Insulin Resistance in Non-Obese Polycystic Ovary Syndrome Subjects and Relation with Family History of Diabetes Mellitus

Obez Olmayan Polikistik Over Sendromlu Olgularda İnsülin Direnci ve Diabetes Mellitus Aile Öyküsü ile İlişkisi

Emre Bozkırlı, Okan Bakıner, Eda Ertörer, İnan Anaforoğlu*, Neslihan Başçıl Tütüncü**, Nilgün Güvener Demirağ***

Başkent University Faculty of Medicine, Department of Endocrinology and Metabolism, Adana, Turkey
*Trabzon Numune Education and Research Hospital, Department of Endocrinology and Metabolism, Trabzon, Turkey
**Başkent University Faculty of Medicine, Department of Endocrinology and Metabolism, Ankara, Turkey
***Başkent University Faculty of Medicine, Department of Endocrinology and Metabolism, İstanbul, Turkey

Abstract

Purpose: Polycystic ovary syndrome (PCOS) is the most common endocrine disorder among women in reproductive age. Conflicting results are reported in the studies examining insulin resistance in lean PCOS subjects. We aimed to observe the controversial presence of insulin resistance in lean PCOS subjects with the gold standard method and assess the impacts of family history of type 2 diabetes mellitus (T2DM) on insulin resistance in these patients.

Material and Method: Nineteen patients with PCOS and nine age-BMI matched control subjects were recruited into the study. Patients with PCOS were divided into two groups according to their FH of T2DM among their first degree relatives (FHneg vs FHpos). Insulin resistance was evaluated with homeostasis model assessment of insulin resistance (HOMA-IR) and hyperinsulinemic euglycemic clamp technique for all participants.

Results: Mean M values were significantly higher in the control group when compared with PCOS patients (p=0.003). There was no statistically significant difference for HOMA-IR and M values when FHneg and FHpos patients were compared. Although HOMA-IR values were similar between all groups, M values were lower in FHneg and FHpos groups compared to the controls (p=0.02 and 0.004 respectively).

Discussion: Lean PCOS patients have evident insulin resistance when compared to healthy subjects, and FH of T2DM seems to not affect insulin resistance. Even non-obese PCOS patients should be encouraged for healthy eating style and exercise to prevent the potential risks associated with insulin resistance. Furthermore these patients can see benefits from medical therapies which improve insulin sensitivity. *Turk Jem 2015; 19: 55-59*

Key words: Polycystic ovary syndrome, insulin resistance, diabetes mellitus

Conflicts of Interest: The authors reported no conflict of interest related to this article.

Özet

Amaç: Polikistik over sendromu (PKOS) üreme çağındaki kadınlarda en sık görülen endokrin hastalıktır. Zayıf PKOS'lu olgularda insülin direncini araştıran çalışmalarda çelişkili sonuçlar bildirilmiştir. Çalışmamızda, zayıf PKOS hastalarında tartışmalı insülin direnci varlığını altın standart yöntem ile değerlendirmeyi ve bu hastalarda ailede tip 2 diabetes mellitus (T2DM) öyküsünün insülin direnci üzerine etkilerini araştırmayı amaçladık.

Gereç ve Yöntem: Çalışmaya 19 PKOS'lu hasta ve 9 yaş-VKİ benzer sağlıklı kontrol alındı. PKOS'lu hastalar birinci derece akrabalarında T2DM öyküsü varlığına göre iki gruba ayrıldı. Tüm katılanlarda insülin direnci "homeostasis model assessment of insulin resistance (HOMA-IR)" ve hiperinsülinemik öglisemik klemp tekniği ile ölçüldü.

Bulgular: Kontrol grubunda ortalama M değerleri PKOS'lu hastalara göre anlamlı derecede yüksekti (p=0,003). Ailesinde T2DM öyküsü olan ve olmayan PKOS'lu hastalar HOMA-IR ve M değerleri ile kıyaslandığında istatistiki anlamlı fark saptanmadı. Tüm gruplarda HOMA-IR değerleri benzer olmakla birlikte, T2DM aile öyküsü olmayan ve olan hastaların M değerleri konrol grubuna göre anlamlı düşüktü (sırasıyla; p=0,02 ve 0,004).

Tartışma: Sağlıklı kişiler ile kıyaslandığında zayıf PKOS hastalarında artmış insülin direnci bulunmaktadır ve ailede T2DM öyküsü varlığı insülin direncini etkilemiyor gözükmektedir. Obez olmayan PKOS'lu hastalar bile insülin direnci ile ilişkili potansiyel risklerden korunmak için sağlıklı beslenme ve egzersiz alışkanlığının edinilmesi yönünden bilinçlendirilmelidir. Ayrıca bu hastalar medikal tedavi seçeneği olarak insülin duyarlılığını arttıran ilaçlardan fayda görebilir. *Turk Jem 2015; 19: 55-59*

Anahtar kelimeler: Polikistik over sendromu, insülin direnci, diabetes mellitus

Çıkar Çatışması: Yazarlar bu makale ile ilgili olarak herhangi bir çıkar çatışması bildirmemiştir.

Introduction

Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders among premenopausal women, with a varying prevalence of 6.1-19.9% depending on the diagnostic criteria used (1). The characteristic features of the syndome are hyperandrogenism and chronic anovulation in the absence of specific diseases of the ovaries, adrenals and pituitary gland. In addition to fertility problems, patients are under risk for obesity, type 2 diabetes mellitus (T2DM), hypertension, dyslipidemia and cardiovascular diseases (2,3).

Besides the β-cell insulin secretory defects, insulin resistance (IR) and hyperinsulinemia play a key role in the pathogenesis of the disease (4). Most of the women with PCOS are insulin-resistant, and they have significantly increased risk for glucose metabolism disorders such as impaired glucose tolerance (IGT) and T2DM. It is well known that obese women with PCOS have evident IR in the base of excess fat tissue. However, conflicting results are reported in the studies examining IR in lean PCOS subjects (5,6).

A family history (FH) of T2DM is an evident increased risk for the development of T2DM in individuals without PCOS; but the question whether this is also acceptable in PCOS has been evaluated in relatively few studies of limited sample size (7,8,9,10). In a recent study; Lerchbaum et al. reported an independent association between FH of T2DM and central fat accumulation, obesity, prediabetes, metabolic syndrome, insulin resistance, low HDL and elevated blood pressure in 714 PCOS women (11). However, most of these studies including Lerchbaum's study were performed with varying patient groups in the mean of body mass indeces and usually homeostasis model assessment of insulin resistance (HOMA-IR) was used instead of "gold standard method" hyperinsulinemic euglycemic clamp (HEC) to evaluate insulin resistance.

In the present study, we aimed to observe the controversial presence of insulin resistance in lean PCOS subjects with HOMA-IR and HEC, and also to assess the impacts of family history of T2DM on insulin resistance. Especially non-obese PCOS patients were recruited to our study for excluding the aggravation of insulin resistance caused by excess fat tissue.

Materials and Methods

Selection of Participants

This study was performed with subjects who referred to Başkent University Endocrinology and Metabolism Diseases Outpatient Clinic and it was designed as a prospective case control study. PCOS patients were diagnosed according to the Rotterdam revised 2003 consensus on diagnostic criteria (12). Patients had to have at least two of the following conditions; 1) oligo- and/or anovulation, 2) clinical and/or biochemical signs of hyperandrogenism, 3) polycystic ovaries and exclusion of other aetiologies (congenital adrenal hyperplasias, androgen-secreting tumours, Cushing's syndrome) (12). Chronic ovulatory dysfunction was defined as intermenstrual intervals of ≥45 days or a total of fewer than eight menses per year (13).

Exclusion criteria for the study were; 1) a prior diagnosis of type 1 or type 2 diabetes mellitus, 2) overweight and obese subjects whose BMI were ≥25 (14), 3) pregnancy, 4) hysterectomy and/or oophorectomy history, 5) thyroid dysfunction, 6) hyperprolactinemia, 7) patients using drugs which affect glucose metabolism and insulin sensitivity like metformin or thiazolidinediones, 8) patients using drugs which may affect reproductive or metabolic functions like oral contraceptives, steroids, beta blockers, anti-androgen drugs, 9) known significant cardiovascular disease, 10) cancer history and 11) evidence of any unresolved medical problem.

Patients were divided into two groups according to their FH of T2DM among their first degree relatives; FH positive (FHpos) versus FH negative (FHneg). Standard (75 grams) oral glucose tolerance test (OGTT) was performed on FHneg patients' first degree relatives to exclude the possibility of unknown T2DM. Age and body mass index (BMI) matched control group was composed of healthy women who had regular menstrual cycles and who were free of any clinical signs of hyperandrogenism. All of the control subjects also did not have a positive FH of T2DM among their first degree relatives.

This study was approved by Başkent University Institutional Review Board and Ethics Committee with project number KA 06/269 and supported by Başkent University Research Fund. A written informed consent was provided from all patients and controls at inclusion.

Methods

Weight and height of the patients and controls were measured with a standard steelyard in the morning after 12 hours of fasting. Body mass index (BMI) was calculated as kg/m2. Waist circumference (WC) was measured at the midpoint between the iliac crest and lower rib margin. All of the subjects were examined for the clinical evidence of hyperandrogenism such as acne, androgenic alopecia, acanthosis nigricans, striae, clitoromegaly and increased muscle mass. The degree of hirsutism was assessed using the modified Ferriman Gallwey (mFG) score in the upper lip, chin, areola and chest, upper back, lower back, upper abdomen, lower abdomen, thighs, and upper arms. Hirsutism was classified as mild (score 8-16), moderate (score 17-24), and severe (score >24). Transabdominal ultrasonography (USG) was performed during the early follicular phase of menstrual cycle. The USG criteria to define polycystic ovaries were presence of 12 or more follicles in each ovary measuring 2 to 9 mm in diameter, and/or increase in ovarian volume (>10 mL) and only one ovary fitting this definition is sufficient for diagnosis (12). All the measurements and physical examinations were performed by the same researcher. A diet containing 300 grams of carbohydrates was given to all subjects for three days before insulin resistance assessments. All of the laboratory tests and HEC were performed after 10-14 hours of fasting. Blood samples were obtained from the forearm brachial veins during the early follicular phase of menstrual cycle. HOMA-IR index was calculated by using the formula; fasting insulin concentration (μIU/mL) x fasting glucose (mg/dL)/405, assuming that normal adults have a score <2.5 (15). Blood glucose was determined with enzymatic colorimetric assay (glucose oxidase) by using Roche Modular Biochemistry Analyzer (Roche Diagnostics, Mannheim, Germany). Insulin levels

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were studied with microparticle enzyme immunoassay by using Axsym Analyzer (Abbott Diagnostics Division, Illinois, USA). HEC was performed in the morning (starting between 08:00-10:00 AM) as described by DeFronzo et al. (16). M value, which has been described as the glucose amount required to maintain euglycemia under steady dose of insulin infusion was calculated. Prolactin and thyroid stimulating hormone (TSH) were assessed in all participants including control subjects by chemiluminescent microparticle immunoassay (CMIA) with Architect Analyzer (Abbott Diagnostics Division, Illinois, USA) to exclude asymptomatic hyperprolactinemia and thyroid dysfunction. Also; follicle stimulating hormone (FSH), luteinizing hormone (LH) and estradiol were studied with CMIA by using Architect Analyzer (Abbott Diagnostics Division, Illinois, USA), free testosterone (fT) and 17 alpha hydroxyprogesterone (17αOHP) leves were evaluated with enzyme immunoassay by using Tecan Sunrise Analyzer (BLK Diagnostics, Badalona, Spain), dehydroepiandrosterone sulfate (DHEAS) was determined with solid phase competitive chemiluminescent enzyme immunoassay by using Immulite 2000 Analyzer (Corporate Offices, Los Angeles, USA) just for patients with PCOS.

Statistical Analysis

Statistical analysis was performed by using SPSS software (Version 17.0, SPSS Inc., Chicago, IL, USA). An assessment of the normality was done initially. All the normally distributed numerical data were expressed as mean values ± standard deviation (SD), while non-normally distributed data were expressed as median with minimum (min) and maximum (max) values. Nonparametric tests were used because of the limited number of patients in the groups. Comparisons between quantitative data were determined with Mann-Whitney U test, while comparisons between qualitative data were studied by using Chi-square test. Independent factors affecting HOMA-IR and M values were assessed with Kruscall Wallis correlation coefficient. Spearmans correlation coefficient was used to evaluate the correlation between HOMA-IR and M values. Correlation coefficients were interpreted as either excellent relationship r≥0.91; good 0.90≤ r ≥0.71; fair 0.970≤ r ≥0.51; weak 0.50≤ r ≥0.31; little or none r ≤0.3. The level for statistical significance was considered as p<0.05. However, while interpreting the difference among three groups Mann-Whitney U test was used to compare groups two-by-two and Bonferroni correction was performed. The level for statistical significance was considered as p<0.017 for these evaluations.

Results

Total of 19 PCOS patients as study group and 9 healthy subjects as a control group were enrolled to the study. Nine patients with PCOS had FH of T2DM among one of their first degree relatives and called as FHpos group, while 10 PCOS patients didn't have any T2DM history among their first degree relatives and called as FHneg group. General characteristics of patients with PCOS and control subjects are summarized in Table 1. In the FHpos group; six patients' mother, two patients' father and only one patient's both of parents were diabetic. This patient's HOMA-IR and M values were calculated as 3.37 and 4.85 respectively in a manner

of exhibiting evident insulin resistance. All of the findings were similar when patients with PCOS were compared with control subjects except for M values. M values were significantly higher in the control group (p=0.003) (Table 1).

Early follicular phase hormone profiles of FHneg and FHpos groups were compared and no statistically significant difference was found (Table 2). Also there was no statistically significant difference in the means of mFG hirsutism scores, menstruation cycles, ovulation status, existence of hyperandrogenism signs and pelvic USG findings between these groups. After that, FHneg and FHpos groups were compared with controls individually. Results are summarized in Table 3. Although HOMA-IR values were similar between groups, M values were lower in FHneg and FHpos groups compared to the controls (p=0.02 and 0.004 respectively).Variance analysis within all PCOS patients revealed

Table 1. General characteristics of patients with Polycystic ovary syndrome and control subjects

	Patients with PCOS (n=19)	Controls (n=9)	p
Age (years)	22.68±3.07	22.78±2.11	NS
Height (cm)	161±5.64	162.22±3.15	NS
Weight (kg)	59.79±8.05	60.11±2.89	NS
BMI (kg/m²)	23.06±2.71	22.85±1.12	NS
Waist circumference (cm)	75.05±7.25	75.56±1.12	NS
Fasting glucose (mg/dL)	86.74±7.67	85.22±5.02	NS
Fasting insulin (μIU/mL)	8.00 (4.50-22.50)	6.70 (3.80-14.90)	NS
HOMA-IR	1.91 (0.91-4.44)	1.59 (0.37-3.58)	NS
M value	5.32 (4.44-10.60)	8.24 (6.10-12.92)	0.003

Normally distributed data are presented as the mean ± SD. Non-normally distributed data are presented with median and minimum-maximum (min-max) values. NS: not significant PCOS: Polycystic ovary syndrome BMI: Body mass index

Table 2. Comparison between FHneg and FHpos groups

	FHneg group (n=10)	FHpos group (n=9)	P
mFG Hirsutism score	16.00±2.36	13.44±3.81	NS
FSH (mIU/mL)	4.82±1.14	4.98±1.21	NS
LH (mIU/mL)	4.14 (2.36-17.90)	6.60 (2.47-10.36)	NS
Estradiol (pg/mL)	36.50 (23-76)	40.00 (18-60)	NS
Free Testosterone (pg/mL)	1.98 (0.86-4.50)	2.59 (1.80-8.40)	NS
TSH (μIU/mL)	1.03 (0.56-3.60)	1.90 (0.65-2.50)	NS
Prolactin (mIU/L)	284 (145-560)	423 (163-884)	NS
17αOHP (ng/mL)	0.80 (0.50-1.60)	1.04 (0.78-1.43)	NS
DHEAS (ng/mL)	2210 (344-3570)	2580 (1922-4350)	NS

Normally distributed data are presented as the mean ± SD. Non-normally distributed data are presented with median and minimum-maximum (min-max) values. NS: not significant

Table 3. Comparisons between groups				
	FHneg (n=10)	FHpos (n=9)	Control (n=9)	p
Age (years)	22.70±3.53	22.67±2.69	22.78±2.11	0.96
BMI (kg/m²)	22.81±2.92	23.33±2.60	22.85±1.12	0.72
WC (cm)	73.90±7.62	76.33±7.04	75.56±2.19	0.55
Fasting glucose (mg/dL)	84.40±7.23	89.33±7.70	85.22±5.02	0.12
Fasting insulin (µIU/mL)	7.45 (4.50-22.50)	8.20 (4.70-15.20)	6.70 (3.80-14.90)	0.84
HOMA-IR	1.63 (0.91-4.44)	1.91 (0.96-3.30)	1.59 (0.37-3.58)	0.84
M value	5.97 (4.91-10.60)	4.92 (4.44-8.29)	8.24 (6.10-12.92)	0.008*

Normally distributed data are presented as the mean ± SD. Non-normally distributed data are presented with median and minimum-maximum (min-max) values. *Only the difference between FHpos and control groups was significant (P=0.004). BMI: Body mass index

that none of the examined factors affected HOMA-IR and M values independently.

Discussion

Hyperinsulinemia and insulin resistance are well known features of PCOS. In the literature, it is shown that 50-60% of patients with PCOS have insulin resistance (17,18). Obese patients with PCOS have decreased insulin sensitivity when compared to normal-weight PCOS patients and obese patients without PCOS (19,20). However, insulin resistance in lean PCOS subjects is still a controversial issue. When we searched the literature assuming that the gold standard method to assess insulin resistance is HEC; we found two relatively big studies with conflicting results. Vrbikova et al. reported similar findings between normal-weight patients and control subjects, while Li et al. reported increased insulin resistance in Chinese normal-weight PCOS women compared to controls (6,21). In a manner of supporting findings of the latter study; we found significantly increased insulin resistance in lean PCOS patients in our study (Table 1). Although HOMA-IR values of PCOS patients and control subjects were similar, there was statistically significant difference among M values. This issue can be explained by the limited number of subjects. When compared to HOMA-IR; HEC is more sensitive and specific, but its application is much more difficult. HOMA-IR assessment is very practical but its sensitivity and specificity is very low in studies with small patient groups, and it seems to be suitable for relatively larger studies (22). Additionally, another reason explaining this issue may be the effect of postprandial hyperinsulinemia. Usually postprandial hyperinsulinemia may be onset before fasting hyperinsulinemia, and can be more important in IR (23). HOMA-IR is a predictor of insulin response to fasting glucose, however HEC test predicts both fasting and postprandial glucose/insulin response.

In the normal population, offsprings with one diabetic parent have 1.6-2.6 times increased risk for diabetes, while this ratio increases to 2.2-3.7 in subjects with two diabetic parents (24). There is no remarkable difference between maternal and paternal inheritance (24). In addition, literature also reveals pancreas β-cell dysfunction and increment in insulin resistance among first degree relatives of PCOS patients (8,25). In another study, Ehrmann et al. reported a close relation between T2DM FH and increased insulin resistance in obese PCOS patients (9). However there are limited and conflicting data concerning T2DM FH among lean PCOS patients.

In our study, we didn't find any statistically significant difference regarding insulin resistance between T2DM FHneg and FHpos patient groups. However both of the patient groups showed increased insulin resistance when compared with the control subjects. This finding was shown with M values assessed by HEC technique. Additionally this difference was more prominent in the FHpos group (p=0.02 for FHneg group versus p=0.004 for FHpos group). Probably depending on the limited number of patients this issue was not confirmed with HOMA-IR evaluations. The difference between FHpos and FHneg patients in the mean of insulin resistance might become more evident with a larger study group. For this reason our study results are not adequate to say that FH of T2DM does not have any impact on insulin resistance in lean PCOS subjects. On the other hand we can say that we didn't observe any apparent impact of T2DM FH on insulin resistance in our study group.

Limitations of this study were; (1) limited number of patients, (2) hormones other than TSH, prolactin and insulin were not studied for control subjects. Small number of participants was due to the difficulty of HEC technique and finding non-obese PCOS patients. Secondly, we didn't assess many hormones in the control group because of the cost. However these hormones are not essential to rule out PCOS diagnosis according to Rotterdam revised 2003 diagnostic criteria (12).

As a conclusion, our study results suggest that lean PCOS patients have evident insulin resistance when compared to healthy subjects. After diagnosis, even lean PCOS patients should be encouraged to apply healthy life style changes like healthy eating style and exercise to prevent future metabolic disorders. Furthermore, lean PCOS patients having fertility problems and irregular menstrual cycles can see benefits from drugs which improve insulin resistance mainly like metformin. According to our findings FH of T2DM seems to not affect insulin resistance in non-obese PCOS women, but studies with larger study groups are required to clarify this issue. Concerning the difficulty of HEC technique, multicentric studies can be planned or data derived from meta-analysis may be more decisive.

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