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ORIGINAL ARTICLE

Evaluation of Serum Levels of Adiponectin, Vitamin D3, Zonulin, and Irisin in Non-Alcoholic Fatty Liver Disease: A Case-Control Study from Nasiriyah Province, Iraq

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KEYWORDS

Non-alcoholic fatty liver disease (NAFLD);
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ABSTRACT: Non-alcoholic fatty liver disease is increasingly recognized as a widespread metabolic abnormality and a leading contributor to insulin resistance. Adiponectin, an adipose tissue-specific adipokine, plays a crucial role in metabolic regulation. Zonulin, a liver-secreted protein, modulates intestinal permeability, while irisin, a hormone produced by skeletal muscle and adipocytes, is implicated in energy homeostasis. This study aimed to evaluate serum levels of vitamin D3, zonulin, irisin, and adiponectin in patients with NAFLD to elucidate their roles in disease pathogenesis and compare findings with a healthy control group. A total of 44 patients with a confirmed diagnosis of NAFLD and 44 apparently healthy controls were recruited. Blood Samples (5 mL) were obtained, and the serum was isolated via centrifugation, then preserved at −80°C for subsequent analysis. Serum levels of irisin, zonulin, vitamin D3, and adiponectin were measured using the ELISA technique: Adiponectin ELISA Kit (Elabscience, USA) and Vitamin D Kit (Sigma-Aldrich, USA). Liver function markers, including ALT and AST, were assessed using photometric methods (ALT and AST kits, LINEAR, Spain). Findings revealed a marked decrease in circulating levels of vitamin D3 in NAFLD patients (13.32±2.90 m m^{-1}) compared to controls (28.14±4.71 m m^{-1}) ($P < 0.05$). Conversely, serum levels of adiponectin, irisin, and zonulin were significantly elevated in NAFLD patients (3.12±1.22 m m^{-1}, 387±77.43 m m^{-1}, 66.42±4.78 m m^{-1}, respectively) compared to controls (1.40±0.91 µg mL^{-1}; 74.34±14.11 ng mL^{-1}; 55.66±5.61 m m^{-1}, respectively) ($P < 0.05$). Additionally, serum ALP levels were significantly higher in NAFLD patients (40±3.28 U L^{-1}) than in controls (32.88±2.56 U L^{-1}) ($P < 0.05$). These findings suggest that adiponectin, vitamin D3, irisin, and zonulin may play critical roles in NAFLD pathogenesis. Alterations in their serum levels could play a key role in unraveling disease mechanisms and facilitating the development of targeted therapies.

INTRODUCTION

NAFLD ranks among the most common liver diseases globally and is frequently linked to diverse metabolic abnormalities, including dyslipidemia, obesity, type 2 diabetes, insulin resistance, hypertension, and cardiovascular diseases (CVD) [1,2]. The disease is marked by the build-up of fat in liver cells unrelated to alcohol use and may evolve into NASH, fibrosis, cirrhosis, and hepatocellular carcinoma [3]. Several factors contribute to NAFLD development, including inflammation, oxidative stress, increased gut

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permeability, and metabolic hormone imbalances [4].

Zonulin, a key regulator of intestinal barrier function, is produced in response to changes in the gut microbiota and increased mucosal permeability [5]. Secreted by both the liver and the intestine, zonulin binds to receptors on enterocytes in the ileum and jejunum, modulating tight junction integrity between intestinal epithelial cells [6]. Elevated serum zonulin levels have been reported in NAFLD patients, with studies indicating a positive correlation between zonulin levels, body mass index (BMI), and obesity [7]. Consequently, zonulin has been proposed as a potential biomarker for diagnosing and predicting NAFLD severity [8].

Irisin, a glycosylated protein hormone secreted by muscles in response to physical activity, plays a crucial role in converting white adipose tissue into brown adipose tissue, increasing energy expenditure, and improving insulin sensitivity [9, 10]. Studies have demonstrated that irisin levels fluctuate in NAFLD patients and may be linked to disease severity and other metabolic parameters [11].

Vitamin D3 is another key regulator of metabolic and immune processes [12]. It possesses anti-inflammatory, antifibrotic, and hepatoprotective properties, potentially preventing NAFLD progression [13]. Several studies have reported vitamin D3 deficiency in individuals with obesity, type 2 diabetes, and hypertension, suggesting that such deficiencies may also be associated with NAFLD severity [14, 15].

Adiponectin helps regulate lipid metabolism by increasing fatty acid oxidation and reducing fat storage. Additionally, the anti-inflammatory potential of this adipokine is primarily attributed to its ability to inhibit pro-inflammatory cytokine production [12, 14]. Zonokin, irisin, and vitamin D3 each play important roles in inflammatory and metabolic processes. Recent studies highlight the significance of vitamin D3 may stimulate the production of irisin, which in turn may have anti-inflammatory effects. Additionally, zonokines are involved in inflammatory responses and may be influenced by both irisin and vitamin D3 [8, 9, and 15].

Simultaneous evaluation of these four biomarkers in Iraqi patients may provide new insights into the diagnosis and management of NAFLD, and this is the innovation of this study. Given the significance of these factors in NAFLD pathogenesis, this study aims to evaluate serum levels of zonulin, adiponectin, vitamin D3, and irisin, along with liver function tests, in Iraqi patients with NAFLD compared to healthy individuals. The findings of this research may enhance our understanding of the underlying mechanisms of NAFLD and aid in identifying novel biomarkers for its diagnosis and monitoring.

MATERIALS AND METHODS

Patients

The presented study is (case-control) involve (88) samples: (44) patients have had nonalcoholic fatty liver disease (NAFLD) and (44) appearantly healthy control matched in regard with the patient's sex and age. The work was started in March, 2024 and finished in Semptember, 2024. Patient samples were collected from private clinics, Nassiyria, Iraq. By using abdominal ultrasonography, fatty liver disease was identified. Five mill (5.0) of blood were taken and Zonulin was measured using (Melsin Medical, China), and lipid profile liver function test was measured by GIESSE Diagnostics kit. All biomarker investigations were carried out in outpatient's lab.s

Statistical analysis

Data from each group was analysed using SPSS version 16. All variables were represented by mean and standard deviation. Statistical significance was defined as a p-value less than 0.05 for all analyses.

RESULTS

In this study, 44 patients with non-alcoholic fatty liver disease (NAFLD) and 44 healthy individuals as the control group were selected. For biochemical analyses, 5 mL of blood was collected from each participant. After serum separation using centrifugation, to ensure stability, the samples were kept at -80°C prior to analysis. Serum concentrations of irisin, zonulin, vitamin D3, and adiponectin were measured using the ELISA method. Specifically, adiponectin levels were assessed using an Adiponectin ELISA kit (Elabscience, USA), while vitamin D levels were determined using a Vitamin D

ELISA kit (Sigma-Aldrich, USA). The evaluation of liver function markers, including ALT and AST, was conducted via photometric methods with the use of enzyme kits (LINEAR, Spain). The results indicated that serum vitamin D3 levels were significantly lower in NAFLD patients compared to healthy individuals, whereas serum concentrations of adiponectin, irisin, and zonulin were markedly elevated in the patient group. Furthermore, the level of alkaline phosphatase (ALP) was significantly higher in NAFLD patients than in the control group. These findings highlight the potential role of these biochemical markers in the pathogenesis of NAFLD and may contribute to a better understanding of disease mechanisms and the development of novel therapeutic strategies.

The results are presented in Tables 1 and 2

Table 1. Some biochemical parameters in NAFLD patients and control.

Parameter	NAFLD	Control	*P* value
Vitamin D3(m m^{-1})	13.32±2.90	28.14±4.71	$P < 0.05$
Adiponectin (m m^{-1})	3.12±1.22	1.40±0.91	$P < 0.05$
Irisin (m m^{-1})	387±77.43	74.34± 14.11	$P < 0.05$
Zonulin (m m^{-1})	66.42±4.78	55.66±5.61	$P < 0.05$

Table 2. Liver function test in NAFLD patients and control.

Liver function test parameter	NAFLD	Control	*P* value
AST, (U/L)	34.21±4.11	31.62±2.98	$P > 0.05$
ALT (U/L)	3.28 ±40	2.56±32.88	$P < 0.05$
ALP	204.62±28.44	198.24±23.58	$P > 0.05$
Total serum bilirubin	0.42± 0.92	0.58± 0.81	$P > 0.05$

According to Table 1, vitamin D3 levels were significantly lower in patients with NAFLD compared to the control group ($P < 0.05$), which may be linked to the actions of vitamin D [17].

Additionally, Table 1 demonstrated a significant increase in adiponectin levels in NAFLD patients compared to the control group (*P* value < 0.05). It has been shown that insulin resistance is modified by adiponectin, ultimately leading to the attenuation of liver inflammation and fibrosis (1).

As shown in Table 1, there was a significant increase in serum irisin levels in NAFLD patients relative to the control group ($P < 0.05$). Since our cohort consisted of obese individuals with NAFLD, and considering that irisin is synthesized in adipose tissues [18, 19], these elevated levels of irisin are positively associated with BMI and obesity [20].

DISCUSSION

Additionally, Table 1 indicated a significant increase in zonulin levels in NAFLD patients relative to the control group ($P < 0.05$). This release of zonulin is linked to an elevation in intestinal permeability, resulting from the degradation of the tight junction protein zonula occludens-1 [21]. Zonulin pathway activation may facilitate innate immunity to variations in the microbiome ecology (bacterial proliferation and/or dysbiosis), serving as a defense mechanism that "flushes out" microorganisms. Increased intestinal permeability causes the passage of bacterial LPS and metabolic materials, ultimately leading to a low-grade inflammatory status [21]. Table (2) showed that most of the parameters of liver function test were normal except serum ALP in which there was significant increase in serum ALP in NAFLD (P value< 0.05**).** Hepatic inflammation and injury of liver lead to increase in ALT hence it used for monitering and assessment of liver function [22-27]. Adiponectin, as an adipokine produced in adipose tissue, plays a significant role in reducing liver inflammation and preventing the progression of liver fibrosis [28]. Therefore, high levels of adiponectin may serve as a biochemical marker for identifying the severity of non-alcoholic fatty liver disease (NAFLD) and predicting its outcomes [29]. Additionally, irisin, a hormone produced in muscle tissue that plays a role in

converting white fat to brown fat, may influence energy metabolism and insulin sensitivity in NAFLD patients [30]. These findings clearly indicate that changes in the levels of irisin and adiponectin can serve as important indicators in the progression of the disease and in evaluating the patients' condition. Zonulin, a protein secreted by the liver that regulates intestinal permeability, has been significantly elevated in NAFLD patients [31]. The oxidative stress plays a significant role in the development and progression of various diseases, including non-alcoholic fatty liver disease, and changes in the serum levels of markers may reflect this [32-37]. This protein may contribute to systemic inflammation and disease progression due to increased intestinal permeability and changes in the gut microbiome. An increase in zonulin levels could be considered a potential marker for identifying and predicting the severity of non-alcoholic fatty liver disease.

CONCLUSIONS

The results of this study can serve as a foundation for future research in evaluating new biochemical parameters in NAFLD and the development of new therapeutic approaches for this disease.

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Conflict of Interest

The authors declare no conflicts of interest that could influence the objectivity of the reported research.

Author Contributions

RA. conducted the experiments. RFA. conceived the study, designed the experiments, and wrote the manuscript. RFA. analyzed the data. RA. contributed reagents, materials, and analysis tools.

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