ORIGINAL ARTICLE

The effect of superoxide dismutase on protein exchange indicators in peritoneal exudate in the terminal phase of peritonitis

RANA KARIMOVA[1], SHAHZADA POLUKHOVA[2], GULNARA GASIMOVA[2], ELNURA MUSAYEVA[2], LEYLA BAGHIROVA[3], AFAG RUSTAMOVA[4], ZENFIRA BAKHSHIYEVA[4], AZIZA RUSTAM-ZADA[4], AFAG KAZIMOVA[2], AZADA RUSTAM-ZADA[4], HUSEYN ABIYEV[5]

[1]Department of Modeling of Pathological Processes, Experimental Surgery and Therapy, Scientific Research Center, Azerbaijan Medical University, Baku, Azerbaijan; [2]Department of Pharmacology, Azerbaijan Medical University, Baku, Azerbaijan; [3]Department of Obstetrics and Gynecology II, Azerbaijan Medical University, Baku, Azerbaijan; [4]Department of Normal Physiology, Azerbaijan Medical University, Baku, Azerbaijan; [5]Department of Medical and Biological Physics, Azerbaijan Medical University, Baku, Azerbaijan.

ABSTRACT

Objective: The aim of the study was to investigate the changes in major indicators of protein metabolism in peritoneal exudate (PE) obtained from patients undergoing surgery in the terminal phase of peritonitis under the influence of the enzyme superoxide dismutase (SOD), and to determine the mechanisms of protein homeostasis disruption and the effect of neutralizing superoxide radicals on these processes during peritonitis.

Methods: The study was conducted at the Scientific Research Center of Azerbaijan Medical University. Protein fractions in peritoneal exudate samples obtained from 30 (17 males and 13 females, aged between 34 and 72 years) patients were measured. Peritoneal exudate samples were divided into aliquots and analyzed with or without SOD after 6 and 24 hours. In samples incubated without SOD, time-dependent alterations in total protein and electrophoretic fractions were observed, indicating that oxidative processes alone can modify the protein profile of peritoneal exudates. SOD was added to the exudate samples and incubated at 10°C for 6 and 24 hours. Total protein, albumin, γ, α1, α2, and β globulins were determined.

Results: In the terminal phase of peritonitis, the mean concentration of total protein in peritoneal exudate was 44±2.55 g/L, and albumin was 25±1.54 g/L. After the addition of SOD, total protein increased by 9.8% at 6 hours and by 27.3% at 24 hours, while albumin levels decreased by 26.7%. γ-globulin levels increased by 11.5% at

Received: 7 October 2025 | Accepted: 19 November 2025

Correspondence: Rana Karimova, MD, PhD, Neonatologist, Senior Researcher / Department of Modeling of Pathological Processes, Experimental Surgery and Therapy, Scientific Research Center, Azerbaijan Medical University, Samad Vurgun street 163, AZ1022, Baku, Azerbaijan / E-mail: dr.renakerimova@mail.ru
ORCID: 0009-0004-4323-9625

6 hours and even more at 24 hours. Simultaneously, decreases were observed in α1, α2, and β-globulin fractions. **Conclusion:** In the terminal phase of peritonitis, significant changes in the protein composition of peritoneal exudate were observed under the influence of SOD. These changes varied depending on the type of protein fraction and reflected the disturbances in protein metabolism and potential for correction. (www.actabiomedica.it)

Key words: peritoneal intoxication, peritoneal exudate, terminal phase, protein metabolism, superoxide dismutase enzyme

Introduction

Peritonitis is one of the most frequently encountered acute surgical diseases of the abdominal cavity and is characterized by a high mortality rate. According to the literature, the contents of the abdominal cavity in cases of peritonitis contain substances of various natures, which not only increase the toxicity of the peritoneal exudate but also disrupt the normal physiological processes of the organism, thereby aggravating the ongoing pathological condition (1-3). One of the main factors in the development of peritoneal intoxication (PI) is the activation of the microbial flora, since peritonitis begins with the disruption of the integrity of the abdominal organs and the gastrointestinal tract. In such cases, the gastrointestinal microflora enters the abdominal cavity and begins to proliferate rapidly. The metabolic products of these microorganisms possess acute toxic properties, which lead to the formation of highly toxic exudate in the abdominal cavity (4). These toxic substances of various origins accumulate in the abdominal cavity and are absorbed into the body through lymphatic and blood vessels, spreading throughout the organism and causing severe pathophysiological disturbances in metabolic processes (5,6). One of the target organs affected by endogenous intoxication during peritonitis is the liver. Toxic substances transported to the liver via the bloodstream impair its functional state and negatively influence various metabolic processes occurring there (7). As a result of the effects of peritoneal intoxication, several synthetic functions of the liver, including protein synthesis, are disrupted (8,9). Clarifying the mechanisms of protein metabolism disorders and their pathogenetic significance in the terminal stage of diffuse peritonitis is of great importance (10,11).

Materials and Methods

The experiments were conducted at the Scientific Research Center of Azerbaijan Medical University. The study investigated changes in protein metabolism indicators in the peritoneal exudate (PE) obtained from the abdominal cavity of 30 patients who underwent surgery during the terminal phase of peritonitis (12,13). All patients underwent a single emergency surgical procedure during the terminal phase of peritonitis. Peritoneal exudate samples were collected intraoperatively, immediately after opening the abdominal cavity and before any lavage or additional manipulation was performed. No repeated surgeries or postoperative lavages were performed prior to sample collection.The study population consisted of 30 patients (17 males and 13 females) aged between 34 and 72 years. Inclusion criteria: Patients aged 34–72 years with terminal phase peritonitis who underwent emergency surgery and provided informed consent. Exclusion criteria: Patients with chronic liver or kidney disease, immunodeficiency, malignancy, or incomplete clinical records.The mean age of the patients was 52.3 ± 11.4 years, ensuring adequate representation of both genders. Simultaneously, the effect of adding the superoxide dismutase (SOD) preparation to the PE on the dynamics of changes in protein metabolism indicators was studied (14,15). These experiments were performed in vitro and incubated in a thermostat at 10°C for 6 and 24 hours. PE aliquots were processed

and incubated under aseptic conditions, but no protease inhibitor cocktail was added prior to incubation. Proteolytic degradation under ex-vivo conditions may therefore have contributed to changes in albumin and some globulin fractions; this is acknowledged as a limitation and is discussed below. Samples were incubated at 10°C to slow enzymatic degradation and microbial proliferation during the in vitro incubation period, allowing controlled observation of SOD-dependent changes in protein fractions while minimizing artifactual proteolysis. We chose to incubate the samples at 10°C to slow down enzymatic degradation and microbial growth, allowing for a controlled assessment of protein metabolism changes without compromising the integrity of the peritoneal exudate during the incubation period. Patients were selected using stratified randomization to ensure homogeneity in age, sex, and disease severity. However, instead of assigning patients to separate groups, each peritoneal exudate (PE) sample was immediately divided into three equal aliquots, allowing within-sample (paired) comparison of the following experimental conditions: baseline (no incubation), incubation at 10 °C without SOD, and incubation at 10 °C with SOD. One aliquot was analyzed immediately (baseline/control), the second aliquot was incubated at 10°C without added enzyme (incubation control), and the third aliquot received the superoxide dismutase (SOD) preparation and was incubated at 10°C (SOD condition). For clarification, although the results are presented as three "groups" (control, incubation, and SOD-treated), these correspond to three experimental conditions applied to aliquots of the same peritoneal exudate sample obtained from each patient, rather than to three separate patient cohorts. The superoxide dismutase (SOD) used in this study was a homogeneous preparation produced in the Russian Federation. The stock solution contained 3000 U/mL and was dissolved in isotonic saline to reach the working concentration in each aliquot of peritoneal exudate. For every 500 mL of exudate, 3 mL of the SOD stock was added and incubated at 10°C for 6 and 24 hours. The preparation was supplied in sealed sterile containers and stored according to the manufacturer's instructions. Patient selection remained balanced for age, sex and disease severity by stratified enrolment, and each patient's three aliquots were processed in parallel. This design reduced selection bias and allowed accurate comparison of all experimental conditions using aliquots derived from the same patient sample.

Group 1 (Control group, n=10): In this group, the peritoneal exudate was analyzed immediately after being collected from the abdominal cavity without incubation or the addition of SOD, and the amounts of total protein, albumin, γ globulin, α1, α2, and β globulin fractions were determined.

Group 2 (Experimental group, n=10): In this group, the peritoneal exudate was incubated without the addition of SOD at 10°C for 6 and 24 hours, and the same protein fractions were measured.

Group 3 (SOD group, n=10): In this group, the SOD preparation was added to the peritoneal exudate and incubated at 10°C for 6 and 24 hours to study its effect on the protein fractions.

Although the results are described as three groups (Group 1, Group 2, Group 3), these do not represent three independent sets of patients. Each of the 30 patients provided one peritoneal exudate (PE) sample, and each sample was immediately divided into three equal aliquots. These aliquots were then subjected to three experimental conditions: (1) baseline analysis, (2) incubation at 10°C without SOD, and (3) incubation at 10°C with SOD. Therefore, the term "group" refers to experimental conditions applied to aliquots from the same patients, not to separate patient cohorts.

The peritoneal content was collected from the following patients:

1. Peritonitis developing as a result of gastric ulcer perforation – 6 patients
2. Peritonitis developing as a result of gallbladder perforation – 6 patients
3. Peritonitis caused by small bowel obstruction – 6 patients
4. Peritonitis developing as a result of appendix-like protrusion perforation – 6 patients
5. Peritonitis developing after surgery on the genital organs – 6 patients

In total, 30 patients participated in the study and were equally divided into 3 groups.

Although the study included a total of 30 patients, they were randomly assigned to three groups

using stratified randomization to ensure balanced distribution with respect to age, sex, and disease severity, thereby minimizing potential confounding factors.

Collection and preparation of peritoneal contents for examination were performed as follows: During surgery, immediately upon opening the abdominal cavity, the contents were aspirated using an electric suction device. Large particles were removed, and the sample was filtered through a sterile filter into a glass container for analysis and determined using a modified Kon sedimentation method.

Determination of α1-globulin concentration

To determine the amount of α1-globulin, 5 ml of filtered PE (peritoneal exudate) is taken and poured into a 10 ml flask. The pH is then measured. Based on the pH level, titration is carried out to adjust the pH to 5.2. Subsequently, 5 ml of 18% ethanol is added to the exudate. The flask is incubated in a thermostat at 5°C for 24 hours. After incubation, the solution is decanted until sediment appears and discarded. Then, 5 ml of physiological saline is added to the sediment and shaken until a primary solution is obtained. From this solution, three separate 1 ml samples are taken using pipettes and transferred into three test tubes. Using a special reagent kit produced in the Czech Republic, the determination is carried out on an FP-marked microanalyzer. The results are collected and averaged by dividing by three.

Determination of α2-globulin concentration

The determination of α2-globulin concentration is performed similarly to the method used for α1-globulin. However, the difference lies in adjusting the pH to 5.8. The flask is shaken until a homogeneous solution is obtained and incubated in a thermostat at 5°C. The subsequent steps are continued in the same manner as described for α1-globulin.

Determination of β- and γ-globulin concentrations

The determination of β- and γ-globulins is performed using the same precipitation method. For this, a 20 ml flask is taken, and 10 ml of filtered PE is added. The pH is adjusted to 6.9 by titration. After that, 10 ml of 25% ethanol is added to the PE in the flask. The flask is incubated in a thermostat at 5°C for 24 hours. Then the solution is decanted until sediment appears. 10 ml of physiological saline is added to the sediment and shaken until a primary solution is obtained. Using six pipettes (1 ml in each), 3 ml of the solution is used to determine β-globulin, and the other 3 ml is used to determine γ-globulin, using specific reagent kits produced in the Czech Republic.

Determination of albumin concentration

For the determination of albumin concentration, 5 ml of filtered PE is poured into a 10 ml flask. The pH is adjusted to 4.8 by titration. Then, 5 ml of 40% ethanol is added, and the flask is shaken until a primary solution is obtained. The solution is incubated in a thermostat at 5°C for 24 hours. After incubation, the flask is removed from the thermostat, and the supernatant is completely aspirated using a pipette. Then, 5 ml of physiological saline is added to the sediment and shaken until it becomes completely homogeneous. From the resulting solution, 1 ml is transferred into each of three test tubes. Then, 1 ml of a specific reagent for albumin determination from a standard reagent kit designed for "protein metabolism analysis" produced in the Czech Republic is added to each test tube. The mixture is analyzed using an FK-500 microanalyzer. The results from the test tubes are added together and averaged by dividing by three.

Protein concentration

The total protein concentration in the peritoneal exudate was determined using a modified biuret method, in accordance with the general procedure for protein quantification. Measurements were performed spectrophotometrically using standard reagent kits, similarly to the method used for albumin determination. The SOD (superoxide dismutase) preparation used in the experiments is a homogeneous preparation stored in a special container and produced in the Russian Federation. The SOD preparation was prepared at a concentration of 3000 U/ml, dissolved in 3 ml of isotonic solution, and added to 500 ml of peritoneal exudate, followed by incubation at 10°C for 6 and 24

hours. We focused on superoxide dismutase due to its established role in neutralizing superoxide radicals that contribute to protein oxidation and imbalance in protein fractions during peritonitis, and based on this mechanism, we selected it. To minimize inter-individual variability, each peritoneal exudate sample obtained from the same 30 patients was immediately divided into three equal aliquots and processed in parallel under three experimental conditions (baseline, incubation without SOD, and incubation with SOD), allowing paired within-sample comparison. Normality of continuous variables was assessed with the Shapiro–Wilk test. Because each patient's PE sample was split into three aliquots and measured at baseline, 6 h and 24 h (paired design), within-sample comparisons across time/conditions were analyzed using the Wilcoxon signed-rank test for two-timepoint paired comparisons and the Friedman test for three-timepoint comparisons. For independent group comparisons (when performed), the Mann–Whitney U test was used. Exact p-values are reported throughout. To account for multiple comparisons across protein fractions and timepoints, Bonferroni correction was applied; the adjusted significance threshold is reported where applicable ($\alpha_{adjusted} = 0.05$ / number of comparisons). Effect sizes (rank-based) and 95% confidence intervals for mean changes were computed and are presented alongside p-values. All analyses were performed using SPSS version 22 and Microsoft Excel 2016.For all reported comparisons we present median (interquartile range) or mean ± SD as appropriate, exact p-values, and 95% CIs. After Bonferroni correction, significance was considered at $p < 0,05$. No formal a priori power calculation was performed for this exploratory ex-vivo study; a convenience sample of 30 patients (30 PE samples; each split into three aliquots) was used to detect biologically meaningful trends. We acknowledge that the sample size limits statistical power for small effect sizes, and larger confirmatory studies are recommended.

Results

Protein levels in peritoneal exudate obtained from the abdominal cavity in the terminal phase of peritonitis were analyzed in samples from 30 patients (n = 30). These samples represent the control (baseline) condition before enzyme addition. Table 1 presents the mean concentrations of total protein and individual protein fractions. To investigate the effect of superoxide dismutase (SOD), the same peritoneal exudate samples were incubated with SOD for 6 and 24 hours. Table 2 presents the dynamics of protein metabolism under three experimental conditions (baseline, incubation without SOD, and incubation with SOD), using aliquots derived from the same 30 patients' peritoneal exudate samples rather than separate patient groups. In order to assess the specific effect of superoxide dismutase (SOD), additional aliquots of the same PE samples were incubated at 10°C for 6 and 24 hours both without and with SOD. Incubation without SOD caused a moderate decline in albumin and α-globulin fractions (approximately 8–12%) and a slight increase in γ-globulin levels, which likely reflect early proteolytic and oxidative changes in vitro (Table 2). In contrast, addition of SOD produced more pronounced shifts—particularly an increase in total protein and γ-globulin concentrations and a decrease in albumin—indicating the modulatory role of superoxide dismutase in protein metabolism under these conditions. Figure 1 provides a visual representation of these findings,

Table 1. Protein levels in peritoneal exudate obtained from the abdominal cavity in the terminal phase of peritonitis (n = 30). Values are shown as mean ± SEM (minimum–maximum). Units: $g \cdot L^{-1}$.

	Proteins, g/l					
Statistical indicators	**Total protein**	**Albumin**	**γ globulin**	**α1- globulin**	**α2- globulin**	**β-qlobulin**
Mean±SEM	44±2,55	25±1,54	18,68±1,32	2,64±0,18	4,56±0,41	7,71±0,73
Min	28	16	12	1,6	2	3,5
Max	61	37	28	4	8,5	15

Values are expressed as Mean ± SEM.

Table 2. Experimental dynamics of protein metabolism in peritoneal exudate from patients with terminal peritonitis after incubation at 10°C with and without superoxide dismutase (SOD).

Proteins	Baseline (from Table 1)	6 h (no SOD)	24 h (no SOD)	6 h (with SOD)	24 h (with SOD)	p-value
Total protein	44 ± 2.55 (28–61)	42 ± 3.10 (30–59)	40 ± 2.85 (28–57)	48.3 ± 5.07 (37–65)	56 ± 4.66 (35–65)	<0.05
Albumin	25 ± 1.54 (16–37)	23 ± 1.90 (15–34)	21 ± 2.10 (13–32)	18.5 ± 2.56 (12–29)	18.3 ± 2.06 (9–23)	<0.05
γ-globulin	18.68 ± 1.32 (12-28)	19.5 ± 1.80 (14–27	20.2 ± 2.00 (15–29)	20.83 ± 2.15 (15–28)	23 ± 2.68 (15–30)	>0.05
α_1-globulin	2.64 ± 0.18 (1.6–4.0)	2.35 ± 0.25 (1.5–3.5)	2.10 ± 0.30 (1.0–3.0)	1.95 ± 0.59 (1.0–3.0)	1.8 ± 0.38 (0.3–3.0)	<0.05
α_2-globulin	4.56 ± 0.41 (2.0–8.5)	4.20 ± 0.50 (2.5–7.0)	3.85 ± 0.55 (2.0–6.5)	3.12 ± 0.45 (2.0–5.0)	3.52 ± 0.59 (3.5–6.5)	<0.05
β-globulin	7.71 ± 0.73 (3.5–15.0)	7.20 ± 0.80 (3.5–12.0)	6.80 ± 0.90 (3.0–10.0)	6.11 ± 0.63 (4.0–8.2)	6.20 ± 1.13 (2.0–10.0)	>0.05

Values are presented as mean ± SEM (min–max); units: g/L.

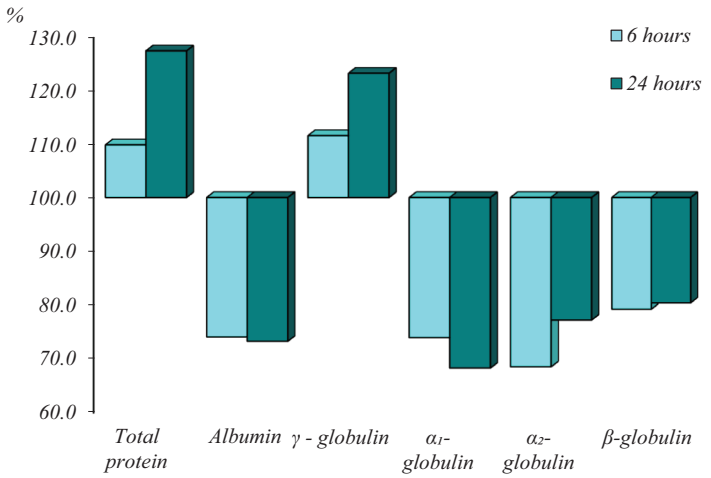

Figure 1. Relative percentage changes in total protein, albumin, and globulin fractions after SOD addition compared with baseline and incubation control samples. The figure illustrates the relative percentage changes in total protein, albumin, and globulin fractions after incubation with and without SOD at 6 and 24 hours. A more pronounced increase in total protein and γ-globulin, along with a significant decrease in albumin, is observed in the SOD-treated samples compared with both baseline and incubation controls.

showing the relative percentage changes in total protein and individual protein fractions after incubation with and without SOD at 6 and 24 hours. (Figure 1).

To provide a complete and reliable comparative analysis of protein metabolism dynamics, an additional series of experiments was conducted using aliquots of the same peritoneal exudate samples obtained from patients in the terminal phase of peritonitis. These aliquots were incubated at 10°C for 6 and 24 hours without the addition of SOD (incubation control) in order to evaluate the intrinsic effect of incubation in the absence of antioxidant protection. The obtained experimental data

demonstrated that, even without SOD, minor biochemical alterations occurred in the peritoneal exudate during incubation, reflecting the natural progression of proteolytic and oxidative processes under ex-vivo conditions. The results of these experiments are summarized in Table 2. As shown, incubation without SOD resulted in a slight decline in total protein and albumin levels (approximately 8–10%) and moderate decreases in α-globulins, which likely indicate early proteolytic activity and partial degradation of low-molecular-weight proteins. In contrast, the addition of SOD led to more pronounced shifts in protein composition, most notably a significant increase in total protein and γ-globulin fractions, suggesting a stabilizing and protective effect of the enzyme on protein structures and the overall metabolic balance of the peritoneal exudate.

After adding the superoxide dismutase preparation to the peritoneal exudate obtained from the abdominal cavity and incubating the solution in a thermostat at 10°C for 6 hours, the total protein amount increased to 9.8%. After 24 hours under the same conditions, the total protein amount increased to 27.3% ($p<0.05$). In the terminal phase of peritonitis, the average quantitative indicator of albumin in the peritoneal exudate obtained from the abdominal cavity was 25±1.54 g/l, with a minimum value of 16 g/l and a maximum value of 37 g/l (Table 2). After 6 hours of incubation with SOD, the mean albumin concentration decreased to 18.5 ± 2.56 g/L ($p < 0.05$), and this value remained essentially unchanged at 24 hours (18.3 ± 2.06 g/L, $p < 0.05$). At the 24th hour, compared to the 6-hour mark, it practically did not change. Table 1 and Table 2 units have been standardized to $g \cdot L^{-1}$ (g/L). All values are presented as mean ± SEM with minimum and maximum values indicated. Exact p-values are reported in the tables. On the day following the surgery, the albumin level in the peritoneal exudate collected from the abdominal cavity was decreased by 26.7% compared to the baseline level ($p<0.05$). Upon the addition of superoxide dismutase, the albumin concentration showed a progressive decrease every 2 hours ($p<0.05$). In the terminal phase of peritonitis, the concentration of γ-globulin in the peritoneal exudate collected from the abdominal cavity reached 18.68±1.31 g/L, with a minimum value of 12 g/L and a maximum value of 28 g/L. After the addition of superoxide dismutase to the peritoneal exudate and incubation in a thermostat for 6 hours, the γ-globulin level increased by 11.5%, with an average concentration of 20.83±2.15 g/L ($p>0.05$), a minimum value of 15 g/L, and a maximum value of 28 g/L. Although some changes in γ-globulin levels were observed after SOD addition, these differences were not statistically significant ($p>0.05$) and should therefore be interpreted as biological trends rather than definitive effects (1,2). By the 24th hour of the experiment, the influence of superoxide dismutase led to a further increase in γ-globulin concentration in the peritoneal exudates (16,17). The mean concentration reached 23±2.68 g/L, with a minimum of 15 g/L and a maximum of 30 g/L (Table 2) .In the terminal stage of peritonitis, the average concentration of α1-globulin in the peritoneal exudate obtained from the abdominal cavity was 2.64±0.18 g/L, with a minimum value of 1.6 g/L and a maximum value of 4 g/L. After the addition of superoxide dismutase, within 6 hours, the mean concentration of α1-globulin in the peritoneal exudate decreased by 26.2%, reaching 1.95±0.37 g/L, with a minimum value of 1 g/L and a maximum value of 3 g/L ($p<0.05$). After incubation in a thermostat for 24 hours, the concentration of α1-globulin in the peritoneal exudate decreased further, reaching an average value of 1.8±0.38 g/L (min. 0.3 g/L, max. 3 g/L). This reduction corresponds to a 31.9% decrease compared to the peritoneal exudate obtained from the abdominal cavity ($p<0.05$) (Figure 1).

Discussion

When comparing the difference in α1-levels, its minimum value decreased by 1.6 times at the 6th hour and by 3.3 times at the 24th hour of the experiment, compared to the exudate before the addition of superoxide dismutase. The maximum value decreased by 1.3 times in both cases. At 6 hours, the α2-globulin concentration decreased by 31.6% compared to baseline (from 4.56 ± 0.41 g/L to 3.12 ± 0.45 g/L). By 24 hours, the concentration partially recovered, showing a 12.8% increase relative to the 6-hour value (3.52 ± 0.59 g/L), while still remaining 22.8% lower than baseline (5,6). In this case, the minimum value dropped to 1.5 g/l, and the maximum value to 5.5 g/l,

meaning the minimum decreased by 1.3 times and the maximum by 1.5 times. In the terminal stage of peritonitis, the average concentration of β-globulin in the peritoneal exudate taken from the abdominal cavity was 7.71 ± 0.73 g/L, with a minimum value of 3.5 g/L and a maximum value of 15 g/L. When superoxide dismutase was added to this exudate and it was incubated in a thermostat at 10°C for 6 hours, the average concentration of β-globulin decreased to 6.11 ± 0.63 g/L, with a minimum of 4 g/L and a maximum of 8.2 g/L. Compared to the intact state at the 6th hour of the experiment, the concentration of β-globulin in the exudate decreased by 20.6%. At the 24th hour of the experiment, a slight increase was observed; however, the level remained lower than the initial state before the addition of superoxide dismutase. This decrease amounted to 19.5%. After 24 hours, the minimum value of β-globulin was 2 g/L, the maximum was 10 g/L, and the average concentration was 6.2 ± 1.13 g/L (18). This study was performed under in vitro conditions, which allowed for the controlled evaluation of biochemical changes in protein fractions upon exposure to superoxide dismutase. However, in vitro models do not fully replicate the complexity of the in vivo inflammatory environment in peritonitis. Therefore, while these findings provide important preliminary insights into the oxidative modulation of protein metabolism, further in vivo and clinical studies are warranted to validate the physiological and therapeutic relevance of these results. We incubated peritoneal exudates at 10°C to reduce rapid enzymatic and microbial changes ex vivo; however, this temperature is not physiological and may alter reaction kinetics and protein–enzyme interactions compared with in vivo conditions. Therefore, the observed dynamics should be interpreted primarily as biochemical trends under the tested ex-vivo conditions, and in vivo confirmation at physiological temperature is warranted. Our findings regarding the decrease in albumin and α-globulin fractions are consistent with previous studies reporting impaired hepatic protein synthesis during peritonitis (10,19). This similarity suggests that oxidative stress may represent a common pathway in protein imbalance in both experimental and clinical settings (10,19).

The observed decrease in albumin following SOD exposure may reflect enhanced albumin oxidation and subsequent proteolytic cleavage or redistribution among exudate compartments; conversely, increases in γ-globulin could be due to relative enrichment following albumin loss or selective stability/aggregation of immunoglobulins under oxidative conditions. These alterations suggest that SOD indirectly modulates protein homeostasis by shifting the oxidative balance. Neutralization of superoxide radicals may accelerate the oxidative modification and degradation of albumin, whereas γ-globulins, due to their structural stability and immunological function, remain less affected or relatively increased. This mechanism reflects a compensatory response of the immune system under oxidative stress and explains the disproportionate preservation of immunoglobulins compared with albumin. These hypotheses are consistent with reports linking oxidative stress to albumin modification and altered protein turnover in severe inflammation (10, 18).

In contrast to previous studies (20,21) who observed only mild γ-globulin changes after SOD treatment in septic patients, our study in terminal peritonitis patients revealed a more pronounced increase, which may be explained by the advanced stage of the disease in our population. These results highlight the dual role of SOD: while it enhances certain protective protein fractions, it may also contribute to albumin depletion, indicating a complex balance between antioxidant activity and protein catabolism (20,21). This study focused on assessing the modulatory influence of superoxide dismutase (SOD) on protein metabolism in peritoneal exudate under ex-vivo conditions, rather than directly measuring its enzymatic activity. Although this design allowed us to evaluate the biochemical effects of SOD on major protein fractions, the absence of direct measurements of residual SOD activity at 6 and 24 hours is recognized as a limitation. Such measurements would have provided additional mechanistic understanding of enzyme stability and its contribution to the regulation of protein homeostasis during peritonitis. Nevertheless, previous reports have shown that SOD retains its catalytic activity for up to 24 hours at comparable incubation temperatures (13,14), supporting the validity of the experimental approach and the interpretation of our findings. These findings suggest that oxidative stress plays a key role in disrupting protein homeostasis in terminal peritonitis.

Although this study was conducted under ex-vivo conditions, timely modulation of oxidative imbalance — including potential antioxidant interventions such as SOD — may have clinical relevance by improving metabolic stability and potentially patient outcomes in severe peritonitis.

Conclusion

After incubating the peritoneal exudate enriched with superoxide dismutase at 10°C for 6 and 24 hours, various changes in protein metabolism were observed (19). The total protein concentration remained at a high level throughout all stages of the experiment. In contrast, the amount of albumin sharply decreased in both cases. Similar to total protein, the level of globulin remained high and intensified further by the 24th hour. However, a decrease in the concentration of all globulin fractions was identified. Thus, after adding superoxide dismutase to the peritoneal exudate collected from the abdominal cavity during the terminal phase of peritonitis and incubating it at 10°C for 6 and 24 hours, the protein composition was altered (22,23). These changes varied depending on the type of protein and the duration of incubation. The authors affirm that all experimental procedures were conducted with methodological rigor and transparency. The study design focused on evaluating the ex-vivo effect of SOD on protein metabolism, without direct measurement of enzymatic activity. The authors take full responsibility for the accuracy of the data and the integrity of the interpretation. All authors reviewed and approved the final version of the manuscript.

Ethic Approval: #07 protocol of Ethics Committee of Azerbaijan Medical University, Baku, Azerbaijan [15.03.2023].

Declaration on the Use of AI: None.

Conflict of Interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article.

Authors Contribution: RK, LB, ZB, AR: experiment, review. ShP, GG, EM, AK: investigation, methodology, editing, resources, writing. HA data curation. AR, AR: statistical calculation, visualization. All authors contributed to the article and approved the submitted version.

References

1. Tang J, Wang D, Chen Y, Feng J. The association between new inflammation markers and frequent peritoneal dialysis-associated peritonitis. BMC Nephrol. 2024 Mar 5;25(1):81. doi: 10.1186/s12882-024-03496-z.
2. Li Y, Liu Y, Xie S, et al. Metabolic response to burn injury: a comprehensive bibliometric study. Front Med (Lausanne). 2025 Jan 3;11:1451371. doi: 10.3389/fmed.2024.1451371.
3. Kim ME, Lee JS. Advances in the regulation of inflammatory mediators in nitric oxide synthase: implications for disease modulation and therapeutic approaches. Int J Mol Sci. 2025 Jan 30;26(3):1204. doi: 10.3390/ijms26031204.
4. Zhao J, Zhang T, Deng Z, Han X, Ma T, Xie K. Evaluation of biomarkers from peritoneal fluid as predictors of severity for abdominal sepsis patients following emergency laparotomy. J Inflamm Res. 2023 Feb 24;16:809-26. doi: 10.2147/JIR.S401428.
5. Hsueh MF, Önnerfjord P, Bolognesi MP, Easley ME, Kraus VB. Analysis of "old" proteins unmasks dynamic gradient of cartilage turnover in human limbs. Sci Adv. 2019 Oct 9;5(10):eaax3203. doi: 10.1126/sciadv.aax3203.
6. Williamson E, Kato H, Volterman KA, Suzuki K, Moore DR. The effect of dietary protein on protein metabolism and performance in endurance-trained males. Med Sci Sports Exerc. 2019 Feb;51(2):352-60. doi: 10.1249/MSS.0000000000001791.
7. Santucci L, Bruschi M, Del Zotto G, et al. Biological surface properties in extracellular vesicles and their effect on cargo proteins. Sci Rep. 2019 Sep 10;9(1):13048. doi: 10.1038/s41598-019-47598-3.
8. Hadadi N, Mohammadi Peyhani H, Miskovic L, Seijo M, Hatzimanikatis V. Enzyme annotation for orphan and novel reactions using knowledge of substrate reactive sites. Proc Natl Acad Sci U S A. 2019 Apr 9;116(15):7298-307. doi: 10.1073/pnas.1818877116.
9. Su HY, Yang JJ, Zou R, et al. Autophagy in peritoneal fibrosis. Front Physiol. 2023 May 15;14:1187207. doi: 10.3389/fphys.2023.1187207.
10. Arı H, Arı M, Ogut S. Oxidative stress and anti-oxidant status in children with sepsis. BMC Pharmacol Toxicol. 2025 Mar 19;26(1):64. doi: 10.1186/s40360-025-00895-2.
11. Meledeo MA, Peltier GC, McIntosh CS, Bynum JA, Corley JB, Cap AP. Coagulation function of never frozen liquid plasma stored for 40 days. Transfusion. 2021 Jul;61 Suppl 1:S111-8. doi: 10.1111/trf.16526.
12. Safavi-Sohi R, Johnson J, Liu Y, et al. Peritoneal cavity-derived small extracellular vesicles from aged tumor-naïve

hosts promote ovarian cancer adhesion and invasion. Cell Commun Signal. 2025 Jul 1;23(1):308. doi: 10.1186/s12964-025-02273-1.
13. Helan M, Malaska J, Tomandl J, et al. Kinetics of biomarkers of oxidative stress in septic shock: a pilot study. Antioxidants (Basel). 2022 Mar 26;11(4):640. doi: 10.3390/antiox11040640.
14. Tian Y, Yao N, Wu Y, Wang F, Zhao Y. Association between plasma level of superoxide dismutase and survival of patients with acute-on-chronic liver failure. BMC Gastroenterol. 2022 Feb 5;22(1):50. doi: 10.1186/s12876-022-02126-y.
15. Lai KJ, Hsieh YP, Chiu PF, Lin PR. Association of albumin and globulin with mortality risk in incident peritoneal dialysis patients. Nutrients. 2022 Jul 12;14(14):2850. doi: 10.3390/nu14142850.
16. Peng F, Sun L, Chen T, et al. Albumin-globulin ratio and mortality in patients on peritoneal dialysis: a retrospective study. BMC Nephrol. 2020 Feb 14;21(1):51. doi: 10.1186/s12882-020-1707-1.
17. Xiao Y, Zhang Y, Cheng X, et al. Association between albumin to globulin ratio and pneumonia in patients with aneurysmal subarachnoid hemorrhage. Sci Rep. 2025 Jul 16;15(1):25731. doi: 10.1038/s41598-025-08466-5.
18. Simões Silva L, Araujo R, Pestana M, Soares-Silva I, Sampaio-Maia B. Peritoneal microbiome in end-stage renal disease patients and the impact of peritoneal dialysis therapy. Microorganisms. 2020 Jan 25;8(2):173. doi: 10.3390/microorganisms8020173.
19. Liu Y, Zhang L, Tian T, et al. Diagnostic value of NLR, PLR and SIRI in peritoneal dialysis-associated peritonitis. Am J Transl Res. 2025 Mar 15;17(3):2250-7. doi: 10.62347/EQGN8207.
20. Bonifazi M, Meessen J, Bernasconi R, Pérez A, et al. Albumin oxidation status in sepsis patients treated with albumin or crystalloids. Front Physiol. 2021 Aug 6;12:682877. doi: 10.3389/fphys.2021.682877.
21. Baştan İ, İrdem Dİ, Kartal YK, Ergin SH, Tunç AS. Oxidative stress in neurological feline infectious peritonitis: cerebrospinal fluid 8-hydroxy-2'-deoxyguanosine and superoxide dismutase levels. Kafkas Univ Vet Fak Derg. 2025;31(4):547-56. doi: 10.9775/kvfd.2025.34251.
22. Xu Y, Liu B, Cheng S, et al. Serum total superoxide dismutase activity as a predictive factor in patients with hepatocellular carcinoma. Hepat Mon. 2024 Apr 5;24(1):e144896. doi: 10.5812/hepatmon-144896.
23. Didziokaite G, Biliute G, Gudaite J, Kvedariene V. Oxidative stress as a potential underlying cause of minimal and mild endometriosis-related infertility. Int J Mol Sci. 2023 Feb 14;24(4):3809. doi: 10.3390/ijms24043809.