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Original Research Article

Effect of continuous blood purification combined with reduced glutathione on endotoxin, inflammatory mediators and severity of liver injury in patients with septic shock

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Abstract

Purpose: *To investigate the impact of continuous blood purification in conjunction with reduced glutathione on endotoxin levels, inflammatory mediators and the severity of liver injury in septic shock patients.*
Methods: *A cohort of 100 septic shock patients admitted at The Second Affiliated Hospital of Hainan Medical University, China between May 2020 and May 2023 were enrolled in this study. They were randomly divided into study and control groups, each comprising 50 patients. Both groups received standard interventions. In addition, control group underwent continuous blood purification, while study group received reduced glutathione therapy for two weeks. Acute physiology score + age point + chronic health point (APACHE II) and sequential organ failure assessment (SOFA) scores, intensive care unit (ICU) and mechanical ventilation duration, oxygenation levels, 28-day mortality, organ injury, serum endotoxin levels, inflammatory markers, as well as serum aspartate aminotransferase (AST) and glutamate aminotransferase (ALT) levels were determined before and after treatment. Adverse events during treatment were documented.*
Results: *Both groups exhibited a significant decrease in APACHE II and SOFA scores, with greater decreases observed in study group ($p < 0.05$). The study group had shorter ICU stays and mechanical ventilation durations. The groups had no significant differences in 28-day mortality or organ injury ($p > 0.05$). Study group demonstrated significantly lower levels of endotoxin, tumor necrosis factor (TNF-α), procalcitoninogen (PCT), ALT and AST in comparison to control group ($p < 0.05$). Adverse reactions were similar between the two groups ($p > 0.05$).*
Conclusion: *Combining continuous blood purification with reduced glutathione therapy reduces endotoxin and inflammatory mediator levels, mitigates liver injury and supports patient recovery in septic shock, with a favorable safety profile. Future studies to accommodate the diverse profiles of septic shock patients from multiple centers will be needed to validate the outcomes of this study.*

Keywords: *Septic shock, Reduced glutathione, Blood purification, Endotoxin, TNF-α, Procalcitoninogen*

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INTRODUCTION

Septic shock, often referred to as infectious shock, represents a systemic inflammatory response triggered by viral, bacterial, fungal and other infections. This condition can affect various tissues and organs, including the liver, kidneys and lungs, leading to transient ischemia, hypoxia, organ failure, immune imbalances and shock [1-3]. Reports indicate that the morbidity and mortality rates for patients with acute and severe septic shock in China exceed 20 %. Among the elderly population whose physical resilience may be compromised, septic shock can lead to severe consequences and a challenging prognosis [4]. The current treatment approach relies on fundamental therapies, including fluid resuscitation and anti-infection measures, mechanical ventilation and blood purification, which can relieve systemic inflammatory response, help patients establish immune balance and improve the survival rate of patients [5]. Continuous hemodialysis is among the contemporary therapeutic approaches utilized in the treatment of infectious diseases, shock and alleviating renal injury [6].

Reduced glutathione is an antioxidant that scavenges reactive oxygen clusters in the body and attenuates cellular damage caused by oxygen free radicals [7]. This study aims to investigate the impact of combining continuous blood purification with reduced glutathione therapy on endotoxin levels, inflammatory mediators and the extent of liver injury in patients with severe septic shock. The findings are presented below.

METHODS

General patient information

A total of 100 cases of patients with septic shock admitted to the hospital for treatment from May 2020 to May 2023 were selected as the study subjects, and they were divided equally into study and control groups by simple random sampling method. All patients included in this study provided informed consent and the study received approval from the Ethics Committee of The Second Affiliated Hospital of Hainan Medical University (approval no. HMU-012). The study procedure followed the guidelines of Declaration of Helsinki [8].

In the study group, there were 50 patients, consisting of 27 men and 23 women, with age ranging from 38 to 62 years (mean, 47.32 ± 7.47 years). The duration of the disease ranged from 0.7 to 2 days (mean, 1.06 ± 0.14 days). Among these patients, 8 had urinary system infections, 26 had pneumonia and 16 had nephritis. In control group, there were 50 patients, including 24 males and 26 females, with agesranging from 35 to 63 years (mean, 47.14 ± 6.95 years). The disease duration in this group ranged from 0.5 to 2 days (mean, 1.02 ± 0.12 days). Among them, 10 had urinary system infections, 27 had pulmonary infections and 13 had nephritis.

Inclusion criteria

The included patients were those who met the criteria of "China Sepsis/Septic Shock Emergency Treatment Guidelines (2018)" [9]; had microbiological culture confirming the type of infectious pathogens; were aged over 18 years; had no prior treatment before admission; had complete data and consent from the patient's family to participate in the study.

Exclusion criteria

The study excluded patients falling within the following categories: Long-term use of drugs including steroid hormones and immunomodulators; concurrent angina pectoris, cardiac insufficiency, or cardiogenic shock; hepatic and renal insufficiency with a history of hemodialysis; coexisting immune system diseases; coagulation dysfunction; patients' unwillingness to cooperate. There were no statistically significant differences in the general information between the two groups. ($p > 0.05$).

Treatments

Patients both groups received conventional basic treatment after admission. The treatment regimen encompassed a range of interventions, such as fluid resuscitation, anti-inflammation measures, anti-infection protocols, vasoactivity enhancement, mechanical ventilation and other relevant approaches. In control group, patients underwent continuous blood purification, which involved the establishment of a transvenous channel in the femoral vein. A continuous blood purification machine (PrisnaFlex) was employed for blood filtration during the treatment period. Sodium bicarbonate served as the daily replacement fluid, supplemented with saline, sterilized water for injection and 5 % glucose injection as needed, based on the patient's condition.

The blood flow rate was set at 200 mL/min, with a replacement volume of 3 L/h and heparin calcium was used for anticoagulation. In study group, a dosage of 1.8 grams of reduced glutathione (from Fu'an Pharmaceuticals,

compliant with China Pharmacopoeia H20183087, at a concentration of 0.6 g) was administered to the patients in this group. It was dissolved in 250 mL of 5 % dextrose injection solution and administered intravenously once daily. The two groups were treated continuously for 2 weeks.

Evaluation of parameters/indices

Endotoxin, tumor necrosis factor (TNF-α) and procalcitoninogen (PCT) levels

Peripheral venous blood was collected at admission and the end of treatment. Serum was centrifuged and analyzed by enzyme-linked immunosorbent assay to determine the levels of endotoxin, TNF-α, and PCT.

Degree of liver injury during treatment

Peripheral venous blood was collected at admission and on the 3rd, 7th and 14th day of treatment and serum aspartate aminotransferase (AST) and glutamate aminotransferase (ALT) levels were determined.

Oxidative stress levels

Superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) levels were determined using the Vazyme kit at admission and after 14 days of treatment; (6) Comparison of adverse effects between the two groups.

APACHE II and SOFA scores

Acute physiology score + age point + chronic health point (APACHE II) and sequential organ failure assessment (SOFA) scores were determined by comparing general indicators and 28-day mortality between the two groups. This includes recording ICU hospitalization time, mechanical ventilation time during treatment, 28-day patient survival and assessments of liver, kidney and lung functions at the end of the treatment to record organ damage.

Statistical analysis

Statistic Package for Social Sciences (SPSS) 27.0 software (IBM, Armonk, NY, USA) was used to analyze the data. The chi-squared (χ^2) test was employed to assess gender differences between the two groups. The 28-day mortality rate and adverse reactions, and *t*-test were used to compare the two groups in terms of APACHE II scores, SOFA scores, ICU length of stay, duration of mechanical ventilation, endotoxin, TNF-α, PCT, AST, ALT, SOD and GSH-Px. *P*-values less than 0.05 indicated that the differences were statistically significant.

RESULTS

APACHE II and SOFA scores

Both groups exhibited significantly reduced APACHE II and SOFA scores compared to their pre-treatment values, with study group demonstrating lower scores than control group ($p < 0.05$; Table 1).

General indices and morbidity and mortality rates

Study group exhibited shorter ICU lengths of stay and reduced mechanical ventilation time compared to control group. However, there were no statistically significant differences in the 28-day mortality rate or the number of damaged organs between the two groups ($p > 0.05$; Table 2).

Table 1: Differences in APACHE II and SOFA scores before and after treatment

Group	APACHE Ⅱ (points)		SOFA (points)	
	Pre-treatment	***Post-treatment***	***Pre-treatment***	***Post-treatment***
Study group	25.54±1.72	11.46±1.10	9.42±0.57	4.52±0.32
Control group	25.78±1.65	17.20±1.33	9.54±0.42	6.88±0.35
T	0.712	23.516	1.198	35.189
P-value	0.478	0.000	0.234	0.000

Table 2: Comparison of general indices and morbidity and mortality

Group	Length of ICU stay (days)	Duration of mechanical ventilation (day)	Damaged organs (pcs)	28-day mortality rate (%)
Study group	10.24±1.83	2.56±0.34	2.34±0.30	4 (8.00)
Control group	13.56±2.01	3.22±0.38	2.48±0.42	3 (6.00)
χ^2/t	8.636	9.153	1.918	0.154
P-value	0.000	0.000	0.058	0.695

Changes in endotoxin and inflammatory mediators

The levels of endotoxin, TNF-α and PCT were lower in both groups after treatment than before treatment, and lower in study group than in control group ($p < 0.05$; Table 3).

Changes in the degree of liver injury during treatment

The difference in AST and ALT between the two groups before treatment was not statistically significant ($p > 0.05$). Both groups showed decreased AST and ALT levels compared to their pre-treatment values on the 3rd, 7th, and 14th days of treatment. The combined group exhibited lower levels than control group on all three time points ($p < 0.05$; Figure 1).

Changes in oxidative stress levels

Following treatment, both groups exhibited significantly elevated levels of SOD and GSH-Px compared to their pre-treatment values, with study group showing higher levels than control group ($p < 0.05$; Table 4).

Adverse reactions

The difference in adverse reactions between the two groups was not statistically significant ($p > 0.05$; Table 5).

Table 3: Changes in endotoxins and inflammatory mediators in patients before and after treatment

Group	Endotoxin (EU/mL)		TNF-α (ng/mL)		PCT (ng/mL)	
	Pre-treatment	***Post-treatment***	***Pre-treatment***	***Post-treatment***	***Pre-treatment***	***Post-treatment***
Study	27.12±3.61	10.45±1.52*	157.20±23.12	64.12±7.16*	13.54±2.05	0.12±0.04*
Control	26.85±3.42	16.42±1.89*	155.75±22.55	96.52±10.67*	13.46±2.11	0.36±0.06*
T	0.394	17.405	0.317	17.829	0.192	23.534
P-value	0.702	0.000	0.752	0.000	0.845	0.000

Note: *$P < 0.05$ vs. pre-treatment

Figure 1: Alterations in the severity of liver injury during treatment in both patient groups. Note: Changes in serum ALT and AST levels for patients in the study and control groups at pre-treatment, 3 days, 7 days and 14 days of treatment. ***Note:*** *$P < 0.05$ vs. pre-treatment; $^{\#}p < 0.05$ vs control

Table 4: Changes in the level of oxidative stress before and after treatment

Group	SOD (U/mL)		GSH-Px (mg/L)	
	Pre-treatment	**Post-treatment**	**Pre-treatment**	**Post-treatment**
Study group	75.54±11.19	157.20±1.33*	41.42±0.57	74.52±9.32*
Control group	77.78±12.23	121.46±1.10*	41.54±0.42	56.88±7.35*
T	0.943	9.034	0.862	10.509
P-value	0.348	0.000	0.391	0.000

Note: *$P < 0.05$ vs. pre-treatment

Table 5: Assessment of variances in adverse reactions

Group	Nausea and vomiting	Stomach ache	Cough	Itchy skin	Total incidence
Study group	2 (4.00)	1 (2.00)	0 (0.00)	1 (2.00)	4 (8.00)
Control group	1 (2.00)	0 (0.00)	1 (2.00)	1 (2.00)	3 (6.00)
X^2					0.154
P-value					0.695

DISCUSSION

Septic shock is characterized by inflammation, tissue and organ damage, ischemia, hypoxia, immune imbalance and other pathological symptoms. Current treatments have limitations, resulting in low patient survival rates. While conventional treatment has improved patient survival rates, early treatment effectiveness still requires enhancement. Additionally, treatment often provides some relief but may not reverse damage to vital organs [9]. Therefore, early detection and preventive treatment during the initial stages of care are crucial for minimizing organ damage and expediting patient recovery.

Continuous blood purification is typically employed for treating renal insufficiency and sepsis-induced renal injury, as well as systemic inflammatory responses [10]. Reduced glutathione, synthesized by the liver, functions as an antioxidant enzyme in the body, effectively mitigating damage caused by reactive oxygen species to cell membranes [11]. While the combined use of reduced glutathione and blood purification for septic shock has limited reported data, this study aims to explore the combined treatment's impact on septic shock.

Clinical assessment commonly utilizes the APACHE II score to evaluate the health status of critically ill patients. A higher score indicates a more severe condition and studies have shown that APACHE II scores hold significant predictive value for septic shock patient prognosis [12]. The SOFA score, another widely used clinical indicator, assesses disease severity based on organ function. It plays a vital role in sepsis diagnosis [13]. In this study, the APACHE II score and SOFA score of the combined group were lower than those of control group, indicating that combined treatment effectively improves the degree of organ tissue damage [14]. In this study, the differences in organ damage and 28-day morbidity and mortality rates between the two groups were not statistically significant while the ICU hospitalization time and mechanical ventilation in study group were smaller than those in control group, suggesting that combined treatment can help patients improve respiratory function and promote faster recovery.

In the initial stages of sepsis development, the body experiences an amplified inflammatory response, leading to tissue damage and immune cell impairment. As the disease advances, the body's pro-inflammatory and anti-inflammatory responses counterbalance, resulting in an immune system imbalance. Endotoxin is a component of the outer membrane of bacteria that induces systemic inflammation, with high levels in the early stages of sepsis, which correlates with bacterial infection and the severity of the disease [15]. The TNF-α is a pivotal molecule in the inflammatory response to sepsis, secreted by activated monocytes and macrophages. It binds to its corresponding receptor, mediating apoptosis [16]. Procalcitoninogen (PCT) on the other hand, is a glycoprotein secreted by parafiltered thyroid cells and neuroendocrine cells. Serum PCT levels are associated with bacterial infections and concentrations are elevated in sepsis patients, correlating with prognosis [17]. In this study, the combined treatment led to decreased levels of endotoxin, TNF-α and PCT compared to control group. This suggests that the combined treatment effectively reduces patient inflammatory responses and promotes immune system recovery. This effect may be attributed to reduced glutathione's ability to stabilize hepatocyte cell membranes, reduce hepatocellular damage, attenuate inflammatory cell infiltration and necrosis, and lower serum TNF-α and PCT levels.

Acute liver injury is among the early organ injuries in sepsis patients as the disease progresses. It can significantly impact the prognosis of septic shock. Timely and effective treatment plays a crucial role in promoting liver function recovery. In patients with sepsis, more endogenous reduced glutathione is consumed due to ischaemia and hypoxia and more reactive oxygen species are generated by the inflammatory response. ALT and AST are commonly used to assess liver function, and the serum levels of ALT and AST were higher than those of control group in rats with sepsis [18]. In this study, the changes in the degree of liver injury at different time points during the treatment period was compared and it was revealed that ALT and AST decreased in both groups after treatment, while ALT and AST levels were lower in the combined group than in control group, suggesting that the combined treatment improved liver function, which may be because reduced glutathione reduced the damage to hepatocytes in septic shock patients by consuming reactive oxygen species in their bodies, and therefore the serum ALT, AST levels were reduced.

Sepsis causes a systemic inflammatory response, producing a large number of reactive oxygen species, causing an imbalance in the body's oxidation and antioxidant mechanisms. In contrast, substances like SOD and malondialdehyde exhibit a significant decrease in sepsis [19]. Under normal circumstances, the

reactive oxygen species produced by the body can serve as important signaling molecules for the homeostasis of the internal environment. Under the safeguard of the antioxidant system, which includes protective substances like peroxidase, glutathione, and vitamins, cells are shielded from the harmful effects of reactive oxygen species. However, in the presence of sepsis, the accumulation of excessive oxidative substances in the body can lead to tissue and organ damage, including brain tissue, lung tissue, heart, liver and kidney damage, due to oxygen deprivation [20]. In this study, the levels of SOD and GSH-Px in study group were higher than those in control group, indicating that reduced glutathione treatment may further promote intracellular antioxidant and oxidative balance by reducing the levels of reactive oxygen species in septic shock patients. In addition, there was no significant difference in the incidence of adverse reactions between the two groups in this study, indicating that this treatment method is safe.

Limitations of this study

This study has several limitations that should be considered when interpreting the results. First, the sample size in this study was relatively small, which may restrict the generalizability of the findings to a broader population of septic shock patients. Additionally, this research was conducted at a single medical center, potentially limiting its ability to capture the diversity of patient populations and treatment protocols seen in different healthcare settings. Another limitation is the relatively short period used in this study. This shorter timeframe might not allow for a comprehensive assessment of long-term outcomes and complications that could be associated with the treatments investigated. Furthermore, control group in this study received continuous blood purification, which may not represent the standard treatment for all septic shock patients.

The study's exclusion criteria also need to be acknowledged, as they may have led to the exclusion of certain patients with specific medical conditions. Consequently, the study's results may not fully represent the diverse profiles of septic shock patients. It is also important to note that there could be unmeasured variables or confounding factors that were not accounted for in the analysis, which could influence the observed outcomes. These limitations should therefore be taken into account when interpreting the results and considering their applicability in clinical practice.

CONCLUSION

Continuous blood purification and reduced glutathione demonstrate promising benefits for patients with severe septic shock. This treatment approach has shown improvements in patient survival rates, reductions in organ damage, mitigation of inflammatory responses, and alleviation of liver injuries. It also exhibits a favorable safety profile. Future studies to accommodate the diverse profiles of septic shock patients from multiple centers and a larger patient population will be needed to validate the outcomes of this study.

DECLARATIONS

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Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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