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The Role of Zonulin as a Prognostic Biomarker in Liver Diseases: A Systematic Review

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Abstract

Liver diseases have become a serious concern in the health care systems worldwide. There is an association between gut permeability and several liver diseases. Zonulin is recognized as a marker of intestinal permeability. Hence, this study summarizes the current knowledge about the role of zonulin as a prognostic biomarker in liver diseases. The present systematic review was performed according to the guidelines of the 2015 Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) statements. Until May 2022, PubMed/Medline, Science Direct, Scopus, and Google Scholar as well as Google databases were searched using the relevant keywords including "Zonulin" and "Liver Diseases", "Fatty Liver", "Hepatitis", "Cirrhosis", "Carcinoma", "Liver Steatosis". Of 279 identified records through database searching, 37 articles were included in this systematic review. The evidence of this review revealed that the level of zonulin as the major biomarker of intestinal permeability increased in various types of liver diseases. Overall, we found that increased level of zonulin is associated with inflammatory responses in liver tissue and development of liver diseases. However, further studies could indicate the diagnostic and therapeutic role of zonulin in liver diseases including non-alcoholic fatty liver disease (NAFLD), Hepatitis, cirrhosis and hepatocellular carcinoma (HCC).

Introduction

The liver is one of the most important organs in the body that performs various physiological functions [1]. The main function of the liver is to purify the blood of the gastrointestinal tract before transferring it to the rest of the body [2]. Detoxification, protein synthesis, production of biochemical compounds, metabolic function and hormone production are other important functions of the liver [3]. Hence, liver dysfunction can lead to a health threatening condition. Mortality and disability due to liver diseases have raised concerns all over the world [4]. Liver diseases impose health and economic burden on the health care system around the world [5]. The number of cases of the liver diseases is estimated at approximately 1.5 billion worldwide [6]. Globally, recent epidemiological data represents that around 2 million deaths per year occur due to the liver diseases [7]. The most common liver diseases are non-alcoholic fatty liver disease (NAFLD), Hepatitis B, Hepatitis C, alcohol-related liver disease (ALD), cirrhosis and hepatocellular carcinoma (HCC) [6]. Cirrhosis and HCC together account for the death of two million people [7]. There is currently no way to compensate for the lack of liver function other than liver transplantation [8]. Several genetic and environmental factors play role in the progression of liver diseases. Emerging data have shown the association between development of the liver diseases and changes in intestinal permeability [9]. Evidence supports the role of the liver– gut axis in the pathogenesis of some of liver diseases including NAFLD [10]. Due to the connection between the gut and the liver through the portal circulation, changes in intestinal permeability can facilitate the entrance of harmful substances to the liver and disrupt metabolic pathways. On the other hand, the interaction between these substances and liver immune cells can lead to inflammation and eventually development of liver disease [9]. Zonulin is a biomarker related to the intestinal barrier integrity of the small intestine [11]. Zonulin is a 47-kDa protein which is identified as the only physiological biomarker for the intestinal permeability [12]. It is known for the reverse regulation of intestinal permeability through disassembling intercellular tight junctions [13]. It has been shown that one of the most important functions of zenolin in the body is controlling the intercellular tight junctions between the intestinal enterocytes and as a result, helping the absorption of nutrients in the body. Furthermore, there are several pieces of evidence about the biological properties of zonulin in activation of EGFR and subsequently the effect of zonulin on transepithelial electrical resistance (TEER) [14]. In fact, zonulin can be considered as a biomarker of gut barrier dysfunction for several diseases including autoimmune, neurodegenerative and liver diseases because of the connection between the liver and the gut means liver–gut axis. Zonulin can also be proposed as a promising therapeutic target for the treatment of various diseases [14]. Hence, this systematic review summarizes the current knowledge about the role of Zonulin as a prognostic biomarker in liver diseases.

Methods

Search strategy

The present systematic review was carried out according to the guidelines of the 2015 Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) statements. Electronic databases such as PubMed/Medline, Science Direct, Scopus, and Google Scholar as well as Google were searched using the following keywords: "Zonulin" and "Liver Diseases", "Fatty Liver", "Hepatitis", "Cirrhosis", "Carcinoma", "Liver Steatosis".

Eligibility criteria

All relevant studies published in the English language until May 2022 were eligible in the current study. The present systematic review included all articles that evaluated the role of zonulin in liver diseases. Articles with insufficient information were not included in this study.

Data extraction and quality assessment

Title/abstract of the studies first were retrieved independently by two reviewers for choosing eligible articles. The studies that could not meet the eligibility criteria were excluded. Then, the extraction of data was carried out by reading the full text of the screened articles. In controversial cases, the debate articles were discussed by the authors and finally decided accordingly.

Discussion

As shown in Figure-1, 279 studies were initially identified after our search. After removal of duplicate articles, 181 were retrieved. Of these, 130 studies that did not meet the inclusion criteria of the study were excluded. Then, 51 studies were chosen based on the research topic. Finally, 37 articles were included in the current systematic review after critical analysis. Table-1 displays the main features of the selected studies. As zonulin is associated with liver diseases, changes in the zonulin level considered as outcome.

Free radicals are toxic products of oxygen metabolism that contain at least one unpaired electron. Excessive production of free radicals during a process called oxidative stress causes damage to different parts of the cell such as proteins, DNA and cell membrane [52-58]. These irreversible damages lead to changes in cell function or cell death. Oxidative damage to vital molecules ultimately leads to chronic diseases such as

heart disease, cancer, diabetes, Alzheimer's, Parkinson's, arthritis and infertility (59-64). The human body needs both types of oxidants (free radicals) and antioxidants for normal metabolism, signal transmission, and regulation of cellular activities [65-69]. Zonulin has been identified as the main indicator of intestinal permeability. It is a protein that participates in the regulation of tight junction of the small intestine through increasing its permeability by disconnection of the zonula occludens-1 protein from tight junctions. Zonulin also involves in the development of intestinal innate immunity [30, 44]. Hepatocytes, enterocytes, adipose tissue, and immune cells are known as the main cells which produce zonulin and small intestine is recognized as only site of zonulin performance [31, 44]. Zonulin could affect intestinal permeability by controlling the binding between the intestinal epithelial cells ([46]. Furthermore, this protein participates in the transmission of biomolecules and immune cells between the intestinal lumen and blood [43]. Zonulin also plays role in the inhibition of the entry of wide range of pathogens including bacteria, antigens, toxins, and other pathogens. This is while facilitating the entry of necessary nutrients [30]. It has been shown that increased plasma levels of zonulin is associated with various types of diseases such as celiac disease, autoimmune diseases, type 1diabetes, fatty liver as well as obesity-associated insulin resistance [18, 43].

However, little knowledge is available regarding the association of zonulin levels with liver disorders. Hence, the aim of the present study was to review systematically the current knowledge about the role of zonulin as a prognostic biomarker in liver diseases. To our best knowledge, the present study for the first time investigated the role of zonulin as a prognostic biomarker in liver diseases through a systematic review of published literature. The findings in the present study suggested that the level of circulating zonulin increases in subjects with liver diseases such as NAFLD, cirrhosis, steatosis, fatty liver, hepatitis, HCC, and other types of liver diseases as mentioned above. Over recent years, a significant correlation has been found between the level of zonulin and intestinal leakage related diseases [67]. Researchers have shown that many diseases originate from the gut. This applies more to liver diseases because of the close anatomical relationship between the liver and the gut. In fact, the intestine communicates with the liver through the portal circulation system, and blood first directly enters the liver from the intestines. The liver also affects the intestine by producing bile. On the other hand, the intestines are known as hosts of various species of bacteria called the intestine microbiota, and any changes in these bacteria have been associated with liver diseases such as fatty liver and cirrhosis [41].

Several studies have revealed the association between the level of zonulin and various types of liver diseases including NAFLD [20, 24]. Pacifico L et al. demonstrated that the level of circulating zonulin elevated in children and adolescents with NAFLD. They concluded that this increase in zonulin level was correlated to the severity of hepatic steatosis [24]. Furthermore, it has been shown that the concentration of zonulin increased in obese subjects which was associated with gut microbiota and total bacteria count. On the other hand, zonulin level could be related to diet fat percentage and fiber intake in association with daily energy consumption [68]. Obesity and NAFLD are closely related to each other. Obesity could induce insulin resistance and finally accumulate fat in the liver. Additionally, obesity is associated with zonulin level through increased intestinal permeability. A possible mechanism which could explain the association between level of zonulin and NAFLD is changes in the gut microbiota following development of obesity in obese subjects. This process has ability to increase intestinal permeability and finally induce high level of zonulin [46]. In line with the other studies, Hendy et al. showed that circulating zonulin could be propose as potential marker and therapeutic target of NAFLD. They understood that increased circulating IL-6 caused by obesity mediated relationship between insulin sensitivity and circulating zonulin. In fact, production of IL-6 and TNF-α, is the primary cause of liver damage in many liver diseases. Researcher have reported the linkage between concentration of zonulin and inflammatory markers in obese patients. This interrelation mediates the role of increased level of zonulin with various diseases including NAFLD, obesity and diabetes [20]. In addition to NAFLD, increased level of zonulin is also observed in other liver diseases such as cirrhosis. Raparelli et al. demonstrated that the level of zonulin has an elevation in cirrhotic patients compared to healthy subjects. They observed that increased gut permeability and subsequently level of zonulin induced enhancing levels of lipopolysaccharides (LPS) and finally liver injury [33]. In line with above study, Hsu et al. indicated that the level of zonulin increased in cirrhotic patients and was associated with carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1) [34]. In another similar study, Voulgaris TA and colleagues found that high level of zonulin was associated with advanced cirrhosis by the presence of ascites [36].

Emerging data have shown conflicting results about the level of zonulin in different liver diseases. For example, Mustafa Kerem Calgin and colleagues showed that the levels of serum zonulin decreased in chronic Hepatitis-B patients. They explained that the possible mechanisms involved in this reduction in the level of

Type of liver disease	Study Model	Participants			Specimen	Method of analysis	Result	Ref
		Total No.	Male	Female				
NAFLD	In vivo (clinical)	140 children (mean age 11 years) (67 children were affected by NAFLD and 73 were control)	56	84	Sera	ELISA	NAFLD patients had significant higher level of zenolin (4.67±2.02 ng/mL) compared to controls (2.07±0.91 ng/mL) (P=0.0001).	[15]
	In vivo (clinical)	Children with NAFLD (n=28) and a healthy control group (n=30)	38	32	Serum	ELISA	Serum level of zenolin increased significantly in NAFLD children (3.3±2.2 ng/mL) compared to control (2.5±1.1 ng/mL). Furthermore, the results indicated that intervention (long-term synbiotic supplementation) could not affect zonulin level.	[16]
	In vivo (clinical and animal)	37 NAFLD patients and 15 control	29	23	Serum	ELISA	Serum zonulin level were significantly higher in patients with NAFLD than in controls.	[17]
	In vivo (clinical)	56 adults subjects with age ranged from 29 to 46 years	22	34	Serum	ELISA	Serum zonulin were significantly elevated in NAFLD and NASH subjects compared to controls. The level of zonulin was positively correlated with HOMA-IR.	[18]
	In vivo (clinical)	211 patients			Serum	ELISA	The level of zonulin increased in NAFLD patients correlated with serum LPS.	[19]
	In vivo (clinical)	56 adult subjects (age range 29–46 years) and 20 healthy controls (12 females and 8 males)	22	34	Serum	ELISA	A significant elevation in serum zonulin were seen in the NAFLD patients compared to the control group (6.48±2.03 ng/mL Vs. 3.65±1.37 ng/mL) (p < 0.001).	[20]
	In vivo (clinical)	32 participants	22	10	Serum	ELISA	Serum zonulin concentration decreased approximately 90% (7.335±13.492 vs. 0.507±0.762 ng/mL, p = 0.001) and correlated with weight reduction and the amount of dietary fiber intake (p = 0.043).	[21]
	In vivo (clinical)	70 participants with NAFLD	40	30	Serum	ELISA	Serum level of zonulin elevated by increasing fibrosis because of NAFLD but not significantly.	[22]
	In vivo (clinical)	108 patients with NAFLD	-	-	Serum	ELISA	Serum level of 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). Furthermore, zonulin levels were not significantly different between patients with simple fatty liver and those with NASH. Higher level of zonulin was associated with liver necroinflammation.	[23]
	In vivo (clinical)	40 obese children with NAFLD and 40 control	25	15	Serum	ELISA	Zonulin level was significantly higher in obese subjects with NAFLD than in those without NAFLD (4.23 ng/mL Vs. 3.31 ng/mL) (p<0.01).	[24]
	In vivo (clinical)	59 obese adolescents (40 NAFLD diagnosis) and 13 control	45.8%	54.2%	Serum	ELISA	Serum level of zonulin increased significantly in obese adolescents compared to control subjects (91.89±3.12 Vs. 15.96±5.1 ng/ml) (p<0.01).	[25]
	In vivo (animal)	24 high-fat diet mice (three groups: control, high-fat diet and coffee + high-fat diet)	24	-	Duodenum	Quantitative RT-PCR	Gene expression of zonulin-1 decreased in high-fat diet mice compared to control group, but not significantly. Furthermore, gene expression of zonulin-1 increased significantly in coffee + high-fat diet compared to high-fat diet group.	[26]
Alcoholic hepatitis	In vivo (clinical)	163 alcoholic hepatitis patients and 23 non- alcoholic control	75%	25%	Serum	ELISA	Zonulin increased significantly in alcoholic hepatitis patients (3.9-70.5 ng/mL) compared to control (1.4-14.9 ng/mL) (P<0.001).	[27]
Cirrhosis	In vivo (clinical)	58 patients (26 in the probiotic group and 32 in the placebo group)	40	18	Stool	ELISA	Probiotics intervention could significantly decrease significantly in treated group compared to control group.	[28]
	In vivo (clinical)	90 cirrhotic patients (50 patients with cirrhosis on long-term proton pump inhibitortherapy and 40 control patients with cirrhosis without proton pump inhibitor therapy)	77	23	Stool	ELISA	Cirrhotic patients with long-term proton pump inhibitortherapy displayed significant increase in faecal zonulin levels compared to patients without PPI use.	[29]
	In vivo (clinical)	24 adult patients (age range 34-93) and 18 control	12	12	Serum Ascites	ELISA	Cirrhotic patients had lower serum zonulin family peptides compared to controls (30.5 vs. 62.0 ng/mL; p< 0:001).	[30]
	In vivo (clinical)	90 patients with HBV-associated liver cirrhosis and 90 healthy controls	65	25	Serum	ELISA	Serum level of zonulin in liver cirrhosis increased compared to control subjects (0.091 ± 0.028 Vs. 0.065 ± 0.002 ng/ml).	[31]
	In vivo (clinical)	21 patients with NAFLD-related cirrhosis and HCC and 20 patients with NAFLD-related cirrhosis without HCC and 20 healthy controls	30	11	Serum	ELISA	Serum level of zonulin increased significantly in Cirrhosis patients compared to control (10.91 Vs. 7.05 ng/ml) (p<0.0001).	[32]
	In vivo (clinical)	69 cirrhotic patients and 30 controls	68%	32%	Serum	ELISA	Cirrhotic patients had a significant higher level of zonulin compared to control (2.54±0.94 Vs. 1.87±0.84 ng/ml) (p<0.006).	[33]
	In vivo (clinical)	15 cirrhotic patients and 20 healthy controls	4	11	Serum	ELISA	Serum level of zonulin increased significantly in Cirrhosis patients compared to control (7.9±0.76 Vs. 1.8±0.22 ng/ml) (p<0.01).	[34]
	In vivo (clinical)	83 consecutive outpatients with LC and 45 control	54	29	Serum	ELISA	Serum level of zonulin was significantly higher than control group (2.19±3.28 ng/mg protein) (p<0.05).	[35]
	In vivo (clinical)	116 cirrhotic patients	71	45	Serum	ELISA	Mean serum zonulin levels were 3.6 ± 1.5 ng/dL. Patients with CTP-B had significantly higher serum zonulin levels compared to those with CTP-A cirrhosis (4.2± 2.4 ng/dL vs 3.5 ± 0.9 ng/dL, P = 0.038).	[36]
	In vivo (clinical)	49 patients with cirrhosis (16 with PVT (portal vein thrombosis) and 33 without PVT)	34	15	Serum	ELISA	Serum level of zonulin (56.3 ± 31.1 ng/ml vs. 69.3 ± 63.1 ng/ml; P = 0.69) were not different between the patients with and without PVT.	[37]
	In vivo (animal)	85 rats	-	-	Feces	qPCR	Zonulin expression increased in cirrhotic rats compared to control. Treatment with obeticholic acid increased the expression of zonulin significantly.	[38]
	In vivo (clinical)	21 patients and 21 control	16	5	Feces	ELISA	The level of zonulin increased in patients compared to healthy control but not significantly.	[39]
Cholelithiasis	In vivo (clinical)	38 patients with diagnosis of gallstone disease and 14 healthy controls	-	-	Stool	ELISA	There was a significant elevation in the level of zonulin in patients with cholelithiasis before and after treatment with surgery and administration of probiotics compared to control group.	[40]
HBV-Associated Chronic Hepatitis	In vivo (clinical)	90 patients with chronic hepatitis and 90 healthy controls	51	39	Serum	ELISA	Serum zonulin level in chronic hepatitis increased compared to control (0.072 ± 0.025 Vs. 0.065 ± 0.002 ng/ml).	[31]
	In vivo (clinical)	30 CHB patients and 17 controls	27	20	Serum	ELISA	The level of serum zonulin was significantly low in CHB patients compared to controls (p<0.001).	[41]
Hepatocellular Carcinoma	In vivo (clinical)	90 patients with HBV-associated hepatocellular carcinoma and 90 healthy controls	45	45	Serum	ELISA	Serum zonulin level in HCC was increased significantly compared to control (0.250 ± 0.108 Vs. 0.065 ± 0.002 ng/ml).	[31]
Liver steatosis	In vivo (clinical)	Twenty-seven obese individuals with mean age 44 years	13	14	Stool	ELISA	Zonulin concentration elevated in patients with steatosis grade 2 and 3 compared to patients without steatosis. Weight reduction decreased zonulin level but not significantly.	[42]
Intrahepatic cholestasis of pregnancy (ICP)	In vivo (clinical)	88 pregnant women (44 patients with ICP and 44 controls)	-	44	Serum	ELISA	Zonulin level increased significantly compared to controls (1.303 ± 0.63 ng/mL vs. 0.728 ± 0.520 ng/mL, p<.001).	[43]
	In vivo (clinical)	88 pregnant women (mean age 26.5 years)	-	88	Sera	ELISA	The plasma zonulin levels in ICP (12.11 ng/mL) were higher than those of the healthy pregnant women (4.77 ng/mL) (p < 0.001).	[44]
Primary sclerosing cholangitis (PSC)	In vivo (clinical)	166 PSC patients and 100 healthy controls	132	34	Serum	ELISA	Zonulin was significantly lower in the patients compared to controls (32 ng/mL vs. 34 ng/mL, p=0.008).	[45]
Fatty liver disease	In vivo (clinical)	140 obese men (mean age=30-60 years, BMI>25 kg/m^2, waist circumference of >90 cm)	140	-	Plasma	ELISA	Zonulin concentrations (ng/mL) increased significantly in the normal, mild fatty liver, and moderate-to-severe fatty liver groups as 0.618, 2.143, and 5.815, respectively (P<0.001).	[46]
Chronic hepatitis (CH)	In vivo (clinical)	Twenty-six patients with chronic liver diseases due to hepatitis C virus (HCV) and hepatitis B virus (HBV)	-	-	Plasma	ELISA	Zonulin level was significantly lower in CH patients in comparison with controls (p<0.001). Furthermore, the concentration of Zonulin were significantly lower in patients with liver cirrhosis in comparison with CH and normal controls (p<0.001).	[47]
Biliary tract infection	In vivo (clinical)	51 patients with BTI and 35 healthy volunteers (controls)	27	24	Serum	ELISA	A non-significant reduction in the serum level of zonulin of patients (9.5 ng/ml) was observed compared to control (10.5 ng/ml) (p=0.274).	[48]
Secondary Sclerosing Cholangitis (SC-CIP)	In vivo (clinical)	18 SC-CIP patients and 21 healthy controls	13	5	Feces	ELISA	Zonulin increased in SC-CIP compared to healthy control group but not significantly.	[39]
	In vivo (clinical)	10 patients with SSC and 21 healthy controls			Stool	ELISA	Fecal zonulin level increased significantly in SSC patients (Median = 289.05 ng/ml; p = 0.048) compared to healthy controls (66.43 ng/ml).	[49]
Alcohol-Related Liver Disease (ALD)	In vivo (clinical)	37 ALD patient and 17 controls	26	11	Serum	ELISA	The serum zonulin decreased in patients with ALD. Zonulin level increased after alcohol withdrawal.	[50]
Autoimmune hepatitis (AH)	-	26 patients and 170 control	-	-	Serum	ELISA	The mean zonulin serum levels in controls was 3.2pg/mg. The serum level of zonulin was 48.5 pg/mg in AH patients.	[51]
Primary biliary cirrhosis (PBC)	-	26 patients and 170 control	-	-	Serum	ELISA	The mean zonulin serum levels in controls was 3.2pg/mg. The serum level of zonulin was 30.7 pg/mg in PBC patients.	[51]

Table 1: Summary of available literature on the role of zonulin in liver diseases.

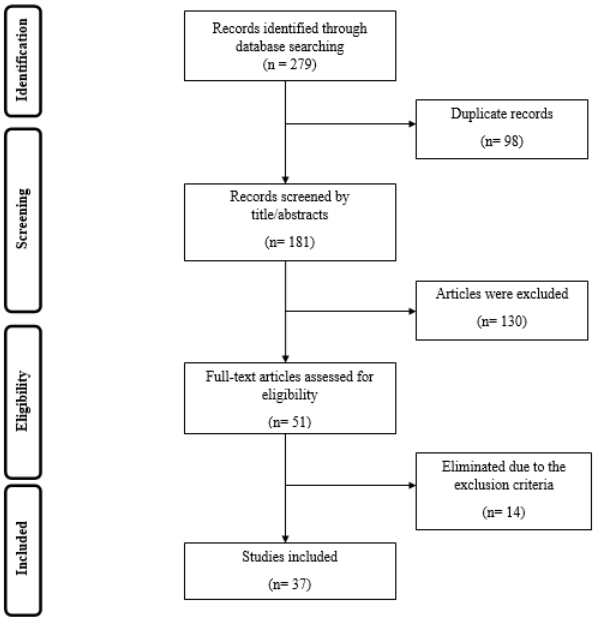

Figure 1: Flowchart of study selection based on PRISMA statements.

zonulin may be associated with intestinal microbiota and viral load in hepatitis patients [41]. This is while an increase in the level of zonulin has been observed in many liver diseases, including biliary tract infection, and cholangitis [48, 49]. Increasing evidence shows that plasma zonulin levels increased in type 1 diabetes [69]. It has been reported that there is a link between zonulin and diabetes in liver disease. The linkage between serum levels of zonulin and diabetes in liver disease could be explain through the association between diabetes and obesity, obesity and alternations in gut microbiome and lately to NAFLD [20]. It seems that the lack of studies on the role of zonulin in liver diseases has caused such contradictory data. The main strength of the current study was that it reviewed the role of zonulin in liver diseases for the first time. The main limitation of the current study was heterogeneity and contradictions in various reports.

Conclusion

Taking together, our findings indicate that changes in the level of zonulin are associated with intestinal permeability. Furthermore, we concluded that zonulin as the main biomarker of intestinal permeability increased in various types of liver diseases. Our results revealed that enhanced level of zonulin as a result of increased intestinal permeability released numerous pathogens, antigens and toxic metals from intestine to liver which trigger immune cells and cytokines and eventually inflammatory responses and subsequently tissue damage in the liver. In conclusion we showed that zonulin could propose as a reliable biomarker associated with liver injury and even a therapeutic marker. However, due to the lack of about the role of zonulin in liver diseases further research could improve information.

Competing Interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

Author Contributions

All the authors contributed equally to the writing of the manuscript.

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