Clin. Lab. 2020;66:705-708

LETTER TO THE EDITOR

Circulating Zonulin is Related to Hepatic Necroinflammation in Patients with Non Alcoholic Fatty Liver Disease

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(Clin. Lab. 2020;66:705-708. DOI: 10.7754/Clin.Lab.2019.190922)

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Letter to the Editor accepted September 27, 2019

KEY WORDS

zonulin, NAFLD, intestinal permeability

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Non-alcoholic fatty liver disease (NAFLD) is becoming the main chronic hepatitis in Western Countries paralleling the increase of obesity and type 2 diabetes. NAFLD encompasses a wide spectrum of liver damage ranging from simple fatty liver (NAFL) to non-alcoholic steatohepatitis (NASH) with or without fibrosis, but the pathogenetic mechanisms involved in the onset and progression of NAFLD are not yet fully elucidated [1]. Emerging data show that the impairment of intestinal barrier, due to alterations in gut microbiota, may play a role in NAFLD pathogenesis as well as in disease progression [2]. In recent years, several studies have focused on the evaluation of serum zonulin as non-invasive marker of intestinal permeability (IP). Zonulin, a 47 kDa protein, is the inactive precursor of haptoglobin-2; it is involved in the regulation of the tight junctions, the main driver of intestinal epithelium homeostasis [3]. Circulating zonulin has been found increased in different pathological conditions such as autism, type 1 and type 2 diabetes, metabolic syndrome, and inflammatory bowel disease [4], often showing a positive relationship with body mass index (BMI), thus indicating the impact of obesity in alterations of IP. In the setting of NAFLD, available data are scanty and often conflicting. Therefore, we investigated the potential role of zonulin in discriminating NASH as well as its association with histological features of NASH in a cohort of 108 biopsy

Table 1. Clinical, biochemical, and histological characteristics of the study cohort (n = 108).

Variables	n = 108
Age, years	46 ± 12
BMI (kg/m^2)	28.4 ± 4.2
Waist (cm)	98 ± 11
Obesity, n (%)	41 (38%)
Diabetes, n (%)	26 (24%)
AST (U/L)	34 (31 - 38)
ALT (U/L)	53 (46 - 64)
GGT (U/L)	63 (51 - 73)
Fasting glucose (mg/dL)	100 (90 - 96)
Fasting insulin (μIU/mL)	15.1 ± 11.3
Triglycerides (mg/dL)	146 (116 - 149)
Total cholesterol (mg/dL)	198 (183 - 203)
HDL-cholesterol (mg/dL)	48 (45 - 49)
Albumin (g/dL)	4.6 ± 0.4
Zonulin (ng/mL)	35 ± 19
Histology	
Fibrosis	
F0/F1	56 (52%)
F2	21 (19%)
F3/F4	31 (29%)
Steatosis	
S1	55 (53%)
S2	35 (34%)
S3	14 (13%)
Ballooning	
0	14 (13%)
1	51 (49%)
2	39 (38%)
Lobular inflammation	
0	24 (23%)
1	70 (67%)
2	10 (10%)
Portal inflammation	
0	57 (55%)
1	44 (42%)
2	3 (3%)
NASH	63 (58%)

Non-alcoholic steatohepatitis is defined as the joint presence of hepatic steatosis, ballooning, and lobular inflammation. Data on hepatic steatosis, ballooning, lobular inflammation, and portal inflammation were available for 104 cases since 4 patients had clinical cirrhosis (F4).

proven NAFLD subjects consecutively enrolled at the Division of Gastroenterology of the University of Torino. All patients signed a written informed consent to participate in the study. The study was approved by the ethics committee of the University Hospital Città della Salute e della Scienza of Torino and was in accordance with the Helsinki Declaration.

Spearman´s correlation test was used for correlations between zonulin and continuous variables. To assess differences between groups, the Kruskal-Wallis test or the Mann-Whitney *U*-test were used as appropriate. Linear and logistic regression analysis were performed to assess the association between circulating zonulin and waist circumference or histological characteristics. Data were analyzed using the Software MedCalc, version 12.0 (Ostend, Belgium).

Overall, serum zonulin increased proportionally to waist circumference ($r = 0.22$, $p = 0.038$; Figure 1A) while no correlation was found with BMI ($r = 0.03$, $p = 0.741$). Furthermore, by multivariable regression analysis including BMI, age and gender, waist circumference resulted independently associated with serum zonulin levels ($t = 2.1$, $p = 0.038$). Despite the lack of magnetic resonance imaging for the quantification of visceral fat is a limitation of the study, waist circumference is considered a good surrogate for the estimation of visceral fat. Conversely, BMI provides an estimate of total body fat. The lack of correlation between zonulin levels and BMI is difficult to explain. Probably the different prevalence of obesity assessed either by waist circumference or by BMI (55% vs. 38%, respectively) may have affected the results. Furthermore, the association between zonulin and higher waist circumference is in accordance with previous data [5].

Concerning histological features, serum zonulin was significantly higher in patients with mild/moderate portal inflammation compared to those without (37 ± 15 ng/mL vs. 46 ± 15 ng/mL, $p = 0.005$, Figure 1B). A similar trend was observed for the degree of ballooning even if the statistical significance was not reached probably due to the small number of cases without this histological characteristic (35 ± 17 ng/mL in patients without compared to 43 ± 16 ng/mL in patients with mild/moderate ballooning, $p = 0.084$, Figure 1C). Interestingly, when we grouped the cases according to NAS, we found that zonulin levels were significantly higher in patients with a more severe necroinflammation (37 ± 15 ng/mL in patients with NAS < 3 vs. 45 ± 16 ng/mL in those with NAS ≥ 4, $p = 0.013$, Figure 1D). Our findings are corroborated by the data reported by Giorgio et al., which demonstrated an association between the results of lactulose and mannitol permeability test (the gold standard test for the IP assessment) and liver disease severity [6].

Finally, we observed that circulating zonulin levels were not significantly different between patients with simple fatty liver and those with NASH ($p = 0.827$). According to our findings, Pacifico et al. did not find a significant correlation between serum zonulin and the

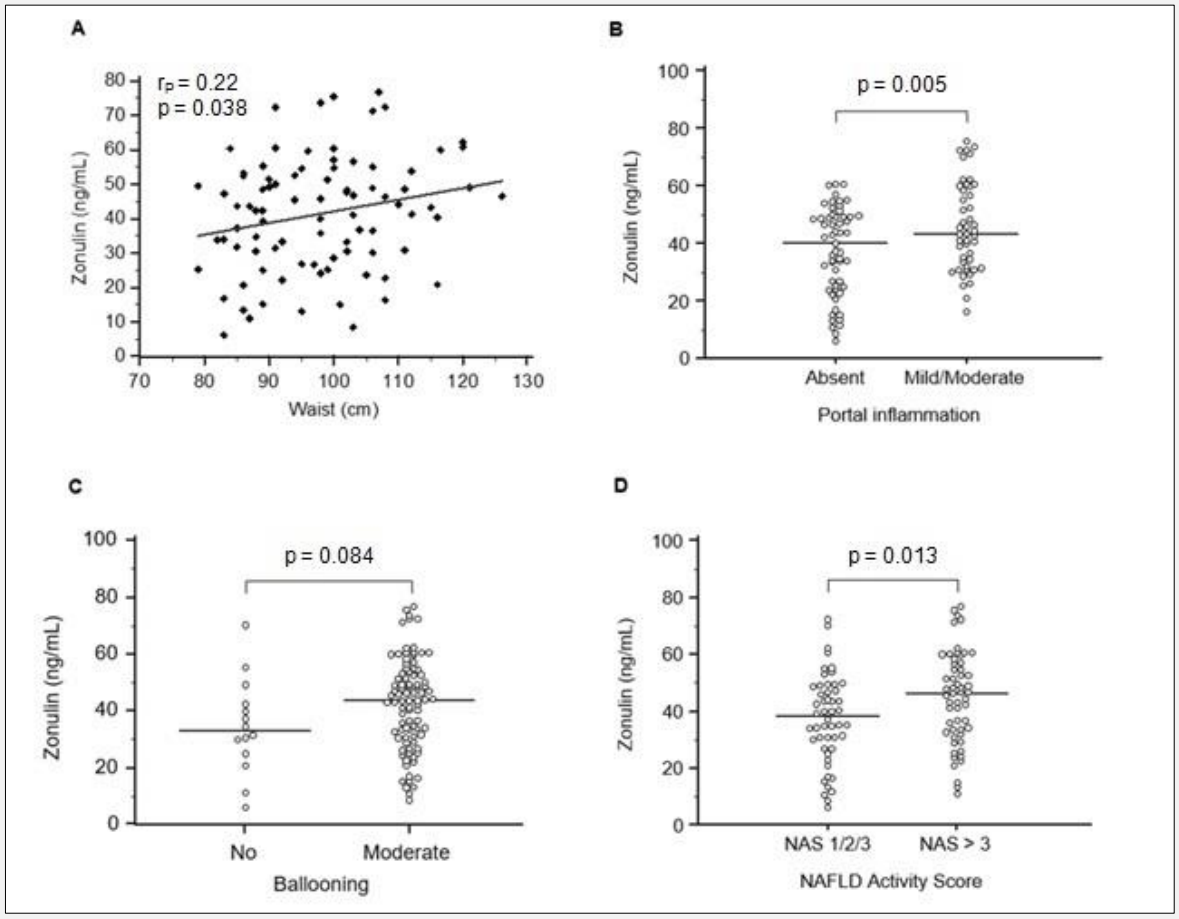

Figure 1. Serum zonulin levels according to waist circumference and the severity of liver disease in patients with non-alcoholic fatty liver disease.

Circulating serum zonulin increases according to waist circumference (A) and to the severity of liver disease: portal inflammation (B), ballooning (C), and NAFLD activity score (D).

presence of NASH (p = 0.170) [7]. Conversely, in the study by Hendy et al., serum zonulin levels were higher in non-obese subjects with NAFLD compared to controls and, in the former group, zonulin was able to discriminate NAFL from NASH (7.60 ± 0.91 ng/mL vs. 4.91 ± 0.46 ng/mL, p < 0.001, respectively) [8]. Different clinical features and inclusion criteria (i.e., diabetes and other characteristics of metabolic syndrome) or, more likely, different analytical methods for the measurement of circulating zonulin may have led to such discrepancies. Indeed, a recent paper by Ajamian et al. raised some concerns regarding the reproducibility of commercial zonulin assays showing that antibodies for the detection of zonulin have different antigen specificity and affinity [9]. Moreover, Vojdani et al. questioned the reliability of a single measurement of serum zonulin for the evaluation of IP due to its short half-life in the bloodstream [10].

CONCLUSION

In our cohort of biopsy-proven NAFLD patients, higher serum zonulin levels were associated with higher waist circumference suggesting that visceral obesity exerts an important effect on the impairment of IP. We could not find distinct zonulin levels among subjects with or without NASH but we found that higher zonulin levels are associated with a more severe liver necroinflammation. Taking into account the potential limitations concerning the analytical methods, further studies are warranted to clarify the role of zonulin in the setting of NAFLD.

Source of Funds:
Chiara Rosso received a research grant from Fondazione Cassa di Risparmio di Torino (2015.2643) and the University of Torino (ROSC_RILO_18_01).

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