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Serum Zonulin as a Marker of Intestinal Mucosal Barrier Function in Chronic Liver Diseases in Pediatrics

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ABSTRACT

Background: The term "chronic liver disease" (CLD) refers to a long-term, permanent alteration in the structure of the liver that may result in consequences including cirrhosis and early mortality. A potential contributing element to the pathophysiology of chronic liver disorders and the development of complications in cirrhosis is the dysfunction of the intestinal epithelial barrier. One indicator of intestinal permeability is zonulin.

Objective: This study aimed to assess serum zonulin levels in CLD with and without cirrhosis in pediatrics.

Patients and methods: The study population consisted of 40 children with CLD with cirrhosis, 40 children with CLD without cirrhosis and 20 apparently healthy children serving as a control group. Serum levels of zonulin was determined.

Results: The main finding of the current study was that serum zonulin levels were significantly higher in CLD with cirrhosis patients than in CLD without cirrhosis patients or healthy controls ($p < 0\ 001$). The median of serum zonulin levels in CLD with cirrhosis patients, CLD without cirrhosis patients, and healthy controls were 264.24, 8.24 and 39.69 ng/mL respectively.

Conclusion: Serum zonulin levels were significantly increased in liver cirrhosis patients.

Keywords: Serum zonulin, Intestinal Mucosal Barrier Function, CLD, Pediatrics.

INTRODUCTION

CLD is defined as the gradual destruction and regeneration of liver parenchyma over at least six months, resulting in fibrosis and cirrhosis. CLD is one of the leading causes of morbidity and mortality in pediatric [1].

The integrity of the intestinal epithelium and the tight connection that closes the paracellular gap play a major part in the intestinal epithelium's vital function of separating luminal contents from the interstitium. The intestine tight junctions exhibit selectivity in permeability, which can be either healthily triggered by luminal nutrients or pathologically induced by pathogens, mucosal immune cells and cytokines, and the enteric nervous system. Numerous clinical disorders, both intestinal and systemic, are linked to compromised intestinal barrier function [2].

A protein called zonulin affects the permeability of the small intestinal wall's tight junction in a reversible manner. Intestinal wall permeability may be accurately and conveniently measured using the serum levels of zonulin [3]. Research points to the migration of bacteria and their byproducts, including endotoxin, from the intestinal lumen into the bloodstream as a potential player in the aetiology of long-term liver disorders and the emergence of problems in cirrhosis. The intestinal epithelial barrier's malfunction may play a significant role in enabling bacterial translocation, in addition to changes in the immune system and gut microbiota [4]. Therefore, The purpose of the research was to evaluate serum zonulin family peptides levels, as a marker of intestinal mucosal barrier function, in CLD with and without cirrhosis in pediatrics.

PATIENTS AND METHODS

Study population:

This was a case control study. The study population consisted of 40 children with CLD with cirrhosis, 40 children with CLD without cirrhosis and 20 apparently healthy children serving as a control group. All patients were recruited during a two-year period (2021-2023) from the Outpatient Clinic and Inpatient Ward of the Paediatric Hepatology, Gastroenterology, and Nutrition Department, National Liver Institute, Menoufia University.

Inclusion criteria: Subjects were matched for age and sex. Cirrhosis was identified by a biopsy or by combining clinical, endoscopic, and radiographic evidence of portal hypertension or cirrhosis.

Exclusion criteria: Children with allergy or sensitivity to gluten or adhere to a gluten-free diet, children with indications of gastrointestinal illness, children with current infection or inflammation, history of immunological abnormalities, and children with diabetes.

History, clinical examination, and investigations:

- Full history taking with stress on family history of similar conditions, consanguinity, presenting symptoms (onset, course, duration and associated symptoms), symptoms of hepatocellular decompensation e.g. ascites and hepatic

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encephalopathy. Symptoms of vascular decompensation e.g. hematemesis and melena.
- Clinical examination with stress on jaundice, pallor, hepatomegaly, splenomegaly, ascites.
- Investigations were recruited from patients' files: Routine laboratory parameters, investigations according to suspected etiology, imaging studies, liver biopsy and esophagogastroduodenoscopy.

Blood Sampling: Initially, each subject's blood samples were centrifuged for 10 minutes at 1500 g. After that, the serum was separated and centrifuged again for three minutes at 4°C at 2000 g. After that, the supernatant was kept at -80 °C.

ELISA Measurements: A double-antibody sandwich ELISA Kit (Shanghai Korain Biotech Co., Ltd.) was used to measure the levels of serum zonulin. Serum zonulin concentrations were measured spectrophotometrically using an ELISA microtiter plate reader (ELX800, Biotech Instruments, Inc., USA, SN: 1502175), and the assay was carried out in accordance with the kit manufacturer's instructions.

Ethical approval: The work has been submitted and approved by Menoufia University's National Liver Institute's Ethics Committee. Each participant's parent gave written consent for the study. Every phase of the study was conducted in accordance with the Helsinki Declaration.

Statistical Analysis

SPSS statistics version 25.0 was used for the statistical analysis. When presenting data for continuous variables, the means ± SD or median values were used. Frequencies and percentages were used to display data for categorical variables. Depending on the kind of data, the ANOVA, Kruskall-Wallis, or Chi-square test were used to identify significant variations in the clinicopathological characteristics across groups. For normally distributed data, post hoc analyses were used to compare the group differences. P-values ≤ 0.05 were deemed statistically significant.

RESULTS

The study consisted of 40 children with CLD with cirrhosis (15 males and 25 females), 40 children with CLD without cirrhosis (18 males and 22 females) and 20 apparently healthy children (10 males and 10 females). The mean ages were11.58 ± 3.31, 11.25 ± 3.39 and 10.90 ± 3.14 years old respectively. There was no difference in age and sex among the three groups. However, hepatomegaly, splenomegaly**,** jaundice and pallor were significantly different among the studied groups (Table 1).

Table (1): Demographic data and clinical data

Parameter	**CLD with cirrhosis** (GI, n= 40)	**CLD without cirrhosis** (GII, n= 40)	**Healthy Controls** (GIII, n= 20)	**P-value**
Age (year) Mean ± SD Range (min-max)	 11.58 ± 3.31 5.00 - 17.00	 11.25 ± 3.39 5.00 - 17.00	 10.90 ± 3.14 6.0 - 17.0	0.751 [a, NS]
Sex [n (%)] Male Female	 15 (37.5) 25 (62.5)	 18 (45.0) 22 (55.0)	 10 (50.0) 10 (50.0)	0.619 [b, NS]
Splenomegaly [n (%)] No Yes	 6 (15.0) 34 (85.0)	 35 (87.5) 5 (12.5)		**<0.001** [b, HS]
Hepatomegaly [n (%)] No Yes	 22 (55.0) 18 (45.0)	 33 (82.5) 7 (17.5)		**0.008** [b, HS]
Jaundice [n (%)] No Yes	 15 (37.5) 25 (62.5)	 32 (80.0) 8 (20.0)		**<0.001** [b, HS]
Pallor [n (%)] No Yes	 29 (72.5) 11 (27.5)	 39 (97.5) 1 (2.5)		**0.002** [b, HS]
%: Percentage within disease group [a]: ANOVA test; [b]: Pearson's X^2-test				

Table (2) is a list of the laboratory features of the individuals under study. The three groups differed considerably in terms of AST, GGT, ALP, total bilirubin, direct bilirubin, albumin haemoglobin percentage, total leucocytic count, platelet count, and INR. AST, ALT, and GGT levels were elevated in CLD with cirrhosis patients relative to CLD without cirrhosis patients and control participants, whereas platelet count and albumin levels were significantly decreased. Patients with CLD and cirrhosis had higher total bilirubin levels and lower haemoglobin levels as compared to other groups. Notably, compared to CLD patients without cirrhosis or healthy controls, blood zonulin levels were considerably greater in patients with CLD who also had cirrhosis (p < 0 001). The range of serum zonulin levels in CLD with cirrhosis patients, CLD without cirrhosis patients, and healthy controls were 155.18 - 499.39, 1.70 - 145.68 and 30.12 - 50.20 ng/mL respectively (Figure 1).

Table (2): Laboratory characteristics in studied groups

Biochemical parameters	**CLD with cirrhosis** (GI, n= 40)	**CLD without cirrhosis** (GII, n= 40)	**Healthy Controls** (GIII, n= 20)	**P-value**	**Pairwise comparisons**
Zonulin (ng/mL) Median (IQR) Range (min-max)	 264.24 (96.87) 155.18 - 499.39	 8.24 (111.33) 1.70 - 145.68	 39.69 (8.79) 30.12 - 50.20	**<0.001** **a, HS**	**p1<0.001** **HS** **p2<0.001** **HS** p3=0.896 NS
ALT (U/L) Median (IQR) Range (min-max)	 103.00 (291.25) 7.00 - 779.00	 43.50 (98.75) 7.00 - 1920.00	 17.00 (6.00) 9.00 - 23.00	**<0.001** a, **HS**	p1=0.059 NS **p2<0.001** **HS** **p3<0.001** **HS**
AST (U/L) Median (IQR) Range (min-max)	 168.50 (311.00) 18.00 - 1393.00	 39.00 (87.75) 17.00 - 1755.00	 21.00 (7.00) 9.00 - 28.00	**<0.001** a, **HS**	**p1<0.001** **HS** **p2<0.001** **HS** **p3<0.001** **HS**
ALP (U/L) Median (IQR) Range (min-max)	 233.50 (188.50) 21.00 - 966.00	 135.50 (122.25) 90.00 - 596.00	 93.50 (86.25) 48.00 - 240.00	**<0.001** **a, HS**	**p1=0.018** **S** **p2<0.001** **HS** **p3<0.001** **HS**
GGT (U/L) Median (IQR) Range (min-max)	 70.00 (72.00) 15.00 - 328.00	 43.50 (29.25) 13.00 - 563.00	 19.50 (9.50) 12.00 - 31.00	**<0.001** **a, HS**	**p1=0.015** **S** **p2<0.001** **HS** **p3<0.001** **HS**
Total bilirubin (mg/dL) Median (IQR) Range (min-max)	 3.90 (4.25) 0.40 - 44.00	 0.80 (0.30) 0.20 - 17.00	 0.67 (0.32) 0.33 - 1.08	**<0.001** **a, HS**	**p1<0.001** **HS** **p2<0.001** **HS** p3=0.335 NS
Direct bilirubin (mg/dL) Median (IQR) Range (min-max)	 2.45 (3.47) 0.09 - 27.00	 0.30 (0.28) 0.00 - 14.70	 0.15 (0.07) 0.10 - 0.21	**<0.001** **a, HS**	**p1<0.001** **HS** **p2<0.001** **HS** **p3<0.001** **HS**
Albumin (g/dL) Median (IQR) Range (min-max)	 3.10 (1.33) 1.60 - 4.40	 4.10 (0.28) 3.70 - 5.00	 4.90 (0.30) 4.00 - 5.10	**<0.001** **a, HS**	**p1<0.001** **HS** **p2<0.001** **HS** **p3<0.001** **HS**
Hb (g/dL) Median (IQR) Range (min-max)	 10.00 (2.98) 7.00 - 14.00	 12.35 (1.38) 8.00- 15.00	 13.05 (1.47) 11.90 - 15.50	**<0.001** **a, HS**	**p1<0.001** **HS** **p2<0.001** **HS** **p3=0.027** **S**
WBCs (10^3 cell/µL) Median (IQR) Range (min-max)	 5.90 (3.75) 1.20 - 13.00	 7.00 (3.08) 4.30 - 13.00	 6.60 (2.38) 4.90 - 9.30	**0.032** **a, S**	**p1=0.041** **S** p2=0.244 NS p3=0.847 NS
Platelets (10^3 cell/µL) Median (IQR) Range (min-max)	 102.00 (77.00) 29.00 - 393.00	 321.50 (145.50) 130.00 - 600.00	 284.00 (119.50) 170.00 - 432.00	**<0.001** **a, HS**	**p1<0.001** **HS** **p2<0.001** **HS** p3=0.839 NS
INR Median (IQR) Range (min-max)	 1.50 (0.50) 0.98 - 3.40	 1.00 (0.05) 0.80 - 1.20	 1.00 (0.07) 0.95 - 1.10	**<0.001** **a, HS**	**p1<0.001** **HS** **p2<0.001** **HS** p3=0.590 NS

IQR: Interquartile range (difference between 1st and 3rd quartile).
a: Kruskal-Wallis test; if significant multiple pairwise comparisons were adjusted by Dunn-Sidak post hoc test.
p1: p-value for the difference between group I and group II
p2: p-value for the difference between group I and group III
p3: p-value for the difference between group II and group III
NS: Non significant at p-value ≥ 0.05 S: significant at p-value < 0.05
HS: Highly significant at p-value < 0.01

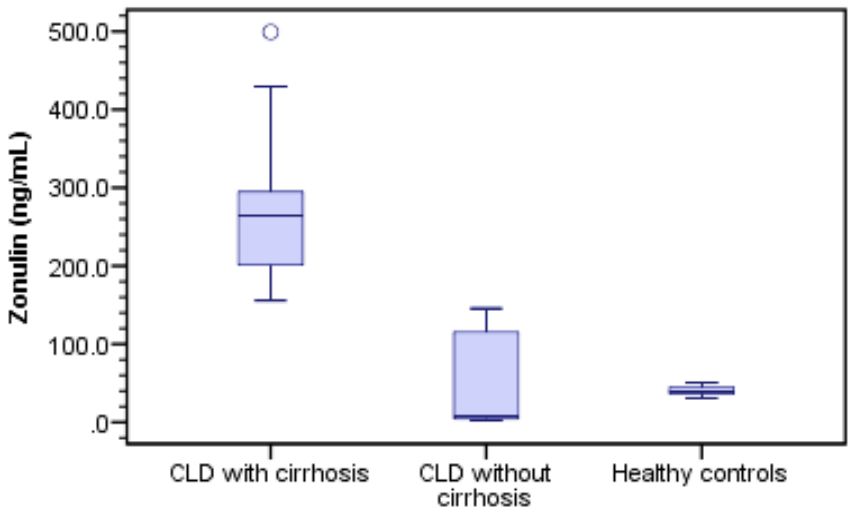

Figure (1): Serum zonulin levels in the studied groups.

DISCUSSION

The key results of the current study is serum zonulin levels were significantly higher in CLD with cirrhosis patients than in CLD without cirrhosis patients or healthy controls ($p < 0\ 001$). The range of serum zonulin levels in CLD with cirrhosis patients, CLD without cirrhosis patients, and healthy controls were 155.18 - 499.39, 1.70 - 145.68 and 30.12 - 50.20 ng/mL respectively.

Chronic liver illnesses have an impact on the growth and incidence of the gut microbiota. Through the "gut-liver axis," changes in the composition and quantity of gut microbiota have a direct impact on the immunological milieu of the liver [5, 6], which in turn affects the body's overall immune condition.

According to the current study, individuals with CLD and cirrhosis had considerably higher intestinal permeability. The breakdown of the intestinal barrier directly led to intestinal bacterial translocation. When pathogenic bacteria and their toxic byproducts, like lipopolysaccharide, enter the portal vein, the liver is immediately exposed to bacterial components that damage the liver [7, 8]. This causes the liver to produce proinflammatory factors like TNF-α, IL-6, and other immune mediators. The endotoxin that the unbalanced bacteria then continually produce causes systemic and intrahepatic inflammation, which in turn aggravates liver damage and dysbacteriosis, creating a vicious cycle that affects both the gut and the liver [9, 10]. Additionally, it was shown that the most severe dysbacteriosis happens during the decompensated phase and that the organisation of the gut microbiota gradually alters as liver cirrhosis progresses [11].

Intestinal permeability is indicated by zonulin. By actively controlling the intercellular tight connection, it aids the intestinal barrier in adapting to different physiological and pathological stimuli [12, 13]. In the presence of bacteria or gluten, the small intestine may exhibit an increase in the pathological secretion of zonulin, which in turn may lead to an increase in intestinal permeability and a disruption of intestinal barrier function. This, in turn, may result in bacterial translocation and aberrant presentation of antigens to the intestinal submucosa, as well as an induction of inflammation and an adaptive immune response [14, 15].

Numerous autoimmune illnesses, malignancies, and neurological disorders, such as inflammatory bowel disease [16], type 1 diabetes [17], and multiple sclerosis [18], are caused by this pathogenic process mediated by zonulin [12, 14]. We found that patients with cirrhosis had significantly higher serum zonulin levels SLD, which may indicate that the breakdown of the intestinal barrier brought on by the zonulin pathway dysfunction is a role in the progression of chronic liver disease. **Wang *et al.*** [19] findings indicated that zonulin levels were greater in liver cirrhosis patients, which are in line with our findings. As well, our results are consistent with **Pietrukaniec *et al.*** [3] who found a statistically significant difference in serum zonulin levels between the group of cirrhotic patients and the healthy controls. Furthermore, our findings are supported by the findings of **Voulgaris *et al.*** [20] who found that individuals with more severe chronic liver disease had greater serum zonulin levels proving the link between increasing intestinal permeability and liver cirrhosis.

In order to track the association between zonulin and chronic liver disease consequences, the current study can be extended by recruiting patients who satisfy the criteria to increase the sample size and by prolonging the follow-up observation period.

In conclusion: Serum zonulin levels were markedly elevated in patients with liver cirrhosis.

Conflict of interest: None.

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