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CLINICAL INVESTIGATION

Effect of Neostigmine on Attenuation of Proinflammatory Cytokines When Given as an Adjuvant Therapy in Septic Shock: A Randomized Control Trial

Mirdhu Bashni T, MD[1]
Nikhil Kothari, MD PhD[1]
Ankur Sharma, MD[2]
Shilpa Goyal, MD[1]
Shrimanjunath Sankanagoudar, MD[3]
Bharat Paliwal, MD[1]
Pradeep Kumar Bhatia, MD[1]

OBJECTIVE: The cholinergic anti-inflammatory pathway (ChAP) is the key regulator of the dysregulated cytokine storm in sepsis, with acetylcholine acting on alpha-7 nicotinic acetylcholine receptors (α7nAChRs) to suppress excessive inflammation. The objective of this study was to evaluate whether neostigmine administration modulates the inflammatory response in sepsis by enhancing cholinergic anti-inflammatory activity.

DESIGN: A single-center, prospective, randomized, double-blinded, placebo-controlled study.

SETTING: One adult ICU at a tertiary academic medical institution.

INTERVENTION: Patients were randomized to receive neostigmine at a fixed rate of 0.2 mg/hr (2 mL/hr) or placebo. Study drug was administered for 5 days.

MEASUREMENTS AND RESULTS: The primary outcome measure was decrease in tumor necrosis factor-alpha levels, in patients treated with neostigmine adjuvant therapy vs. the standard therapy. The secondary outcomes were hemodynamic parameters, septic shock reversal, changes in procalcitonin levels, and organ failure scores. The mean tumor necrosis factor-alpha levels were significantly lower in the neostigmine group (40 ± 36 pg/mL, mean ± SD) on day 5 as compared with the control group (67 ± 43 pg/mL; $p = 0.002$). There was a significant reduction in Sequential Organ Failure Assessment scores from day 1 to day 5 ($p < 0.001$) and 28-day mortality was also lower in the neostigmine group (26%) as compared with control group (54%, $p = 0.02$).

CONCLUSIONS: The neostigmine infusion modulates the ChAP by potentiating the acetylcholine release leading to reduced systemic inflammation and decreased cytokine levels in septic shock patients. (Clinical Trial Registry of India number: CTRI/2023/07/ 055054).

KEYWORDS: cholinergic anti-inflammatory pathway; neostigmine; septic shock; tumor necrosis factor-α

Sepsis remains a major global health challenge, with an estimated 48.9 million cases and over 11 million deaths reported worldwide in 2017 (1). Although age-standardized incidence and mortality rates have declined modestly over the past decade, the burden remains disproportionately high in low- and middle-income countries with resource-limited healthcare systems (2).

The pathogenesis of sepsis is driven by a dysregulated host response to infection, often described as a "cytokine storm," leading to organ dysfunction and

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KEY POINTS

Question: Does the cholinergic anti-inflammatory pathway (ChAP) mediated by nicotinic acetylcholine receptors have any role in modulating excessive inflammation in septic shock?

Findings: Neostigmine enhances ChAP signals, leading to significant reduction in tumor necrosis factor-alpha levels, suppression of cytokine storm, and improved clinical outcomes. It was tested by infusing low-dose neostigmine for 5 days to evaluate its immunomodulatory potential via sustained acetylcholine release.

Meaning: Targeting ChAP through neostigmine infusion may represent a novel therapeutic strategy, conferring survival benefits in critically ill septic shock patients.

high mortality (3). Excessive release of proinflammatory cytokines—such as tumor necrosis factor-alpha (TNF-α), interleukin-1 (IL-1), interleukin-6 (IL-6), and interleukin-8 (IL-8)—contributes to tissue injury, endothelial dysfunction, and disruption of the blood–brain barrier, thereby exacerbating organ failure. Although these cytokines are essential for pathogen clearance and immune cell recruitment, their uncontrolled overproduction is now recognized as a key driver of morbidity and mortality in sepsis (4).

Existing immunomodulatory strategies, including corticosteroids and IV immunoglobulins, aim to attenuate excessive inflammation, but their widespread adoption is hindered by limited high-quality evidence. The cholinergic anti-inflammatory pathway (ChAP) offers a novel, neuro-immunological approach to inflammation control. Mediated predominantly via the α7 nicotinic acetylcholine receptor (α7nAChR), ChAP involves vagus nerve stimulation to suppress proinflammatory cytokine release. Unlike systemic pharmacological immunosuppression, this mechanism provides precise, targeted modulation of the immune response, potentially minimizing side effects (5).

Preclinical and early clinical studies have highlighted the role of the autonomic nervous system, particularly the cholinergic system, in sepsis regulation. Borovikova et al (6) first described ChAP, demonstrating that vagus nerve stimulation can prevent excessive cytokine release. Neostigmine, an acetylcholinesterase inhibitor, augments cholinergic signaling by inhibiting acetylcholine breakdown, thereby enhancing ChAP activation. In experimental sepsis models, ChAP stimulation—via vagus nerve or pharmacologic agents like neostigmine—has been shown to reduce cytokine levels and improve survival (7, 8).

El-Tamalawy et al (9) evaluated neostigmine's effects in sepsis, using the change in Sequential Organ Failure Assessment (ΔSOFA) score as the primary endpoint, with secondary outcomes including 7-day and 28-day mortality, ICU length of stay, and shock reversal. Their findings suggested therapeutic promise but left important gaps, particularly regarding neostigmine's impact on specific inflammatory biomarkers such as TNF-α, procalcitonin (PCT), and serum lactate. TNF-α, an early-response cytokine released by monocytes/macrophages upon pathogen recognition through toll-like receptors, acts as a master regulator of downstream cytokines (IL-1, IL-6, IL-8, IFN-γ) and is central to sepsis pathophysiology.

Recognizing the pivotal role of TNF-α in sepsis-associated inflammation, we sought to determine whether adding neostigmine to standard therapy could modulate the inflammatory response and enhance clinical outcomes in septic shock. To date, no human study has evaluated the immunomodulatory effect of neostigmine on inflammatory cytokines in patients with septic shock. To address this, we performed a randomized controlled trial evaluating the impact of neostigmine infusion on serum TNF-α concentrations, whereas assessing clinical outcomes such as reversal of septic shock and organ dysfunction.

METHODS

Study Design and Setting

We conducted a prospective, double-blinded, randomized, placebo-controlled trial to evaluate the effects of continuous IV neostigmine infusion (0.2 mg/hr for 120 hr) as an adjunctive therapy in patients with septic shock, in addition to standard care.

Both patients and outcome assessors were blinded to group allocation. Randomization was implemented using numbered, opaque, sealed plastic envelopes prepared by an independent investigator. Each sealed envelope contained five pre-filled 50-mL coded syringes. For the treatment group, syringes

contained neostigmine 0.1 mg/mL, prepared by diluting 5 mg neostigmine (Myostigmin 0.5% vial, NEON Laboratories, Mumbai, India) with normal saline to a total volume of 50 mL, with instructions to infuse at a fixed rate of 2 mL/hr (0.2 mg/hr) for 5 days via a syringe pump (Fresenius Fresenius Kabi India Pvt. Ltd Abbkine Scientific Co. Ltd, Atlanta, GA). For the control group, syringes contained 50 mL of normal saline as placebo (standard of care), identically labeled and administered at the same infusion rate.

Ethical Considerations

The study adhered to the Declaration of Helsinki, Good Clinical Practice guidelines of the International Council for Harmonisation, and institutional research regulations (10). Approval was obtained from the Institutional Ethics Committee (AIIMS/IEC/2023/4456; dated March 18, 2023). Written informed consent was obtained from the legally authorized representatives of all participants before enrollment.

Patient Population

All patients admitted to the adult ICU (AICU) with a diagnosis of sepsis were screened for eligibility. Septic shock was defined as the need for vasopressor support to maintain mean arterial pressure greater than or equal to 65 mm Hg despite adequate fluid resuscitation (30 mL/kg balanced crystalloids within the first hour), consistent with the Surviving Sepsis Campaign 2024 criteria (11). Point-of-care ultrasound was used to assess inferior vena cava collapsibility to confirm fluid responsiveness and to exclude cardiogenic shock. Inclusion criteria comprised adults 18–80 years old with septic shock, enrolled within 6 hours of diagnosis. Key exclusion criteria included known contraindications to neostigmine (e.g., bradycardia, asthma, intestinal obstruction, etc.), pregnancy, preexisting conduction abnormalities, and significant chronic organ dysfunction not related to sepsis. A total of 82 patients were randomized in a 1:1 ratio to receive either neostigmine infusion (treatment arm, $n = 41$) or normal saline (control arm, $n = 41$) in addition to standard care.

Randomization and Blinding

The simple randomization was performed using computer-generated random numbers, implemented via sequentially numbered, opaque, sealed envelopes. The envelopes contained coded syringes to ensure allocation concealment. Blinding integrity was maintained throughout the trial, with identical appearance and labeling of study syringes in both groups. The randomization was done at the time of diagnosis of septic shock, with persistent hypotension and the need for vasopressors to maintain mean arterial pressure above 65 mm Hg.

Intervention Protocol

Patients in the treatment arm received a continuous IV infusion of neostigmine (0.2 mg/hr; 2 mL/hr of 0.1 mg/mL solution) for 120hours. This dosage and duration were adapted from El-Tamalawy et al (9) due to the absence of established dosing guidelines for this indication. Control group patients received 2 mL/hr of normal saline infusion for the same duration. All patients were managed per institutional sepsis guidelines, including source control, fluid resuscitation, vasopressors titrated to mean arterial pressure (MAP) greater than or equal to 65 mm Hg, and antimicrobials per Surviving Sepsis Campaign recommendations (12).

Data Collection and Baseline Assessment

At enrollment, demographic data, baseline physiologic parameters, and illness severity scores (Acute Physiology and Chronic Health Evaluation [APACHE] II, SOFA, and Glasgow Coma Scale) were recorded. Venous blood samples were collected via a central venous catheter using aseptic technique for the determination of TNF-α, PCT, lactate, and other routine laboratory parameters (full blood count, renal and liver function tests, serum electrolytes, arterial blood gases, and cultures). The probability of missing data was minimized by having a single-center study and with frequent follow-ups of the patients on day 1, day 3, and day 5, and weekly follow-up for 1 month.

TNF-α Assay

For TNF-α measurement, venous blood was allowed to clot at room temperature (22–24°C) for 30 min, centrifuged at 1,500 g for 15 min, and serum aliquots were stored at –80°C until batch analysis. TNF-α levels were quantified using the Elikine Human TNF-α

enzyme-linked immunosorbent assay Kit (Abbkine), based on a double-antibody sandwich method, with a detection range of 7.8–500 pg/mL. Assays were performed in a single batch to minimize inter-assay variability.

Study Outcomes

Primary outcome: Change in serum TNF-α concentrations from baseline to 72 hours and 120 hours after initiating the intervention. Secondary outcomes: Variation in heart rate (HR) and vasopressor dosing requirements. Changes in organ dysfunction scores (SOFA and APACHE II) between baseline and day 5. Changes in PCT and lactate levels within 120 hours and 28-day all-cause mortality. Follow-up visits were scheduled on days 1, 3, and 5, with weekly monitoring up to 28 days post-enrollment. A predefined subgroup analysis was performed for survivors vs non-survivors.

Safety Monitoring

An independent safety monitoring committee reviewed adverse events. Safety endpoints included the incidence of bradycardia (HR < 50 beats/min), hypotension (MAP < 65 mm Hg), hyperglycemia (> 180 mg/dL or new insulin requirement after enrollment), and serious allergic reactions (e.g., anaphylaxis). Administration of atropine for bradycardia was recorded.

Statistical Analysis

The primary efficacy analysis was conducted under the null hypothesis, which stated that "continuous neostigmine infusion (0.2 mg/hr for 5 d) would not affect outcomes in patients with septic shock, as compared with placebo (standard of care)." Sample size estimation was based on data from the preliminary cases (**Supplementary Tables 1–3**, https://links.lww.com/CCM/H895): the mean difference in TNF-α levels between the intervention and the control groups at day 5 was 24.8 ± 23.3 pg/mL, yielding an estimated effect size of 0.63 (mean difference/SD) and a power of 90% at a two-sided alpha level of 0.05. Based on these findings, the sample size was estimated to be 74 participants, allowing for a 10% attrition rate; the final sample size was set at 82.

There was no loss to follow-up, and none of the patients switched groups or stopped taking the treatment. The intention-to-treat data analysis approach was used, and the data of

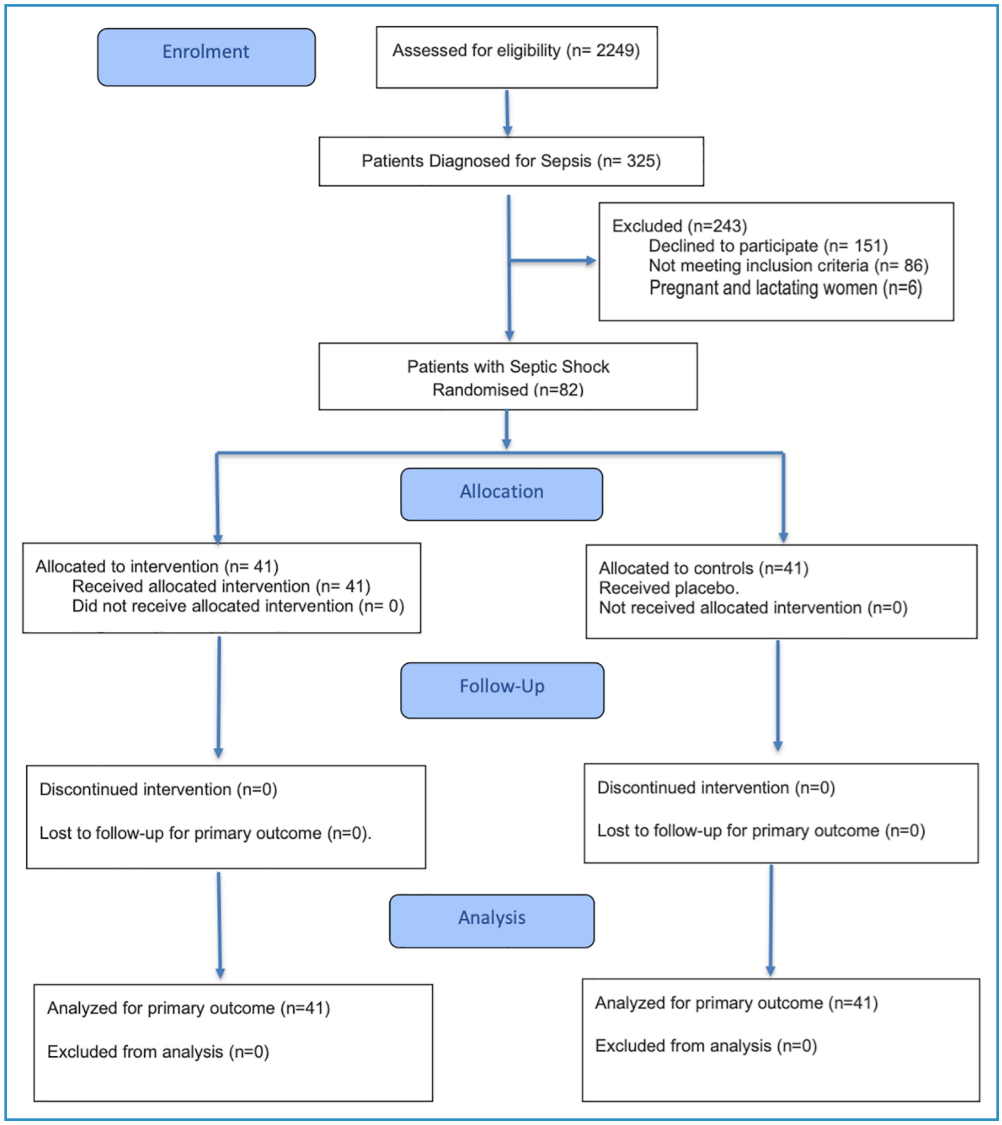

Figure 1. Consolidated Standards of Reporting Trials flowchart.

TABLE 1.
Demographic Data and the Comorbidities

Parameters	Neostigmine Group ($n = 41$)	Control Group ($n = 41$)
Age (mean ± SD)	53.6 ± 15.3	51.3 ± 18.0
Male	26 (63)	28 (68)
Female	15 (37)	13 (32)
Hypertension	13 (32)	10 (24)
Diabetes	12 (29)	15 (37)
Chronic kidney disease	3 (7)	5 (12)

The data are represented as *n* (percentage).

TABLE 2.
Effect of Adjunctive Neostigmine Infusion on Serum Biomarkers, Organ Support Parameters, and Clinical Outcomes in Septic Shock

Parameters	Neostigmine Group ($n = 41$)	Control Group ($n = 41$)	*p*
TNF-α day 1	88 (62.73 to 127.51)	66 (44.35 to 93.65)	< 0.001
TNF-α day 3	42.3 (22.1 to 80.5)	57.1 (43.95 to 85)	0.049
TNF-α day 5	23 (9.80 to 65.76)	59.5 (31.85 to 91.39)	< 0.001
TNF day 5–day 1	−56.4 (−25.95 to80.8)	−6.9 (−42.65 to 24.75)	< 0.001
PCT day 1	3.6 (2.08 to 7.85)	4.24 (1.47 to 11.27)	0.892
PCT day 3	2.69 (0.82 to 7.21)	4.88 (0.68 to 10.82)	0.488
PCT day 5	1.08 (0.15 to 4.49)	5.05 (1.32 to 11.26)	0.012
HR day 1	112.17 ± 19.72	110.92 ± 19.29	0.784
HR day 3	101.97 ± 16.10	102.04 ± 15.98	0.567
HR day 5	94.48 ± 18.08	102.63 ± 15.47	0.767
Noradr day 1	0.35 ± 0.22	0.24 ± 0.14	0.006
Noradr day 3	0.25 ± 0.21	0.22 ± 0.16	0.434
Noradr day 5	0.18 ± 0.21	0.24 ± 0.19	0.213
Vaso day 1	0.5 ± 0.84	0.64 ± 0.88	0.444
Vaso day 3	0.38 ± 0.73	0.7 ± 0.89	0.077
Vaso day 5	0.18 ± 0.6	1.22 ± 1.12	< 0.001
S.Cr day 1	2.92 ± 1.12	2.3 ± 2.03	0.307
S.Cr day 3	1.82 ± 1.14	2.08 ± 1.55	0.391
S.Cr day 5	1.29 ± 0.69	2.25 ± 1.26	< 0.001
28 Days mortality	11 (27%)	22 (54%)	0.02

HR = heart rate, Noradr = noradrenaline, PCT = procalcitonin, S.Cr = serum creatinine, TNF-α = tumor necrosis factor-α, Vaso = vasopressin.

The median values of TNF-α (pg/mL), PCT (pg/mL), and serum lactates (mmol/L). The data are represented as median and interquartile range. The values of HRs as beats/min (mean ± SD), Noradrenaline is in microgram/kg/min (mean ± SD), vasopressin in units/h (mean ± SD), serum creatinine as mg/dL (mean ± SD). TNF day 5–day 1 is the difference in TNF-α levels between control and the treatment group on day 5 and day 1.

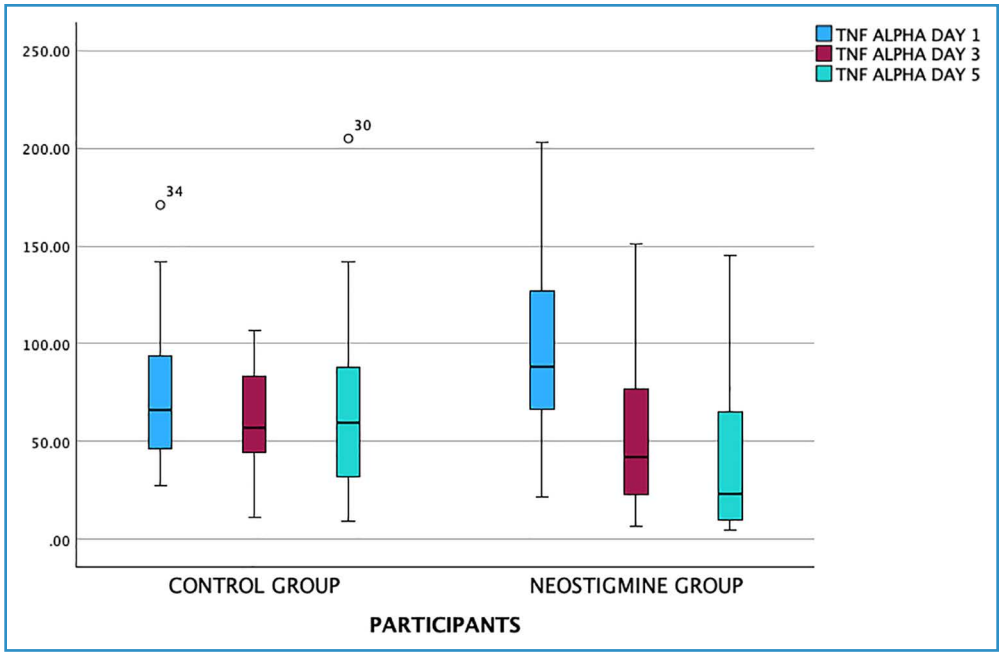

Figure 2. Change in tumor necrosis factor (TNF)-α levels in control and neostigmine groups between day 1 and day 5 ($p < 0.001$ in neostigmine group).

all 82 patients were used for the analysis. The analyses were performed using IBM SPSS Statistics, Version 30.0.0 (Armonk, NY). The primary and key secondary endpoints were analyzed using a prespecified, sequential, hierarchical testing procedure, with a two-sided significance threshold of 0.05 for each test. Secondary endpoints were evaluated in hierarchical order contingent on the significance of the preceding endpoint.

Given the non-normal distribution of cytokine data, results are presented as medians with interquartile ranges (IQR). Between-group comparisons were made using the Mann-Whitney *U* test, and statistical significance was defined as a two-sided *p* value of less than 0.05, reported to three decimal places. Changes in TNF-α levels over time (baseline, 72 hr, and 120 hr) were evaluated using the Friedman test for repeated measures.

RESULTS

Study Population

The trial was performed in the AICU of a tertiary care academic hospital as part of a postgraduate thesis project, following the approved protocol. During the study period, 2249 patients were admitted to the ICU, of whom 325 were diagnosed with sepsis. Consent to participate was declined by the families of 151 patients. The remaining patients were screened for eligibility, and 92 were excluded due to absolute or relative contraindications to cholinesterase inhibitors (e.g., bradycardia, cardiac conduction disturbances, bronchial asthma, pregnancy, or lactation). A total of 82 patients met the inclusion criteria and were enrolled (**Fig. 1**).

Baseline Characteristics

Most participants were between 40 and 70 years old; 28.05% were younger than 40 years, and 18.29% were older than 70 years. The mean age was 53.6 ± 15.3 years in the neostigmine group and 51.3 ± 18.0 years in the control group ($p = 0.54$), indicating no significant age difference. The male-to-female ratio was 1.73 in the neostigmine group and 2.15 in the control group ($p = 0.82$), with no statistically significant difference (**Table 1**).

Primary Outcome

In the neostigmine group, median TNF-α decreased from 88 pg/mL on day 1 to 23 pg/mL on day 5 ($p < 0.001$). In contrast, the control group showed a nonsignificant decline from 66 pg/mL to 59.5 pg/mL ($p = 0.75$; **Table 2**, **Fig. 2**). Within-group comparison over 5 days using the Friedman test confirmed a significant TNF-α reduction in the neostigmine group ($p = 0.04$) but not in controls (Table 2; and **Supplementary Table 4**, https://links.lww.com/CCM/H895).

Secondary Outcomes

1) *Biomarker*. Baseline PCT levels were similar between groups (3.6 pg/mL in neostigmine vs. 4.3 pg/mL in control, $p = 0.89$). By day 5, PCT decreased significantly to 1.1 pg/mL in the neostigmine group ($p < 0.001$), whereas it increased to 5.1 pg/mL in controls (Table 2).
2) *Hemodynamic Parameters*. On day 1, mean HR was 112.3 ± 19.7 beats/min in the neostigmine group and 110.9 ± 19.3 beats/min in controls ($p = 0.78$). After 120

hours, HRs decreased to 94.5 ± 18.1 beats/min (neostigmine) and 102.6 ± 15.5 beats/min (control), without a statistically significant difference (p = 0.77; **Fig. 3**, and **Supplementary Tables 7–12**, https://links.lww.com/CCM/H895 and **Supplementary Fig. 1**, https://links.lww.com/CCM/H895).

3) *Safety Outcomes*. Continuous low-dose neostigmine infusion (0.2 mg/h; ≤5 mg/d) for 5 days was well tolerated in patients receiving noradrenaline for septic shock. No infusions required, early termination, or rate adjustment. Adverse event monitoring revealed no episodes of bradycardia (< 50 beats/min), and HR trends were comparable between groups (p = 0.78). The low-dose regimen did not precipitate abrupt bradycardia, and HR data were normally distributed in both groups. Besides this, no other adverse events like hypotension, hypoglycemia, or anaphylaxis were observed.
4) *Organ Dysfunction*. Median SOFA score in the neostigmine group decreased from 11 (IQR 10–13) on days 1–9 (IQR 7–10.5) on day 5, a statistically significant reduction (Wilcoxon Signed-Rank test). No significant change was observed in the control group (**Fig. 4**).
5) *Illness Severity*. Baseline APACHE II scores were 23.1 ± 5.2 (neostigmine) and 21.9 ± 5.9 (control) (p = 0.38). By day 5, scores declined significantly in the neostigmine group to 18.3 ± 6.3 (p < 0.001), whereas remaining unchanged in controls (21.1 ± 5.9). The mean change from baseline was –4.8 ± 6.6 in the neostigmine group vs. –0.9 ± 5.9 in controls, a statistically significant difference (Fig. 4).
6) *Survival Outcomes*. Kaplan-Meier analysis showed a 28-day survival rate of 73.2% in the neostigmine group vs. 46.3% in controls (**Fig. 5**). Chi-square comparison of mortality rates demonstrated a statistically significant difference between groups (p = 0.02).

DISCUSSION

To our knowledge, this randomized controlled trial is the first human study to demonstrate a significant reduction in circulating TNF-α levels following continuous neostigmine infusion in patients with septic shock, providing clinical evidence of in vivo activation of the ChAP. Given that TNF-α is a pivotal upstream cytokine that drives the cascade of sepsis-related inflammation, its modulation has long been considered a therapeutically desirable yet elusive target (13). Despite advances in antimicrobial therapy and supportive care, contemporary management of septic shock continues to rely heavily on timely source control, appropriate antibiotic selection, hemodynamic stabilization through fluid resuscitation, and vasopressor therapy. However, these interventions do not directly mitigate the overwhelming inflammatory response that characterizes septic shock. In this context, the robust TNF-α reduction observed in our study introduces a promising adjunctive therapeutic avenue.

Preclinical literature strongly supports the mechanistic plausibility of our findings. In a seminal murine model, Hofer et al (14) demonstrated that cholinergic agonists—including nicotine, physostigmine, and neostigmine—substantially decreased TNF-α, IL-1β, and IL-6 after cecal ligation and puncture–induced sepsis. This intervention significantly reduced circulating proinflammatory cytokines TNF-α, IL-1β, and IL-6 ($p \leq 0.05$). Similarly, Bencherif et al (15) emphasized the critical role of the ChAP in suppressing TNF-α production through vagal nerve stimulation (16). Across multiple animal studies, activation of the ChAP reliably attenuates inflammatory cytokine

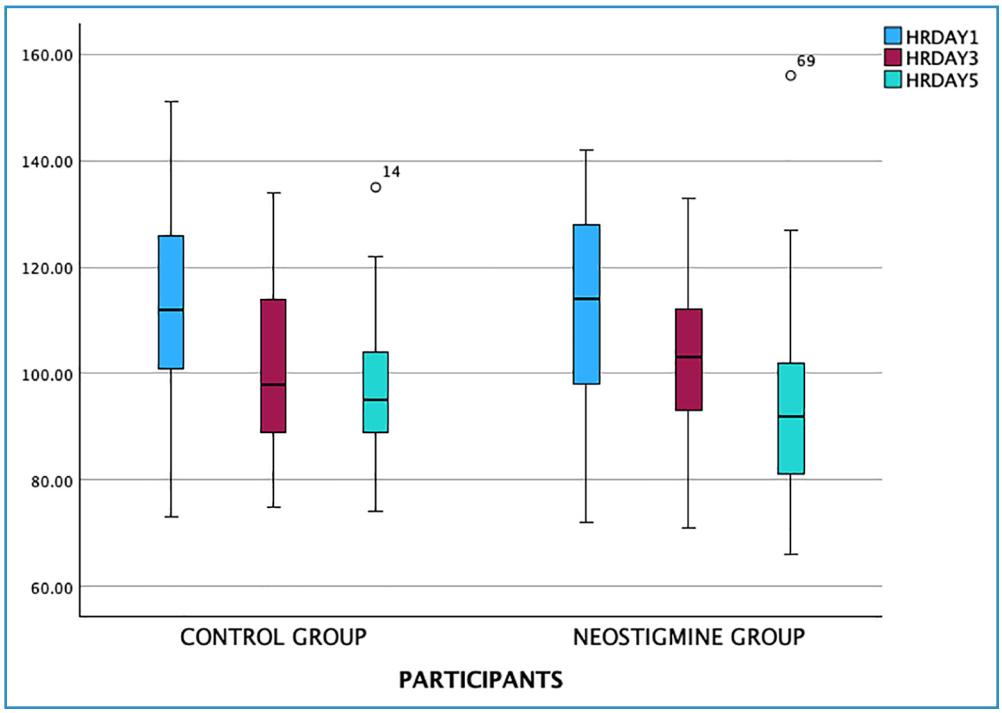

Figure 3. Change in heart rate (HR) in control and neostigmine groups between day 1 and day 5 ($p > 0.05$).

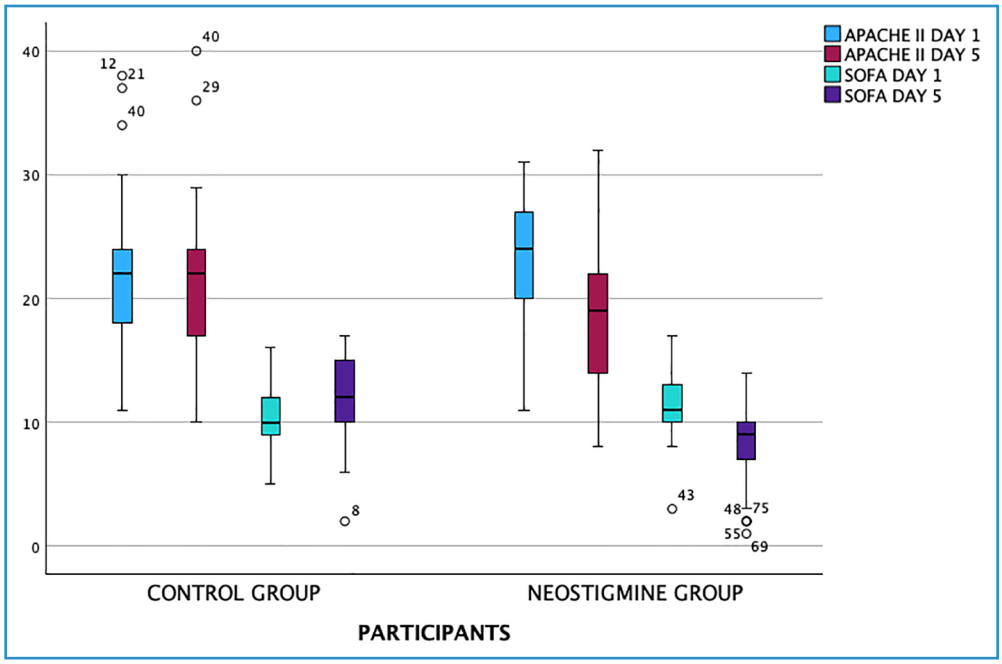

Figure 4. Change in Acute Physiology and Chronic Health Evaluation (APACHE) II and Sequential Organ Failure Assessment (SOFA) scores in control and neostigmine groups between day 1 and day 5 ($p < 0.05$ in both the groups).

release, improves hemodynamics, and reduces organ injury. Our findings translate these mechanistic insights into the clinical realm, offering the first human evidence that pharmacologic augmentation of acetylcholine availability via acetylcholinesterase inhibition can effectively reduce TNF-α levels in established septic shock (17–19).

A relevant comparator is the trial by El-Tamalawy et al (9), which also evaluated neostigmine as an adjuvant therapy in sepsis. Although the trial by El-Tamalawy et al (9) focused on a narrower subset of patients and short-term outcomes, the present study included a broader population, examined the effect of neostigmine on cytokine reduction, and assessed longer-term composite endpoints, including 28-day mortality (20–22). Although both studies demonstrated improvements in hemodynamic stability and inflammatory markers, our findings extend the existing evidence in several important ways. First, our study included a broader and more heterogeneous septic shock population, better reflecting real-world clinical settings. Second, unlike the trial by El-Tamalawy (9), which focused primarily on short-term physiologic outcomes, our investigation incorporated serial cytokine profiling, with TNF-α as a predefined biomarker of interest. Third, our trial assessed composite clinical outcomes, including 28-day mortality, providing more meaningful prognostic insight. The alignment between these two independent studies supports the reproducibility of neostigmine's therapeutic potential, yet also underscores the necessity for standardized dosing regimens, uniform cytokine assessment protocols, and harmonized eligibility criteria in future research (23–25).

Unlike the study by El-Tamalawy et al (9), which did not evaluate cytokine modulation, we found a substantial reduction in TNF-α levels from day 1 to day 5. Among survivors in the neostigmine group. Notably, even nonsurvivors in this group, those who died within 28 days, also exhibited significant TNF-α reduction. These findings suggest that neostigmine attenuates systemic inflammation regardless of survival outcome, with more pronounced benefits in survivors (26). In contrast, the control group showed minimal cytokine changes in both subgroups, underscoring neostigmine's modulatory effect ($p > 0.05$).

The clinical implications of these biochemical changes were reflected in survival analyses. Kaplan-Meier curves demonstrated a notably lower 28-day mortality in the neostigmine group (26.83%) compared with controls (53.66%). Although our study was not powered primarily for mortality and the findings should be interpreted cautiously, this trend aligns with the mortality reductions reported by El-Tamalawy et al (9), albeit without statistical significance in their smaller cohort. Collectively, these observations raise a compelling hypothesis: TNF-α attenuation through ChAP activation may translate into improved survival in septic shock, a hypothesis that warrants rigorous evaluation in larger multicenter trials (27, 28).

From a practical standpoint, neostigmine possesses several characteristics that make it an attractive candidate for integration into sepsis care pathways. It is

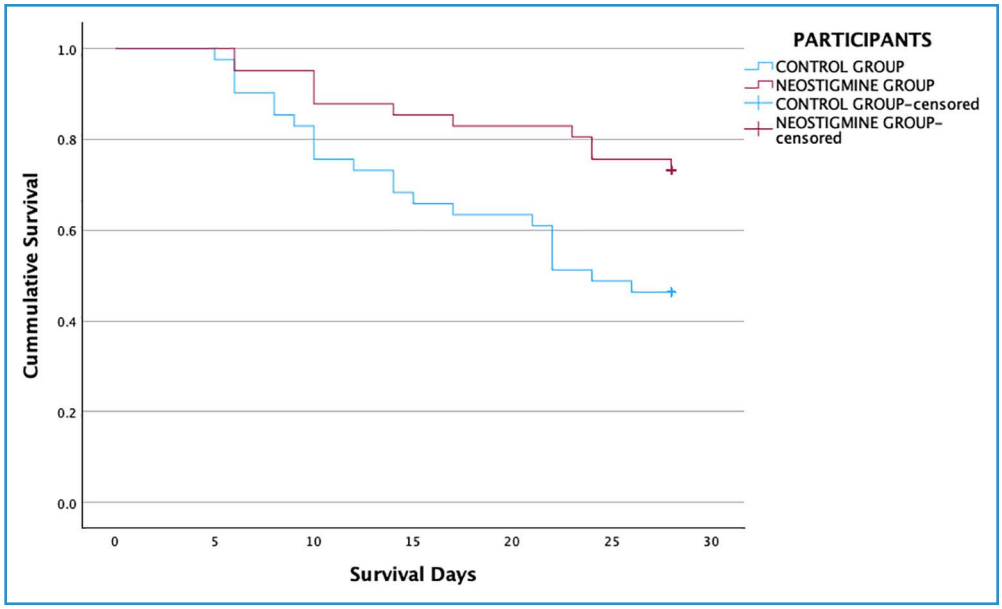

Figure 5. The Kaplan-Meier Survival analysis showing less 28 days mortality in the neostigmine group as compared with the control group.

widely available, inexpensive, and well-established within hospital formularies globally. Its safety profile is well characterized from decades of use in anesthesia and neuromuscular disorders (29). Nevertheless, potential contraindications, including bradycardia, conduction abnormalities, active bronchospasm, hypersensitivity to cholinergic agents, and gastrointestinal obstruction, necessitate careful patient selection and continuous monitoring. Its parenteral administration is compatible with existing critical care workflows, suggesting that protocol implementation would be feasible in centers with established intensive care infrastructure.

However, several barriers to widespread adoption remain. Concerns regarding cholinergic side effects, limited clinician familiarity with acetylcholinesterase inhibitors in sepsis, and the need for continuous cardiac monitoring may temper enthusiasm. In addition, institutional inertia and the absence of formal guideline recommendations make it unlikely that neostigmine will be rapidly incorporated into standard practice without further compelling evidence. Most critically, the current evidence base—though promising—is not yet definitive.

In summary, our study provides the first clinical demonstration that neostigmine significantly reduces TNF-α levels in septic shock, offering translational confirmation of ChAP activation in humans. The associated improvements in inflammatory markers, hemodynamics, and survival trends highlight neostigmine's potential role as a novel adjunct in sepsis therapy. Although these findings are encouraging, robust multicenter trials remain essential before neostigmine can be integrated into clinical guidelines or routine sepsis management.

This study has several limitations. First, baseline imbalances between groups—particularly higher initial TNF-α levels in the intervention arm may have influenced observed effects and complicated interpretation of inflammatory trajectories. Second, being a single-center study with narrow inclusion criteria limits its generalizability; multicenter validation with broader populations is warranted. In addition, reliance on TNF-α as the primary biomarker provided an incomplete picture of immune modulation; inclusion of other cytokines such as IL-6 and IL-10 could offer a more comprehensive view. The fixed neostigmine dose, not adjusted for patient characteristics, may also have influenced outcomes. Third, the absence of long-term follow-up precludes conclusions about the durability and safety of neostigmine beyond the acute phase. Fourth, the limited sample size may have underpowered detection of differences in secondary outcomes, increasing the risk of type II error. Finally, the power calculation assumed a TNF-α difference of 24.8 pg/mL based on unpublished internal pilot data. We acknowledge that this large effect size may not reflect the general population, likely underpowering the trial for mortality endpoints.

CONCLUSIONS

Given the accumulating evidence supporting the immunomodulatory properties of neostigmine via the ChAP,

its integration into therapeutic strategies for septic shock may represent a potential shift in current management paradigms. In this study, adjunctive neostigmine was associated with a significant reduction in inflammatory biomarkers, specifically TNF-α and PCT, over 5 days in patients with septic shock. Concurrent trends toward improved organ function, reduced illness severity, and lower mortality were observed, although these secondary outcomes did not reach definitive statistical significance. Although these preliminary findings are promising, they warrant validation in larger, multicenter trials with adequate power to detect clinically meaningful differences in outcomes. If corroborated, neostigmine could emerge as an accessible, cost-effective immunomodulatory adjunct in the treatment of septic shock. Nonetheless, the translational potential and generalizability of these results require confirmation across diverse patient populations and healthcare settings. This investigation highlights the clinical relevance of these observations and underscores their implications for advancing septic shock management.

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1 *Department of Anaesthesiology and Critical Care, All India Institute of Medical Sciences (AIIMS), Jodhpur, Rajasthan, India.*

2 *Department of Trauma and Emergency, All India Institute of Medical Sciences (AIIMS), Jodhpur, Rajasthan, India.*

3 *Department of Biochemistry, All India Institute of Medical Sciences (AIIMS), Jodhpur, Rajasthan, India.*

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The authors have disclosed that they do not have any potential conflicts of interest

For information regarding this article, E-mail: drnikhilkothari@gmail.com; kotharin@aiimsjodhpur.edu.in

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