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RESEARCH

Insulin resistance and cardiovascular risk factors in childhood metabolic dysfunction-associated steatotic liver disease

Çocukluk çağı metabolik disfonksiyonla ilişkili yağlı karaciğer hastalığında insülin direnci ve kardiyovasküler risk faktörleri

Meltem Gümüş[1], Alaaddin Yorulmaz[2], Hakan Candan[3], Anna Carina Ergani[1], Reyhan Kaya[1], Fuat Buğrul[4], Hüsamettin Vatansev[5], Halil Haldun Emiroğlu[1]

[1]Selçuk University Medical School, Department of Pediatric Gastroenterology, [2]Department of Pediatrics, [5]Department of Biochemistry, Konya, Turkey
[3]Department of Pediatri, Beyhekim Hospital, Konya, Turkey
[4]Department of Pediatric Endocrinology, Necip Fazıl City Hospital, Kahramanmaraş, Turkey

Abstract

Purpose: The objectives of the present study were to develop biochemical indices as a more practical way for the early diagnosis of cases with suspected metabolic dysfunction-assosiated steatotic liver disease (MASLD) and to develop easy biomarkers to enable clinicians to recognize MASLD in obese children.
Materials and Methods: A total of 90 patients who had MASLD and 70 healthy volunteering children between the ages of 6-18 who were diagnosed with reference to the ESPGHAN 2012 Guideline between January 2020 and March 2023 were included. Age, gender, Anthropometric measurements and biochemical analysis were determined. Some Biochemical ratios such as HOMA-IR, HOMA-β, FGIR, QUICKI, AIP etc were calculated.
Results: There were 40 (44.4%) girls and 50 (55.6%) boys in the patient group.A positive correlation was found between AST, GGT, TSH, LDL, TG, total cholesterol, HDL, FAS, insulin, HOMA-IR, HOMA-β, QUICKI score, FGIR, MHR, LHR, LKR, THR ALT/AST ratios and Systemic Immun Inflammatory Index values in the analysis. The ROC analysis results of the HOMA-IR value was taken as 2.94, the specificity of the diagnostic value was found to be 52.20% and the sensitivity was 80.0%.
Conclusion: MASLD has an increasing trend today.Based on the design of the present study, it was concluded that almost all of the biochemical parameters and biomarkers obtained are among the most accurate and useful indices to determine MASLD and IR and predict complications.
Keywords: Metabolic-dysfunction associated liver disease, biomarker, child

Öz

Amaç: Bu çalışmanın amaçları: Metabolik disfonksiyon-ilişkili yağlı karaciğer hastalığı (MASLD) varlığından şüphelenilen olguların erken tanısı için daha pratik bir yol olması amacıyla biyokimyasal indeksler geliştirmek ve obez çocuklarda MASLD gibi önemli bir hastalığı tanımamızı sağlayacak kolay biyobelirteçler geliştirmektir.
Gereç ve Yöntem: Ocak 2020-Mart 2023 tarihleri arasında ESPGHAN 2012 kılavuzu referans alınarak tanı konulan 6-18 yaş arası MASLD tanısı konulan 90 hasta ve 70 sağlıklı gönüllü çocuk dâhil edilmiştir. Yaş, cinsiyet, antropometrik ölçümler ve biyokimyasal analizler belirlendi. HOMA-IR, HOMA-β, FGIR, QUICKI, AIP, vb. gibi bazı biyokimyasal oranlar hesaplandı.
Bulgular: Hasta grubunda 40 (%44) kız ve 50 (%55.6) erkek vardı. AST, GGT, TSH, LDL, TG, Total Kolesterol, HDL, FAS, İnsülin, HOMA-IR, HOMA-β, QUICKI skoru, FGIR, MHO, LHO, LKO, THO, AST/ALT oranları ve Sistemik immun inflamatuar indeks değerleri arasında pozitif korelasyon bulunmuştur. HOMA-IR değerinin ROC analizi sonuçları 2,94 olarak alınmış, tanı değerinin özgüllüğü %52,20 ve duyarlılığı %80,0 olarak bulunmuştur.
Sonuç: MASLD günümüzde giderek artan bir seyir göstermektedir. Biyokimyasal parametrelerin ve elde ettiğimiz biyobelirteçlerin MASLD ile insülin direncini belirlemek ve komplikasyonları tahmin etmek için doğru ve faydalı indeksler arasında olduğu sonucuna varılmıştır.

Anahtar kelimeler: Metabolik disfonksiyon- ilişkili yağlı karaciğer hastalığı, biyobelirteç, çocuk

Address for Correspondence: Meltem Gümüş, Department of Pediatric Gastroenterology, Selçuk University Medical School, Konya, Turkey **E**-mail: meltemdorum@gmail.com
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INTRODUCTION

The condition of hepatic steatosis, hepatocyte damage, liver inflammation, and fibrosis, which has long been associated with overweight or obese people, was published in 1980 by Jurgen Ludwig under the term 'non-alcoholic steatohepatitis'. It was thought that this definition did not fully elucidate the etiology, was stigmatizing and contributed to health inequalities. In the article published by Eslam et al. in 2020, the terminology of metabolic dysfunction-related steatotic liver disease (MASLD) was proposed. The nomenclature was changed in 2023 by the Association for the Study of Liver Diseases (AASLD) and the European Association for the Study of Liver Diseases (EASL) in the hope that this would improve awareness and patient identification. While the developing world is still trying to find ways and means to decrease the burden of childhood malnutrition, it has been thrown unprepared into the childhood obesity epidemic[1]. According to the 2016 World Health Organization (WHO) data, it was predicted that more than 340 million children between the ages of 5 and 19 and more than 42 million under the age of 5 were obese[2]. In a study that was conducted in Turkey, 8.2% of the children who were aged 6-18 years were obese, 14.3% were slightly obese, 5.9% of children under 5 years old were obese, and 14.6% were overweight[3]. It is already known that childhood obesity is a serious risk factor for adult obesity[1].

Obesity increases the risks of Cardiovascular Disease (CVD) and Diabetes Mellitus (DM) by triggering Insulin Resistance and impaired glucose tolerance, leading to hypertension, dyslipidemia, and MASLD[4].

MASLD is a chronic liver disease detected frequently in the childhood age group as the accumulation of more than 5% fat in the liver cells without metabolic or autoimmune diseases, and chronic drug and substance use[5]. It has been reported that 5-20% of patients develop Steatohepatitis, and 10-20% of these progress to Fibrosis[6]. A Liver Biopsy is recommended as the gold standard for the diagnosis and staging of Fibrosis in patients who have MASLD. It is difficult to apply in children because it is an invasive procedure[7]. There is a need for improvement in non-invasive screening tests based on easily accessible data in clinical practice.

It is vital to recognize the complications which may emerge because of obesity and to intervene and prevent the problems that may follow. Of course, the etiological investigation of obesity and its complications necessitates the attention of researchers, and for this purpose, every meaningful effort is needed to provide detailed scientific data and analyses on the prevention and care of obesity. The objectives of the present study were; To develop anthropometric and biochemical indices as a more practical way for the early diagnosis of cases with suspected MASLD and to develop easy biomarkers to enable clinicians to recognize an important disease such as MASLD in obese children in hospital conditions.

MATERIALS AND METHODS

Sample

A total of 90 patients who had MASLD and 70 healthy volunteering children between the ages of 6-18 who were diagnosed with reference to the ESPGHAN 2012 Guideline, Pediatric Hepatology Clinic of the Faculty of Medicine of Selçuk University between January 2020 and March 2023 were included in the present study. Two expert hepatologists determined the suitability of the patients for the study. Patients included who were diagnosed with MASLD, detected to have adiposity on Ultrasonography, with excluded autoimmune, metabolic, and infectious causes, and who were not known to have drug and toxin exposure. The control group consisted of healthy children who met the inclusion criteria after semi-structured diagnostic interviews.

The study was carried out after receiving approval from Ethical Committee of the Medical Faculty of Selçuk University (number: E-70632468-050.01.04-556898. 18.07.2023/360).

Anthropometric measurements

The gender and age characteristics of all patients who were included in the study were recorded. By performing physical examinations and using an electronic scale, the Body Weight (BW) was calculated after the shoes were removed with the least clothes in kilogram (kg), height was measured feet together and bare in Harpenden Stadiometer in centimeters (cm) when back, hips and heels touching each other and standing upright; the BMI was calculated with the formula of Weight/Height^2 (kg/m^2); and the abdominal circumference was

measured with a tape measure at the widest point in cm. Age and gender-appropriate growth curves that were prepared for Turkish children were used to determine BW and height percentiles[8]. The BMI values were compared with age- and gender-appropriate BMI curves. A BMI of 19-24.9 was considered normal, a BMI of 25-30 was considered overweight, and a BMI of ≥30 was considered obese. Those with BMI ≥ 95th percentile for age and gender were considered obese. Standard Deviation Scores (SDS) of height, weight, and BMI values were also calculated.

Biochemical analysis

Blood samples were taken after 8 hours of fasting in the patient and control groups. Fasting Blood Glucose (FBC), Triglyceride (TG), serum total cholesterol, LDL cholesterol, HDL cholesterol, AST, ALT, and uric acid levels were determined with the ROCHE Cobas C-702 Device (Spectrophotometric Method), Fasting blood insulin was studied in a ROCHE Cobas 8000 e602 Autoanalyzer (Electrochemiluminescent Method) in the Biochemistry Laboratory of the University Medical Faculty Hospital.

In obese children, serum total cholesterol level ≥200mg/dL, triglyceride level ≥100mg/dL for those under 10 years of age, ≥130mg/dL for those over 10 years old, ≥130mg/dL LDL cholesterol level or ≤35mg/dL HDL cholesterol level was considered dyslipidemia[9].

Calculation of biochemical biomarkers

1. FGIR was calculated by dividing Fasting Glucose (mg/dL) by Fasting Insulin U/mL.
2. The Homeostasis Model Assessment (HOMA-IR) score used for Insulin Resistance in obese children and adolescents was calculated with the following formula as defined by Matthews[10].

   HOMA-IR: (Fasting serum Insulin [μU/mL] X Fasting Plasma Glucose [mmol/L]/22.5 or

   HOMA-IR: Fasting Insulin (mU/L) x Fasting Glucose (mg/dL)/405.

   Prepubertal and pubertal reference values determined by Kurtoğlu et al. were used for HOMA-IR. Values above 2.5 in the prepubertal age group and above 3.16 in the pubertal age group were considered positive for Insulin Resistance[11].
3. HOMA β Cell Index was calculated with the following formula as defined by Matthews.

   HOMA β Cell (%) Index=(20xFasting Serum Insulin [μU/mL])/(Fasting Plasma Glucose [mmol/L]-3.5)[10].
4. QUICK1-Quantitative Insulin Sensitivity Check Index was calculated as follows[12].

   QUICK1-1/ Log Fasting Plasma Glucose (mg/dL)+Log Fasting Insulin (mU/L).
5. Atherogenic Index and Plasma (AIP) values were shown with 4 different formulas.

   AIP1: Log (TG/HDL). An AIP value of <0.11 was considered low risk, a value between 0.11 and 0.21 was considered medium risk, and a value >0.21 was considered high risk according to the references of previous studies[13].
6. AIP2: (TG-HDL)/HDL[14].
7. AIP3 was calculated as TG/HDL.
8. AIP4 was calculated as LDL/HDL[15].
9. TyG Index=Log(Fasting Triglyceride [mg/dL]x Fasting Glucose [mg/dL])/2 [16].
10. TyG*BMI Index was obtained by multiplying the TyG Index and BMI.
11. THR was obtained by dividing the Triglyceride Value by the HDL cholesterol value.
12. LCR was obtained by dividing the LDL cholesterol value by total cholesterol.
13. ALT/AST was obtained by dividing the ALT value by the AST value.
14. Systemic Immun Inflammatory Index (SII): platelet count × neutrophil-to-lymphocyte ratio

Radiological examination

In all cases, as a diagnostic method, USI was initially performed by using a convex 3.5-5.0 MHz Probe on the same device (GE, LOGIC 500). Ultrasonographia Imaging (USI) was performed blindly by a single experienced radiologist, unaware of the purpose of the study and laboratory values. The researchers initially applied USI as a diagnostic method in all cases.

Statistical analysis

The data were entered into the SPSS 23.0 program. The findings on the categorical variables were planned to be presented as frequency (n) and percentage (%). In the descriptive statistics, normally distributed parameters were shown as Mean ± Standard Deviation for the continuous variables, while numerical parameters that did not show normal distribution were shown as Median (minimum-maximum). The categorical variables were rpresented as numbers and percentages. The compliance with normal distribution was evaluated with the Kolmogorov-Smirnov Test of Normality. In the comparison of numerical parameters in the two groups, the Student T-Test was used for those with normal distribution, and the Mann-Whitney U Test was used for those without normal distribution. The Kruskal Wallis Test was used to compare the medians in groups of more than two. The ANOVA Test was used to compare the numerical parameters that had normal distribution in more than two independent groups and the Tukey Test was used for Post-Hoc Analysis. The Chi-Square or Fischer's Exact Tests were used to compare the categorical variables. The correlation of the numerical variables was evaluated with the Spearman Correlation Test and $p<0.05$ was accepted as the significance level in all analyses.

RESULTS

A total of 160 patients, 90 of whom had MASLD and 70 were in the control group, were included in the study. Among the patients who were included in the study, 40 (44.4%) were female and 50 (55.6%) were male. When the gender distribution of the patients who were included in the study was examined in the patient and control groups, no statistically significant differences were detected (p:0.185). Distribution of demographic characteristics, anthropometric measurements and biochemical values of the patients participating in the study according to the patient and control groups are shown in Table 1.

When the biochemical values were examined, although the ALT value of the patients was found to be 30.11±22.54 U/L in the patient group, it was 13.46±6.29 U/L in the control group. When the ALT values were compared in the patient and control groups, statistically significant differences were detected ($p<0.001$). When the AST, GGT, TSH, LDL, TG, total cholesterol, HDL, FAS, insulin, HOMA-IR, HOMA-β, QUICKI score, FGIR, MHR, LHR, LKR, THR, ALT/AST ratios, and SII values of the patients were compared to the control group, statistically significant differences were detected. However, no statistically significant differences were detected between the groups in terms of albumin, HbA1c, vitamin B12, free T4, APRI, and PNI scores. The distribution of the values of the biochemical and biochemical indices of the patients according to the control and patient groups is given in Table 2.

When the HOMA-β value of the patients who were included in the study was compared according to age groups, it was 173.88±106.32 under 10 years of age, 247.86±217.02 in children aged 11-15, and 246.67±184.68 in children over 15 years of age.When the HOMA-β value was compared according to age groups, no significant differences were found (p:0.385). Although the HOMA-β value was 151±164.97 in patients with a BMI below 24.9, it was 248.35±165.49 in children with a BMI between 25.0-29.9, and 335.12±221.37 in children with a BMI above 30. When HOMA-β levels were compared statistically according to BMI groups, significant differences were found (p:<0.001). The distribution of biochemical index values of patients according to BMI are given in Table 3.

When liver USIs were examined, 70 (43.8%) of the patients were found to be Grade 0, 79 (49.4%) were Grade 1, and 11 (6.9%) were Grade 2-3. When the HOMA-β level of the patients was examined, the grade value indicating the level of steatosis measured with liver USI was found to be 144.77±113.21 in patients with Grade 0, 294.95±193.37 in patients with Grade 1, and 361.71±308.93 in patients with Grade 2-3. When the levels of HOMA-β and liver hepatosteatosis were compared statistically, significant differences were detected between the groups (p:<0.001). The biochemical indices, BMI values, and distribution of the patients included in the study according to liver grading are given in Table 4.

When the ROC analysis results of the values of the biochemical indexes for the diagnostic value of patients with MASLD were examined, when the cut-off value of the HOMA-IR value was taken as 2.94, the specificity of the diagnostic value was found to be 52.20% and the sensitivity was 80.0%. The Area Under the Curve (AUC) was measured as 0.676 (95% CI: 0.967-1.0). The positive likelihood ratio was calculated as 1.67. The ROC Analysis results of the biochemical index values for the diagnostic value of patients with MASLD are given in Table 5.

Multivariate Logistic Regression Analysis was made for the diagnostic value of patients who were diagnosed with MASLD. The parameters that were independent risk factors in the Univariate Analysis were included in the Multivariate Logistic Regression Analysis Model for the diagnosis of MASLD. Four different models were created in this respect. Odds Ratio, specificity (%), sensitivity (%), and Nagelkerke R Square Values were calculated for each model. The Logistic Regression Analysis results are given in Table 6.

Table 1. Distribution of demographic characteristics, anthropometric measurements and biochemical values of the patients participating in the study according to the patient and control groups.

Variable		**Patient Group**		**Control Group**		
		n	**%**	**N**	**%**	**p**
Gender	Boy	50	55.6	33	47.1	0.185
	Girl	40	44.4	37	52.9	
Age Group	<10	16	55.2	13	44.8	0.513
	10.1-15.0	38	52.1	35	47.9	
	>15.1	36	62.1	22	37.9	
BMI	<24.9	10	16.1	52	83.9	**<0.001**
	25.0-29.9	37	82.2	8	17.8	
	>30.0	41	91.1	4	8.9	
Trygliceride (mg/dL)	Normal	26	31.7	56	68.3	**<0.001**
	High	64	85.3	11	14.7	
Cholesterol (mg/dL)	Normal	62	48.8	65	51.2	**<0.001**
	High	28	93.3	2	6.7	
LDL (mg/dL)	Normal	75	53.6	65	46.4	**0.005**
	High	15	88.2	2	11.8	
HDL (mg/dL)	Low	17	68.0	8	32.0	0.170
	Normal	73	55.3	59	44.7	
ALT (u/L)	Normal	67	53.6	58	46.4	**<0.001**
	High	23	95.8	1	4.2	
HOMA-IR	<2.5	14	31.1	31	68.9	**<0.001**
	>2.5	76	66.7	38	33.3	
		Mean±SD	Median (Min-max)	Mean±SD	Median (Min-max)	p
Age (year)		13.31 ± 3.08	14.01 (7.02 - 17.8)	12.76 ± 2.93	13.06 (7.1 - 17.02)	0.204
Body Weight (kg)		82.98 ± 25.09	83.6 (36 - 152)	53.35 ± 16.5	51.95 (22.8 - 88.6)	**<0.001**
BW Persentil		98.34 ± 4.91	100 (61 - 100)	60.54 ± 34.4	70 (1 - 100)	**<0.001**
BW SDS		3.31 ± 1.57	2.92 (0.27 - 9.08)	0.45 ± 1.46	0.53 (-2.48 - 3.72)	**<0.001**
Height (cm)		161.04 ± 15.48	163.2 (120 - 193.3)	154.85 ± 15.24	157.5 (123 - 182)	**0.013**
Height Persentil		72.05 ± 27.48	83 (10 - 100)	51.18 ± 33.03	51.5 (1 - 100)	**<0.001**
Height SDS		0.97 ± 1.32	0.94 (-1.29 - 5.61)	0.06 ± 1.29	0.04 (-2.59 - 3.56)	**<0.001**
BMI		31.36 ± 6.54	29.55 (22.11 - 52.9)	21.65 ± 4.43	20.94 (14.2 - 35.49)	**<0.001**
BMI Persentil		98.01 ± 3.95	100 (76 - 100)	61 ± 34.5	72 (1 - 100)	**<0.001**
BMI SDS		2.78 ± 1.11	2.66 (0.72 - 6.93)	0.49 ± 1.31	0.58 (-2.86 - 3.56)	**<0.001**

Table 2. Distribution of biochemical values and values of biochemical indices according to patients and control group

	Patient Group		Control Group		
	Mean±SD	**Median (Min-max)**	**Mean±SD**	**Median (Min-max)**	**p**
Uric acid (mg/dL)	5.451 ± 1.369	5.4 (3.1 - 10.10)	4.269 ± 1.10	4.0 (2.30 - 8.7)	<0.001
ALT (u/L)	30.112 ± 22.541	22.9 (6.7 - 149)	13.462 ± 6.296	13 (6.3 - 48.9)	<0.001
AST(u/L)	23.364 ± 11.958	21.05 (11 - 93.9)	18.923 ± 5.104	18.2 (9.6 - 34)	<0.001
GGT (U/L)	25.494 ± 24.696	18 (7.0 - 176)	11.39 ± 4.327	10 (6.0 - 28)	<0.001
Albümin (g/dL)	4.684 ± 0.322	4.7 (3.3 - 5.4)	4.662 ± 0.247	4.7 (3.9 - 5.15)	0.383
LDL (mg/dL)	96.028 ± 27.901	96.16 (34.16 - 189)	71.892 ± 19.928	68.2 (32.6 - 140.8)	<0.001
Trygliceride (mg/dL)	152.121 ± 60.561	143.5 (53 - 324.2)	92.355 ± 34.218	85 (35.6 - 166)	<0.001
Cholesterol (mg/dL)	168.817 ± 31.509	167.55 (104.1 - 265)	140.936 ± 23.213	137 (94 - 218)	<0.001
HDL (mg/dL)	44.006 ± 10.246	43.25 (28.2 - 92)	49.582 ± 11.076	50 (27.8 - 75)	<0.001
HBA1C	5.353 ± 0.375	5.3 (4.7 - 7)	5.394 ± 0.317	5.4 (4.6 - 6)	0.095
Glucose (mg/dL)	95.278 ± 14.892	90.05 (71.6 - 154)	102.918 ± 14.102	105.705 (74.9 - 131.24)	<0.001
İnsülin (U/mL)	23.323 ± 14.014	20.5 (6.71 - 78.1)	14.336 ± 9.404	11.4 (2.88 - 41.5)	<0.001
FGIR	5.434 ± 2.922	4.781 (1.39 - 12.809)	10.834 ± 7.034	8.942 (2.056 - 28.031)	<0.001
HOMA-IR	5.612 ± 3.916	4.971 (1.31 - 24.182)	3.666 ± 2.414	2.886 (0.547 - 9.559)	<0.001
HOMA-β	303.109 ± 209.708	243.598 (62.824 - 1158.545)	144.771 ± 113.215	112.262 (26.986 - 669.355)	<0.001
QUICKI	0.307 ± 0.025	0.30 (0.25 - 0.37)	0.329 ± 0.035	0.33 (0.28 - 0.43)	<0.001
AIP1	0.155 ± 0.219	0.184 (-0.366 - 0.641)	-0.108 ± 0.219	-0.109 (-0.566 - 0.407)	<0.001
AIP2	167.817 ± 31.509	166.55 (103.1 - 264)	139.936 ± 23.213	136 (93 - 217)	<0.001
AIP3	3.964 ± 0.891	3.932 (1.718 - 6.662)	2.971 ± 0.842	2.754 (1.887 - 6.939)	<0.001
AIP4	2.262 ± 0.731	2.184 (0.564 - 4.562)	1.537 ± 0.667	1.392 (0.687 - 4.77)	<0.001
TyG	3.822 ± 0.18	3.835 (3.325 - 4.284)	3.645 ± 0.177	3.668 (3.217 - 4.01)	<0.001
TyG*BMI	119.961 ± 26.088	113.23 (80.132 - 203.151)	79.033 ± 17.51	76.236 (48.647 - 136.981)	<0.001
LDL/Cholesterol ratio	0.561 ± 0.085	0.583 (0.313 - 0.713)	0.504 ± 0.074	0.507 (0.322 - 0.687)	<0.001
Trygliceride/HDL ratio	3.691 ± 1.786	3.502 (0.986 - 10.032)	2.025 ± 1.072	1.785 (0.622 - 5.845)	<0.001
ALT/AST ratio	1.276 ± 0.742	1.074 (0.28 - 6.278)	0.727 ± 0.329	0.667 (0.324 - 2.778)	<0.001

ALT: Alanin aminotransferase; AST: Aspartat Aminotransferase; GGT: Gama Glutamil TransferaseL; LDL: Low Dansity Lipoprotein HDL: High Dansity Lipoprotein, FGIR: Fasting Glucose/fasting İnsulin; HOMA-IR: The Homeostasis Model Assesment Score; QUICK: Quantitative Insulin Sensitivity Check İndex; AIP: Aterogenic Index and Plasma values; TyG index:Log(Fasting triglyceride xFasting Glucose; BMI: Body Mass Index;

Table 3. Distribution of biochemical index values of patients according to BMI

BMI	<24.9		25.0-29.9		>30.0		
	Mean±SD	Median (Min-max)	Mean± SD	Median (Min-max)	Mean±SD	Median (Min-max)	p
HBA1C	5.38 ± 0.29	5.4 (4.7 - 6)	5.33 ± 0.31	5.3 (4.6 - 6.1)	5.38 ± 0.46	5.3 (4.7 - 7)	0.535
Glucose	100.73 ± 14.64	103.98 (74 - 125.5)	96.54 ± 12.88	95.1 (74 - 131.24)	99.03 ± 17.36	94 (71.6 - 154)	0.254
İnsülin	13.27 ± 8.76[a]	10.05 (2.88 - 35.4)	19.55 ± 9.19[b]	17.7 (6.71 - 42.1)	27.79 ± 16.46[c]	23.3 (7.55 - 78.1)	<0.001
FGIR	11.34 ± 7.16[a]	9.75 (2.09 - 28.03)	6.15 ± 3.02[b]	5.63 (2.06 - 13.7)	4.64 ± 2.42[b]	3.9 (1.39 - 11.51)	<0.001
LDL	74.63 ± 25.06[a]	72.2 (32.6 - 140.8)	94.78 ± 31.87[b]	85 (45.66 - 189)	94.01 ± 20.23[b]	92.34 (46.28 - 138)	<0.001
Trygliceride	97.08 ± 38.55[a]	89.3 (35.6 - 188)	151.63 ± 66.15[b]	147 (39 - 324.2)	143.19 ± 59.9[b]	139 (65 - 321)	<0.001
Cholesterol	143.6 ± 28.95[a]	138.5 (94 - 218)	168.99 ± 34.92[b]	163.3 (112.8 - 265)	165.8 ± 23.91[b]	165 (125.3 - 221)	<0.001
HDL	49.29 ± 11.46[a]	49 (27.8 - 75)	45.38 ± 11.76	43.6 (29.3 - 92)[b]	43.72 ± 8.45[b]	43.8 (28.2 - 66)	0.031
HOMA-IR	3.3 ± 2.15[a]	2.48 (0.55 - 8.38)	4.66 ± 2.26[b]	4.17 (1.32 - 10.91)	6.97 ± 4.77[c]	5.73 (1.61 - 24.18)	<0.001
HOMA-β	155.26 ± 165.15[a]	112.66 (26.99-1158.55)	244.42 ± 165.17[b]	184.62 (52.76-710.4)	326.98 ± 209.38[c]	289.38 (86 - 1000.47)	<0.001
QUICKI	0.33 ± 0.03[a]	0.33 (0.28 - 0.43)	0.31 ± 0.02[b]	0.31 (0.27 - 0.37)	0.30 ± 0.02[b]	0.30 (0.25 - 0.36)	<0.001
AIP1	-0.09 ± 0.23[a]	-0.1 (-0.57 - 0.41)	0.13 ± 0.25[b]	0.15 (-0.52 - 0.64)	0.13 ± 0.22[b]	0.15 (-0.37 - 0.54)	<0.001
AIP2	142.6 ± 28.95[a]	137.5 (93 - 217)	167.99 ± 34.92[b]	162.3 (111.8 - 264)	164.8 ± 23.91[b]	164 (124.3 - 220)	<0.001
AIP3	3.04 ± 0.89[a]	2.93 (1.72 - 6.94)	3.89 ± 1.05[b]	3.85 (2.34 - 6.66)	3.91 ± 0.8[b]	3.94 (1.9 - 5.5)	<0.001
AIP4	1.6 ± 0.72[a]	1.48 (0.56 - 4.77)	2.2 ± 0.88[b]	2.06 (1.04 - 4.56)	2.23 ± 0.6[b]	2.12 (0.7 - 3.59)	<0.001
TyG	3.65 ± 0.18[a]	3.67 (3.22 - 4.01)	3.82 ± 0.2[b]	3.83 (3.29 - 4.25)	3.81 ± 0.18[b]	3.81 (3.39 - 4.28)	<0.001
TyG*BMI	74.88 ± 11.63[a]	75.6 (48.65 - 93.84)	105.37 ± 8.09[b]	106.9 (84.73 - 120.76)	138.28 ± 23.49[c]	134.29 (101.04 - 203.15)	<0.001
THR	2.15 ± 1.2[a]	1.83 (0.62 - 5.85)	3.62 ± 1.98[b]	3.27 (0.7 - 10.03)	3.48 ± 1.7[b]	3.23 (0.99 - 7.89)	<0.001
LCR	0.51 ± 0.08[a]	0.5 (0.32 - 0.69)	0.55 ± 0.09[b]	0.56 (0.31 - 0.71)	0.56 ± 0.07[b]	0.57 (0.37 - 0.67)	<0.001
ALT/AST Ratio	0.75 ± 0.39[a]	0.66 (0.32 - 2.78)	1.23 ± 0.86[b]	1.05 (0.4 - 6.28)	1.29 ± 0.6[b]	1.19 (0.28 - 3.89)	<0.001
Uric Acid	4.32 ± 1.18[a]	4 (2.3 - 8.7)	5.36 ± 1.5[b]	5.35 (2.6 - 10.1)	5.36 ± 1.27[b]	5.1 (3.7 - 8.7)	<0.001
ALT	14.65 ± 9.93[a]	12.2 (6.3 - 67.3)	28.01 ± 19.94[b]	22.5 (7 - 113)	30.14 ± 24.91[b]	23 (7 - 149)	<0.001
AST	19.28 ± 5.22	18.2 (9.6 - 34)	23.13 ± 12.97	21.1 (11 - 93.9)	22.28 ± 11.27	20 (11.4 - 86)	0.158
GGT	11.72 ± 4.82[a]	10 (6 - 28)	20.05 ± 10.81[b]	18 (8 - 60)	29.7 ± 31.96[c]	18 (7 - 176)	<0.001

BMI: Body Mass Index; FGIR: Fasting Glucose/fasting İnsulin; LDL: Low Dansity Lipoprotein, HDL: High Dansity Lipoprotein, HOMA-IR: The Homeostasis Model Assesment Score; QUICK: Quantitative Insulin Sensitivity Check İndex; AIP: Aterogenic Index and Plasma values; TyG index:Log(Fasting triglyceride xFasting Glucose; THR: Triglyceride/HDL Cholesterol; LCR:LDL Cholesterol/total Cholesterol; ALT: Alanin aminotransferase ; AST: Aspartat Aminotransferase; GGT: Gama Glutamil Transferase,

Table 4. The biochemical indices, BMI values, and distribution of the patients included in the study according to liver grades

	Grade 0 n:70 (%43.8)		Grade 1 n:79 (%49.4)		Grade 2-3 n: 11 (%6.9)		
	Mean±SD	Median (Min-max)	Mean±SD	Median (Min-max)	Mean±SD	Median (Min-max)	p
BW SDS	0.45 ± 1.46	0.53 (-2.48 - 3.72)	3.2 ± 1.45	2.89 (0.27 - 8.19)	4.1 ± 2.16	3.11 (2.11 - 9.08)	<0.001
BMI SDS	0.49 ± 1.31	0.58 (-2.86 - 3.56)	2.72 ± 1.05	2.66 (0.72 - 6.93)	3.18 ± 1.48	2.31 (1.85 - 6.5)	<0.001
HBA1C	5.39 ± 0.32	5.4 (4.6 - 6)	5.31 ± 0.28	5.3 (4.7 - 6.1)	5.68 ± 0.7	5.6 (4.9 - 7)	0.053
Glucose (mg/dl)	102.92 ± 14.1[a]	105.71 (74.9 - 131.24)	93.74 ± 12.85[b]	90 (71.6 - 129)	106.3 ± 23.09[a]	105 (74 - 154)	<0.001
İnsülin	14.34 ± 9.4[a]	11.4 (2.88 - 41.5)	22.04 ± 12.95[b]	19.2 (6.71 - 73.8)	32.53 ± 18.24[c]	24 (17.7 - 78.1)	<0.001
FGIR	10.83 ± 7.03[a]	8.94 (2.06 - 28.03)	5.64 ± 3.01[b]	5.12 (1.39 - 12.81)	3.93 ± 1.63[b]	4.01 (1.61 - 6.44)	<0.001
LDL (mg/dL)	71.89 ± 19.93[a]	68.2 (32.6 - 140.8)	95.3 ± 27.97[b]	95.92 (34.16 - 189)	101.24 ± 28.12[b]	107.56 (64 - 161)	<0.001
Trygliceride (mg/dL)	92.36 ± 34.22[a]	85 (35.6 - 166)	147.19 ± 57.47[b]	142.9 (53 - 324.2)	187.5 ± 72.86[c]	188 (90 - 311)	<0.001
Cholesterol (mg/dL)	140.94 ± 23.21[a]	137 (94 - 218)	167.51 ± 31.39[b]	165 (104.1 - 265)	178.23 ± 32.25[b]	182 (136 - 233)	<0.001
HDL(mg/dL)	49.58 ± 11.08[a]	50 (27.8 - 75)	44.28 ± 10.43[b]	43.4 (28.2 - 92)	42.03 ± 9.01[b]	42.8 (31 - 54.2)	<0.001
HOMA-IR	3.67 ± 2.41[a]	2.89 (0.55 - 9.56)	5.17 ± 3.31[b]	4.31 (1.31 - 18.7)	8.81 ± 6.2[c]	6.76 (4.17 - 24.18)	<0.001
HOMA-β	144.77±113.21[a]	112.26 (26.99-669.35)	294.95±193.37[b]	240.93 (62.82-1000.47)	361.71±308.93[c]	289.38 (124.94-1158.55)	<0.001
QUICKI	0.33 ± 0.04[a]	0.33 (0.28 - 0.43)	0.31 ± 0.02[b]	0.31 (0.26 - 0.37)	0.29 ± 0.02[c]	0.29 (0.25 - 0.31)	<0.001
AIP1	-0.11 ± 0.22[a]	-0.11 (-0.57 - 0.41)	0.14 ± 0.21[b]	0.17 (-0.37 - 0.6)	0.27 ± 0.23[c]	0.31 (-0.14 - 0.64)	<0.001
AIP2	139.94 ± 23.21[a]	136 (93 - 217)	166.51 ± 31.39[b]	164 (103.1 - 264)	177.23 ± 32.25[b]	181 (135 - 232)	<0.001
AIP3	2.97 ± 0.84[a]	2.75 (1.89 - 6.94)	3.91 ± 0.89[b]	3.86 (1.72 - 6.66)	4.34 ± 0.82[b]	4.37 (2.7 - 5.7)	<0.001
AIP4	1.54 ± 0.67[a]	1.39 (0.69 - 4.77)	2.23 ± 0.73[b]	2.18 (0.56 - 4.56)	2.47 ± 0.72[b]	2.31 (1.37 - 3.66)	<0.001
TyG	3.65 ± 0.18[a]	3.67 (3.22 - 4.01)	3.8 ± 0.17[b]	3.82 (3.33 - 4.12)	3.96 ± 0.17[c]	3.94 (3.75 - 4.28)	<0.001
TyG*BMI	79.03 ± 17.51[a]	76.24 (48.65 - 136.98)	117.39 ± 23.18[b]	113.21 (80.13 - 199.83)	137.98 ± 37.82[c]	129.3 (91.45 - 203.15)	<0.001
THR	2.02 ± 1.07[a]	1.79 (0.62 - 5.85)	3.54 ± 1.66[b]	3.38 (0.99 - 9.11)	4.77 ± 2.31[c]	4.7 (1.66 - 10.03)	<0.001
LCR	0.5 ± 0.07[a]	0.51 (0.32 - 0.69)	0.56 ± 0.09[b]	0.58 (0.31 - 0.71)	0.56 ± 0.07[b]	0.53 (0.47 - 0.69)	<0.001
ALT/AST	0.73 ± 0.33[a]	0.67 (0.32 - 2.78)	1.22 ± 0.76[b]	1.05 (0.28 - 6.28)	1.67 ± 0.41[c]	1.63 (1.06 - 2.66)	<0.001
Uric acid	4.27 ± 1.1[a]	4 (2.3 - 8.7)	5.36 ± 1.3[b]	5.4 (3.1 - 10.1)	6.08 ± 1.76[b]	5.75 (3.8 - 8.7)	<0.001
ALT(IU/mL)	13.46 ± 6.3[a]	13 (6.3 - 48.9)	28.14 ± 22.22[b]	22.3 (6.7 - 149)	44.26 ± 20.49[c]	38.5 (17 - 92.9)	<0.001
AST(IU/mL)	18.92 ± 5.1[a]	18.2 (9.6 - 34)	23.02 ± 12.49[b]	20.6 (11 - 93.9)	25.81 ± 7.03[b]	24.2 (16 - 36)	<0.001
GGT	11.39 ± 4.33[a]	10 (6 - 28)	23.24 ± 18.9[b]	18 (7 - 124)	39.82 ± 46.4[c]	25 (15 - 176)	<0.001
Albümin	4.66 ± 0.25	4.7 (3.9 - 5.15)	4.71 ± 0.33	4.76 (3.3 - 5.4)	4.54 ± 0.2	4.51 (4.3 - 4.8)	0.079

BW: Body Weigt, BMI: Body Mass Index; FGIR: Fasting Glucose/fasting İnsulin; HOMA-IR: The Homeostasis Model Assesment Score; QUICK: Quantitative Insulin Sensitivity Check İndex; AIP: Aterogenic Index and Plasma values; TyG index:Log(Fasting triglyceride xFasting Glucose; THR: Triglyceride/HDL Cholesterol; LCR:LDL Cholesterol/total Cholesterol LCR: LDL/Cholesterol Ratio; THR: Trygliceride/HDL Ratio; ALT:Alanin Aminotransferase AST:Aspartat Aminotransferase

Table 5. ROC analysis results of biochemical values for the diagnostic value of patients with MASLD

	AUC (%95 CI)	Cut Off	p	Sensitivity (%)	Specificity (%)	+LR	- LR	PPV (%)	NPV (%)	Accuracy (%)
BW	0.832 (0.770-0.895)	67.30	<0.001	84.38	75.00	3.38	0.21	71.05	86.84	78.95
BW Percentile	0.918 (0.867-0.970)	92.15	<0.001	79.69	95.45	17.53	0.21	92.73	86.60	82.70
BW SDS	0.928 (0.884-0.971)	2.15	<0.001	90.62	80.68	8.60	0.12	77.33	92.21	78.17
BMI	0.910 (0.861-0.958)	24.31	<0.001	79.69	90.91	8.77	0.22	86.44	86.02	79.66
BMI Percentile	0.918 (0.870-0.965)	95.50	<0.001	85.94	89.77	8.40	0.16	85.94	89.77	81.93
BMI SDS	0.922 (0.878-0.966)	1.73	<0.001	85.94	89.77	8.40	0.16	85.94	89.77	81.93
İnsülin	0.725 (0.645-0.805	12.85	<0.001	56.52	81.11	2.99	0.54	69.64	70.87	62.70
FGIR	0.242 (0.166-0.318)	6.99	<0.001	37.68	25.56	0.51	2.44	27.96	34.85	23.75
LDL	0.765 (0.690-0.840)	94.47	<0.001	91.04	54.44	6.07	0.16	59.80	89.09	62.25
Trygliceride	0.809 (0.742-0.876)	110.50	<0.001	89.55	76.10	3.11	0.24	54.55	85.11	55.65
Cholesterol	0.765 (0.692-0.839)	149.50	<0.001	68.66	92.20	6.55	0.43	64.79	75.58	62.92
HDL	0.338 (0.250-0.426)	37.20	<0.001	80.60	26.67	1.10	0.73	45.00	64.86	41.61
HOMA-IR	0.676 (0.591-0.762)	2.94	<0.001	52.20	80.00	2.61	0.60	66.67	68.57	60.07
HOMA-β	0.789 (0.719-0.859)	114.57	<0.001	52.17	91.10	1.90	0.99	29.03	71.30	49.37
QUICKI	0.327 (0.241-0.413)	0.285	<0.001	10.14	85.60	0.95	1.05	35.00	55.40	44.77
AIP1	0.799 (0.730-0.868)	0.089	<0.001	82.20	66.70	3.72	0.27	64.71	83.33	73.25
AIP2	0.765 (0.692-0.839)	168.25	<0.001	91.10	70.00	5.58	0.18	57.55	88.24	67.52
AIP3	0.824 (0.754-0.893)	3.037	<0.001	71.60	87.80	5.86	0.32	81.36	80.61	80.89
AIP4	0.805 (0.733-0.877)	1.875	<0.001	83.60	73.30	4.46	0.22	70.00	85.71	77.71
TyG	0.761 (0.687-0.836)	3.75	<0.001	74.60	73.13	2.80	0.37	66.22	78.31	72.61
TyG*BMI	0.922 (0.879-0966)	91.28	<0.001	80.65	89.80	7.89	0.22	84.75	86.81	86.00
THR	0.799 (0.730-0.868)	2.82	<0.001	82.10	66.70	3.72	0.27	64.71	83.33	73.25
LCR	0.720 (0.639-0.801)	0.57	<0.001	88.10	52.20	4.37	0.29	57.00	82.46	66.24
ALT/AST	0.863 (0.801-0.925)	0.88	<0.001	89.71	80.00	7.77	0.13	77.22	91.14	84.18
ALT	0.852 (0.792-0.912)	16.10	<0.001	83.82	77.80	4.80	0.21	74.03	86.42	80.38
AST	0.641 (0.555-0.727)	21.50	0.002	73.91	48.90	1.87	0.56	51.52	70.00	58.49
GGT	0.848 (0.783-0.912)	14.50	<0.001	83.10	79.00	4.66	0.21	74.24	86.49	80.71
Uric Acid	0.765 (0.678-0.853)	4.85	<0.001	80.40	65.50	3.31	0.30	65.08	80.39	71.93

AUC: Area under the curve; 95%CI: %95 Confidence Interval; +LR: pozitivite likelihood ratio

Table 6. Logistic regression analysis results

Multivariate Analysis									Univariate Analysis	
		p	**OR (%95 CI)**	**-2 Log likelihood**	**Nagelkerke R Square**	**Accuracy**	**Sensivity**	**Specifity**	**p**	
MODEL 1	BW SDS	<0.001	4.236 (1.805-9.941)	43.487	0.817	93.1	88.4	96.6	<0.001	5.625 (3.2 - 9.998)
	FGIR	0.294	0.829 (0.584-1.177)						<0.001	0.783 (0.7 - 0.863)
	AIP1	0.043	117.247 (1.161-11843.84)						<0.001	194.031 (31.8 - 1184.006)
	ALT/AST	0.107	12.391 (0.579-265.22)						<0.001	1.258 (1.1 - 1.439)
	HOMA-IR	0.023	0.693 (0.505-0.951)						<0.001	1.008 (1 - 1.011)
	GGT	0.863	0.989 (0.868-1.)125						<0.001	1.281 (1.2 - 1.412)
	Uric Acid	0.046	2.195 (1.013-4.759)						<0.001	2.334 (1.6 - 3.473)
	Constant	0.005								
MODEL 2	BWPercentil	0.183	1.946 (0.731-5.183)	50.021	0.793	88.7	84.4	91.8	<0.001	1.218 (1.1 - 1.32)
	AIP2	0.037	1.029 (1.002-1.056)						<0.001	1.038 (1 - 1.054)
	HOMA-IR	0.813	0.971 (0.758-1.243)						<0.001	1.008 (1 - 1.011)
	TyG*BMI	0.261	1.036 (0.974-1.102)						<0.001	1.112 (1.076-1.150)
	ALT	0.033	1.116 (1.009-1.235)						<0.001	1.191 (1.116-1.271)
	Uric Acid	0.119	1.632 (0.881-3.021)						<0.001	2.334 (1.6 - 3.473)
	Constant	<0.001								
MODEL 3	BMI SDS	0.002	4.068 (1.701-9.731)	47.727	0.805	91.5	88.9	93.4	<0.001	7.964 (3.9 - 16.183)
	FGIR	0.374	0.855 (0.604-1.209)						<0.001	0.783 (0.7 - 0.863)
	AIP3	0.044	2.969 (1.256 - 8.966)						<0.001	4.094 (2.5 - 6.749)
	HOMA-IR	0.151	0.786 (0.566-1.092)						<0.001	1.008 (1 - 1.011)
	ALT/AST	0.081	14.790 (0.718-304.74)						<0.001	1.258 (1.1 - 1.439)
	Uric Acid	0.046	2.007 (1.011-3.984)						<0.001	2.334 (1.6 - 3.473)
	Constant	0.009								
MODEL 4	BMI SDS	0.002	5.302 (1.845-15.235)	42.439	0.831	92.5	91.1	93.4	<0.001	7.964 (3.9 - 16.183)
	HOMA-IR	0.038	0.700 (0.500-0.980)						<0.001	1.008 (1 - 1.011)
	HOMA-B	0.028	1.009 (1.001-1.018)						<0.001	1.008 (1.005-1.011)
	AIP4	0.006	10.017 (1.919-52.289)						<0.001	5.116 (2.8 - 9.478)
	ALT	0.186	1.053 (0.976-1.136)						<0.001	1.191 (1.116-1.271)
	Uric Acid	0.084	1.869 (0.919-3.800)						<0.001	2.334 (1.6 - 3.473)
	Constant	<0.001								

BW: Body Weigt, FGIR: Fasting Glucose/fasting İnsulin; AIP: Aterogenic Index and Plasma values; HOMA-IR: The Homeostasis Model Assesment Score; GGT: Gama Glutamil Transferase, TyG index:Log(Fasting triglyceride xFasting Glucose; ALT: Alanin aminotransferase; AST: Aspartat Aminotransferase; ; BMI: Body Mass Index;SDS: Standart Deviation Score

DISCUSSION

MASLD prevalence continues to increase in many countries in line with the worldwide obesity epidemic. It is considered to be the most common cause of liver disease in the pediatric population in the developed world, with an estimated average prevalence of 7.6% in the general pediatric population and 34.25% in obese children[17]. The frequency of MASLD was found to be 23-62% in obese children in our country[18].

Although the pathogenesis of MASLD has not been understood fully, it is multifactorial, and genetic and genetic factors have a great effect on the development and progression of the disease. Most patients have sedentary lifestyles and an unhealthy diet. Fatty liver reflects an imbalance in the liver's fatty acid uptake and synthesis and oxidation of fatty acids, and Insulin Resistance resulting in increased insulin levels[19]. Free fatty acids accumulate in the liver with Insulin Resistance (IR). In addition to these, MS has also been blamed for the pathogenesis of fatty liver disease[20]. MASLD is often associated with MS (Diabetes Mellitus, IR, visceral obesity, dyslipidemia, arterial hypertension)[21]. Children are at risk for the development of MASLD because of the high prevalence of MS. MS was detected in 88% of MASLD patients in a previous study[22]. In another study, it was shown that the prevalence of MS was three-fold higher in obese children with MASLD than in those without liver disease[23]. The incidence of each component of MS was also significantly higher in MASLD patients.

In a previous study conducted by Pirgon et al., which showed that HOMA-IR is an important predictor of MASLD, in obese adolescents and control groups, a significant difference was detected between fasting insulin and HOMA-IR values of those with and without MASLD[24]. In another study conducted by Denzer et al. in 532 obese children, a significant difference was detected between the hepatosteatosis group and the control group in terms of HOMA-IR values[25]. In a study conducted by Marchesini et al., they found the mean HOMA value to be 3.3 in the group with MASLD and 1.8 in the control group[26]. In another similar study, the mean HOMA value of 64 patients with MASLD was reported to be 2.7[27]. In the present study, the HOMA-IR value was 5.612±3.916 in the MASLD patient group and 3.666±2.414 in the healthy control group. When the patient groups were compared with the control group, HOMA values were found to be significantly higher in the patient group, and QUICKI values were significantly lower in the patient group. These findings show that IR is significantly higher in patient groups than in healthy individuals, as reported in other studies. Also, the fact that HOMA-β values in our patients were higher in the patient group than in the healthy control group suggests that they are candidates for Type 2 DM in the future.

Studies conducted with MASLD patients show an almost universal association of HOMA-IR, which makes it a significant parameter for the diagnosis of MASLD, which has not shown any specific markers to date despite its high prevalence in the general population. However, one of the common results obtained in previous studies conducted by using HOMA and QUICKI methods was that there is no single method that can give accurate results in every patient group. Different methods and different lower limit values are used in different patient groups. There is currently no universal lower bound value for the HOMA and QUICKI. It increases in cases with Insulin Resistance and >2.7 is considered resistance in general[28]. In a previous study conducted by Gökçel et al., they found the lower limit of the HOMA value for IR to be 2.2 and the QUICKI value to be 0.3469[29]. When examined with the ROC Analysis in the present study, when the HOMA-IR value was taken as 2.94 (cut-off), the AUC value was calculated as 0.676, the specificity as 80.00%, and the sensitivity as 52.20%. When the QUICKI value was taken as 0.285 (cut-off), the AUC value was found to be 0.327. Also, the cut-off value for the HOMA-IR value to predict the diagnosis of MS was found to be 2.84 and the AUC was calculated as 0.877, which made us think that MASLD is a part of MS.

Logistic Regression was applied to estimate the MASLD. In the present study, the researchers tried to predict MASLD around 80% by applying 4 models. The importance of this is that it is not possible to perform a biopsy in societies such as our country. In some centers, there is even no experienced radiologist who can detect fatty liver disease. For this reason, each physician should be able to diagnose 80-90% of MASLD with the parameters the researchers used in the present study.

As a general rule, the higher the BMI, the greater the person's Insulin Resistance. In a study conducted by De Luis et al., a significant positive correlation was detected between BMI and HOMA-IR, and the HOMA-IR levels of those who were obese were

found to be higher than those with normal BMI[30]. In another study, a significant negative correlation was reported between BMI and QUICKI values to determine the effects of BMI on IR [31]. In our study, HOMA-β and HOMA-IR levels were significantly higher in the obese group when compared to the healthy control group, and QUICKI values were found to be lower. Also, a moderate correlation was detected between HOMA-IR values and positive and negative QUICKI values and BMI in the obese group. Again in the present study, the researchers found a significant difference between HOMA-IR, HOMA-β, and QUICKI index values according to USI liver grades. Glucose and insulin values of the patients increased in direct proportion to the degree of hepatosteatosis.

In a previous study, it was shown that ALT levels were 61.8% higher in patients with MASLD according to USIs of patients who applied to obesity clinics[32]. In a study, 1118 patients who had elevated liver enzymes were evaluated, and MASLD was found to be the cause of enzyme elevation in 26% of these patients. Two or more liver enzymes were found to be elevated in 40.7% of patients with MASLD, and GGT was found in 70%, ALT in 51%, and AST in 26%[33]. In the present study, however, ALT and GGT levels were significantly higher, consistent with the literature data. On the other hand, contrary to the studies in the literature, a significant relationship was detected between AST values and MASLD in the present study. Also, it was found in the present study that ALT, AST, and GGT values increased in proportion to the severity of fatty liver. In a study conducted by Bishnu et al., patients who were diagnosed with MASLD with elevated liver enzymes and USI and divided into groups according to liver grade grading were compared. As a result of the study, a positive correlation was reported between elevation in ALT levels and the stage of hepatosteatosis, but no significant correlation was detected between AST and GGT levels[34]. In the present study, the researchers found a correlation between the groups according to liver grading.

A previous study showed that only 55% of overweight or obese children referred to a gastroenterologist for elevated serum ALT had MASLD. Also, high serum ALT, defined as twice the upper limit of normal, was shown to have a specificity of 71% and a sensitivity of 57% for MASLD in obese and overweight children[35]. In the present study, its sensitivity was found to be 83.82% and specificity as 77.80, based on a cut-off value of 16.10 in ALT value by ROC Analysis. The high ALT level of the patients increases the probability of MASLD by 4.8 times. Although AST and GGT elevation were not found to be associated with MASLD in some studies, a significant relationship was found in the present study. In light of these data, it was revealed that pediatric patients with high ALT, AST, and GGT levels may have MASLD.

The presence of IR in most patients who have MASLD causes increased uric acid levels. In their study, Lee et al. found a significant relationship between the degree of steatosis detected ultrasonographically and serum uric acid concentrations[36]. In the study conducted by Fu et al., they found increased uric acid levels in patients diagnosed with MASLD[37]. In our study, a significant increase was found in uric acid levels between the patient and the healthy control group in accordance with the literature data.

One of the most important risk factors for CVD is dyslipidemia the frequency of which in obese children and adolescents varies between 43% and 69%. In a study investigating dyslipidemia, Sozua et al. found dyslipidemia in 20-80% of MASLD cases, and hypertriglyceridemia was mostly observed[38]. In the study of Madan et al., high triglyceride and LDL levels showed a significant relationship with the degree of liver inflammation[39]. In their study, Freedman et al. compared the current data of 1142 adult participants with the childhood data, and determined that childhood BMI values were positively associated with the risk of Cardiovascular Disease, regardless of the BMI values of adults[40]. In another study, patients who had MASLD had higher VA, BMI, serum LDL and insulin levels, and HOMA-IR scores, and a higher frequency of MASLD in patients with IR, which indicates that obesity, hyperlipidemia, and IR have roles in the development of MASLD[41]. As another independent risk factor for MASLD, the frequency of dyslipidemia is found to be increased in obese patients.

Nigam et al. found that HDL levels were significantly lower in patients with MASLD when compared to healthy controls[42]. In the study of Ozhan et al., TG and cholesterol values were found to be higher and HDL values to be significantly lower in the MASLD patient group when compared to the healthy control group, and no significant difference was detected in LDL levels[43]. In their study in which Toledo et al.

examined the relationship between different degrees of fatty liver and dyslipidemia, they found increased serum TG levels and decreased HDL levels in patients with moderate and severe steatosis when compared to the healthy control group. No statistically significant differences were detected between the patient groups with Grade 2 and 3 Fatty Liver Disease[44]. In our study, increased serum cholesterol, LDL, and TG levels and decreased HDL values were found in the individuals in the case group when compared to the individuals in the healthy group, which were statistically significant. Also in the present study, the researchers found a statistically significant relationship between the severity of adiposity and LDL, TG, and HDL levels in the case group.

Although MASLD is known to be a major risk factor for Cardiovascular Disease, little research has been conducted on AIP values, especially in the pediatric age group[45]. Various AIP values have been used in different studies none of which have proven superiority over the other. In the present study, the researchers examined 4 different AIP values in terms of their effects on Insulin Resistance, their place in the prediction of MASLD, and their superiority over each other. In the present study, AIP values were found to be significantly higher in all patients with MASLD when compared to the healthy control group. The cut-off values of each AIP value were determined in the ROC Analysis. These values showed us that the AUC value of each AIP was higher to predict the development of MASLD and AIP values may be more significant than other biomarkers.

In a study conducted in China, the LDL-HDL ratio was found to be associated with the development of new MASLD[46]. In this study, the researchers named the LDL/HDL ratio AIP4. According to our study results, the AUC value was 0.805, the sensitivity was 83.60% and the specificity was 73.30% based on the cut-off value of 1.875 for the HDL/LDL ratio. According to the results of this study, the researchers think that there is a relationship between HDL-LDL ratio and MASLD and it can be used to predict MASLD, as well as to determine IR and cardiovascular risks in pediatric patients.

Nobili et al. reported that MASLD activity and Fibrosis Scores showed a significant and positive relation with TG/HDL ratios in children with MASLD confirmed by liver biopsy[47]. It was also reported that the TG/HDL ratio most accurately predicts Advanced Liver Disease when compared with other lipid ratios (Cholesterol/HDL and LDL/HDL). It has been reported that the TG/HDL ratio can be a useful index in identifying obese children with Insulin Resistance, dyslipidemia, hypertension, and MS risk from different ethnic origins[47]. The TG/HDL ratio is higher and in parallel with the increased TG levels and shows a significant relationship with HOMA indices. Olson et al. showed that there is a relationship between TG/HDL ratio and HOMA and QUICKI in children[48]. Di Bonito et al. conducted a study to determine MASLD and found a high TG/HDL ratio of >2 and reported that this value is useful to predicting MASLD in clinical practice[49]. Giannini et al. determined the cut-off value for the TG/HDL ratio as 2.27 to estimate the IR[50]. In the present study, the researchers determined the cut-off value for the AIP3 ratio as 3.037 and AUC was calculated as 0.799, specificity 66.70%, and sensitivity 82.10%. The results suggest that the TG/HDL ratio can be a good predictor of MASLD in children and can be used in determining the IR.

Other biomarkers used in the study were Triglyceride-Glucose Index (TyG) and TyG*BMI. TyG Index based on triglycerides and fasting glucose is a simple and reliable biomarker in the diagnosis of IR compared to the gold standard Euglycemic Hyperinsulinemic Clamp. However, it was revealed that the TyG Index outperformed HOMA-IR in evaluating Insulin Resistance in clinical practice, regardless of diabetes status[51]. Wang et al. reported that the TyG Index can predict cardiovascular events in patients with DM[52]. However, few studies have been conducted to evaluate the roles of IR as assessed by the TyG Index and HOMA-IR on arterial stiffness in the Type 2 DM population at high risk of increased arterial stiffness. Also, the researchers did not find any studies on this in pediatric patients. According to the results of the present study, the researchers determined a cut-off value of 3.75 for the TyG Index, and the AUC value for this value was 0.761 with a specificity of 73.13 and a sensitivity of 74.60%. These values show us that it can be used in pediatric patients to determine IR and predict cardiovascular risks.

The study had some limitations. Firstly, it had a retrospective and cross-sectional design. Secondly, the results may not be representative of the entire population because it was a single-center study. Verification studies with multicenter studies are needed. Thirdly, the Hyperinsulinemic Euglycemic

Clamp, which is the gold standard for evaluating insulin sensitivity and insulin secretion, could not be used.

In conclusion, MASLD has an increasing trend today. The rapidly advancing technology and life in this environment have limited our movements today. Also, industrialized foods and environmental factors cause diseases of our age, such as obesity and diabetes, which are associated with MASLD. It was observed that the rate of obese children increased in studies conducted on children, especially in developed countries, increasing the high Insulin Resistance values of obese children due to MASLD. We would like to emphasize that childhood obesity will significantly affect morbidity and mortality in adulthood if left untreated. For this reason, there is an urgent need for public healthcare measures to prevent the complications of the ongoing obesity epidemic. However, there are few studies conducted to identify CVD risks, therefore, the findings of the present study should be supported by future studies. Pediatricians have important roles in determining obesity and complications. No matter what disease the patient comes to the clinician, s/he should screen for obesity in children as much as possible. In light of the data found in the present study, the researchers believe that patients who are considered risky should be more sensitive about a referral to a higher center.

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