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Evaluation of the relationship between non-alcoholic fatty liver disease and serum c-peptide, c-peptide to glucose and c-peptide to HbA1C ratio in obese children

Obez çocuklarda alkolsüz yağlı karaciğer hastalığı ile serum c-peptit, c-peptit-glikoz ve c-peptit-HbA1C oranı arasındaki ilişkinin değerlendirilmesi

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Abstract

Aim: Obesity-related complications such as metabolic syndrome, insulin resistance and non-alcoholic fatty liver disease (NAFLD) have increased in childhood. The aim of this study is to investigate the fasting c-peptide, c-peptide to glucose and c-peptide to HbA1C ratios in obese children with NAFLD.

Methods: This case-control study was conducted from August through November 2018. A total of 60 obese children, 40 with and 20 without NAFLD, were included in the study. Patients with BMI > 2 z-score were considered obese. The ultrasonographic characteristics of NAFLD were identified with liver contrast and brightness in echogenicity. Serum fasting c-peptide levels of patients were compared.

Results: Of the 60 patients included in the study, 37 (61.7%) were male and the mean age was 11.9 (2.9) years. The mean ALT, weight BMI, waist circumference, waist to height ratio, serum c-peptide, fasting c-peptide to glucose ratio, fasting c-peptide to HbA1C ratio and insulin levels were considerably higher in patients with NAFLD (P<0.05 for each). When the c-peptide levels of all the patients was evaluated by ROC analysis, the area under the curve in patients with NAFLD was 0.70 (95% CL: 0.725-0.955) and the c-peptide cut off value was 2.62 ng/ml (sensitivity 60%, specificity 75%, P=0.011). Logistic regression analysis results showed that the risk of NAFLD was significantly higher in obese children with c-peptide levels greater than 2.62 ng/ml (OR: 4.52 95% CI: 1.65-3.25) (P<0.001).

Conclusion: In our study, a significant relationship was found between NAFLD and serum c-peptide level, c-peptide to glucose and c-peptide to HbA1C ratios. Even though BMI, waist circumference, waist to height ratio, HbA1c and insulin are better parameters in determining insulin resistance in NAFLD patients, c-peptide can be used as an inexpensive method for screening.

Keywords: Childhood, C-peptide, C-peptide to glucose ratio, C-peptide to HbA1C ratio, Non-alcoholic fatty liver disease, Obesity

Öz

Amaç: Obeziteye bağlı görülen metabolik sendrom, insulin direnci ve non-alkolik yağlı karaciğer hastalığı (NAYKH)gibi komplikasyonlar çocukluk çağında da sıklığı giderek artmaktadır. Bu çalışmanın amacı, NAYKH ile ilişkili obez çocuklarda açlık c-peptit, c-peptit-glukoz ve c-peptit-HbA1C oranlarını araştırmaktır.

Yöntemler: Bu vaka-kontrol çalışması Ağustos-Kasım 2018 arasında gerçekleştirildi. Çalışmaya 40'ı NAYKH ile ilişkili ve 20'si NAYKH olmayan toplam 60 obez çocuk alındı. VKİ> 2 z-skoru olan hastalar obez olarak kabul edildi. NAYKH'nin ultrasonografik özellikleri, karaciğer kontrastı ve ekojenitede parlaklık olarak tanımlandı. Hastaların serum açlık c-peptit düzeyleri karşılaştırıldı.

Bulgular: Çalışmaya dahil edilen 60 hastanın 37'si (%61,7) erkekti ve ortalama yaş 11,9 (2,9) yıldı. NAYKH'li hastalarda ortalama ALT, ağırlık, BMI, bel çevresi, bel çevresi-boy oranı, serum c-peptit, açlık c-peptit-glikoz oranı, açlık c-peptit-HbA1C oranı ve insülin seviyeleri, NAYKH olmayan obez çocuklara göre anlamlı olarak daha yüksekti. (her biri için P<0,05).

Bütün hastalar içinde ROC analizi ile değerlendirildiğinde, NAYKH'li hastalar için eğri altındaki alan 0,70 (%95 CI: 0,725-0,955) ve c peptit kesme değeri 2,62 ng / ml (duyarlılık %60, özgüllük %75, P=0,011) idi. Lojistik regresyon analizi ile, c-peptit seviyesi 2.62 ng / ml'den yüksek olan obez çocuklarda NAYKH riski anlamlı derecede daha yüksekti (OR: 4,52 %95 CI: 1,65-3,25) (P<0,001).

Sonuç: Bu çalışmada, NAYKH ile serum c-peptit düzeyi, c-peptit-glukoz ve c-peptit-HbA1C oranları arasında anlamlı bir ilişki bulunmuştur. NAYKH hastalarında insülin direncini belirlemede BMI, bel çevresi, bel-boy oranı, HbA1c ve insülin daha iyi standart parametreler olsa da, c-peptit tarama için pahalı olmayan bir yöntem olarak kullanılabilir.

Anahtar kelimeler: Çocukluk çağı, C-peptit, C-peptit-glikoz oranı, C-peptit-HbA1C oranı non-alkolik yağlı karaciğer hastalığı, Obezite

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Introduction

Obesity is a common public health problem among non-communicable diseases worldwide. Comorbidities such as glucose intolerance, hypertension, dyslipidemia, non-alcoholic fatty liver disease (NAFLD) and ischemic heart disease are associated with obesity [1]. Obesity-related NAFLD is the most common cause of chronic liver disease in children in developed countries. NAFLD is characterized by excessive fat accumulation in hepatocytes and covers a wide spectrum of diseases ranging from simple non-alcoholic fatty liver (NAFL) disease to non-alcoholic steatohepatitis (NASH), cirrhosis and end stage liver disease [2].

The prevalence of NAFLD in childhood is about 7% and may reach 34% among obese children [3]. Recently, a meta-analysis showed that the global prevalence of NAFLD is 25.24% with the highest prevalence in the Middle East and South America and the lowest in Africa for the year of 2016 [4].

Obesity and insulin resistance are the leading causes of NAFLD in childhood. In the diagnosis, evaluation and staging of obesity, waist circumference (WC), and waist to height ratio, and body mass index (BMI) are utilized [5]. The role of insulin resistance, hemoglobin A1c (HbA1c), insulin level, and fasting glucose level have been demonstrated in the development of NAFLD.

The level of c-peptide in blood is a good indicator of beta cell activity and endogenous insulin secretion. Once the polypeptide structure – a preprohormone, produced in pancreatic beta cells, is released into the blood, the c-peptide part is separated, and the remainder is the insulin hormone [6]. Serum c-peptide level is positively correlated with fat distribution in nondiabetic subjects and increased levels of c-peptide is an indicator of insulin hypersecretion and resistance in healthy and diabetic subjects [7]. In addition, the serum c-peptide level is a risk factor for cardiovascular disease, metabolic syndrome and NAFLD in adult studies. [8-9].

According to the literature, the serum c-peptide levels and fasting c-peptide to glucose ratio, as well as c-peptide to HbA1C ratio in children with NAFLD have not been evaluated previously. The aim of this study was to determine the relationship between serum fasting c-peptide levels and NAFLD in children with obesity.

Materials and methods

Patients

This study was conducted between August 2018 and November 2018 in the Department of Pediatric Gastroenterology Hepatology and Nutrition. Sociodemographic characteristics of the patients together with physical (BMI, WC, waist to height ratio) and laboratory findings (venous, 8 h-fasting glucose level, lipid panel, aspartate aminotransferase [AST normal values: Females: 8–46 U/L, males: 8–40 U/L], alanine aminotransferase [ALT normal values: Females: 0–35 U/L, males: 0–40 U/L], insulin, 8h-fasting c-peptide level, HbA1C, HOMA index), as well as follow-up periods were recorded. In our study, NAFLD is defined as: (1) Moderate to severe hepatic steatosis on ultrasound (hepatorenal echo contrast, liver brightness, deep attenuation, and vascular blurring), (2) no history of chronic liver diseases, (3) not infected with hepatitis B or hepatitis C. Other leading causes of NAFLD such as infectious, autoimmune, metabolic, and endocrine reasons were excluded by hepatitis A, B, C and autoimmune markers, serum α-1-antitrypsin and ceruloplasmin level tests.

The calculation of percentiles and z-scores of weight, height, and body mass index (BMI) were performed using WHO Anthroplus v 1.0.4 software [10]. Patients with BMI > 2 z-score were considered obese.

C-peptide measurement

Serum fasting c-peptide (IMMULITE 2000® DPC, USA) is a solid phase, competitive chemiluminescent enzyme immunoassay. Incubation Cycles: 1 × 30 minutes. Storage: Assay within 2-3 hours or store frozen at –20°C for 1 week.

The calculation of ratios of c-peptide to glucose and c-peptide to HbA1C was performed by dividing fasting c-peptide (ng/ml) to fasting plasma glucose (mg/dl) x100 and fasting plasma HbA1C, respectively. The formula of fasting insulin (μU/ml) x fasting plasma glucose (mg/dl) / 405 was used for the estimation of homeostatic model assessment of insulin resistance (HOMA-IR).

The study was approved by the Ethics Committee of Ondokuz Mayis University on 01.03.2019 (Clinical research ethics committee decision number: 2019/192). Written informed consent were obtained from the parents and/or legal guardians of all patients included in the study.

Statistical analysis

Statistical analyses were performed using SPSS v. 22.0 software (Statistical Package for Social Sciences, Inc.). Power analysis revealed that at least 20 patients were required for each group. Normally distributed data were stated as mean (standard deviation) and non-normally distributed data were stated as median (range) values. Comparisons of independent binary groups with normal distribution were made with the t test, and ANOVA variance analysis was applied to hypervariable groups. The Mann-Whitney U test was used to compare over-variant groups, and two groups of non-normally distributed data. For the comparison of percentages of qualitative data, the Paired chi-square test and z-test were applied. When interpreting the association based on Pearson correlation coefficients, reference ranges were adopted as follows: $0.00 < r < 0.25$-very weak; $0.26 < r < 0.49$-weak; $0.50 < r < 0.69$-moderate; $0.70 < r < 0.8$-high; and $0.90 < r < 1.00$-very high. A value of $P<0.05$ was considered statistically significant. Performance of different models was assessed by the area under the receiver operating characteristic (ROC) curve.

Results

This study comprised 37 males (61.7%), and 23 females (%38.3), with a mean age of 11.9 (2.9) years, and NAFLD was present in 40 (66.7%) out of 60 patients.

The mean weight z-score, BMI z-score and waist circumference were 2.6 (0.4), 2.58 (0.21), and 94.3 (17.4) cm, respectively.

Of the 40 obese children with NAFLD, 23 (57.5%) were male and the mean age was 12.9 (2.5) years. Their mean fasting c-peptide level, fasting c-peptide to fasting glucose ratio, fasting c-peptide to HbA1C ratio, and insulin levels were 4.05 (0.49)

ng/ml, 4.866 (0.62), 0.81 (0.10) (range: 0.06-1.1), and 22.8 (3.2) µU/ml, respectively. Of the patients with NAFLD, 23 (57.5%) had high ALT levels and a mean fasting c-peptide level of 4.29 (0.23) ng/ml.

Among 20 obese children without NAFLD, 14 (70%) were male and the mean age was 9.9 (2.5) years. The mean serum c-peptide level, fasting c-peptide to fasting glucose ratio, fasting c-peptide to HbA1C ratio, and insulin levels were 2.4 (0.32) ng/ml, 2.771 (0.33), 0.42 (0.02), and 18.6 (3.5) µU/ml, respectively. Demographic and laboratory parameters of the patients are shown in Table 1.

Table 1: Comparison of demographic findings of children with obesity

Total (n = 60)	Hepatosteatosis (n = 40)	Non-hepatosteatosis (n = 20)	*P*-value
Age (years)	12.9 (2.5)	9.9 (2.5)	0.530
Gender (Female/Male)	17/23	6/14	0.257
Height z-score	0.57 (0.17)	0.77 (0.19)	0.320
Weight (kg)	69.6 (5.02)	51.2 (3.4)	*0.039*
Weight z-core	2.7 (0.6)	2.3(0.13)	0.146
BMI (kg/m^2)	32.3 (1.4)	26.9(0.96)	< 0.001
BMI z Score	2.6 (0.21)	2.1(0.19)	0.145
Waist circumference (cm)	99.03 (18.2)	84.8 (10.3)	*0.001*
Waist-to-height ratio	0.67 (0.23)	0.59(0.05)	*0.015*
Fasting C-peptide(ng/ml)	4.05(0.49)	2.4(0.32)	*0.011*
C-peptide-to- glucose ratio	4.866 (0.62)	2.771(0.33)	*0.005*
C-peptide-to-HbA1C ratio	0.81(0.10)	0.42(0.02)	*0.003*
AST U/L	34.2 (14)	22.7(5.9)	0.252
ALT U/L	54.04(9.3)	20.4(9.7)	*0.016*
Fasting glucose (mg/dl)	83.9(10.1)	85.0(8.9)	0.210
Insulin	22.8(3.2)	18.6(3.5)	*0.031*
HOMA-IR	4.8(0.68)	4.55(0.55)	0.113
HbA1c	5.2(0.35)	5.3(0.33)	0.691
Triglyceride (mg/dl)	127.7(11.09)	124.4(7.5)	0.426
Total cholesterol (mg/dl)	158.2(34.8)	159.5(22.6)	0.877
HDL (mg/dl)	42.2(9.8)	44.7(7.8)	0.145
LDL (mg/dl)	92.1(20.2)	90.5(19.4)	0.961

F: female, M: male, AST: Aspartate aminotransferase ALT: Alanine aminotransferase, HDL: High density lipoprotein, LDL: Low density lipoprotein, BMI: Body mass index, HOMA-IR: homeostatic model assessment of insulin resistance (Normal values; AST, 8–46 U/L (female), 8–40 U/L (male); ALT, 0–35 U/L (female), 0–40 U/L (male); triglyceride, 0-200 mg/dl; total cholesterol,0-200 mg/dl; HDL, 35-75 mg/dl; LDL, 0-165 mg/dl; fasting glucose, 70-110 mg/dL; insulin, 3-30; HbA1c, 4-6; fasting c-peptide, (0.0-7.1) ng/ml)

The BMI, weight, waist circumference, waist to height ratio, ALT, c-peptide (Figure 1) fasting c-peptide to fasting glucose ratio, fasting c-peptide to HbA1C ratio and insulin levels of NAFLD patients were significantly higher than those without NAFLD (*P*<0.001, *P*=0.039, *P*=0.001, *P*=0.015, *P*=0.016, *P*=0.011, *P*=0.005, *P*=0.003 and *P*=0.031, respectively).

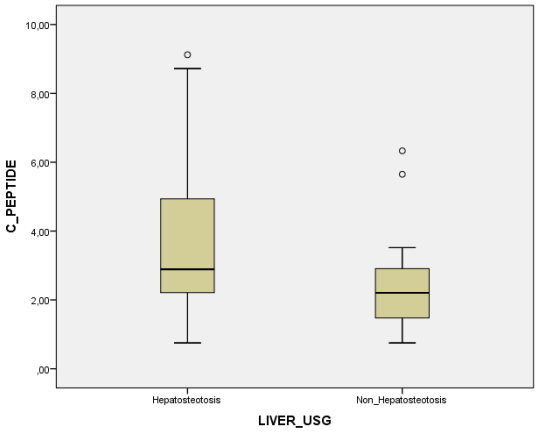

Figure 1: Comparison of C-peptide levels between the groups

A positive correlation was found between fasting c-peptide level and waist circumference (r = 0.333, *P*=0.009), BMI (r = 0.378, *P*=0.003) insulin (r = 0.845, *P*<0.001) and HOMA-IR (r = 0.785, *P*<0.001). When the c-peptide levels of all the patients were evaluated by ROC analysis, the area under the curve in patients with NAFLD was 0.70 (95% CL: 0.725-0.955) and the c-peptide cut off value was 2.62 ng/ml (sensitivity 60%, specificity 75%, *P*=0.011) (Figure 2). When evaluated by logistic regression analysis (OR: 4.52 95% CI: 1.65-3.25), the risk of fatty liver was significantly higher in obese children with c-peptide levels greater than 2.62 ng/ml (*P*<0.001).

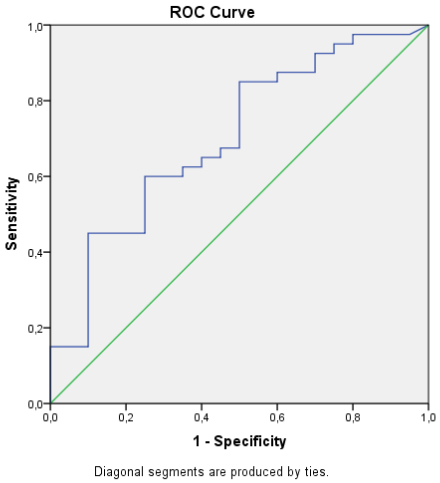

Figure 2: Cut-off C-peptide = 2.62 ng / mL level for NAFLD diagnosis with 60% sensitivity and 75% specificity

Discussion

Non-alcoholic fatty liver disease is one of the most common liver disorders. It is characterized by fat accumulation in liver (steatosis) and includes chronic liver diseases ranging from simple fatty infiltration to inflammation and fibrosis [2]. It is well known that pediatric NAFLD is associated with increased insulin resistance, dyslipidemia, cardiovascular disease, and most importantly, visceral adiposity [11-12]. The relationship between NAFLD and c-peptide has been described in adult studies [13-14].

Insulin resistance is the most common metabolic abnormality associated with NAFLD and it is related with disease severity and progression [15]. In clinical practice, while insulin resistance is routinely diagnosed with fasting plasma glucose, HbA1c and insulin levels, c-peptide is not commonly used [16]. In this study, the fasting c-peptide level of children with NAFLD was significantly higher than the non-NAFLD group. The risk of NAFLD was 4.52 times (95% CI: 1.65-3.25) higher in children with increased c-peptide levels. C-peptide is a widely used method for evaluating pancreatic beta cell function [17]. The pancreatic beta cells secrete proinsulin which cleaves into c-peptide and insulin prior to secretion. Even though the quantity of c-peptide is equal to the produced insulin level, the half-life of c-peptide (20-30 min) is higher when compared to insulin (3-5 min). That characteristic renders c-peptide a reliable method to test the functionality of beta cells [18].

In this study, c-peptide to HbA1C and c-peptide to fasting glucose ratios of NAFLD patients were considerably higher when compared to non-NAFLD patients. El-Koofy et al. [19] demonstrated that serum fasting insulin and c-peptide of obese patients were significantly higher than those of the control group. While the ratio of serum c-peptide to glucose was used in adult studies to determine the efficacy of treatment, no studies have investigated the relationship between peptide to fasting glucose, c-peptide to HbA1C ratio and hepatic steatosis [20]. In this study, the association between NAFLD and c-peptide to

fasting glucose and c-peptide to HbA1C ratios showed that the loss of β cell mass in obese children with fatty liver might be due to increased free fatty acids, oxidative stress, and inflammation.

In adult studies, the elevation of serum c-peptide in patients with obesity- and diabetes-associated NAFLD has been demonstrated [21]. The study of 542 patients by Francque et al. [22] reported a direct relationship between NAFLD patients and c-peptide levels. In a recent study, c-peptide was an independent risk factor for NAFLD and utilized to assess the insulin resistance in these patients [13]. Tricò et al. [21] found that in patients without NAFLD, high fasting glucose and c-peptide levels were risk factors for NAFLD development in follow-up periods.

In our study, the values of waist circumference, waist to height ratio and BMI of NAFLD patients were substantially higher than non-NAFLD patients. Manco et al. [23] reported that 92% of patients with NAFLD had a higher BMI and 84% had a wider waist circumference. It has been shown that every 5 cm increment in the waist circumference of obese children or adolescents increases the probability of ultrasound to detect fatty liver by 1.4 times [15]. In addition, a cross-sectional study involving 145 pediatric patients reported a significant relationship between the incidence of NAFLD and waist circumference, total fat mass, and intraabdominal adipose tissue [24]. For this reason, waist circumference can be utilized as a credible screening modality in pediatric NAFLD patients. In our study, waist circumference and waist to height ratio were significantly elevated in NAFLD patients, which indicate that patients with NAFLD accumulate more visceral adipose tissue than non-NAFLD ones. NAFLD patients are more insulin resistant than non-NAFLD patients. Both BMI and waist circumference are considered important parameters in predicting NAFLD severity and steatosis [24].

Increased ALT levels are frequently seen in pediatric patients with NAFLD [25]. Generally, slight elevation of aminotransferases (1.5-2 times the upper limit of normal) is observed [2]. In this study, the ALT levels of obese children with NAFLD were significantly higher than patients without NAFLD. The study by Arslan et al. [26] presented that BMI, AST, ALT, GGT and triglyceride values were remarkably high in obese children with NAFLD compared to non-NAFLD ones. Based on the serum ALT > 30 U/L threshold, the National Health and Nutrition Examination Survey in the United States estimated a prevalence of 8% NAFLD in a study of adolescents [27]. Central obesity has also been shown to reliably predict the evidence of ultrasonographic characteristics and elevated aminotransferase levels in NAFLD in more than 11,000 children with obesity aged 6-18 years [28].

Limitations

Only ultrasound was utilized for diagnosis in our study, however, liver biopsy is the most important method in diagnosing NAFLD. Another limitation is the lack of usage of elastography, which has increased diagnostic efficiency for it can determine tissue stiffness. Additionally, the sparse number of patients and absence of genetic examination for obesity are amongst constraints. Although the fasting c-peptide and c-peptide to glucose ratio of the patients were evaluated, they were not compared with postprandial values.

Conclusions

Obesity has become an increasingly common public health problem in childhood. The most common etiology is obesity-related insulin resistance. In this study, fasting c-peptide level besides ALT, BMI, waist circumference and weight to height ratio were main factors associated with NAFLD. In patients with NAFLD, serum fasting c-peptide level measurement was considered as significant as fasting insulin level. Even though BMI, waist circumference, waist to height ratio, HbA1c and insulin levels are better standard parameters in determining insulin resistance in NAFLD patients, c-peptide can also be used for screening as a non-invasive and inexpensive method.

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