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Serum Zonulin and Growth Differentiation Factor-15 Levels Support Link between Coronary Artery Disease: The Liver-Heart Axis

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Abstract
Objectives: This study aimed to evaluate associations between serum zonulin and GSF-15 levels in coronary arteries with NAFLD patients and to study their correlations with different parameters.
Methods: In this case-control study, 90 subjects were collected, consisting of 45 coronary artery disease with non-alcoholic fatty liver (grade one) patients and 45 controls, clinical demographic information, including family history of coronary disease, lifestyle factors including hypertension and diabetes, and certain blood parameters data (lipid profile, liver functions test).
Results: The patients' mean age was 55.64 ± 8.462 years. Among all participants, 64 (71.1%) were male and 26 (28.9%) female. Zonulin, GDF-15, triglycerides, ALT, AST, ALP, and LDL levels were significantly higher in the patients ($P \leq 0.001$, $P \leq 0.001$, $P = 0.022$, $P \leq 0.001$, $P = 0.035$, $P \leq 0.001$, $P \leq 0.001$, respectively). In comparison to the controls, HDL and total cholesterol were considerably higher ($P \leq 0.001$ and $P \leq 0.001$).
Conclusion: Patients who suffer from non-alcoholic fatty liver disease and coronary arteries had higher levels of zonulin and GDF-15.
Keywords: Zonulin, GDF-15, Coronary Artery Disease, Non-Alcoholic Fatty Liver

Introduction

Coronary artery disease (CAD) is one of the most prevalent cardiovascular diseases affecting people globally. According to research, both in industrialized and developing nations, this condition is the primary factor in deaths. Cardiovascular disease development is influenced by environmental, genetic, and lifestyle factors.[1] A stenotic condition of the right coronary artery (RCA), left circumflex artery (LCX), or left anterior descending artery (LAD) is a sign of coronary artery disease (CAD).[2]

The primary cause of CVD is atherosclerosis. It happens when plaque builds up on the inner wall of a blood vessel and blocks the vessel. Plaque develops as a result of the accumulation of fatty substances and cellular waste, cholesterol, calcium, and fibrin. The main causes of atherosclerosis include elevated cholesterol levels, diabetes, hypertension, and smoking. Stress and sleep apnea can also lead to cardiovascular problems. In CAD, plaque that has been accumulated along the arteries blocks the flow of oxygen-rich blood. Blood clots develop upon plaque rupture and result in thrombosis. Myocardial infarction (MI) results from it.[3]

Around 30% of adults worldwide suffer from non-alcoholic fatty liver disease (NAFLD), which significantly affects morbidity and death associated to extrahepatic and liver disease. The leading cause of death for those with NAFLD is cardiovascular disease, followed by problems connected to the liver and extrahepatic malignancies.[4] In the absence of secondary causes of hepatic steatosis caused by considerable alcohol use, nonalcoholic fatty liver disease (NAFLD) is determined by the fat buildup in 5% of hepatocytes. The range of NAFLD, histologically speaking, starts with simple steatosis (SS), which Some individuals may experience the progression of cirrhosis, severe fibrosis, non-alcoholic steatohepatitis (NASH), hepatocellular carcinoma (HCC), and liver failure.[5]

Zonulin, a prehaptoglobulin protein, is a modulator of intercellular tight junctions and was first reported as a key regulator of intestinal permeability by disrupting tight junctions. It has been suggested that zonulin plays a part in the etiology of a number of chronic inflammatory disorders, including autoimmune, metabolic, and allergy conditions. Previous studies have demonstrated that an increase in serum zonulin level is associated with obesity, hyperlipidemia, and atopic dermatitis.[6]

A member of the transforming growth factor (TGF) (cytokine) superfamily, growth differentiation factor 15 (GDF-15), often referred to as macrophage inhibitory factor-1, is a cytokine. According to research, GDF-15 is a stress-responsive cytokine that is only infrequently expressed in cardiac tissues and only significantly expressed in the placenta and prostate under physiologically normal circumstances.[7] GDF15 is present as a 25 kDa dimer connected by a single disulfide connection between the chains. A signal peptide, propeptide, and mature peptide are used in its synthesis to create the 308a a peptide.[8] Increased GDF-15 has been shown to support angiogenesis and inflammation, the physiological and pathological involvement of the GDF-15 system in atherosclerosis and CAD is supported by clinical and experimental research.[9]

Materials and Methods

The present research included a case-control study for a group of 90 samples: (in which 45 patient samples of coronary artery with non-alcoholic fatty liver), and another 45 samples as apparently healthy control people matched the patients in sex and age. The work was started in September 2022 and finished in May 2023. Patient samples were collected from Kerbala Heart Center at Al-Hussein medical city. The diagnosis of chronic coronary artery disease was identified based on signs

and symptoms, evaluation of echo, CCTA, and Catheterization. The presence of fatty liver disease was confirmed by abdominal ultrasound. The World Medical Association Declaration of Helsinki guided the conduct of this investigation. It has been approved by the Kerbala University/College of Medicine's ethical committee. Participants' informed consent was gained when the study's outline was explained to them.

A sterile disposable syringe was used to draw five milliliters of venous blood from volunteers who were fasting. For clotting, blood it was put in a gel tube and kept at room temperature for roughly 5 minutes, then separated into four Eppendorf tubes and stored at –20°C in the deep freezer. Two tubes were used to measure serum zonulin and GDF-15 by using the enzyme-linked immune sorbent assay (ELISA) technique, one tube was used to measure lipid profile, and one tube was to measure liver function test by using the enzymatic colorimetric method.

Zonulin was measured using (Melsin Medical, China) kit, GDF-15 was measured by (Shanghai YL Biont, China) kit, and lipid profile/liver function test was measured using GIESSE Diagnostics kit.

The following equation was used to compute body mass index (BMI):

$$BMI\ (kg/m^2) = weight/(height)^2$$

Patients were divided into three groups based on their BMIs: normal (18.5–24.9), overweight (25–29.9), and obese (30–34.9).[10]

Exclusion Criteria

Patients with the presence of renal diseases, cirrhosis, stroke, skeletal muscle injury, malignancy, and ongoing infectious diseases were excluded from this study. The relationship between illness and biochemical indicators was assessed. The effectiveness of the markers as diagnostic tools was evaluated using receiver operating characteristic (ROC) curves in patient populations.

Results

The descriptive table depicts the adjustment of additional risk factors that were obtained via self-reporting (questionnaire), such as the medical history, lipid profile, and liver function test.

The level of Zonulin was high in the patient compared to the control group, the means in the patient and control were 94.800 and 54.597 respectively. Significantly increased GDF-15 levels have been observed in CAD patients with fatty liver. ($P \leq 0.001$). Once inflammation occurs in the liver, the GDF-15 level begins to increase Table 1.

Table 2 showed the correlation between zonulin and BMI, total cholesterol, triglycerides, HDL, LDL, albumin, AST, ALT, ALP, and total bilirubin in the patient group. there was a strongly significant positive correlation between zonulin and BMI [$r = 0.758$, $P \leq 0.001$], and WC [$r = 0.428$, $P = 0.009$].

Table 3 showed that correlation between GDF-15 and BMI, total cholesterol, triglycerides, HDL, LDL, albumin, AST, ALT, ALP, and total bilirubin in the patient group. Significantly, there was a positive correlation between GDF-15 and BMI [$r = 0.763$, $P \leq 0.001$] and WC [$r = 0.397$, $P = 0.016$], and a weakly significant positive correlation between GDF-15 and AST [$r = 0.369$, $P = 0.013$], and ALT [$r = 0.317$, $P = 0.034$].

Table 1. **Demographic characteristics of the study participants**

Characteristics		Patient Mean ± SD	Control Mean ± SD
Demographic	Age	55.64 ± 8.462	54.06 ± 9.100
	Sex	(32/13)	(32/13)
	BMI	30.011 ± 4.011	25.06 ± 9.100
	WC	109.33 ± 17.505	91.29 ± 7.270
Medical history	Smoking	(11/34)	–
	DM	(23/22)	–
	HT	(25/20)	–
Biochemical parameters	Zonulin, ng/l	94.800 ± 18.355	54.597 ± 5.136
	GDF-15, ng/l	271.269 ± 54.304	185.136 ± 29.470
Lipid profile	Total Cholesterol, mg/dl	119.03 ± 26.278	130.35 ± 19.900
	Triglycerides, mg/dl	131.60 ± 58.531	124.71 ± 34.942
	HDL-C, mg/dl	46.89 ± 3.437	56.76 ± 9.608
	LDL-C, mg/dl	85.702 ± 22.377	65.506 ± 19.709
Liver function test	Albumin, g/dl	4.303 ± 0.223	4.176 ± 0.134
	ALT activity, U/L	45.39 ± 25.795	27.59 ± 11.948
	AST activity, U/L	30.14 ± 13.747	27.35 ± 7.123
	ALP, U/L	210.11 ± 51.205	184.88 ± 38.067
	Total serum bilirubin, mg/dl	0.674 ± 0.388	0.687 ± 0.288

BMI; Body mass index, WC; Waist circumference, DM; Diabetes mellitus, HP; Hypertension, GDF-15; Growth differentiation factor, HDL; High-density lipoprotein, LDL; Low-density lipoprotein, ALT; Alanine aminotransferase, AST; Aspartate aminotransferase, ALP; Alkaline phosphatase.

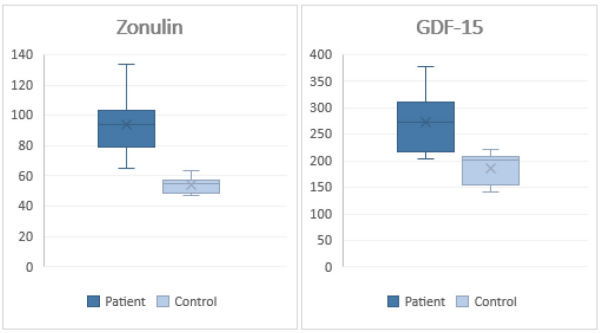

Fig. 1 **Boxplot of the Distribution of serum zonulin and growth differentiation factor in patient and control groups.**

Table 2. **Correlation between zonulin and parameters in patients**

Parameters	Zonulin	
	Coronary artery with fatty liver	
	r	*P*
BMI, kg/m^2	0.758**	<0.001
WC	0.428**	0.009
Total Cholesterol, mg/dl	0.232	0.126
Triglycerides, mg/dl	0.007	0.962
HDL-C, mg/dl	0.1	0.802
LDL-C, mg/dl	0.1	0.145
VLDL-C, mg/dl	0.007	0.962
Albumin, g/dl	0.123	0.474
AST activity, U/L	–0.1	0.606
ALT activity, U/L	–0.127	0.407
ALP activity, U/L	–0.234	0.123
Total bilirubin, mg/dl	0.1	0.661

Table 3. **Correlation between GDF-15 and parameters in patients**

Parameters	GDF-15	
	Coronary artery with fatty liver	
	r	*P*
BMI, kg/m^2	0.763**	≤0.001
WC	0.397	0.016
Total cholesterol, mg/dl	0.108	0.414
Triglycerides, mg/dl	0.218	0.106
HDL-C, mg/dl	–0.140	0.357
LDL-C, mg/dl	0.037	0.807
VLDL-C, mg/dl	0.041	0.788
Albumin, g/dl	0.369*	0.013
AST activity, U/L	0.317*	0.034
ALT activity, U/L	–0.349	0.019
ALP activity, U/L	0.030	0.844

Receiver Operating Characteristics of Diagnostic Markers of CAD and NAFLD

In the patient group, the levels of zonulin and GDF-15 were plotted using ROC curves. The cut-off values and AUC were derived based on the specificity and sensitivity of the test. In the patient group, zonulin had the highest AUC, which was 0.941 [95% CI (confidence interval) = 0.907–0.975], sensitivity % = 0.9, specificity % = 0.9, cut-off points = 69.79.

GDF-15 had a high AUC, which was 0.816 [95% CI (confidence interval) = 0.749–0.884], sensitivity % = 0.6, specificity % = 0.9, cut-off points = 250.605, (Table 4, Figures 2 and 3).

Discussion

According to some publications, there are variations between the sexes in CAD patients because male patients had greater rates of infection. These sex differences are primarily related to social behavior and biological factors. Men's higher smoking prevalence is one of the societal factors that is thought to explain it. Women are often somewhat protected against cardiovascular conditions before menopause, and after menopause, the risk for heart disease in women significantly increases. It has been demonstrated that the deficiency of sex hormones, which occurs with advancing age, has a significant role in the onset of CVD. When compared to males of the same age, premenopausal women have a generally reduced incidence of CVD, which is attributed to estrogen, which is frequently acknowledged for its cardioprotective properties. Males have also been demonstrated to benefit from the cardioprotective effects of estrogen. According to one study, the progressive drop in estrogen levels after puberty makes men 10–15 years more likely than women for developing heart disease.[11]

When compared to women of reproductive age, men have a greater incidence and severity of NAFLD. However, Since NAFLD is more prevalent in women after menopause, estrogen may have preventive effects. There are also gender disparities for the key NAFLD risk factors. In general, animal models of NAFLD replicate the sex differences seen in people, males had higher amounts of proinflammatory/profibrotic cytokines, more severe steatosis and steatohepatitis, and a higher prevalence of liver cancers.[12]

The majority of NAFLD patients have normal or nearly normal LFTs. As fibrosis advances to cirrhosis, the ALT normally decreases (although the AST may increase).[13]

Zonulin levels in CAD patients were found to be considerably greater. According to the findings, increased zonulin in atherosclerosis may enhance intestinal IP and allow bacteria movement from the intestine to the blood. The mechanisms behind bacteria's role in atherosclerosis are multifaceted. Previous research primarily focused on indirect pathways. Microbial activation of these innate immune receptors induces inflammation, which dampens reverse cholesterol transport and hence increases insulin resistance, hyperlipidemia, and vascular inflammation.[14]

Table 4. **AUC, optimal threshold, sensitivity, and specificity of zonulin obtained by the ROC curves**

	AUC	*P*	Sensitivity	Specificity	Cut-off	CI (95%)	Youden index	PPV	VPP	Accuracy
Zonulin	0.941	≤0.001	0.9	0.9	69.79	(0.907–0.975)	0.755	1	0.937	0.966

The current investigation found a link between GDF15 plasma level and NAFLD observed that the degree of change in GDF15 corresponded to the degree of change in intrahepatic fat concentration over time supports the concept that GDF15 production may increase with an increase in lipid synthesis in the liver.[15] In GDF15 concentrations were shown to be higher in the liver of a mouse model with enhanced lipid synthesis.[16] The analysis by the experts found that GDF15 may have a protective impact against the inflammatory insult caused by viruses and bacteria, and they came to the conclusion that GDF15 attenuates the inflammatory response by modifying lipid metabolism.

Furthermore, Later studies have demonstrated that GDF15 expression in the liver can significantly boost hepatocytes' ability to beta-oxidation fatty acids and undergo ketogenesis., thus protecting against hepatic steatosis and inflammation.[17,18]

When pro-inflammatory cytokines including interleukin 1, interleukin 2, and macrophage colony-stimulating factor are present, activated macrophages such as those in the body's immune system create GDF-15.[19] It helps to prevent inflammation and the final phases of macrophage activation. These results suggested that it might be connected to long-term inflammatory diseases. Although GDF-15's impact on CAD is unknown, the circulation amount of GDF-15 has been seen to increase quickly in response to cardiovascular injury, such as pressure overload, heart failure, ischemia/reperfusion, and atherosclerosis.[20] Indeed, greater plasma GDF-15 levels have been documented in patients with cardiovascular diseases such as CAD in various studies.

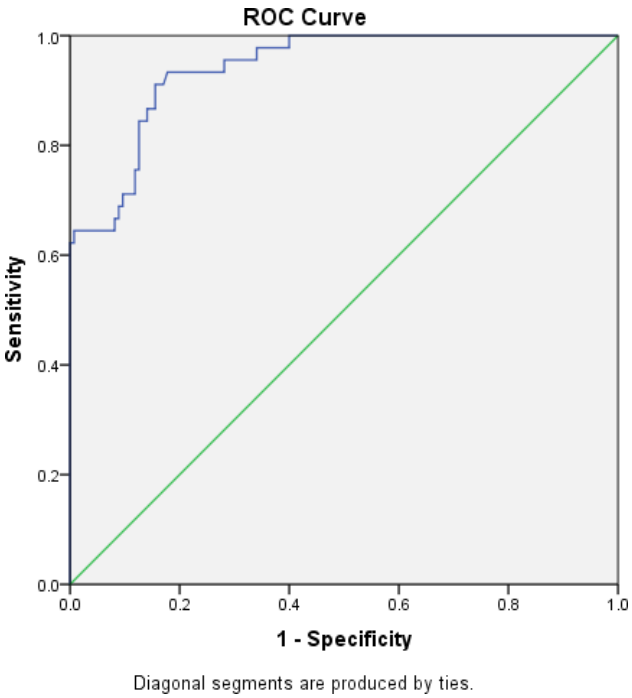

Fig. 2 **Receiver operating characteristic (ROC) of zonulin.**

Statistical Analysis

Statistics were applied to the data from each group using descriptive methods. Scale variables were represented by mean and standard deviation, whilst values for categorical variables were expressed as *n* (%). The Shapiro-Wilk test was employed to determine whether the data distribution was normal.

The univariate analysis for anomalous distributions was carried out by an independent Kruskal Wallis Test for data that is continuous. The Pearson rank test was used to compare biomarkers in order to determine the association within the case study.

Significant differences in the parameters in categorical variables were found by analytical statistical studies. A *P*-value of 0.05 (two-sided) or above was considered statistically significant for all hypothesis tests.

Using receiver operating characteristic (ROC) analysis, an optimal threshold with high specificity and sensitivity for crucial instances were identified. It was discovered that all *P* values were two-sided, and a *P* value of 0.05 was considered statistically significant.

Conclusion

Increased zonulin and GDF-15 levels were associated with coronary arteries and non-alcoholic fatty liver patients. Zonulin may be used as a useful marker for diagnosis of coronary artery with fatty liver.

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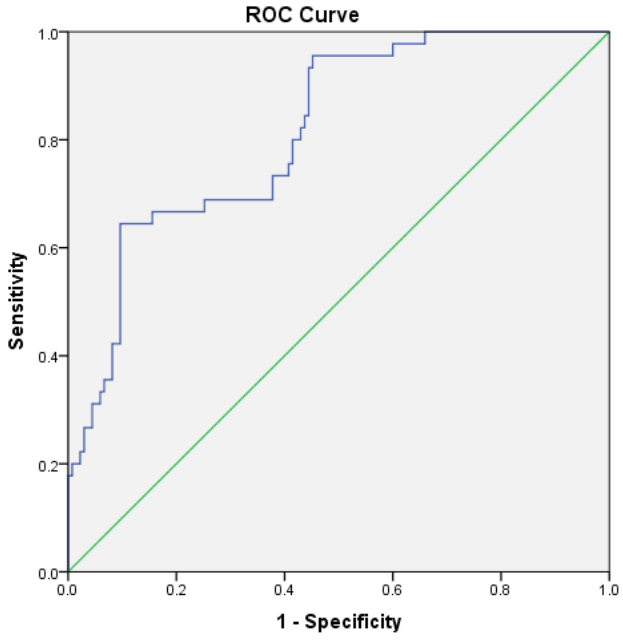

Fig. 3 **Receiver operating characteristic (ROC) of GDF-15.**

Table 5. **AUC, optimal threshold, sensitivity, and specificity of GDF-15 obtained by the ROC curves**

	AUC	*P*	Sensitivity	Specificity	Cut-off	CI (95%)	Youden index	PPV	NPV	Accuracy
GDF-15	0.816	≤0.001	0.6	0.9	250.605	(0.749–0.884)	0.548	0.878	0.719	0.777

(Al-Hussein Medical City) in Kerbala, Iraq. This study would not have been done without the patient's cooperation and aid in obtaining the sample.

Declaration of Interest

There are no potential biases or other competing interests that need to be disclosed by the authors.

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