severe Sepsis and Septic Shock: a Retrospective Before-After Study. *Chest*. 2017;151(6):1229–1238. doi:10.1016/j.chest.2016.11.036
15. Fujii T, Luethi N, Young PJ, et al. Effect of Vitamin C, Hydrocortisone, and Thiamine vs Hydrocortisone Alone on Time Alive and Free of Vasopressor Support among Patients with Septic Shock: the VITAMINS Randomized Clinical Trial. *JAMA*. 2020;323(5):423–431. doi:10.1001/jama.2019.22176
16. Cha J, Roomi MW, Ivanov V, Kalinovsky T, Niedzwiecki A, Rath M. Ascorbate supplementation inhibits growth and metastasis of B16FO melanoma and 4T1 breast cancer cells in vitamin C-deficient mice. *Int J Oncol*. 2013;42(1):55–64. doi:10.3892/ijo.2012.1712
17. Tarallo S, Beltramo E, Berrone E, Dentelli P, Porta M. Effects of high glucose and thiamine on the balance between matrix metalloproteinases and their tissue inhibitors in vascular cells. *Acta Diabetol*. 2010;47(2):105–111. doi:10.1007/s00592-009-0124-5
18. Nagaset H, Woessner JF. Matrix metalloproteinases. *J Biol Chem*. 1999;274:21491–21494. doi:10.1074/jbc.274.31.21491
19. Opdenakker G, Van Den Steen PE, Van Damme J, Gelatinase B. A tuner and amplifier of immune functions. *Trends Immunol*. 2001;22:571–579. doi:10.1016/S1471-4906(01)02023-3
20. Maitra SR, Shapiro MJ, Bhaduri S, El-Maghrabi MR. Effect of chemically modified tetracycline on transforming growth factor-β1 and caspase-3 activation in liver of septic rats. *Crit Care Med*. 2005;33(7):1577–1581. doi:10.1097/01.CCM.0000169880.82060.F7
21. Takeshita S, Tokutomi T, Kawase H, et al. Elevated serum levels of matrix metalloproteinase-9 (MMP-9) in Kawasaki disease. *Clin Exp Immunol*. 2001;125(2):340. doi:10.1046/j.1365-2249.2001.01608.x
22. Maitra SR, Jacob A, Zhou M, Wang P. Modulation of matrix metalloproteinase-9 and tissue inhibitor of matrix metalloproteinase-1 in sepsis. *Int J Clin Exp Med*. 2010;3(3):180–185.
23. Hoffmann U, Bertsch T, Dvortsak E, et al. Matrix-metalloproteinases and their inhibitors are elevated in severe sepsis: prognostic value of TIMP-1 in severe sepsis. *Scand J Infect Dis*. 2006;38(10):867–872. doi:10.1080/00365540600702058
24. Lalu MM, Cena J, Chowdhury R, Lam A, Schulz R. Matrix metalloproteinases contribute to endotoxin and interleukin-1β induced vascular dysfunction. *Br J Pharmacol*. 2006;149(1):31–42. doi:10.1038/sj.bjp.0706823
25. Maitra SR, Bhaduri S, Valane PD, Tervahartiala T, Sorsa T, Ramamurthy N. Inhibition of matrix metalloproteinases by chemically modified tetracyclines in sepsis. *Shock*. 2003;20(3):280–285. doi:10.1097/00024382-200309000-00014
26. Sivula M, Hästbacka J, Kuitunen A, et al. Systemic matrix metalloproteinase-8 and tissue inhibitor of metalloproteinases-1 levels in severe sepsis-associated coagulopathy. *Acta Anaesthesiol Scand*. 2015;59(2):176–184. doi:10.1111/aas.12423

27. Wohlschlaeger J, Stubbe HD, Schmitz KJ, et al. Roles of MMP-2/-9 in cardiac dysfunction during early multiple organ failure in an ovine animal model. *Pathol Res Pract*. 2005;201(12):809–817. doi:10.1016/j.prp.2005.08.009
28. Lalu MM, Gao CQ, Schulz R. Matrix metalloproteinase inhibitors attenuate endotoxemia induced cardiac dysfunction: a potential role for MMP-9. *Mol Cell Biochem*. 2003;251(1–2):61–66. doi:10.1023/A:1025417529167
29. Biswas MHU, Almeida S, Lopez-Gonzalez R, et al. MMP-9 and MMP-2 Contribute to Neuronal Cell Death in iPSC Models of Frontotemporal Dementia with MAPT Mutations. *Stem Cell Rep*. 2016;7(3):316–324. doi:10.1016/j.stemcr.2016.08.006
30. Chen Y, Wang W, Liu F, Tang L, Tang R, Li W. Apoptotic effect of matrix metalloproteinases 9 in the development of diabetic retinopathy. *Int J Clin Exp Pathol*. 2015;8(9):10452–10459.
31. Visse R, Nagase H. Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. *Circ Res*. 2003;92:827–839. doi:10.1161/01.RES.0000070112.80711.3D
32. Iglesias J, Vassallo AV, Patel VV, Sullivan JB, Cavanaugh J, Elbaga Y. Outcomes of Metabolic Resuscitation Using Ascorbic Acid, Thiamine, and Glucocorticoids in the Early Treatment of Sepsis: the ORANGES Trial. *Chest*. 2020;158(1):164–173. doi:10.1016/j.chest.2020.02.049