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Association of Biopsy-proven Nonalcoholic Fatty Liver Disease with Obesity and Apolipoproteins

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ABSTRACT

Background & Aims: Insulin resistance is considered the most important key mechanism in the development of nonalcoholic fatty liver disease (NAFLD). Some studies have reported that hyperinsulinemia decreases the hepatic secretion of apolipoprotein (Apo) B. Chronic hyperinsulinemia in NAFLD may be responsible for the accumulation of triglycerides in hepatocytes. We aimed to investigate whether apolipoproteins are related to histological findings in patients with biopsy-proven NAFLD. We also aimed to evaluate the effects of obesity on apolipoproteins and the pathogenesis of NAFLD.

Methods: In this cross-sectional study, 91 patients with biopsy-proven NAFLD were included. The control group consisted of 39 healthy subjects who had no history of liver disease or alcohol consumption and were matched for age, gender and smoking. Apoliprotein A1 and Apo B were measured via an immunoturbidimetric method with commercially available OSR6142 Apo A1 and OSR6143 Apo B immunoassay kits on an Olympus AU2700 analyzer.

Results: Age, gender, and smoking distribution were similar among nonalcoholic steatohepatitis patients, simple steatosis patients, and controls. The differences in the mean Apo A1 and Apo B levels and the Apo B/A1 ratio among non-alcoholic steatosis, simple steatosis, and control subjects did not reach statistical significance. In addition, patients with obese NAFLD had higher steatosis scores than patients with nonobese NAFLD ($p<0.05$).

Conclusions: Apo A1 and B levels and the B/A1 ratio were not associated with histopathological findings in patients with NAFLD. Fibrosis and ApoB1/A were found to be independent risk factors for metabolic associated fatty liver disease. In addition, obesity increases the grade of hepatic steatosis but does not cause lobular inflammation, ballooning or fibrosis.

Key words: NAFLD – obesity – apolipoproteins – insulin resistance – fatty liver.

Abbreviations: ALT: alanine aminotransferase; Apo: apolipoprotein; AST: Aspartate aminotransferase; BMI: body mass index; DBS: diastolic blood pressure; DM: diabetes mellitus; FPG: fasting plasma glucose; GGT: γ-glutamyltransferase; HbA1c: glycated hemoglobin; HDL: high-density lipoprotein; HDL-C: HDL cholesterol; HOMA-IR: homeostasis model assessment-insulin resistance; hs-CRP: hypersensitive C-reactive protein; HT: hypertension; LDL: low-density lipoprotein; MAFLD: metabolic dysfunction-associated fatty liver disease; NAFLD: nonalcoholic fatty liver disease; NAS: NAFLD activity score; NASH: non-alcoholic steatohepatitis; OGTT: oral glucose tolerance test; SBP: systolic blood pressure; SS: simple steatosis; TG: triglycerides; VLDL: very-low-density lipoprotein; WC: waist circumference.

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is a clinicopathological entity characterized by fatty infiltration of the liver in the absence of excessive alcohol consumption. Although NAFLD is strongly associated with obesity, diabetes mellitus (DM) and metabolic syndrome, the underlying pathological mechanism of NAFLD is poorly understood. Insulin resistance is considered the most important key mechanism leading to hepatic steatosis and steatohepatitis. Indeed, a key molecular event in the pathogenesis of NAFLD is the accumulation of triglycerides in hepatocytes. The causes of this accumulation are thought to be elevated de novo synthesis of fatty acids and delivery of free fatty acids from peripheral tissues to the liver as well

as impaired lipoprotein secretion [1]. Reduced absolute apolipoprotein (Apo) B secretion has been demonstrated in patients with NAFLD and may contribute to increased hepatocellular lipid content [2, 3]. In addition, Musso et al. [4] reported that defects in postprandial hepatic Apo B secretion may lead to the development of steatohepatitis by modulating triglyceride accumulation and antioxidant activity in liver cells, as well as indirectly by affecting insulin sensitivity. Taken together, these findings suggest that alterations in lipoprotein metabolism may be the causative mechanism for lipid accumulation in hepatic steatosis.

Apolipoproteins are important proteins in lipid metabolism that transport lipids in the blood and serum. They bind to lipids to form emulsified lipoprotein particles. These particles are composed of phospholipids, free cholesterol, triglycerides, cholesterol esters, and apolipoproteins. Apolipoprotein A1 is the major component of high-density lipoprotein (HDL), while Apo B is the endpoint of very-low-density lipoprotein (VLDL) catabolism and the major apolipoprotein of low-density lipoprotein (LDL) [5]. Indeed, apolipoproteins are primarily measured to evaluate cardiovascular risk. High ApoA1 levels have a cardioprotective effect, whereas high Apo B levels are atherogenic [6]. Recent clinical studies suggested that the Apo B/A1 ratio was a more useful indicator for predicting cardiovascular risk than LDL cholesterol [7]. This feature may be attributed to the supposition that Apo B reflects the total number of potentially atherogenic particles. In addition, some studies have reported that hyperinsulinemia decreased the hepatic secretion of Apo B [8, 9]. Therefore, chronic hyperinsulinemia in NAFLD may be responsible for the accumulation of triglycerides in hepatocytes.

Although many studies have investigated the relationship between apolipoproteins and NAFLD, the results remain controversial [10-12]. Therefore, we aimed to investigate whether there is a relationship between apolipoproteins and histological findings in patients with biopsy-proven NAFLD. We also aimed to evaluate the effects of obesity on apolipoproteins and the pathogenesis of NAFLD.

METHODS

Subjects

This cross-sectional study was carried out in the Department of Gastroenterology, Gulhane School of Medicine, Ankara, Turkey. Patients who had been admitted to our outpatient clinic with elevated liver enzyme levels were evaluated for the study between March 2016 and May 2019. Among these patients, those in which a biopsy was performed to determine the etiology and in which NAFLD was detected in the biopsy were included in the study. A total of 91 patients with biopsy-proven NAFLD were enrolled. The inclusion criteria were as follows: (a) elevated aminotransferases for more than 6 months; (b) ultrasonographic presence of a bright liver without any other liver or biliary tract disease; and (c) evidence of NAFLD on liver biopsy. The exclusion criteria included those with a history of alcohol consumption >20 g/day or >140 g/week, seropositive for hepatitis B or C virus, and other known causes of chronic liver disease (i.e., autoimmune hepatitis, hemochromatosis, primary biliary cirrhosis, Wilson's disease, or α1-antitrypsin deficiency). The control group consisted of 39 healthy subjects who had no history of liver disease or alcohol consumption were matched for age, gender and smoking. The study was approved by the Ethics Committee of Gulhane School of Medicine (Approval number: 1491-1198-10/1539). Written informed consent was obtained from all participants. We declare that our study was conducted in accordance with the ethical principles of the Declaration of Helsinki.

Clinical and Laboratory Measurements

A detailed medical history and smoking status were obtained from each participant via a self-administered questionnaire. A regular smoker was defined as a subject who currently smokes at least one cigarette per day. Obesity was defined as a body mass index (BMI) ≥ 30 kg/m^2. Waist circumference (WC) was measured after expiration at the midpoint between the lowest rib and the iliac crest. Hypertension (HT) was defined as a blood pressure higher than 140/90 mmHg or reported use of regular antihypertensive medication.

Our patient group was divided into two groups according to meeting the criteria for metabolic dysfunction-associated fatty liver disease (MAFLD), which has been proposed as a more appropriate new term, independent of alcohol use, to describe liver disease associated with known metabolic dysfunction. The definition of MAFLD is based on the presence of ultrasound-confirmed hepatic steatosis in the presence of one of the following: DM or overweight/obesity (BMI ≥25 kg/m^2). In addition, the diagnosis of MAFLD in non-overweight/obese individuals requires the presence of at least two of the following criteria: 1) WC ≥102 cm in men and 88 cm in women. 2) pre-diabetes [glycated hemoglobin (HbA1c) of 5.7–6.4%, or fasting plasma glucose (FPG) of 5.6–6.9 mmol/L, or 2-hour post-load glucose levels of 7.8–11.0 mmol/L]. 3) blood pressure ≥130/85 mmHg or under anti-hypertension therapy. 4) high-density lipoprotein cholesterol (HDL-C) <1.0 mmol/L for males and <1.3 mmol/L for females. 5) triglyceride (TG) ≥1.70 mmol/L or specific drug treatment. 6) homeostasis model assessment-insulin resistance (HOMA-IR) score ≥2.5; and 7) hypersensitive C-reactive protein (hs-CRP) level >2 mg/L [13].

Laboratory assessments were performed on blood taken from the antecubital vein after fasting overnight. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyltransferase (GGT), triglyceride (TG), HDL, fasting plasma glucose (FPG), uric acid, and creatinine levels were measured via enzymatic colorimetric methods via commercially available Olympus reagents (Beckman Coulter Inc. CA, USA) on an Olympus AU2700 autoanalyzer (Beckman Coulter, USA). LDL cholesterol was calculated with Friedewald's formula [14]. Fasting venous blood was collected for apolipoprotein measurements on the same day as the liver biopsy. Blood samples were centrifuged at 3000 g for 10 minutes and stored at –80°C until the biochemical analyses were performed. Apoliprotein A1 and apolipoprotein B were measured via the immunoturbidimetric method with commercially available OSR6142 Apolipoprotein A1 and OSR6143 Apolipoprotein B immunoassay kits (Beckman Coulter, Inc., CA, USA) on an Olympus AU2700 analyzer (Beckman-Coulter, USA).

Patients and controls underwent a standard 75 g oral glucose tolerance test (OGTT). Measurements of glucose were performed at 0 and 120 min. Type 2 DM and increased risk of diabetes (prediabetes) were defined according to the definition and description of DM by the American Diabetes Association [15]. Fasting serum insulin levels were measured with an ADVIA Centaur XP analyzer (Siemens Diagnostics, Tarrytown, NY). The homeostatic model assessment of insulin resistance index (HOMA-IR) was defined by the following equation: fasting plasma insulin (mU/ml) × fasting plasma glucose (mg/dl)/405 [16].

Liver Histology

To determine the diagnosis of NAFLD, liver biopsy was obtained with ultrasonography guidance by the same gastroenterologist for all patients with NAFLD. All biopsies were at least 2 cm in length and contained a minimum of 8 portal tracts. Slides were stained with hematoxylin and eosin, and an experienced hepatopathologist who was blinded to the subjects' details scored each liver biopsy specimen via the semiquantitative classification of Kleiner et al. [17]. Briefly, the degree of steatosis, liver injury, and inflammatory activity were scored on an 8-point scale (steatosis 0–3; lobular inflammation 0–3; ballooning hepatocyte degeneration 0–2). Histopathological features were graded according to the NAFLD activity score (NAS), where a score of ≥5 was defined as non-alcoholic steatohepatitis (NASH). The stage of fibrosis was graded on a 6-point scale as follows: 0=no fibrosis; 1a, b= mild (1a)/moderate (1b) zone 3 and perisinusoidal fibrosis; 1c=portal fibrosis only; 2= zone 3 and portal/periportal fibrosis; 3=bridging fibrosis; and 4=cirrhosis.

Statistical Analysis

The SPSS (Statistical Package for the Social Sciences ver. 17.0, SPSS Inc., Chicago, IL, USA) computer program was used for all the statistical calculations. The results are reported as the means ± standard deviations (SDs), frequencies and percentages. The Kolmogorov-Smirnov test was used to determine the distribution characteristics of continuous variables. Normally distributed variables were compared with one-way ANOVA for multiple group comparisons and Tukey's post hoc test. The Kruskal-Wallis test was used for multiple group comparisons of variables without a normal distribution, and a Bonferroni-adjusted Mann-Whitney U test was used for post hoc analysis. Categorical variables were compared via the chi-square test or Fisher's exact test. Pearson correlation analysis was used to evaluate the relationships between continuous variables. Statistical significance was defined as $p<0.05$.

RESULTS

The flow diagram of study is represented in Fig. 1. The baseline characteristics of the study population are shown in Table I. Age, gender, and smoking distribution were similar among NASH, simple steatosis (SS), and control subjects. There was no difference in terms of FPG, HDL, LDL, or creatinine levels or the prevalence of HT or DM among the three groups. Body mass index, WC, diastolic blood pressure (DBP), GGT, TG, insulin, and HOMA-IR levels were greater in patients with NAFLD than in control subjects. However, no significant difference in these variables was found between the NASH and SS groups. Patients with NASH had higher systolic blood pressure (SBP), uric acid, AST, ALT, and OGTT levels than both SS patients and control subjects. No significant differences in SBP, uric acid, or OGTT results were found between the SS patients and the controls (Table I).

The differences in the mean Apo A1 and Apo B levels and the Apo B/A1 ratio among NASH, SS, and control subjects did not reach statistical significance (Table I).

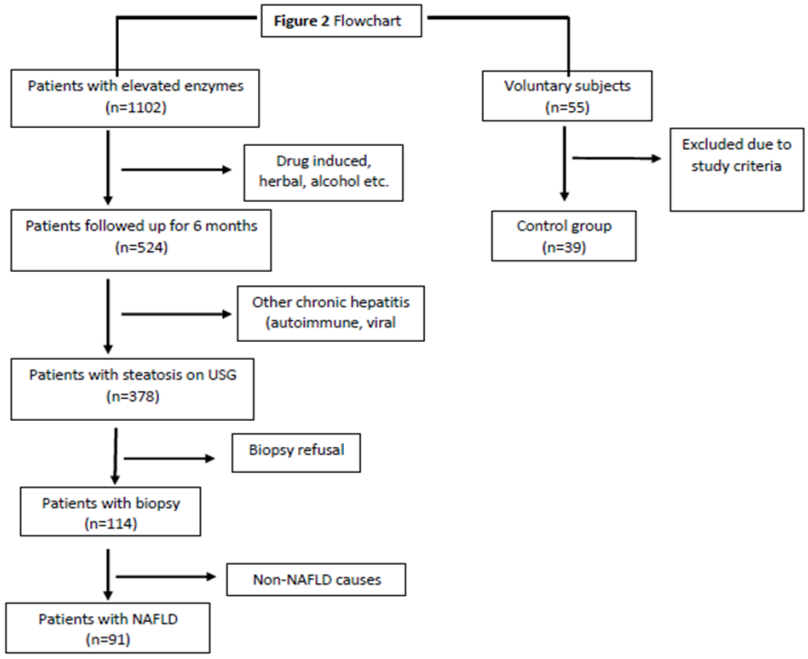

Fig. 1. A flow diagram of study.

Table I. The clinical and biochemical characteristics of study population

Variables	NASH (n=64)	SS (n=27)	Controls (n=39)	P
Age (year)	36.2±7.5	37.7±8.6	36.9±10.1	0.936
Smoking (%)	35.7	60	36.8	0.225
Male(%)	93.5	100	94.9	0.438
HT (%)	7.8	3.7	2.5	0.124
DM (%)	12.5	3.7	5.1	0.179
WC (cm)	103.3±7.7	100.5±8.1	90.4±10.2	<0.001
BMI (kg/m^2)	29.3±2.8	28.4±3.2	24.9±3.6	<0.001
SBP (mmHg)	128.8±16.5	119.6±29.9	116.3±10.3	0.001
DBP (mmHg)	79.5±10.3	78.6±6.8	71.4±9.9	0.001
AST (U/L)	64.4±39.7	45.3±18.5	21.9±4.7	<0.001
ALT (U/L)	117.1±55.1	86.5±31.1	21.5±7.9	<0.001
GGT (U/L)	74.5±41.4	90.2±92.5	22.9±14.8	<0.001
FPG (mg/dl)	100.5±12.5	102.6±21.3	95.3±16.5	0.149
2-h OGTT (mg/dl)	128.6±41.8	101.1±28.2	93.5±20.5	<0.001
Creatinine (mg/dl)	1.00±0.1	1.01±0.1	0.96±0.1	0.209
Uric acid (md/dl)	6.7±1.3	6.6±1.2	5.9±1.3	0.011
LDL-C (mg/dl)	123.7±37.7	138.1±35	117.4±33	0.97
HDL-C (mg/dl)	40.7±8	47.2±15.2	44.1±8.6	0.26
TG (mg/dl)	182.6±103.6	169.9±88.7	113.5±49.9	0.001
Ferritin (ng/ml)	176.1±130.3	125.8±85.9	75.9±58.8	<0.001
Insulin (mU/ml)	17.1±10.3	14.6±9.2	8.5±4.5	<0.001
HOMA-IR	4.3±2.9	3.7±2.3	2.1±01.2	<0.001
APO A-1 (mg/dl)	112.1±16.6	113.7±16.9	114.1±14.3	0.830
APO B (mg/dl)	105.1±30.7	106.4±24.3	100.3±25.2	0.621
B/A-1 (%)	0.95±0.29	0.96±0.28	0.89±0.23	0.481
NAS (0-8)	5.4±0.9	3.3±0.5	-	<0.001
Fibrosis (0-4)	1	0	-	<0.001
Steatosis	2.3±0.6	1.3±0.4	-	<0.001
Lobular inflammation	1.7±0.5	1±0.4		<0.001
Ballooning	1.4±0.5	1		<0.001

NAFLD: nonalcoholic fatty liver disease; SS: simple steatosis; NASH: steatohepatitis; DM: diabetes mellitu; HT: hypertension; WC: waist circumference; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; AST: aspartat aminotransferase; ALT: alanine aminotransferase; GGT: γ-glutamyltransferase; FPG: fasting plasma glucose; OGTT: oral glucose tolerance testing; LDL-C: low density lipoprotein cholesterol; HDL-C: high density lipoprotein cholesterol; TG: triglyceride; HOMA-IR: homeostasis model assessment of insulin resistance; APO: apolipoprotein, NAS NAFLD activity score; APO: apolipoprotein; NAS: NAFLD activity score.

In addition, patients with NAFLD were divided into two groups according to the presence of fibrosis. There was no difference in Apo A1 or Apo B between the groups (Fig. 2). No differences in metabolic or histopathological parameters were observed after regression analysis for possible confounders.

Patients with NAFLD were classified into subgroups according to BMI as follows: obese NAFLD (BMI>30 kg/m^2),

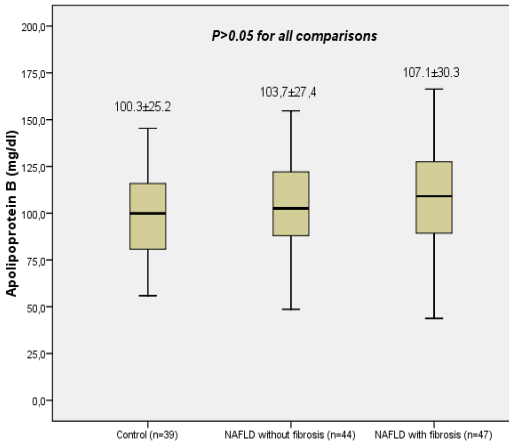

Fig. 2A. The association of apolipoprotein B with fibrosis.

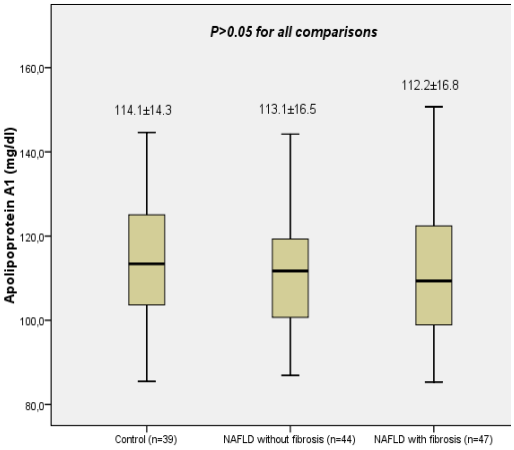

Fig. 2B. The association of apolipoprotein A1 with fibrosis.

nonobese NAFLD (BMI<30 kg/m^2), and control. There were no significant differences among the three groups in terms of age, sex, smoking status, or frequency of HT or DM. The levels of FPG, LDL, HDL, creatinine, Apo A1, and Apo B and the Apo B/A1 ratio were similar among the three groups. Body mass index, WC, and SBP were greater in patients with obese NAFLD than in patients with nonobese NAFLD and control subjects. Compared with controls, patients with NAFLD had higher DBP, AST, ALT, GGT, TG, insulin, and HOMA-IR values, whereas no significant difference existed between patients with and without obese NAFLD. Patients with obese NAFLD had higher uric acid levels than nonobese NAFLD patients and control subjects. Moreover, patients with obese NAFLD had higher steatosis scores on histopathological assessment than patients with nonobese NAFLD. However, the NAS and fibrosis, ballooning, and lobular inflammation scores in NAFLD patients were similar between obese and nonobese patients (Table II).

In patients with NAFLD, we performed a subgroup analysis on the basis of the presence of MAFLD. While there was no significant difference between the groups in terms of Apo A1 or Apo B (P>0.05), MAFLD patients had significantly higher levels of ApoB/A1 (P<0.05). Although the levels of NAS were similar between the groups, the fibrosis scores were higher in the patients with MAFLD than in those without MAFLD (Table

Table II. Association of obesity with apolipoprotein in NAFLD

Variables	Obese NAFLD (n=45)	Nonobese NAFLD (n=46)	Controls (n=39)	P
Age (year)	36.8±7.6	36.5±8.2	36.9±10.1	0.978
Smoking (%)	40	41.4	36.8	0.926
Male(%)	95.6	95.7	94.9	0.983
HT (%)	6.6	6.5	2.5	0.645
DM (%)	8.8	8.6	5.1	0.254
WC (cm)	108.8±5.1	97.3±5.6	90.4±10.2	<0.001
BMI (kg/m^2)	32.2±1.7	26.4±1.7	24.9±3.6	<0.001
SBP (mmHg)	132.4±14.7	119.6±24.3	116.3±10.3	<0.001
DBP (mmHg)	81.1±8.9	77.2±9.4	71.4±9.9	<0.001
AST (U/L)	62.1±43.7	54.3±24.3	21.9±4.7	<0.001
ALT (U/L)	102.4±41.2	112.2±57.7	21.5±7.9	<0.001
GGT (U/L)	87.6±71.1	67.4±43.8	22.9±14.8	<0.001
FPG (mg/dl)	102.7±18.9	98.8±10.2	95.3±16.5	0.100
2-h OGTT (mg/dl)	116±35.8	124.9±43.5	93.5±20.5	0.011
Creatinine (mg/dl)	1.00±0.1	1.01±0.1	0.96±0.1	0.162
Uric acid (md/dl)	6.9±1.3	6.4±1.1	5.9±1.3	0.001
LDL-C (mg/dl)	128.1±29.5	127.2±43	117.4±33	0.343
HDL-C (mg/dl)	40.7±11.3	43.9±9.9	44.1±8.6	0.229
TG (mg/dl)	190.1±110.8	169.8±83	113.5±49.9	<0.001
Ferritin (ng/ml)	147.1±82.1	174.1±146.9	75.9±58.8	<0.001
Insulin (mU/ml)	18.2±11.9	14.3±6.9	8.5±4.5	<0.001
HOMA-IR	4.6±3.3	3.5±1.9	2.1±01.2	<0.001
APO A-1 (mg/dl)	109.4±16	115.4±16.2	114.1±14.3	0.162
APO B (mg/dl)	106.5±26.5	105.1±30.6	100.3±25.2	0.570
B/A-1 (%)	0.98±0.27	0.92±0.3	0.89±0.23	0.259
NAS (0-8)	4.9±1.2	4.8±1.3	-	0.727
Fibrosis (0-4)	0.6±0.6	0.5±0.6	-	0.697
Steatosis	2.1±0.6	1.9±0.8	-	0.036
Lobular inflammation	1.5±0.6	1.5±0.5	-	0.474
Ballooning	1.3±0.5	1.3±0.4	-	0.580

For abbreviations see Table I.

Table III. Comparison of metabolic and histopathological parameters according to MAFLD

Variables	Non-MAFLD (n=21)	MAFLD (n=70)	P
APO A-1 (mg/dl)	116.7±15.5	111.2±16.4	0.176
APO B (mg/dl)	97.6±28.8	108.3±28.2	0.133
B/A-1 (%)	0.84±0.25	0.99±0.29	0.038
NAS (0-8)	4.5±1.1	4.9±1.3	0.22
Fibrosis (0-4)	0.3±0.4	0.7±0.6	0.013

MAFLD: metabolic dysfunction-associated fatty liver disease. For the rest of abbreviations see Table I.

Table IV. Correlation analyses of Apoliproteins with NAS and metabolic parameters

	B/A-1		APO A-1		APO B	
	r	p	r	p	r	p
NAS	0.027	0.801	0.002	0.986	0.045	0.677
Fibrosis	0.080	0.464	-0.027	0.805	0.079	0.465
BMI	0.192	0.029	-0.017	0.845	0.196	0.025
WC	0.178	0.048	-0.057	0.533	0.171	0.059
FPG	0.083	0.350	0.000	0.992	0.082	0.356
LDL-c	0.708	<0.001	0.094	0.303	0.837	<0.001
HDL-c	-0.303	<0.001	0.701	<0.001	0.021	0.815
TG	0.549	<0.001	-0.312	<0.001	0.399	<0.001
Insulin	0.134	0.132	-0.062	0.488	0.100	0.259
HOMA-IR	0.136	0.126	-0.057	0.524	0.107	0.230

For abbreviations see Table I

III). Multivariate regression analysis revealed that fibrosis and Apo B/A1 were independent risk factors for MAFLD ($p<0.025$ and $p<0.033$, respectively).

According to the correlation analyses, Apo B was positively correlated with LDL, TG, and BMI. Apolipoprotein A1 was positively correlated with HDL and negatively correlated with TG. The Apo B/A1 ratio was negatively correlated with HDL and positively correlated with BMI, WC, LDL, and TG. No significant correlation was found between apolipoprotein parameters and histopathological findings. In addition, there was no association between apolipoproteins and insulin (Table IV).

DISCUSSION

In the present study, we demonstrated that Apo A1, Apo B, and the Apo B/A1 ratio in patients with NAFLD were not associated with histopathological findings of NAFLD or insulin resistance. We showed that obesity did not affect apolipoprotein metabolism in NAFLD patients. Obesity was associated with the degree of hepatic steatosis but not with the degree of inflammation or ballooning in the liver. In addition, fibrosis and ApoB1/A were found to be independent risk factors for MAFLD in this study. To the best of our knowledge, this is the most comprehensive study of the associations of apolipoproteins with obesity and histopathological findings in patients with NAFLD.

The pathogenesis of NAFLD has not been fully elucidated. However, insulin resistance is considered the most widely supported theory in the development of NAFLD. Insulin resistance occurs frequently in patients with obesity and DM type 2. However, it also occurs in patients with NAFLD without obesity or DM. On the other hand, some patients with NAFLD may have normal insulin levels [18, 19]. This suggests that rather than a single mechanism, as in the multi-hit theory, many different causes, such as lipoprotein metabolism disorders and hepatic oxidative injury, may contribute to the pathogenesis of NAFLD. Some researchers have focused on the role of apolipoproteins in the pathogenesis of NAFLD and have proposed several hypotheses. Recently, Charlton et al. [20] reported that Apo B synthesis is reduced due to hyperinsulinemia in patients with NASH and that diminished Apo B synthesis may contribute to the net retention of lipids [20]. In contrast, Koruk et al. [21] reported an increase in Apo B and a decrease in Apo A1 in patients with NASH and

abnormalities in lipid metabolism, suggesting that these features may be primarily responsible for the development of NASH rather than insulin resistance. Together, these two previous studies suggested that the ApoB/ApoA1 ratio was an independent predictor of NAFLD [22, 23]. In the present study, we found that Apo B, Apo A1, and Apo B/Apo A1 levels were not significantly different between patients with NAFLD and controls and were not associated with histopathological findings or insulin resistance. Consistent with previous studies, we demonstrated that the ApoB/ApoA1 ratio was an independent predictor of MAFLD. However, MAFLD was not associated with Apo A1 or Apo B. When the abovementioned studies were analyzed separately, in contrast to our study, patients with NAFLD had an increase in LDL and a decrease in HDL compared with the control subjects. Because of their effects on apolipoproteins, it is possible that these differences in lipid profiles may have led to different results in the trials. Moreover, the fact that the ApoB/A1 ratio was found to be significant in MAFLD patients but not NAFLD patients may be explained by the fact that multiple factors are more effective in histopathology when combined, as in the multi-hit theory. The common finding of all three studies suggests that the ApoB/A1 ratio is a stronger predictor in patients with NAFLD than ApoA1 and ApoB, as in coronary heart disease.

It is well known that there is a strong association between NAFLD and obesity, with NAFLD found in up to 80% of patients with obesity [24]. Initially, the relationship between NAFLD and obesity was thought to be a benign reversible change. However, expanded adipose tissue has been shown to have more complex endocrine functions, primarily mediated by the production of numerous pro- and anti-inflammatory cytokines, including adiponectin, leptin, resistin, visfatin, tumor necrosis alpha, and interleukin-6 [25]. One of these cytokines, adiponectin, is an insulin-sensitizing hormone secreted from adipocytes, and its circulating levels are inversely proportional to body mass index. Many studies have shown that low adiponectin levels contribute to the development of NAFLD in obese individuals through its effects on chronic inflammation and insulin resistance [26-27]. In contrast, obesity does not increase the risk of death related to coronary heart disease or chronic liver disease, especially in patients with NAFLD [28-29]. In this study, obese NAFLD patients had higher insulin and HOMA levels than nonobese NAFLD patients and controls. When the effect of obesity on NAFLD histopathology was analyzed in our study, we found that while hepatic steatosis was increased, obesity did not alter chronic inflammatory processes, including lobular inflammation and ballooning. On the basis of our findings and the studies above, it can be speculated that obesity may not be a strong factor in progression and chronicity. However, the lack of an effect of obesity on inflammation and fibrosis in NAFLD can be explained by the low prevalence of DM and the mild histopathology of the patients in our study. We therefore assume that the duration of the disease was too short for the effects of the inflammatory mediators of obesity to manifest themselves because the patients with NAFLD in our study were younger than those in previous studies.

Our study has several limitations that should be noted. First, as our study was cross-sectional, it does not allow for causality assessment; therefore, a longitudinal study is needed to determine the effects of obesity and apolipoproteins on NAFLD. Second, the number of NAFLD patients with advanced fibrosis was very small. Therefore, it was not possible to assess the relationship between obesity and fibrosis in NAFLD patients. Finally, we used fasting insulin and HOMA-IR rather than the gold standard clamp technique to evaluate insulin resistance. Considering that a linear correlation exists between HOMA-IR and measures of insulin sensitivity via the glucose clamp technique, this commonly used index is routinely used to provide a noninvasive assessment of insulin sensitivity.

CONCLUSIONS

The present study revealed that Apo A1, B, and the B/A1 ratio are not associated with histopathological findings in patients with NAFLD. We also showed that fibrosis and high ApoB/A1 are independent risk factors for MAFLD. This finding suggests that patients with NAFLD who have multiple metabolic disorders, such as MAFLD, have an increased risk of developing atherosclerosis and chronic liver damage. In addition, obesity increases the grade of hepatic steatosis but does not cause lobular inflammation, ballooning or fibrosis. Thus, we suggest that weight loss and physical activity may reverse the histological findings of NAFLD in patients without advanced fibrosis. To evaluate the progression of hepatic steatosis to steatohepatitis and cirrhosis in obese individuals, prospective longitudinal studies are warranted.

Conflicts of interest: None to declare.

Authors' contributions: K.O., A.U., O.K. conceived and designed the study. Y.K. analysed the liver histology. A.T. and T.O. performed the laboratory investigations. O.K. collected data. T.T. performed the statistical analysis. C.O. interpreted the results. O.K. drafted the manuscript K.O. H.D., A.U., O.K. critically revised the manuscript. All authors critically revised the manuscript, approved the final version to be published, and agree to be accountable for all aspects of the work.

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