RESEARCH ARTICLE / ARAŞTIRMA MAKALESİ

DOI: 10.4274/atfm.galenos.2023.50479
Ankara Üniversitesi Tıp Fakültesi Mecmuası 2023;76(4):328-332

MEDICAL SCIENCES / DAHİLİ TIP BİLİMLERİ

Zonulin is not Useful for the Diagnosis of Intestinal Permeability in Cirrhotic

Zonulin Sirozlu Hastalarda İntestinal Geçirgenlik Tanısında Kullanışlı Değildir

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Abstract

Objectives: Increased intestinal permeability is an important cause of serious complications in cirrhotic. In addition, intestinal permeability can promote impaired liver functions. Zonulin, well-known intestinal permeability marker, has never studied sufficiently before in cirrhotic as a potential marker of increased intestinal permeability.

Materials and Methods: This is a cross-sectional study. Forty-one cirrhotic patients (11 patients in compensated phase and 30 patients in decompensated phase) were enrolled into the study. Meanwhile forty-one healthy controls were also enrolled. Zonulin was assessed by enzyme-linked immunosorbent assay technique.

Results: Zonulin levels was not significantly different between patients with cirrhotic and healthy individuals; however lower levels was observed in cirrhotic (p=0.068). In patients with cirrhotic, zonulin level was significantly different between child-pugh A and child-pugh B (p=0.05). Also zonulin was significantly lower in child-pugh C compared to child-pugh-A and child-pugh-B (p=0.011 and p=0.026, respectively). In all patients with cirrhotic, MELD score was positively correlated with zonulin levels (p=0.001, R=0.358).

Conclusion: This study reveals that serum zonulin levels were decreased, especially in decompensated phase in cirrhotic compared to healthy individuals. It is possible to speculate that zonulin is not a causal factor for intestinal permeability in cirrhotic, instead of this, its synthesis effects negatively in cirrhotic situation.

Key Words: Cirrhotic, Intestinal Permeability, Zonulin

Öz

Amaç: Sirozlu hastalarda artmış intestinal geçirgenlik ciddi komplikasyonların önemli bir nedenidir. Ek olarak artmış intestinal geçirgenlik karaciğer fonksiyonlarında kötüleşmeye de yol açar. Zonulin, intestinal geçirgenlik için iyi tanımlanmış bir belirteç olsa da, sirozlu hastalarda artmış intestinal geçirgenlik varlığının tanısında yeterince çalışılmamıştır.

Gereç ve Yöntem: Bu çalışma kesitsel bir çalışmadır. Kırk bir siroz tanılı hasta (11 tanesi kompanse, 30 tanesi ise dekompanse fazda olan) çalışmaya dahil edilmiştir. Eş zamanlı 41 sağlıklı kontrol grubu da çalışmaya alınmıştır. Zonulin enzyme-linked immunosorbent assay ile çalışılmıştır.

Bulgular: Zonulin seviyeleri sirozlu hastalar ve sağlıklı bireyler arasında önemli ölçüde farklı değildi; ancak sirozda daha düşük seviyeler gözlendi (p=0,068). Sirozlu hastalarda zonulin düzeyi, child-pugh A ve child-pugh B arasında anlamlı olarak farklıydı (p=0,05). Ayrıca zonulin, child-pugh C'de child-pugh-A ve child-pugh-B'ye göre anlamlı olarak daha düşüktü (sırasıyla p=0,011 ve p=0,026). Tüm sirozlu hastalarda MELD skoru zonulin seviyeleri ile pozitif korelasyon gösterdi (p=0,001, R=0,358).

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Geliş Tarihi/Received: 27.03.2021 **Kabul Tarihi/Accepted:** 18.10.2023

Öz

Sonuç: Bu çalışma, sağlıklı bireylere göre sirozda özellikle dekompanse fazda serum zonulin düzeylerinin azaldığını ortaya koymaktadır. Sirozda zonülinin İP için nedensel bir faktör olmadığı, bunun yerine sentezinin sirotik durumda olumsuz etkilendiği söylenebilir.

Anahtar Kelimeler: Siroz, İntestinal Geçirgenlik, Zonulin

Introduction

Liver cirrhotic is still an important cause of morbidity in mortality worldwide. In recent years many medical, interventional and endoscopic therapeutic strategies were used successfully in terms of treatment of underlying disease and treatment of complications (1,2). Still, there are many potential complications which are not having an enough therapeutic assessment yet. Intestinal permeability (IP) and related conditions is one of them. IP is a potential source of many complications in cirrhotic. Bacterial translocation (BT) results from IP and induces systemic inflammation, which negatively affects the liver (3). Also BT is a well-known precipitating factor of spontaneous bacterial peritonitis and hepatic encephalopathy attacks (4).

Tight-junction (TJ) is an intercellular located proteins which is control IP. Several proteins control TJ; in this group zonulin is the only measurable protein that controls TJ interaction and as a result, IP (5). Binding to its receptors, epidermal growth factor receptor and protease-activated receptor 2-, TJ gets a separate position (6). In recent years both IP and zonulin were investigated under the etiopathogenesis of various diseases, especially in autoimmune disorders such as type-1 diabetes and celiac disease (7,8).

Although IP was suspicious as a possible cause of many complications in cirrhotic, serum zonulin was not investigated sufficiently in cirrhotic patients. In this observational study our aim was to investigate serum zonulin levels in cirrhotic, and also investigate possible relationship between zonulin and severity of cirrhotic.

Materials and Methods

Design and Study Cohort

This is a prospective, cross sectional study which was conducted in Clinic of Gastroenterology of Keçiören Training and Research Hospital between 01 January 2019-01 April 2019. In this time period cirrhotic patients either newly diagnosed or under routine control were enrolled into this study. In their admission time to the study, in addition to etiological causes, Child-Pugh (CP) score and MELD score, presence of ascites, presence or history of hepatic encephalopathy, presence or history of variceal bleeding were noted. Also their blood samples including transaminase levels, albumin and bilirubin levels and international normalized ratio values were collected.

Simultaneously, healthy individuals were enrolled into the study. Healthy individuals who have liver disease history, chronic alcohol consumption, hepatitis B virus (HBV) or hepatitis C virus (HCV) infection history were excluded.

Assessment of Zonulin

Two milliliters of blood was collected from subjects who had read and signed the informed consent form to participate in the study. The samples were collected and centrifuged for 15 min at 2,500 g within a period of 30 min of collection. The blood samples were stored at -80 °C until the time of analysis.

The concentration of zonulin was measured using the enzyme-linked immunosorbent assay (ELISA) method. We used the human Zonulin ELISA kit (Elabscience, Elabscience Biotechnology Inc, Texas, USA). The procedure for the ELISA method was performed according to the instructions provided by the manufacturer. Absorbance was measured at at a wavelength of 450 nm using the ELISA reader. The Zonulin levels were presented as ng/mL. The intra-assay and inter-assay coefficients of variation were <%10.

Stastical Analysis

Statistical analyses were performed using the computer program SPSS 22.0 (IBM, USA). Kolmogorov-Smirnov normality tests were used to test the distribution of variables. Independent samples t-test was used for comparison of two group means. One-Way ANOVA was used for comparison of more than two group means. Pearson correlation was used to evaluate the linear relationship between the tested biomarkers. Data were presented as means $\pm$ standard or number and percentage according to their type and distribution. Differences were considered significant at $p<0.05$.

Ethical Considerations

The study protocol was approved by the Ethics Committee of Keçiören Training and Research Hospital (approval no: 2012-KAEK-15/1825, date: 13.02.2019). All individuals gave written informed consent.

Results

A total of 41 cirrhotic patients and 41 healthy individuals were enrolled into the study. Patients' mean age was 58.9±12.01, 27 patients were male and 14 patients were female. When we look the etiological causes of cirrhotic, most common cause was viral hepatitis, either HBV (n=16, 39.0%) and HCV (n=2, 4.9%). The second most common cause was cryptogenic cirrhotic (n=10, %24,4) followed by alcoholic cirrhotic, non-alcohol related steatohepatitis and autoimmune hepatitis. Eleven patients were in compensated phase with CP score A, nevertheless 30 patients were decompensated either by having ascites, variceal bleeding and/or hyperbilirubinemia (n=15 in CP score B and n=15 in CP score C). The mean MELD score was 15.6±5.83. Demographic, biochemical, and clinical characteristics of cirrhotic patients were listed in Table 1.

Zonulin levels were found as 17.5±1.04 ng/mL in cirrhotic patients and 19.7±0.65 ng/mL in healthy individuals. Although zonulin levels were lower than heathy controls, there was no significant difference between groups (p=0.068) (Table 2). In cirrhotic patients zonulin levels were significantly different between CP group A, B and C (p=0.05 for CP A and B, p=0.11 for CP A and C, p=0.026 for CP B and C) (Table 3).

After these results, we investigated the correlation between zonulin and biomarkers. Also, we investigated the correlation between the MELD score and zonulin. In laboratory parameters, no correlation was found between zonulin and patients' baseline laboratory values. However, zonulin was significantly correlated with MELD score (R=0.358, p=0.001) (Table 4).

Discussion

In our results, we found that zonulin levels were not statistically significant compared to healthy controls; however lower levels were observed in patients with cirrhotic. In patients with cirrhotic, zonulin level was significantly different between CP-A and CP- B. In addition, zonulin was significantly lower in CP-C compared to CP-A and CP-B. In all patients with cirrhotic, MELD score was positively correlated with zonulin levels.

Zonulin was studied in many clinical conditions other than cirrhotic in which increased IP plays a role in the physiopathology of the disease. In type-1 diabetes mellitus and celiac disease, elevated zonulin level was found according to the literature and elevated zonulin level is a predictor of IP (9). In the field of hepatology, few studies can be found according to the literature. Some of these studies investigated zonulin in chronic hepatitis patients and they found that zonulin level was significantly decreased in chronic hepatitis compared to healthy controls (10,11).

Table 1: Demographic, biochemical and clinical characteristics of cirrhotic patients

	Cirrhotic patients (n=41)
Age (years) (mean ± SD)	58.9±12.01
Sex % (M/F)	65.9/34.1
Etiology %	
HBV	(16) 39.0%
HCV	(2) 4.9%
Cryptogenic	(10) 24.4%
Autoimmune	(2) 4.9%
Alcoholic	(6) 14.6%
PBC	(1) 2.4%
NASH	(2) 4.9%
Budd-Chiari	(1) 2.4%
Wilson disease	(1) 2.4%
Complications Variceal bleeding (%)	(5) 12.2%
Hepatic encephalopathy (%)	(16) 39.0%
Ascites (%)	(34) 82.9%
Mortality (%)	(8) 19.5%
Child-Pugh Score	
A	(11) 26.8%
B	(15) 36.6%
C	(15) 36.6%
MELD Score	15.6±5.83
Hemoglobin (g/dL)	10.7±2.11
ALT (U/L)	30.3±24.98
AST (U/L)	50.6±33.37
GGT (U/L)	97.3±121.34
ALP (U/L)	152.5±92.82
Total protein (g/dL)	6.4±0.89
Albumin (g/dL)	2.9±0.57
Total bilirubin (mg/dL)	2.9±2.35
Direct bilirubin (mg/dL)	1.7±1.64
Creatinine (µmol/L)	0.97±0.41
Sodium (mEq/L)	130.7±21.41
WBC (x10^9/L)	6.0±2.31
Platelet (x10^3/L)	907.1±409.2
INR	1.6±0.48
Zonulin (ng/mL)	17.5±1.04

M/F: Male/Female, SD: Standard deviation, HBV: Hepatitis B virus, HCV: Hepatitis C virus, PBS: Primary biliary cholangitis, NASH: Non-alcoholic steatohepatitis, MELD: Model for end-stage liver disease, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, GGT: Gamma-glutamyl transferase, ALP: Alkaline phosphatase, WBC: White blood cells, INR: International normalized ratio

Table 2: Comparison of serum zonulin levels in cirrhotic patients and healthy controls

	Cirrhotic patients (n=41)	Healthy control (n=41)	p*
Zonulin (ng/mL)	17.5±1.04	19.7±0.65	0.068

*t-test was used for analysis

Table 3: Serum zonulin levels in cirrhotic patients according to Child-Pugh classification

	Child-Pugh score			
	a (n=11)	b (n=15)	c (n=15)	p*
Zonulin (ng/mL)	20.3±1.96	18.2±1.16	14.7±2.01	$p_1<0.05$, **$p_2=0.011$, $p_3=0.026$**

*One-Way ANOVA was used for analysis. p_1 is between a and b, p_2 is between a and c, and p_3 is between b and c

Table 4: Correlations between serum zonulin levels and selected biomarkers in cirrhotic patients

	Zonulin (ng/mL)	
	R	p*
MELD score	0.358	**0.001**
Hemoglobin (g/dL)	0.202	>0.05
ALT (U/L)	0.087	>0.05
AST (U/L)	0.024	>0.05
GGT (U/L)	-0.054	>0.05
ALP (U/L)	-0.160	>0.05
Total protein (g/dL)	-0.060	>0.05
Albumin (g/dL)	0.269	>0.05
Total bilirubin (mg/dL)	0.082	>0.05
Direct bilirubin (mg/dL)	0.055	>0.05
Creatinine (µmol/L)	-0.002	>0.05
Sodium (mEq/L)	-0.104	>0.05
WBC (x10^9/L)	0.163	>0.05
Platelet (x10^3/L)	0.132	>0.05
INR	-0.028	>0.05

*Pearson correlation test was used for analysis
MELD: Model for end-stage liver disease, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, GGT: Gamma-glutamyl transferase, ALP: Alkaline phosphatase, WBC: White blood cells, INR: International normalized ratio

Histopathologic studies of cirrhotic in humans and also in experimental models demonstrate important changes like a reduced number of microvilli, and shorter and atrophic mucosa in the intestine (12). In addition to these structural alterations, cirrhotic is a condition to promote IP in many ways related to its natural disease course. One of them is increased intestinal bacterial overgrowth. Increased oxidative stress either by increased bacterial overgrowth and intestinal hypoperfusion related to portal hypertension is a potential damage mechanism for intestinal integrity (13). Also increased TNF-alpha by monocytes and mesenteric lymph nodes induces IP, which in turns stimulates hepatic encephalopathy and spontaneous bacterial peritonitis (14). Nevertheless, beside this information, TJ proteins, zonulin and molecular pathways were not sufficiently investigated in cirrhotic.

Initially of our study period, our expectation is to observe elevated zonulin level in patients with cirrhotic because that IP is a natural property of cirrhotic. In contrast with this expectation we found nearly equal level of zonulin between cirrhotic and healthy controls. In addition, when cirrhotic situation gets worse, zonulin was also decreased. A possible explanation of this result is the biochemical property of zonulin. Zonulin was identified as a pre-haptoglobulin 2, precursor of haptoglobulin (15,16). It is well-known that the primary synthesis location of haptoglobulin is liver. As an explanation of our results, it possible to say that like other liver related proteins, zonulin levels gets below the normal in patients with cirrhotic. This result can contribute some speculations according to us. Firstly, zonulin is not a proper marker to investigate IP in patients with cirrhotic and secondly, zonulin is not play a role in the physiopathology of cirrhotic related IP.

Occludin and claudin proteins are the most investigated TJ proteins. Assimakopoulos et al. (17) found that these proteins were significantly reduced in cirrhotic patients both in compensated and decompensated compared to healthy controls. Also, In 2014, Pijls et al. (18) investigated IP in the compensated phase via claudin-3, and claudin-4 gene expressions and occludin expression and they found that occluding expression was significantly increased in the duodenum and sigmoid colon tissues but there is not any difference about claudin. Results of these studies are confusing especially in compensated phase. In our study, we did not find a significantly difference of zonulin levels between healthy control and cirrhotic in compensated phase. Based on these data's, it is possible to speculate that compensated phase is not a starting point of IP in cirrhotic.

Study Limitations

Our study has some limitations. Study population is small to exact evaluation of zonulin. However our results, especially in decompensated cirrhotic, show significantly difference and marked correlations. Another limitation is to lack of follow-up of patients with cirrhotic. It is important to evaluate relationship between zonulin level and complications of cirrhotic and death. Overall, zonulin is not a proper biomarker to investigate IP in cirrhotic, but follow-up studies can evaluate its possible role to predict complications and death in cirrhotic.

Conclusion

According to our study, zonulin is not a correct and useful marker in terms of diagnosis of intestinal permeability in cirrhotic.

Ethics

Ethics Committee Approval: The study protocol was approved by the Ethics Committee of Keçiören Training and Research Hospital (approval no: 2012-KAEK-15/1825, date: 13.02.2019).

Informed Consent: All individuals gave written informed consent.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: M.Y.A., Design: M.Y.A., Data Collection and Processing: V.G., M.U., E.K.A., F.S., Y.N., Analysis or Interpretation Ö.C.D., Literature Search: Z.G., Writing: M.Y.A.

Conflict of Interest: The authors declared that there was no conflict of interest during the preparation and publication of this article.

Financial Disclosure: The authors declared that they did not receive any financial support during the research and authoring of this article.

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