

## Investigation of Adiponectin, Leptin, Retinol Binding Protein-4 and Resistin Levels in Non-Diabetic and Non-Obese Patients With Non-Alcoholic Fatty Liver Disease

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### ABSTRACT

**AIMS:** This study aimed to investigate the levels of adiponectin, leptin, resistin and RBP-4 in the serum of NAFLD with non-obese and non-diabetic cases.

**METHODS:** A total of 81 people aged between 25-72 were included in the study. 56 obese and non-diabetic NAFLD cases with fatty liver detected by ultrasonography (US) were compared with a control group of 25. Liver function tests and lipid profile analysis of all cases were performed, insulin resistance (HOMA -IR) was evaluated. Liver biopsy was performed in 18 patients. Biopsy patients were staged according to their groups. Serum values of adiponectin, leptin,

resistin and RBP-4 were measured in all cases by ELISA method.

**RESULTS:** Waist circumference, body mass index (BMI), alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transferase (GGT) and HOMA-IR values were higher in the NAFLD group and were considered statistically significant. Lipid profiles and adipokine levels were similar in both groups. There was no statistically significant difference between the groups. The mean values of adiponectin, resistin, leptin and RBP-4 were compared according to the biopsy stage, and the mean of leptin and resistin was not associated with the biopsy stage. A positive correlation was found between HOMA-IR and biopsy stage, and a negative correlation between averages of adiponectin and RBP-4.

**CONCLUSION:** Unfortunately, not being overweight or fit doesn't always lead to better health. Obese and non-diabetic individuals may also be at risk of fatty liver and steatohepatitis. Adipokines, which have roles in the pathogenesis of NAFLD, are increasingly important in detecting steohepatitis at an early stage. However, in our study, we did not find any significant differences between the groups in terms of adipokine profiles. For this purpose, studies with larger biopsy-based cases are needed.

**Key words:** Non-alcoholic fatty liver disease; Adiponectin; Leptin; Resistin; Retinol binding protein-4

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### INTRODUCTION

Non-Alcoholic Fatty Liver Disease (NAFLD) is a pathological condition characterized by morphological features observed in alcohol-related liver disease, especially in patients without a significant alcohol use history<sup>[1]</sup>. The global prevalence of NAFLD is currently estimated to be around 24%<sup>[2]</sup>. Higher rates are reported

in the Middle East (32%), South America (31%), Asia (27%), the United States (24%) and Europe (23%), respectively. NAFLD is less common in Africa (14%)<sup>[2]</sup>. Alcohol-free fatty liver disease is often associated with obesity, whereas the clinical NAFLD picture that occurs in non-obese people is called “lean NAFLD” and their body mass index is considered within the normal range<sup>[3]</sup>.

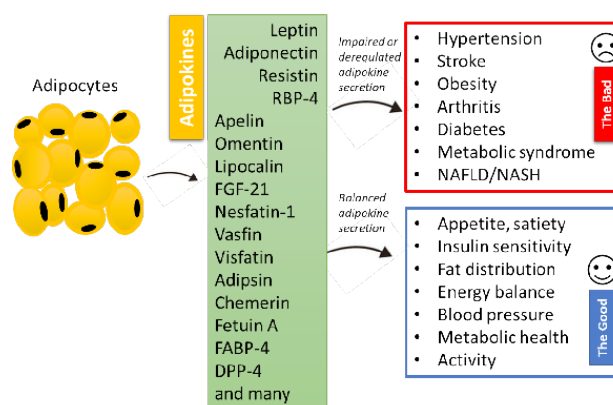
When we consider to liver damage, come to mind in a wide spectrum that can progress from simple steatosis to inflammatory steatohepatitis, advanced fibrosis, cirrhosis and even liver cancer. Currently, NAFLD which continues to be one of the most common liver diseases, in this condition liver lipid content increases without secondary factors leading to fat accumulation in liver cells<sup>[3,4]</sup>. NAFLD has been reported to be responsible for 90% of this condition in people with impaired liver function tests, such as viral hepatitis, alcohol use, and hereditary liver diseases<sup>[5]</sup>. Throughout the life of non-alcohol steatohepatitis (NASH) patients has been shown that 20% of cirrhosis has developed<sup>[6]</sup>. The importance of this disease has increased gradually with the publication of new researches showing that NAFLD can progress to malignancy<sup>[7]</sup>.

The pathophysiology of NAFLD and NASH is very complex and a large number of intermediary molecules are involved in the disease mechanism<sup>[3,4]</sup>. Although the pieces of the puzzle are assembled and new pieces are illuminated every year in NAFLD, the big picture is not yet complete.

The most known risk factor for NAFLD is diabetes and obesity<sup>[7]</sup>. 60-90% of overweight people (body mass index, BMI > 30) have fatty liver disease<sup>[8]</sup>. Therefore, NAFLD is now common in society and is considered one of the elements of metabolic syndrome such as type 2 diabetes, insulin resistance, obesity. There are studies that detect fatty liver in 75% of diabetic patients, 30% of obese men and 40% of obese women<sup>[9,10]</sup>. In parallel with the increase in obesity and diabetes cases, NAFLD has become an important health problem in the society today as one of the main causes of chronic liver disease<sup>[11]</sup>.

In addition to obesity, insulin resistance, oxidative damage and inflammatory processes are other factors that are thought to play a key role in the pathogenesis and progression of NAFLD<sup>[12]</sup>. Furthermore, adipose tissue secreted cytokines, adipokine molecules such as leptin, adiponectin, resistin, retinol binding protein-4 (RBP4), interleukin-6 (IL-6), tumor necrosis factor alpha (TNF- $\alpha$ ) and visfatin are known to cause low-grade inflammation on the liver<sup>[10]</sup>. It is believed that adipokines, which are thought to play a key role in the transition from NAFLD to NASH, are released from hepatocytes and contribute to liver damage. Considering that necroinflammation and fibrosis occur as a result of liver fat accumulation in the pathogenesis of NASH with multiple steps, the idea that adipokines play roles in NAFLD and NASH is stronger<sup>[12,13]</sup>. However if we consider that the number of all known adipokines is more than 600, it is likely that imbalances in adipokine secretion contribute to diseases associated with NAFLD (Figure 1)<sup>[13,15]</sup>. Therefore, over the past few years, the number of newly discovered adipokines has increased significantly and has become a current topic in obesity, diabetes and liver research.

Hence, in recent years, with the awareness of local, peripheral and central effects of adipokines, called the cytokines and hormones of adipose tissue, such as leptin, adiponectin, resistin, retinol binding protein-4 (RBP-4), apelin, omentin, lipocalin, visfatin and nesfatin, adipose tissues are now considering as an important endocrine organ that plays a key role in body energy hemostasis<sup>[16-19]</sup>. Adiponectin, the best studied adipokine in NAFLD, named after adipose tissue, is an anti-inflammatory molecule and inversely proportional to fat mass. This has metabolic effects on insulin resistance in insulin-dependent tissues, such as skeletal muscle and liver<sup>[11]</sup>. One of the major targets



**Figure 1** As a adipose tissue secretome, effects of adipokines in health and disorders. DPP-4: dipeptidyl peptidase-4 enzyme; FABP-4: fatty acid binding protein-4; FGF: fibroblast growth factor.

of the resistin, another adipokine named after insulin resistance, is the liver and can cause insulin resistance in the metabolic organs<sup>[16-19]</sup>.

It is obvious that urgent need a non-invasive diagnostic tests and molecular markers due to the scarcity of methods other than biopsy which shows the progression from NAFLD to NASH. Also known as satiety hormone, leptin is believed to contribute to the formation of insulin resistance is an essential mediator for liver fibrosis<sup>[10]</sup>. In recent years, intensive studies have been conducted on adipokines, which are believed to have an important role in the pathogenesis of NAFLD may be early markers of necroinflammation. For example, high insulin and leptin values in the bloodstream as a result of inflammation are thought to be an ectopic symptom of NAFLD<sup>[20]</sup>.

Herein, start from aforementioned points, we aimed to investigate and evaluate whether adiponectin, leptin, resistin and RBP-4 adipokines have a role in NAFLD pathogenesis in patients with fatty liver, but without diabetes and obesity.

## MATERIALS AND METHODS

### Study design and sampling method

This study was carried out on patients who were diagnosed with fatty liver in US without diabetes and obesity. Patients applied to Fatih University Hospital, Internal Medicine and Gastroenterology outpatient clinics with various complaints from May 2009 to June 2010. The local hospital ethical committee approved the study protocol, and all patients signed an informed consent. The criteria for inclusion and exclusion of patients are shown in Table 1.

The study included 81 cases, aged between 25 and 71, meeting the criteria in Table 1. Patients’ ages, genders, weights, waist circumference were recorded, and consent forms were filled. Venous blood samples were taken after detailed physical examinations.

Weight measurement was determined with classical scale, waist, hip and height measurement were measured with an inelastic tape measure. In all cases, measurements were made in casual clothes, on an empty stomach, without shoes. Body Mass Index: BMI = Weight (kg)/Length<sup>2</sup> (m<sup>2</sup>) was calculated. As the waist circumference, the narrowest diameter between the archus costarum and the proessus spina iliaca anterior superior was considered.

### Laboratory Tests

Venous blood samples of the participants were taken in the morning after 8-12 hours of fasting. Blood were centrifuged for 5 minutes at 4000 cycles at + 4°C within 30 minutes and their serum was separated and stored at -80°C. Blood glucose value was measured

with the Bosch Integra 800 device using the Time-endpoint method. Normal value 70-110 mg/dl was accepted. AST (n: 1-32 uu/L) and GGT (n: 5-40 uu/L) enzymatic method, ALT (n: 1-31 uu/L) and ALP (n: 40-129 uu/L) kinetic method measured with Bosch Integra 800 device. Hepatitis markers were measured with the Roche E170 modular system device using the insulin electrochemiluminescence method.

Serum basal cholesterol, triglyceride, HDL and LDL values were measured with Bosch Integra 800 device after an overnight fast to evaluate lipid profile in patients. Serum adiponectin, resistin, leptin and RBP-4 levels were analyzed using ELISA microplate strip washer (ELX 50; BioTek Instruments, USA) and ELISA microplate reader (ELX 808; BioTek Instruments, USA). Leptin adipokine was studied using DRG Instruments GmbH (Marburg, Germany) kits. Test sensitivity was 1.0 ng/ml. Sample variability was between 8.95-8.66%. Adiponectin and resistin was evaluated Biovendor, Modrice, Czech Republic; Epitope Diagnostics Inc. with San Diego, US kits. In this sampling system, the coefficient of variation between measurements and within measurements is always below 10%. Serum RBP-4 levels were studied with ELISA kits from Immunodiagnostic AG, Bensheim, Germany. The detection limit of the kit was 0.90 µg/mL. For RBP-4, the intra- and intra-measurement variation coefficients were 9.7% and 5%, respectively. Insulin resistance was calculated using the Homeostasis Model Assessment (HOMA) method. In the calculation, the formula  $HOMA = \text{fasting plasma glucose mg/dL} \times \text{fasting insulin level } \mu\text{U/mL} / 405$  was used.

### Ultrasonography

The hepatobiliary ultrasonography of the patients was performed in the Radiology Clinic based on the following criteria in the determination of fatty liver. The degree of liver fattening was determined as seen in Table 2. In our study, patients who did not have fatty liver based on ultrasonography were accepted as the control group. Patients with different levels of fatty liver in ultrasonography were grouped after ruled out alcohol use, chronic viral hepatitis B and/or C, autoimmune hepatitis or metabolic liver diseases.

### Liver biopsy

Hepatic biopsy was performed to those who were found to have fatty liver on ultrasonography and accepted the procedure, and he was performed percutaneously with hepatic needle in the Interventional Radiology Department. At least 15 mm sized samples were tried to be taken. Samples were examined in the Department of Pathology. The obtained biopsies were fixed with formalin and blocked in paraffin, and serial sections were obtained. Histochemically, it was stained with PAS, DPAS, masson-trichrom, Iron, silver, immunohistochemically with HbsAg, HbcAg. Pathology preparations were evaluated in terms of fattening according to the Brunt Classification Scoring system.

### Statistical Analysis

SPSS for Windows 15.0 statistical package program was used in the statistical analysis of the data. Results of all parameters of the cases were given as mean  $\pm$  standard deviation. Student's t, Mann whitney u, ANOVA Kruskal wallis, Pearson correlation t and chi-square tests were used in comparisons.  $p < 0.05$  value was considered statistically significant.

## RESULTS

81 cases, whose ages ranged between 25-72 (mean age  $45.12 \pm 11.8$  years) were included for the study. 25 of them were women

**Table 1** Inclusion (A) and exclusion criteria (B) of patients into the study.

A	
Patients (18-75 aged)	
Body mass index (under 30)	
Abdominal ultrasonography with steatohepatit	
Informed consent	
Ruled out outer liver diseases	
B	
Alcohol consumption	
Pregnancy	
Patients with systemic diseases (cardiovascular disease, kidney disease, cerebrovascular disease)	
Oncology patients	
Major operation in last two months	
Receiving parenteral nutrition	
Other liver disease	
Hepatotoxic drugs usage	

**Table 2** Degree of fatty liver.

Low fatty liver (grade I)	Minimal diffuse fat accumulation, normal intrahepatic vessel and diaphragm appearance
Medium fatty liver (grade II)	Medium diffuse fat accumulation, anormal intrahepatic vessel and diaphragm appearance
High fatty liver (grade III)	High level of diffuse fat accumulation, no sound penetration at posterior segment of right liver, intrahepatic vessel and diaphragm non appearance

**Table 3** Demographic and laboratory results.

	Control (n = 25)	NAFLD (n = 56)	P value	Normal range
Sex (female/male)	9/16	16/40	0.52	-
Age (mean)	43.3	46.1	0.34	-
Body mass index (kg/m <sup>2</sup> )	25.6 $\pm$ 2.3	27.5 $\pm$ 2.9	0.03	-
Waist (cm)	86.2 $\pm$ 7.2	94.6 $\pm$ 8.4	0.38	-
HOMA-IR	1.5 $\pm$ 1.4	3.2 $\pm$ 2.6	0.03	0-2.5
ALT (IU/L)	18.6 $\pm$ 7.7	26.3 $\pm$ 12	<0.01	0-45
AST (IU/L)	18.8 $\pm$ 8.2	27.8 $\pm$ 11.2	<0.01	0-45
GGT (IU/L)	22 $\pm$ 13.1	40.9 $\pm$ 30.7	0.45	0-55
Total cholesterol (mg/dL)	185 $\pm$ 44	198 $\pm$ 34	0.19	120-200
LDL (mg/dL)	115 $\pm$ 30.4	126 $\pm$ 30.8	0.14	85-125
Triglycerid (mg/dL)	117 $\pm$ 61	160 $\pm$ 104	0.24	50-200

and 56 were men. The BMI of the patients and control groups was  $< 30$ . Fatty liver was graded by ultrasonography. The study group was considered as 56 patients with fatty liver and 25 age- and sex-matched patients without steatosis determined as control group. The liver fat level was graded as following; 22 (27.1%) were mild, 16 (20%) were medium and 18 (22.1%) high. Liver biopsy was performed in patients with heavy liver fat accumulation.

Gender distributions between the groups were considered to be homogeneous. BMI, waist circumference and HOMA-IR averages were higher in those with NAFLD than controls. HOMA-IR was evaluated higher in patients with advanced fibrosis.  $p < 0.05$  value was considered statistically significant. While there was no difference in lipid profile (triglyceride, LDL, total cholesterol) values, ALT, AST; GGT values especially were found to be significantly higher in the NAFLD group compared to the controls (Table 3).

Leptin, adiponectin, resistin and RBP-4 values were similar in both groups. The difference was not considered statistically significant ( $p > 0.05$ , Table 4). The relationship of adipokines (leptin, adiponectin, resistin and RBP-4) with the stage of fibrosis was evaluated. Since the number of patients with biopsy is insufficient and the number between the groups is not homogeneous, no statistically significant result was obtained (Table 5). When we looked at the average values of adipokines, there was no difference between the groups in leptin value. Adiponectin level decreased as the stage of fibrosis increased, but no significant relationship could be established in the value of the resistin. RBP-4 mean was inversely proportional to the stage of fibrosis. When the prevalence of NAFLD is distributed by age, it was found to be 58,1% in 25-30 years old, 76% in 40-49 years old, 72% in 50-71 years old.

## DISCUSSION

The present study investigated level of four adipokines in NAFLD patients. Recently has been considered a part of the metabolic syndrome, NAFLD, appears to be a serious health problem owing to potential cause of liver failure, insulin resistance and comorbidity with type 2 diabetes, obesity and coronary artery disease<sup>[20-22]</sup>.

Adipose tissue-derived molecules may favor NAFLD progression towards severe histological stages such as NASH fibrosis, cirrhosis and a substantial risk for developing hepatocellular carcinoma. Indeed, the prevalence of NAFLD is 10-30% and it is suggested that the average incidence is around 20%<sup>[21]</sup>. Calculating that the prevalence is between 50% and 70% in obese people in USA, making NAFLD the most common cause of elevated liver enzymes<sup>[21-22]</sup>.

NAFLD alone it is thought that there is no risk factor for fibrosis, recent studies contradict this assumption. For example, in one study, 22% of participants with NAFLD developed fibrosis over time and 44% developed NASH<sup>[23]</sup>.

Because of increasing and accumulating effects on world health, there is a great interest in NAFLD and its advanced NASH stage. In USA alone, the number of NAFLD cases is estimated to increase from 83.1 million in 2015 to 100.9 million in 2030. In addition, 2.3 billion adults of the world population are overweight and at least 700 million are obese. The severity of the condition is obvious, since the incidence of NAFLD and type 2 diabetes comorbidity is likely to increase<sup>[24]</sup>.

Although NAFLD is more common in Asian obese men than women, the general opinion is that it is seen equally in both sexes<sup>[25,28]</sup>. When the cases participating in our study were evaluated by age; the prevalence of NAFLD was found to be 58,1% at 25-30 years old, 76% at 40-49 years and 72% at 50-71 years old. Although BMI was < 30 in both groups, the BMI was higher in the NAFLD group than in the others. As the BMI increased, an increase in the rate of fatty liver was detected. It was observed that waist circumference increased in both sexes as the age progressed after the third decade.

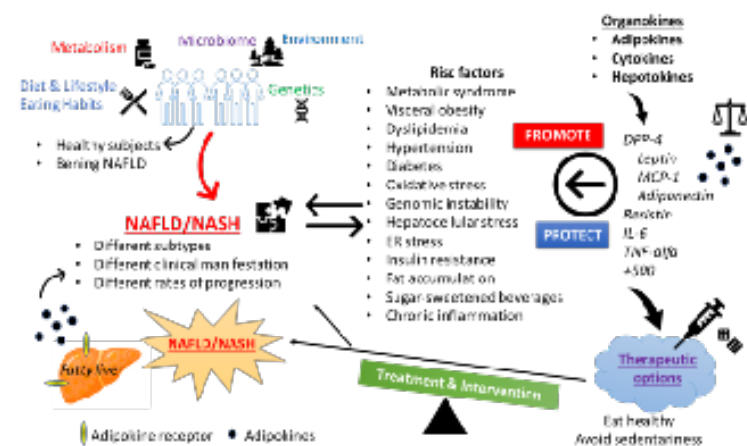
Generally, the most common abnormality detected as laboratory test in NAFLD is high ALT and AST level, which does not exceed 2-3 times the normal range. Generally AST/ALT ratio is < 1. The current accepted view is that the AST/ALT ratio exceeding one is a risk factor for progress to fibrosis. In our study, the AST/ALT ratio was not different in the patient and control groups, and this ratio was below 2 (Table 3). ALT and GGT elevation was detected in 46% of our patients. In another study conducted by our clinic, in patients with metabolic syndrome, ALT, AST and GGT levels were ALT = 30.6 U/L; AST = 24.0 U/L; GGT = 43.8 U/L on average. Lipid values and GGT levels were found to be higher in patients with hyperlipidemia than in normal cases<sup>[29]</sup>.

**Table 4** Plasma levels of leptin, adiponectin, resistin and RBP-4.

Adipokines	Control (n = 25)	NAFLD (n = 56)	P value
Leptin (ng/mL)	1.9 ± 12.7	14.7 ± 14.3	0.68
Adiponectin (µg/mL)	18 ± 9.2	17.2 ± 9.3	0.65
Resistin (ng/mL)	21.6 ± 12	22.8 ± 12	0.82
RBP-4 (mg/mL)	86.2 ± 7.2	94.6 ± 8.4	0.15

**Table 5** Relation between adipokines and fibrosis level.

Adipokines	Fibrosis level 1 (n = 4)	Fibrosis level 2 (n = 6)	Fibrosis level 3 (n = 8)
Leptin (ng/ml)	6.5 ± 5.6	25.3 ± 23.2	14.6 ± 11.1
Adiponectin (µg/ml)	21 ± 9.8	15.9 ± 8.5	13.7 ± 2.6
Resistin (ng/ml)	21.1 ± 10.3	15.3 ± 3.8	19.2 ± 9.1
RBP-4 (mg/ml)	81.4 ± 20	71.5 ± 21.3	61.6 ± 37.6
HOMA-IR	2.1 ± 0.8	2.2 ± 0.4	2.9 ± 1.7



**Figure 2** Adipokines and possible involving in NAFLD pathology.

However, it should not be forgotten that there is not always a positive correlation between high liver function tests and fatty liver. In patients, liver fat deposition is detected by US during routine controls. Interestingly although some patients develop NAFLD, liver function tests are normal. In our NAFLD group, ALT, AST, GGT mean values were found as 26.3 U/L, 27.8 U/L and 40.9 U/L, respectively, and these values were higher than the control group. Although 16 patients were diagnosed with NAFLD, ALT and AST levels were within normal limits, whereas in GGT, some patients had a moderate high. Although liver function tests were normal in 2 patients, liver biopsy results showed stage 1 fibrosis in one patient and stage 2 in another patient.

It has been emphasized for many years that the main event in the pathogenesis of NAFLD is insulin resistance<sup>[21,24]</sup>. Thus, different methods have been proposed in the quantitative measurement of insulin resistance. Among these, although the glucose clamp technique is still the gold standard, this method is expensive and difficult<sup>[30,31]</sup>. From the perspective of the clinician, it is generally considered to evaluate the insulin resistance with homeostasis model assessment (HOMA) technique which is easier in large-scale clinical studies. In our study, the mean of HOMA-IR in those with NAFLD was found to be 3.2 ± 2.6 and 1.5 ± 1.4 in the control group. The difference was considered statistically significant ( $p = 0.03$ ). In one study conducted by Marchesini and his group, the mean HOMA value in the NAFLD group was 3.3 ± 1.0 (2.2-5.6), while this value was 1.8 ± 0.6 (0.9-2.4) in the control group. ( $p < 0.001$ )<sup>[32]</sup>. Comert and his colleagues detected severe insulin resistance in patients with obese and non-diabetic NAFLD<sup>[31]</sup>.

When the lipid profiles of the our cases were evaluated, there was no difference between the groups in terms of total cholesterol and LDL cholesterol. HDL cholesterol levels decreased statistically significantly as the level of liver fatty deposition increased, while triglyceride levels increased. To evaluate the relationship between NASH and hyperlipidemia, hyperlipidemia was detected in patients with NASH at a rate of 20-81%, and it was shown that there was a relationship especially between high triglyceride level and NASH<sup>[33,34]</sup>.

In another study, hyperlipidemia was detected in 55.8% (BMI:  $30 \pm 2.3$ ) and 52% (BMI:  $28.3 \pm 3.4$ ) of cases<sup>[35]</sup>. In our study, hyperlipidemia was detected in 45.7% (BMI:  $27.5 \pm 2.9$ ) of the NAFLD group and in 32% (BMI:  $25.6 \pm 2.3$ ) of the control group. Observing an increase in hyperlipidemia in parallel with the increase in body mass index supports the hypothesis that NAFLD is an element of metabolic syndrome.

Although the gold standard in NASH diagnosis is still biopsy, this non-invasive methods are becoming increasingly important due to the fact that biopsy is an invasive procedure, the possibility of complications and difficulty in persuading patients to this procedure.

For this reasons, one of the non-invasive parameters used in recent years is adipokine molecules, which are thought to play a key role in the pathogenesis of NASH<sup>[36]</sup>. Adipokines are also called organokines together with myokines and hepatokines (Figure 2). Accumulating evidence links obesity with inflammation which may emerge from adipose tissue derived pro- and anti-inflammatory cytokines called adipokines.

The first adipokine molecule discovered is leptin which was discovered in 1994 and launched as a satiety hormone. But until now, very contradictory results have been obtained regarding leptin. The beneficial and harmful effects of leptin have been reported in NAFLD<sup>[36]</sup>. In a study of 47 patients and 47 control groups, high leptin levels in NASH patients correlated with the severity of liver steatosis, whereas inflammation and fibrosis did not correlate<sup>[37]</sup>. In different studies, no significant difference was found in leptin levels, and independent association with liver fibrosis could not be demonstrated<sup>[38-40]</sup>. Tsochatzis and his colleagues reported that leptin levels were significantly higher in patients with hepatosteatosis compared with patients with chronic viral hepatitis<sup>[41]</sup>.

In our study there was no statistically significant difference between the patient and control groups in terms of leptin level. There was no correlation between necroinflammation and fibrosis stages. These different leptin results obtained in patients with NAFLD suggest that the subject should be clarified by studies containing more homogeneously selected cases.

Adiponectin, discovered in 1995, just one year after the leptin, inhibits gluconeogenesis and lipogenesis in the liver and is known as a protective adipokine in metabolic syndrome and type 2 diabetes<sup>[11,19,20]</sup>. Adiponectin has been the focus of attention owing to its insulin sensitivity, anti-inflammatory and antiapoptotic effects. Hui and his colleagues compared the level of adiponectin in patients with NASH with respect to age, gender and BMI levels and showed that patients with NASH were significantly lower than those with control and simple steatosis<sup>[42]</sup>. An increase in coronary artery disease and atherosclerotic events also seen at an early age has been reported in patients with low adiponectin levels<sup>[43,44]</sup>. Even in non-diabetic patients, the decrease in adiponectin levels has been shown to be associated with impaired glucose tolerance<sup>[43]</sup>. In addition, an increase in coronary artery disease and atherosclerotic events seen at an early age in patients with low adiponectin levels has been reported<sup>[44]</sup>.

Important steps have been taken to use adiponectin as a non-

invasive marker to distinguish between NAFLD and NASH. 66 cases with early stage NASH (Brunt scale 1-2) were compared with 19 cases with simple fatty liver, evaluated together with serum HOMA-IR, adiponectin and serum collagen type 4 and early stage NASH was diagnosed with 94% sensitivity and 74% specificity<sup>[45]</sup>. In this study, the area under the ROC (receiver operating characteristic) curve was reported to be 0.79 when the adiponectin and HOMA-DR were combined, and the area under the ROC curve was 0.765 when adiponectin was used alone to separate the fatty liver from NASH. When looking at adiponectin and HOMA together to distinguish steohepatitis from fatty liver, HOMA-IR > 3 and adiponectin < 10  $\mu\text{g/ml}$  were found in 77% of patients with NASH, and it was found normal in 33% simple steatosis patients.

In our study, 0.44 ( $p = 0.39$ ) under the curve for adiponectin in the NAFLD group was not considered statistically significant. The subcurved area 0.70 (0.56-0.82) ( $p = 0.005$ ) for HOMA-IR distinguished fatty liver with sensitivity 89% specificity 48%. In a study with 101 obese and NASH patients, adiponectin and resistin diagnosis were 95% sensitivity and 75% specificity<sup>[46]</sup>. Thirty patients with untreated hepatitis C compared with the control group, the total adiponectin and high molecular weight adiponectin levels decreased in patients with steohepatitis whereas low-molecular weight adiponectin levels was unchanged<sup>[47]</sup>. But, there was no statistically significant difference between NAFLD and control group in terms of adiponectin level. However, as the stage of fibrosis increased, an inversely proportional decrease was observed in the level of adiponectin (Table 5).

Apart from the level of adiponectin, the measurement of the expression of adiponectin receptors (ADIPOR1 and ADIPOR2) in adipose and liver tissue revealed contradictory results. Adiponectin exerts many of its functions via this distinct receptors that elicit AMP kinase signaling. Biopsy results with 103 patients showed low adiponectin receptor expression in fatty tissues and high in liver tissue have been associated with liver damage<sup>[48]</sup>. This "over production" of the receptors probably appears as a compensatory response to hypoadiponectinemia. In 2 smaller studies with contradictory results, adiponectin mRNA level and AdipoR2 receptor expression were shown to be significantly lower in patients with NASH than in fatty liver patients<sup>[49,50]</sup>. In another study, single nucleotide adiponectin polymorphism was studied in 70 obese and non-diabetic NAFLD cases whose lipid levels were measured within normal limits, and interestingly, 45 TT and 276GT polymorphisms were significantly higher in NAFLD patients than in the general population<sup>[51]</sup>.

But it is not possible immediately to transfer the data of such polymorphism studies to the clinic. Nevertheless, genetic testing with a diagnostic and prognostic value as a non-invasive method appears to be used in the hepatic manifestation of the metabolic syndrome, NAFLD, management.

Resistin, another adipokine and first described in 2004, has been reported to play a role in glucose and lipid metabolism, thereby contributing to the development of insulin resistance and obesity<sup>[52-54]</sup>. However, in a study conducted with 80 patients with NAFLD, no significant difference was observed between the groups with NASH and NAFLD in the levels of resistin<sup>[55]</sup>. In a small study with 45 NAFLD and 50 control groups with a BMI > 45, the level of resistin and adiponectin was not found to be significantly different between the groups, while the TNF-alpha level was found to be significantly different between the groups. The level of resistin was similar between NASH and simple liver fattening<sup>[56]</sup>.

Similar results were obtained in our study; when the NAFLD group was compared with the control group, there was no significant

difference between them in terms of resistin.

Studies on animal models have been found that resistin is the main target of the liver<sup>[57]</sup>. The resistin with high and low molecular isoforms probably plays a role in the pathogenesis of liver insulin resistance and aggravates the clinical outcome in patients with NAFLD<sup>[58]</sup>. The expression of the resistin observed in NAFLD in the inflammatory histiocytes infiltrate, in Kupffer cells and in histiocytes surrounding hepatocytes. Histologically, a positive correlation was found between the expression of the resistin and the severity of NAFLD<sup>[58]</sup>.

In another study by Tsochatzis<sup>[41]</sup> the level of resistin was found to be related to the severity of fibrosis. In the study conducted by Pagano<sup>[59]</sup> the level of resistin was found higher in patients with NAFLD compared to the control group, and a positive correlation with inflammation was found.

But, in our study, there was no significant difference between the groups. There are other studies in NAFLD patients where there is no difference in serum levels of the resistin compared to controls<sup>[55]</sup>. Although these observations suggest that the possible role of resistin in the pathogenesis of NAFLD may be limited, the differences in the measurements may also origin from different circulating resistin isoforms in peripheral blood.

Another adipokine RBP-4, a peptide hormone secreted by hepatocytes and adipose tissue, has been shown to induce insulin resistance and increased plasma RBP-4 values has been found in type 2 diabetes, obesity, metabolic syndrome and cardiovascular disease<sup>[60]</sup>. RBP-4 was identified as an adipokine in 2005 and is released mainly from visceral fat tissue and liver. RBP-4 has been recognized for years as a vitamin A transport protein from the liver to peripheral tissues<sup>[61,62]</sup>.

Polyzo<sup>[63]</sup> investigated the levels of resistin and RBP-4 in NAFLD and NASH patient serums, no significant differences were found between the groups. However, in the study of Seo and their groups RBP-4 levels were found to be high in NASH patients<sup>[64]</sup>. In the study conducted by Zwolak and his group RBP-4 was also found to be high in all NAFLD patients, especially obese<sup>[65]</sup>. 42 obese children with NAFLD with an average age of 12.2 years (BMI: 29.5 ± 3.73) were compared with a control group of 12 people with similar BMI values and no difference was observed in terms of RBP-4 level<sup>[66]</sup>. In this case, circulating RBP-4 levels may not be associated with NAFLD<sup>[67]</sup>.

In these studies, contradictory and different results may arise from different and interlocking mechanisms of NAFLD and inflammatory NASH. It has been found that circulating RBP-4 is reduced during medical interventions that lead to improved metabolic picture such as diet, exercise, oral antidiabetic drugs, and hypolipidemic agents<sup>[68]</sup>. However, further attempt to seek the metabolic roles of RBP-4 are needed, because RBP-4 also affected by some non-metabolic conditions such as kidney failure, acute illness, injury and liver failure.

In our study, as the biopsy stage increased adiponectin and RBP-4 levels decreased in NASH cases diagnosed by biopsy (Table 5). No relationship was observed with leptin and resistin levels. Although it is known that these adipokines may play a role in pathogenesis, large-scale studies with biopsy-proven cases will be more guiding.

Studies on adipokines are mostly performed with obese (BMI > 30), hyperlipidemic, insulin resistant and diabetic patients. Although NAFLD is accepted to be associated with these groups, it is known that steatosis may occur in obese and non-diabetic cases. As a result, biopsy is still the gold standard diagnostic method in the diagnosis of NASH. Non-invasive diagnostic methods are needed due to difficulty in persuading and complications of biopsy. Various studies

have shown that the levels of adipokines listed above vary in NASH, NAFLD and healthy controls.

## CONCLUSION

There is still a lot of uncertainty and heterogeneity between studies around the pathophysiological relationship between adipokines and NAFLD. Hence, apart from adipose tissue, many different cell groups can produce more than one adipokine in a constantly changing metabolic environment. Because of the hepatic effects of these hundreds of adipokine molecules with different receptors and different isoforms, circulation levels may not be reflected in the reality. Gender-specific issues are also of interest.

For these aforementioned reasons, although the discovery of biomarkers such as adiponectins are promising parameters for the future, it is obvious that larger studies are needed in these subjects.

In conclusion, our investigation demonstrated that four adipokines concentrations are not significantly changed in non-diabetic and non-obese NAFLD participants suggesting that a possible imbalanced adipokines contribution to NAFLD is controversial.

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