European Journal of Trauma and Emergency Surgery
https://doi.org/10.1007/s00068-023-02298-6

ORIGINAL ARTICLE

Gut barrier protein levels in serial blood samples from critically ill trauma patients during and after intensive care unit stay

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Received: 9 February 2023 / Accepted: 29 May 2023

Abstract

Purpose In an effort to better manage critically ill patients hospitalised in the intensive care unit (ICU) after experiencing multiple traumas, the present study aimed to assess whether plasma levels of intestinal epithelial cell barrier proteins, including occludin, claudin-1, junctional adhesion molecule (JAM-1), tricellulin and zonulin, could be used as novel biomarkers. Additional potential markers such as intestinal fatty acid-binding protein (I-FABP), D-lactate, lipopolysaccharide (LPS) and citrulline were also evaluated. We also aimed to determine the possible relationships between the clinical, laboratory, and nutritional status of patients and the measured marker levels.
Methods Plasma samples from 29 patients (first, second, fifth and tenth days in the ICU and on days 7, 30 and 60 after hospital discharge) and 23 controls were subjected to commercial enzyme-linked immunosorbent assay (ELISA) testing.
Results On first day (admission) and on the second day, plasma I-FABP, D-lactate, citrulline, occludin, claudin-1, tricellulin and zonulin levels were high in trauma patients and positively correlated with lactate, C-reactive protein (CRP), number of days of ICU hospitalisation, Acute Physiology and Chronic Health Evaluation II (APACHE II) score and daily Sequential Organ Failure Assessment (SOFA) scores ($P < 0.05$–$P < 0.01$).
Conclusion The results of the present study showed that occludin, claudin-1, tricellulin and zonulin proteins, as well as I-FABP, D-lactate and citrulline, may be used as promising biomarkers for the evaluation of disease severity in critically ill trauma patients, despite the complexity of the analysis of various barrier markers. However, our results should be supported by future studies.

Keywords Citrulline · Claudin-1 · D-lactate · Intensive care · I-FABP · JAM-1 · LPS · Occludin · Tricellulin · Zonulin

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Abbreviations

APACHE II	Acute Physiology and Chronic Health Evaluation II
ASPEN	American Society of Enteral and Parenteral Nutrition
BMI	Body mass index
CRP	C-reactive protein
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
EN	Enteral nutrition
ESPEN	European Society for Clinical Nutrition and Metabolism
IBD	Inflammatory bowel disease
ICU	Intensive care unit
I-FABP	Intestinal fatty acid-binding protein
Ig	Immunoglobulin
IQR	Interquartile range

JAM	Junctional adhesion molecule
LPS	Lipopolysaccharide
mNUTRIC	Modified nutrition risk in the critically ill
PN	Parenteral nutrition
ROC	Receiver operating characteristic
SOFA	Sequential Organ Failure Assessment
TRISS	Trauma and Injury Severity Score

Introduction

Due to malfunctions in digestion and absorption, damage to mucosal integrity, changes in the microbiome and the development of various infections throughout the gastrointestinal system, the intestine may remain vulnerable in patients suffering from critical illness after experiencing trauma, which is a leading cause of death among people under the age of 50 years. Intestinal hypoperfusion can damage the mucosal barrier by disrupting tissue oxygenation, after which toxic substances in the intestine mix into the blood and lymph circulation. This can cause bacterial translocation, sepsis, multiple organ failure and death [1–5]. Gastrointestinal dysfunction is observed in up to 50% of patients hospitalised in the intensive care unit (ICU) and plays an important role in the prognosis of these patients [5–8].

The pathophysiology of gastrointestinal disorders has not been fully elucidated. However, the use of biomarkers such as intestinal fatty acid-binding protein (I-FABP), D-lactate, lipopolysaccharide (LPS) and citrulline is recommended for the diagnosis and follow-up of gastrointestinal disorders in critically ill patients [5, 9–19]. Due to the inadequacy of current parameters, supportive laboratory analysis for the early management of critically ill trauma patients still needs to be developed.

In addition to the aforementioned potential biomarkers, proteins located in tight junctions (zonula occludens) and desmosome regions (belt desmosomes = zonula adherens and spot desmosomes = macula adherens), which hold epithelial cells in the intestinal mucosa, are likely to serve as biomarkers. Tight junctions ensure the integrity of the intestinal barrier by filling spaces between cells. Proteins such as occludin, claudin, junctional adhesion molecule (JAM), tricellulin, angulin, zonulin and cingulin function in the intestinal barrier [3, 20–26].

The aim of the present study was to determine whether the plasma levels of intestinal epithelial cell barrier proteins, including occludin, claudin-1, JAM-1, tricellulin and zonulin, can be used as biomarkers to assess gastrointestinal dysfunction in critically ill patients hospitalised for five or more days in the ICU after experiencing multiple traumas. I-FABP, D-lactate, LPS and citrulline were evaluated as potential biomarkers. In the present study, we also aimed to determine the possible relationships between the clinical, laboratory and nutritional statuses of patients and the measured marker levels.

Methods

Study design and ethical approval

The present prospective study was conducted between October 2018 and October 2019. Data were collected from critically ill multi-trauma patients admitted for five or more days to the anaesthesia, internal and surgical intensive care units of the University Hospital with multiple traumas (extremities, external, chest, face, head and neck) due to motor vehicle accidents, falls from height, stab wounds and/or firearm injuries. Patients aged 18 years and older who had no gastrointestinal injury and were expected to stay in the ICU for at least five days were included in the present study. Patients who had abdominal (internal organs, such as the intestines, liver and spleen, which may affect plasma tight junction protein levels) and/or neurological injuries, an additional chronic condition (hypertension, heart disease, or diabetes mellitus), inflammatory bowel disease, gastrointestinal surgery, kidney disease, pregnancy or lactation, or any prior cancer diagnosis were excluded from the present study (Fig. 1).

Patients were consecutively included in the study within the first 24 h after trauma, depending on the inclusion and exclusion criteria. The patients were observed in the ICU and discharged from the hospital. The control group consisted of individuals without chronic diseases, of the same age and sex as the patient group (Fig. 1).

The protocol for the present study was approved by the University Medical Faculty Research Ethics Committee (No. 2018/525) and conducted in accordance with the Declaration of Helsinki and local laws, which afforded greater protection to the participants. Written informed consent for the use of blood samples was obtained from each patient (or a relative of the patient) and healthy volunteers before the collection of blood samples.

Clinical evaluation and data collection

Data regarding age, sex, body mass index (BMI), Acute Physiology and Chronic Health Evaluation II (APACHE II) score, Trauma and Injury Severity Score (TRISS), organ affected by trauma and cause of trauma were obtained when patients were admitted to the ICU. During their stay in the ICU, data on the daily Sequential Organ Failure Assessment (SOFA) score, Glasgow score, need for mechanical ventilation, need for vasopressors, presence of infection, C-reactive protein (CRP) level, shock state and lactate level were recorded. Information on the

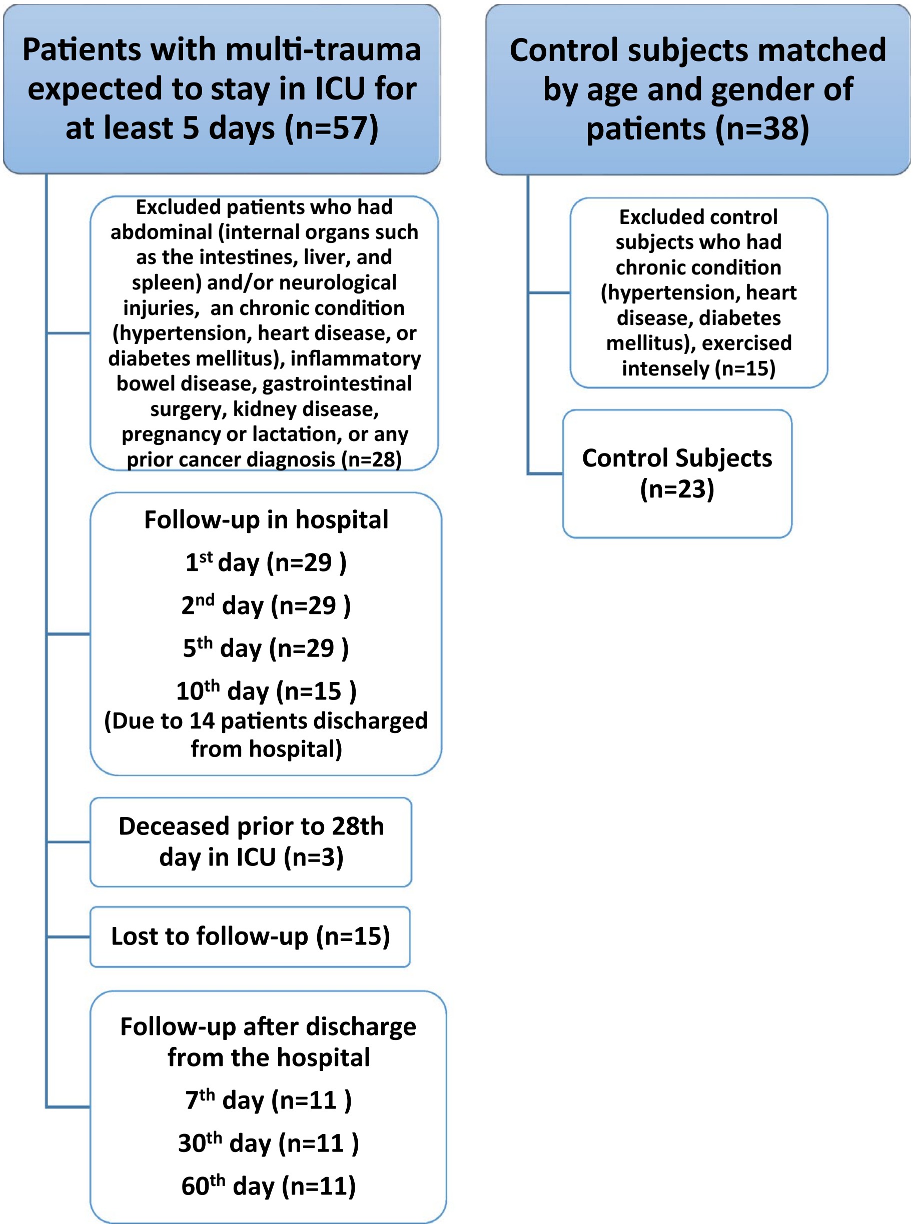

Fig. 1 Flow chart of participants' selection and follow-up

number of days of stay on a mechanical ventilator in the ICU, the total number of days of hospital stay and 28-day mortality were obtained. Age and BMI data were collected for the control group.

On the 1st day (admission) of each patient's ICU stay and again on the second (48th hour), fifth and tenth days of the stay and on days 7, 30 and 60 after discharge from the hospital (ICU or service/s), blood samples were drawn into tubes with ethylenediaminetetraacetic acid (EDTA) (BD Vacutainer K2EDTA, Becton, Dickinson and Company, Franklin Lakes, NJ, USA). Blood samples were collected once from the control group. All samples were centrifuged at 1500×*g* for 10 min. Separated plasma samples were stored at −80 °C until analysis.

Nutritional evaluation and data collection

Each patient's Modified Nutrition Risk in the Critically Ill (mNUTRIC) score was obtained on first day in the ICU and the target caloric intake was calculated using a simple weight-based equation (25–30 kcal/kg/d) [27]. The patients were fed according to the recommendations of the European Society for Clinical Nutrition and Metabolism (ESPEN) and American Society of Enteral and Parenteral Nutrition (ASPEN) guidelines [27, 28]. During the ICU stay, information on the type of feeding, calories ingested and time of feeding was obtained and the percentage of target calories reached was recorded [29].

Plasma protein and molecule measurements

The proteins and molecules (I-FABP, D-lactate, LPS, citrulline, occludin, claudin-1, JAM-1, tricellulin and zonulin) in the plasma samples were measured using the same manufacturer's enzyme-linked immunosorbent assay (ELISA) kits (YL Biont, Shanghai, China). These kits are solid-phase enzyme-linked immunosorbent tests based on the sandwich principle for in vitro quantitative measurements. The plates were washed with a microplate washer (Biotek-ELX50, Santa Clara, CA, USA) and the absorbance of the samples was measured at 450 nm using an ELISA reader (Biotek-ELX800, Santa Clara, CA, USA). The protein and molecular concentrations in each plasma sample from both patients and controls were calculated using a standard curve. Mean values obtained from three independent experiments were used to confirm the results. The intra- and interassay coefficients of variation (CV) for the ELISA kit were $< 8\%$ and $< 10\%$, respectively.

Statistical analysis

Data analysis was performed using the Statistical Package for the Social Sciences (IBM SPSS Statistics V22.0, Armonk, NY, USA). The Kolmogorov–Smirnov test was used to test the normality of the distribution of continuous data. Continuous variables were expressed as medians (interquartile range [IQR]: 25–75%) and categorical variables were expressed as numbers and percentages. The Mann–Whitney *U*, Kruskal–Wallis and Friedman tests were used to analysis continuous variables. Pearson's chi-square and Fisher's exact tests were used to evaluate the differences between categorical variables. Receiver operating characteristic (ROC) curve analysis was used to determine the duration of ICU stay in relation to the nutritional status of the patients. The relationships between the protein and molecular levels measured in the plasma samples of patients during treatment in the hospital and after discharge from the hospital and those of control subjects with age, BMI, lactate, CRP, number of days on a mechanical ventilator, number of days of ICU hospitalisation, TRISS, APACHE II and SOFA scores were evaluated using Pearson's correlation analysis. Statistical significance was set at $P < 0.05$.

Results

Characteristics of the participants

A total of 52 individuals, 29 patients and 23 controls were included in this study. The demographic and clinical variables of the participants are presented in Table 1. The patients' clinical and laboratory data during their ICU stay and follow-up period are provided in Table 2 and information on their nutritional status is shown in Table 3.

Intestinal markers

Levels of some intestinal markers (I-FABP, D-lactate and citrulline) in the plasma samples obtained from trauma patients treated in the ICU showed significant increases on the first and/or second day compared to the control group, except for LPS ($P < 0.05$–$P < 0.01$) (Fig. 2). Additionally, when these markers were evaluated during the follow-up of the patients, statistically significant differences were found between the plasma citrulline and LPS levels during the 60-day follow-up of the patients ($P < 0.05$ and $P < 0.01$, respectively) (Suppl. Table 1). The plasma I-FABP, D-lactate and citrulline levels of the patients on the 60th day after discharge from the hospital decreased to values similar to those in the control group ($P > 0.05$) (Fig. 2; Suppl. Table 1).

Tight junction markers

Tight junction protein (occludin, claudin-1, tricellulin and zonulin) levels in plasma samples obtained from patients treated in the ICU showed significant increases on the first and/or second day compared to the control group, except for JAM-1 ($P < 0.01$) (Fig. 3). Additionally, when these proteins were evaluated during the follow-up period, statistically significant differences were observed between the plasma levels of occludin, JAM-1 and zonulin ($P < 0.05$, $P < 0.05$ and $P < 0.01$, respectively) (Suppl. Table 1). Plasma occludin, claudin-1, tricellulin and zonulin levels in patients on the seventh, 30th and 60th days after discharge from the hospital, however, were returned to values close to those in the control group ($P > 0.05$) (Fig. 3; Suppl. Table 1).

Correlations between plasma protein and molecule levels and variables of patients

There were negative correlations between age and JAM-1 on the fifth day in the hospital ($r = -0.376$; $P < 0.01$), occludin on the seventh day after discharge from the hospital ($r = -0.676$; $P < 0.05$) and BMI and D-lactate on the 30th day after discharge from the hospital ($r = -0.729$; $P < 0.05$) in the patients.

No statistically significant correlation was found between the number of days on the mechanical ventilator or TRISS and plasma protein and molecular levels in patients ($P > 0.05$).

The number of days of the ICU hospitalisation and the first day plasma protein and molecule (I-FABP, D-lactate, LPS, citrulline, occludin, claudin-1, JAM-1 and tricellulin) levels, except for zonulin, were positively correlated ($r = 0.552$, $P < 0.01$; $r = 0.576$, $P < 0.01$; $r = 0.535$, $P < 0.01$;

Table 1 Demographic and clinical characteristics of all participants

Variables	Patient Group $n=29$	Control Group $n=23$	*P* value
Age (years), median (IQR)	30 (19–50)	30 (19–50)	0.956
Gender (female/male), *n* (%)	7 (24)/22(76)	7 (30)/16(70)	0.615
BMI (kg/m^2), median (IQR)	24.8 (23.6–26.0)	25.7 (24.0–28.0)	0.619
Cause of injury			
Motor vehicle accident, *n* (%)	23 (80)		
Fall from height, *n* (%)	4 (14)		
Stab wound, *n* (%)	1 (3)		
Firearm injuries, *n* (%)	1 (3)		
mNUTRIC score, median (IQR)	1 (0–2)		
Days on mechanical ventilation, median (IQR)	1 (0–6.5)		
Days in the hospital, median (IQR)	18 (10.5–27)		
Days of in the ICU stay, median (IQR)	15 (8.5–22)		
Shock state, *n* (%)	15 (51)		
APACHE II score, median (IQR)	10 (5.5–18)		
First day SOFA score, median (IQR)	3 (1.5–5.5)		
First day GCS score, median (IQR)	11 (4–15)		
TRISS median (IQR)	74.1 (64.0–96.2)		
ISS, median (IQR)	35.7 (24.5–42)		
RTS, median (IQR)	6.5 (5.5–7.8)		
Mortality in 28 days (in 13, 25, and 27 days), *n* (%)	3 (11)		

APACHE II acute physiology and chronic health evaluation II; *BMI* body mass index; *GCS* Glasgow Coma Scale; *ICU* intensive care unit; *IQR* interquartile range (25–75%); *ISS* injury severity score; *mNUTRIC* modified nutrition risk in critically ill; *RTS* revised trauma score; *SOFA* sequential organ failure assessment; *TRISS* trauma and injury severity score

Table 2 Clinical and laboratory data of patients followed up during the intensive care unit (ICU) period

Variables	First day (admission) $n=29$	Second day $n=29$	Fifth day $n=29$	Tenth day $n=15$
CGS score, median (IQR)	11(4–15)	14 (5.5–15)	15 (6.5–15)	14.5 (9.75–15)
SOFA score, median (IQR)	3 (1.5–5.5)	4 (2–6.5)	2 (1–4.5)	2 (0.5–4.5)
CRP (mg/L), median (IQR)	11.74 (2.14–33.78)	120.00 (92.11–200.00)	125.82 (74.90–223.00)	93.37 (32.23–109.62)
Lactate (mmol/L), median (IQR)	2.08 (1.46–3.32)	1.35 (0.97–2.13)	1.30 (1.08–1.91)	1.54 (0.91–2.67)
New infection, *n* (%)	0 (0)	1 (3)	3 (10)	6 (21)
Vasopressor usage, *n* (%)	10 (34)	6 (20)	3 (10)	4 (22)

CRP C-reactive protein; *GCS* Glasgow coma scale; *IQR* interquartile range (25–75%); *SOFA* sequential organ failure assessment

$r=0.523$, $P<0.01$; $r=0.526$, $P<0.01$; $r=0.489$, $P<0.01$; $r=0.478$, $P<0.01$; and $r=0.511$, $P<0.01$, respectively) (Suppl. Figure 1). Positive correlations were also found between the number of days of ICU hospitalisation and the second day I-FABP ($r=0.446$; $P<0.05$) and the fifth day I-FABP ($r=0.501$; $P<0.01$), LPS ($r=0.494$; $P<0.01$), occludin ($r=0.482$; $p<0.01$) and tricellulin ($r=0.505$; $P<0.01$) levels of the patients.

Positive correlations were found between first day serum lactate levels and first day D-lactate ($r=0.396$; $P<0.05$), LPS ($r=0.398$; $P<0.05$), citrulline ($r=0.368$; $P<0.05$), occludin ($r=0.486$; $P<0.01$), claudin-1 ($r=0.555$; $P<0.01$), JAM-1 ($r=0.492$; $P<0.01$) and tricellulin ($r=0.403$, $P<0.05$) levels of the patients. Serum lactate values on the fifth day were positively correlated with LPS ($r=0.450$, $P<0.05$) and tricellulin ($r=0.379$, $P<0.05$) on the fifth day.

The first day CRP values of the patients were negatively correlated with first day JAM-1 levels ($r=-0.394$; $P<0.05$), although positive correlations were found between CRP values on the fifth day and all patient plasma protein and molecule (I-FABP, D-lactate, LPS, citrulline, occludin, claudin-1, JAM-1, tricellulin and zonulin) levels on the fifth day ($r=0.857$, $P<0.01$; $r=0.728$, $P<0.05$; $r=0.860$, $P<0.01$;

$r = 0.832$, $P < 0.01$; $r = 0.917$, $P < 0.01$; $r = 0.779$, $P < 0.01$; $r = 0.778$, $P < 0.01$; $r = 0.749$, $P < 0.05$; and $r = 0.816$, $P < 0.01$, respectively).

Positive correlations were found between the patients' first day APACHE II scores and the first day plasma protein and molecule (I-FABP, D-lactate, LPS, citrulline, occludin, claudin-1, JAM-1 and tricellulin) levels, except for zonulin ($r = 0.412$, $P < 0.05$; $r = 0.491$, $P < 0.01$; $r = 0.446$, $P < 0.05$; $r = 0.465$, $P < 0.05$; $r = 0.526$, $P < 0.01$; $r = 0.556$, $P < 0.01$; $r = 0.559$, $P < 0.01$; and $r = 0.528$, $P < 0.01$, respectively) (Suppl. Figure 2).

Positive correlations were found between patients' first day SOFA scores and first day D-lactate ($r = 0.446$; $P < 0.05$), citrulline ($r = 0.398$; $P < 0.05$), occludin ($r = 0.427$; $P < 0.05$), JAM-1 ($r = 0.426$; $P < 0.05$), tricellulin ($r = 0.456$; $P < 0.05$) and zonulin ($r = 0.380$; $P < 0.05$) levels.

Table 3 Nutritional status of the patients followed up during the intensive care unit (ICU) period

Variables	Patient group *n* = 29
Feeding start time (h), median (IQR)	49 (9–79)
Calculated target calorie, median (IQR)	998 (910–1187)
The reaching the target calorie, *n* (%)	
Patients of hypocaloric feeding	16 (55)
Patients of hypercaloric feeding	13 (45)
Type of feeding (0–2 days), *n* (%)	
Oral	11 (38)
Only EN	6 (21)
Only PN	5 (17)
Dextrose	7 (24)
Type of feeding (0–5 days), *n* (%)	
Oral	15 (52)
Only EN	11 (38)
Only PN	2 (7)
Dextrose	1 (3)

EN enteral nutrition; *IQR* interquartile range (25–75%); *PN* parenteral nutrition

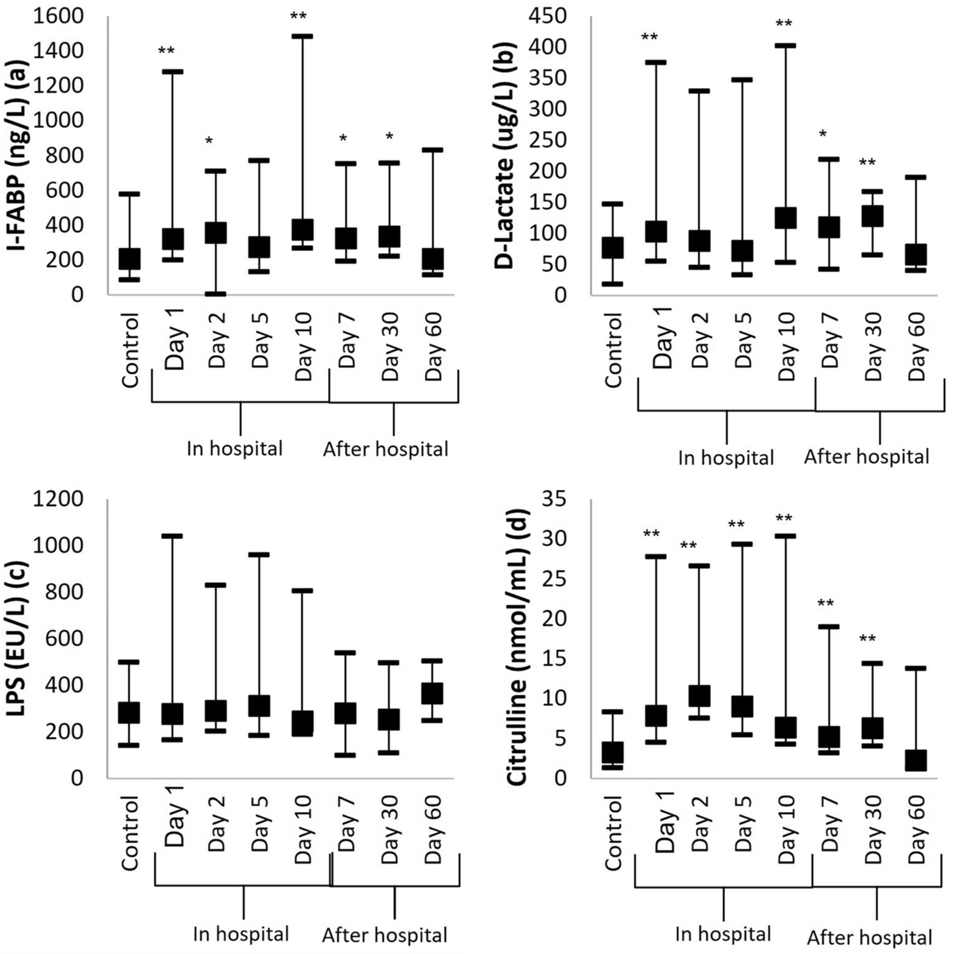

Fig. 2 The comparison of plasma intestinal marker levels in patient and control groups. Plasma intestinal fatty acid-binding protein (I-FABP) (ng/L) (**a**), D-lactate (µg/L) (**b**), lipopolysaccharide (LPS) (EU/L) (**c**), and citrulline (nmol/mL) (**d**) levels of critically ill trauma patients treated in the intensive care unit (ICU). Each bar represents the median (interquartile range: IQR; 25–75%) from patient and control subjects. Significance level is representing by * ($P < 0.05$) and **($P < 0.01$)

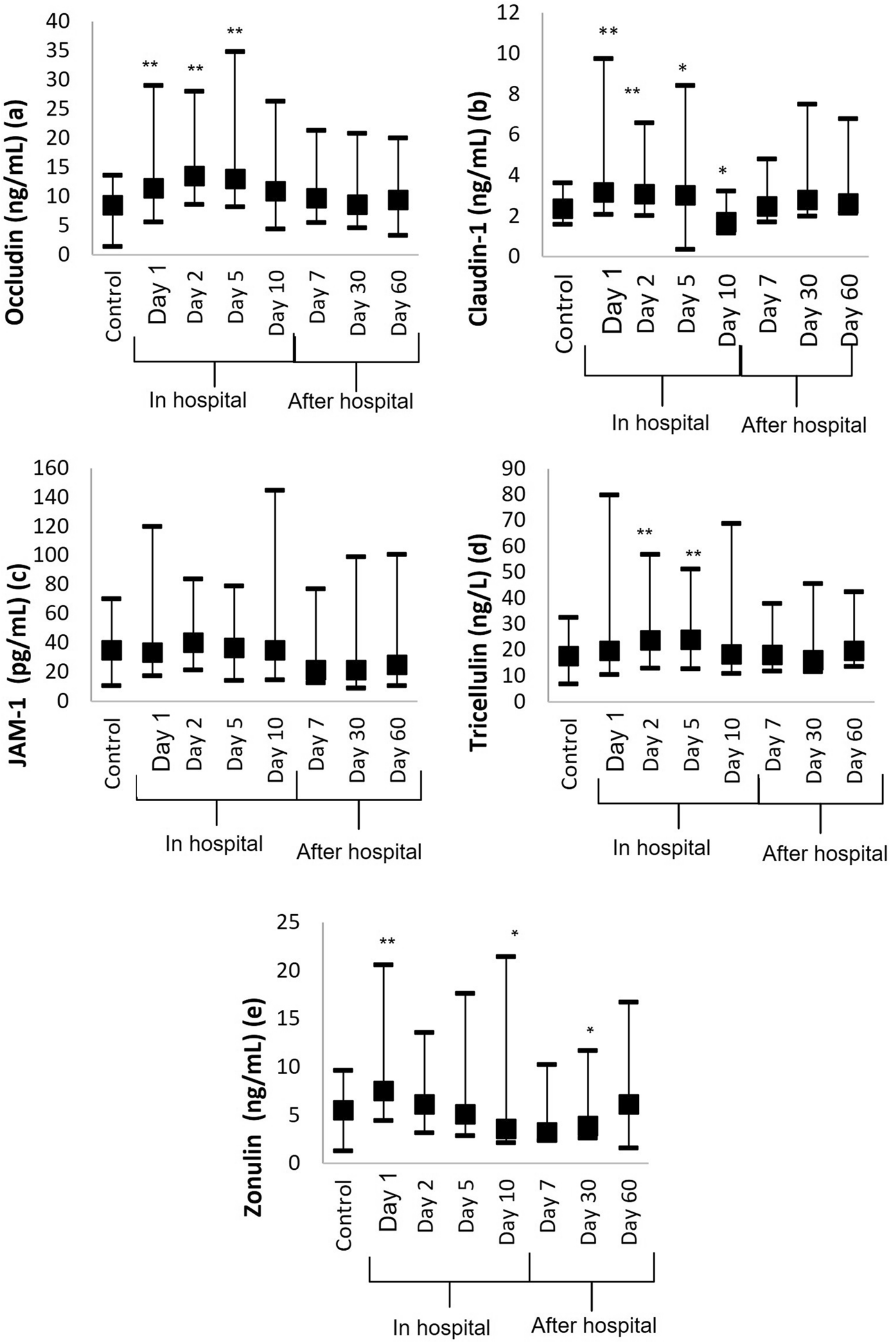

Fig. 3 The comparison of plasma tight junction protein levels in patient and control groups. Plasma occludin (ng/mL) (**a**), claudin-1 (ng/mL) (**b**), junctional adhesion molecule-1 (JAM-1) (pg/mL) (**c**), tricellulin (ng/L) (**d**), and zonulin (ng/mL) (**e**) levels of critically ill trauma patients treated in the intensive care unit (ICU). Each bar represents median (interquartile range: IQR; 25–75%) from patient and control subjects. Significance level is representing by *($P < 0.05$) and **($P < 0.01$)

Plasma protein and molecule levels according to the shock state, nutritional status, time to start feeding and reached the target calorie of patients

Based on the shock state of the patients, the levels of I-FABP on the second day, LPS on the first and fifth days, citrulline on the fifth day, occludin on the 10th day, tricellulin on the second and 10th days and zonulin on the fifth day were found to be higher in patients in shock ($P < 0.05$).

When looking at each patient's nutritional status, there was no statistically significant difference between the mNUTRIC scores based on > 15 and > 7 days of ICU stay ($P > 0.05$). No statistically significant differences were found between the Injury Severity Score (ISS), Revised Trauma Score (RTS) and TRISS scores based on the severity of trauma and injury in patients fed only oral or enteral nutrition (EN), parenteral nutrition (PN) only, or dextrose in both the first 2 days and in the first 5 days ($P > 0.05$). There was also no statistically significant difference between any plasma protein and molecular levels for patients fed orally with EN, PN and dextrose, both within the first two days and within the first five days ($P > 0.05$).

There was no significant difference between plasma protein and molecular levels at the time to start feeding of the patients ($P > 0.05$). Among the patients who reached the target calorie level in the first 3 days, there was no significant difference between the patients on hypocaloric and hypercaloric diets in terms of all plasma protein and molecular levels ($P > 0.05$).

Discussion

Previous studies have recommended the use of biomarkers such as I-FABP, D-lactate, LPS and citrulline in blood samples collected at regular intervals from patients with acute gastrointestinal injury to evaluate intestinal barrier function [5, 9, 12, 15, 16, 18, 19]. However, there is no consensus regarding the best method for measuring the severity of gastrointestinal dysfunction. The present study analysed certain tight junction proteins as potential biomarkers, as well as I-FABP, D-lactate, LPS and citrulline, in the plasma samples of critically ill trauma patients housed in the ICU using ELISA. Our results showed that plasma occludin, claudin-1, tricellulin and zonulin levels could be used as biomarkers to assess gastrointestinal dysfunction in critically ill patients with trauma. Furthermore, serial analysis of various barrier markers in critically ill trauma patients housed for five or more days in the ICU, including both in the hospital and after discharge from the hospital, is a complex assessment and was addressed for the first time in view of the long-term observation of patients.

The results of the present study may be considered similar to those of previous studies [5, 9, 11, 18, 19, 26, 30] in terms of high first day plasma I-FABP, D-lactate and citrulline levels in critically ill trauma patients treated in the ICU and their positive correlation with the clinical characteristics of the patients. But, intestinal failure is associated with low plasma citrulline levels in critically ill patients and an increase in plasma citrulline levels is a potential indicator of renal failure [9, 16]. In the present study, however, the levels of plasma citrulline in patients with trauma during their stay in the ICU were higher than those in controls, although patients with kidney disease were not included. This increase is questionable because patients with abdominal or gastrointestinal injuries were excluded from this study. Additionally, the hypocaloric feeding of patients reaching the target calories may also account for the persistence of elevated levels of I-FABP, D-lactate and citrulline. As such, the results of the present study indicate that plasma I-FABP, D-lactate and citrulline, but not LPS, are suitable intestinal markers for the follow-up of trauma patients in the ICU.

As they form the intestinal barrier, the transmembrane proteins (occludin, claudin, JAM, tricellulin and angulin) in the tight junction associate with zonulin and cingulin proteins, which bind to intracellular actin filaments [3, 20–26, 31, 32]. Tight junction protein complexes regulate paracellular transport in epithelial cells. The disruption of tight junctions leads to an increase in intestinal permeability. These tight junction proteins may leak into the systemic circulation of trauma patients. In the present study, we simultaneously evaluated plasma occludin, claudin-1, JAM-1, tricellulin and zonulin levels in easily and consecutively obtainable blood samples during the follow-up of critically ill trauma patients in the hospital and after discharge from the hospital and assessed whether these proteins could be used as potential tools to monitor gastrointestinal dysfunction in these patients.

Occludin plays functional and structural roles in the regulation of paracellular permeability and intestinal barrier function. Inflammatory bowel disease (IBD) is a gastrointestinal disease caused by uncontrolled inflammation. Previous studies have shown that intestinal epithelial occludin expression is downregulated in tissue samples and biopsies from patients with Crohn's disease and ulcerative colitis, two subtypes of IBD [33, 34]. However, we have shown an increase in plasma occludin levels in the early days in trauma patients treated in the ICU and positive correlations between first day occludin levels and first day lactate levels, APACHE II scores and SOFA scores. This indicated that occludin loss from tight junctions may lead to intestinal permeability by causing an increase in plasma and a decrease in tissue after trauma.

It has been suggested that the expression of claudin, another transmembrane protein in tight junctions, increases because of inflammation, unlike occludin, in biopsies from patients with Crohn's disease and ulcerative colitis [34, 35]. Claudins are a large family, comprised of at least 27 members, that show specific localisation in the gastrointestinal tract [36, 37]. Plasma claudin-5 levels, a part of the endothelial tight junction, are increased in patients with haemorrhagic shock in the early course after polytrauma [26]. Furthermore, claudin-1 and -2 expressions have been reported to be elevated in cases of active IBD using immunohistochemical staining and correlated positively with inflammatory activity [38]. However, despite the widespread expression of claudin-1 in intestinal epithelial cells, role of claudin-1 in the intestinal epithelial cell barrier in critically ill patients with trauma remains unknown. Therefore, plasma claudin-1 levels were assessed as barrier builders and found to increase significantly during the early days of hospitalisation in trauma patients treated in the ICU. In addition, the positive correlations between first day claudin-1 levels and first day lactate levels and APACHE II scores may be a sign of increased paracellular permeability and impaired tight junctions after trauma.

Unlike claudins and occludins, JAMs comprise a single transmembrane chain. JAM-1 (JAM-A), a member of the immunoglobulin (Ig) superfamily, regulates tight junction barrier function [25, 26]. A previous study showed increased plasma JAM-1 concentrations in patients after polytrauma and plasma JAM-1 levels were associated with APACHE II and SOFA scores [3]. However, we observed slightly increased plasma JAM-1 levels in critically ill trauma patients treated in the ICU compared to those in the control group, which was not statistically significant. The reason for the minimal increase in JAM-1 levels may be that patients with abdominal injuries were excluded from the study. In contrast, first day JAM-1 levels correlated with first day lactate levels and APACHE II and SOFA scores, indicating a disruption of tight junctions in critically ill trauma patients.

Tricellulin is localised at three-cell contacts to regulate barrier properties but also plays a key role in two-cell contacts [39, 40]. Downregulation of tricellulin expression has been observed in biopsies from patients with ulcerative colitis, resulting in increased macromolecule permeability, but not in patients with Crohn's disease [41]. We also found that plasma tricellulin levels increased significantly on the second and fifth days in critically ill trauma patients treated in the ICU. This increase may contribute to an increase in epithelial permeability in two-cell junctions, depending on tight junction barrier disruption due to trauma.

Zonulin is a key modulator that connects transmembrane proteins in tight junction complexes with intracellular actin filaments. Dysregulated zonulin pathways and increased intestinal permeability have been implicated in many chronic inflammatory diseases including celiac disease, diabetes, IBD and irritable bowel syndrome [42–45]. Previous studies have shown that plasma zonulin levels increase in patients with obesity and sepsis, consequently increasing intestinal permeability increases [46–48]. Our data also showed that plasma zonulin levels in critically ill trauma patients treated in the ICU increased on first day but decreased in the remaining days of ICU stay and first day zonulin levels correlated with the first day SOFA scores. Therefore, increased plasma zonulin levels may be useful in determining intestinal permeability in critically ill patients with trauma and altered tight junction integrity.

Many junctional proteins are present not only in epithelial cells but also in other cell types, such as endothelial cells. Furthermore, tight junction proteins may also be associated with damage to other organs, such as the relationship between I-FABP and acute kidney, traumatic brain and abdominal injuries [49]. Therefore, in the present study, we excluded polytrauma patients with liver, spleen, intestinal, or neurological injuries, considering that tight junction protein levels may be affected by these injuries.

Assessing gastrointestinal dysfunction in critically ill trauma patients is complicated. Gastrointestinal dysfunction in critical illnesses is likely multifactorial. However, no clinical score can cover all the functions of the gastrointestinal tract, including endocrine, immune and barrier function [50]. Moreover, the current markers do not adequately reflect intestinal cell damage. However, high levels of tight junction proteins and intestinal markers may be useful in assessing the prognosis of critically ill patients with trauma.

One limitation of the present study, beyond the relatively small number of patients and healthy controls in the study groups, was the lack of tissue sampling. If this study had included more patients, significant differences based on the nutritional status of patients could have been observed. We attempted to increase the strength of the study by serially collecting blood samples from trauma patients both in the hospital and after discharge. Despite the regular decreases in patient parameters during hospital follow-up, some increases were observed in patients after hospitalisation, such as zonulin and occludin levels, which may be due to the use of home medication and nutrition. Although tight junctions play a key role in intestinal epithelial barrier formation, it is possible that the barrier markers investigated are not highly specific to the gut and therefore could also indicate extra-abdominal barrier problems.

Conclusion

In the present study, we found that the plasma levels of the tight junction proteins occludin, claudin-1, tricellulin and zonulin in multi-trauma patients hospitalised in the ICU increased and were positively correlated with lactate, CRP, number of days of ICU hospitalisation, APACHE II scores and daily SOFA scores. Therefore, it may be proposed that circulating occludin, claudin-1, tricellulin and zonulin, in addition to I-FABP, D-lactate and citrulline could be used as possible biomarkers of intestinal injury from easily obtainable blood samples to evaluate disease severity (prognosis) in critically ill trauma patients, if the results of the present study are supported by larger studies. Additionally, serial analysis of many barrier markers for gastrointestinal dysfunction in critically ill trauma patients treated in the ICU could be instructive for clinicians in the long-term observation of these patients, although it is complex and difficult to assess at the patient level.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00068-023-02298-6.

Author contributions HD-A, SSE, PA-C, KB, HE, RU and KG: conception, design, and development of the study; acquisition, analysis

and interpretation of the data; interpretation of results and writing and critical review of the manuscript. GGS, NTO, ST, TBA, AE, RCY and MS: acquisition, analysis, and interpretation of the data; writing and critical review of the manuscript.

Funding This work was funded by the Scientific Research Projects Coordination Unit of Erciyes University (Project ID: 8633).

Availability of data and materials The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest All the authors declare that they have no competing interests.

Ethics approval Study design and protocol were approved by the University Medical Faculty Research Ethics Committee (No. 2018/525).

Consent for publication Written informed consent for the use of blood samples was obtained from each patient (or a relative of the patient) and healthy volunteers before the collection of blood samples.

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