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High-dose vitamin C improves norepinephrine level in patients with septic shock

A single-center, prospective, randomized controlled trial

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Abstract

Background: The effects of vitamin C supplementation on patients with septic shock remain controversial. We aimed to evaluate the effects of different vitamin C dosages on norepinephrine (NE) synthesis in adult patients with septic shock.

Methods: A total of 58 patients with septic shock admitted to our intensive care unit (ICU) between July 2021 and December 2022 were included. Patients were randomly divided into 3 groups: high-dose vitamin C (150 mg/kg/d, group A), low-dose vitamin C (50 mg/kg/d, group B), and placebo (group C). NE synthesis-related indicators (dopamine-β-hydroxylase [DβH], tyrosine hydroxylase [TH], tetrahydrobiopterin [BH4], and dopamine [DA]), plasma NE, and vitamin C levels were measured every 24 hours and analyzed. All-cause mortality within 28 days and other clinical outcomes (including Acute Physiology and Chronic Health Evaluation [APACHE], Sequential Organ Failure Assessment [SOFA], and Multiple-Organ Dysfunction Syndrome [MODS] scores) were compared.

Results: Changes in TH, BH4, and DβH levels at 96 hours in groups A and B were greater than those in group C. These differences became more pronounced over the course of the intravenous vitamin C administration. Significant differences between groups A and C were detected at 96-hours TH, 72-hours BH4, 96-hours BH4, 96-hours DA, and DβH levels every 24 hours. The 96-hours TH, 96-hours BH4, and 48-hours DβH in group B were significantly higher than those in group C. The NE levels every 24 hours in groups A and B were higher than those in group C, group A and group C had a statistically significant difference. The 96-hours exogenous NE dosage in groups A and B was significantly lower than that in group C. No significant reductions in APACHE, SOFA, or MODS scores were observed in the vitamin C group, including the duration of ICU stay and mechanical ventilation. The 28-days mortality was lower in groups A and B than in group C (0%, 10%, and 16.67%, $P = .187$), but the difference was not significant.

Conclusion: For patients with septic shock, treatment with vitamin C significantly increased TH, BH4, and DβH levels and reduced the exogenous NE dosage, but did not significantly improve clinical outcomes.

Abbreviations: ALT = alanine aminotransferase, APACHE = acute physiology and chronic health evaluation, BH4 = tetrahydrobiopterin, Cre = creatinine, CRP = C reaction protein, DA = dopamine, DβH = dopamine-β-hydroxylase, ICU = intensive care unit, Lac = lactic acid, MAP = mean arterial pressure, MODS = multiple-organ dysfunction syndrome, NE = norepinephrine, NO = nitric oxide, PCT = procalcitonin, RCT = randomized controlled trial, ROS = reactive oxygen species, SD = standard deviation, SOFA = sequential organ failure assessment, TH = tyrosine hydroxylase.

Keywords: mortality, norepinephrine, septic shock, vitamin C

WL and RZ contributed equally to this article.

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This manuscript has been read and approved by all the authors, and each author believes that the manuscript represents honest work.

The authors have no conflicts of interest to disclose.

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

All procedures involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. This study was approved by the Ethics Committee of the First Affiliated Hospital of Dalian Medical University. All participants agreed to participate and written informed consent was obtained from all participants.

Supplemental Digital Content is available for this article.

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1. Introduction

Sepsis is a life-threatening condition in critically ill patients and accounts for 19.7% of all deaths worldwide.[1] Septic shock leads to circulatory and cellular metabolic abnormalities, manifesting as deterioration in tissue perfusion and decreased peripheral vascular resistance, which substantially increases mortality. Norepinephrine (NE) is the first-line treatment for septic shock, according to the Surviving Sepsis Campaign guidelines.[2] However, potential adverse effects of NE, such as immunosuppression and peripheral vascular necrosis, may result in other undesirable consequences.[3] Therefore, we aimed to improve tissue perfusion using a limited amount of NE.

Vitamin C is a potent antioxidant that prevents vascular endothelial damage and maintains the microvascular integrity. According to in vitro and animal studies, it can alleviate oxidative injury-induced microcirculatory impairment, restore vascular responsiveness to vasoconstrictors, and preserve the endothelial barrier.[4,5] Additionally, high-dose vitamin C can enhance antibacterial defense. These pathophysiological protective effects appear to relieve organ injury and dysfunction in patients with sepsis or septic shock. Vitamin C deficiency was frequently observed in these patients.[6]

Therefore, adjunctive vitamin C administration regimens for patients with septic shock have received considerable attention in real-world clinical practice.[7] Several studies have shown that intensive care unit (ICU) stay is shortened and the dosage and duration of exogenous NE combined with high-dose vitamin C are limited.[8,9] However, there is no consensus regarding whether vitamin C supplementation improves mortality or prognosis.[10] No large randomized controlled trial (RCT) has demonstrated a significant reduction in long-term all-cause mortality.[11] Recently, several RCTs have demonstrated no significant differences in outcome parameters with vitamin C supplementation.[12] Notably, the Lessening Organ Dysfunction with Vitamin C (LOVIT) trial, an international prospective RCT of 872 patients with sepsis, questioned the role of vitamin C in sepsis.[13] The reason for this inconsistency between clinical outcomes and pathophysiological theory of vitamin C is unknown. In addition, the mechanisms underlying the effect of vitamin C on NE synthesis in patients with septic shock remain unclear. To date, there is a lack of biological evidence regarding the treatment effect of intravenous vitamin C in patients with sepsis, and the potential explanations for these differences are unclear.

We evaluated the effect of high-dose vitamin C on NE synthesis in 58 septic shock patients. This study aimed to determine the influence of different vitamin C doses on plasma NE concentrations, biological indicators of NE synthesis, inflammatory biomarkers, hemodynamic parameters, and clinical outcomes. We focused on the concentration of plasma NE and its synthesis-related enzymes to determine the potential effects of vitamin C on septic shock. We hypothesized that vitamin C increases NE levels in patients with septic shock.

2. Materials and methods

2.1. Patients

This was a single-center, prospective, double-blind RCT of 58 consecutive patients with septic shock who were admitted to our ICU between July 2021 and December 2022. This study was approved by the Ethics Committee of the First Affiliated Hospital of Dalian Medical University (PJ-KY-2020-01).

The inclusion criteria were as follows: fulfilling the Sepsis-3 definition of septic shock according to The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3)[14]; age ≥ 18 years; treatment in the ICU for at least 96 hours; and agreement to participate in this study and signed informed consent from the patient or a representative. The exclusion criteria were as follows: ICU stay < 96 hours; a history of vitamin C allergy or contraindications to vitamin C infusion (such as thromboembolic diseases, sideroblastic anemia, gout, glucose-6-phosphate dehydrogenase deficiency, paroxysmal nocturnal hemoglobinuria, or hyperoxaluric acidosis); treatment with continuous renal replacement therapy within 96 hours of admission to the ICU; history of tumor or immunodeficiency disease; glucocorticoid use within the last 3 months; pregnancy or lactation; and refusal to participate in this trial. Enrolled patients who met the exclusion criteria after enrollment (e.g., survival time < 96 h, received continuous renal replacement therapy, etc) will be excluded and not included in the final analysis.

All enrolled patients were randomly divided into 3 groups using a random number generator in SPSS (IBM, Armonk, NY, USA) and administered high-dose vitamin C (150 mg/kg/d, group A), low-dose vitamin C (50 mg/kg/d, group B), or placebo (control group: the same amount of sterile water as the study group, group C) (Fig. 1).

2.2. Interventions

For the treatment groups A (high-dose vitamin C [150 mg/kg/d] and B (low-dose vitamin C [50 mg/kg/d]), vitamin C was diluted in sterile water to a total volume of 30 mL and infused using an infusion pump. In the control group (Group C), 30 mL of sterile water was infused using an infusion pump as a placebo at a speed identical to that of the treatment group. Vitamin C or placebo was infused every 6 hours for a maximum of 30 minutes. The intervention lasted for 4 days. All patients received guideline-adherent medications or therapeutic regimens including supportive care, fluid resuscitation, antibacterial drugs, mechanical ventilation, and treatment of the primary disease.[2] For patients with previous hypertension, the mean arterial pressure (MAP) was maintained at ≥80 mm Hg. For the remaining patients, the MAP was maintained above 65 mm Hg.

Independent research staff prepared the study drug, which was identical and labeled as "glucose." All research data were extracted from the electronic medical record system by the research staff. All data were entered by doctors who were blinded to study allocation. The laboratory team that measured all biomarkers was blinded to the treatment allocation.

2.3. Study parameters

The baseline data collected included age, sex, vital signs, comorbidities, and the site of infection. Plasma NE and vitamin C concentrations, NE synthesis-related indicators, inflammatory biomarkers, and hemodynamic parameters were measured on ICU admission (Appendix 1, Supplemental Digital Content 1, http://links.lww.com/MD/M197). NE synthesis-related biological indicators included plasma dopamine-β-hydroxylase (DβH), tyrosine hydroxylase (TH), tetrahydrobiopterin (BH4), and dopamine (DA). The baseline levels and changes in procalcitonin (PCT), C reaction protein (CRP), MAP, arterial lactic acid (Lac), and urine volume were measured. Changes in alanine aminotransferase (ALT), total bilirubin, creatinine levels, extra NE dose administered, and the rehydration fluid volume were also recorded. The study parameters at each 24-hour intervals (24, 48, 72, and 96 h) were recorded and analyzed.

2.4. Primary and secondary outcomes

The primary outcome was all-cause mortality within 28 days of randomization. The secondary outcomes included changes in the

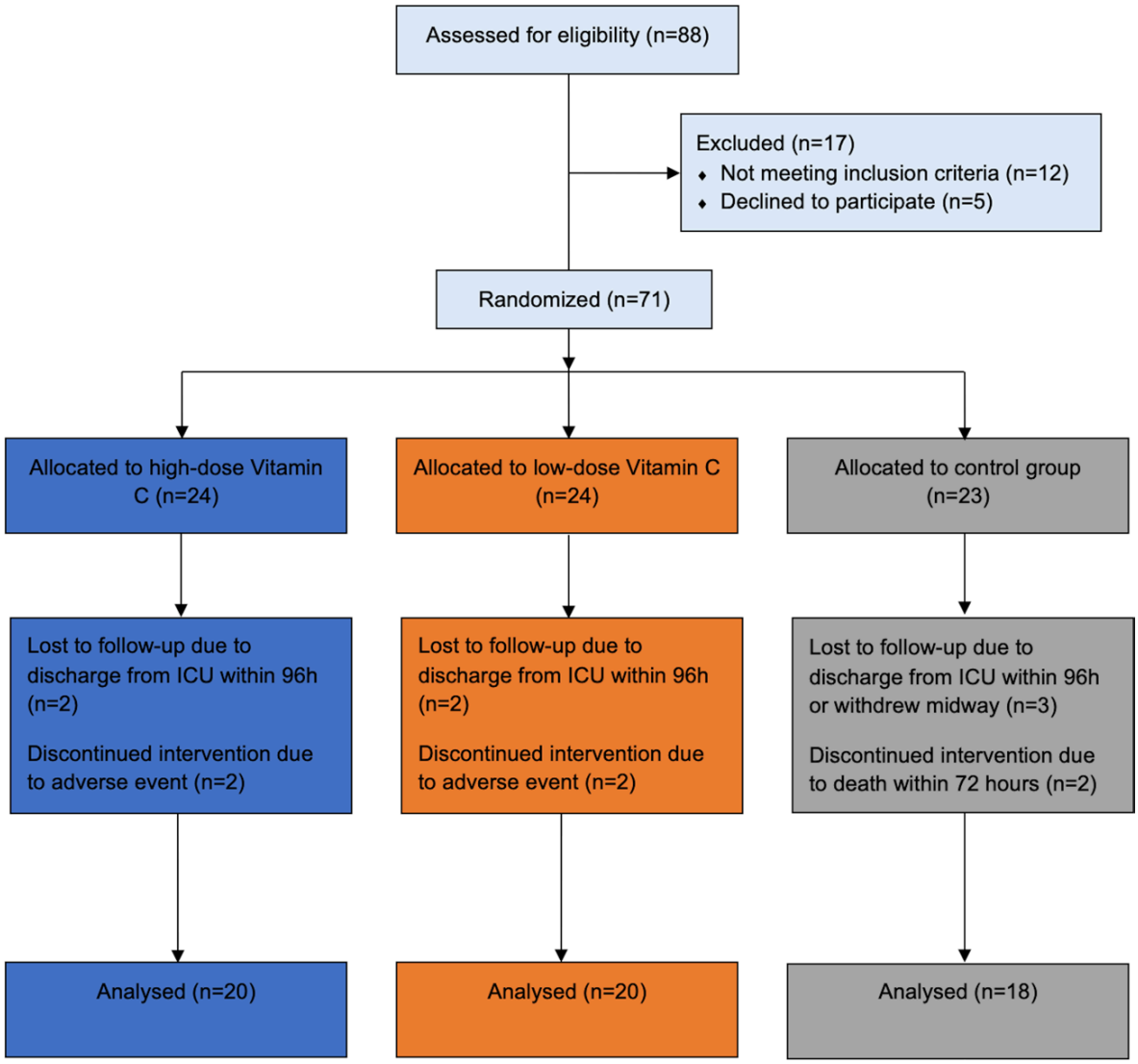

Figure 1. Study flowchart.

Acute Physiology and Chronic Health Evaluation (APACHE), Sequential Organ Failure Assessment (SOFA), and Multiple-Organ Dysfunction Syndrome (MODS) scores, duration of ICU stay, duration of mechanical ventilation, and total length of hospital stay.

2.5. Safety outcomes

Vitamin C-related adverse events, such as severe nausea and vomiting, syncope, rash, abdominal pain, abnormal blood glucose values, severe cardiac arrhythmia, and venous thrombosis, were recorded. If adverse events occur, vitamin C administration will be immediately stopped and the trial will be completed.

2.6. Statistical analysis

All analyses were performed using SPSS version 26.0 (SPSS Inc., Chicago, IL, USA). Continuous variables were expressed as mean ± standard deviation (SD) if normally distributed. One-way analysis of variance (ANOVA) with Bonferroni correction was used for comparisons between the 3 groups. For skewed data, the Mann–Whitney U test was used for comparison, and these variables were expressed as the median and interquartile range (25th–75th percentile). For categorical variables, the χ^2 test or Fisher exact test was used. Kaplan–Meier analysis with the Wilcoxon test was used to show the effect of vitamin C on survival probability. A 2-tailed P-value < .05 was considered statistically significant.

3. Results

3.1. Baseline clinical characteristics

The baseline clinical characteristics of the 3 groups are summarized in Table 1. A total of 58 patients with septic shock were included (20 in Group A, 20 in Group B, and 18 in Group C). The primary etiology of sepsis was in the lungs (32, 55.17%), followed by the bloodstream (8, 13.80%), biliary tract (10, 17.24%), and abdomen (8, 13.80%).

There were no significant differences between the 3 groups in terms of age, sex, comorbidities, vital signs, arterial blood gas values, PCT levels, SOFA scores, or other baseline values. However, baseline Cre and DβH levels in group C were higher than those in group B.

Table 1

Baseline characteristics of patients.

	Group A (n = 20)	Group B (n = 20)	Group C (n = 018)	*P*
Age (yrs)	72.00 ± 14.67	63.30 ± 17.13	67.78 ± 13.53	.454
Female, n (%)	6 (30.00)	8 (40.00)	2 (11.11)	.450
Weight (kg)	70.10 ± 9.86	73.00 ± 25.87	70.78 ± 6.40	.988
Sepsis etiology, n (%)				
Lungs	8 (40.00)	12 (60.00)	12 (66.67)	.196
Biliary tract	6 (30.00)	2 (10.00)	2 (11.11)	.185
Blood	4 (20.00)	2 (10.00)	2 (11.11)	.754
Abdomen	2 (10.00)	4 (20.00)	2 (11.11)	.754
Comorbidities, n (%)				
Hypertension	8 (40.00)	4 (20.00)	12 (66.67)	.327
Diabetes mellitus	8 (40.00)	4 (20.00)	2 (11.11)	.321
Cerebrovascular diseases	4 (20.00)	2 (10.00)	2 (11.11)	.574
Temperature (°C)	36.99 ± 0.79	36.88 ± 0.75	36.86 ± 0.60	1.000
Heart rate (BPM)	94.30 ± 13.47	112.00 ± 15.33	96.56 ± 25.46	.065
Respiratory rate (BPM)	21.80 ± 6.11	19.50 ± 4.28	24.44 ± 6.33	.107
MAP (mm Hg)	61.90 ± 1.52	63.20 ± 2.04	62.67 ± 2.50	.125
Lac (mmol/L)	3.26 ± 1.11	3.29 ± 1.77	3.08 ± 1.81	.902
APACHE II score	20.80 ± 5.41	17.60 ± 7.32	19.67 ± 4.64	.216
MODS score	6.90 ± 2.42	6.00 ± 1.76	7.00 ± 2.29	.274
SOFA score	8.50 ± 3.50	9.00 ± 4.74	10.22 ± 3.42	.376
PCT (μg/L)	0.88 (0.32, 34.49)	0.59 (0.12, 3.34)	1.25 (0.37, 22.71)	.797
CRP (mg/L)	123.29 ± 54.82	100.62 ± 65.46	103.16 ± 61.75	.130
LVEF (%)	55.20 ± 7.69	56.10 ± 4.58	54.67 ± 5.07	.748
ALT (U/L)	121.40 ± 71.79	97.40 ± 47.68	114.33 ± 90.03	.633
Tbil (μmol/L)	47.96 ± 38.00	50.74 ± 37.37	41.84 ± 33.31	.739
Cre (μmol/L)	139.70 ± 80.37	107.90 ± 74.16	188.00 ± 121.03	.030
Urea (mmol/L)	16.92 ± 8.50	13.51 ± 4.46	20.47 ± 13.80	.075
WBC ($\times 10^9$/L)	14.40 ± 6.51	17.03 ± 4.12	13.90 ± 3.65	.154
PLT ($\times 10^9$/L)	196.00 ± 178.83	195.10 ± 120.34	170.89 ± 123.76	.988
pH	7.44 ± 0.09	7.63 ± 0.66	7.39 ± 0.08	.214
Oxygenation index (mm Hg)	197.60 ± 95.59	220.10 ± 86.80	208.11 ± 79.47	.912
Vitamin C (μmol/L)	17.30 ± 2.13	18.61 ± 3.10	23.48 ± 11.46	.014
DA (nmol/L)	96.27 ± 13.55	95.84 ± 14.22	95.60 ± 9.46	.986
BH_4 (ng/L)	68.52 ± 11.17	66.76 ± 12.25	63.72 ± 12.55	.448
NE (ng/L)	220.50 ± 39.12	232.60 ± 46.31	243.92 ± 41.65	.227
TH (U/L)	142.39 ± 24.11	128.77 ± 23.63	141.62 ± 21.69	.114
DβH (U/L)	40.04 ± 4.98	39.10 ± 6.86	44.62 ± 8.11	.028

ALT = alanine aminotransferase, BH4 = tetrahydrobiopterin, BPM = beats per minute, Cre = creatinine, CRP = C reaction protein, DA = dopamine, DβH = dopamine-β-hydroxylase, Lac = arterial lactic acid, LVEF = left ventricular ejection fraction, MAP = mean arterial pressure, NE = norepinephrine, PCT = procalcitonin, PLT = platelets, Tbil = total bilirubin, TH = tyrosine hydroxylase, WBC = white blood cells.

3.2. Norepinephrine-synthesis-related biological indicators and vitamin C levels

There were no significant differences in baseline NE, DA, TH, or BH4 levels among the 3 groups. The baseline DβH levels in group C (44.62 ± 8.11 U/L) were higher than those in group B (39.10 ± 6.86 U/L, $P = .036$). Significant differences between groups A and B were detected at 72-hours TH, 96-hours TH, 72-hours BH4, and 96-hours BH4. Significant differences between groups A and C were detected at 96-hours TH, 72-hours BH4, 96-hours BH4, 96-hours DA, and DβH levels every 24 hours. The 96-hours TH, 96-hours BH4, and 48-hours DβH levels in group B were significantly higher than those in group C. The differences in BH4, TH, and DβH levels between the 3 groups became more pronounced over the course of vitamin C administration (Figure 2a, b, d). However, no significant differences in DA levels were detected among the 3 groups (Table 2 and Figure 2c). The ratios of the values at 96 hours to the baseline values (Δ96 h) of TH and DβH in groups A and B were considerably higher than those in group C (Table 2 and Figure 3a). The Δ96 hours of BH4 in group A was significantly higher than those in groups B and C, but no significant difference was detected between groups B and C.

All enrolled patients' average plasma vitamin C levels were subnormal at 19.67 ± 7.01 μmol/L. The baseline vitamin C levels in group C (23.48 ± 11.46 μmol/L) were higher than those in group A (17.30 ± 2.13 μmol/L, $P = .016$). The vitamin C concentrations at 24, 48, 72, and 96 hours in the 3 groups were significantly different (all $P < .001$). The 24-hours vitamin C concentration in group A (46.84 ± 8.78 μmol/L) was significantly greater than that in groups B (33.75 ± 7.58 μmol/L, $P < .001$) and C (28.71 ± 3.93 μmol/L, $P < .001$). The difference between groups B and C was not statistically significant ($P = .09$). The 48-hours, 72-hours, and 96-hours vitamin C levels in groups A and B were significantly greater than those in group C (Figure 3b and Appendix 1, Supplemental Digital Content 2, http://links.lww.com/MD/M198, Additional Table 1).

3.3. Plasma norepinephrine concentrations and exogenous norepinephrine dosage

The plasma NE concentrations every 24 hours in groups A and B were higher than those in group C, but only groups A and C showed a statistically significant difference. The 24-hours, 72-hours, and 96-hours NE in group A were significantly higher than those in group B. The 96-hours NE showed a significant difference (318.72 ± 40.95, 267.22 ± 59.19, 227.06 ± 43.17 ng/L, $P < .001$, respectively) between the 3 groups (Table 3 and Appendix 1, Supplemental Digital Content 3, http://links.lww.com/MD/M199, Additional Figure 1). Correspondingly, the exogenous NE dosages in groups A and B were lower than those in group C, but only the 96-hours exogenous NE dosage was reduced significantly (0.11 ± 0.09, 0.19 ± 0.15, 0.26 ± 0.11 μg/(kg·min), $P = .038$). There was no statistically significant difference in plasma NE concentration or exogenous NE dosage at any 24-hours mark between groups B and C, except for 96-hours NE levels (267.22 ± 59.19 vs 227.06 ± 43.17 ng/L $P = .035$).

3.4. Renal and liver function, hemodynamics, procalcitonin and C reaction protein

There was no significant difference in ALT or total bilirubin levels between the 3 groups every 24 hours. The 96-hours creatinine in groups A (66.00 ± 21.55 μmol/L) and B (69.80 ± 38.33 μmol/L) was significantly lower than that in group C (120.22 ± 76.99 μmol/L, $P = .002$), whereas its change was not different between the groups (–0.44 ± 0.22, –0.24 ± 0.34, –0.27 ± 0.26, $P = .063$) (Appendix 1, Supplemental Digital Content 2, http://links.lww.com/MD/M198, Additional Table 2). There was no significant difference in Lac and rehydration volume every 24 hours among the 3 groups, except for the 96-hours Lac (Appendix 1, Supplemental Digital Content 2, http://links.lww.com/MD/M198, Additional Table 3). The 24-hours, 48-hours, and 72-hours MAP in groups A and B were higher than those in group C, but the 96-hours MAP showed no significant difference. Compared with group C, the urine volumes at 72 and 96 hours in groups A and B were significantly higher. PCT and CRP levels decreased after treatment in all patients, but the differences among the 3 groups were not significant, except for 72-hours CRP (Appendix 1, Supplemental Digital Content 2, http://links.lww.com/MD/M198, Additional Table 4).

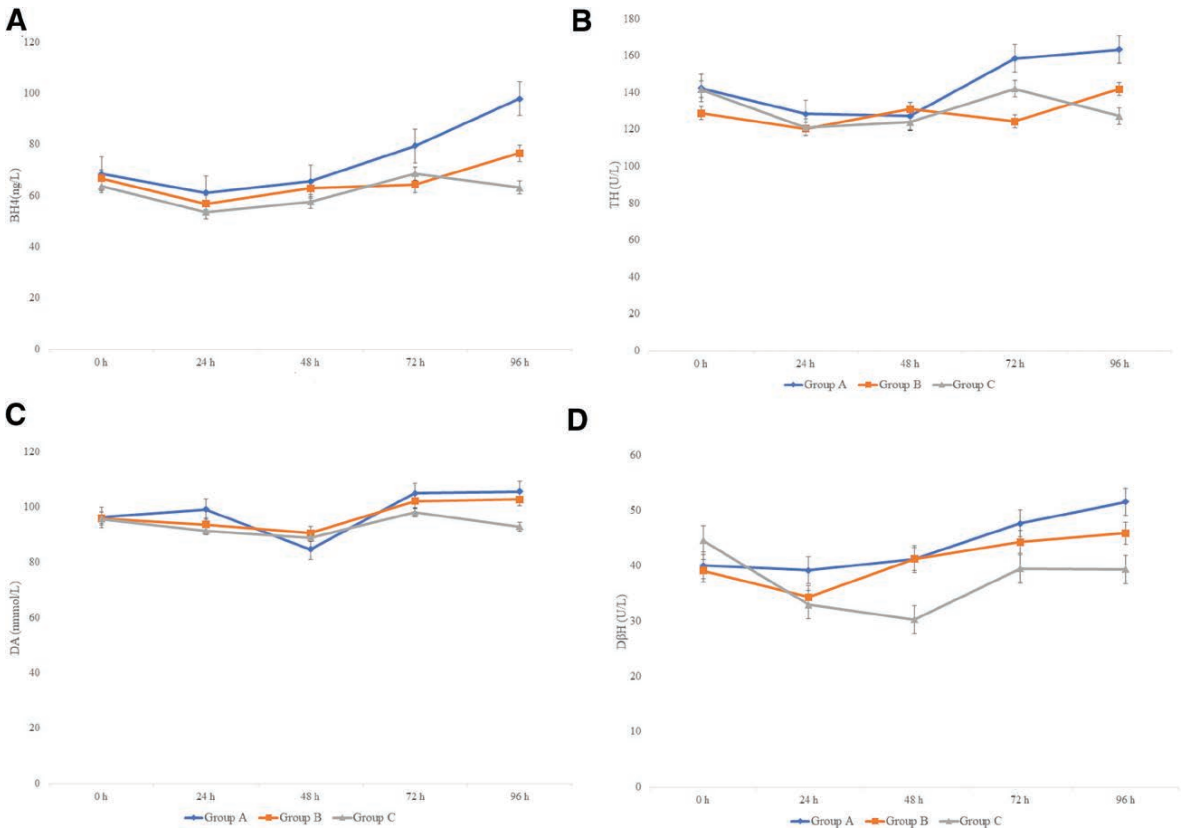

Figure 2. Line graphs of changes in BH4, TH, DA, and DβH. BH4 = tetrahydrobiopterin, DA = dopamine, DβH = dopamine-β-hydroxylase, TH = tyrosine hydroxylase.

Table 2

BH4, TH, DA, and DβH.

	Time (h)	Group A (n = 20)	Group B (n = 20)	Group C (n = 18)	*P*	P_{A-B}	P_{A-C}	P_{B-C}
BH_4 (ng/L)	0	68.52 ± 11.17	66.76 ± 12.25	63.72 ± 12.55	.448	1.000	0.631	1.000
	24	61.09 ± 16.70	56.89 ± 12.13	53.59 ± 13.25	.252	1.000	0.300	1.000
	48	65.52 ± 10.59	62.92 ± 10.04	57.70 ± 9.84	.056	1.000	0.055	0.331
	72	79.40 ± 12.25	64.42 ± 14.66	68.64 ± 12.63	.002	0.002	0.038	0.954
	96	97.83 ± 7.84	76.55 ± 14.24	63.16 ± 14.77	<.001	<0.001	<0.001	0.004
	Δ96	0.47 ± 0.30	0.19 ± 0.36	0.03 ± 0.28	<.001	0.017	<0.001	0.304
TH (U/L)	0	142.39 ± 24.11	128.77 ± 23.63	141.62 ± 21.69	.114	0.185	1.000	0.256
	24	128.36 ± 36.73	120.31 ± 18.58	121.10 ± 23.20	.582	1.000	1.000	1.000
	48	127.23 ± 21.34	130.93 ± 27.26	123.82 ± 23.20	.648	1.000	1.000	1.000
	72	158.47 ± 32.49	124.31 ± 20.92	142.03 ± 23.92	<.001	<0.001	0.159	0.113
	96	163.33 ± 22.16	141.83 ± 14.51	127.28 ± 15.43	<.001	<0.001	<0.001	0.037
	Δ96	0.19 ± 0.33	0.16 ± 0.21	−0.07 ± 0.23	.001	1.000	0.002	0.017
DA (nmol/L)	0	96.27 ± 13.55	95.84 ± 14.22	95.60 ± 9.46	.986	1.000	1.000	1.000
	24	99.16 ± 12.02	93.67 ± 14.20	91.45 ± 16.18	.21	0.636	0.270	1.000
	48	84.74 ± 9.65	90.49 ± 8.21	88.96 ± 12.19	.168	0.206	0.571	1.000
	72	104.95 ± 9.94	102.07 ± 16.71	97.98 ± 11.54	.25	1.000	0.294	0.982
	96	105.57 ± 12.58	102.77 ± 20.55	92.93 ± 12.00	.035	1.000	0.040	0.155
	Δ96	0.12 ± 0.23	0.09 ± 0.26	0.02 ± 0.18	.142	1.000	0.173	0.439
DβH (U/L)	0	40.04 ± 4.98	39.10 ± 6.86	44.62 ± 8.11	.028	1.000	0.106	0.036
	24	39.18 ± 6.87	34.35 ± 7.46	32.95 ± 6.47	.015	0.084	0.019	1.000
	48	41.18 ± 8.28	41.22 ± 7.16	30.30 ± 7.07	<.001	1.000	<0.001	<0.001
	72	47.62 ± 6.58	44.29 ± 4.73	39.51 ± 7.09	<.001	0.256	<0.001	0.053
	96	51.54 ± 9.63	45.87 ± 9.39	39.38 ± 7.76	<.001	0.137	<0.001	0.079
	Δ96	0.31 ± 0.31	0.21 ± 0.33	−0.08 ± 0.29	<.001	0.830	<0.001	0.018

BH4 = tetrahydrobiopterin, DA = dopamine, DβH = dopamine-β-hydroxylase, TH = tyrosine hydroxylase.

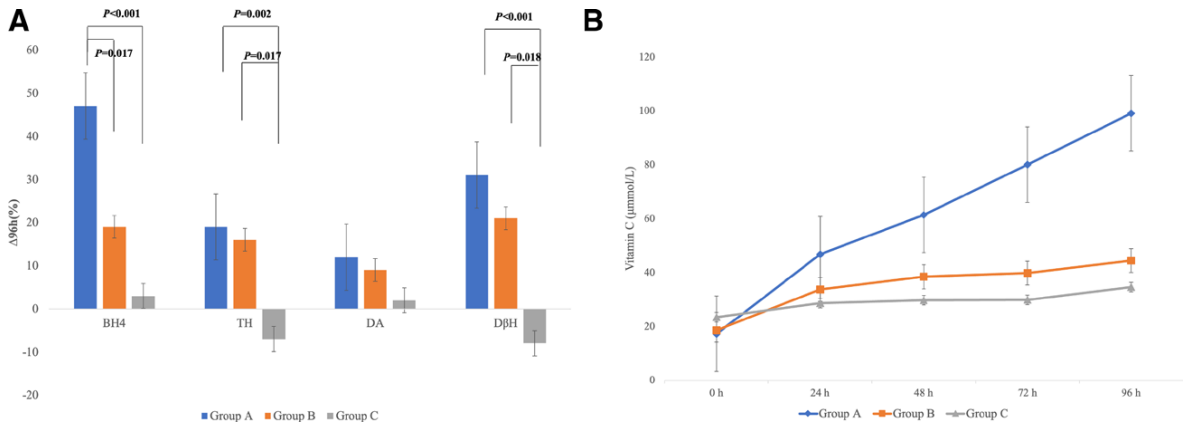

Figure 3. Δ96 of NE synthesis-related indicators and Vitamin C levels. NE = norepinephrine.

Table 3

NE levels and NE dosage.

	Time (h)	Group A (n = 20)	Group B (n = 20)	Group C (n = 18)	*P*	P_{A-B}	P_{A-C}	P_{B-C}
NE (ng/L)	0	220.50 ± 39.12	232.60 ± 46.31	243.92 ± 41.65	.227	1.000	0.260	1.000
	24	239.13 ± 47.64	203.24 ± 38.83	197.92 ± 30.95	.003	0.015	0.006	1.000
	48	238.12 ± 48.28	233.03 ± 56.62	192.30 ± 54.55	.017	1.000	0.026	0.056
	72	272.73 ± 59.87	233.05 ± 27.99	237.76 ± 38.84	.01	0.016	0.047	1.000
	96	318.72 ± 40.95	267.22 ± 59.19	227.06 ± 43.17	<.001	0.003	<0.001	0.035
	Δ96	0.49 ± 0.35	0.16 ± 0.23	-0.03 ± 0.31	<.001	0.002	<0.001	0.135
NE dosage (μg/(kg·min))	0	0.19 ± 0.11	0.16 ± 0.08	0.19 ± 0.08	.876	0.348	1.000	0.256
	24	0.17 ± 0.09	0.20 ± 0.10	0.22 ± 0.10	.653	0.278	0.185	0.987
	48	0.20 ± 0.13	0.26 ± 0.21	0.29 ± 0.19	.467	0.376	0.234	0.432
	72	0.16 ± 0.10	0.21 ± 0.10	0.26 ± 0.13	.087	0.254	0.051	0.189
	96	0.11 ± 0.09	0.19 ± 0.15	0.26 ± 0.11	.038	0.083	0.005	0.167
	Δ96	−0.34 ± 0.70	0.04 ± 0.58	0.34 ± 0.38	.035	0.087	0.002	0.181

Δ96 = ratio of increased NE at 96 hours to the baseline value. NE = norepinephrine.

3.5. Primary and secondary outcomes

As the treatment continued, the APACHE, SOFA, and MODS scores gradually decreased in all patients; however, no significant reduction was observed in the vitamin C treatment groups (Appendix 1, Supplemental Digital Content 2, http://links.lww.com/MD/M198, Additional Tables 5, 6, and 7). Although the duration of ICU stay, mechanical ventilation, and total length of hospital stay were not reduced in the vitamin C group, the 28-days mortality was lower than that in the non-vitamin C group (0%, 10%, 16.67%, *P* = .187) (Appendix 1, Supplemental Digital Content 2, http://links.lww.com/MD/M198, Additional Table 8); however, this difference was not significant (Appendix 1, Supplemental Digital Content 3, http://links.lww.com/MD/M199, Additional Figure 2). No vitamin C-related adverse events were observed.

4. Discussion

In the present study, we found that high-dose vitamin C significantly increased plasma NE, DβH, TH, and BH4 concentrations but had a weak influence on DA levels. Vitamin C can promote NE synthesis and reduce the dose of exogenous NE, consequently reducing the adverse effects of NE in septic shock patients. However, similar to previous studies, our study did not observe a considerable reduction in clinical outcomes or mortality with high-dose vitamin C..

4.1. Vitamin C and norepinephrine synthesis

Vitamin C is a physiological antioxidant required for 2 the steps of catecholamines (DA, epinephrine, and NE) biosynthetic pathway.[15,16] It is a cofactor for DβH, which employs oxygen to introduce a hydroxyl group to dopamine to form NE. Subsequently, the methyltransferase-catalyzed methylation of the amine group of NE forms epinephrine. Moreover, vitamin C may promote reprocessing of BH4, thereby accelerating the rate-limiting step in the synthesis of DA.[17] BH4, along with molecular oxygen and iron, acts as a cofactor for TH and is responsible for catalyzing the conversion of the amino acid L-tyrosine to L-3,4-dihydroxyphenylalanine. L-3,4-dihydroxyphenylalanine is a precursor of DA, which in turn is a precursor of NE and epinephrine. In addition, vitamin C enhances TH synthesis and α- and β-adrenergic receptor activities.[18] Vitamin C promotes NE production by enhancing the activity of NE synthesis-related enzymes that do not rely on its antioxidant properties. Saturated vitamin C is necessary in endothelial cells to protect BH4 from oxidation and provide optimal conditions for cellular nitric oxide synthesis. Radical scavenging by vitamin C can provide beneficial vascular effects and protect nitric oxide from inactivation. In the present study, the Δ96 h values of TH and DβH in the vitamin C treatment groups were noticeably higher than those in the control group. This effect became more pronounced over the course of vitamin C administration; however, DA synthesis was only weakly affected. The reason for this difference is unclear; however, it may be that DA is the last step in NE synthesis and is susceptible to other factors. The small sample size in this study may be another reason.

4.2. Vitamin C and exogenous vasopressor

As demonstrated in our study, Vitamin C deficiency was frequently observed in critically ill patients. Excessive amounts

of reactive oxygen species (ROS), inadequate supplementary exogenous vitamin C, high metabolic expenditure, and dilution-related deficiency following fluid resuscitation may lead to a shortage of vitamin C in patients with septic shock. In terms of pathophysiology, vitamin C supplementation may reduce ROS-induced damage to the endothelial cells, improve tissue perfusion and oxygenation, and alleviate organ dysfunction. These pathophysiological protective effects appear to relieve organ injury and dysfunction in patients with sepsis or septic shock.

Several clinical studies have focused on the effect of vitamin C, and the results demonstrated that vitamin C could reduce the dosage of vasopressors.[8–10] A very high-dose of vitamin C (30 g over 1 h followed by 30 g over 5 h) limits the dose and duration of vasopressor treatment and results in rapid reductions over time.[19] However, a pilot RCT revealed no significant decrease in the mean dose or duration of vasopressor infusion of intravenous vitamin C (25 mg/kg every 6 h).[20] These inconsistent findings may be related to differences in the vitamin C dosage and duration of administration. Some studies have suggested that patients with sepsis in developing countries are more likely to benefit from intravenous vitamin C.[21] In our study, we demonstrated a higher plasma NE level and a reduction in exogenous NE dosage with vitamin C treatment, especially in the high-dose vitamin C group. However, no significant improvement was observed in the duration of ICU stay, mechanical ventilation, or the length of hospital stay.

4.3. Vitamin C and clinical outcomes in patients with sepsis

Although vitamin C has been demonstrated to reduce the dosage of vasopressors and shorten the ICU stay and the need for mechanical ventilation, its clinical improvement is currently in question.[8–10,22] Marik et al reported that the early utilization of intravenous vitamin C, together with hydrocortisone and thiamin, effectively prevents progressive organ dysfunction and reduces septic shock mortality.[23,24] A significant reduction in 28-days all-cause mortality was observed in the CITRIS-ALI trial, which focused on the effect of intravenous vitamin C infusion in patients with sepsis-induced acute respiratory distress syndrome.[25] However, vitamin C did not reduce the SOFA score or biomarker levels even at high doses. Compared with other studies on sepsis, the delayed administration of vitamin C infusion in this study due to the delay between the onset of shock and the development of acute respiratory distress syndrome may have limited its effect on the SOFA score and biomarkers. In an RCT of 216 patients with septic shock, Fujii et al revealed that adjunctive vitamin C treatment did not significantly decrease vasopressor-free time over 7 days or short-term or long-term mortality.[26] Apart from the change in SOFA score at 3 days, there was no significant improvement in vasopressor dosage, length of ICU stay, or length of hospital stay. However, as the authors noted, the trial was underpowered to detect differences in mortality and the possible individual effects of vitamin C were not assessed separately. Unlike these early trials, most trials did not find a marked reduction in mortality from vitamin C. Like most studies, we did not observe a marked decrease in APACHE, SOFA, or MODS score or 28-days mortality in the vitamin C group. However, 3 patients in the control group died during follow-up, which was higher than that in the intervention group.

In 2022, the LOVIT (Lessening Organ Dysfunction with Vitamin C) trial, the largest trial to date addressing this question in 863 patients with sepsis requiring vasopressors, reported that high-dose vitamin C (200 mg/kg/d for 96 h) may be harmful to patients with severe sepsis.[13] The primary outcome was the composite of death and persistent organ dysfunction on 28-days. Unexpectedly, the primary outcome increased in the vitamin C group (risk ratio, 1.21; 95% CI, 1.04–1.40; $P = .01$), but no difference was detected after adjusting for age, sex, illness severity, steroid use, or time to randomization. No differences in secondary outcomes (including length of ICU stay, SOFA score, or death within 6-month) were observed between the intervention and placebo groups. A Bayesian reanalysis of the LOVIT trial showed that vitamin C is associated with a high probability of harm in patients with sepsis.[27] However, we still should not completely dismiss the role of vitamin C in patients with sepsis. In fact, a concurrently published meta-analysis of 41 relevant RCTs involving 4915 patients, including the LOVIT trial, reported that vitamin C might reduce mortality with low certainty evidence.[28] A potential explanation for the harmful effects of vitamin C in the LOVIT trial is that vitamin C was administered at a pH as low as 5.0, which is 3 to 4-fold higher than that used in previous RCTs.[29]

Heterogeneity in the treatment effects of intravenous vitamin C may be due to a multitude of causes. Moreover, the effects of vitamin C may also be associated with certain conditions. To date, the dose, initiation, and duration of vitamin C administration and its combination with other drugs have varied between trials. Scholz et al reported no reduction in mortality in patients treated with vitamin C.[11] However, the timing of the pooled mortality assessment indicated a reduction in short-term mortality. Interestingly, vitamin C treatment for 3 to 4 days significantly improved survival compared to treatment for only 1 to 2 or > 5 days. Intravenous vitamin C intake for ≥5 days was significantly associated with decreased hospital and 90-d mortality in sepsis patients.[30] Septic shock patients with more severe conditions, such as hypoalbuminemia or severe organ failure, might benefit more from early administration within 6 hours after septic shock recognition.[31] In most studies, the dose of vitamin C was 1.5 g or 25 to 50 mg/kg every 6 hours.[11] Megadose vitamin C (30 g over 1 h followed by 30 g over 5 h) induced a significant increase in urine volume and a greater reduction in the vasopressor dose and SOFA score over time than other agents.[19] In our study, the dosage of vitamin C was 50 mg/kg/d for 4 days in the low-dose group and 150 mg/kg/d for 4 days in the high-dose group, which was consistent with the doses used in many trials. We also detected an increase in the urine volume in the vitamin C group. However, this could not be completely ruled out as an effect of the different baseline renal functions. In addition, no significant improvement in clinical outcomes was detected in our study and no adverse events were observed in the high- or low-vitamin C groups.

However, whether the effect of vitamin C depends on the type of sepsis remains unclear. An animal study demonstrated that vitamin C reversed pathophysiological and behavioral responses to gram-negative sepsis without adverse side effects.[32] Compared with peritonitis-related sepsis models, machine learning and murine models demonstrated minimal improvement in pneumonia caused by hydrocortisone, ascorbic acid, and thiamin therapy.[33] Vitamin C may be associated with a decrease in the 28-days mortality in ventilated patients with sepsis shock.[34] In summary, an individual and targeted vitamin C dosing regimen is essential for maximizing the protective effects of vitamin C in patients with septic shock. In future studies, it will be crucial to determine the optimal dose, treatment initiation time, and duration of vitamin C administration.

5. Limitations

This study has some limitations. First, the sample size was small; therefore, this trial was underpowered to detect the differences in mortality and other outcomes. However, each NE synthesis indicator was tested every 24 hours and the trends were clear. Second, the starting time and duration of antibiotic administration were not collected; however, all the patients received guideline-adherent treatments. Third, data on auxiliary medications were not collected, which may have led to the biased

results. Fourth, none of the patients in the high-dose vitamin C group died; however, the strength of its strong survival effect may have been weakened by its small size.

6. Conclusion

In patients with septic shock, treatment with intravenous vitamin C increased TH, BH4, and DβH levels but had a weak influence on DA levels. Vitamin C can reduce exogenous NE dosage; however, it does not significantly improve the clinical outcomes.

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