ORIGINAL RESEARCH

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Clinical Utility of Hepassocin and TXNDC5 in Patients with Non-alcoholic Fatty Liver Disease and/or Type 2 Diabetes

Alkolik Olmayan Yağlı Karaciğer Hastalığı ve/veya Tip 2 Diyabet Hastalarında Hepassosin ve TXNDC5'in Klinik Yararı

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Abstract

Objective: The prevalence of non-alcoholic fatty liver disease (NAFLD) is high both in the general population and in people with type 2 diabetes mellitus (T2DM), while studies on its etiopathogenesis are still ongoing. The aim of this study is to investigate the association between the ultrasound grade of liver steatosis and serum levels of hepassocin (HPS) and thioredoxin domain-containing protein 5 (TXNDC5) in patients with T2DM.

Method: The cross-sectional study included 156 participants who were divided into four groups: isolated NAFLD, isolated T2DM, both NAFLD and T2DM, and healthy controls. The demographic data as well as the physical characteristics, laboratory findings, and ultrasonographic grades of liver steatosis of the participants were evaluated between all groups.

Results: According to ultrasound examinations, HPS values were significantly higher in patients with grade 1 and 2 liver steatosis than in patients without liver steatosis. HPS levels were significantly higher in the vast majority of participants, including healthy controls than in those with isolated T2DM. No significant differences were found between HPS and diabetes. There was no significant correlation between TXNDC5 serum levels and ultrasound results in all groups.

Conclusion: In the present study, our results show that serum HPS levels were higher in individuals with liver steatosis than in individuals without liver steatosis. These results provide further evidence for the association

Öz

Amaç: Alkolik olmayan yağlı karaciğer hastalığının (NAFLD) prevalansı hem genel popülasyonda hem de tip 2 diyabeti (T2DM) olan kişilerde yüksek olmakla birlikte; etiyopatogenezi üzerine çalışmalar halen devam etmektedir. Çalışmamızın amacı, T2DM'li hastalarda karaciğer yağlanmasının ultrasonografik derecesi ile hepassosin (HPS) ve tiyoredoksin domain içeren protein 5 (TXNDC5) serum düzeyleri arasındaki ilişkiyi araştırmaktır.

Yöntem: Bu kesitsel çalışmaya 156 katılımcı dahil edilmiş ve izole NAFLD, izole T2DM, hem NAFLD hem de T2DM ve sağlıklı kontrol olmak üzere dört grup oluşturulmuştur. Katılımcıların demografik verilerinin yanı sıra fiziksel özellikleri, laboratuvar bulguları ve karaciğer yağlanmasının ultrasonografik dereceleri tüm gruplar arasında değerlendirilmiştir.

Bulgular: Ultrasonografik incelemeye göre, 1. ve 2. derece karaciğer yağlanması olan hastalarda HPS seviyeleri, karaciğer yağlanması olmayanlara göre anlamlı derecede yüksekti. HPS seviyeleri, sağlıklı kontroller de dahil olmak üzere katılımcıların büyük çoğunluğunda izole T2DM'li olanlardan anlamlı derecede yüksekti. HPS ile diyabet arasında önemli farklılıklar bulunmamıştır. Ayrıca, tüm gruplarda serum TXNDC5 düzeyleri ile ultrason sonuçları arasında anlamlı bir ilişki bulunmamıştır.

Sonuç: Bu çalışmada, bulgularımız karaciğer yağlanması olanlarda serum HPS düzeylerinin karaciğer yağlanması olmayanlara göre daha yüksek olduğunu göstermektedir. Bu sonuçlar, HPS'nin NAFLD ile

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Abstract

of HPS with NAFLD and expand our understanding of its potential role in the pathogenesis of NAFLD. In addition, our study can be considered one of the first studies in the literature to investigate the association between ultrasonographic hepatic steatosis and serum HPS levels.

Keywords: Hepassocin, non-alcoholic fatty liver disease, TXNDC5, type 2 diabetes mellitus, ultrasonography

Öz

ilişkisi için daha fazla kanıt sağlamakta ve NAFLD patogenezindeki olası rolü hakkındaki anlayışımızı genişletmektedir. Ayrıca, çalışmamız ultrasonografik karaciğer yağlanması ile serum HPS düzeyleri arasındaki ilişkiye dair literatürdeki ilk araştırmalardan biri olarak kabul edilebilir.

Anahtar kelimeler: Alkolik olmayan yağlı karaciğer hastalığı, hepassosin, tip 2 diyabet, TXNDC5, ultrasonografi

Introduction

Non-alcoholic fatty liver disease (NAFLD) is one of the most common causes of liver disease in the world population. NAFLD encompasses a range of damage, from a mildly abnormal accumulation of liver triglycerides to non-alcoholic steatohepatitis (NASH), which can lead to fibrosis and potentially irreversible cirrhosis (1,2). NAFLD is a growing health problem in Western countries, the most common form of liver disease, and the leading cause of end-stage liver disease (3).

NAFLD is associated with various metabolic disorders such as hyperlipidemia, visceral obesity, insulin resistance (IR), type 2 diabetes mellitus (T2DM), and hypertension and is thought to be a manifestation of metabolic syndrome in the liver (4). The study by Chiloiro et al. (5) showed that fatty liver is associated with metabolic syndrome in overweight and obese patients. As a result of these studies, the Asia-Pacific consensus report by Eslam et al. (6) suggested updating the nomenclature from NAFLD to metabolic (dysfunction) associated fatty liver disease. Excessive adiposity is associated with *de novo* lipogenesis in the liver leading to accumulation of triglycerides in the liver and increased lipid flux to the liver (7). NAFLD is also cited as a significant factor in the onset and development of cardiovascular disease and as the first marker for atherosclerosis, particularly in patients with T2DM (8).

The prevalence of T2DM in patients with NAFLD has been reported to be as high as 70%, as T2DM makes the development of NAFLD more likely (9). Since oxidative stress (OS) is critical to the pathogenesis of diabetes and liver disease (10), methods to reduce the effects of OS (11) have been shown to be effective in preserving liver function in diabetes (12). OS and lipotoxicity-induced endoplasmic reticulum (ER) stress are the main causes of liver damage in NAFLD (13). Studies have shown that ER stress is involved in the pathogenesis of various liver diseases such as NAFLD, viral hepatitis, and cirrhosis (14).

Hepassocin (HPS), also called hepatocyte-derived fibrinogen-related protein and fibrinogen-like 1, is a hepatokine and a liver-specific expression gene that has DNA synthesis-stimulating activity in hepatocytes and plays an important role in the regulation of hepatocyte proliferation (15,16). HPS has been shown to be a strong regulator of liver cell growth not only in rats but also in humans (15). HPS expression is reduced in patients with hepatocellular carcinoma (17). A recent study has revealed a causal relationship between HPS and NAFLD. According to this study, HPS plays a crucial role in NAFLD and causes the accumulation of liver lipids via an ERK1/2-dependent pathway (18). Although the functions of HPS have been established in patients with liver failure and hepatocellular carcinoma, its role in NAFLD is still unknown and several clinical studies are needed.

Thioredoxin domain holding protein 5 (TXNDC5), also called resident endoplasmic reticulum 46 (Erp46), 15th member of protein disulfide isomerase family A, thioredoxin-related protein in the cytoplasm or endo-PDI, is a protein disulfide isomerase (19). TXNDC5, which is affected by liver fat and associated with the regulation of ER stress, is thought to contribute significantly to the control of apolipoprotein B and the development of steatosis (20). Under these conditions, high fatty acid levels, calcium deficiency, and IR have been observed to cause damage to ER homeostasis through improperly packaged proteins and OS (20). There is ample evidence that dysregulation of its expression can cause cell aging, oxidative stress, and many pathological conditions such as arthritis, cancer, diabetes, vitiligo, and viral infections. Altered expression in pancreatic cells could alter insulin folding and adiponectin response, which could be a new etiology of diabetes (19).

In the present study, we investigated serum levels of HPS and TXNDC5 in patients with isolated NAFLD, with isolated diabetes, and with NAFLD and T2DM compared to healthy controls. A further aim was to compare the levels of these markers with biochemical, metabolic, and anthropometric

parameters associated with NAFLD and T2DM and with the ultrasonographic grade of liver steatosis.

Materials and Methods

Patients and Study Design

In our study, patients were selected in the following order: To determine whether liver fat was present, a hepatobiliary ultrasound was performed to identify those who were admitted to the radiology department for various reasons. Subsequently, patients with and without hepatic steatosis, patients previously diagnosed with T2DM, and those found to be at risk of T2DM were referred to the internal medicine and diabetes outpatient clinic to be screened for the study. Eligible participants were screened for T2DM according to the American Diabetes Association diagnostic criteria (fasting blood glucose ≥126 mg/dL, 2-hour postprandial glucose ≥200 mg/dL after the 75 g oral glucose tolerance test, or HbA1c ≥6.5%) (21). All participants read and signed a written informed consent, which was added to the study.

The age of the study participants was between 18 to 65 years, with no ethnic difference between the patients and the control group. Physical examination findings, age, gender, and anthropometric measurements [body mass index (BMI), waist circumference (WC), hip circumference (HC)]; cardiovascular risk factors including family history, hypertension, dyslipidemia, diabetes, hyperuricemia, and ongoing medication use were recorded.

The inclusion criteria and the division of the groups were defined as follows: Group 1, patients with isolated NAFLD; Group 2, patients with isolated T2DM without liver steatosis; Group 3, patients with both NAFLD and T2DM; and Group 4, healthy controls. Patients with one or more of the following conditions that may affect metabolic parameters were excluded from the study: Hyper-hypothyroidism, renal failure, hepatic failure, heart failure, alcoholism, malignancies, pregnancy, pancreatic disease, and medications associated with steatohepatitis (corticosteroids, valproic acid, amiodarone, estrogen, tamoxifen, diltiazem, etc.). Serologic tests showed that the patients had no active or chronic viral hepatitis.

Laboratory Measurement

Serum concentrations of HPS/FGL1 and TXNDC5 were assayed in duplicate using enzyme-linked immunoassay kits (Hepassocin: catalog no: SED022Hu, Cloud-Clone Corp., TX, USA; TXNDC5, catalog no: SRB-T-86174, Shanghai Sunred Biological Technology Co., Ltd., PRC) according to the manufacturer's protocol. The intra- and inter-assay coefficients of variation were <10% and <12% for HPS (detection range 0.156-10 ng/mL and sensitivity 0.071 ng/mL), respectively. The intra- and inter-assay coefficients of variation were <10% and <12%, respectively, for TXNDC5 (detection range 0.2-30 ng/mL and sensitivity 0.136 ng/mL). To obtain a standard optical density (OD) curve for the concentration of both markers (HPS and TXNDC5), we introduced samples, standard samples, and biotin-labeled antibodies into micropores pre-coated with both marker antibodies. Subsequently, the OD values of the standard samples and samples were measured using a microplate spectrophotometer (Smart Microplate Reader; USCN KIT INC.) at a wavelength of 450 nm. The concentrations of HPS and TXNDC5 were then determined by comparing the OD values of the samples with the standard curve.

Radiological Measurement

The same radiologist at the hospital's radiology clinic performed the hepatobiliary ultrasound examination of the patients using a grayscale ultrasound machine (Mindray DC-7) and a convex multihertz probe that can vary between 3.3-5 mHz. The grade of liver steatosis in all participants was determined after 10 hours of fasting based on the increase in echo in the liver parenchyma and the ultrasound image of liver fat infiltration. The degree of ultrasonographic adiposity was classified as follows: Grade 0: patients without adiposity; Grade 1: minimal diffuse increase in liver echogenicity (mild adiposity); Grade 2: moderate increase in liver echogenicity (moderate adiposity); Grade 3: significant increase (severe) in liver echogenicity as inability of sound to penetrate the posterior part of the right lobe of the liver or inability to see hepatic vessels and diaphragm (severe adiposity).

Ethics Issues

Before to conducting the study, ethical approval was obtained from the Clinical Research Ethics Committee of University of Health Sciences Turkey, İstanbul Bağcılar Training and Research Hospital with protocol number date: IRB number: 2019.03.3.05.034 (March 29, 2019). All participants acknowledged and signed their written informed consent. The authors declare that there are no potential conflicts of interest relevant to this article.

Statistical Analysis

Number Cruncher Statistical System 2007 (Kaysville, UT, USA) was preferred for the statistical analysis. Descriptive statistical methods and the data distribution were analyzed using the Shapiro-Wilk test. The Kruskal-Wallis test was used to compare the quantitative data of more than two

groups, and the Mann-Whitney U test was preferred to compare two groups. The chi-square test was used to determine the relationship between qualitative data. Evaluation of the significance was carried out at the levels of p<0.01 and p<0.05.

Results

Participants' Characteristics

One hundred fifty-six participants were included in the study [48.7% (n=76) male and 51.3% (n=80) female]. The distribution of the study groups was determined as 24.4% (n=38) Group 1; 25% (n=39) Group 2; 25.6% (n=40) Group 3 and 25% (n=39) Group 4. According to ultrasound findings were found to have 50% (n=78) no liver steatosis (grade 0) (39 from group 2 and 37 from group 4), 26.9% (n=42) mild liver steatosis (grade 1) (25 from group 1 and 17 from group 3) and 23.1% (n=36) moderate liver steatosis (grade 2) (13 from group 1 and 23 from group 3) (Table 1). There was no patient with grade 3 ultrasonographic liver steatosis.

Comparison of Demographic Features, Anthropometric Measurements, and Physical Examination Findings

When comparing the age distribution of the study groups, the mean age of the controls was lower than the other groups (p=0.001) (Table 2).

The BMI, WC, HC, and WC/HC ratio (WHR) values in the control group were found to be significantly lower than all other groups (p=0.001), while the BMI, WC, and HC values of group 3 were significantly higher than those of group 1 and group 2 (p=0.001) (Table 2). In terms of systolic blood pressure values, the values of the control group were significantly lower than those of Group 2 and Group 3, and the values of Group 1 were lower than those of Group 2 (p=0.001).

Comparison of Laboratory Findings

Serum HPS levels of the groups showed statistically significant differences (p=0.001). The HPS levels of group 1, group 3, and group 4 were higher than those of group 2 (p=0.001). There was no significant difference in TXNDC5 serum levels between the groups (p=0.109) (Table 3).

The liver-related enzymes [aspartate transaminase (AST), alanine transaminase (ALT), gamma-glutamyl transferase (GGT), except alkaline phosphatase (ALP)] of the groups containing NAFLD were significantly higher than those without it, and co-existing diabetes increased this (p=0.002, p=0.001, p=0.001 and p=0.507; respectively). The AST/ALT ratio of the control group was higher than that of all other groups (p=0.001) (Table 3).

Comparison of the Patient Groups in Terms of the Use of Antidiabetic Drugs

A significant relationship was detected between the use of antidiabetic drugs in the study groups (p=0.001). Besides, the antidiabetic drug users were found to be statistically significantly lower than the non-users in group 3 (p=0.001). It also indicated to be statistically significant that the

Table 1. Distribution of participants according to ultrasonographic grades of the liver steatosis

	Isolated NAFLD (Group 1)	Isolated T2DM (Group 2)	NAFLD + T2DM (Group 3)	Controls (Group 4)
Grade 0 (n=78)	0	39	0	37
Grade 1 (n=42)	25	0	17	0
Grade 2 (n=36)	13	0	23	0

NAFLD: Non-alcoholic fatty liver disease, T2DM: Type 2 diabetes mellitus

Table 2. Comparison of age and anthropometric measurements according to study groups

	Isolated NAFLD (Group 1) n=38	Isolated T2DM (Group 2) n=39	NAFLD + T2DM (Group 3) n=40	Controls (Group 4) n=39	p
			Mean ± SD		
Age	38.82±11.3	50.18±9.08	47.83±9.43	30.38±9.5	**0.001****
BMI	31.68±6.02	28.36±4.53	33.18±4.58	26.12±4.98	**0.001****
WC	94.42±10.52	94.48±9.03	98.69±8.04	82.08±12.38	**0.001****
HP	105.42±13.43	100.48±9.18	108.74±8.15	96.44±10.5	**0.001****
WHR	0.9±0.08	0.95±0.1	0.91±0.07	0.85±0.09	**0.001****

SD: Standard deviation, NAFLD: Non-alcoholic fatty liver disease, T2DM: Type 2 diabetes mellitus, BMI: Body mass index, WC: Waist circumference, HC: Hip circumference, WHR: Waist/hip ratio, ANOVA test, *p<0.05, **p<0.01

antidiabetic drug users were higher than non-users in Group 2 (p=0.001) (Table 4).

Comparison of Anthropometric Measurements According to the Ultrasound Results

As the grade of liver steatosis increased, the BMI, WC, and HC values also significantly increased (p=0.001). In line with the ultrasound results, age was not significantly different within all ultrasound patterns (p=0.314) (Table 5).

Comparison of Laboratory Findings Based on the Ultrasound Results

According to the ultrasound results, serum HPS levels demonstrated a significant difference (p=0.002). The serum HPS levels of those without liver steatosis (grade 0) were significantly lower than those with grades 1 and 2 liver steatosis (p=0.001). In line with the ultrasound results, serum TXNDC5 levels were not significantly different within all ultrasound patterns (p=0.154) (Table 6).

In patients with liver steatosis (grade 1-2), serum levels of AST, ALT, and AST/ALT ratio were significantly higher than those without it (p=0.001, p=0.001, and p=0.018; respectively) (Table 6).

Discussion

In the present study, we found that HPS levels are significantly higher in patients with fatty liver. There was no significant difference in TXNDC5 serum levels between the ultrasound groups. The liver-related enzymes (AST, ALT, GGT), BMI, WC and HC levels of the groups with hepatic

Table 3. Comparison of laboratory measurements by study groups

	Isolated NAFLD (Group 1) n=38	Isolated T2DM (Group 2) n=39	NAFLD + T2DM (Group 3) n=40	Controls (Group 4) n=39	p
			Min-max (median) or mean ± SD		
Hepassocin	0.79-4.91 (2.09)	0.16-8.99 (1.18)	0.42-9.85 (2.08)	0.16-4.01 (1.69)	**0.001**[a]
TXNDC5	0.63-18.09 (0.79)	0.54-16.82 (0.88)	0.43-16 (0.77)	0.4-15.9 (1.44)	0.109[a]
AST	12-50 (23)	9-75 (18)	12-52 (23)	13-61 (20)	**0.002**[a]
ALT	7-100 (27)	9-109 (17)	11-72 (26)	7-67 (17)	**0.001**[a]
AST/ALT ratio	0.97±0.38	0.95±0.46	0.88±0.36	1.26±0.5	**0.001**[b]
ALP	73.74±18.83	83±39.63	79.78±25.49	77.68±16.86	0.507[b]

NAFLD: Non-alcoholic fatty liver disease, T2DM: Type 2 diabetes mellitus, TXNDC5: Thioredoxin domain containing protein 5, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, SD: Standard deviation, ALP: Alkaline phosphatase, [a]Kruskall-Wallis test (min-max/median), [b]ANOVA test (mean ± SD), *p<0.05, **p<0.01

Table 4. Comparison of the use of antidiabetic drugs according to study groups

	Isolated NAFLD (Group 1) n=38	Isolated T2DM (Group 2) n=39	NAFLD + T2DM (Group 3) n=40	Controls (Group 4) n=39	p
Antidiabetic drug use					
Yes	0	23 (59%)	15 (37.5%)	0	**0.001****
No	38 (100%)	16 (41%)	25 (62.5%)	39 (100%)	

Chi-square test, **p<0.01, NAFLD: Non-alcoholic fatty liver disease, T2DM: Type 2 diabetes mellitus

Table 5. Comparison of age and anthropometric measurements according to the ultrasonographic grades of the liver steatosis

	Grade 0 liver steatosis n=78	Grade 1 liver steatosis n=42	Grade 2 liver steatosis n=36	p
		Mean ± SD		
Age	40.28±13.58	43±10.57	43.94±12.16	0.314
BMI	27.24±4.85	31.01±5.02	33.82±5.47	**0.001****
WC	87.82±12.53	94.29±9.96	99.57±8.11	**0.001****
HP	98.31±10.04	105.18±10.47	109.6±11.49	**0.001****
WHR	0.89±0.11	0.9±0.08	0.91±0.08	0.700

BMI: Body mass index, SD: Standard deviation, WC: Waist circumference, HC: Hip circumference, WHR: Waist/hip ratio, ANOVA test, *p<0.05, **p<0.01

Table 6. Comparison of laboratory measurements according to the ultrasonographic grades of the liver steatosis

	Grade 0 liver steatosis n=78	Grade 1 liver steatosis n=42	Grade 2 liver steatosis n=36	p
	Min-max (median) or mean ± SD			
Hepassocin	0.16-8.99 (1.4)	0.79-9.85 (2.23)	0.42-6.19 (2)	**0.002**[a]
TXNDC5	0.4-16.82 (0.91)	0.5-18.09 (0.78)	0.43-16.34 (0.81)	0.154[a]
AST	9-75 (19)	12-50 (21)	12-52 (24)	**0.001**[a]
ALT	7-109 (17)	7-98 (25)	11-100 (28.5)	**0.001**[a]
AST/ALT ratio	1.1±0.5	0.98±0.4	0.85±0.31	**0.018***[b]
ALP	80.19±29.73	77.38±21.51	75.85±23.64	0.736[b]

NAFLD: Non-alcoholic fatty liver disease, T2DM: Type 2 diabetes mellitus, TXNDC5: Thioredoxin domain containing protein 5, SD: Standard deviation, AST: Aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase, [a]Kruskall-Wallis test (min-max/median), [b]ANOVA test (mean ± SD), *p<0.05, **p<0.01

steatosis were significantly higher than those without hepatic steatosis.

NAFLD is strongly associated with T2DM and IR. According to the study by Xie et al. (22), hepatic steatosis is an independent determinant of increased IR and is associated with increased insulin secretion. In T2DM patients without liver steatosis detected by ultrasound, ALT and AST are associated with hyperinsulinemia and IR in the study by Esteghamati et al. (23). In the literature, results show that HPS induces hepatic lipid accumulation in NAFLD (18), may be involved in the development of NAFLD (24), and may facilitate the accumulation of elevated hepatic lipids in NAFLD and T2DM (25). Our results are in line with the consensus in the literature on the interaction between HPS and NAFLD. First, as the most important finding of our study, when evaluated between study groups, those with the healthy control group, those with both NAFLD and T2DM, and those with isolated NAFLD had higher serum HPS levels than those with isolated T2DM. In other words, those with isolated T2DM had the lowest serum HPS levels compared to the other groups. Second, when evaluated according to the extent of ultrasound fatty liver, serum HPS levels were found to be higher in steatosis. Importantly, the results show that this study is the first to demonstrate an incremental relationship between HPS and ultrasonographic hepatic steatosis. This finding suggests that HPS levels can predict ultrasonographic steatosis. Through a more comprehensive planned study with defined HPS cut-off points, the ultrasonographic degree of liver steatosis can be estimated based on HPS values. Although the results of the study contribute to the literature, prospective research is needed to prove the association between HPS and NAFLD.

An interesting aspect that emerged from the current analysis was that the HPS scores of the groups with isolated T2DM were the lowest compared to the other groups, including the control group. The results, which differ from those of the researchers (26), suggest that elevated HPS levels may be a risk factor for the development of diabetes and IR and that HPS could also be used as a biomarker for the diagnosis of prediabetes. Few studies have examined the causal relationship of HPS with IR, obesity, and diabetes (25-29), and the current study partially contradicted them with respect to diabetes. The contradiction revealed by the current study on diabetes and HPS might be due to confounding factors highlighted by Giorda et al. (3). To summarize these proposed reasons: a reasonable proportion of patients recovered from fatty liver disease, while lower IR (less abdominal obesity, dyslipidemia, hypertension, renal damage) facilitated this; older patients with higher LDL and HbA1c levels had a lower likelihood of resolving NAFLD status; pharmacological treatment of diabetes has been shown to be a notable probable factor for progression or regression of NAFLD. The fact that the diabetic patients in our study received different diabetes treatments was considered an important factor among all these proposed reasons. The HPS score may have been lower because those with isolated T2DM were taking more diabetes medications than others.

Although there was an indication that TXNDC5 contributes to the development of steatosis and seems to be a marker for hepatic steatosis in the absence of apoE (20), there were not enough studies aimed at this, so no association was found in our study either. Many studies have reported that ER stress is involved in the pathophysiologic development of NAFLD. In a study aimed at this, glucagon-like peptide-1 (GLP-1) was shown to ameliorate NAFLD by reducing hepatic ER stress and subsequent apoptosis, and that treatment with GLP-1 analogs may contribute significantly to the maintenance of NAFLD (30). In the same study,

it was suggested that the anti-lipotoxic effect of GLP-1 might have an impact on the activation of the Erp46 (TXNDC5) signaling pathway. However, the link between T2DM and TXNDC5 could not be established in this study. In this regard, our study showed no association between TXNDC5 and diabetes. The association between serum levels of TXNDC5 and the parameters of the current study was not statistically significant in all analyzes.

Study Limitations

The limitations of our study, the use of antidiabetic drugs and the different age distribution between the groups proved to be possible confounding factors. In addition, the fact that the study was conducted in a tertiary care hospital prevents generalization of the results. There is a need for studies that include newly diagnosed diabetics who have not yet received antidiabetic medication and the age differences between the groups that comprise the entire population. The study cohort is limited by the absence of grade 3 steatosis, which limits the availability of data for the worst grade of steatosis with hepassocin.

Conclusion

Our study has shown the association of HPS with NAFLD, confirming previous studies and contributing to the literature on its involvement in the pathogenesis of NAFLD. Our study is originally based on the fact that it is the first study to demonstrate the association between HPS and ultrasonographic grade of liver steatosis. No comparable association was demonstrated between HPS and T2DM and, as an additional finding, no significant association was found between TXNDC5 and NAFLD and T2DM, either individually or together. More comprehensive prospective research is needed to confirm the results of the current study, taking into account potential limitations.

Ethics

Ethics Committee Approval: Before to conducting the study, ethical approval was obtained from the Clinical Research Ethics Committee of University of Health Sciences Turkey, İstanbul Bağcılar Training and Research Hospital with protocol number date: IRB number: 2019.03.3.05.034 (March 29, 2019).

Informed Consent: All participants acknowledged and signed their written informed consent.

Peer-review: Internally and externally peer-reviewed.

Authorship Contributions

Concept: E.D., E.S., İ.D., Design: E.D., E.S., İ.D., Data Collection or Processing: E.D., E.S., M.D., Analysis or Interpretation: İ.D., M.D., Ş.A., Drafting Manuscript: E.D., E.S., İ.D., Ş.A., Critical Revision of Manuscript: E.D., E.S., M.D., Final Approval and Accountability: E.D., E.S., İ.D., M.D., Ş.A., Technical or Material Support: E.D., E.S., İ.D., Supervision: E.D., E.S., Writing: E.D., E.S., İ.D., M.D., Ş.A.

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