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Original Article

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Investigation of Blood Betatrophin Levels in Obese Children with Non-Alcoholic Fatty Liver Disease

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Purpose: The prevalence of obesity has significantly increased among children and adolescents worldwide and is becoming an important health care problem in parallel with the increased prevalence of obesity pediatric non-alcoholic fatty liver disease. Betatrophin is a newly define hormone that is commonly secreted by liver and plays role in glucose tolerance. This study aimed to investigate the relationship between serum betatrophin levels and non-alcoholic fatty liver disease in obese children.

Methods: The study included 40 obese children with a body mass index (BMI) above 95th centile, and 35 non-obese subjects with a BMI 3-85th centile, whose age and gender were similar to those of the patient group. For the evaluation of metabolic parameters fasting serum glucose, insulin, alanine aminotransferase, aspartate aminotransferase, lipid profile and serum betatrophin levels were measured. Total cholesterol: high-density lipoprotein cholesterol and low-density lipoprotein cholesterol: high-density lipoprotein cholesterol ratios were calculated as "atherogenic indices."

Results: Serum betatrophin levels of the obese subjects were similar to that of non-obese subjects (p=0.90). Betatrophin levels were not correlated with the metabolic parameters.

Conclusion: In the present study, levels of betatrophin are not different between obese and insulin resistant children and non-obese subjects, and they are not correlated with atherogenic indices. To elucidate the exact role of betatrophin in obesity, further studies are required to identify the betatrophin receptor and/or other possible cofactors.

Key Words: Betatrophin, Child, Obesity, Liver

INTRODUCTION

The prevalence of obesity has significantly increased among children and adolescents worldwide and is becoming an important health care problem. Recently, in parallel with the increased prevalence of

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obesity pediatric non-alcoholic fatty liver disease (NAFLD) has also become widespread both in developed and developing countries and also an important medical problem worldwide [1,2]. Increased prevalence of NAFLD has been reported in various studies, it is suggested that increased incidence of NAFLD is a consequence of the increased incidence of obesity and metabolic syndrome in Western world [3]. The prevalence of pediatric NAFLD is estimated between 2.6% and 9.6% and associated with age, sex and ethnicity [4]. Excess lipid accumulation in adipose tissue can lead to ectopic fat deposition in non-adipose tissues, such as muscle, liver and pancreas [5]. Furthermore, as a key metabolic organ of the body, the liver has a critical role in lipid metabolism [6]. Moreover, NAFLD is one of the most common complications of pediatric obesity strongly associated with the clinical features of insulin resistance, especially including type 2 diabetes mellitus (T2DM) and metabolic syndrome in obese youth [7]. It was shown that metabolic changes are associated with leptin, adiponectin, irisin, fibroblast-like growth factor and some cytokines in obesity [8,9].

Betatrophin is a newly define hormone that is commonly secreted by liver but also from adipose tissue and leads proliferation and development of pancreas β cells and plays role in glucose tolerance [10]. Accumulation of free fatty acids in the hepatocytes of patients with NAFLD might be associated with the levels of betatrophin in insulin-resistant obesity. The studies about effects of betatrophin are in obese or diabetic adults and in experimental animals [11,12]. There are only a few studies about betatrophin levels in obese children [13]. In the present study we aimed to introduce serum betatrophin levels in obese children with NAFLD.

MATERIALS AND METHODS

Ethics statement

This prospective study was approved by the local ethics committee of Çanakkale Onsekiz Mart University Faculty of Medicine (IRB no. EK-2014-172) and conducted in accordance with the principles of the Declaration of Helsinki. An equal number of pediatric patients diagnosed with exogenous obesity and healthy control subjects were recruited for the study after informed consent was obtained from their parents.

Study population

The study was conducted at the Pediatric Unit of the Çanakkale Onsekiz Mart University, Faculty of Medicine, from December 2014 to July 2015. The study included 58 obese children and adolescents with a body mass index (BMI) above 95th centile, according to reference curves for Turkish children [14]. All children had diagnosis of exogenous obesity and the exclusion criteria included hepatic virus infections (hepatitis A, B, C, cytomegalovirus and Epstein-Barr virus infections), history of parenteral nutrition, alcohol consumption, and drug history that affect carbohydrate metabolism and body weight and induce steatosis (e.g., valproic acid, amiodarone, L-asparaginase, glucocorticoids). Metabolic and autoimmune, α-1-antitrypsin associated liver disease and Wilson's disease were ruled out based on standard clinical and laboratory criteria. Familial history of obesity and diabetes was questioned. None of the patients had a familial history of T2DM. The exclusion criteria also included a previous diagnosis of any disease affecting the endocrine system (e.g., hypothyroidism, Cushing's disease), any syndrome associated with obesity (e.g., Prader-Willi and Laurence-Moon-Biedle syndromes), other systemic disorders, and/or a history of drug use. Exogenous obesity was defined as no endocrine, metabolic, or genetic causes of obesity. Twenty-four healthy individuals with a BMI 3-85th centile, whose age and gender were similar to those of the patient group were enrolled as normal weight group.

Subjects and study design

The subjects were divided into three group.

NAFLD obese group consisted of 16 girls and 16 boys with mean age: 13.31 years, mean BMI 30 kg/m^2 ($\pm$6) and ultrasound evidence of fatty changes in the liver (Table 1).

Table 1. The Clinical and Laboratory Characteristics of Study Groups

Characteristic	Groups			*p*-value
	Obese without NAFLD	Obese with NAFLD	Control	
Age (y)	12.82±3.32	13.31±2.83	13.09±3.27	0.164*
Gender (female/male)	19/7	16/16	16/8	0.145‡
BMI (kg/m^2)	29±5	30±6	19±2	<0.001*
WC (cm)	84 (55-117)	96 (57-126)§	63 (52-70)	<0.001†
HC (cm)	94 (62-160)	105.5 (75-135)§	79 (69-97)	<0.001†
WC/HC	0.87 (0.54-1.34)	0.89 (0.55-1.17)‖	0.8 (0.72-0.88)	<0.001†
Systolic BP (mmHg)	111 (90-160)	120 (90-136)	100 (85-117)	<0.001†
Diastolic BP (mmHg)	70 (50-100)	70 (60-97)	60 (45-70)	<0.001†
Betatropin (ng/mL)	10 (5-116)	9 (4-115)	10 (6-68)	0.98†
Glucose (mg/dL)	91±13	90±8	87±7	0.365*
Insulin (μU/mL)	17.40 (2-43)	20 (2-67.6)	4.13 (2-21.6)	<0.001†
HOMA-IR score	4.08 (0.33-12.1)	4.34 (0.45-14.86)	0.82 (0.4-4.85)	<0.001†
Total cholesterol (mg/dL)	165 (110-239)	164 (108-271)	153 (126-180)	0.651†
Triglyceride (mg/dL)	99.5±44.4	108.4±48	90±45	0.312*
LDL-C (mg/dL)	101 (46-191)	103 (49-216)	88 (68-108)	0.913†
HDL-C (mg/dL)	55 (20-113)	48 (36-83)	53 (41-65)	0.602†
AST (U/L)	23 (16-37)	27 (15-76)	21 (10-32)	<0.001†
ALT (U/L)	17 (10-48)	37 (31-78)	13 (7-39)	<0.001†

Values are presented as mean±standard deviation, number only, or median (range).
NAFLD: non-alcoholic fatty liver disease, BMI: body mass ındex, WC: waist circumference, HC: hip circumference, BP: blood pressure, HOMA-IR: homeostasis model assessment-insulin resistance index, LDL-C: low-density lipoprotein cholesterol, HDL-C: high-density lipoprotein cholesterol, AST: aspartate aminotransferase, ALT: alanine aminotransferase.
*ANOVA, †Kruskal-Wallis test, ‡chi-square test. §$p<0.016$ (Bonferroni correction) comparison between obese group without NAFLD and obese group with NAFLD. ‖$p>0.016$ (Bonferroni correction) comparison between obese group without NAFLD and obese group with NAFLD.

Non-NAFLD obese group consisted of 19 girls and 7 boys with mean age 12.82 years, mean BMI 29 kg/m^2 (±5) and with no ultrasound evidence of fatty changes in the liver (Table 1).

Non-obese (normal weight) control group included 16 girls and 8 boys with mean age: 13.09 years, mean BMI 19 kg/m^2 (±2) (Table 1). This control group consisted of healthy subjects presented to the hospital for minor illnesses such as conjunctivitis, common cold or other similar conditions. None of the subjects was vegetarian.

Anthropometric measurement

Height was measured to the nearest 0.5 cm with the subject naked feet, eyes looking straight ahead, back against the wall. Weight was measured in the post absorptive state, using a standard lever scale, sensitive to 100 g and BMI was calculated as weight in kilograms divided by the square of height in meters. The same investigator (FB) measured body weight and height using a stadiometer (Seca 703, accurate to 100 g; Seca GmBH&Co. Kg, Hamburg, Germany).

Blood pressure

After resting for at least 5 minutes, diastolic and systolic pressure (mmHg) were measured with children in a sitting position, using a cuff appropriate for body size and a mercury-gravity manometer.

Biochemical analysis

Following overnight fasting, blood samples were collected from obese children in the morning using venous puncture technique into Vacutainer® serum tubes with gel separator (BD Vacutainer, Plymouth, UK). The samples were then analyzed on the same day for serum lipid profile (total cholesterol [TC], high-density lipoprotein cholesterol [HDL-C], low-

density lipoprotein cholesterol [LDL-C], and triglycerides [TG]), and plasma glucose by enzymatic methods using Roche Cobas c 501 analyzer (Roche Diagnostics, Indianapolis, IN, USA) and for insulin measurement by electrochemiluminescence immunoassay using Roche Cobas e 601 analyzer (Roche Diagnostics). Insulin resistance was assessed through the homeostasis model assessment-insulin resistance index (HOMA-IR). The status of insulin resistance was determined using different cut-off values for prepubertal (>2.5) and pubertal (>4) stages [15].

Measurement of betatrophin levels

Serum samples collected for betatrophin were kept at -80^{o} until analysis. Serum levels of betatrophin were measured with enzyme-linked immunosorbent assay kit in accordance with the manufacturer's instructions (Cat. no: YHB3586Hu, Yehua Biological Technology, Shangai, China). The intra- and inter-assay coefficients of variations were $<10\%$ and $<12\%$ for betatrophin (ng/L) respectively.

Liver ultrasonography

All patients with abnormally high transaminase levels and those having abnormal liver images on ultrasound were screened for other liver conditions (hepatitis B surface antigen, hepatitis C antibody, serum iron level, total iron-binding capacity, prothrombin time, ferritin, and antinuclear antibodies) which were all negative. Liver ultrasonography was carried out by an experienced operator (NA) who was not aware of clinical and laboratory characteristics of the subjects. Scans were performed with a Toshiba Aplio XG model ultrasonography using 3.5 MHz prob. The presence of NAFLD was evaluated according to the scoring system defined by Singh et al. [16] based on the visibility of vascular structures, hyperechogenicity of liver tissue and difference in echogenicity between liver and diaphragm. Pediatric NAFLD is defined as chronic hepatic steatosis in children aged less than 18 years without an etiology of genetic or metabolic disorders, medications, ethanol consumption, infections or malnutrition [17].

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics ver. 21.0 (IBM Co., Armonk, NY, USA). Normality of the variables was evaluated through visual (histograms, probability plots) and analytical methods (Shapiro-Wilk's test). According to tests of normality, either the Student's t-test or Mann-Whitney U-test was used depending on the results of normality tests in order to compare differences between NAFLD with obese and non-obese children. Pearson's correlation was used to investigate the correlations between the independent parameters. The $p<0.05$ values were considered statistically significant.

RESULTS

Clinical and laboratory characteristics of the study population

The clinical and laboratory characteristics of the children are summarized in Table 1. NAFLD obese subjects had significantly higher values of BMI, systolic blood pressure (SBP), and diastolic blood pressure (DBP) compared with gender- and age-matched non-obese subjects. No significant differences were detected in serum betatrophin levels between three subgroups.

Furthermore, there were not statistically significant differences between non-obese and NAFLD

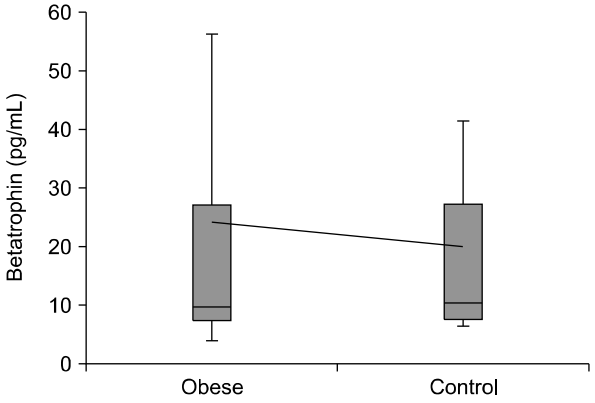

Fig. 1. Comparison of serum betatrophin concentration between obese and non-obese subjects.

Table 2. The Correlations between Betatrophin Levels and Clinical and Laboratory Parameters in the Study Groups

	Obese without NAFLD		Obese with NAFLD		Control	
	r	*p*	r	*p*	r	*p*
Weight*	−0.026	0.898	0.043	0.817	0.292	0.156
Height*	0.1	0.626	0.198	0.276	0.220	0.290
BMI†	−0.034	0.868	−0.128	0.484	0.1	0.635
Systolic BP*	−0.114	0.581	0.129	0.481	0.251	0.226
Diastolic BP*	0.036	0.862	−0.058	0.751	0.316	0.124
WC*	0.062	0.764	0.201	0.271	0.313	0.128
HC*	−0.041	0.843	0.021	0.908	0.237	0.253
WC/HC*	−0.116	0.572	0.351	0.049	0.115	0.585
HOMA-IR*	0.106	0.605	0.266	0.141	−0.052	0.804
Insulin*	0.041	0.842	0.273	0.131	−0.070	0.740
Glucose†	0.287	0.155	0.023	0.902	0.099	0.638
Cholesterol*	−0.143	0.485	−0.036	0.844	−0.018	0.930
LDL-C*	−0.021	0.919	0.16	0.383	0.368	0.070
HDL-C*	0.006	0.975	−0.335	0.061	−0.272	0.189
TG†	−0.163	0.428	−0.009	0.962	−0.145	0.490
AST*	0.188	0.358	−0.071	0.697	−0.091	0.665
ALT*	−0.215	0.291	−0.334	0.062	−0.042	0.843

NAFLD: non-alcoholic fatty liver disease, BMI: body mass ındex, BP: blood pressure, WC: waist circumference, HC: hip circumference, HOMA-IR: homeostasis model assessment-insulin resistance index, LDL-C: low-density lipoprotein cholesterol, HDL-C: high-density lipoprotein cholesterol, TG: triglyceride, AST: aspartate aminotransferase, ALT: alanine aminotransferase.
*Spearman correlation, †Pearson correlation.

obese groups regarding the TG, TC, HDL-C, LDL-C, HOMA-IR, serum insulin, and betatrophin (Table 1, Fig. 1). Serum betatrophin level was similar in the obese subjects with NAFLD compared to the non-NAFLD group (median, 9 [4-115]; median, 10 [5-116]; $p=0.90$, respectively).

Correlation of betatrophin with clinical parameters

In study groups, serum betatrophin levels were not correlated with the glucose, fasting serum insulin, HOMA-IR, TG, TC, HDL-C, LDL-C, BMI, SBP, DBP, alanine aminotransferase, and aspartate aminotransferase ($p>0.05$) (Table 2).

There was also no correlation between degree of hepatosteatosis and serum betatrophin levels (r=0.139, $p=0.449$).

DISCUSSION

In the present study there were no difference of serum betatrophin levels of obese and control groups. There are only a few studies in the childhood or adolescence about the relations between circulating betatrophin concentrations and metabolic parameters. Gómez-Ambrosi et al. [11] showed decreased betatrophin levels, while Fu et al. [10] demonstrated increased betatrophin levels in T2DM patients. On the other hand, Fenzl et al. [18] did not find any correlation between serum glucose and/or insulin levels and circulating betatrophin concentrations in the obese adult patients. Wu et al. [13] showed that betatrophin levels were higher in insulin-resistant patients compared to non-insulin resistant subjects. However, in that study, betatrophin levels were not increased significantly in the obese children compared with age- and gender-matched normal-weight children. Hu et al. [19] found a negative correlation between betatrophin levels and insulin sensitivity, Gómez-Ambrosi et al. [11] found a positive correlation between these two parameters in T2DM patients. A more recent study with a large sample size of T2DM patients, reported no significant correlation between betatrophin levels and insulin resistance [20]. In our

study, we could not find any correlation between betatrophin and HOMA-IR in obese and control persons. The differences among published reports may be due to the design and sample size and variations in demographics and ethnic characteristics of the studies. The variations among the studies in terms of betatrophin levels in these metabolic disorders may also be related to the inflammation degree. Calan et al. [21] reported a strong positive correlation between betatrophin and serum high sensitivity C-reactive protein (hs-CRP) which is an inflammatory marker. Moreover, several multiple linear regression analyses indicated that betatrophin levels are affected by hs-CRP independently. Thus, further human studies are warranted to clarify the relationship between betatrophin levels and insulin resistance.

Additionally, betatrophin is involved in the regulation of lipid metabolism by reducing triglyceride clearance [12,22]. Moreover, lipid metabolism is known to be affected by the variations in betatrophin sequence [23]. Although there is an evidence showing a strong association between betatrophin and lipid homeostasis, human studies on this issue are not sufficient. Betatrophin has been reported to be positively correlated with both LDL-C and TC [18]. Gao et al. [24] also reported a positive correlation between betatrophin and TG. On the other hand, in another study, betatrophin levels were found to be positively correlated with HDL-C and negatively correlated with TG [11]. In addition, Chen et al. [25] reported a negative correlation between betatrophin and TC, LDL-C and HDL-C. In our study, obese children did not exhibit dyslipidemia compared with controls. No significant correlations were found between betatrophin levels and atherogenic lipid profiles and also no significant correlation with HDL-C was detected in obesity and control groups.

The present study has some limitations. First, our study sample included small number of children. Second limitation of the study is the technique used to evaluate insulin resistance. Although it is inferior to the clamp technique, we used the less invasive HOMA-IR method due to ethical considerations. This should be taken into consideration in future studies about betatrophin levels. Finally, participants were not screened for the variations in betatrophin sequence.

In summary, the present work is of importance because we demonstrated that levels of betatrophin did not increase in obese and insulin resistant children compared to non-obese subjects, and they are not correlated with atherogenic indices. However, further studies are required to identify the betatrophin receptor and/or other possible cofactors in order to elucidate the exact role of betatrophin in obesity and/or insulin resistance.

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