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Association of circulating preptin with non-alcoholic fatty liver disease: A case-control study

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Ethics Committee Approval
The study protocol was approved by the Medipol University Ethics Committee (10840098-604.01.01-E.175 number:1191).
All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

Conflict of Interest
No conflict of interest was declared by the authors.

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Abstract

Background/Aim: Non-alcoholic fatty liver disease (NAFLD) is the hepatic component of metabolic disorders and identifying patients with a high risk of fibrosis is crucial. A scoring system, the FIB-4 score, based on clinical and biochemical parameters was developed to predict fibrosis. NAFLD is associated with various peptide hormones. However, the relationship of preptin, a newly discovered peptide critical for regulating energy metabolism, with NAFLD remains unclear. Therefore, we aimed to determine the relationship of preptin with NAFLD and evaluate whether there was an association between serum preptin levels and FIB-4 score.

Methods: In this prospective study, serum biochemical parameters and serum preptin levels of 51 patients with grade 2-3 hepatosteatosis proven by ultrasonography and 35 healthy controls with similar demographic characteristics were compared. Serum preptin levels were measured using ELISA. The FIB-4 scores were calculated and recorded.

Results: The serum preptin levels were higher in the NAFLD group than in the control cases (P<0.001). Among the patients, the sensitivity, specificity, positive predictive, and negative predictive values of preptin above a cut-off value of ≥62.3 were 85.7%, 56.9%, 85.3%, and 57.7%, respectively, with an accuracy of 68.6%. The preptin level was negatively correlated with the FIB-4 score and AST in the patient group (P=0.004 and P=0.014, respectively). The linear regression model revealed that there was a significant relationship between the FIB-4 score and preptin (P<0.05). Each 1 unit increase in the FIB-4 score induced a decrease of 86.37 units in preptin values (R^2=%8.5).

Conclusion: We demonstrated that the serum preptin levels were higher in patients with NAFLD than healthy individuals and negatively correlated with the FIB-4 score and AST levels. Preptin may have a role in the pathogenesis of NAFLD, is likely to distinguish patients with mild and severe liver damage and may be a useful marker in predicting a high risk for progression to fibrosis. Further studies establishing a stratification system including plasma preptin level and the FIB-4 score and its verification with liver biopsies are required.

Keywords: Preptin, Hepatosteatosis, Non-alcoholic fatty liver disease

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Introduction

Non-alcoholic fatty liver disease (NAFLD), defined as liver dysfunction with excessive fat deposition in the liver parenchyma without evidence of significant alcohol consumption or other secondary etiologies of liver disease, is acknowledged as the most common cause and form of chronic liver disease and estimated to affect 20-30% of the general population [1]. NAFLD encompasses simple steatosis (SS), non-alcoholic steatohepatitis (NASH), liver cirrhosis, and hepatocellular carcinoma (HCC). SS is the most common type and has been considered the most benign presentation of the disease [2]. Unfortunately, about 30 percent of patients with SS progress to NASH, which is defined as the presence of NAFL plus inflammation with hepatocyte damage, fibrosis, and cirrhosis, or HCC [3, 4]. Therefore, it is important to identify groups of patients with a higher risk of advanced fibrosis to optimally conduct follow-up and treatment.

Although liver biopsy is the gold standard for diagnosing and staging liver fibrosis, various non-invasive estimation methods have been developed based on the clinical and biochemical parameters to assess fibrosis without a liver biopsy. Based on this idea, the FIB-4 score consisting of age, liver function tests, and platelet counts of the patient has been established to forecast advanced fibrosis in patients with NAFLD [5]. However, the complexity of NAFLD pathogenesis and various biological deflections make it difficult to distinguish NASH and SS with a single biomarker.

Recently, NAFLD was found to be associated with various peptide hormones synthesized in a multitude of tissues [6-8]. Preptin, the importance of which is just beginning to be understood, is a 34-amino acid peptide derived from proinsulin-like growth factor II (pro-IGF-II) [9]. This peptide hormone is synthesized in pancreatic β-cells and co-secreted from cells with insulin in response to glucose [10]. Preptin has also been found in the salivary glands, breast tissue, and kidneys [11]. Previous studies have demonstrated that preptin induces insulin secretion via a variety of biochemical pathways. In experimental studies, intravenous preptin infusion in rats has been shown to cause a decrease in blood glucose level associated with insulin secretion during glucose loading. Glibenclamide and preptin, which stimulate insulin secretion by blocking ATP-sensitive potassium channels in pancreatic β-cells, have similar effects on insulin secretion [12]. Additionally, human studies showed that preptin levels were increased in metabolic disorders such as gestational diabetes mellitus (GDM), polycystic ovary syndrome (PCOS), diabetes mellitus (DM), and high blood pressure, and there was a significant relationship between preptin and body mass index (BMI) [11-19].

Despite the serious damage caused by NAFLD, the underlying pathological mechanism has not yet been fully elucidated. Although the effects of metabolic factors, reactive oxygen metabolites, cytokines, endotoxins, and mitochondrial changes have been proven in the etiopathogenesis of fatty liver disease [20], the effects of peptide hormones are still a matter of debate. In this study, we aimed to determine the relationship between preptin and NAFLD and evaluate the possible association between serum preptin levels and the FIB-4 score in this patient group.

Materials and methods

Study design

This single-centered study was planned and conducted prospectively with 86 patients aged 17 to 70 years who presented to the internal medicine outpatient clinic of Medipol Mega University Hospital between January 2020 and April 2020. The patient group of our study consisted of 51 patients with grade 2-3 hepatosteatosis according to liver ultrasonography. Thirty-five healthy individuals with similar demographics were included in the study as a control group. To avoid possible effects of blood glucose disorders, patients with insulin resistance and diabetes mellitus were excluded, as well as patients with a history of hypertension, kidney diseases, and malignancies, viral/autoimmune hepatitis, Wilson's disease, hemochromatosis, alpha-1 antitrypsin deficiency, primary sclerosing cholangitis, biliary system diseases, an alcohol consumption of >20 g/day, those using hepatotoxic drugs, herbal products, those receiving hormone replacement therapy or antidiabetic medication and pregnant women.

Ethical approval and patient consent

All patients were given detailed information that the study was not part of their treatment, and informed consent of all participants was obtained. The study protocol was approved by Medipol University Ethics Committee (10840098-604.01.01-E.175 number:1191) and conducted per the principles of the Declaration of Helsinki.

Blood sample test

Blood samples were taken from the patients after 10 to 12 hours of fasting. Laboratory data, including the levels of serum glucose, HbA1c, total cholesterol, low-density lipoprotein cholesterol (LDL), triglyceride, high-density lipoprotein-cholesterol (HDL), urea, creatinine, aspartate transaminase (AST), alanine transaminase (ALT) levels were analyzed using routine methods with an autoanalyzer. During routine blood tests, an extra tube of blood was collected from the participants into EDTA tubes and centrifuged at 1,000g for 15 min, and the obtained sera were stored at -80 °C until preptin analysis.

The height and weight of the participants were measured, and the value obtained by dividing the body weight by the square of height was recorded as BMI (kg/m²). In all cases, the blood pressure, measured with a sphygmomanometer after 10 minutes of rest, was recorded.

Measurement of serum preptin by ELISA

Serum preptin levels were measured using an enzyme-linked immunosorbent test kit in a human double antibody sandwich (catalog number E-EL-H0913 96T; Donghu Hi-Tech Development Area, Wuhan, Hubei, China). Measurement with preptin immunosorbent test kits was performed by the ELISA method per the manufacturer's protocol. The results were expressed as picograms per milliliter (pg/mL). The coefficient of variation (CV) is <10 %, sensitivity is 37.50 pg/ml, and detection range is 50-4.000 pg/mL for the preptin ELISA kit.

Ultrasound examination protocol

The liver of all patients was evaluated by the same radiologist using an X brand ultrasonography device after at least

eight hours of fasting. The degree of hepatosteatosis was divided into four groups: Normal (grade 0), mild (grade 1), moderate (grade 2), and severe (grade 3), depending on the increase in the parenchymal echo, the thickness of intrahepatic vascular structures, diaphragm and gallbladder wall, and whether there was clear visualization of the fatty liver with an increased parenchymal echo.

Calculation of the FIB-4 score

The following formula was used to calculate the FIB-4 score: [age (years) × (AST)] / [platelet counts ($\times 10^9$/L) × √ALT U/L].

Statistical analysis

The power analysis for the mean preptin level differences between the two groups was performed using G power 3.1.6 for Windows. The minimum required size of the study population was calculated as 90 subjects for a large effect size at a 95% confidence interval for α=0.05. The data were collected, transferred to the Microsoft Excel program, organized, cleaned, and rendered suitable for analysis. Mann Whitney U, T-Test, ROC Curve Analysis, Spearman Correlation Tests, and Linear Regression Analysis were used for assessment. Data were tested using the IBM SPSS Statistics 26.0 (Statistical Package for Social Science) package programs. A *P*-value of <0.05 was considered statistically significant.

Results

The demographical and clinical characteristics of the participants are shown in Table 1. The study was conducted with 86 patients as four out of ninety participants reported that they wanted to leave the study. Fifty-one patients diagnosed with grade 2-3 hepatosteatosis and 35 healthy control cases were included.

Table 1: Demographic and disease-related characteristics

	Hepatosteatosis group (n = 51)	Control group (n = 35)	*P*-value
Age (years) Mean (SD)	40.69 (9.19)	36.23 (11.94)	0.054
Male	27 (52.9)	19 (54.3)	0.539
Gender			
Female	24 (47.1)	16 (45.7)	
BMI (kg/m²) Mean (SD)	25.15 (1.81)	25.13 (1.05)	0.951
SBP (mmHg) Mean (SD)	122.88 (8.93)	120.03 (10.86)	0.186
DBP (mmHg) Mean (SD)	75.02 (6.3)	74.63 (6.62)	0.782
FIB-4	0.58 (0.24)	-	-

BMI: Body mass index, SBP: Systolic blood pressure, DBP: Diastolic blood pressure

The laboratory data of the participants are summarized in Table 2. The serum preptin, AST, ALT, and triglyceride levels of the NAFLD group were significantly higher than those of the control cases (*P*<0.001, and *P*<0.01 for the latter three, respectively). In addition, serum HDL levels were significantly lower in the NAFLD group compared to the control group (*P*<0.001).

Table 2: Laboratory findings of the NAFLD and control groups

	NAFLD group		Control group		*P*-value
	Mean (SD)	Range	Mean (SD)	Range	
Glucose (mg/dl)	92.19 (4.37)	92-16.6	92.04 (4.34)	92-15	0.873
Urea (mg/dl)	26.65 (5.74)	26.4-28.2	25.31 (6.05)	25-20.5	0.299
Creatinine (mg/dl)	0.92 (0.14)	0.93-0.59	0.86 (0.18)	0.86-0.72	0.129
AST (IU/L)	23.95 (9.43)	22.8-45.1	18.31 (12.93)	16-76	<0.001*
ALT (IU/L)	44.69 (25.19)	39.5-126.2	17.31 (8.9)	16-40.7	<0.001*
TC (mg/dl)	197.37(39.22)	196.6-181.4	188.42 (30.36)	186-134	0.259
LDL-C (mg/dl)	119.14 (37.34)	123-166	113.54 (28.04)	109-114	0.454
HDL-C (mg/dl)	42.36 (9.23)	39.9-38	55.09 (11.76)	56-42	<0.001*
TG (mg/dl)	194.16 (102.35)	167.1-517.4	99.68 (45.53)	92-214	<0.001*
HbA1c (%)	5.25 (0.32)	5.3-1.46	5.23 (0.3)	5.22-1.25	0.824
Preptin (pg/ml)	83.73 (71.11)	64.01-472.32	56.52 (8.08)	54.24-40.83	<0.001*

*Statistically significant at 0.05, AST: aspartate aminotransferase, ALT: alanine aminotransferase, TC: total cholesterol, LDL-C: low-density lipoprotein cholesterol, HDL-C: high-density lipoprotein cholesterol, TG: triglyceride.

The minimum-maximum, 25-75% percentile, and median values of serum preptin levels were higher in the patient group compared to the control group (Figure 1). In the patient group, the serum preptin level's area under the curve (AUC) in ROC analysis, its cut-off, sensitivity, specificity, positive and negative predictive values, and accuracy were 0.767 (95% CI 0.668-0.866) (Figure 2), ≥62.3, 85.7%, 56.9%, 85.3%, 57.7%, and 68.6%, respectively (Table 3).

Figure 1: Minimum, maximum, 25-75% percentile and median values of serum preptin levels of the participants

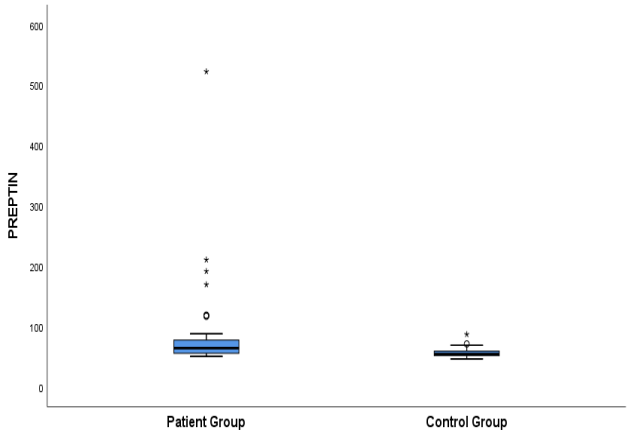


Figure 2: The receiver operating characteristic curve for determining the serum preptin level of the patient group (AUC):0.767 %95 CI 0.668-0.866

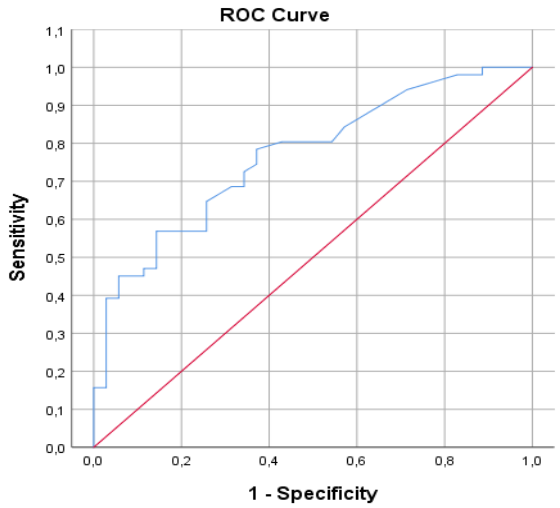


Table 3: The sensitivity, specificity, PPV and NPV of preptin to predict NAFLD

	Cut-off	Sensitivity	Specificity	PPV	NPV	Accuracy
Preptin	62.33	85.7%	56.9%	85.3%	57.7%	68.6%

PPV: positive predictive value, NPV: negative predictive value

The preptin level was significantly negatively correlated with the FIB-4 score and AST level in the patient group (*P*=0.004 and *P*=0.014, respectively), while in the control group, preptin was significantly negatively correlated with urea and creatinine and positively correlated with triglyceride (*P*=0.029, *P*=0.034 and *P*=0.038, respectively) (Table 4).

The results of the linear regression model are given in detail in Table 5. Accordingly, the FIB-4 score was significantly related to preptin (*P*<0.05). One unit increase in FIB-4 values induces a decrease of 86.37 units in preptin values (R^2=%8.5) (Table 5) (Figure 3).

Table 4: Spearman's correlation analyses between preptin and other variables

	General	Hepatosteatosis	Control
Age (years)	0.00 (*P*=0.973)	-0.25 (*P*=0.079)	0.06 (*P*=0.723)
Glucose (mg/dl)	-0.02 (*P*=0.841)	-0.06 (*P*=0.668)	0.04 (*P*=0.823)
Urea (mg/dl)	-0.18 (*P*=0.099)	-0.18 (*P*=0.210)	-0.37 (*P*=0.029*)
Creatinine (mg/dl)	0.03 (*P*=0.753)	0.19 (*P*=0.177)	-0.36 (*P*=0.034*)
AST (IU/L)	-0.04 (*P*=0.746)	-0.34 (*P*=0.014*)	-0.17 (*P*=0.340)
ALT (IU/L)	0.19 (*P*=0.079)	-0.18 (*P*=0.197)	-0.22 (*P*=0.211)
TC (mg/dl)	0.06 (*P*=0.611)	-0.10 (*P*=0.496)	0.11 (*P*=0.527)
LDL-C (mg/dl)	0.03 (*P*=0.764)	-0.05 (*P*=0.740)	0.05 (*P*=0.777)
HDL-C (mg/dl)	-0.37 (*P*<0.001*)	-0.18 (*P*=0.206)	-0.10 (*P*=0.551)
TG (mg/dl)	0.50 (*P*<0.001*)	0.22 (*P*=0.129)	0.35 (*P*=0.038*)
BMI (kg(m²)	0.05 (*P*=0.651)	0.18 (*P*=0.203)	-0.22 (*P*=0.203)
SBP (mmHg)	0.12 (*P*=0.275)	0.06 (*P*=0.652)	0.19 (*P*=0.267)
DBP (mmHg)	0.15 (*P*=0.180)	0.15 (*P*=0.302)	0.16 (*P*=0.344)
HBA1C (%)	0.27 (*P*=0.012*)	0.23 (*P*=0.104)	0.24 (*P*=0.170)
FIB-4	-0.40 (*P*=0.004*)	-0.40 (*P*=0.004*)	

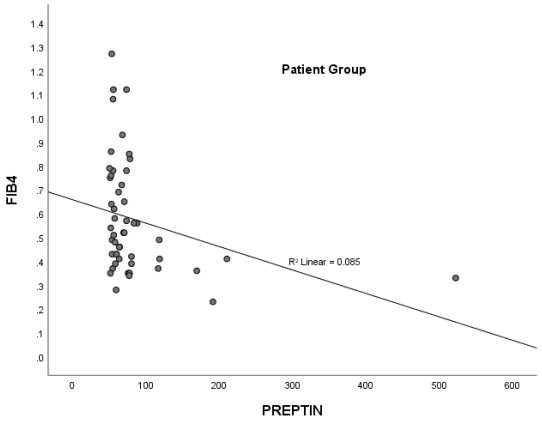
*Statistically significant at 0.05. Spearman correlation analyses, BMI: Body mass index, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, TC: Total cholesterol, LDL-C: Low-density lipoprotein cholesterol, HDL-C: High-density lipoprotein cholesterol, TG: Triglyceride.

Table 5: Regression analysis for preptin levels of the NAFLD group (dependent: preptin, independent: investigated variables)

	B	t	*P*-value	R Square	Durbin-Watson
Constant	133.482	5.286	<0.001*	0.085	2.308
FIB-4	-86.367	-2.131	0.038*		

*Statistically significant at 0.05, B: Backward method

Figure 3: Correlation analysis between serum preptin levels and FIB-4 score in the hepatosteatosis group



Discussion

In this study, we aimed to investigate preptin levels in patients with grade 2-3 hepatosteatosis. We primarily showed that the serum preptin levels in patients with hepatosteatosis were increased compared to the healthy individuals. Our second aim was to determine the possible relationship between preptin and the FIB-4 score, a non-invasive method that was developed to assess fibrosis without a liver biopsy. We demonstrated that the preptin level had a significant negative correlation with the FIB-4 and AST levels in NAFLD. To the best of our knowledge, this is the first study investigating the relationship between the serum levels of preptin and NAFLD.

NAFLD is of great importance because it is the most common chronic liver disease and the hepatic component of metabolic syndrome. The mechanism of the development of both NAFLD and metabolic syndrome is based on glucose metabolism disorders [2]. Glucose metabolism is arranged by various enzymes and hormones. A newly discovered hormone, preptin, has a role in the carbohydrate mechanism by moderating the release of glucose-mediated insulin [10]. In immunohistochemical studies, the demonstration of the presence of pro-IGF-II and insulin at the same location in secretory granules of the pancreas indicates that pancreatic β-cells synthesize not only insulin but also preptin [12]. In an experimental study, the effect of preptin on glucose metabolism was demonstrated with the IGF-II receptor and activation of the protein kinase C and phospholipase C pathways. Furthermore, preptin was found to enhance, but not initiate, insulin secretion in a calcium-dependent manner under high glucose levels [10].

In the literature, there are a limited number of human studies on preptin levels. In these studies, the common characteristic of metabolic disorders is increased insulin levels and/or insulin resistance. In a study on patients with T2DM, preptin levels were higher than in healthy individuals [16]. The other two studies showed increased preptin levels in the serum and colostrum of mothers with GDM as well as in the fetal cord blood [11, 13]. Furthermore, Celik et al. [14] determined that the serum preptin levels were higher in patients with PCOS than in healthy volunteers. In our study, in which insulin resistance and diabetes patients were excluded, preptin levels were significantly higher in the NAFLD group compared to the controls. The result of our study suggests that hepatosteatosis increases preptin levels regardless of insulin resistance.

We also expected the increase in preptin levels to be more pronounced in correlation with the increase of FIB-4 score. Surprisingly, we found that preptin had a statistically negative correlation with the FIB-4 score and AST level in the patient group. In addition, using the factors that significantly determine the preptin level in the univariate analysis, we constructed a logistic regression model, and the FIB-4 score was the most significant factor. This negative correlation between the FIB-4 score and preptin can be explained by several mechanisms. First, the decreased preptin level in patients with NAFLD may be a result of reduced secretion and/or increased catabolism of preptin. Since the liver is the major organ of the synthesis of plasma proteins such as pro-IGF-II, from which preptin derivates, the synthesis of preptin is likely to decrease due to the loss of liver function with the development of fibrosis.

Second, although the mechanisms leading to NAFLD have not yet been clarified, the role of chronic inflammation is evident in the development and progression of NAFLD. Reduction of pro-IGF-II expression caused by inflammatory cytokines may be involved not only with the development of NAFLD but also the grade of NAFLD progression. In addition to all these probabilities, inflammatory cytokines themselves may have caused a decrease in preptin levels, as well as a decrease in protein synthesis in the liver. Unfortunately, there are only limited data on the association of preptin with inflammation. In a clinical study of Dogan et al. [21], the preptin levels were lower in psoriasis and Behçet's disease, both inflammatory diseases. Additionally, in their study, even when they excluded patients with insulin resistance to prevent metabolic parameters from influencing outcomes, the decrease in serum preptin levels in the patient group was unchanged. These findings support our thesis of the probability of a relationship between inflammation and preptin.

In our study, while preptin levels were affected by urea, creatinine, and triglyceride levels in healthy individuals, these three parameters did not affect the preptin level of NAFLD patients. We found this result is worth mentioning as it indicates that the FIB-4 score is the only factor affecting the preptin levels in NAFLD patients.

Our main aim was to compare serum preptin levels in patients with NAFLD and determine whether there was a

diagnostic difference compared to healthy individuals. We found that the preptin levels achieved a diagnostic accuracy of 68.6% in NAFLD. Although not very high, we could not find any report in the literature that we could compare this value with; thus, we decided to present this as a contribution to the literature. In addition, we determined the sensitivity of preptin as 85.7%, while its specificity was 56.9%. According to these results, preptin is effective in identifying the patients with NAFLD and can be considered a new marker with its disease-diagnostic feature in this specific patient group. The low specificity can be explained by the presence of other undiagnosed diseases in the NAFLD group that may increase the preptin level. We could not make any comparisons, since sensitivity and specificity analyses were not conducted for preptin in any of the previous studies.

Studies have shown that there is an association between plasma preptin levels and blood pressure. In a study, preptin levels were positively correlated with systolic blood pressure in patients with T1DM [17]. Similarly, Yang et al. [16] found that plasma preptin levels were positively correlated with diastolic blood pressure in patients with T2DM. Cai et al. [18] determined that patients with essential hypertension had lower plasma preptin levels compared to the control group. Conversely, Wang et al. [22] did not observe a significant difference between the preptin levels between women with preeclampsia and those with normal pregnancies. Similarly, in our study, we did not find any significant differences between the serum preptin levels and blood pressure. Due to the previous studies in which controversial results have been obtained, further investigations regarding circulating preptin levels in hypertension need to be carried out.

In previous studies, an association between serum preptin and BMI has been reported [19, 23, 24]. Nevertheless, we did not determine a correlation between preptin and BMI. Yang et al. [16], who examined patients with T2DM, reported that plasma preptin levels were lower in men compared to women. However, in our study, we did not observe any significant differences between the preptin levels of males and females.

Some limitations of this study must be considered. First, although we excluded some diseases that could affect preptin levels, various other diseases with unknown preptin expression may have been overlooked in our study population. In addition, the patient group has been lacking liver biopsy protocol, which is the only method to demonstrate the real-time severity of the damage. Lastly, pro-inflammatory cytokines and the correlation of oxidant-antioxidant status with preptin were not studied. On the other hand, the superiority of our study is that we have shown that preptin can distinguish fibrosis in NAFLD and may be a useful marker in predicting its progression. In fact, with the finding that the increase of the FIB-4 score associated with the severity of NAFLD leads to a decrease in serum preptin levels, the idea of creating a new classification that includes preptin levels in NAFLD scoring seems worthwhile. However, to obtain more accurate information about this issue, further studies should be planned with a greater number of cases to compare the results with liver biopsy findings. Despite these limitations, we believe that this study contributes to the understanding of the association between NAFLD and preptin.

Conclusion

This study provides a new insight that higher circulating preptin levels are associated with an increased risk of NAFLD. The results of this study demonstrated that the serum preptin levels were significantly higher in patients with NAFLD than healthy individuals. We also determined that the preptin level was significantly negatively correlated with the FIB-4 and AST levels in NAFLD. These findings suggest that preptin plays a potential role in the pathogenesis of NAFLD, is likely to distinguish patients with mild and severe liver damage and may be a useful marker in predicting elevated risk for progression to fibrosis. In patients with NAFLD, which is common in clinical practice and mostly asymptomatic, creating a risk profile for predicting severe liver damage should be the main goal. For this purpose, further studies establishing a stratification system with serum preptin levels and the FIB-4 score and verification with liver biopsy are required.

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