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Safety and Efficacy of Vitamin C, Vitamin B1, and Hydrocortisone in clinical outcome of septic shock receiving standard care: A quasi experimental randomized open label two arm parallel group study

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Abstract:

Aim: Current study designed to identify whether the combination of vitamin C, hydrocortisone, and thiamine, to decrease the mortality and free of vasopressor administration in patients with septic shock.

Method: An open-label, randomized Quasi experimental study conducted in 240 cases of sepsis at intensive care unit for the duration of 3 years. Standard care group consists 120 and Interventional consists 120 (Standard care with Hydrocortisone, Vitamin C, and Thiamine). The primary endpoint hospital survival and secondary outcome the duration of vasopressor therapy and other outcomes were measured.

Result: Among 240 patients who were randomized, the primary outcome measurement recorded as 3cases (1.25%) in Interventional and 31cases (12.91%) in Standard groups. All patients in the interventional group were weaned off vasopressors with a mean of 17.5±10.2 hrs after starting treatment with the vitaminC+thiamine+hydrocortisone infusion. The mean duration of vasopressor use was 35.8±21.5 h in the standard group (p=0.001); 25% patients in the standard group received higher dose of vasopressors and died due to refractory septic shock. The mean duration of vasopressor treatment is 61.4± 33.8 h in the control patients who died compared with 39.5±12.5h in those who survived. The median length of Hospital stay is 13.64 ± 9.04 (4-80) days in the interventional group compared with 10.28 ± 5.93 (2-28) days in the control group. Conclusion: Early use of intravenous vitamin C, together with hydrocortisone and thiamine, may prove to be effective in the reduction of vasopressors dosage and mortality of patients with sepsis and septic shock.

Keywords: ***septic shock, hydrocortisone, vitamin C, thiamine***

1. INTRODUCTION

Sepsis affects an estimated 15 to 19 million cases per year worldwide incorporating approximately 20% deaths worldwide [1]. The vast majority of these cases occur in low-income countries. Sepsis results due to abnormal host immune response involving both pro- and anti-inflammatory reactions at earliest phase. These abnormal immune responses predominates in initial phase and causes host injury which in later phase is characterized through hypofunction of immune cells and opportunistic superinfections.

Although survival rate of sepsis patients has improved due to recent advances in its supportive management, yet the related morbidity and mortality remains high[2]. Therefore, timely diagnosis and improvements in supportive care has led to a decrease in mortality rates by 25% [3]. The mortality from sepsis/septic shock in low-income countries is approximately 60% [4]. Sepsis following cardiac surgeries has been known to have catastrophic consequences. Affordable and effective treatment is the need of the hour to reduce mortality and financial burden.

Over the last 3 decades, >100 phase-2 and phase-3 clinical trials performed incorporating several novel pharmacologic agents and therapeutic interventions in an attempt to improve the outcome of patients with sepsis and septic shock. All of these efforts ultimately failed to produce a novel pharmacologic agent that improved the outcome of sepsis.

Studies have shown that vitamin C, which is a cofactor for the production of catecholamines and cortisol, hormones needed for the survival of shock is depleted during sepsis [5-8]. To compound the problems, cardiac failure is also known to be a state of catecholamine depletion. Studies have shown that vitamin C given in a dose of 6 g per day is safe and devoid of side effects. The doses as high as 100–150 g have safely administered to patients with burns and malignancies [9]. Intravenous thiamine (vitamin B1) is added to vitamin C for preventing renal side-effect using higher doses of vitamin C and Hydrocortisone which increases endogenous production of catecholamine. A recent study found decreased mortality in patients with sepsis treated with high-dose vitamin C, hydrocortisone and thiamine [10].

Newer therapeutic approaches are needed to treat sepsis urgently. The combination of thiamine (vitamin B1), ascorbic acid (vitamin C), and corticosteroids (hydrocortisone) is a promising new therapeutic option for sepsis resuscitation but currently lacks robust

evidences to support its widespread use.

On the basis of the literature reported safety and potential benefit of this therapeutic intervention, the combination of intravenous vitamin C and corticosteroid became routinely used as adjunctive therapy for the treatment of sepsis and septic shock in ICU. The combination of thiamine, ascorbic acid, and corticosteroids is a promising new therapy for sepsis resuscitation.

This prospective interventional study aimed to identify the safety and efficacy of the Hydrocortisone, Vitamin C and Thiamine as an add on treatment on biochemical and clinical outcomes in Sepsis and Septic Shock patients receiving standard care in a randomized open label two arm parallel group approach.

2. Materials and methods

Ethics: The study protocol was approved by the Institutional Ethics committee, Narayana Medical College, Nellore, A.P. INDIA**.**

- A randomized open label two arm parallel group (Quasi experimental design) study carried out for 3 years duration in Sepsis and Septic Shock patients.

Inclusion criteria are 18-65 years **of a**ged patients, Diagnosis of sepsis or septic shock, Procalcitonin (PCT) level $\geq$ 2 ng/mL within 24 h of admission.

Exclusion criteria are Patients < 18 years of aged Patients, pregnant patients, patients with limitations of care, and patients on steroids.

Standard care group: 120;

Interventional group: 120 (Standard care with Hydrocortisone, Vitamin C, and Thiamine).

Instruments for data collection

Biochemistry Analyzer: Serum creatinine, WBC, platelet count, total bilirubin, PCT and lactate levels, vitamin C, hydrocortisone and thiamine and other biochemical parameters.

Medication and Usage:

We administer 6 g of vitamin C per day, in four equal doses over 30-60 min infusion in 100 mL dextrose 5% (D5W) or normal saline. Intravenous thiamine was given as a piggyback in 50 mL of D5W or normal saline for 30-min infusion (Figure 1).

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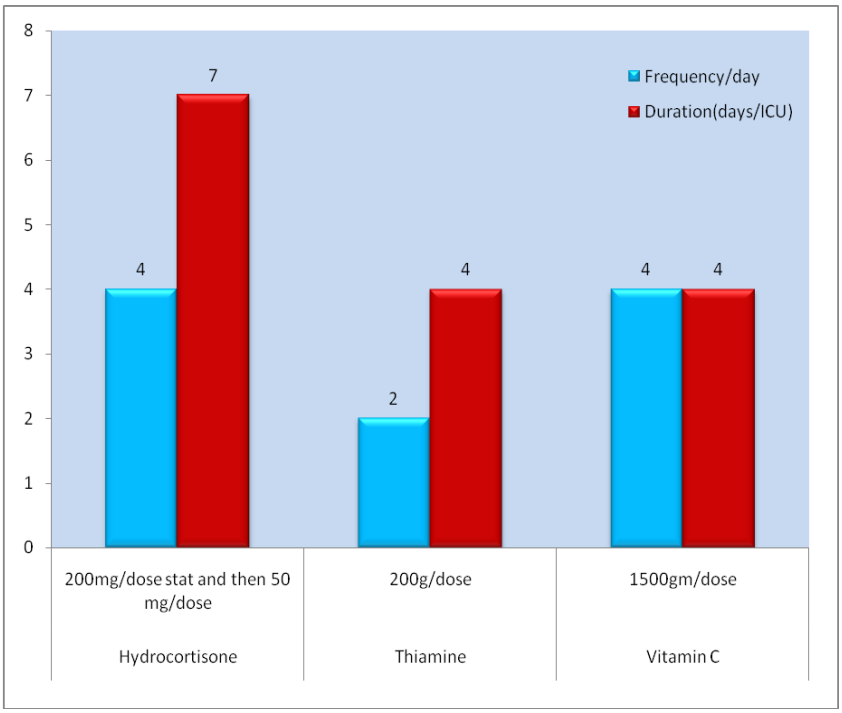

Figure 1. Combination approach of using hydrocortisone, thiamine and vitamin C for treatment of sepsis.

Primary outcome measurements

The primary endpoint was hospital survival (Time Frame: 30 days).

Secondary outcome measurements

1. Duration of vasopressor therapy (Time Frame: 72 hr)
2. ICU length of stay (4 days)
3. Change of APACH II score over the first 72 hours.

   APACH II score (total 0~100) is a score to evaluate the mortality rate of ICU patients, higher values represent a worse outcome. It includes:

   (A) PaO_2 (depending on FiO_2), Temperature (rectal), Mean arterial pressure, pH arterial, Heart rate, Respiratory rate, Sodium (serum), Potassium (serum), Creatinine, Hematocrit, White blood cell count.

   (B) Age points (score: 0~6).

   (C) Chronic health problems (liver cirrhosis, dialysis, COPD, congestive heart failure, immunocompromised).

   PaO2: mm Hg; Glasgow Coma Scale: points 15: 0 13-14: +1 10-12: +2 6-9: +3 <6: +4; Bilirubin: mg/dL; Mean arterial pressure: mmHg; Creatinine: mg/dL.

Data collection: APACHE II score was calculated for 0 hrs and 24 hrs. SOFA (Sepsis-Related Organ Failure Assessment) scores were calculated daily for 4 days.

Statistical analysis: Clinical data were presented as mean, SD, medians and interquartile range, or percentages as appropriate. One-way ANOVA was used to compare data between the treatment and control groups. Statistical significance was declared for probability values of 0.05 or less. Statistical analysis was performed with GraphPad Prism (version V) software. Binary logistic regression with propensity score adjustment was then performed to assess the odds ratio for mortality by treatment group. This analysis was then repeated with both age and propensity score adjustments to assess the odds ratio for mortality.

3. Results

Demographics: The present study enrolled a total of 120 patients in interventional group and 120 patients in standard treatment group. There were 87 (62.14%) of males and 53 (37.85%) of females in Interventional and 63 (63.00%) were of males and 37 (37.00%) of females recorded in standard group without significant difference between them ($p>0.05$). The mean age was 52.51±17.40 for Interventional and 56.31±16.58 for standard group.

Mean arterial pressure (MAP): The observed values at baseline for MAP max were 127.8±33.61 and MAP min was 77.39±16.27 in interventional group while it was reported 133.6±28.97 and 80.82±16.48 in standard treatment group without significant difference. The similar observations were recorded after 24h of treatment in both the groups (MAPmax: 125.3±24.27 and MAPmin: 80.67±16.87 in interventional group vs. MAP max: 126.5±24.88 and MAPmin: 80.92±17.06 in standard group. The similar findings were reported in gender-wise analysis in both male and females without significant difference ($p>0.05$).

Temperature: The temperature in interventional group at baseline was 99.31±1.05 and after 24h was 99.83±1.33, while standard treatment group showed mean temperature value of 99.51±1.51 at baseline and 99.97±1.56 after 24h of treatment without significant change ($p>0.05$).

Heart rate (HR): The mean heart rate in interventional group at baseline was 109.90±21.51 and after 24h was 96.58±29.04, while standard treatment group showed mean HR value of 111.70±27.70 at baseline and 94.19±29.88 after 24h of treatment without significant change ($p>0.05$).

Respiratory rate (RR): The mean RR in interventional group at baseline was 26.98±7.85 and after 24h was 21.36 ± 5.80, while standard treatment group showed mean RR value of 25.82 ± 7.88 at baseline and 21.26 ± 6.11 after 24h of treatment without significant change.

Table: 1 Values of pH, HCO_3, Na and K in interventional and standard treatment groups at baseline and after 24h.

Parameters	**Interventional group (N=120) Mean ± SD**	**Standard group (N=120) Mean ± SD**	**95 % CI**	***p* value**
pH 0h	7.20±0.16	7.31±0.14	-3.645 to 3.568	>0.05
pH 24h	7.33±0.11	7.33±0.13	-3.613 to 3.600	>0.05
HCO_3 0h	19.22±6.04	20.36±7.65	-4.746 to 2.466	>0.05
HCO_3 24h	19.69±5.68	20.95±8.10	-4.871 to 2.342	>0.05
Na 0h	137.80±5.99	137.10±6.74	-2.837 to 4.375	>0.05
Na 24h	140.90±23.87	138.30±6.84	-1.049 to 6.163	>0.05
K 0h	4.31±1.00	4.23±0.78	-3.526 to 3.686	>0.05
K 24h	4.11±0.71	4.12±0.75	-3.619 to 3.593	>0.05

Table: 2 Values of Hb, WBC and CRP in interventional and standard treatment groups at baseline and at day 6

Parameters	**Interventional group (N=120) Mean±SD**	**Standard group (N=120) Mean±SD**	**95 % CI**	***p* value**
Hb 0h	11.31±4.50	11.14±2.40	-2571 to 2571	>0.05
Hb 24h	10.24±2.92	10.39±2.18	-2568 to 2567	>0.05
WBC 0h	17450±10070	15030±7859	-154.9 to 4980	>0.05
WBC 24h	18220±12360	16620±8228	-970.9 to 4164	>0.05
CRP Day 0	216.20±122.70	208.20±117.60	-34.99 to 51.01	>0.05
CRP Day 6	137.50±120.70	195.90±136.00	-101.4 to -15.40	>0.05

Procalcitonin levels: Procalcitonin levels were observed at baseline as 7.40±12.79 and 20.21±34.63 in interventional and standard groups respectively showing significant difference (p=0.0001). Similarly at day 6, procalcitonin levels were decreased drastically as

3.76±9.49 and 13.24±25.28 in both the groups respectively and had significant difference between the groups (*p*=0.0001).

Table: 3 Values of procalcitonin and lactate in interventional and standard treatment group at baseline and at day 6

Parameters	**Interventional group (N=120) Mean±SD**	**Standard group (N=120) Mean±SD**	**95 % CI**	***p* value**
Procalcitonin Day 0	7.40±12.79	20.21±34.63	-19.05 to -6.565	**0.0001**
Procalcitonin Day 6	3.76±9.49	13.24±25.28	-15.71 to -3.229	**0.0001**
Lactate 0h	3.11±2.55	3.36±2.66	-6.490 to 5.994	>0.05
Lactate 24h	2.88±2.20	3.34±2.67	-6.808 to 5.695	>0.05

Calcium (Ca): Calcium levels were observed as 8.18±1.03 and 8.05±0.93 in interventional and standard groups respectively without significant difference.

Magnesium (Mg): Mg levels were observed as 1.98±0.37 and 1.94±0.49 in interventional and standard groups respectively without significant difference.

Prothrombin time (PT): PT was observed as 22.13±8.52 and 23.64±10.29 in interventional and standard groups respectively without significant difference.

INR: INR levelswere observed as 1.72±1.10 and 1.85±1.10 in interventional and standard groups respectively without significant difference.

APTT: APTT levelswere observed as 35.46±17.58 and 38.38±21.76 in interventional and standard groups respectively without significant difference.

SGOT: SGOT levels were observed as 179.40±436.60 and 102.70±234.10 in interventional and standard groups respectively without significant difference.

SGPT: SGPT levels were observed as 110.40±289.40 and 72.45±219.60 in interventional and standard groups respectively without significant difference.

ALP: ALP levels were observed as 88.03±177.00 and 114.00±195.40 in interventional and standard groups respectively without significant.

Albumin: ALB levels were observed as high (247.30±331.60) in interventional group and low (203.40±243.50) in standard group without statistical significant difference.

Table: 4 Values of urea and creatinine levels in interventional and standard treatment groups at baseline and after 24h of treatment

Parameters	**Interventional group (N=120) Mean±SD**	**Standard group (N=120) Mean±SD**	**95 % CI**	***p* value**
Urea 0h	75.62±58.68	78.46±55.57	-21.36 to 15.67	>0.05
Urea 24h	77.24±48.28	81.68±50.06	-22.96 to 14.08	>0.05
Cr 0h	2.66±3.10	2.53±2.06	-2567 to 2567	>0.05
Cr 24h	2.34±2.14	2.51±1.82	-2568 to 2567	>0.05

Table: 5 Values of SPO_2 and UOP levels in interventional and standard treatment groups

Parameters	**Interventional group (N=120) Mean ± SD**	**Standard group (N=120) Mean ± SD**	**95 % CI**	***p* value**
SPO_2	96.83±4.87	97.13±4.11	-519.8 to 519.2	>0.05
UOP 24h	1363±722.10	1143±719.90	-312.1 to 752.3	>0.05
UOP 72h	1545±1588	1613±2957	-605.6 to 470.5	>0.05

Table: 6 Values of platelet count and total builirubin levels in interventional and standard treatment groups at baseline and after 72h of treatment

Parameters	**Interventional group (N=120) Mean±SD**	**Standard group (N=120) Mean±SD**	**95 % CI**	***p* value**
Platelets 0h	214000±135600	200600±122700	-29620 to 56360	>0.05
Platelets 72h	194900±120900	178500±111700	-26560 to 59420	>0.05
Bilirubin 0h	1.823±2.927	1.983±3.667	-1.215 to 0.8951	>0.05
Bilirubin 72h	1.713±2.327	2.165±3.378	-1.507 to 0.6030	>0.05

Risk factors: Among different risk factors, smoking and alcoholics were observed highest as 53 (44.17%) and 45 (37.5%) in interventional and standard groups.

Table: 7 Percentages of patients with primary diagnosis

Primary diagnosis No (%)	Interventional group (N = 120)	Standard group (N = 120)	*p* value
Pneumonia	37.5%	44.17%	0.36
Urosepsis	15.83%	10%	0.65
Bacteraemia	6.67%	8.33%	0.90
GI/biliary	27.5%	20%	0.25
Other (meningitis, deceased before cultures, etc.)	10%	8.33%	0.75
Unknown	5%	10%	0.55

Critical illness scores:

The 24h SOFA score was 9.899±4.289 in the interventional group compared with 10.099±4.249 in the standard group (p=0.915). The Day 3 SOFA score was 7.5714±4.0788 in the interventional group compared with 9.25±4.063 in the standard group (p=0.850). None of the patients in the interventional group developed new organ failure (due to because of increase in their SOFA score) requiring an escalation of therapy. The time course of the SOFA score in the interventional group, the standard patients who died, and the control patients who survived. The trends in the SOFA score between both the groups were assessed and were found to be comparable at all-time intervals.

The time course of the APACHE score in the interventional group, the standard patients who died, and the control patients who survived. The 0 hr APACHE score was 22 ± 9.7024 in the interventional group compared with 25.428±7.645 in the standard group respectively (p=0.758). The 24h APACHE score was 21.322 ± 8.637 the interventional group compared with 27.755 ± 7.949 in the standard group (p=0.756). None of the patients in the interventional group required the escalation of therapy. The time course of the APACHE score in the interventional group, the standard patients who died, and the control patients who survived.

The 0 hr GCS score was 11 ± 4.645 in the interventional group compared with 10±4.949 in the standard group respectively (p=0.855). The 24hr GCS score was decreased than baseline, 10±4.89 in the interventional group compared with 8±4.739 in the standard group (p=0.658). None of the patients in the interventional group required the escalation of therapy. The

median Day 3 PCT clearance was 82% (79.5%-90.5%) in the interventional group compared with 32% (–45.4% to 59.5%) in the standard group (p=0.001); the time course of the PCT over the first 4 days.

Table: 8 Critical illness scores and mortality in interventional and standard treatment groups

Illness scores	**Interventional group (N = 120)**	**Standard group (N = 120)**	***p* value**
Day 1 SOFA, Mean ± SD	9.899 ± 4.289	10.099 ± 4.24	0.915
Day 3 SOFA, Mean ± SD	7.5714 ± 4.078	9.25 ± 4.063	0.850
APACHEII 0h, Mean ± SD	22 ± 9.70	25.428 ± 7.645	0.758
APACHEII 24h, Mean ± SD	21.322 ± 8.63	27.75 ± 7.949	0.756
GCS- SCORE 0h, Mean ± SD	11±4.645	10 ± 4.949	0.855
GCS- SCORE 24h, Mean ± SD	10±4.89	8 ± 4.739	0.658
MODS N (%)	64 (26.66%)	80 (33.33%)	**0.045**
Mortality N (%)	3 (1.25%)in total 240	31 (12.91%)in total 240	**0.000156**

Comorbidities:

Most patients had multiple comorbidities. The distribution of infections was similar between the two groups, with the lung being the most common site of infection. Blood cultures were positive in 36% and 35 % in interventional and standard treatment groups respectively. *Escherichia coli* (n=12) and gram-positive organisms (n=6) were the commonest blood isolates in the interventional group, while gram-positive organisms (n= 12) and *E coli* (n= 8) were the commonest isolates in the standard treatment group.

Mortality:

The hospital mortality was 2.52% in the interventional group compared with 25.61% in the standard group (p=0.000156). The propensity adjusted odds of mortality in patients treated with the vitamin C+ Thiamine + Hydrocortisone protocol was 0.13 (95% CI, 0.05-0.58; p=0.005). Two of the patients in the interventional group died due to sepsis complication. These patients survived their ICU stay, received standard treatment, and died of complications of their underlying disease (advanced).

Secondary outcome:

79 patients (66.38%) in the interventional group met the criteria for AKI compared with 77 (63.63%) in the standard treatment group (not significantly different, p=0.78). The 24h fluid balance was 2.1±3.2 L in the interventional group compared with 1.8±2.5 L in the standard

group (not significant). Similarly, the 72h fluid balance was 1.9±3.7 L in the interventional group compared with 1.6±3.3 L in the standard group. There was no difference in time to vasopressor independence between interventional and standard groups. 32% patients with AKI in the interventional group required RRT compared to 38% patients in the standard group (p=0.5).

All patients in the interventional group were weaned off vasopressors a mean of 17.5±10.2 hrs after starting treatment with the vitaminC+thiamine+hyrdocortisone protocol. The dose of vasopressors was predictably reduced between 2 and 4 hrs after the first infusion of vitamin C+thiamine+hydrocortisone. The mean duration of vasopressor use was 35.8±21.5 h in the standard group (p=0.001); 25% patients in the standard group received higher dose of vasopressors and died due to refractory septic shock. The mean duration of vasopressor treatment was 61.4± 33.8 h in the control patients who died compared with 39.5±12.5 h in those who survived. The time course of the vasopressor dose (in norepinephrine equivalents) in the interventional group, and the control patients who died, and the control patients who survived.

Table: 9 Secondary outcome measures in interventional and standard treatment groups

Variables	Interventional group (N = 120)	Standard group (N = 120)	*p* value
Tracheostomy	17	18	0.90
Vasopressors, No (%)	100	100	1.00
Vasopressor time	17.5± 10.2 h	35.8 ± 21.5 h	**0.001**
Acute kidney injury, No (%)	79 (66.38%)	77 (63.63%)	0.780
Blood cultures, No (%)	36%	35%	0.950

ICU and hospital stay:

The mean Ventilator days was 7.258±7.92 (2-26) days in the interventional group compared with 7.53±4.45 (1-18) days in the standard group (p=0.9, not significantly different). The mean Noradrenaline infusion days was 4.28 ± 3.8 (1-16) days in the interventional group compared with 4.05±2.37 (1-12) days in the standard group (p=0.89, not significantly different). The mean Vasopressin infusion days was 4.06±3.4(1-16) days in the interventional group compared with 3.71±2.4 (1-13) days in the standard group (p=0.45, not significantly different) .

The median length of ICU stay was 8.52±7.63 (2-27) days in the interventional group compared with 8.04 ± 4.3 (2-25) days in the standard group (p=0.89, not significantly different). The median length of hospital stay was 13.64±9.04 (4-80) days in the interventional group compared with 10.28 ± 5.93 (2-28) days in the control group (p=0.3, not significantly different).

Trend in vasopressor requirements:

The dose of noradrenaline and vasopressin over 5 days was lower in interventional group compared to standard group during the study period. The difference in vasopressin and noradrenalin from baseline to various time intervals showing improvement was observed in interventional group population at all-time points.

Table: 10 Trend in ICU and hospital stay and vasopressor requirements in interventional and standard treatment groups

Variable	Interventional group (N = 120)		Standard group (N = 120)		P value
	Mean±SD	CI		CI	
Ventilator days	7.258±7.92	2-26	7.53±4.452	1-18	0.90
Noradrenaline Days	4.280898876±3.8670	1-16	4.051948052±2.3780	1-12	0.89
Vasopressin Days	4.06557377±3.453832	1-16	3.719298246±2.454	1-13	0.45
HD/SLED N (%)	57 (40.71%)		47 (47.00%)		0.15
Hospital stay	13.64705882±9.040950	4-80	10.2892562±5.9307	2-28	0.30
ICU stay	8.529411765±7.634470	2-27	8.041322314±4.342	2-25	0.89

APACHE: Acute Physiology and Chronic Health Evaluation; CVA: cerebrovascular accident; CRF: chronic respiratory failure; IQR: interquartile range; SOFA: Sepsis-Related Organ Failure Assessment; ICU: intensive care unit; AKI: acute kidney injury; RRT: renal replacement therapy; LOS: length of stay; IQR: interquartile range; PCT: procalcitonin.

4. Discussion

For initial revival of sepsis patients, arterial blood pressure (ABP) has been considered a fundamental measure for monitoring and guiding the hemodynamic therapy [11-13]. Hence, mean arterial pressure (MAP) was considered as one of the parameter to evaluate the host response in sepsis during tri-therapy in interventional group. In sepsis patient's metabolic alkalosis is the most often due to the treatment given to correct hypotension, shock and acidosis [14]. Further metabolic acidosis is another risk factor in sepsis which can be the

result of either reduced bicarbonate or increased hydrogen ion (H^+) concentrations [15]. In sepsis, disturbances in electrolytes has been one of the most common clinical manifestation encountered in ICU and is related with the increased morbidity and mortality of sepsis patients [16].

Several mechanisms including increased destruction and reduced production of red blood cells have been discovered that contribute to the acute reduction in haemoglobin (Hb) levels in sepsis patients [17]. Many septic patients often develop leucocytosis in a delayed fashion [18]. C-reactive protein (CRP) has been a well-established marker of inflammation in infectious diseases [19].

Several studies have demonstrated the discriminative ability of serum Procalcitonin (PCT) and Lactate levels in bacterial sepsis [20]. In our study, we observed slight decrease in PCT levels at both baseline and after day 6 of treatment in interventional group as compared to standard treatment group. Studies have demonstrated that the serum PCT level rises rapidly as compared to CRP and peaks in a very short time. Furthermore, if patient responds properly to the treatment, the level of PCT returns to normal range earlier than CRP which makes it a better biomarker for sepsis [21]. The reduced levels of PCT in interventional group receiving Vitamin C, Thiamine (Vitamin B1), and Hydrocortisone showed better host response of sepsis patients as compared to standard care. The similar observations were made for males both at baseline and after 6 days of tri-therapy. However, PCT values didn't differ in female sepsis patients in interventional group after 6 days of treatment. Collectively these data indicates that tri-therapy can provide better and faster normalization of PCT levels than standard care and can be considered as better treatment strategy.

Hypocalcemia and hypomagnesemia have been the most common in critically ill sepsis patients with significantly increased hospitalization, prolonged ICU stay and mortality. In addition to this, during sepsis coagulation system becomes diffusely activated which results in Disseminated Intravascular Coagulation (DIC) representing higher mortality rate [22]. Thus, we compared Ca, Mg, and components of DIC including prothrombin time (PT), International Normalized Ratio (INR), and APTT levels. We didn't observe any significant change in these parameters in interventional group of patients as compared to standard care both at baseline and during the treatment. Thus these findings revealed that tri-therapy is safe and equally efficacious to standard care which can be opted further in larger cohort studies.

Approximately 19% of sepsis patients develop liver dysfunction. More specifically early hepatic failure leads to increased in-hospital mortality of sepsis patients [23]. Hence, in this

study we compared SGOT, SGPT, ALP, ALB, Bilirubin, Urea and Creatinine in sepsis patients in interventional group with standard care. Our study didn't show significant change in any of these liver function tests in interventional group as compared to standard care representing that tri-therapy is safe and equally effective in sepsis patients irrespective of gender.

In our study interventional group of patients didn't show significant change at baseline and after 72 hours of treatment duration and were comparable to the standard treatment group. Thus these data represent safety and tolerability of tri-therapy in sepsis patients of both the genders.

Among different risk factors, smoking and alcoholics were observed as highest in interventional (37.85%) and standard groups (45%). The most common infection in both the groups was pneumonia (Interventional: 38% vs. Standard: 45%), followed by gastrointestinal and biliary infection (Interventional: 28% vs. standard: 20%). 10% patients in the interventional group and 8% patients in the standard group had positive blood cultures.

The primary outcome was measured in terms of patient's mortality in both the groups. None of the patients in the interventional group developed new organ failure (due to because of increase in their SOFA score) requiring an escalation of therapy. The hospital mortality was 2.52% in the interventional group compared with 25.61% in the standard group (p=0.000156). Two of the patients in the interventional group died due to sepsis complication. These patients survived their ICU stay, received standard treatment, and died of complications of their underlying disease (advanced).

Both the SOFA and APACHE score didn't vary in interventional group with respect to standard treatment group. None of the patients in the interventional group required the escalation of therapy. The median Day 3 PCT clearance was 82% (79.5%-90.5%) in the interventional group compared with 32% (–45.4% to 59.5%) in the standard group (p=0.001); the time course of the PCT over the first 4 days. The distribution of infections was similar between the two groups, with the lung being the most common site of infection. Blood cultures were positive in 36% and 35 % in interventional and standard treatment groups respectively. Escherichia coli (n=12) and gram-positive organisms (n=6) were the commonest blood isolates in the interventional group, while gram-positive organisms (n=12) and E coli (n=8) were the commonest isolates in the standard treatment group.

Fowler et al (2014) showed 86 hrs of mean duration of vasopressor dependency after treatment with vitamin C. Similarly effect of Intravenous Vitamin C, Thiamine, and

Hydrocortisone on early weaning from Vasopressors in sepsis patients has been described recently by Masood et al (2019)[24],[25]. In our study, all patients in the interventional group were weaned off vasopressors a mean of 17.5±10.2 hrs after starting treatment with the vitamin C + thiamine + hydrocortisone protocol. The dose of vasopressors was predictably reduced between 2 and 4 hrs after the first infusion of vitamin C + thiamine + hydrocortisone. The dose of noradrenaline and vasopressin over 5 days was lower in interventional group compared to standard group during the study period. The difference in vasopressin and noradrenalin from baseline to various time intervals showing improvement was observed in interventional group population at all-time points.

While the dosing strategy for corticosteroids (hydrocortisone) in patients with sepsis and septic shock has been well studied, that for vitamin C is more uncertain. When high dosages of vitamin are given intravenously, metabolic conversion to oxalate increases. Oxalic acid is normally excreted by the kidney, and serum levels will increase with renal impairment. In patients with renal impairment receiving megadose vitamin C, super saturation of serum with oxalate may result in tissue deposition as well as crystallization in the kidney. Worsening renal function is therefore a concern with mega dose vitamin C. Thiamine deficiency increases the conversion of glyoxylate to oxalate. Thiamine deficiency is common in septic patients and is associated with an increased risk of death. For these reasons, thiamine was included in our vitamin C protocol.

In addition, the safety of hydrocortisone, vitamin C, and thiamine is supported by previous studies. In conclusion, the results of our study suggest that the early use of intravenous vitamin C, together with hydrocortisone and thiamine, may prove to be effective in preventing progressive organ dysfunction, including acute kidney injury, and reducing the mortality of patients with sepsis and septic shock.

Table: 11 Comparative studies

	Katsenos et al [26](2014)	**Fowler et al (2014)**	**Donnino et al (2016)[34]**	**Marik et al (2016)[10]**
Design	Non-randomized, prospective, longitudinal	Placebo-controlled, double- blind, randomized, phase I trial	Two-center, placebo-controlled, double-blind, randomized, pilot	Retrospective, before-after study
Population	Septic shock adults; norepinephrine >0.5 mcg/kg/min	sepsis	Sepsis + lactate >3 mmol/L + hypotension + vasopressors	sepsis or septic shock + procalcitonin >2 ng/mL

Intervention	IV hydrocortisone 50 mg q6h x 7d Early <9 hours (n=46) Late >9 hours (n=124)		IV vitamin C divided q6h Placebo (n=8) Lo-AscA: 50 mg/kg/d (n=8) Hi-AscA: 200 mg/kg/d (n=8)		Thiamine 200 mg q12h x 7d Placebo (n=45) Thiamine (n=43)		Triple therapy* Control (n=47) Triple therapy (n=47)	
Baseline	APACHE II SOFA	26.3 vs 23.9 11.5 vs 10.7	APACHE II SOFA	20.4 vs 20.4 vs 24 13.3 vs 10.1 vs 10.8	APACHE II SOFA	26.5 vs 25.7 10.2 vs 10.1	APACHE II SOFA	22.6 vs 22.1 8.7 vs 8.3
Outcomes	⇓vasopressor duration ⇓mortality		⇓SOFA score ⇓c-reactive protein ⇓procalcitonin		⇓mortality in deficient patients		⇓vasopressor duration ⇓mortality ⇓SOFA score ⇓procalcitonin	

*Triple therapy= IV hydrocortisone 50 mg q6h x 7d + IV vitamin C 50 mg q6h x 4d + IV thiamine 200 mg q12h x 4

	Katsenos 2014[26]		**Fowler 2014[24]**		**Donnino 2016**		**Marik 2016**	
Design	Non-randomized, prospective, longitudinal		Placebo-controlled, double- blind, randomized, phase I trial		Two-center, placebo-controlled, double-blind, randomized, pilot		Retrospective, before-after study	
Population	Septic shock adults; norepinephrine >0.5 mcg/kg/min		sepsis		Sepsis + lactate >3 mmol/L + hypotension + vasopressors		sepsis or septic shock + procalcitonin >2 ng/mL	
Intervention	IV hydrocortisone 50 mg q6h x 7d Early <9 hours (n=46) Late >9 hours (n=124)		IV vitamin C divided q6h Placebo (n=8) Lo-AscA: 50 mg/kg/d (n=8) Hi-AscA: 200 mg/kg/d (n=8)		Thiamine 200 mg q12h x 7d Placebo (n=45) Thiamine (n=43)		Triple therapy* Control (n=47) Triple therapy (n=47)	
Baseline	APACHE II SOFA	26.3 vs 23.9 11.5 vs 10.7	APACHE II SOFA	20.4 vs 20.4 vs 24 13.3 vs 10.1 vs 10.8	APACHE II SOFA	26.5 vs 25.7 10.2 vs 10.1	APACHE II SOFA	22.6 vs 22.1 8.7 vs 8.3
Outcomes	⇓vasopressor duration ⇓mortality		⇓SOFA score ⇓c-reactive protein ⇓procalcitonin		⇓mortality in deficient patients		⇓vasopressor duration ⇓mortality ⇓SOFA score ⇓procalcitonin	

*Triple therapy= IV hydrocortisone 50 mg q6h x 7d + IV vitamin C 50 mg q6h x 4d + IV thiamine 200 mg q12h x 4

5. Conclusion

In conclusion, vitamin C treatment reduces dose and duration of vasopressors as compared to placebo treatment. Early use of intravenous vitamin C, along with hydrocortisone and thiamine, may prove to be effective in the reduction of vasopressors dosage and mortality of patients with sepsis and septic shock.

Standard (regular) treatment protocol did not decrease the need for RRT initiation in patients with AKI, nor did it shorten ICU length of stay, hospital LOS, or the duration of vasopressor therapy when compared to SC.

References

[1] Adhikari NK, Fowler RA, Bhagwanjee S, Rubenfeld GD. Critical care and the global burden of critical illness in adults. Lancet 2010; 376:1339-46.

[2] Kaukonen KM, Bailey M, Suzuki S, Pilcher D, Bellomo R. Mortality related to severe sepsis and septic shock among critically ill patients in Australia and New Zealand 2000-2012. JAMA 2014; 311:1308-16.

[3] Gaieski DF, Edwards JM, Kallan MJ, Carr BG. Benchmarking the incidence and mortality of severe sepsis in the United States. Crit Care Med 2013;41:1167-74.

[4] Kadri SS, Rhee C, Strich JR, Morales MK, Hohmann S, Menchaca J, et al. Estimating ten-year trends in septic shock incidence and mortality in United States Academic Medical Centers using clinical data. Chest 2017;151:278-85.

[5] Long CL, Maull KI, Krishnan RS, Laws HL, Geiger JW, Borghesi L, et al. Ascorbic acid dynamics in the seriously ill and injured. J Surg Res 2003;109:144-8.

[6] De Grooth HJ, Choo WP, Spoelstra-de Man AM, Swart EL, Oudemans-van Straaten HM. Pharmacokinetics of four high-dose regimes of intravenous vitamin-C in critically ill patients. Intensive Care Med Exp 2016; 4:A52.

[7] De Grooth HJ, Choo WP, Spoelstra-de Man AM, Swart EL, Oudemans-van Straaten HM. Pharmacokinetics of four high-dose regimes of intravenous vitamin-C in critically ill patients. Intensive Care Med Exp 2016; 4:A52.

[8] Tanaka H, Matsuda T, Miyagantani Y, Yukioka T, Matsuda H, Shimazaki S. Reduction of resuscitation fluid volumes in severely burned patients using ascorbic acid administration: A randomized, prospective study. Arch Surg2000; 135:326-31.

[9] Wacker C, Prkno A, Brunkhorst FM, Schlattmann P. Procalcitonin as a diagnostic marker for sepsis: A systematic review and meta-analysis. Lancet Infect Dis 2013; 13:426-35.

[10] Marik PE, Khangoora V, Rivera R, Hooper MH, Catravas J. Hydrocortisone, Vitamin C, and Thiamine for the treatment of severe sepsis and septic shock: A retrospective before-after study. Chest 2017; 151:1229-38.

[11] Angus DC, van der Poll T. Severe sepsis and septic shock. N Engl J Med 2013; 369:840-51.

[12] Vincent J.L., Moreno R., Takala J., Willatts S., De Mendonça A., Bruining H., Reinhart C.K., Suter P.M., Thijs L.G. The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure. On behalf of the Working Group on Sepsis-Related Problems of the European Society of Intensive Care Medicine. Intensive Care Med. 1996;22:707–710.

[13] Levy MM, Evans LE, Rhodes A: The Surviving Sepsis Campaign Bundle: 2018 Update. Crit Care Med 2018; 46:997–1000.

[14] Ganesh K, Sharma RN, Varghese J, Pillai MG. A profile of metabolic acidosis in patients with sepsis in an Intensive Care Unit setting. Int J Crit IllnInj Sci. 2016;6(4):178-181.

[15] Kellum JA. Metabolic acidosis in patients with sepsis: Epiphenomenon or part of the pathophysiology? Crit Care Resusc. 2004;6:197–203.

[16] Lee JW. Fluid and Electrolyte Disturbances in Critically Ill Patients. Electrolyte Blood Press. 2010 Dec; 8(2): 72–81.

[17] Jung SM, Kim YJ, Ryoo SM, Kim WY. Relationship between low hemoglobin levels and mortality in patients with septic shock. Acute and Critical Care 2019; 34(2): 141-147.

[18] Seigel TA, Cocchi MN, Salciccioli J, et al. Inadequacy of temperature and white blood cell count in predicting bacteremia in patients with suspected infection. J Emerg Med 2012;42:254-9.

[19] Lobo S, Higashiama E, Rezende D Jr, Sivieiro E, Sanches L. Serum C-reactive protein levels in patients with sepsis varies according to the site of infection. *Crit Care*. 2005;9(Suppl 1):P173.

[20] Castelli GP, Pognani C, Meisner M, Stuani A, Bellomi D, Sgarbi L. Procalcitonin and C-reactive protein during systemic inflammatory response syndrome, sepsis and organ dysfunction. *Crit Care*. 2004;8(4):R234-R242.

[21] Standage SW, Wong HR. Biomarkers for paediatric sepsis and septic shock. Expert Rev Anti-Infect Ther. 2011;9(1):71–9.

[22] Simmons J, Pittet JF. The coagulopathy of acute sepsis. *CurrOpinAnaesthesiol*. 2015;28(2):227-236.

[23] Bakker J, Grover R, McLuckie A, Holzapfel L, Andersson J, Lodato R, Watson D, Grossman S, Donaldson J, Takala J: Administration of the nitric oxide synthase inhibitor NG-methyl-L-arginine hydrochloride (546C88) by intravenous infusion for up to 72 hours can promote the resolution of shock in patients with severe sepsis: results of a randomized, double-blind, placebo-controlled multicenter study (study no. 144–002). Crit Care Med 2004, 32: 1-12.

[24] Fowler AA, 3rd, Syed AA, Knowlson S, Sculthorpe R, Farthing D, DeWilde C, et al. Phase I safety trial of intravenous ascorbic acid in patients with severe sepsis. J Transl Med. 2014; 12:32.

[25] Masood H, Burki AM, Sultan A, et al. Effect of Intravenous Vitamin C, Thiamine, and Hydrocortisone (The Metabolic Resuscitation Protocol) on Early Weaning from Vasopressors in Patients with Septic Shock. A Descriptive Case Series Study. *Cureus*. 2019;11(6):e5016.

[26] Katsenos CS, Antonopoulou AN, Apostolidou EN, Ioakeimidou A, Kalpakou GT, Papanikolaou MN, Pistiki AC, Mpalla MC, Paraschos MD, Patrani MA, Pratikaki ME, Retsas TA, Savva AA, Vassiliagkou SD, Lekkou AA, Dimopoulou I, Routsi C, Mandragos KE; Hellenic Sepsis Study Group. Early administration of hydrocortisone replacement after the advent of septic shock: impact on survival and immune response. Crit Care Med. 2014 Jul;42(7):1651-7.

[27] Donnino MW, Andersen LW, Chase M, et al. Randomized, Double-Blind, Placebo-Controlled Trial of Thiamine as a Metabolic Resuscitator in Septic Shock: A Pilot Study. *Crit Care Med*. 2016;44(2):360-367.