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Original Research

Relationship between betatrophin levels and metabolic parameters in patients with polycystic ovary syndrome

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Abstract: To evaluate the status of serum betatrophin levels and potential relations between metabolic parameters and betatrophin levels in patients with polycystic ovary syndrome. We included patients newly diagnosed with PCOS in our study. Fifty-seven female patients (30 patients with PCOS and 27 healthy control subjects) were enrolled in this study. Serum betatrophin levels were measured using a betatrophin enzyme-linked immunosorbent assay kit. Insulin resistance was calculated using the homeostasis model of the assessment-insulin resistance index formula. The betatrophin level was 1538,85 ng/L in the patient group and 2440,46 ng/L in the control group, and the difference was statistically significant (p=0.003). A significantly negative correlation was found between betatrophin level and insulin, HOMA-IR, and BMI. Betatrophin levels in patients with PCOS are lower than those without PCOS and inversely related to insulin resistance.

Key words: Betatrophin, insulin resistance, polycystic ovary syndrome.

Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine pathology in women of reproductive age; approximately 18% are affected (1). PCOS is characterized by ovulatory dysfunction and hyperandrogenism and leads to metabolic disorders (2). The occurrence of metabolic syndrome, obesity, dyslipidemia, and the susceptibility to diabetes is increased in patients with PCOS, and it has been shown that these metabolic disturbances are associated with insulin resistance (3-5). Insulin resistance and hyperandrogenism have also been found to affect adipose cell functions. Insulin resistance facilitates the differentiation of preadipocytes from adipocytes, which causes abdominal obesity and facilitates visceral obesity (6). Some adipose tissue hormones function as an endocrine organ, causing the release of cytokines and adipokines and playing a role in glucose and fat metabolism (7,8). Although the exact etiology of PCOS is unknown, PCOS is considered a common and complex disease caused by the interaction between genetic and environmental factors (9).

Betatrophin, also known as lipasin (10,11), atypical angiopoietin-like protein 8 (ANGPTL8) (12), refeeding induced fat and liver protein (12), and chromosome 19 open reading frame 80, is primarily released from the liver and adipose tissue (12-14). Animal studies have shown that betatrophin increases pancreatic beta-cell proliferation and beta-cell mass and also improves glucose tolerance in animal models with insulin resistance (14).

The pathogenesis of betatrophin's effects on insulin secretion and glucose homeostasis is still not completely known. Clinical studies have shown that obese type 1 and type 2 diabetic patients have increased levels of betatrophin (15-20), decreased levels (21), or levels that remain unchanged (22).

In this study, we evaluated the status of serum betatrophin levels and evaluated the potential relations between metabolic parameters and betatrophin levels in patients with PCOS.

Materials and Methods

Fifty-seven female patients (30 patients with PCOS and 27 healthy control subjects) were enrolled in this study. Each participant signed an informed consent form in accordance with the Declaration of Helsinki**.** The study was approved by the local ethics committee of Canakkale Onsekiz Mart University (2015/03-01).

We included patients newly diagnosed with PCOS in our study. Diagnosis of PCOS was based on the 2003 Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group diagnostic criteria as follows: oligo or anovulation, clinical and/or biochemical hyperandrogenism, or positive ultrasound presentation of polycystic ovaries determined by transvaginal scan and/or abdominal scan and defined as the presence of 12 or more follicles measuring 2–9 mm in diameter in each ovary and /or an ovarian volume of >10 mL (23). Our exclusion criteria were a history of cardiovascular disease, cancer, systemic infection, diabetes mellitus, liver disease, renal disease, hematological disease, thyroid dysfunction, congenital adrenal hyperplasia, hyperprolactinemia, or those who had received medical treatment and those who smoked or used alcohol. The con-

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trol group was composed of healthy female volunteers. Demographic, anthropometric, and clinical variables were also recorded.

Venous blood samples of 5 mL were drawn simultaneously from the cases between 09:00 AM and 10:00 AM during 3–5 days of the follicular phase. All patients and controls fasted one night prior to the sample collection. Blood samples were drawn into vacutainer EDTA aprotinin tubes for betatrophin and stored at –80 °C until the time of analysis. Serum betatrophin levels were measured using a betatrophin enzyme-linked immunosorbent assay (ELISA) kit (Cat. no: 201-12-5327; Sunred Biological Technology, Shangai, China) according to the manufacturer's instructions. The intra-assay and inter-assay coefficients of variations were <10% and <12%, respectively, for betatrophin (ng/L). Insulin resistance was calculated using the homeostasis model of assessment-insulin resistance index (HOMA-IR) formula: fasting blood glucose (FBG; mmol/L) × fasting insulin (mU/L)/22.5.

SPSS version 19.0 (IBM, Chicago, IL, USA) was used for statistical analysis, and a p value of <0.05 was considered statistically significant. Continuous variables were expressed as mean ± standard deviation. The *t* test and Mann–Whitney *U* test were used for parametric and non-parametric variables, respectively. Correlation between the parameters was analyzed by the Pearson and Spearman methods for parametric and non-parametric variables, respectively. Differences were considered significant at p<0.05. The determinants of the existence of betatrophin, (independent variables: BMI, insulin, and HOMA-IR) were evaluated separately using multiple linear regression analyses with enter method.

Results

The average age of women with PCOS was 21.56 years and in the control group was 21.66 years; this difference was not statistically significant (p=0.105). The body mass index (BMI) was 26,86 in women with PCOS and 21,39 in the control group; this difference was statistically significant (p<0.000). The HOMA-IR level in the patient group (3.59) was significantly higher than that of the control group (2.15; p=0.001). Demographic characteristics and biochemical values of the PCOS patients and healthy controls are shown in Table 1.

The betatrophin level was 1538,85 ng/L in the patient group and 2440,46 ng/L in the control group, and the difference was statistically significant (p=0.003) (figure 1). A significantly negative correlation was found between betatrophin level and BMI, insulin, and HOMA-IR levels (p=0.03; p=0.04; p=0.02, respectively). Correlation values between betatrophin and other parameters are given in Table 2.

Multiple linear regression analyses were performed to assess the independent variables (BMI, insulin, and HOMA-IR) affecting the dependent variable (Betatrophin). The effects of these variables on Betatrophin were not significant (Table 3).

Discussion

We found betatrophin levels in PCOS patients to be lower than levels in the control group. We also showed a significant negative correlation between betatrophin level and insulin, BMI, and HOMA-IR level. We revealed through multiple regression analyses that PCOS is the only factor having an effect on the decline of

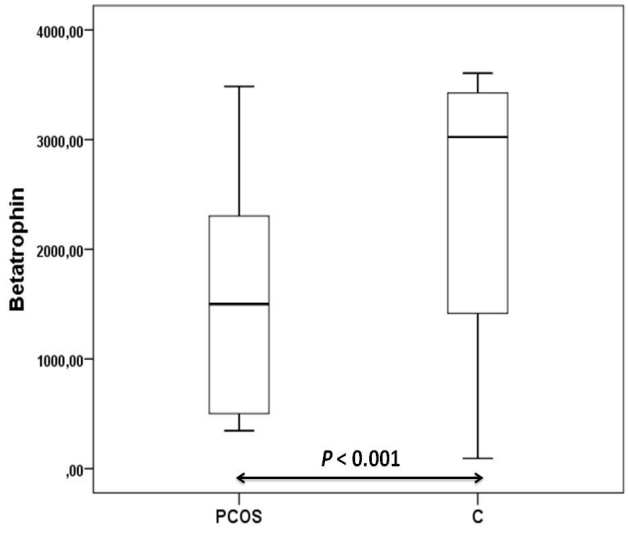

Figure 1. Box plot presentation of serum levels of Betatrophin in study participants.

Table 1. Demographical characteristics and biochemical values of controls and PCOS patients.

	PCOS Group (N:30)	Control Group (N:27)	P Value
Age(years)	21,56±0,79	21,66±0,31	0,105
BMI(kg/m²)	26,86±1,31	21,39±0,53	**0,000**
Glucose(mg/dl)	92,82±1,55	88,62±1,18	**0,03**
İnsulin(mU/L)	15,56±1,7	9,75±1,15	**0,007**
AST	17,87±1,5	14,55±0,51	**0,01**
ALT	18,2±4,01	12,51±0,75	0,44
LDL(mg/dl)	108,6±7,24	90,84±5,83	**0,06**
HDL(mg/dl)	58±3,9	70,24±3,72	**0,02**
TG(mg/dl)	98,68±11,89	61,77±4,04	**0,02**
TSH	2,4±0,21	1,9±0,17	0,08
HOMA-IR	3,59±0,38	2,15±0,25	**0,001**
Betatrophin(ng/L)	1538,85±1051,59	2440,46±1104,34	**0,003**

Abbreviations: BMI; body mass index, LDL; low density lipoprotein, TG; triglycerides, HDL; High density lipoprotein, AST; aspartate aminotransferase, ALT; Alanine aminotransferase, TSH; thyroid stimulating hormone, HOMA-IR; Homeostatic Model Assessment-Insulin Resitance.

Table 2. Correlation analysis between Betatrophin and other parameters in the whole study groups.

	r	p
Age	-0,04	0,76
BMI	-0,28	**0,03**
Glucose	0.07	0,95
Insulin	-0,27	**0,04**
TSH	-0,02	0,88
LDL	-0,24	0,07
HDL	0,16	0,27
TG	-0,15	0,24
HOMA-IR	-0,29	**0,02**

Table 3. Results of multiple regression analysis for dependent variable Betatrophin of the whole population (Model 1).

	B	ß	*p-value*	*95 % CI*
Model 1				
BMI	47,25	0,316	0,746	-251,99-346,50
HOMA-IR	2653,24	4,595	0,217	-1679,33-6985,80
Insulin	-586,80	-4,555	0,197	-1501,73-328,11

Dependent variable: Betatrophin Independent variables: BMI, HOMA-IR and insulin

BMI; Body Mass Index, HOMA-IR; homeostasis model of assessment-insulin resistance index

betatrophin level.

Published reports of betatrophin show conflicting data. While many studies have documented an increase in betatrophin levels in type 2 diabetic patients (15-17), Gomez-Ambrosini et al. showed the opposite (22). Mohamed Abu-Farha et al. in a large sample study (1,047 with type 2 diabetes and 556 without diabetes) found a significantly high level of betatrophin in patients with type 2 diabetes. In the same study, betatrophin levels showed a strong correlation with age in both groups, but the correlation with BMI, FBG, TG, and HOMA-IR was found only in the non-diabetic group. They also found that patients with type 2 diabetes had a negative correlation between betatrophin level and low-density lipoprotein and total cholesterol (24). In our study, we found no correlation between betatrophin level and age.

In conditions with increased insulin resistance, such as obesity, pregnancy, and PCOS, compensatory expansion of the beta-cell mass and insulin secretion is observed, but the underlying mechanisms are largely unknown. Betatrophin is a potential stimulator of beta-cell mass expansion and stimulates the proliferation of mice B cells (14); however, betatrophin has a limited impact on the proliferation of human B cells (25). The pathophysiologic role of betatrophin on metabolism and human B-cell function is still unknown (26). Hepatic betatrophin expression is increased with insulin receptor antagonists in mouse models with increased insulin sensitivity (14), and betatrophin concentration has been found to decrease with obesity and is negatively associated with insulin resistance (22). These results support the hypothesis that betatrophin level is regulated not only by insulin deficiency but also by insulin resistance. In our study, while the correlation between the level of FBG and betatrophin level was absent, betatrophin was found to correlate negatively with BMI, insulin, and HOMA-IR, similar to other studies. In our study, the patients with PCOS were significantly more obese than those in the control group. The insulin resistance of the patients with PCOS was higher than that of the control group, as it is supposed to be. We performed multiple regression analyses since obesity and insulin resistance, which have an important effect on betatrophin is confounder for the findings of our study. Consequently, we determined that betatrophin level isn't affected by BMI and HOMA-IR.

Studies conducted in individuals with type 1(27) and type 2 diabetes (17) suggest that impaired insulin secretion potentially increases the level of circulating betatrophin. Tokumato et al. (26) found that while there is a negative correlation between the concentration of insulin secretion and betatrophin concentration, there is a positive correlation with the duration of type 2 diabetes. They found no relation between the betatrophin concentration and BMI, HbA1c, or TGs and lipids, such as high-density lipoprotein cholesterol. In our study, we found a negative correlation between betatrophin level and HOMA-IR and insulin levels. However, unlike other studies, we found no relation between plasma betatrophin concentrations and age.

Betatrophin is secreted by liver and adipose tissue at high levels, and while its level increases significantly with nutrition, it is suppressed by hunger (11-13). Yang Wang et al. showed that in mice that are lacking betatrophin, weight gain is slower compared to wild-type littermates, lower levels of betatrophin while fasting increased after feeding. In response to food intake in mice, betatrophin plays an important role in the transition of TGs to peripheral tissues. Yang Wang et al. suggested that betatrophin inhibition might be useful in the treatment of dyslipidemia in mice lacking betatrophin due to a failure to store TGs in adipose tissue (28). In our study, we found no relation between betatrophin and TG levels. These different results between studies may be due to the use of different ELISA kits, age, ethnic groups, or differences in sample size.

It was revealed in the study performed by Calan et al. (29) that betatrophin level was higher than that of the control group. Similarly,it was revealed in this study that in addition to BMI not affecting betatrophin, HOMA-IR has positive effects on the betatrophin level. We couldn't explain this difference. However, betatrophin level was detected as declined in obesity and HOMA-IR, in many betatrophin-related studies. Differ-

ent ethnic groups, study design, and different number of patients, are considered the reasons for this difference. But, betatrophin levels in our study declined in accordance with other obesity and HOMA-IR study findings.

Limitations in our study included the small number of patients, blood being drawn while fasting in patients and control group, and betatrophin levels not being measured after feeding.

Betatrophin levels in PCOS patients are lower than those without PCOS and inversely related to insulin resistance. PCOS is generally evaluated in clinics as a result of cosmetic problems, such as hirsutism, and menstrual dysfunction or infertility and can be approached as a public health problem due to its frequency and the long-term health risks it carries. The etiology of the syndrome is not yet fully known, and the debate about diagnostic criteria is continuing. Similarly, there is no standard treatment regimen, and treatment of the syndrome is planned according to the individual patient. Elucidation of the molecular mechanisms of the changes in the signaling pathways of the postreceptor that causes a reduction in insulin influence will undoubtedly play an important role in better understanding the pathophysiology of PCOS and in developing new treatment options.

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