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ORIGINAL ARTICLE

Evaluation of Novel Markers in Predicting Nonalcoholic Fatty Liver: Uric Acid–High-Density Lipoprotein Ratio

Non-alkolik Karaciğer Yağlanmasının Değerlendirilmesinde Kullanılabilecek Yeni Bir Belirteç: Ürik Asidin Yüksek Dansiteli Lipoproteine Oranı

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Abstract

Introduction: The aim of this study was to evaluate the association between the existence of nonalcoholic fatty liver (NAFL) with uric acid–high-density lipoprotein (HDL) ratio (UHR) and vitamin B12 levels and the effectiveness of these markers in predicting the severity of NAFL.

Methods: This retrospective observational study was conducted in a university hospital between November 2021 and April 2022. The patients who had hepatobiliary ultrasonography examinations with the suspicion of fatty liver were enrolled. Patients who had fatty liver were divided into three groups after an ultrasonographic examination.

Results: Of the 575 patients enrolled in the study, NAFL was found in 314 (54.6%) participants. Fatty liver was evaluated as grade 1 in 158 (27.5%), grade 2 in 123 (21.4%), and grade 3 in 33 (5.7%). UHR was higher in patients who had NAFL than in those who did not have NAFL. In addition, UHR levels increased as the severity of NAFL increased. Receiver operating characteristic (ROC) curve analysis revealed that, among parameters inspected in the study, the most effective parameter to predict NAFL was UHR. A UHR value of 9.47 revealed a sensitivity of 78% and a specificity of 53%.

Discussion and Conclusion: According to the results of this study, UHR can be used to predict the existence and severity of NAFL. There was no association between vitamin B12 levels and NAFL. UHR may be a noninvasive, available, and very simple inflammatory marker that may predict the existence of NAFL in suspected individuals.

Keywords: High-density lipoprotein; Nonalcoholic fatty liver disease; Uric acid

Nonalcoholic fatty liver (NAFL) is characterized by the deposition of lipids for more than 5% of total cell mass in hepatocytes that eventually ends up with fatty liver, which is the most frequent reason for chronic liver disease in the population.[1] NAFL affects almost one-third of the adult population in developed countries and causes chronic inflammation and fibrosis in liver tissue, which may progress to liver cirrhosis.[2] Chronic inflammation is the key point in the development of NAFL.[3] Once lipid accumulation exceeds tolerable limits, inflammation is triggered inevitably.[4] The severity of inflammation in hepatocytes is directly associated with the degree of fatty liver.[5]

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One of the accused factors in the pathogenesis of NAFL and the injury in liver tissue is the disturbances in the function of the mitochondrion. Vitamin B12 has an important role in maintaining normal mitochondrial functions.[6] In addition, the liver is the main localization of storage for vitamin B12. Interestingly, studies inspecting the association of vitamin B12 with NAFL reveal conflicting results. In a study inspecting the patients who underwent liver biopsies, it was reported that the degree of deficiency of vitamin B12 was associated with the severity of fatty liver.[7] On the other hand, another study reported no association between NAFL and vitamin B12 levels.[8]

Today, it is well documented that high-density lipoprotein (HDL) protects the endothelium from oxidative damage related to low-density lipoprotein levels.[9] Increased uric acid levels are associated with increased inflammations,[10] and previous studies report associations between elevated uric acid levels and the existence of NAFL.[11] When the uric acid levels increase, mitochondrial oxidative stress also increases and results in the development of more severe NAFL.[12] Uric acid–HDL ratio (UHR) is a better, novel inflammatory marker in predicting metabolic syndrome when compared with other inflammatory markers.[13] Recent studies with special patient groups that do not have concomitant diseases or obesity revealed associations between UHR and the severity of NAFL.[14,15] However, the information about the relationship between UHR and NAFL in the general population is not satisfactory.

Due to the aforementioned issues, this study was designed to evaluate the association between the existence of NAFL with UHR and vitamin B12 levels and the effectiveness of these markers in predicting the severity of NAFL.

Materials and Methods

This study is a retrospective observational study conducted in a university hospital in Ankara with patients who applied to the internal medicine outpatient clinic between November 2021 and April 2022. Patients who had hepatobiliary ultrasonography examinations and laboratory tests with the suspicion of fatty liver were enrolled. The patient records in the hospital data management system were inspected, and the patients who had complete records that were necessary for the study were included in the study. This study was approved by the local ethics committee (approval no.: 2022070), and it was conducted in compliance with good clinical practices guidelines and the Declaration of Helsinki.

Patients with potential confounding factors, such as patients who had liver diseases, malignancies, acute infections, who consumed alcohol over 20 g daily, and who were under medication for hyperlipidemia and under the age of 18 years, were excluded.

Ultrasonography of all participants was performed by a 15-year experienced radiologist on GE Voluson 730 (GE Medical Systems, Kretztechnik GmbH, Austria) ultrasonography device. As described previously, patients who had fatty liver were divided into three groups after the ultrasonographic examination.[16]

Uric acid, alanine aminotransferase (ALT), aspartate aminotransferase (AST), HDL, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), thyroid-stimulating hormone (TSH), and vitamin B12 levels were obtained from all participants. UHR was calculated by dividing uric acid levels (mmol/L) by HDL levels (mmol/L) and multiplying with 100. TSH and vitamin B12 levels were analyzed by Roche Hitachi Cobas 601 (Switzerland) device. In addition to lipid levels, ALT, AST, uric acid, HDL, and CRP levels (by immunoturbidimetry method) were analyzed using Roche Hitachi Cobas 501 (Switzerland) device. ESR was studied in Biosed 100 (Italy) device. All laboratory devices are being regularly controlled, and calibrations were performed when necessary according to good laboratory practices guidelines and hospital laboratory management regulations monitored by internal and external supervisors.

Statistical Analysis

SPSS for Windows 25.0 statistical software package (SPSS, Inc., Armonk, NY, USA) was used for the statistical analysis of the data. Data distributions or normality tests were evaluated using the Shapiro–Wilk test. The data were presented as mean±standard deviation for normally distributed variables. The comparisons between groups were evaluated by independent t-test, one-way ANOVA test, and Chi-squared test. A value of $p<0.05$ was considered significant. Receiver operating characteristic (ROC) curve analysis was performed by calculating the area under the curve, and it was used to determine the diagnostic power of parameters in predicting NAFL.

Results

UHR was higher in patients who had NAFL than in those who did not have NAFL. In addition, UHR levels were increasing as the severity of NAFL increased. Besides advanced age, uric acid levels, ALT, and AST levels were higher in the NAFL group, HDL levels were lower (Table 1). ALT, AST, CRP, and uric acid levels were associated with the severity of NAFL in the NAFL group and the mean age between groups was similar (Table 2).

Table 1. Laboratory parameters and mean age of participants in study groups according to the existence of NAFL

	NAFL+ (n=314)	NAFL– (n=261)	p
Age (years)	51.04±12.34	44.82±15.48	<**0.001***
Gender (male/female ratio)	156/158	147/114	0.112†
Uric acid (mmol/L)	0.32±0.08	0.27±0.07	<**0.001***
Aspartate aminotransferase (IU/L)	20.45±9.42	17.96±6.48	<**0.001***
Alanine aminotransferase (IU/L)	28.85±21.41	19.99±16.12	<**0.001***
High-density lipoprotein (mmol/L)	1.15±0.28	1.32±0.34	<**0.001***
C-reactive protein (mg/L)	40.85±43.05	29.90±46.95	**0.004***
Erythrocyte sedimentation rate (mm/h)	17.42±12.87	15.79±14.36	0.155
Thyroid-stimulating hormone (mIU/L)	2.30±1.75	2.26±2.00	0.795
Vitamin B12 (pmol/L)	306.96±138.61	303.21±148.34	0.755
UHR (%)	13.04±4.73	9.81±4.88	<**0.001***

NAFL: Nonalcoholic fatty liver; UHR: Uric acid–high-density lipoprotein ratio; *: Independent sample t-test; †: Chi-squared test.

Table 2. Laboratory parameters and mean age of participants in study groups according to the grades of NAFL

	Grade 1 (n=158)	Grade 2 (n=123)	Grade 3 (n=33)	p
Age (years)	50.32±13.13	52.25±10.96	49.97±13.31	0.373
Gender (male/female ratio)	79/79	63/60	14/19	0.664
Uric acid (mmol/L)	0.30±0.08	0.34±0.08	0.36±0.07	<**0.001***,†
Aspartate aminotransferase (IU/L)	17.90±6.37	21.34±8.04	29.36±17.45	<**0.001***,‡
Alanine aminotransferase (IU/L)	22.12±12.54	31.45±19.48	51.42±38.59	<**0.001***,§
High-density lipoprotein (mmol/L)	1.18±0.30	1.12±0.26	1.10±0.32	0.145
C-reactive protein (mg/L)	35.33±40.48	41.62±38.95	66.57±60.67	**0.001***,‖
Erythrocyte sedimentation rate (mm/h)	16.97±13.72	17.61±12.13	19.22±11.11	0.688
Thyroid-stimulating hormone (mIU/L)	2.26±1.54	2.26±1.42	2.61±3.24	0.565
Vitamin B12 (pmol/L)	314.31±135.63	292.20±142.14	328.01±138.24	0.280
UHR (%)	11.91±4.36	13.94±4.80	15.11±4.94	<**0.001***,¶

NAFL: Nonalcoholic fatty liver; UHR: Uric acid–high-density lipoprotein ratio; *: One-way ANOVA test; †: Groups 1–2, p<0.001; Groups 1–3, p=0.001; ‡: Groups 1–2, p=0.004; Groups 1–3, p<0.001; Groups 2–3, p<0.001; §: All groups, p<0.001; ‖: Groups 1–3, p=0.001; Groups 2–3, p=0.012; ¶: Groups 1–2, p=0.001; Groups 2–3, p=0.001.

Table 3. Laboratory parameters and mean age of participants in study groups according to the existence of NAFL

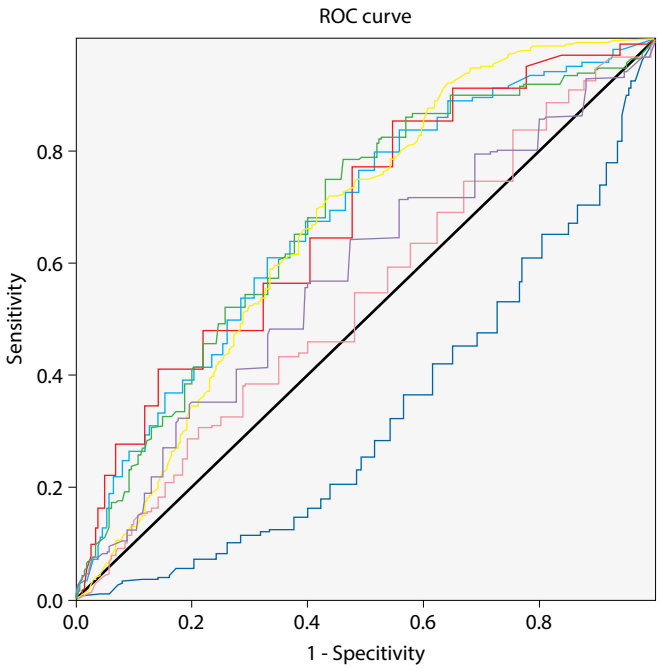
	NAFL+ (n=314)		NAFL– (n=261)		p
	n	%	n	%	
Diabetes mellitus	69	22.0	19	7.3	<**0.001***
Hypertension	103	32.8	46	17.6	<**0.001***
Coronary artery disease	25	8.0	9	3.5	**0.032***
Hyperlipidemia	26	8.3	6	2.3	**0.002***
Chronic obstructive pulmonary disease	16	5.1	7	2.7	0.103

NAFL: Nonalcoholic fatty liver; *: Chi-squared test.

Of the total 575 patients enrolled in the study, 303 (52.7%) were males and 272 (47.3%) were females. The mean age of the participants was 48.22±14.18 years (males 45.94±13.96, females 50.75±14.03). NAFL was found in 314 (54.6%) participants. Fatty liver was evaluated as grade 1 in 158 (27.5%), grade 2 in 123 (21.4%), and as grade 3 in 33 (5.7%).

Hypertension was the most frequent concomitant disease among the participants. Except for chronic obstructive pulmonary disease, all concomitant diseases were more frequent in the NAFL group (Table 3).

ROC analysis revealed that, among the other parameters inspected in the study, the most effective parameter to



	AUC	95% CI	p
Erythrocyte sedimentation rate	0.56	0.52–0.60	<0.018
C-reactive protein	0.67	0.63–0.71	<0.001
Uric asid	0.69	0.65–0.72	<0.001
Aspartate aminotransferase	0.60	0.56–0.64	<0.001
Alaline aminotransferase	0.68	0.65–0.72	<0.001
Uric asid to high density lipoprotein ratio	0.71	0.67–0.74	<0.001
High density lipoprotein	0.34	0.30–0.38	<0.001

AUC: Area under curve; CI: Confidence interval.

Figure 1. Receiver operating characteristic curve analysis of study parameters for predicting NAFL.

AUC: Area under curve; CI: Confidence interval; NAFL: Nonalcoholic fatty liver.

predict NAFL was UHR. A UHR value of 9.47 revealed a sensitivity of 78% and a specificity of 53% (Fig. 1).

Discussion

The results of the study conclude that UHR can be used to predict the existence and severity of NAFL. There was no association between vitamin B12 levels and NAFL. UHR is superior to other inflammatory markers in the diagnosis of metabolic syndrome.[13] Fatty liver is a well-known companion to metabolic syndrome.[17] This presumption brings the idea that UHR might also be elevated in fatty liver. There are few studies evaluating the relationship between UHR and NAFL in the literature. In a study from China, 6285 patients whose body mass index was below 24 were examined, and UHR was found to be associated with NAFL.[14] Another study compared 60 patients who were diagnosed with NAFL with 57 healthy participants who applied to the hospital for routine checkup examinations. UHR in the NAFL group was significantly higher than in the control group.[15] The present study reports that UHR values are significantly higher in the NAFL group than in the group that did not have NAFL. In addition, distinguished from the aforementioned studies, the present study reports that UHR is also associated with the severity of NAFL. As inflammation developed on the basis of NAFL results in an increase in uric acid levels and a decrease in HDL levels, UHR increases eventually.

Uric acid is an end product of purine metabolism produced mainly in the liver. The relationship between uric acid and NAFL was first reported in 2002.[18] Some other studies supported this finding.[11,12] Increased uric acid levels trigger the development of fatty liver by increased oxidative stress, impaired fructose metabolism, and increased insulin-resistance mechanisms.[19] In concordance with these studies, the present study reports elevated uric acid levels in patients with fatty liver.

HDL levels were reported to be decreased in NAFL.[20] In a previous study conducted by the authors of this study, 329 patients were inspected. Advanced age and increased ALT and AST levels were found to be associated with the existence of NAFL.[21] AST and ALT levels are closely related to liver injury and are markers that reflect increased inflammation and are associated with fatty liver.[22] Congruently, the present study reports the association between NAFL and decreased HDL levels, advanced age, and increased AST and ALT levels.

Zhu et al.[23] reported that increased CRP levels were associated with NAFL. The present study also reveals similar results: CRP levels were higher in the NAFL group, and as the severity of NAFL increased, CRP levels also increased correlatively. Because inflammation has an important role in the pathogenesis of NAFL, increased CRP levels are expected in severe NAFL.[3,4] In addition, as the severity of NAFL increases, the magnitude of concomitant inflammation also increases.[5]

This study has some limitations. First, it has a retrospective design. The existence of fatty liver was determined by ultrasonography, which has low sensitivity in the diagnosis of fatty liver. However, it has the advantage of simplicity and availability even in basic health care facilities. It is also a noninvasive procedure. Another issue is the ethical issue that prevents the golden standard procedure of liver

biopsy from being used easily in evaluating fatty liver. The unavailability of ultrasound elastography in the radiology unit was also a handicap of this study. One important limitation of this study is that UHR levels were not followed after treatment. Changes in UHR levels after proper treatment for NAFL may reveal important findings.

Conclusion

UHR was found to be the most effective inflammatory marker in this study to predict the existence and severity of fatty liver. UHR may be a noninvasive, available, and very simple inflammatory marker that may predict the existence of NAFL in suspected individuals. Further studies in which the existence of NAFL was confirmed with liver biopsies are necessary to evaluate the efficiency of UHR in NAFL. In addition, prospective studies that evaluate the changes in UHR levels after treatment for NAFL may bring important information for the efficacy of this novel marker.

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