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Value of zonulin as a diagnostic and prognostic marker in different degrees of nonalcoholic fatty liver disease

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Abstract

Background Nonalcoholic fatty liver disease (NAFLD) is a group of hepatic disorders ranging between simple form of accumulation of fat in hepatocytes (hepatic steatosis) and inflammation of liver internal tissues and injury of hepatocytes that is known as nonalcoholic steatohepatitis (NASH) with increasing levels of fibrosis and cirrhosis and hepatocellular carcinoma (HCC). The composition of one's gut microbiota has a role in both the onset and progression of chronic liver disorders. One indicator of intestinal permeability is zonulin. In this study, we aimed to detect the value of zonulin as a diagnostic and prognostic marker in patients with different degrees of nonalcoholic fatty liver disease (steatosis, steatohepatitis, cirrhosis).

This case–control study was conducted on 60 subjects in Gastroenterology and Bariatric Surgery Departments at Ain Shams University Hospitals who were divided into 3 groups: Group A: 20 patients underwent bariatric surgery and have mild NASH, Group B: 20 patients underwent bariatric surgery and have moderate-to-severe NASH, and Group C: 20 healthy controls, during a period 1 of year.

Results There was high statistically significant difference between the studied groups; as regard zonulin concentration, zonulin showed high diagnostic accuracy in diagnosis of NASH among hepatic patients with total accuracy of 81.7%, sensitivity of 72.5%, and specificity of 100.

Conclusion Serum zonulin levels increase with steatosis severity in patients with NAFLD. This explains the high diagnostic accuracy of zonulin in diagnosis and prognosis of NASH among patients.

Keywords Zonulin, Cirrhosis, Intestinal barrier, Bacterial translocation, Permeability

Background

The intestine is an organ that not only breaks down food and absorbs nutrients but also protects the body from potentially dangerous external substances. The intestinal epithelial cells make a tight junction with the surrounding cells, and this gap serves as a key barrier against bacteria, viruses, toxins, and allergens that enter the body via the digestive tract [1].

Obesity and fatty liver disease are associated. As key risk factor for cirrhosis and liver cancer, fatty liver is characterized by an abnormal composition of fat in the

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liver. Major metabolic disorders including insulin resistance, diabetes, hypertension, and dyslipidemia are also strongly linked to obesity [2].

Several techniques exist for measuring intestinal permeability. Since plasma zonulin concentrations are readily evaluated with blood tests, they are widely used to evaluate intestinal permeability [3]. Intestinal permeability is regulated in a reversible fashion by the protein zonulin, which regulates the binding of intestinal mucosal epithelial cells [4].

Celiac disease and type 1 diabetes patients have increased expression of this gene because of its role in innate gut immunity [4–6]. The lactulose-mannitol ratio, once employed to assess intestinal permeability in clinical settings, is highly linked with plasma zonulin concentrations [7].

Both gluten and bacteria may stimulate the release of zonulin, and this protein's capacity to promote intestinal permeability aids in the gut's natural ability to flush away bacterial colonization [3].

Non-autoimmune diseases, such as type 2 diabetes and obesity, have also been linked to increased levels of circulating zonulin. Glucose levels, dyslipidemia, inflammation, and insulin resistance have all been demonstrated to be correlated with its concentration. Contradictory findings on the effect of food on zonulin secretion have prevented any firm conclusions from being reached [8].

The aim is to study the value of zonulin as a diagnostic and prognostic marker in different degrees of nonalcoholic fatty liver disease (steatosis, steatohepatitis, and cirrhosis) in patients with and without diabetes mellitus.

Methods

Patient selection

This case–control study was conducted in Gastroenterology and Bariatric Surgery Departments, Ain Shams University Hospitals. It was conducted on 60 subjects.

Study design

This study was performed in accordance with the ethical standards of Ain Shams University Research Committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The Ain Shams University Faculty of Medicine Research Ethics Committee (REC) FWA 000017585 has approved the study protocol with approval number (MD 03/2019).

After eligibility was confirmed and after obtaining the patients' written informed consent, patients were assigned into three groups according to abdominal ultrasound:

- *Group A*: Twenty patients with mild NASH
- *Group B*: Twenty patients with moderate-to-severe NASH
- *Group C*: Twenty healthy controls

Patients in group A and B are obese patients with BMI ≥ 30 and have NASH by clinical criteria and liver biopsy. Patients more than 60 or less than 18 years, patients with history of psychiatric illness, patients with known chronic liver disease, intestinal disorders (malabsorption, inflammatory bowel disease, celiac disease, food allergy), chronic kidney disease, or cardiac diseases were excluded from the study.

Groups A and B were subjected to the following: full history taking and clinical examination with special emphasis on weight and body mass index (BMI).

Routine preoperative investigations were done as complete blood count, random blood sugar, liver function tests, lipid profile, and glycosylated hemoglobin (HBA1c). Serum zonulin levels ELISA technique was used for the determination of human zonulin concentrations quantitatively in serum and abdominal ultrasound. Liver biopsy was taken intraoperative.

Group C was subjected to the following: Pelviabdominal ultrasound to exclude fatty liver, zonulin serum levels and liver enzymes, and nonalcoholic fatty liver disease fibrosis (NAFLD) score.

Serum zonulin was measured by an ELISA technique used for the quantitative determination of human zonulin concentrations in serum, plasma, and tissue homogenates. The kit was provided by MyBioSource (San Diego, CA, USA). The principle of the assay is as follows: This assay employs the quantitative sandwich enzyme immunoassay technique. Antibody specific for zonulin has been pre-coated onto a microplate. Standards and samples are pipetted into the wells, and any zonulin present is bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for zonulin is added to the wells. After washing, avidin-conjugated horseradish peroxidase (HRP) is added to the wells to remove any unbound avidin-enzyme reagent. Then, a substrate solution is added to the wells, and color develops in proportion to the amount of zonulin bound in the initial step. The color development is stopped, and the intensity of the color is measured. This assay has high sensitivity and excellent specificity for detection of human zonulin. No significant cross-reactivity or interference between human zonulin and analogues was observed. The minimum detectable dose of human zonulin is typically less than 0.156 ng/mL. Intra- and interassay coefficients of variation were 2–7% and 4–10%, respectively.

Sample size is as follows: Using pass program, set alpha error at 5% and power at 90%. The result from previous

study [9] showed the mean zonulin in normal, mild, and moderate-to-severe nonalcoholic steatohepatitis was 0.618, 2.14, and 5.8, respectively, with assumed common standard deviation of 4. Based on this, the needed sample is 20 mild, 20 moderate to severe, and 20 controls.

A written informed consent was obtained from all participants.

Statistical analysis

The Statistical Package for Social Sciences (SPSS version 20.0 for windows) software was used for data analysis. We used frequencies and percentages to express qualitative data. We also used mean and standard deviation to describe quantitative data if normally distributed and median and interquartile range if nonparametric. We used the Kolmogorov–Smirnov test to test the normality of distribution of numerical variables. Chi-square test was used to test the association between categorical variables. Fissure exact test was used in case of violation of the assumptions. We also used one-way ANOVA to test the difference between more than two groups concerning numerical parametric variables. Kruskal–Wallis test was used in case of nonparametric numerical variables. A post hoc analysis was conducted to test the difference between each of the two groups concerning significant numerical parameters.

Results

This study included 60 subjects divided into three groups. Twenty subjects are in each group: *Group A*: 20 patients with mild NASH, *Group B*: 20 patients with moderate-to-severe NASH, and *Group C*: 20 healthy controls.

Comparison between study groups regarding sociodemographic characteristics shows that there was no significant difference between study groups regarding age and gender distribution ($p=0.466$, $p=0.592$) respectively. In addition, we found that most participants in all groups were nonsmokers ($p=0.969$) as shown in Table 1.

Regarding associated comorbidities, we found that less than 50% of included patients in both group A and group B were either diabetic or hypertensive. We also found that BMI was significantly higher among patients with NASH when compared to control group ($p<0.001$) as shown in Table 1.

We compared between study groups concerning lab results and found that there was no significant difference between study groups concerning Hb levels, TLC, and platelet count ($p=0.163$, $p=0.802$, $p=0.955$) respectively.

Regarding liver functions, we found that both ALT and AST serum levels were significantly higher among patients with severe NASH when compared to other groups ($p=0.007$, $p=0.023$) respectively. On the other hand, both GGT and total bilirubin serum levels were not significantly different between study groups ($p=0.643$, $p=0.893$) respectively. We also found that serum albumin was significantly lower among patients with severe NASH when compared to other groups ($p<0.001$) as shown in Table 2.

Regarding lipid profile, we found that both cholesterol and triglycerides serum levels were not significantly different when compared between studied groups ($p=0.3$, $p=0.879$). On the other hand, HDL serum levels were significantly higher among control group, unlike LDL serum levels which were significantly high among patients with severe NASH when compared to other groups ($p=0.019$) as shown in Table 2.

All patients were examined via ultrasonography. We found that all patients had steatosis. However, all

Table 1 Comparison between study groups concerning sociodemographic characteristics

	Group A ($n=20$)	Group B ($n=20$)	Group C ($n=20$)	*p*-value
Age	38.05 ± 7.54	36.05 ± 6.74	35.45 ± 6.44	0.466A
Gender				
Male	5 (25)	7 (35)	8 (40)	0.592F
Female	15 (75)	13 (65)	12 (60)	
Smoking status				
Current	3 (15)	3 (15)	4 (20)	0.969F
Ex-smoker	2 (10)	4 (20)	3 (15)	
Nonsmoker	15 (75)	13 (65)	13 (65)	
Diabetes mellitus	6 (30)	7 (35)	0	0.015F
Hypertension	7 (35)	7 (35)	0	0.011F
BMI (kg/m^2)	35.54 ± 3.77	36.75 ± 2.91	27.35 ± 3.27	< 0.001A
	p1 = 0.258, p2 < 0.001, p3 < 0.001			

[A] One-way ANOVA. [F]Fissure exact test. p1, Group A versus Group B. p2, Group A versus Group C. p3, Group B versus Group C

Table 2 Difference between study groups concerning laboratory results

	Group A ($n=20$)	Group B ($n=20$)	Group C ($n=20$)	p-value
Hb (mg/dl)	12.05 ± 1.11	11.54 ± 1.23	12.26 ± 1.31	0.163A
TLC	5.63 ± 1.29	5.35 ± 1.43	5.52 ± 1.3	0.802A
PLT	250 ± 56.38	255.7 ± 65.22	253.65 ± 58.88	0.955A
ALT	37.5 (28–49)	41.5 (29–57.25)	29 (14–33)	0.007A
	p1 = 0.445, p2 = 0.009, p3 = 0.004			
AST	32.5 (25.5–44.25)	35.5 (25.5–48.25)	28.5 (13.75–33.25)	0.023A
	p1 = 0.429, p2 = 0.030, p3 = 0.013			
GGT	75 (45–123.5)	87.5 (45.75–114.5)	66 (36.75–91)	0.643A
Total bilirubin	0.89 (0.56–1.1)	0.87 (0.44–1.19)	0.85 (0.55–1.03)	0.893
Albumin	4.17 ± 0.45	3.72 ± 0.43	4.61 ± 0.61	< 0.001
	p1 = 0.006, p2 = 0.008, p3 < 0.001			
Cholesterol	201.6 ± 37.59	190.5 ± 34.72	182.65 ± 42.54	0.3
Triglycerides	150.65 ± 64.35	156.45 ± 35.66	145.8 ± 88.11	0.879
HDL	49.45 ± 7.77	41.95 ± 7.24	51.8 ± 8.15	< 0.001
	p1 = 0.003, p2 = 0.340, p3 < 0.001			
LDL	124.6 ± 35.34	127.75 ± 26.41	102.5 ± 26.89	0.019
	p1 = 0.740, p2 = 0.023, p3 = 0.010			
HbA1C	5.72 ± 0.78	6.07 ± 0.94	5.17 ± 0.28	< 0.001
	p1 = 0.031, p2 = 0.54, p3 = 0.005			

[A] One-way ANOVA. [F]Fissure exact test. p1, Group A versus Group B. p2, Group A versus Group C. p3, Group B versus Group C

Table 3 Comparison between studied cases according to ultrasonographic findings

	Group A ($n=20$)		Group B ($n=20$)		p
Steatosis					
Grade 1	20	100.0	0	0.0	
Grade 2	0	0.0	15	75.0	< 0.001
Grade 3	0	0.0	5	25.0	

Table 4 Comparison between studied cases according to fibroscan findings

	Group A ($n=20$)	Group B ($n=20$)	p
Result (Kpa)	5.25 ± 1.59	7.35 ± 1.50	< 0.001*
Score			
F0 to F1	18 (90%)	1 (5%)	
F2	2 (10%)	14 (70%)	< 0.001
F3	0	5 (25%)	

* Independent sample t-test

patients with mild NASH had grade 1. Unlike those with severe NASH, 25% of them had grade 3 steatosis as shown in Table 3.

All patients underwent fibroscan study. We found that steatosis was more significantly observed among patients with severe NASH when compared to those with mild degree ($p < 0.001$). In addition, we found that 25% of patients with severe fatty liver disease had F3 classification. However, those with mild degree fatty liver lied in F0 to F1 classification as shown in Table 4 and Fig. 1.

We also compared between study groups concerning NAFLD fibrosis scores and found that NAFLD fibrosis median scores were significantly low among patients with severe NASH when compared to others ($p < 0.001$) as shown in Table 5. When interpreting their scores, we found that 10% of patients with severe NASH had higher fibrosis scores when compared to those with mild NASH degree; among whom, no one had high fibrosis score. This was statistically significant ($p < 0.001$) (Table 5).

A biopsy was taken from all cases and found that fibrosis and steatosis were more significantly present among patients with severe NASH when compared to those with mild form ($p < 0.001$) as shown in Table 6.

We measured zonulin serum levels among all participants and found that its median serum levels were significantly higher among patients with severe NASH when compared to others. This was statistically significant ($p < 0.001$) as shown in Table 7 and Fig. 2.

We found that there was a moderate positive correlation between serum zonulin and NAFLD fibrosis score ($r = 0.431$, $p = 0.001$) as shown in Table 8.

We also tested the diagnostic accuracy of zonulin in diagnosis of NAFLD and found that above serum concentration of 209.35, it showed a sensitivity of 72.5% and

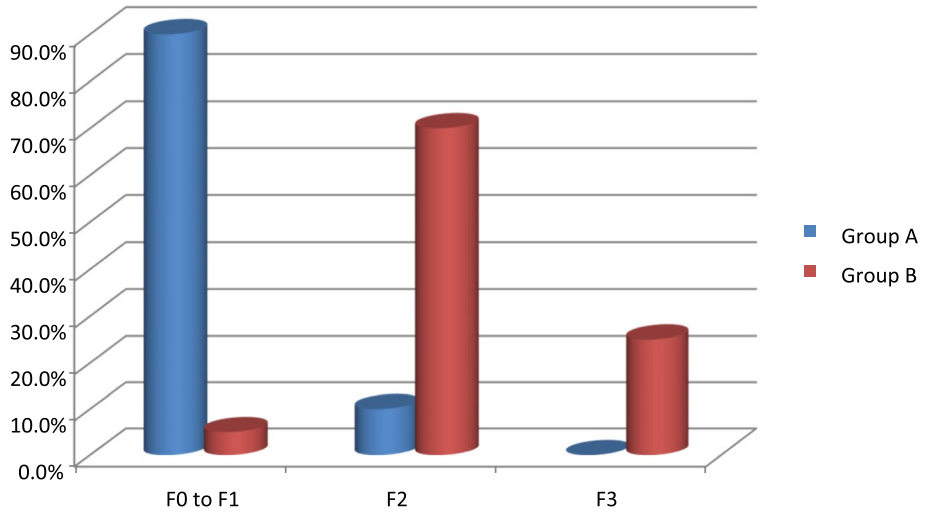

Fig. 1 Comparison between study groups concerning fibroscan

Table 5 Comparison between studied cases according to NAFLD fibrosis score

	Group A ($n=20$)	Group B ($n=20$)	Group C ($n=20$)	*p*
NAFLD fibrosis score	−1.76 (−2.25 to −1.2) p1 = 0.640, p2 < 0.001, p3 < 0.001	−1.31 (−2.62 to −0.58)	−3.02 (−3.63 to −2.35)	< 0.001
Interpretation				
Low score (lower than −1.455)	14 (70%)	10 (50%)	20 (100%)	
Intermediate score (between −1.455 and 0.676)	6 (30%)	8 (40%)	0	0.005*
High score (higher than 0.676)	0	2 (10%)	0	

* Chi-square test. p1, Group A versus Group B. p2, Group A versus Group C. p3, Group B versus Group C

Table 6 Comparison between studied cases according to liver biopsy

	Group A ($n=20$)	Group B ($n=20$)	*p*-value
Finding			
No fibrosis	18 (90%)	8 (40%)	< 0.001*
Fibrosis present	2 (10%)	12 (60%)	
Steatosis grade 1 (mild)	20 (100%)	0 (0%)	
Steatosis grade 2 (moderate)	0 (0%)	15 (75%)	
Steatosis grade 3 (severe)	0 (0%)	5 (25%)	
Ishak score			
0	18 (90%)	8 (40%)	
1	1 (5%)	2 (10%)	0.009*
2	1 (5%)	7 (35%)	
3	0 (0%)	3 (15%)	

Chi-square test

*highly significant

specificity of 100% with a total accuracy of 81.7% in diagnosis of NAFLD among participants as shown in Table 9 and Fig. 3.

Discussion

Recently, changes in gut microbiota followed by compromised intestinal wall integrity have been proposed as

Table 7 Comparison between studied groups according to zonulin level

	Group A ($n=20$)	Group B ($n=20$)	Group C ($n=20$)	*p*
Zonulin	208.9 (75.3–414.6) p1 = 0.031, p2 = 0.005, p3 < 0.001	566 (328.83–697.95)	71.6 (65.38–118.93)	< 0.001*

Kruskal–Wallis test. p1, Group A versus Group B. p2, Group A versus Group C. p3, Group B versus Group C

*highly significant

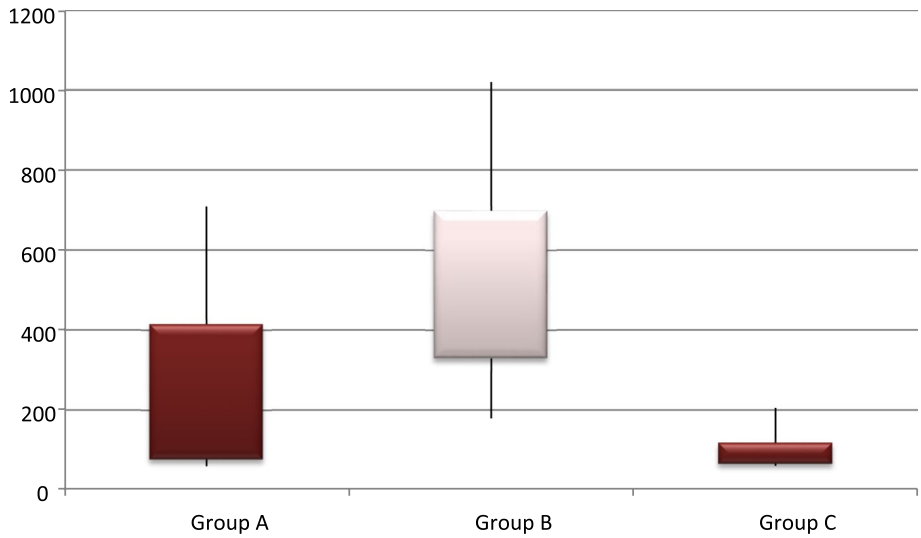

Fig. 2 Comparison between study groups concerning zonulin serum levels

Table 8 Correlation between NAFLD fibrosis score and zonulin level

	Zonulin level	
	R	*P*
NAFLD fibrosis score	0.431	0.001*

*highly significant

potential contributors to NAFLD etiology. Specifically, disruption of tight connections between intestinal cells seems to be the root cause of the permeability increase. The intestinal tract serves as a barrier to foreign substances while also aiding in the digestion and absorption of food [10].

As intestinal permeability rises, the gut loses its basic barrier function, which may cause a wide range of clinical symptoms. Conditions of intestinal diseases, such as celiac disease, are associated with a dramatic rise in intestinal permeability. Intestinal permeability may also be increased by metabolic disorders including obesity and insulin resistance, as well as by liver ailments. Therefore, researchers have paid a lot of attention to intestinal permeability [11].

Tight junctions are regulated by zonulin, a family of peptides which are produced by cells in the intestine and liver. Since zonulin alters the junctions present between the gut epithelial cells, increased serum zonulin levels have been associated with improved permeability of the intestine. Zonulin, which makes the gut permeable by opening enterocytes, is primarily triggered by bacteria and the gluten protein (gliadin) [12].

In our study, we found that the mean age of the studied groups was 38.05 ± 7.54, 36.05 ± 6.74, and 35.45 ± 6.44 years old in groups A, B, and C respectively, and there was non-significant differences between studied groups as regard each of age and gender distribution, while there was a significant difference between patients as regard associated

Table 9 ROC curve analysis for the use of zonulin to discriminate between cases and controls

	Cutoff	AUC	Sens%	Spec%	PPV%	NPV%	Accuracy %
Zonulin	209.35	0.880	72.5	100.0	100.0	64.5	81.7

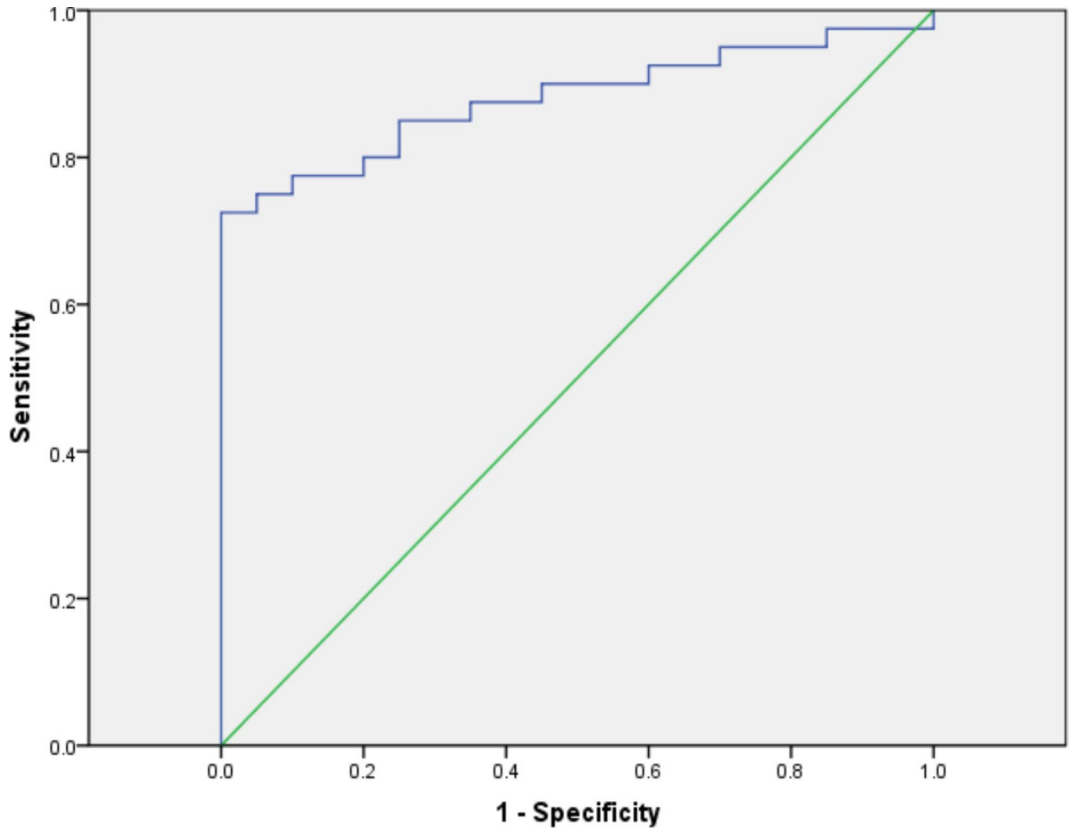

Fig. 3 ROC curve analysis for the use of zonulin to discriminate between cases and controls

comorbidities in the form DM and HTN which were not present among control group.

In agreement with our findings, the study of *Kim and Ko* was conducted on 140 participants divided into 3 groups; 89 were assigned to the mild group, 34 were included in the moderate to severe group, and the remaining 17 patients were assigned to normal control group.

They reported that their mean age of the studied groups was 44.5 ± 9.5, 44.8 ± 8.9, and 44.7 ± 9.2 years old in the three groups respectively. Also this study is in contrary to our findings as regard comorbidities where they reported no difference regarding either dyslipidemia hypertension or diabetes. This difference may be explained by the differences between two studies as regard sample size and inclusion and exclusion criteria [9].

In our study, we found that patients with NAFLD had higher blood pressure recordings compared to normal individuals. Our results were consistent with *Tahir* et al. who reported that more than 60% of included patients had comorbidities among which hypertension was the commonest [13].

Unlike *Hendy* et al. who found that both diastolic and systolic blood pressure recordings were nearly the same when compared between study groups [14]. This difference may be related to patients' inclusion criteria difference between the studies.

We also found that anthropometric measures as body mass index (BMI) were higher among patients with NAFLD. This may be due to that patients with NAFLD are often in their 40 s or 50 s, overweight, or obese.

Regarding CBC parameters, we found either Hb serum levels, WBC count, or platelet count were nearly the same when compared between study groups. In agreement with our findings, in the study of *Öztürk* et al., they used data from 66 patients with nonalcoholic steatohepatitis confirmed through biopsy, 49 healthy individuals, and 34 patients with simple degree of steatosis and to study the association between parameters of atherosclerosis, CBC findings, and results of liver biopsy in those suffering from nonalcoholic fatty liver disease [15].

They found no significant differences between the groups in terms of RBCs and mean platelet count. While the subgroups of people with NAFLD had significantly lower hemoglobin levels than the controls, there was no significant difference between themselves [15].

It is known that liver enzymes are often the first-line investigation for any suspected liver disease with or

without imaging, and in our study, both ALT and AST serum levels were significantly higher among patients with fatty liver disease; furthermore, both enzymes were higher in Group B due to effect of severity of fatty diseases on the liver.

In comparison with our findings, *Kim and Ko* reported that liver enzymes were similar between studied groups [9].

Also, we are supported by *Pacifico* et al. who showed that as expected, patients with NAFLD had higher liver enzymes compared to healthy controls [16]. Additionally, *Hendy* et al. showed that the albumin serum levels were similar between study groups. On the contrary, in liver function tests, patients had higher liver enzymes [14]. In addition in a study by Xie et al. in which participants with NAFLD had higher triglyceride, cholesterol, HbA1c, liver enzymes; AST, ALT, (ALP), (GGT), and lower HDL-cholesterol levels compared to healthy controls [17].

It is known that NAFLD is associated with dyslipidemia. HDL and LDL levels were significantly higher in the NAFLD group compared to the healthy control groups. In Mansour-Ghanaei et al. study, patients with NAFLD had lower HDL but high levels of LDL, TC, and TC/HDL ratios. Moreover, TG was shown to have a significant correlation with NAFLD, but LDL lacked such a correlation [18].

Also, Santhoshakumari et al. reported that NAFLD patients had higher LDL, TC, and TG but lower serum levels of HDL compared to the normal healthy individuals [19].

In our study, we found that among NAFLD group had higher random blood sugar levels in addition to greater HbA1C serum levels. Previous clinical study by *Chen* et al. called attention to the hepatic component of metabolic syndrome known as nonalcoholic fatty liver disease (NAFLD). Moreover, it was associated with type 2 diabetes development [20].

In addition to the above findings, we found that steatosis was significantly higher among patients in Group B when our findings can be explained by the findings of *Cengiz* et al. which illustrated that NAFLD disease encompasses a spectrum of liver diseases that progress from NAFLD to nonalcoholic steatohepatitis, fibrosis, and cirrhosis in the absence of heavy alcohol use [21].

A comprehensive clinical history in addition to pathological correlations is the gold standard for diagnosing NAFLD. After determining that alcohol usage is not a contributing factor, a liver biopsy may establish the presence of steatosis [22]. Liver biopsies are the gold standard for identifying fibrosis, defining its etiology, and establishing whether or not it has advanced to cirrhosis [23].

Elwan et al. collaborate our findings by reporting a positive correlation between fibrosis score and ultrasound findings; specifically, they found that changes in hepatic echo pattern were associated with an increase in fibrosis levels in both fibroscan and liver biopsy [24].

In our study, there was a high significant difference as regard zonulin concentration which was of higher level among NAFLD groups than in healthy control group.

Serum zonulin was shown to be higher in patients with NAFLD and in patients with NASH compared to those with simple steatosis, indicating a potentially important role for serum zonulin in the development of NAFLD and NASH.

Moreover, we found that there was significant positive correlation between zonulin concentration and both NAFLD Fibrosis score ($r=0.431$) and biopsy grades (Ishak score) ($r=0.423$).

In agreement with our study, Pacifico et al. reported that participants who were both obese with NAFLD had higher zonulin levels. The degree of steatosis was correlated with an increase in zonulin concentrations in NAFLD patient population [16].

In a trial to study the mechanism that might account for the connection between zonulin serum levels and the nonalcoholic fatty liver disease severity, to begin, the gut microbiota changes that occur in obese persons lead to intestinal permeability increase. This change in intestinal permeability is a potential cause of the increased zonulin serum levels. Secondly, alterations in the microbiota may potentially result in an increase in the production of cytokines that are pro-inflammatory. To a tolerable extent, the lipopolysaccharide (LPS) is secreted by gut bacteria which operate as toll-like receptor (TLR) ligands to unduly excite TLRs [25].

Zak-Gob et al. reported a higher gut permeability that showed a positive correlation with the liver disease severity in children with NAFLD diagnosed through biopsy, lending support to the idea that serum zonulin levels are positively correlated with the severity of fibrosis, steatosis, and lobular inflammation in NASH [26].

Noteworthy is the fact that the findings of Miele et al. and Pacifico et al. both rejected the idea that there is any relation between intestinal permeability and the severity of NAFLD or the course of the disease. Instead, they approached the two conditions as two distinct disease entities.

However, the studies that were discussed earlier examined intestinal permeability based on a variety of modalities. These modalities were definitely impacted by the clinical and metabolic features of the patients, as well as the qualities of the varied dietary variations. In contrast, in order to steer clear of these potential problems, the researchers in this investigation relied on serum zonulin

levels as a proxy for measuring intestinal permeability [16, 27].

Another study by *Kim and Ko* reported there was a significant positive correlation between the plasma zonulin concentrations and fatty liver disease severity [9].

In the current study, above 324.05 pc/mL, it can discriminate between the probability of advanced fibrosis (intermediate and high score) and the absence of advanced fibrosis (low score) with AUC of 0.807, level of sensitivity 81.3%, specificity 77.3%, *PPV* 56.5%, *NPV* 91.9%, and accuracy 78.3%.

Our study is in agreement with the study of *Parkhomenko* et al. which reported a higher serum zonulin levels in obese adolescents compared to healthy individuals. Using zonulin, it was shown that above 209.35 pc/mL, it can discriminate between cases and control with AUC of 0.880, level of sensitivity 72.5%, specificity 100%, *PPV* 100%, *NPV* 64.5%, and accuracy 81.7% [28].

According to the findings of Voulgaris and colleagues, the capacity of serum zonulin levels to study liver disease progression to reach the stage of decompensated liver disease was substantial, though poor (*AUROC*: 0.723); however, serum zonulin levels that were lower than 3.65 pc/mL at the time of the first evaluation had a negative predictive value of 84% for the development of liver decompensation over the subsequent year [29].

While in a study of *Hendy* et al., they found that zonulin could achieve a 100% sensitivity and specificity in early diagnosis of NASH with a cutoff value 8.3 pc/mL, ($AUR = 1.000$, $p < 0.001$). That is why it should be noted that a probable involvement in the etiology of NAFLD incidence and development is suggested by the rising zonulin levels in NAFLD patients, especially those in the NASH group [14].

There are a few drawbacks to this research, the most notable of which are that it does not characterize the gut microbiota and does not examine the levels of endotoxin. In addition, there were no biopsies of intestinal wall performed to study the correlation between zonulin levels and the small intestine tight junctions integrity. Another limitation of our study was the small sample size of the cases that were investigated, and because of this, we recommend that additional research be conducted with a larger sample size and on a larger geographical scale to emphasize our findings.

Conclusion

We found that the degree of steatosis is correlated with the serum zonulin levels in individuals with NAFLD. Our study's cross-sectional method, however, prevents us from drawing any firm conclusions about the temporal or causative links between elevated zonulin concentrations and the degree of steatosis in those with NASH. Additional research may be necessary to fully understand the underlying processes and establish a causal link between causes and effects.

Abbreviations

NAFLD	Nonalcoholic fatty liver disease
NASH	Nonalcoholic steatohepatitis
HCC	Hepatocellular carcinoma
BMI	Body mass index
HBA1c	Glycosylated hemoglobin
SPSS	Statistical Package for Social Sciences
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
CBC	Complete blood count
INR	International normalized ratio
TLR	Toll-like receptor
DM	Diabetes mellitus
HTN	Hypertension
GGT	Gamma glutamyl transferase
HDL	High-density lipoprotein
LDL	Low-density lipoprotein
ELISA	Enzyme-linked immunosorbent assay
ANOVA	Analysis of variance
ACE	Angiotensin-converting enzyme

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Authors' contributions

AME and EMB made the design for the study, MMS and MSM selected the cases from Gastroenterology and Bariatric Surgery Departments, Ain Shams University Hospitals, AMME contributed to data collection, MSE is the bariatric surgeon who did the bariatric surgery for the cases and took the liver biopsy, and AME, NMT, and SAE were responsible for data analysis and manuscript editing. All authors provided critical feedback and helped shape the research, analysis, and manuscript. All authors have read and approved the manuscript.

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Availability of data and materials

The authors confirm that the data supporting the finding of this study are available within the article.

Declarations

Ethics approval and consent to participate

A written informed consent was obtained from all participants.

Consent for publication

Informed consent to publish patient's data was signed by all participants prior to the beginning of the research.

Competing interests

The authors declare that they have no competing interests.

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