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Effect of vitamin C on coagulation factors and endothelium function in patients with sepsis

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Abstract: **Objective:** Sepsis is one of the leading causes of mortality in intensive care unit. Despite advances in its management, its mortality rate remains high. Recently, high dose of vitamin C in sepsis treatment has attracted the attention of researchers. In the current study, the impacts of 25 mg/kg of vitamin C every 6 hours as a bolus for 3 days were assessed in septic patients in intensive care unit (ICU).

Methods: This was a prospective cohort study that was performed on adult patients with diagnosis of sepsis. Patients were assigned to control group (administration of placebo) or intervention group, i.e., those receiving a 25 mg/kg dose of vitamin C every 6 hours as a bolus for 3 days. Clinical data were recorded before and after the experiment. Also, plasma levels of antithrombin III, syndecan-1, fibrin degradation product (FDP), D-dimer, and C-reactive protein (CRP) were measured at 0, 24, 48, and 72 hours.

Results: In septic patients receiving vitamin C, a significant upregulation of antithrombin III and significant decreases in the levels of syndecan-1 (at 48 hours; P-value=0.046 and at 72 hours; P-value=0.007), D-dimer and CRP were observed compared to the control. Reductions in sequential organ failure assessment (SOFA) score, in-hospital mortality, and ICU length of stay were seen in septic patients receiving vitamin C.

Conclusion: Prescribing high dose of intravenous vitamin C can reduce the mortality of sepsis patients and reduce the length of stay in the ICU.

Keywords: Acid Ascorbic; Blood Coagulation; Endothelium; Sepsis; Syndecan-1; Vitamin C

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1. Introduction

Sepsis is one of the major causes of mortality, which imposes a high burden on health systems. Accordingly, the world health organization (WHO) reports revealed that sepsis affects 30 million people worldwide every year and causes the death of about 6 million patients, while the incidence rate is increasing. Besides, patients who survived sepsis encountered functional disorders of various body organs, including kidney failure, cognitive disorders, respiratory disorders, etc. Despite the outstanding advances in understanding its pathophysiology and improvement in its treatment strategies, the management of this disease is still considered a serious issue (1).

It has been found that the endothelium is the target of damage in the initial phase of inflammation and its damage causes protein leakage, tissue edema, increased adhesion of leukocytes, and coagulation dysfunction (2). Glycocalyx is a complex network of plasma-soluble components, which covers the endothelial surface and acts as an important factor in regulating the function of endothelial cells (3). Syndecan-1 is one of the components of the glycocalyx, and its plasma level increases due to endothelium destruction, so it can be used as a sensitive biomarker for endothelial disorders (4). Syndecan-1 is structurally connected to the endothelial cell membrane and glycosaminoglycans such as heparan sulfate can attach to it (5). Syndecan-1 has been found to be degraded by a variety of mediators such as cytokines or reactive oxygen species (ROS) during inflammatory diseases (5). A recent investigation revealed that high serum level of syndecan-1 is correlated with more severe damage to the endothelium and it can be used as a biomarker to predict mortality in sepsis patients (6).

Clinical diagnosis of disseminated intravascular coagulation (DIC) is difficult, especially in sepsis, and several scoring methods are used. For example, Toshika et al. proposed a new scoring system based on thrombin and antithrombin for prediction of DIC occurrence in septic patients (7). Antithrombin inhibits several coagulation factors, including X, IX, VII, XI, and XII factors, and the level of antithrombin usually decreases due to its consumption and disruption in synthesis. By binding to glycosaminoglycans, thrombin can ex-

ert anti-inflammatory effects on the surface of endothelial cells and exert its protective effects through the inactivation of cytotoxic thrombin as well as maintaining the function of vascular endothelium (8). Hence, antithrombin III can be a marker for severity of sepsis. In addition, high level of plasma thrombomodulin is also associated with endothelial damage. Vitamin C has been considered as a part of sepsis treatment due to its antioxidant effects, anti-inflammatory effect and probable protective effects on vascular endothelium (9,10). The results of a research indicated that vitamin C upregulates norepinephrine synthesis (11). It has been shown that the administration of this vitamin can be useful in septic patients due to the improvement of micro-vascular function (12). Vitamin C seems to play a vital role in preventing the endothelium destruction and the apoptosis in septic shock (12). In a study conducted by Marik et al., vitamin C (along with hydrocortisone and thiamine) was able to reduce sepsis-related mortality (13). However, to the best of our knowledge, no study has assessed the influence of vitamin C on endothelial markers and coagulation factors in sepsis. In the current study, the effects of high dose vitamin C on coagulation factors (fibrin degradation product (FDP), D-dimer and antithrombin III) were investigated. Meanwhile, the protective effect of vitamin C on vascular endothelium was studied.

2. Methods

2.1. Study design

The current study is a single-center double-blind randomized controlled clinical trial (RCT) that was conducted in the intensive care units (ICUs) of Sina Hospital, a tertiary care center in Tehran, Iran. Written consent was obtained from all patients and all their information was kept confidential.
The researchers adhered to the declaration of Helsinki during conducting the trial. Also, the current research was approved by the Ethics Committee of Tehran University of Medical Sciences (IR.TUMS.VCR.REC.1399.158).

2.2. Study participants

The sample size was calculated to be 58 patients based on Cochran's formula. A random sampling method was used to select the sample. Adult patients with diagnosis of sepsis, based on the criteria stated in the 2016 surviving sepsis campaign guidelines, who were admitted to intensive care unit were included (14). Exclusion criteria were as follows: age more than 80 years, pregnancy, any kind of preexisting kidney failure based on the definition of kidney disease improving global outcomes (KDIGO) guidelines, administration of other antioxidants since 24 hours before and during the study, n-acetylcysteine (NAC), melatonin and selenium) administration of intravenous fibrinogen or prothrombin complex concentrates (PCC), administration of heparin with a therapeutic dose, international normalized ratio (INR)>1.5, thrombocytopenia (platelet count<100000/μl), administration of warfarin, hemochromatosis patients, and more than 48 hours passing from the diagnosis of sepsis.
Clinical data such as age, gender, comorbidities, and initial sequential organ failure assessment (SOFA) and acute physiology and chronic health evaluation (APACHE) II scores were recorded. Also, time to antibiotic administration and steroids administration were recorded. Time to antibiotic administration was assessed in four subgroups of <1, 1-<3, 3-6 and >6 hours and the data of both groups were statistically compared. Also, the number of patients who received steroids during the trial was recorded.
During the experiment, 3 acute kidney injury (AKI) patients were diagnosed and were excluded. Also, 2 patients received fibrinogen and 3 patients died and were excluded from the experiment. Therefore, the final sample size was 50 patients (Figure 1).

2.3. Study protocol

If the patient, or his/her legal guardian signed the consent form, they were randomly assigned to one of following groups:
Control group: these patients received distilled water+5% dextrose water in addition to the standard treatment (n=25).
Vitamin C group: for these patients, 25 mg/kg dose of vitamin C was prescribed every 6 hours as a bolus for 3 days (n=25) in addition to the standard treatment.
All other treatment protocols, like deep vein thrombosis (DVT) prophylaxis, acid suppression, fluid therapy, antibiotic therapy, etc. were all the same between two groups.
Three patients in the control group and 2 patients in the vitamin C group were transferred from the ICU and were excluded from the study (Figure 1).

2.4. Outcome assessment

Primary outcome of the study was serum syndecan-1 levels. Secondary outcome was the level of other endothelial biomarkers and coagulation factors. Demographic data of patients such as age, gender, and other clinical data like cause of hospitalization, comorbidities, need for vasopressors, daily urine output (for 3 days of intervention), fluid balance after 24 and 72 hours, and length of stay in ICU were recorded. SOFA score of the patient was recorded every day of ICU stay. International society of thrombosis and hemostasis (ISTH) scoring system was used to measure DIC (15). Other information about their admission course was recorded.

2.5. Sampling for coagulation factors and endothelial biomarkers

Blood samples were taken from patients in both treatment and control groups at 0, 24, 48, and 72 hours. In order to prepare the samples, first the blood samples were kept in the tube for 60 minutes at room temperature to clot and then the serum and plasma were separated using a centrifuge. Then the samples were kept at -70°C until further analyses. Serum creatinine, C-reactive protein (CRP), D-Dimer

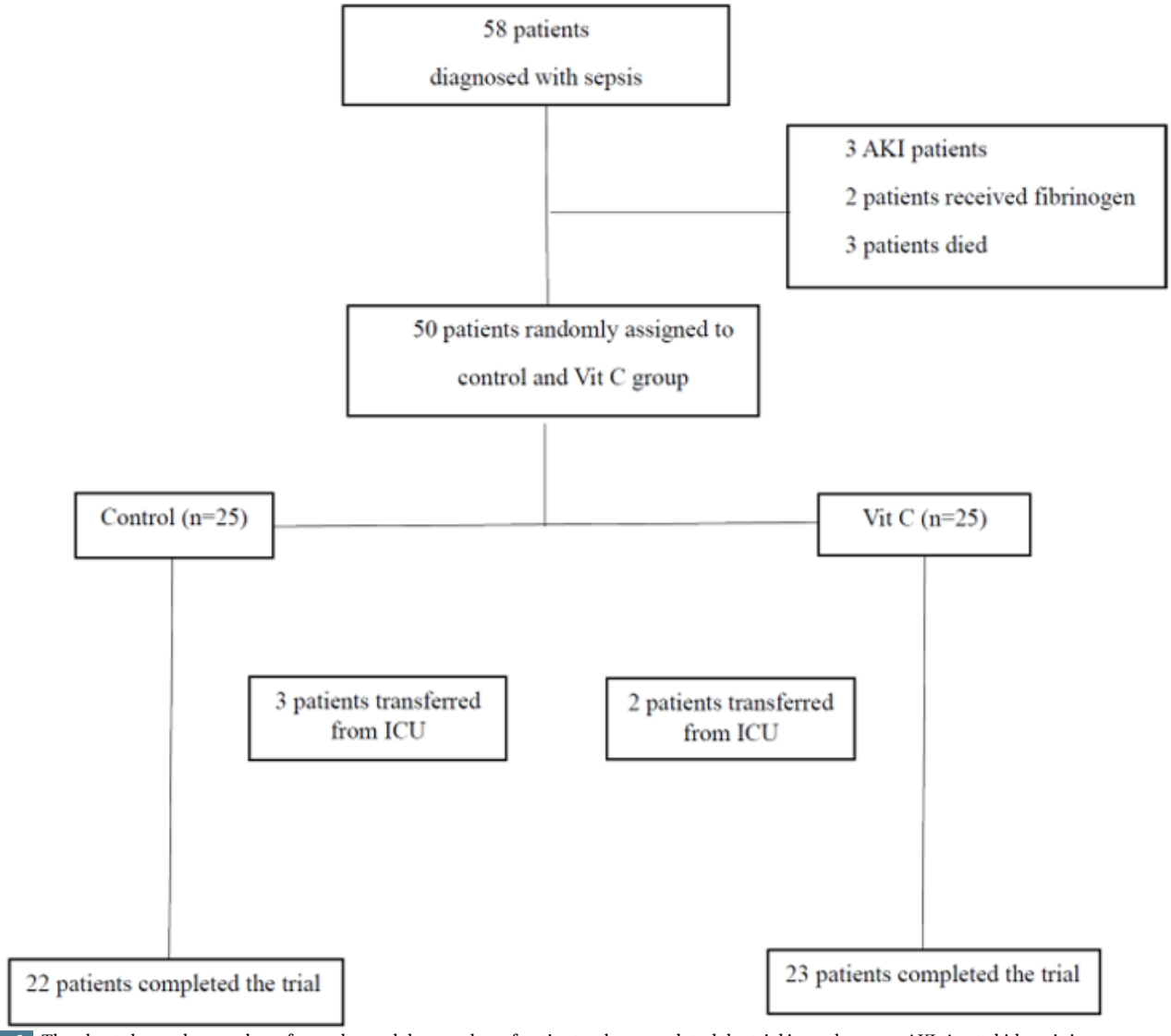

Figure 1 The chart shows the number of samples and the number of patients who completed the trial in each group; AKI: Acute kidney injury; Vit C: Vitamin C; ICU: Intensive care unit

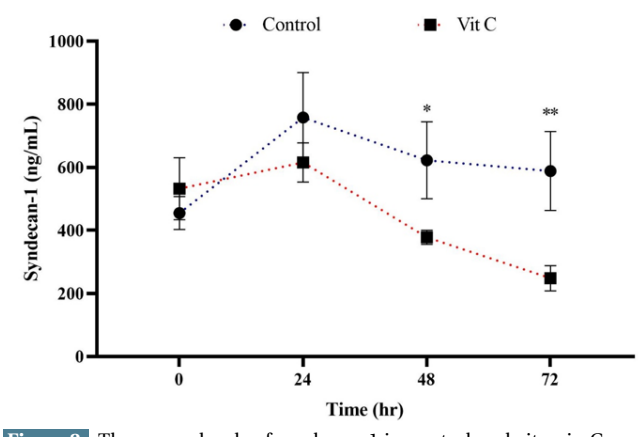

Figure 2 The serum levels of syndecan-1 in control and vitamin C (vit C) groups at 0, 24, 48, and 72 hours

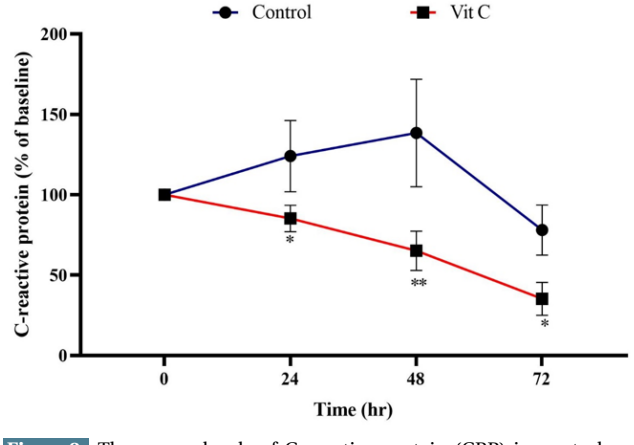

Figure 3 The serum levels of C-reactive protein (CRP) in control and vitamin C (vit C) groups at 0, 24, 48, and 72 hours

Table 1 Demographic and baseline clinical data of the patients

Characteristics	Control (n=25)	Vitamin C (n=25)	P-Value
Age (years), mean ± SD	68.9±14.7	69.2±15.8	0.922
Gender, n (%)			
Male	14 (56.0)	15 (60.0)	0.912
Female	11 (44.0)	10 (40.0)	0.988
Comorbidities, n (%)			
Hypertension	6 (24.0)	6 (24.0)	1
Stroke	6 (24.0)	5 (20.0)	0.962
Diabetes mellitus	3 (12.0)	5 (20.0)	0.122
Myocardial infarction	3 (12.0)	4 (16.0)	0.922
Influenza	4 (16.0)	3 (12.0)	0.815
Chronic pulmonary disease	2 (8.0)	1 (4.0)	0.814
Cancer	1 (4.0)	1 (4.0)	1
SOFA score, mean±SD	10±5.78	11±4.28	0.755
APACHE II score, mean ±SD	26.8±7.22	24.22±8.36	0.611
Time (hours) to antibiotic administration, n (%)			
<1	13 (52.0)	11 (44.0)	0.734
1-<3	7 (28.0)	8 (32.0)	0.933
3-6	4 (16.0)	4 (16.0)	1
>6	1 (4.0)	2 (8.0)	0.922
Steroid administration, n (%)	12 (48.0)	10 (40.0)	0.196
Mechanical ventilation, n (%)	14 (64.0)	11 (44.0)	0.744

SOFA: Sequential organ failure assessment; APACHE: Acute physiology and chronic health evaluation; SD: Standard deviation

and FDP were recorded daily for 3 days. Antithrombin III (Cat# ab222507, abcam, UK), fibrinogen (Cat# ab108842, abcam, UK) and syndecan-1 (Cat#ab46506, abcam, UK) were measured using kits. All samples were run in duplicate. The manufactures' instructions were followed during conducting the enzyme-linked immunosorbent assay (ELISA) using a microplate reader.

2.6. Statistical analysis

Kolmogorov–Smirnov test was used to evaluate the normal distribution of data. Non-parametric Mann-Whitney U-test (non-normal data) and t-test (normal data) were used to compare the mean of homeostatic parameters between the control and vitamin C groups. Chi-squared test was used to compare qualitative variables between the two groups. A significance level of P<0.05 was considered and all analyzes were performed in GraphPad Prism V.8 software.

3. Results

3.1. Study participants

The demographic data and clinical characteristics of septic patients in the control group and intervention group are given in table 1.

There was no significant difference regarding these features between the two groups (P>0.05). The mean age in the control group was 68.9±14.7 years and in the vitamin C group, it was 69.2±15.8 years (P=0.922). In both groups, the number of men was more than women, and hypertension and stroke were the most common comorbidities. SOFA score was 10±5.78 in the control group and 11± 4.28 in the vitamin C group (P=0.755).

3.2. Effects of vitamin C on syndecan-1

Syndecane-1 levels reached their maximum value one day after the start of the experiment in both groups of patients. Nevertheless, a decreasing trend was observed during the subsequent times (48 and 72 hours) and its lowest level was seen after 72 hours in septic patients who received vitamin C. The results revelaed that there was a significant difference between the two groups regarding level of syndecan-1 at different times (at 48 hours; P-value=0.046, and at 72 hours; P-value=0.007), which is depicted in figure 2.

3.3. Effects of vitamin C on serum CRP

As shown in figure 3, administration of 25 mg/kg dose of vitamin C every 6 hours for 3 days has led to a significant decrease in serum CRP levels in septic patients compared to controls. Also, administration of vitamin C prevented the increase in the levels of this inflammatory biomarker in the first 48 hours.

3.4. Effects of vitamin C on coagulation factors

Vitamin C administration led to a significant upregulation in antithrombin III levels in sepsis patients admitted to ICU (Figure 4a). Significant differences in antithrombin III levels were observed at 24 (P=0.008), 48 (P<0.001) and 72 (P=0.032) hours.

In terms of D-dimer, there were no significant differences between the two groups after 24 and 48 hours (Figure 4b). However, the D-dimer level in sepsis patients receiving vitamin C was significantly lower than the control group at 72 hours (P=0.043; Figure 4b). FDP (P=0.385) and fibrinogen (P=0.658) levels were not significantly different between the two groups

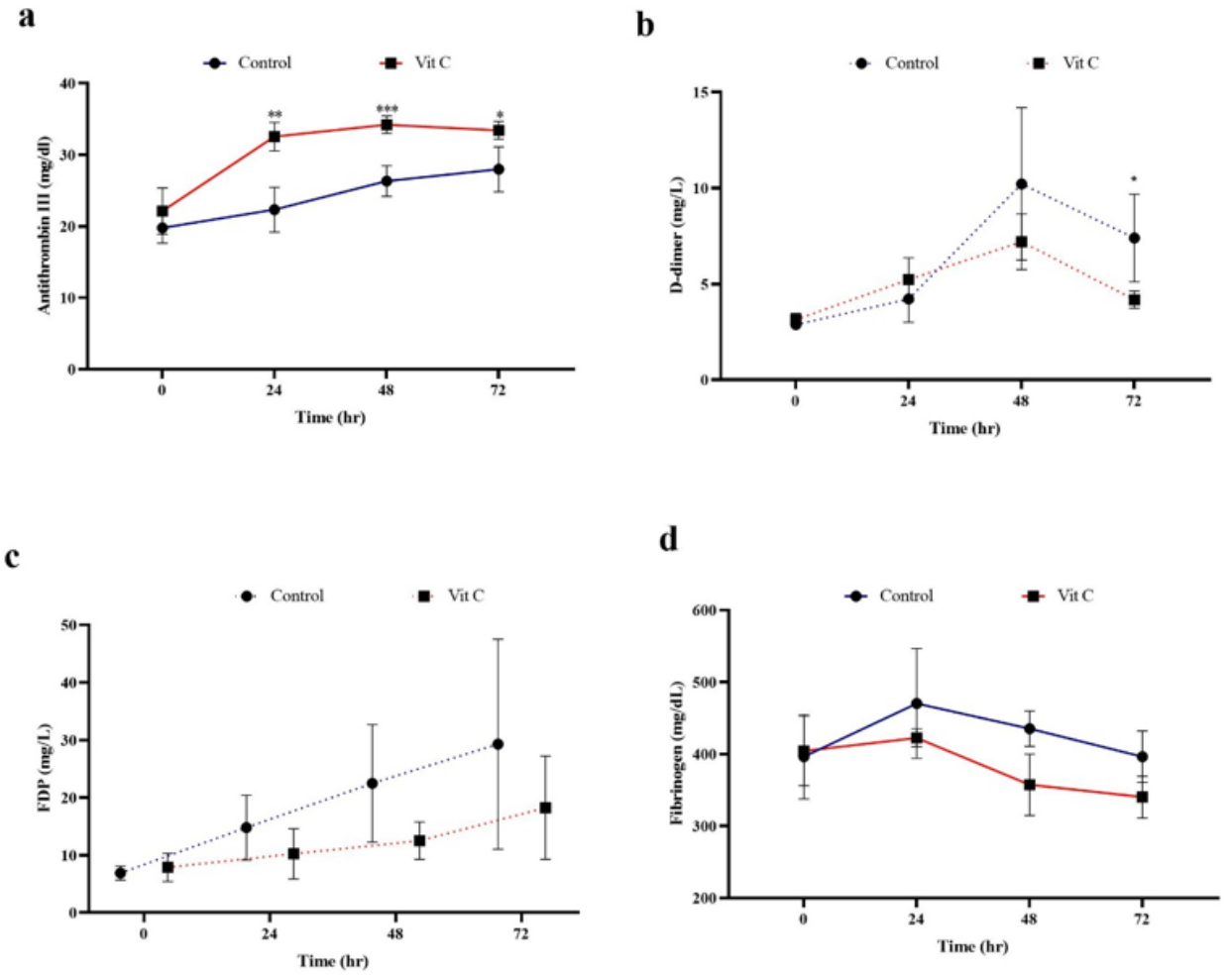

Figure 4 The plasma levels of antithrombin III (a), D-dimer (b), fibrinogen degradation products (FDP)(c), and fibrinogen (d) in control and vitamin C (vit C) groups

(Figure 4c, d).

3.5. Effects of vitamin C on other outcomes

Administering vitamin C every 6 hours as a bolus for 3 days to sepsis patients hospitalized in ICU, in comparison with the control group, led to a decrease in SOFA score (△ SOFA score of control: 0.520±0.200; △ SOFA score of vitamin C: -2.43±0.55), in-hospital mortality (20% in vitamin C group vs. 28% in control group), and the length of stay in ICU (control: 12.8± 2.8 d; vitamin C: 8.6±1.6 d; P=0.048). Regarding the concern of nephrotoxicity of ascorbic acid, the serum levels of creatinine was not significantly different between septic patients receiving vitamin C and the control group (control: 1.96±0.10 mg/d; vitamin C: 1.58±0.40 mg/dL) (Figure 5).

4. Discussion

The findings of the current study showed that the administration of 25 mg/kg vitamin C every 6 hours as a bolus for 3 day leads to a decrease in serum levels of syndecan-1, CRP, D-dimer and antithrombin III. Also outcomes like decresing trend of SOFA score, in-hospital mortality, and length of stay in ICU, were improved in the intervention group. These results indicated that vitamin C administration can have a positive effect on coagulopathy in septic patients.

Some studies have shown that vitamin C level decreases in septic patients (16). This vitamin is a cofactor for the dopamine beta-hydroxylase enzyme, which plays a vital role in the synthesis of norepinephrine from dopamine. Therefore, the reduction in serum levels of vitamin C is directly related to the reduction of norepinephrine levels (17,18). Low levels of this hormone have been reported in sepsis patients (19). Hence, one of the protective mechanisms of vitamin C in sepsis conditions can be the increase of norepinephrine levels (20).

In scientific literature, there are conflicting reports regarding the effect of vitamin C on sepsis patients (16). It seems that the dose of this vitamin, and the duration and the route of administration of this vitamin play an important role in the improvement of critically ill patients. Administering high doses of vitamin C (>3g) intravenously is recommended (21). Nevertheless, the administration of these doses can lead to an in-

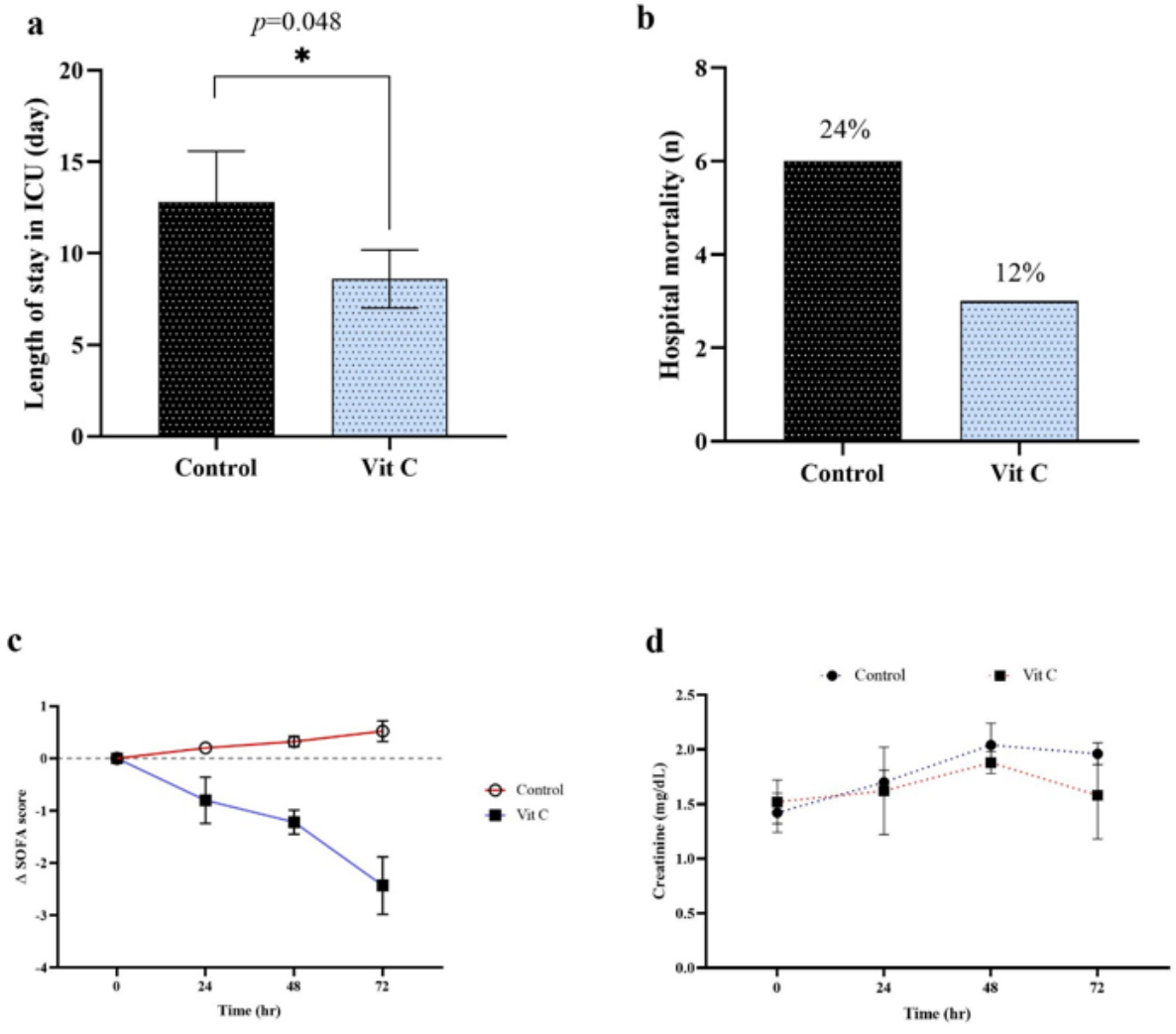

Figure 5 Length of stay in intensive care unit (ICU) (a), in-hospital mortality (b), △SOFA score (c) and serum creatinine changes during 3 days of intervention

crease in serum oxalate levels and its deposition in the kidney and other tissues (22) and so, the kidney function is compromised. Therefore, in the present study, to prevent this, the dose of 25 mg/kg was considered and the results showed that it can be effective in the recovery of septic patients. This is supported by the reduction of SOFA score and mortality of septic patients compared to the control group in the present study.

Recently, syndecan-1 has been reported as a biomarker for endothelial damage and the onset of organ failure (6). In sepsis, it was revealed that the level of this biomarker are correlated with the severity of the disease, organ failure, and high mortality of patients (6). In the present investigation, it was proved that the administration of vitamin C to septic patients hospitalized in ICU can reduce the plasma levels of syndecan-1, which indicates the reduction of damage to the endothelium. In septic patients, it has been shown that serum level of syndecan-1 is increased (23) and its level was much higher in non-survivors compared to survivors (6). A significant positive correlation was also reported between the expression levels of this biomarker with inflammatory cytokines and high mortality (6). Therefore, it seems that in the present study, the reduction of syndecan-1 level as a result of the administration of vitamin C has probably inhibited the systemic inflammatory process in sepsis patients.

In the current study, the levels of FDP, D-dimer and antithrombin III were studied in septic patients and the results showed that there were increases in the levels of FDP and D-dimer and a decrease in the level of antithrombin III. This indicates a condition of coagulopathy that can be caused by endothelium damage (24), which increases the likelihood of DIC. Nevertheless, the administration of vitamin C to septic patients led to a significant increase in antithrombin III and a significant decrease in D-dimer, which can indicate an anticoagulation effect. A decrease in antithrombin III levels can be caused by damage to the liver (25), which is the main site of its synthesis. A decrease in antithrombin III level has been reported as a predictor of poor outcome and DIC in sepsis

(26). Therefore, vitamin C prevents coagulopathy by increasing the levels of antithrombin III and decreasing the levels of coagulation factors such as D-dimer and FDP.

Among the limitations of the current research, we can mention the small sample size, being a single-center study, and lack of longer follow-up for the patients. Also, the treatments have been done in different seasons. Genetic variation and polymorphism in alpha, beta, and vasopressin receptors, as well as angiopoietin-2, lactate and renin levels may have influenced our present data and findings. The future point of care assay would allow targeted resuscitative effort toward personalization. However, the results of the present research is supported by other experimental studies. We believe that this study can provide good insight for designing future sepsis treatment protocols.

5. Conclusion

Prescribing 25 mg/kg vitamin C every 6 hours as a bolus for 3 days can have protective effects on vascular endothelium and prevent coagulopathy, and can thus play a vital role in reducing the burden of sepsis.

6. Declarations

6.1. Acknowledgement

None.

6.2. Authors' contribution

All the authors passed four criteria for authorship contribution based on recommendations of the International Committee of Medical Journal Editors.

6.3. Conflict of interest

We hereby state that there is no conflict of interest for any of the authors of this manuscript.

6.4. Funding

There was no funding from any company or pharmaceutical manufacturer.

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