ORIGINAL ARTICLE ÖZGÜN ARAŞTIRMA

Can Asprosin be Used as a Diagnostic Biomarker for Non-Alcoholic Fatty Liver Disease in Obese Children?

Asprosin, Obez Çocuklarda Alkole Bağlı Olmayan Karaciğer Yağlanması için Tanısal Bir Biyobelirteç Olarak Kullanılabilir Mi?

*Hale Tuhan (0000-0002-7637-9630), Berna Singin (0000-0002-2204-5336), *Zeynep Donbaloğlu (0000-0003-0605-3229), **Sebahat Özdem (0000-0002-0619-1405), *Mesut Parlak (0000-0002-3550-1425)

*Akdeniz University Hospital, Clinic of Pediatric Endocrinology, Akdeniz University Hospital, Antalya, Türkiye

**Akdeniz University Hospital, Clinic of of Biochemistry, Akdeniz University Hospital, Antalya, Türkiye

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Abstract

Introduction: As obesity rates rise among children, non-alcholic fatty liver disease (NAFLD) is becoming more prevalent. Although liver biopsy is considered the gold standard for diagnosing NAFLD, it is an invasive procedure and not practical for screening. Asprosin, a recently unveiled adipokine, is released in response to fasting. In this study, we aimed to investigate the diagnostic value of asprosin for NAFLD in obese patients.

Materials and Methods: A total of 142 participants (71 obese, 71 control) aged between 6 and 18 years were included in the study. Obese patients were also divided to subgroup as NAFLD (+) and NAFLD (-) according to hepatobiliary ultrasonopgraphic features and compared with each other regarding their clinical and laboratory features, including asprosin level.

Results: When comparing obese and non-obese patients, asprosin levels were significantly higher in the obese group (P=0.034). NAFLD was diagnosed in 35 (49.2%) of the obese cases. While body mass index (BMI) were similar; waist circumference and insulin resistance were higher in patients with NAFLD. When comparing Asprosin, interleukine-6 (IL-6), and tumor necrosing factor alpha (TNF-α) levels, no statistically significant differences were observed between the NAFLD (+) and NAFLD (-) subgroups. The level of Asprosin showed a positive correlation with IL-6 and TNF-α levels, while no significant relationship was found between the asprosin and any clinical or laboratory parameters in obese patients with NAFLD.

Conclusion: Serum asprosin levels were elevated in obese children. However, there were no significant findings to support the use of asprosin levels as a non-traumatic diagnostic indicator for NAFLD diagnosis in the pediatric age.

Keywords
Asprosin, obesity, fatty liver, adipokine

Anahtar kelimeler
Asprosin, obezite, karaciğer yağlanması, adipokin

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Address for Correspondence/Yazışma Adresi: Zeynep Donbaloğlu, Akdeniz University Hospital, Clinic of Pediatric Endocrinology, Akdeniz University Hospital, Antalya, Türkiye
E-mail: drzeynepdonbaloglu@gmail.com

Öz

Giriş: Çocuklar arasında obezite oranlarının artmasıyla birlikte, alkole bağlı olmayan karaciğer yağlanması hastalığı (NAFLD) daha yaygın hale gelmektedir. Karaciğer biyopsisi NAFLD tanısında altın standart olarak kabul edilse de, invaziv bir işlem olması nedeniyle tarama amaçlı kullanımı pratik değildir. Yakın zamanda keşfedilen bir adipokin olan asprosin, açlık durumunda salınmaktadır. Bu çalışmada, obez hastalarda NAFLD tanısında asprosinin tanısal değerini araştırmayı amaçladık.

Gereç ve Yöntem: Çalışmaya yaşları 6 ile 18 arasında değişen toplam 142 katılımcı (71 obez, 71 kontrol) dahil edildi. Obez hastalar, hepatobilier ultrasonografik bulgulara göre NAFLD (+) ve NAFLD (-) olarak iki alt gruba ayrıldı ve klinik ve laboratuvar bulguları (asprosin düzeyi dahil) açısından karşılaştırıldı.

Bulgular: Obez ve obez olmayan hastalar karşılaştırıldığında, obez grupta asprosin düzeyleri anlamlı derecede daha yüksekti (P=0.034). Obez olguların 35'ine (%49,2) NAFLD tanısı konuldu. Vücut kitle indeksi (VKİ) benzer olmasına rağmen, bel çevresi ve insülin direnci NAFLD olan hastalarda daha yüksekti. Asprosin, interlökin-6 (IL-6) ve tümör nekroz faktörü alfa (TNF-α) düzeyleri karşılaştırıldığında, NAFLD (+) ve NAFLD (-) alt grupları arasında istatistiksel olarak anlamlı bir fark bulunmadı. Asprosin düzeyi, IL-6 ve TNF-α düzeyleriyle pozitif korelasyon gösterdi; ancak NAFLD'li obez hastalarda asprosin ile herhangi bir klinik veya laboratuvar parametresi arasında anlamlı bir ilişki saptanmadı.
Sonuç: Serum asprosin düzeyleri obez çocuklarda artmış olarak bulundu. Ancak, asprosin düzeylerinin çocukluk çağında NAFLD tanısı için travmatik olmayan bir tanı aracı olarak kullanılmasını destekleyecek anlamlı bulgular elde edilemedi.

Introduction

Obesity is a chronic metabolic disease associated with numerous comorbidities, which has increased worldwide more than threefold in the last 20 years among childhood and adolescence. In many studies, obesity has been shown to cause serious complications such as hypertension, dyslipidemia, non-alcoholic fatty liver disease (NAFLD), type 2 diabetes, and cancer later in life (1-3). As obesity rates rise among children, NAFLD is becoming more prevalent, potentially resulting in liver inflammation, fibrosis, and, in severe cases, cirrhosis. NAFLD is closely linked to metabolic syndrome traits and can be viewed as a hepatic indication of insulin resistance (4). Liver biopsy, the gold standard for assessing NAFLD, is the only reliable way to differentiate between non-alcoholic steatohepatitis and simple steatosis. Although liver biopsy is considered the gold standard for diagnosing NAFLD, it is an invasive procedure and not practical for screening a large number of high-risk patients or for monitoring individuals with NAFLD post-treatment. Considering the importance of NAFLD in clinical practice and the drawbacks associated with liver biopsy, there is an increasing demand for precise, non-invasive imaging methods for assessment (5). Several techniques, including ultrasonography (US), magnetic resonance imaging, computed tomography, and magnetic resonance spectroscopy, have been employed to evaluate individuals with NAFLD, primarily focusing on measuring the extent of hepatic steatosis.

White adipose tissue not only functions as a tissue that provides energy reserve but also acts as a dynamic organ responsible for the synthesis and secretion of many adipokines, growth factors, and inflammatory markers such as leptin, adiponectin, tumor necrosis factor-α (TNF-α), resistin, and interleukin-6 (IL-6) (6). A changed secretion profile of adipokines from adipose tissue depots often marks obesity and related comorbidities. Findings from studies on metabolic hormones indicate that obesity is associated with elevated levels of leptin, TNF-α, and IL-6, while ghrelin levels tend to decrease (7-9).

The count of recognized adipokines has rapidly surged over the last 20 years (10). Among these, asprosin, a recently unveiled adipokine, is released in response to fasting (11). Multiple studies in the adult population have indicated a notable increase in circulating asprosin levels among individuals with higher body weight, encompassing both overweight and obese individuals (12-14). A recent investigation by Ke et al. (15) demonstrated a significant elevation in serum asprosin levels in untreated adult patients with NAFLD. Research on asprosin within the pediatric age group, however, remains scarce.

In light of these findings, this study aims to comprehensively investigate the relationship between asprosin, IL-6, TNF-α, and biochemical and clinical parameters in subjects with and without obesity and obese subjects with and without NAFLD in pediatric and adolescent age groups.

Material and Methods

Study Design and Participants

This study was designed as a cross-sectional, single-center, case-control research. A total of 142 participants aged between 6 and 18 years were included in the study. Among these participants who were admitted to the pediatric endocrinology clinic of our hospital, 71 cases diagnosed with obesity were assigned to the obese group. The control group comprised 71 healthy non-obese volunteers of the same age and sex who visited the hospital where the study was conducted for routine health checkups, including services such as vaccination. Exclusion criteria for the obese group encompassed a prior diagnosis of any chronic disease affecting the endocrine system (e.g., Cushing's disease, hypothyroidism), syndromes associated with obesity (e.g., Prader-Willi, Bardet-Biedl syndromes), other systemic disorders, and a history of drug use.

The obese patients were compared with control subjects regarding their clinical and laboratory features. Obese patients were also divided into subgroups NAFLD (+) and

NAFLD (-) according to hepatobiliary ultrasonographic features and compared with each other. The correlations between clinical/biochemical parameters and Asprosin, TNF-α, and IL-6 were also investigated among obese patients with NAFLD.

Clinical Investigations and Anthropometric Data

Height measurements in both an upright standing position and during deep inspiration were obtained using a wall-mounted stadiometer. Body Mass Index (BMI) was calculated by dividing the weight by the square of height (kg/m2). Standard Deviation Scores (SDS) for height, weight, and BMI were determined based on reference values for Turkish children (16). Individuals with a BMI exceeding the 95th percentile for their age and sex, as per Turkish children's reference values, were classified as obese and included in the obese group. Participants with a BMI between the 3rd and 85th percentiles were categorized as a non-obese group, and overweight cases were not included in this group. Waist circumference was measured with a tape measure at the level of the umbilicus. Measurements were taken from children with their abdomen freely exposed while standing upright. Upper arm circumference was measured between the acromial process in the shoulder and the olecranon process at the elbow, with the elbow bent at a 90-degree angle. The measurements were recorded in centimeters (cm) and analyzed. Triceps skinfold (TSF) thickness measurement was conducted while the arm was freely hanging by the side of the body. A caliper was utilized to measure the midpoint of the anterior surface of the forearm. The Tanita BC-418 equipment from Tokyo, Japan, was employed for bioelectrical impedance analysis to assess the fat mass and percentage of body fat (PBF). Blood pressure measurements followed a validated protocol, with systolic blood pressure (SBP) and diastolic blood pressure (DBP) recorded twice on the right arm after a 10-minute rest in the supine position, using a calibrated sphygmomanometer and conducted by one of the investigators. The average of the two blood pressure readings was considered. Pubertal status was determined based on the Tanner classification and categorized as pubertal or prepubertal (17).

Laboratory Investigations and Sonographic Evaluations

Peripheral blood samples were collected in the morning (between 8:00 and 9:00 a.m.) following a 10-hour fasting period. Fasting glucose levels were determined using the hexokinase method while fasting insulin levels were measured using the radioimmunoassay technique. Insulin resistance (IR) was assessed using the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) formula: fasting insulin (μU/mL) × fasting glucose (mg/L)/405. Subjects with HOMA-IR values above >4 in the pubertal stage and >2.5 in the prepubertal stage were considered IR (18). Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were measured using the spectrophotometric method. Triglyceride (TG), total cholesterol (TC), and high-density lipoprotein cholesterol (HDL-C) concentrations were determined enzymatically using DP Modular Systems (Roche Diagnostic Corp., Indianapolis, IN). Low-density lipoprotein cholesterol (LDL-C) levels were calculated using the Friedewald formula when plasma TG was < 400mg/dL. The diagnosis of NAFLD was based on ultrasonographic evidence of hepatic steatosis, following the criteria outlined in the ESPGHAN Hepatology Committee's guidelines (19). The same qualified radiologist conducted all examinations.

The measurement methods and kit details for adipokines are as follows;

Asprosin: Serum samples were analyzed using the ELISA (Enzyme-Linked Immunosorbent Assay) method with a Sunred kit (SunRed Biotechnology, China) (Kit catalog no: 201-12-7193, reference no: DZE201127193, lot no: 202304). The intra-assay coefficient of variation (CV) was <% 10, and the inter-assay CV was <% 12. The kit sensitivity was 1.0 ng/mL, and measurement linearity ranged from 1 to 300 ng/mL. The kit was stored at 2-8°C until use.

IL-6: Serum samples were analyzed using the ELISA method with a Sunred kit (SunRed Biotechnology, China) (Kit catalog no: 201-12-0091, reference no: DZE201120091, lot no: 202304). Intra-assay CV was <% 10, and inter-assay CV was <% 12. The kit sensitivity was 2.11 ng/L, and measurement linearity ranged from 3 to 600 ng/L. The kit was stored at 2-8°C until use.

TNF-α: Serum samples were analyzed using the ELISA method with a Sunred kit (SunRed Biotechnology, China) (Kit catalog no: 201-12-0083, reference no: DZE201127193, lot no: 202304). Intra-assay CV was <% 10, and inter-assay CV was <% 12. The kit sensitivity was 2.827 ng/L, and measurement linearity ranged from 3 to 900 ng/L. The kit was stored at 2-8°C until use.

Statistical Analysis

We conducted the statistical analysis using The Statistical Package for the Social Sciences (SPSS for Windows, Version 23.0, Chicago, IL, USA). Continuous measurements were reported as either median [Interquartile range (IQR)] or

mean ± standard deviation (SD), while categorical data were presented as counts and percentages. We employed Pearson's chi-square and Fisher's exact tests to compare categorical variables. The Shapiro-Wilk test was used to assess normality, and distribution was also checked when comparing continuous measurements. Normally distributed parameters were compared using the t-Test, while non-normally distributed parameters were compared using the Mann-Whitney U test. The Pearson correlation test was utilized to explore relationships among normally distributed variables, and the Spearman correlation was employed for variables that did not adhere to a normal distribution. A p-value less than 0.05 was considered indicative of statistical significance.

Ethics

Approval was obtained from the Ethics Committee prior to the commencement of the study (protocol number: 70904504/103 dated March 09, 2022). Informed consent was acquired from the parents of all participants before their involvement. The study strictly adhered to the principles outlined in the Declaration of Helsinki and followed ethical guidelines.

Results

The clinical and laboratory characteristics of obese and non-obese subjects are summarized in Table 1.

The age, sex, and pubertal status were similar in the two groups. The BMI, BMI SDS, waist and upper arm circumferences, TSF thickness, total body fat mass, PBF, and

Table 1. The clinical and laboratory characteristics of obese and non-obese subjects

	Obese Subjects (n=71)	Non-obese Subjects (n=71)	P
Age (year)	11.9 ± 3.0	11.3±2.8	0.233
Male (%)	42.2	39.4	0.733
Pubertal (%)	77.4	76.0	1.000
BMI (kg/m^2)	31.8 (7.3)	17.6 (3.3)	<0.001
BMI SDS	2.7 (0.7)	-0.1 (1.3)	<0.001
Waist circumference (cm)	93.0 (18.8)	62.0 (8.8)	<0.001
Upper arm circumference (cm)	32.0 (6.8)	22.0 (3.8)	<0.001
TSF thickness (mm)	20.6 (9.1)	6.9 (6.5)	<0.001
Fat mass (kg)	28.5 (15.3)	7.2 (4.6)	<0.001
PBF (%)	37.0 (6.7)	20.3 (5.6)	<0.001
SBP (mmHg)	120 (10)	100 (20)	<0.001
DBP (mmHg)	80 (10)	70 (10)	<0.001
Glucose (mg/dL)	88.1± 6.7	85.0 ± 6.0	0.004
Insulin (uIU/mL)	20.3 (19.2)	7.0 (5.6)	<0.001
HOMA-IR	4.4 (4.6)	1.4 (1.3)	<0.001
ALT (U/L)	20 (16)	12 (3)	<0.001
AST (U/L)	19 (10)	22 (8)	0.359
Triglyceride (mg/dL)	90.0 (48)	63.5 (25)	<0.001
TC (mg/dL)	156.8 ± 27.0	156.1 ± 24.5	0.805
LDL-C (mg/dL)	95.0 ± 24.3	87.3 ± 18.9	0.033
HDL-C (mg/dL)	46.8 (14.1)	60.7 (21.3)	<0.001
Asprosin (ng/mL)	42.7 (31.1)	39.0 (23.3)	0.034
TNF-α (ng/L)	300.9 (195.9)	315.8 (127.8)	0.317
IL-6 (ng/L)	47.1 (39.0)	53.5 (25.1)	0.185

Data are given mean±SD, median (IQR) or n(%)

BMI: body mass index; BMI-SDS: standard deviation score of body mass index; TSF: triceps skinfold; PBF: percentage of body fat; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; HOMA-IR: homeostasis model assessment of insulin resistance; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; LDL-C: low density lipoprotein cholesterol; TC: total cholesterol; HDL-C: high density lipoprotein cholesterol; TNF- α: tumor necrosis factor-α; IL-6: interleukine 6

SBP and DBP were significantly higher in the obese group (P<0.001). When laboratory parameters were examined, fasting glucose, HOMA-IR, ALT, triglyceride, and LDL-C levels were significantly higher in obese patients while HDL-C was lower (P<0.001) (Table 2).

When comparing obese and non-obese patients, IL-6 and TNF-alpha levels were similar, whereas Asprosin levels were significantly higher in the obese group (P=0.317; P=0.185; P=0.034; Figure 1).

Out of the 35 patients with NAFLD, 25 (71.4 %) had grade 1, 8 (22.8 %) had grade 2, and 2 (5.7 %) had grade 3 hepatosteatosis. Comparisons of the clinical and laboratory characteristics of obese subjects with and without NAFLD are demonstrated in Table 2. In patients with and without NAFLD, similar age, sex, and pubertal status were observed. While BMI and BMI SDS were similar, waist circumference was higher in patients with NAFLD (P=0.066; P=0.384; P=0.011). DBP and HOMA-IR were also higher in patients with NAFLD (P=0.029; P=0.032). Lipid profiles and liver enzymes were similar in both groups. No statistically significant differences were observed between the groups when comparing Asprosin, IL-6, and TNF-α levels.

Correlation analysis was performed, and the relationships between Asprosin, TNF-α, IL-6, and clinical and laboratory parameters in obese patients with NAFLD are presented in Table 3. The level of Asprosin showed a positive correlation with IL-6 and TNF-α levels, while no significant relationship was found between the adipokines and any clinical or laboratory parameters.

Table 2. Comparison of the clinical and laboratory characteristics of obese subjects with and without NAFLD

	NAFLD (+) Obese subjects (n=35)	NAFLD (-) Obese subjects (n=36)	P
Age (year)	12.4 ± 2.7	11.4 ± 3.3	0.170
Male (%)	48.5	36.1	0.411
Pubertal (%)	82.8	72.2	0.481
BMI (kg/m^2)	31.9 (7.5)	30.7 (6.7)	0.066
BMI SDS	2.9 (0.8)	2.6 (0.5)	0.384
Waist circumference (cm)	98 (16.5)	84.5 (21)	0.011
Upper arm circumference (cm)	33 (5.5)	31 (7.5)	0.050
TSF thickness (mm)	22.2 (8.9)	19.9 (8.6)	0.205
Fat mass (kg)	30.8 (13.9)	27.5 (17.2)	0.113
PBF (%)	37.0 (6.5)	36.6 (6.3)	0.904
SBP (mmHg)	120 (13.8)	120 (21.3)	0.306
DBP (mmHg)	80 (10)	75 (16.3)	0.029
Glucose (mg/dL)	88.8 ± 6.5	87.4 ± 6.9	0.361
Insulin (uIU/mL)	24.8 (18.4)	17.3 (20.8)	0.033
HOMA-IR	5.1 (3.7)	3.7 (4.3)	0.032
ALT (U/L)	20.5 (17.5)	16.0 (14.3)	0.461
AST (U/L)	18.5 (9.3)	19.5 (7.3)	0.782
Triglyceride (mg/dL)	89.0 (36.5)	91.5 (54.8)	0.932
TC (mg/dL)	155.4 ± 26.1	158.3 ± 28.1	0.657
LDL-C (mg/dL)	95.0 ± 22.3	94.9 ± 26.4	0.988
HDL-C (mg/dL)	43.9 (11.5)	50.1 (14.8)	0.112
Asprosin (ng/mL)	40.3 (17.6)	47.6 (51.1)	0.543
TNF-α (ng/L)	286.5 (90.9)	306.4 (261.9)	0.240
IL-6 (ng/L)	41.0 (23.8)	51.9 (56.3)	0.104

Data are given mean±SD, median (IQR) or n(%)

NAFLD: non-alcholic fatty liver disease; BMI: body mass index; BMI-SDS: standard deviation score of body mass index; TSF: triceps skinfold; PBF: percentage of body fat; SBP: systolic blood pressure; DBP: diastolic blood pressure; HOMA-IR: homeostasis model assessment of insulin resistance; ALT: alanine aminotransferase; AST: aspartate aminotransferase; LDL-C: low density lipoprotein cholesterol; TC: total cholesterol; HDL-C: high density lipoprotein cholesterol; TNF-α: tumor necrosis factor-α; IL-6: interleukine 6

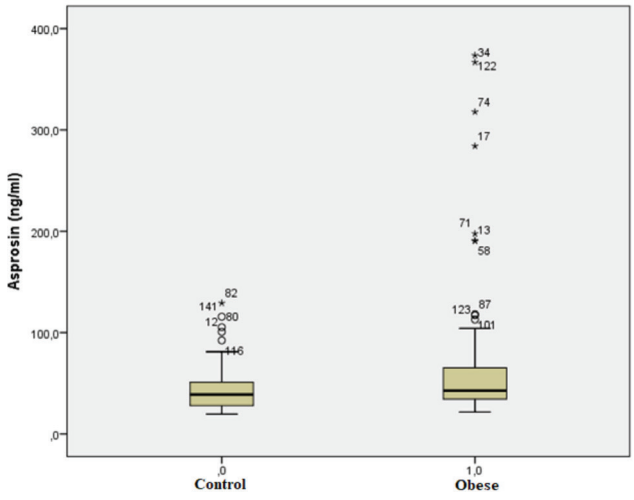

Figure 1. The distribution of asprosin levels in obese and non-obese subjects

Discussion

Obesity represents a significant global public health concern, leading to elevated rates of illness and death. It can stem from genetic, metabolic, or endocrine imbalances and, if left untreated, significantly reduces life expectancy while diminishing overall quality of life. (20) Researchers have conducted various studies to better understand the role of hormones and mediators released in the body in body weight and energy balance. Adipokines secreted from adipose tissue are also a significant focus of interest. Recent research has uncovered the intricate and paradoxical role of asprosin in obesity. Numerous studies have indicated elevated levels of asprosin in both humans and mice suffering from obesity. Pathologically high serum levels of asprosin have been observed in obese adults, children, and mice. In contrast, obese mice exhibited reduced body weight and food intake when treated with a specific asprosin antibody (13,21,22).

Additionally, a separate study highlighted the synthesis of asprosin in human salivary glands. A study conducted by Ugur et al. (14) found that as the BMI of subjects increased, the levels of LDL-C and asprosin in both saliva and blood also increased.

In our study, when we compared obese patients with healthy individuals of the same age group, matched for pubertal stage and gender ratio with the obese patients, we also found that the levels of asprosin were statistically significantly higher in the obese group. However, this difference was not markedly pronounced. While most studies investigating the relationship between asprosin and obesity are conducted in the adult age group, a study comparing obese

Table 3. The relationship between Asprosin, TNF-α, IL-6 and clinical and laboratory parameters in the obese patients with NAFLD

	Asprosin	TNF-α	IL-6
Age (year)	P:0.897 R:-0.023	P: 0.209 R:-0.218	P: 0.239 R:-0.204
BMI (kg/m^2)	P:0.520 R:-0.112	P: 0.312 R: -0.176	P: 0.570 R: -0.099
BMI SDS	P:0.754 R:0.055	P: 0.973 R: 0.06	P: 0.570 R: -0.099
Waist circumference (cm)	P: 0.427 R:-0.139	P: 0.318 R:-0.174	P: 0.541 R:-0.107
Upper arm circumference (cm)	P: 0.997 R:-0.001	P: 0.565 R:-0.101	P: 0.915 R:-0.019
TSF thickness (mm)	P: 0.332 R:0.174	P: 0.427 R: 0.143	P: 0.334 R: 0.174
Fat mass (kg)	P:0.428 R:-0.141	P:0.132 R:-0.264	P:0.336 R:-0.170
PBF (%)	P:0.099 R:-0.288	P:0.160 R:-0.247	P:0.396 R:-0.150
SBP (mmHg)	P: 0.702 R:-0.074	P: 0.505 R:-0.229	P: 0.598 R: -0.102
DBP (mmHg)	P: 0.071 R:0.340	P: 0.428 R: 0.153	P: 0.118 R: -0.177
HOMA-IR	P: 0.771 R:-0.051	P: 0.070 R:-0.203	P: 0.248 R:-0.200
ALT (U/L)	P:0.755 R:-0.055	P: 0.989 R: 0.003	P: 0.819 R: -0.040
AST (U/L)	P:0.933 R:0.015	P:0.295 R:0.182	P:0.576 R:0.098
Triglyceride (mg/dL)	P:0.831 R:0.037	P:0.476 R:-0.125	P:0.644 R:-0.081
TC (mg/dL)	P:0.543 R:-0.106	P:0.212 R:0.216	P:0.085 R:-0.295
LDL-C (mg/dL)	P:0.409 R:-0.144	P:0.183 R:-0.230	P:0.071 R:-0.309
HDL-C (mg/dL)	P:0.949 R:-0.011	P:0.127 R:-0.264	P:0.054 R:-0.328
Asprosin (ng/mL)	-	P<0.001 R:0.624	P<0.001 R:0.593
TNF-α (ng/L)	P<0.001 R:0.624	-	P<0.001 R:0.818
IL-6 (ng/L)	P<0.001 R:0.593	P<0.001 R:0.818	-

*Statistically significant correlation.

BMI: body mass index; BMI-SDS: standard deviation score of body mass index; TSF: triceps skinfold; PBF: percentage of body fat; SBP: systolic blood pressure; DBP: diastolic blood pressure; HOMA-IR: homeostasis model assessment of insulin resistance; ALT: alanine aminotransferase; AST: aspartate aminotransferase; LDL-C: low density lipoprotein cholesterol; TC: total cholesterol; HDL-C: high density lipoprotein cholesterol; TNF- α: tumor necrosis factor-α; IL-6: interleukine 6

patients aged between 6-14 years with healthy individuals of normal body weight reported a negative correlation between asprosin levels and age- and gender-adjusted BMI. The findings of this study differ from the majority of studies in the literature, suggesting a complex relationship between asprosin and obesity (23). The degree of obesity may also be an essential factor affecting asprosin levels. In a study conducted by Romero et al. (11) in adult patients, the average BMI was >35 kg/m² (approximately equivalent to >+3 SD), and the patients' asprosin levels were significantly higher compared to the control group. Our study's median BMI SDS was 2.7, and the asprosin level was slightly higher than the control group. However, in the research conducted by Long et al. (23), the obese group exhibited relatively lower BMI standard deviations (2.09±0.47 in males and 2.22±1.08 in females) compared to previously mentioned studies. Surprisingly, their levels of asprosin were lower than those in the control group. Furthermore, the obese patients in this study had significantly lower insulin and HOMA-IR levels, indicating that they might be in a metabolically healthy 'honeymoon phase (24)'. This finding holds significance in light of the dose-dependent relationship between BMI and adverse health outcomes, particularly insulin resistance, which closely ties in with the role of asprosin (11, 24). Serum asprosin levels were found to be significantly different between groups in another study: 70.9 ± 17.4 ng/mL, 79.7 ± 29.5 ng/mL, and 106.2 ± 122.6 ng/mL in normal weight, overweight, and obese children, respectively, which is compatible with dose-dependent relationship between BMI and asprosin levels (25).

Non-alcoholic fatty liver disease is the most common chronic liver complication among adult and pediatric obese subjects (26). The relationship between asprosin and NAFLD in obese patients, as well as its role in the pathogenesis, remains under investigation. The role of asprosin in the pathogenesis of NAFLD has not yet been fully elucidated. Several hypotheses have been proposed in this regard. One of the proposed mechanisms is that excessive asprosin in obese patients increases hepatic glucose release, resulting in partial or systemic insulin resistance (11,27). Hyperinsulinemia and hyperglycemia induced by IR have been reported to create a lipid input-to-output imbalance, promoting hepatic steatosis (28). Another hypothesis suggests that the accumulation of fat in the liver is significantly influenced by de novo lipogenesis, a process involving the production and storage of elevated glucose in the form of triglycerides through glycolysis.

Additionally, a recent study highlighted the role of recombinant asprosin in promoting metabolic disorders, triggering inflammation through the TLR4/JNK pathway (29). Notably, Ke et al. (15) found significantly higher levels of serum asprosin in adult NAFLD patients, which was also corroborated in the pediatric age group (30). However, when we divided our obese patients into those with and without NAFLD, no difference in asprosin levels was observed. Obesity manifests in various forms, such as whole-body obesity and abdominal obesity. Abdominal obesity, in particular, is strongly linked to visceral obesity and NAFLD (31). In our research, we observed that obese children exhibited a broader waist circumference, indicating a predominance of abdominal obesity among our study participants.

Additionally, patients with NAFLD had wider waist circumferences compared to those without NAFLD. Compared to insulin resistance, which plays a crucial role in the pathogenesis of NAFLD, patients with NAFLD similarly exhibited higher fasting insulin levels and HOMA-IR index in cases with NAFLD. However, when we compared the levels of ALT, a well-known marker for NAFLD, between obese individuals with and without NAFLD, there was no statistically significant difference. Similarly, the levels of TNF-alpha and IL-6, markers of chronic inflammation, were not significantly increased in NAFLD patients. This may be explained by the higher number of cases in our patient population in the early stage of hepatosteatosis (grade 1, 71.8%). In the future, when a more chronic period develops and the degree of inflammation increases, asprosin levels may increase. Serum asprosin levels were positively correlated with TNF-alpha and IL-6. This finding suggests that asprosin may also play a role in chronic inflammation similar to proinflammatory cytokines. However, our study found no significant correlation between asprosin levels and the other parameters examined.

Study Limitations

There are certain limitations in our study. Being a case-control study precludes establishing a causal relationship between asprosin, obesity, and NAFLD. Secondly, we opted for liver ultrasonography as a diagnostic method for NAFLD, which, although non-invasive, has a slightly lower diagnostic accuracy than liver biopsy. Additionally, a post-hoc power analysis for the comparison of asprosin levels between obese subjects with and without NAFLD indicated a small effect size (Cohen's $d = 0.19$) and low statistical power (12.4% at $\alpha = 0.05$), suggesting that our study was underpowered to detect small differences between groups.

Conclusion

In conclusion, our results indicate that serum asprosin levels were elevated in obese children. However, when

comparing obese patients with and without NAFLD, similar levels of asprosin were observed. There were no significant findings to support the use of asprosin levels as a non-traumatic diagnostic indicator for NAFLD diagnosis in the pediatric and adolescent age group. Further studies are needed to explore the molecular mechanism of asprosin in childhood obesity and NAFLD.

Ethics

Ethical Approval: Approval was obtained from the Ethics Committee prior to the commencement of the study (protocol number: 70904504/103 dated March 09, 2022). Informed consent was acquired from the parents of all participants before their involvement. The study strictly adhered to the principles outlined in the Declaration of Helsinki and followed ethical guidelines.

Footnotes

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

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