ORIGINAL RESEARCH

Vitamin C Therapy for Routine Care in Septic Shock (ViCTOR) Trial: Effect of Intravenous Vitamin C, Thiamine, and Hydrocortisone Administration on Inpatient Mortality among Patients with Septic Shock

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Abstract

Background: Sepsis remains a leading cause of death worldwide despite advances in management strategies. Preclinical and observational studies have found mortality benefit with high-dose vitamin C in sepsis. Our study aims to prospectively evaluate the effect of intravenous hydrocortisone, vitamin C [ascorbic acid (AA)], and thiamine (HAT) administration in reducing inpatient all-cause mortality among patients with septic shock.

Materials and methods: Our single-center, prospective, open-label, randomized controlled trial recruited patients with admitting diagnosis of septic shock and assigned eligible patients (1:1) into either intervention (HAT) or control group (routine). The HAT group received intravenous combination of vitamin C (1.5 g every 6 hours), thiamine (200 mg every 12 hours), and hydrocortisone (50 mg every 6 hours) within 6 hours of onset of septic shock admission. The treatment was continued for at least 4 days, in addition to the routine standard of care provided to the control group. Thiamine and hydrocortisone use in control arm was not restricted. Vitamin C levels were estimated at baseline and at the end of the 4 days of treatment for both groups. The primary outcome evaluated was mortality during inpatient stay.

Results: Among 90 patients enrolled, 88 patients completed the study protocol. The baseline characteristics between the HAT ($n = 45$) and the routine ($n = 43$) groups were comparable. The all-cause mortality in the HAT cohort was 57% (26/45) compared to 53% (23/43) in the routine care group ($p = 0.4$, OR 1.19, 95% CI 0.51–2.76). The time to reversal of septic shock was significantly lower in the HAT (34.58 $\pm$ 22.63 hours) in comparison to the routine care (45.42 $\pm$ 24.4 hours) ($p = 0.03$, mean difference −10.84, 95% CI −20.8 to −0.87). No significant difference was observed between the HAT and the routine care with respect to changes in sequential organ failure assessment (SOFA) scores at 72 hours (2.23 $\pm$ 2.4 vs 1.38 $\pm$ 3.1), the use of mechanical ventilation (48% vs 46%), and mean Vasoactive Inotropic Score (7.77 $\pm$ 12.12 vs 8.86 $\pm$ 12.5).

Conclusion: Intravenous administration of vitamin C, thiamine, and hydrocortisone did not significantly improve the inpatient all-cause mortality among patients with septic shock.

Clinical significance: HAT protocol does not reduce hospital mortality but decreases time to shock reversal in septic shock.

Keywords: Ascorbic acid, HAT protocol, Mortality, Sepsis, Septic shock, Vitamin C.

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Introduction

Sepsis remains as a life-threatening medical condition of dysregulated immune response with a mortality rate exceeding 50% in septic shock. More than 5 million deaths globally could be attributed to sepsis.[1,2] Given the high burden of sepsis worldwide, the search continues for effective targeted therapies in improving sepsis outcomes, especially in developing countries with a focus on inexpensive, safe, and accessible interventions.[3,4] Besides the short-term mortality, septic patients suffer from numerous short- and long-term complications, with a reduced quality of life and an increased risk of death following acute event.[5]

Sepsis is the result of a complex interaction between the host response and infecting organism resulting in severe organ dysfunction.[6] Widespread distribution of proinflammatory mediators plays an important role in the pathogenesis and high morbidity and mortality associated with sepsis. High cellular production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) contributes to oxidative stress, leading to consequent tissue and organ damage. Vitamin C is an essential water-soluble

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nutrient which plays a role in mediating inflammation through antioxidant activities and is also important in the synthesis of cortisol, catecholamines, and vasopressin, which are key mediators in the disease process.[7] It is understood to synergistically act with steroids to protect against and reverse vascular endothelial dysfunction caused by the endotoxin released during sepsis. The high dose of vitamin C is also thought to increase the risk of kidney damage by leading to the formation of oxalate crystals. Oxidation of the oxalate to carbon dioxide occurs by a series of steps that include the enzyme glyoxylate aminotransferase which requires thiamine pyrophosphate as a coenzyme.

Recent evidence-based research has suggested the beneficial impact of vitamin C administration on patients with severe sepsis and septic shock. Preliminary studies indicated positive effect on multiple organ failure and endothelial injury.[8,9] The administration of thiamine and hydrocortisone has been independently reported to improve sepsis outcomes, such as lactate clearance and shock reversal.[10,11] Reduction of sequential organ failure assessment (SOFA) scores, time to shock resolution, and proinflammatory biomarkers have been reported with Vit C administration.[8,12] VITAMINS trial did not observe any improvement in time alive and vasopressor-free duration.[13] Ambiguity existed in relation to the beneficial effects of vitamin C as several recent randomized clinical trials failed to demonstrate a positive impact on clinical outcomes, warranting further research. Accordingly, our randomized clinical trial investigated the effect of hydrocortisone, vitamin C [ascorbic acid (AA)], and thiamine (HAT) intravenous administration on improving all-cause mortality among patient with septic shock.

Materials and Methods

Study Design and Setting

This Vitamin C Therapy Or Routine care in septic shock (ViCTOR) Trial is an investigator initiated, prospective, single-site, open-label, randomized controlled trial conducted at an academic tertiary care center in South India. The study was approved by the Institutional Research Ethics Committee (MD/MS2017/56) and registered with CTRI: (CTRI/2018/07/014787). Patient enrolment into the study commenced from June 2018 and continued till August 2019. Informed consent was obtained from patients or legal surrogate prior to study recruitment.

Study Participants

All patients admitted to the participating intensive care units (ICUs) of the tertiary referral teaching hospital were identified by an alert from the primary physician/nurse. Adult non-pregnant patients with septic shock (Surviving Sepsis Campaign 2016 guidelines[14]) and within 6 hours of initiation of inotropic support were eligible for recruitment. Patients with burns, limitations of care due to terminal illness or acute liver failure were excluded from the study.

Randomization

Once consent was obtained, patients were randomly assigned in 1:1 ratio into either the HAT or the routine group. Patient enrolment proceeded according to a preformed randomization list generated through an online randomization software, which employed random permuted block sizes of length 6 to ensure balance over time.

Study Intervention

Following an informed consent, the randomized patients in the HAT cohort received intravenous hydrocortisone (50 mg every 6 hours), vitamin C (AA) (1.5 g every 6 hours), and thiamine (200 mg every 12 hours) for 4 days, with the first doses of the drugs administered within 6 hours of onset of septic shock/admission, with routine care. Vitamin C was infused over 60 minutes using a photolysis preventing infusion set. Routine group received the standard of care for septic shock. The use of hydrocortisone and vitamin supplements in the control group was at the treating physician's discretion.

Data Collection

The data collected included basic demographics — Age, sex, admitting diagnosis, comorbidities, complete blood count, renal and liver function profile, procalcitonin, details of vasopressor use (hourly dosage) PaO_2/FiO_2, Glasgow Coma Scale (GCS), need for mechanical ventilation, length of ICU and hospital stay, acute kidney injury (AKI), blood culture, lactate, fluids administered, and SOFA score. ΔSOFA was calculated as the absolute difference between the SOFA scores at admission (ASOFA) and at 72 hours. Initial fluid resuscitation was performed with normal saline as per surviving sepsis campaign guidelines. New onset of AKI was defined as the increase in serum creatinine to ≥1.5 times the baseline value after initiation of the HAT protocol.[15] The Inotrope Score (IS) was calculated by adding dopamine dose (μg/kg/minute) + dobutamine dose (μg/kg/minute) + 100 × epinephrine dose (μg/kg/minute). The Vasoactive Inotropic Score (VIS) is IS + 10 × milrinone dose (μg/kg/minute) + 10,000 × vasopressin dose (units/kg/minute) + 100 × norepinephrine dose (μg/kg/minute).[16] The vitamin C level at baseline (prior to therapy) and after 4 days was collected for both groups. Data collected were compiled in a spreadsheet by a trained medical staff and data of 30 randomly selected patients were independently cross-checked and verified for data fidelity by another investigator.

Outcome Measures

The primary outcome evaluated was inpatient all-cause mortality between the HAT and the routine groups. The secondary endpoints assessed in our study included time to shock reversal [time to shock reversal is defined as the amount of time (in hours) required to withdraw all vasoactive medication supports from recruitment in study] change in SOFA score over 72 hours, need for mechanical ventilation (institution of invasive mechanical ventilation for respiratory support), incidence of new onset of AKI, ICU, and hospital length of stay (LOS).

Study Measurements

AA Measurements in Plasma

Two blood samples were collected per patient for vitamin C assay from both the study groups. The initial sample was collected before the first dose of vitamin C was administered and the final sample between 2 and 6 hours after the last dose of vitamin C on day 4. Concentration of AA in plasma was determined using a validated

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high-performance liquid chromatography mass spectrometry (HPLC-MS/MS) method.[17] Protein precipitation extraction (PPE) technique was used to separate AA from the complex biological matrix. All the sample treatment and extraction of AA from plasma were carried out under monochromatic light and water bath (2–8°C). Ascorbic acid 13C6 was used as an internal standard for this analysis.

Samples were treated with dithiothreitol and metaphosphoric acid solution to increase the stability of AA in blood/plasma. For extracting AA from plasma, thawed 50 µL plasma aliquots added with 20 µL of internal standard solution and 1 mL of 2% formic acid each were centrifuged and 300 µL of the supernatant was added with 300 µL of 2 mM of ammonium formate solution. Final samples were transferred to autosampler of HPLC for analysis. Ascorbic acid was detected in the electrospray ionization (ESI) negative ion mode. Eight point calibration curves were constructed in drug-free plasma for AA, concentration ranging from 0.5 to 80 µg/mL. The complete protocol in detail for AA estimation in blood/plasma is available in Appendix I.

Study Sample Size Determination

Based on the mortality rates in control and interventional groups reported by Marik et al., a minimum sample size of 84 patients was calculated using nMaster© software with an estimated risk difference of 32% at 80% power and alpha error of 0.05.[18]

Statistical Analysis

Descriptive statistics were used to summarize the key outcome measures. The baseline characteristics and treatment outcomes were compared between the routine care and the HAT groups. Continuous variables were analyzed using Student's *t* or Mann–Whitney *U* tests and categorical variables were compared using Chi-square or Fisher's exact tests. Outcomes observed to be statistically significant across the study arms were reanalyzed by adjusting for corticosteroid therapy.[19] Kaplan–Meier plots were used to evaluate the survival of patients in each group. A log-rank test was run to determine if there were differences in the survival distribution between the HAT and the routine groups. Statistical software SPSS 20.0 version (IBM, USA) was used for the analysis. A two-sided *p* value of <0.05 was considered statistically significant.

Results

Patient Characteristics

The CONSORT (consolidated standards of reporting trials) diagram of patient enrolment is provided in Flowchart 1. A total of 2,297 patients were screened among the two participating ICUs in the hospital, between June 2018 and August 2019. Three hundred and one patients were found to be eligible and 90 were randomized for the study after consent, with 45 in each group. Two patients were excluded from the routine care group for final per-protocol analysis as they received vitamin C inadvertently. Baseline characteristics on ICU admission were comparable between the groups and are enumerated in Table 1. Pneumonia (42%) was the major focus of infection for sepsis in our study cohort. Primary outcomes and secondary outcomes were assessed for the study cohort according to per-protocol analysis and intention-to-treat (ITT) analysis.

Study Outcomes

Primary Endpoint

The primary endpoint of all-cause mortality was 53% (23/43) and 57% (26/45) in the routine care and the HAT groups, respectively, and was not significantly different (Table 2). The survival distributions as per Kaplan–Meier analysis did not exhibit a significant difference between the HAT and the routine study groups (log-rank test: $p = 0.4$) (Fig. 1A).

Secondary Endpoints

The mean time to shock reversal was significantly lower in the HAT group (34.58 ± 22.63 hours) in comparison to the routine group (45.42 ± 24.4) ($p = 0.03$, mean difference −10.84, 95% CI −20.8 to −0.87). However, no significant difference was observed between the HAT and the routine groups in survival distributions with respect to mean time to shock reversal as per Kaplan–Meier plots (log rank test: $p = 0.05$) (Fig. 1B). The time to shock reversal, which was found to be significant ($p = 0.03$) between the HAT and the control arms, was reanalyzed after adjustment to steroid use. Hours to shock reversal remained significant ($p = 0.04$) after regression methods where steroid use was considered as a covariate. Shock reversal within 12 hours of enrolment was significantly higher in the HAT group ($n = 14$, 31%) as compared to the control group ($n = 4$, 9%) ($p = 0.01$). Similar results were observed for shock reversal at 36 hours ($p < 0.001$).

Average length of hospital stay was significantly higher in the HAT group (20.9 ± 15.01:31.58 ± 31.06, $p = 0.04$). After adjusting for outliers, the average LOS between the study groups did not significantly differ ($p = 0.19$). Baseline procalcitonin at ICU admission ($p = 0.18$) and procalcitonin clearance ($p = 0.1$) did not significantly differ between the study groups. Other secondary outcomes including change in SOFA over 72 hours, length of ICU stay, duration of mechanical ventilation, and the highest VIS score did not exhibit a significant difference between the study groups. New onset of AKI was observed to be 7 (15%) in the HAT and 5 (12%) in the routine groups (Table 2). The mean VIS score among the groups over the 4 days were comparable and is charted in Figure 2.

SOFA Score Subgroup Analysis

SOFA score on admission was 10.89 ± 3.82 in the routine group and 11.2 ± 2.99 in the HAT group. Distribution of patients across the SOFA score categories (2–7, 8–11, >11) were also comparable between the two groups ($p = 0.3$). A subgroup analysis revealed that the survival proportions of patients were significantly different between the HAT and the routine groups with respect to SOFA score categories ($p = 0.005$). The proportion of patients belonging to SOFA category of 2–7 in the HAT group (75%) who survived was higher than patients belonging to the same SOFA category in the routine group (50%). An increasing survival trend was observed for patients in the HAT group in comparison to the routine group as SOFA score severity decreased (Fig. 3).

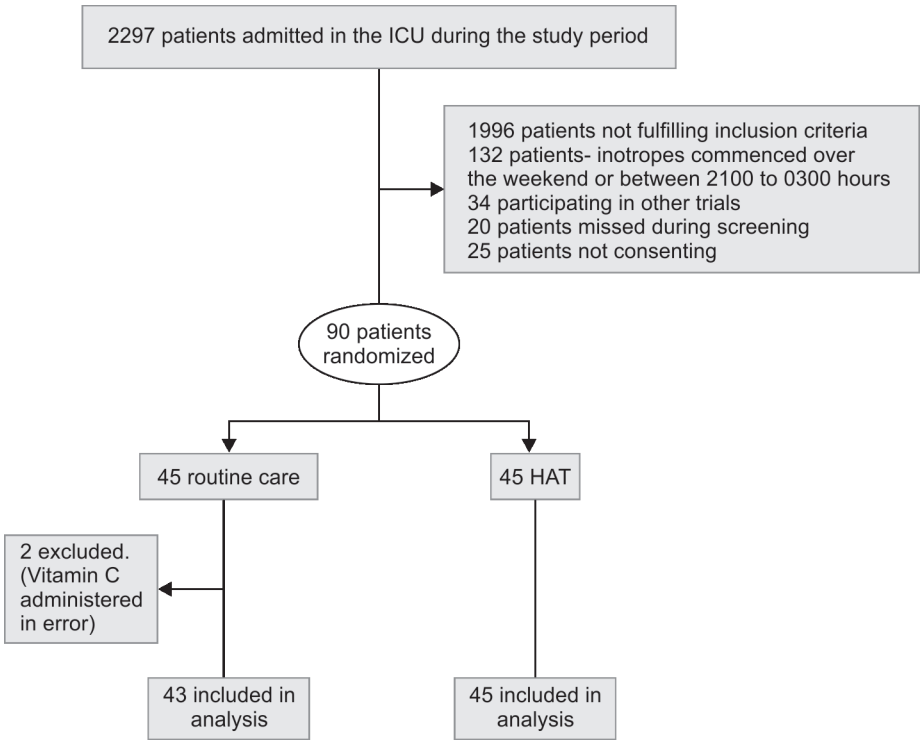
Microbiological Profile

The microbiological profile of the routine group and the HAT group is provided in Table 3. The proportion of multidrug resistant (MDR) bacteria in the routine and the HAT groups was 55.58 and 55.5%, respectively. Gram-negative organisms, mainly *Klebsiella* (35%) and *E. coli* (18%), predominated the positive cultures. Of note, the proportion of cultures positive for *Klebsiella* (68%) ($p = 0.01$) and *Candida* (64%) ($p = 0.28$) was observed to be higher in the HAT group.

AA Levels

Mean baseline values of vitamin C did not significantly differ between the study groups, with 1.49 ± 1.39 and 2.1 ± 2.61 µg/mL in the routine and the HAT groups, respectively ($p = 0.6$).

Flowchart 1: Patient recruitment flowchart



Posttreatment regimen, the vitamin C levels measured 2.2 ± 4.1 and 94.2 ± 107.42 µg/mL in the routine and the HAT groups, respectively ($p < 0.001$). Outliers (>90th percentile) and inconsistent AA measurements reported for nine patients were removed from the final analysis. No adverse events attributable to HAT therapy were recorded during the study.

ITT Analysis

A total of 90 patients randomized in the study into 45 patients each in the routine and the HAT groups were evaluated for primary and secondary outcomes as per ITT. Inpatient mortality was reported to be 49% (25) and 51% (26) in the routine and the HAT groups, respectively, and no significant difference was observed. The mean time to shock reversal was significantly higher in the routine group (45.42 ± 23.8) in comparison to the HAT group (34.58 ± 22.63) ($p = 0.03$). The major secondary outcomes including the change in SOFA scores over 72 hours, length of ICU stay, and the highest VIS score did not exhibit a significant difference between the study groups.

Discussion

In our randomized controlled trial, we found that the HAT protocol (combination of hydrocortisone, AA, and thiamine) within 6 hours of diagnosis of septic shock, did not reduce all-cause in-hospital mortality in patients with septic shock. Our results are consistent with other recently published randomized controlled trials that explored the use of HAT protocol in sepsis and septic shock.[12,13,20,21]

The ViCTOR trial was performed at a tertiary care teaching center in South India. A comparison of other five randomized controlled trials that investigated the use of AA (and its combination) in sepsis or septic shock is presented in Table 4. The trial which had looked at similar outcomes was VITAMINS trial but they had initiated the HAT protocol later in the course of sepsis after a mean time of 23 hours of commencement of inotropes, which was perceived as the reason for the lack of benefit of the HAT protocol. The delay in the initiation of the HAT protocol was addressed in our protocol in which HAT was strictly initiated within 6 hours but clear mortality benefit could not be demonstrated. The lack of mortality benefit might be due to the fact that our HAT cohort was sicker as evidenced by higher mean ASOFA scores (11.22 ± 2.99) compared to the study cohort described in Marik et al. (mean ASOFA 8.3) and VITAMINS trial (mean ASOFA 8.4). However, our cohort was sicker than the cohort of VITAMINS trial as evidenced by the higher ASOFA with a considerable proportion of them falling into ASOFA >11 category (33%). Our cohort had high prevalence of Gram-negative and MDR organisms. Our mortality is consistent with those published from the subcontinent in patients with septic shock.[22] There was higher incidence of culture positivity for *Klebsiella* and *Candida* in the HAT group which could have played a role in tilting the balance of the survival rates in the HAT group. Similarly, the LOS was observed to be significantly higher in the HAT cohort when compared to the routine group. However, no significant difference was observed in LOS between the HAT and the routine groups when adjusted for outliers in LOS for the whole cohort by removing outliers greater than 90th percentile ($p = 0.19$) and 1.5 times interquartile range (IQR) ($p = 0.49$). The mean SOFA scores at admission were also higher in the HAT cohort (11.22 ± 2.99) relative to the routine group (10.89 ± 3.82) without statistical significance ($p = 0.64$) and higher SOFA scores are reported to be associated with prolonged LOS.[23,24]

The mean time for shock reversal showed significant reduction in the HAT group as compared to the routine group. The early shock reversal observed in the HAT group was observed to be significant even after adjustment to use of corticosteroid therapy. Although

Table 1: Baseline characteristics of study cohort

Characteristics	*Routine (43)*	*HAT (45)*
Age, mean (range)	59.37 ± 15.01	58.69 ± 14.89
Male	32 (74)	31 (69)
Weight	66.35 ± 12.5	63.8 ± 12.4
Hypertension	21 (48)	21 (46)
Diabetes mellitus	25 (58)	22 (48)
CAD	10 (20)	4 (8)
CKD	11 (25)	6 (14)
CLD	13 (30)	12 (26)
COPD	2 (4)	1 (2)
CVA	2 (4)	1 (2)
Malignancy	10 (20)	12 (25)
Immunosuppression	6 (13)	4 (8)
Pneumonia	16 (37)	21 (46)
Urosepsis	9 (21)	12 (26)
Abdomen	14 (32)	17 (37)
Skin and soft tissue sepsis	6 (14)	2 (4)
Multidrug resistance	23 (47)	26 (53)
PaO_2/FiO_2	292.2 ± 201.4	311.1 ± 255.5
HR (/minute)	100 ± 22.9	102.8 ± 23.4
RR (/minute)	23.14 ± 5.9	23.02 ± 6.3
GCS	15 (2–15)	15 (2–15)
CRP (mg/dL)	143.57 ± 99.0	134.6 ± 22.1
Procalcitonin (ng/mL)	26.84 ± 34.18	16.38 ± 28.21
SOFA	10.89 ± 3.82	11.22 ± 2.99
Bilirubin (mg/dL)	4.69 ± 6.5	3.5 ± 6.7
Creatinine (mg/dL)	2.5 ± 1.9	2.8 ± 1.6
INR	1.84 ± 1.3	1.7 ± 0.8
Lactate (mmol/L)	3.99 ± 3.24	3.42 ± 2.20

these could not be translated into a mortality benefit, early time to shock reversal could make the HAT protocol a potential adjunct therapy in sepsis to provide a window of hemodynamic stability for the patient.

Hypovitaminosis is defined as an AA level of 23 µmol/L and severe AA deficiency defined as an AA level 11.3 µmol/L.[12] The measured vitamin C levels in the ORANGES trial, CITRIS-ALI and by Marik et al. were 21.7 ± 14.8, 17.9 ± 2.4, and 14.7 ± 11.8 µmol/L, respectively.[12,18,25] Our analysis of vitamin C levels in these patients presents an interesting picture of baseline hypovitaminosis (Vit C in the control group was 1.49 ± 1.39 and in the HAT group was 2.1 ± 2.61 µg/dL).

We see that although HAT protocol seems to be safe and is helpful in reducing the inflammatory markers and provides early shock reversal, mortality benefit was observed only in a *post hoc* subset of patients with a lower SOFA score. Chang and colleagues showed in their prespecified subgroup analysis that the HAT protocol may produce a mortality benefit when administered within 48 hours of diagnosis of sepsis.[21] There are signals from our study, ORANGES trial and from Chang and colleagues (HYVCTTSSS trial) that if the HAT protocol is initiated early, before circulatory and other organ failure ensues, it could be a useful adjunct therapy by reducing circulatory failure. However, this needs to be independently verified.

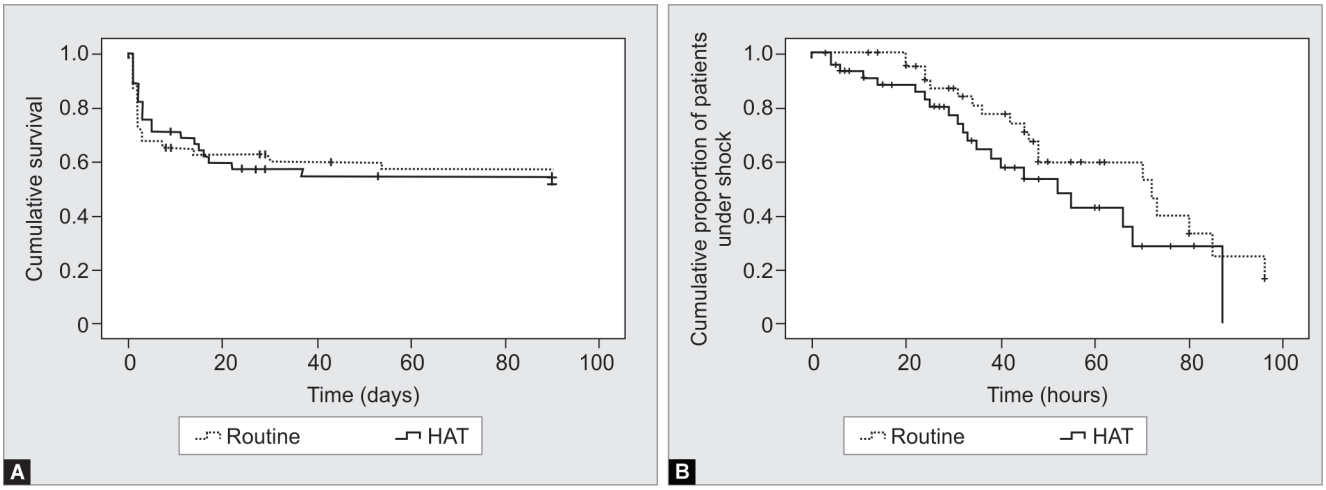
No adverse event attributable to vitamin C therapy was recorded in our study. New onset of AKI in the cohort had no significant association between the HAT and the routine groups. The drugs and dose we used for the trial intervention is the one used by Marik et al. in his study.[18] Whether a different dosage can produce a different effect cannot be determined.

Conclusion

We conclude that intravenous vitamin C, thiamine, and hydrocortisone in the dose and duration used did not affect hospital mortality in patients with septic shock. Vitamin C therapy seems to be safe. There is evidence of reduction in the inflammatory process

Table 2: Major outcomes of study cohort

Characteristics	*n = 88*	*Routine (43)*	*HAT (45)*	*p value*
Mortality (%)	49 (55)	23 (53)	26 (57)	0.4
Time to reversal of septic shock (hours)	39.8 ± 23.9	45.42 ± 24.4	34.58 ± 22.63	0.03
Mean vasoactive inotropic score on admission	8.39 ± 10.47	7.89 ± 10.66	8.86 ± 10.38	0.66
Highest vasoactive inotropic score	45.9 ± 71.5	45.4 ± 64.1	46.3 ± 78.7	0.93
Mean vasoactive inotropic score	8.3 ± 12.4	8.86 ± 12.5	7.77 ± 12.12	0.6
Mechanical ventilation	44 (50%)	20 (46.5)	22 (48.8)	0.4
Ascorbic acid levels				
Before regimen	1.8 ± 2.07	1.49 ± 1.39	2.1 ± 2.61	0.15
After regimen	51.8 ± 91.1	2.2 ± 4.1	94.2 ± 107.42	<0.001
SOFA@ 72 hours	9.11 ± 3.7	9.3 ± 3.8	8.9 ± 3.6	0.7
ΔSOFA	1.83 ± 2.7	1.38 ± 3.1	2.23 ± 2.4	0.22
New onset of AKI	12 (14)	5 (12)	7 (15)	0.4
Procalcitonin on day 3	12.38 ± 21.4	18.97 ± 27.90	6.73 ± 11.5	0.03
Procalcitonin clearance at 72 hours	682.8 ± 2288.72	152.7 ± 478.2	1118.3 ± 3012.9	0.1
Lactate on day 3	1.65 ± 0.91	1.79 ± 1.11	1.63 ± 0.85	0.9
Lactate clearance at 24 hours	5.98 ± 58.04	10.38 ± 44.14	2.1 ± 68.26	0.5
ICU LOS	10.49 ± 11.78	8.44 ± 8.16	12.44 ± 14.2	0.1
Hospital LOS	26.38 ± 25.0	20.9 ± 15.01	31.58 ± 31.06	0.043



Figs 1A and B: (A) Kaplan–Meier survival distributions for the two study groups with respect to mortality; (B) Kaplan–Meier survival distributions for the two study groups with respect to septic shock reversal

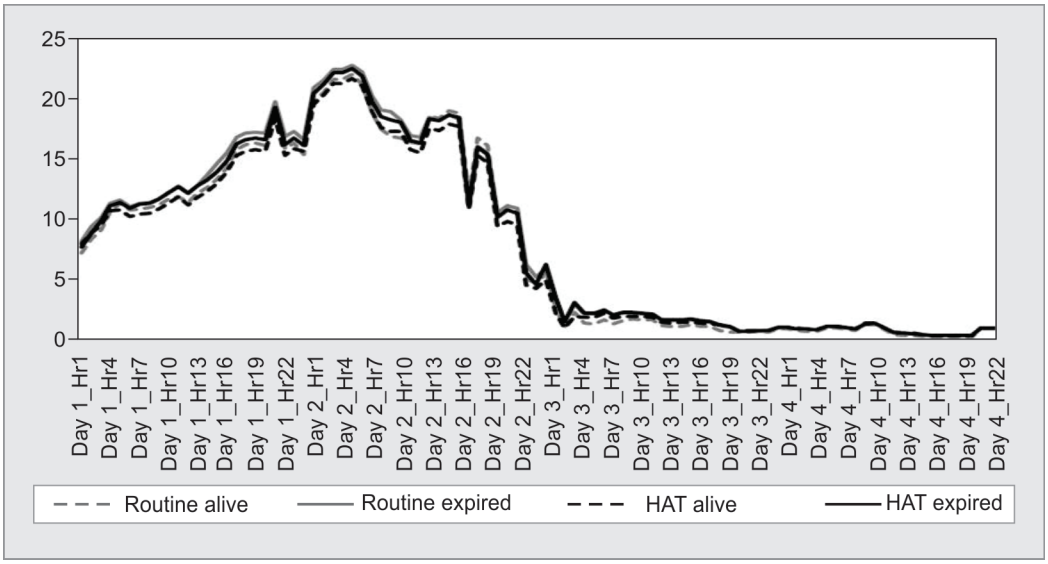


Fig. 2: Distribution of mean Vasoactive Inotropic Scores (VIS) of study groups over the treatment period

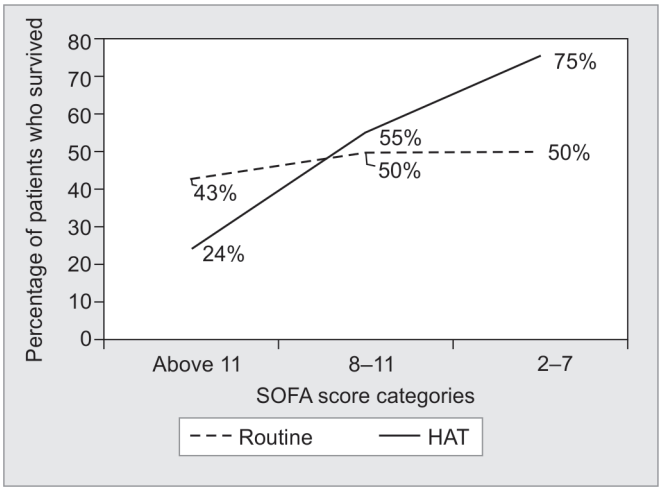


Fig. 3: Distribution of survived patient proportions among SOFA score categories in study groups

Table 3: Microbiological profile

Characteristics	*n = 88 (%)*	*MDR (%)*	*Control arm (43)*	*Vitamin C arm (45)*
Organisms				
Klebsiella pneumonia	31 (35)	24(77)	10 (32)	21 (68)
E. coli	16 (18)	12 (75)	7 (44)	8 (57)
Candida	11 (13)	7 (64)	4 (36)	7 (64)
Enterococcus	6 (8)	4 (67)	3 (50)	3 (50)
Pseudomonas	7 (8)	5 (71)	3 (43)	4 (57)
Acinetobacter	3 (3)	3 (100)	2 (67)	1 (33)

and therefore, the effect of earlier commencement of HAT protocol in reducing the mortality in sepsis needs further evaluation. Attributable mortality to septic shock was not determined for our cohort.

Table 4: Comparison of mortality and major outcomes across RCTs on use of ascorbic acid

Outcomes	*ViCTOR*	*CITRIS-ALI*	*Vitamins*	*Wani2020*	*HYVCTTSSS*	*ORANGES*
Country	India	USA	Australia, NZ, Brazil	India	China	USA
Primary outcome	All-cause mortality	Change in mSOFA @96 hours	Duration of time alive and free of vasopressor administration up to day 7	In-hospital mortality	28-day all-cause mortality	Resolution of shock and change in SOFA
Sample size	43:45 septic shock	83:84	107:109 septic shock	50:50 sepsis and septic shock	40:40 sepsis and septic shock	69:68 septic and septic shock
SOFA (enrolment)	10.9 ± 3.8:11.2 ± 3	10.3 ± 3.1:9.8 ± 3.2	8.4 ± 2.7:8.6 ± 2.7	9.36 ± 3.66:9.22 ± 3.54	10.1 + 4.0:9.6 + 4.5	7.9 ± 3.5:8.3 ± 3
Mortality (%)	49:51:00	46.3:29.8*	24.5:28.6	42:40:00	35:27.5%	19:16
Shock reversal (hours)	45.4 ± 24.4:34.58 ± 22.63*			96.13 ± 40.50:75.72 ± 30.29*	58.5 [28–104]:46 [23.8–102.5]*	53 ± 38:27 ± 22*
AKI (%)	47:53:00		72.1:69.2			75:79
ΔSOFA	1.38 ± 3.1:2.23 ± 2.4	3.5:3 (96 hours)	−1 (−3 to 0):−2 (−4 to 0)*	6.62 ± 3.94:5.64 ± 3.55	1.8+3.0:3.5+3.3*	1.93 ± 3.5:2.9 ± 3.3
LOS (ICU) (days)	8.44 ± 8.16:12.44 ± 14.2				7.5[4–11.8]:7.5[4–12.8]	4.66 ± 3.45:4.76 ± 4.3
LOS (H) (days)	20.9 ± 15.01:31.58 ± 31.06*		12.3 (6.2–26.1):12.3 (6.2–26.0)	10.70 ± 6.39:11.82 ± 7.36		11 ± 6.2:11.5 ± 6.8
Mechanical ventilation (%)	46.5:48.8		62.5:61.7	6:06		
Comment	62% control received steroid	68–72% in septic shock	Control:Hydrocort 50 mg every 6 hours			75% in septic shock at enrolment. 41% in control arm received steroids

*Implies statistical significance

Clinical Significance

HAT protocol does not reduce hospital mortality but decreases time to shock reversal in septic shock.

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Appendix I

Ascorbic Acid Measurements in Plasma

Concentration of AA in plasma was determined using a validated HPLC-MS/MS method. The method was developed and validated by our CRO partner, Biointegrity Consulting. Protein precipitation extraction (PPE) technique was used to separate AA from the complex biological matrix. All the sample treatment and extraction of AA from plasma were carried out under monochromatic light and water bath (2–8°C). Ascorbic acid 13C6 was used as an internal standard for this analysis.

Sample Treatment

To increase the stability of AA in blood/plasma, 5 µL of 1M of dithiothreitol was added to the blood per 1 mL and plasma was separated by using refrigerated centrifuge (4,000 rpm for 10 minutes at 5°C). To the obtained plasma, 100 µL of 1% metaphosphoric acid solution was added to per 1 mL of plasma. 1 M of dithiothreitol and 1% metaphosphoric acid solution were also added to calibration curve samples and quality control samples in same proportion as added in the biological samples after collection and stored in freezer (−80°C).

Extraction of AA from Plasma

Samples were retrieved from deep freezer (−80°C) and allowed to thaw at room temperature. Samples were vortexed for 30 seconds and centrifuged for 5 minutes. Twenty microliters of internal standard solution were added in 50 µL of each plasma sample and 1 mL of 2% formic acid in acetonitrile solution was added in each sample. Samples were vortexed for 5 minutes, followed by centrifuged for 15 minutes at 4,000 rpm at 5°C. From the centrifuged solution, 300 µL of supernatant was taken and added to 300 µL of 2 mM of ammonium formate solution, pH 3.05 taken in prelabeled HPLC vials. Final samples were vortexed for 10 seconds and transferred to autosampler of HPLC for analysis.

Sample Analysis

The LC-MS/MS instrument includes HPLC from Schimadzu and MS/MS (API-4000 model) from Applied Biosystems Sciex, USA. The HPLC system is equipped with autosampler with thermos at 5°C. The column used was Thermo Scientific, Syncronis C18, 150 × 4.6 mm, 5 µm, heated at 30°C. The mobile phase was a mixture of acetonitrile and 2 mm ammonium formate solution (pH 3.00) in ratio of 70:30 (v/v). Injection volume was 2 µL and runtime was 4.20 minutes. Ascorbic acid was detected in the electrospray ionization (ESI) negative ion mode. Eight point calibration curves were constructed in drug-free plasma for AA, concentration ranging from 0.5 to 80 µg/mL.

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