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Metabolic Characteristics In The Subgroups Of Polycystic Ovary Syndrome

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ABSTRACT

Aim: Polycystic ovary syndrome (PCOS) is a heterogenous disease that is characterized with chronic anovulation, menstrual irregularities and hyperandrogenism. In the present study we aimed to compare the metabolic features of PCOS'subgroups based on revised Rotterdam diagnostic criterias.

Materials and Methods: Ninety-five women with PCOS were enrolled into the study and divided into four groups. Group 1; oligomenorrhea and/or anovulation with biochemical hyperandrogenemia and/or hyperandrogenism, group 2; biochemical hyperandrogenemia and/or hyperandrogenism with polycystic ovaries, group 3; oligomenorrhea and/or anovulation with polycystic ovaries and group 4; polycystic ovaries with oligomenorrhea and/or anovulation with biochemical hyperandrogenaemia and/or hyperandrogenism. Body mass indeces (kg/m2) and waist to hip ratios were calculated in all study patients. Fasting glucose, lipid levels, kidney and liver function tests were measured after 12 hours fasting. Oral glucose tolerance test (OGTT) was performed to evaluate the responses of glucose and insülin. Hormone levels were measured during the follicular phase of the menstrual cycle. Adrenocorticotrophic hormone (ACTH) (0.5 mg Synacthen IM) and gonadotrophin-releasing hormone agonist (buserelin) tests were performed in order to evaluate adrenal and ovarian hyperandrogenemia. Ovarian ultrasonography was performed on all study patients to define PCO morphology.

Results: The frequency of the classical PCOS phenotype was higher than the non-classical PCOS phenotype (74.7% and 25.2%, respectively). Body mass indexes, waist-hip ratios, serum total, LDL and HDL-cholesterol levels were similar in all groups. Serum triglyceride levels were found to be significantly lower in group 2 compared to other groups ($p< 0.01$). Peak insulin and AUC insulin levels were significantly lower in group 2 ($p< 0.05$). The prevalence of impaired fasting glucose and impaired glucose tolerance was similar in all groups. Free testosterone and androstenedione levels were significantly lower in group 3 compared to group 4.

Conclusion: Metabolic disturbances of patients with PCOS without hyperandrogenism (OA+PCO; group 3) were similar to the classical PCOS groups contrary to the expectations. The metabolic disturbances found in patients with PCOS without menstrual dysfunction (HA+PCO; group 2) were found to be milder in terms of lipid levels and insulin resistance. These findings support that newly developed HA+PCO and OA+PCO groups are the part of wide PCOS spectrum and also it supports that PCO morphology is one of the diagnostic criterias of PCOS.

Keywords: Polycystic Ovary Syndrome, Hyperandrogenism, Metabolic Differences, Insulin Resistance

Polikistik Over Sendromu'nun Alt Gruplarında Metabolik Özellikler

Süreç

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ÖZ

Amaç: Polikistik over sendromu (PKOS), kronik anovülasyon, adet düzensizlikleri ve hiperandrojenizm ile karakterize heterojen bir hastalıktır. Bu çalışmada revize edilmiş Rotterdam tanı kriterlerine göre PKOS alt gruplarının metabolik özelliklerini karşılaştırmayı amaçladık.

Materyal ve Metod: PKOS'lu 95 kadın çalışmaya dahil edildi ve dört gruba ayrıldı. Grup 1; biyokimyasal hiperandrojenemi ve/veya hiperandrojenizm ile oligomenore ve/veya anovülasyon, grup 2; polikistik over ile biyokimyasal hiperandrojenemi ve/veya hiperandrojenizm, grup 3; polikistik over ile oligomenore ve/veya anovulasyon ve grup 4; polikistik over ile biyokimyasal hiperandrojenemi ve/veya hiperandrojenizm ile oligomenore ve/veya anovulasyon. Çalışmaya alınan tüm hastaların vücut kitle indeksleri (kg/m2) ve bel-kalça oranları hesaplandı. 12 saatlik açlıktan sonra açlık glukozu, lipid düzeyleri, böbrek ve karaciğer fonksiyon testleri ölçüldü. Glukoz ve insülin yanıtlarını değerlendirmek için oral glukoz tolerans testi (OGTT) yapıldı. Adet döngüsünün foliküler fazında hormon seviyeleri ölçüldü. Adrenal ve over hiperandrojenemisini değerlendirmek için adrenokortikotropik hormon (ACTH) (0,5 mg Synacthen IM) ve gonadotropin salgılatıcı hormon agonisti (buserelin) testleri yapıldı. PKO morfolojisini tanımlamak için tüm çalışma hastalara ultrasonografi yapıldı.

Bulgular: Klasik PKOS fenotipinin sıklığı, klasik olmayan PKOS fenotipinden daha yüksekti (sırasıyla %74,7 ve %25,2). Vücut kitle indeksleri, bel-kalça oranları tüm gruplarda benzerdi. Serum trigliserit düzeyleri grup 2'de diğer gruplara göre anlamlı olarak düşük bulundu ($p< 0.01$). Pik insülin ve AUC insülin seviyeleri grup 2'de anlamlı olarak düşüktü ($p< 0.05$). Bozulmuş açlık glukozu ve bozulmuş glukoz toleransı prevalansı tüm gruplarda benzerdi. Serbest testosteron ve androstenedion düzeyleri grup 3'te grup 4'e göre anlamlı olarak düşüktü.

Sonuç: Hiperandrojenizmi olmayan PKOS'lu hastaların (OA+PCO; grup 3) metabolik bozuklukları beklenenin aksine klasik PKOS gruplarına benzerdi. Menstrüel disfonksiyonu olmayan PKOS'lu hastalarda (HA+PCO; grup 2) saptanan metabolik bozuklukların lipid düzeyleri ve insülin direnci açısından daha ılımlı olduğu saptandı. Bu bulgular yeni gelişen HA+PKO ve OA+PKO gruplarının geniş PKOS spektrumunun bir parçası olduğunu ve PKO morfolojisinin PKOS tanı kriterlerinden biri olduğunu desteklemektedir.

Anahtar sözcükler: Polikistik Over Sendromu, Hiperandrojenizm, Metabolik Farklılıklar, İnsülin Direnci

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Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrinologic disorder in women of reproductive age and its classic symptoms are anovulation with hyperandrogenism [1,2]. PCOS is a clinical picture with a heterogeneous etiology that requires differential diagnosis with other etiologic factors such as Cushing's syndrome, hyperprolactinemia, thyroid disease, and androgen-producing tumors. The definition of PCOS, having metabolic, cardiovascular, and reproductive risks, is still uncertain. In the 2003 ESHRE (European Society of Human Reproduction and Embryology)/ASRM (American Society for Reproductive Medicine) consensus held in Rotterdam in 2003, it was recommended that after excluding other etiologic causes, the syndrome should be diagnosed with the combination of two of the following three criteria [3]; 1. Oligo-anovulation (OA), 2. Clinical and/or biochemical hyperandrogenism findings (HA), 3. Polycystic ovaries in ultrasonography (PCO). Furthermore, as per the criteria proposed by the National Institutes of Health (NIH, 2009), the diagnosis of PCOS includes the detection of clinical or biochemical hyperandrogenism and ovulation disorders.

These diagnostic criteria add new phenotypes to the definition of PCOS (e.g. a patient without hyperandrogenism can also be diagnosed with PCOS). Further studies are needed to determine whether these new phenotypes represent the syndrome and to determine their common and different aspects with the classical definition of PCOS. The aim of the present study was to evaluate whether PCOS subgroups differ from each other in terms of metabolic parameters such as insulin resistance, glucose intolerance, and dyslipidemia.

Materials and Methods

This prospective study was conducted on a group of 95 patients with PCOS who applied to the Endocrinology Outpatient Clinic of the Department of Internal Medicine, Erciyes University Faculty of Medicine (E.U.T.F) between January 2006 and March 2010. The study protocol was approved by the Ethics Committee of the Faculty of Medicine and consent was obtained from all the patients.

Patients with a history of medication use due to PCOS in the last six months were excluded from the study.

The age of the patients was between 16 and 40 years old. The patients were divided in 4 groups: Group 1; oligomenorrhea and/or anovulation with biochemical hyperandrogenemia and/or hyperandrogenism (OA+HA), group 2; biochemical hyperandrogenemia and/or hyperandrogenism with polycystic ovaries (HA+PCO), group 3; oligomenorrhea and/or anovulation with polycystic ovaries (OA+PCO), and group 4; polycystic ovaries with oligomenorrhea and/or anovulation with biochemical hyperandrogenaemia and/or hyperandrogenism (HA+OA+PCO). The presence of oligomenorrhea (six or less menstrual periods per year) or amenorrhea (two or less menstrual periods per year), hirsutism, menstruation, fertility history, marital status, and medication history of the patients were questioned. Waist/hip ratios and BMI (Body mass indices) were calculated. Hirsutizm was assessed by the mFG (modified Ferriman-Gallwey) method. Patients with acne, oily skin, voice thickening, weight gain, and androgenic alopecia were considered as clinical hyperandrogenism.

Fasting blood glucose and lipid levels were measured after 12 hours of fasting. Glucose and insulin responses were then evaluated with 75-gram OGTT. Insulin resistance index was calculated by HOMA-IR. According to the 2013 criteria of the American Diabetes Association (ADA), the diagnosis of DM, IGT (impaired glucose tolerance), and impaired fasting glucose in the patients were defined [4]. Also, the area obtained in OGTT (AUC: Area under the curve) was calculated using the mathematical trapezoidal formula.

AUC(glucose):
[(Glu0+Glu30)x30+(Glu30+Glu60)x30+(Glu60+Glu90)x30+(Glu90+Glu120)x30]/2
0^{th}, 30^{th}, 60^{th}, 90^{th}, 120^{th} min. glucose at Glu0,30,60,90,120 :OGTT (mg/dL),
AUC(insulin):
[(Ins0+Ins30)x30+(Ins30+Ins60)x30+(Ins60+Ins90)x30+(Ins90+Ins120)x30]/2
0^{th}, 30^{th}, 60^{th}, 90^{th}, 120^{th} min. insulin at Ins0,30,60,90,120: OGTT (µIU/mL).

Despite was expected in patients with regular menstruation, blood samples were taken for the laboratory tests after induced menstruation with medroxyprogesterone acetate in patients with irregular menstruation. In the early follicular phase of the menstrual cycle (days 2-5 of the menstrual cycle), hormone levels were measured, and the Free Androgen Index (FAI=Total T nmol/L x 100/SHBG nmol/L) was calculated. Progesterone levels were examined in the luteal phase (on days 22-24 of menstruation) to determine whether ovulation was present; those below 4ng/mL (10 nmol/L) were considered anovulatory [5]. During the follicular period, ACTH stimulation (0.5 mg Synacthen IM) test was performed to evaluate the adrenocortical response and buserelin test was performed to evaluate the ovarian response.

To rule out Cushing's disease, patients underwent a 1mg dexamethasone suppression test. Acromegaly was excluded according to anamnesis and phenotypic features of the patients. The presence of 12 or more follicles with a diameter of 2 - 9 mm and/or increased ovarian volume (> 10 mL) on ultrasonographic examination was defined as polycystic ovary. The presence of this finding in a single ovary was considered sufficient [3].

Hyperandrogenemia was defined as the presence of any one or more androgen levels higher than normal according to laboratory references.

Statistical Methods: All data were uploaded to the computer via the SPSS program and statistically evaluated. The distribution of the data meeting the

parametric condition was defined as X±SD. Normality analysis of the data was conducted by Shapiro-Wilk Test. Comparison of the four independent groups fitting the normal distribution was performed by one-way analysis of variance. Pairwise comparisons of the groups that differed were made with Tukey Test. In the comparisons of non-normally distributed groups, median values were determined using the non-parametric test (Kruskal-Wallis) and defined as OD (min-max). Dunn's test was used for pairwise comparisons of the groups in which differences were found. Chi-Square test was used to compare qualitative variables. Significance level was accepted as $p<0.05$.

Results

The study included 95 patients diagnosed with PCOS. These patients were divided into four groups representing sub-phenotypes. Of 95 patients, 14 (14.7%) were in group 1, 13 (13.7%) in group 2, 11 (11.6%) in group 3 and 57 (60%) in group 4. Fifty-three patients (55.8%) presented with hirsutism and 28 patients (29.5%) with menstrual irregularities. When married patients were evaluated in terms of infertility, it was found to be statistically significantly lower in group 2 when compared to other groups ($p< 0.05$) (Table 1).

Table 1. Comparison of the symptoms in PCOS subgroups

Symptoms	Group 1 (HA+OA)	Group 2 (HA+PCO)	Group 3 (PCO+OA)	Group 4 (OA+HA+PCO)	Chi-Square	p
Acne	9 patients (64.3%)	5 patients (38.5%)	4 patients (36.4%)	21 patients (36.8%)	3,676	NS
Weight Gain	7 patients (50%)	3 patients (23.2%)	7 patients (63.6%)	36 patients (63.2%)	7,360	NS
Hair Loss	9 patients (64.3%)	7 patients (53.8%)	7 patients (63.6%)	34 patients (59.6%)	0,376	NS
Infertility	3 patients (75%) (total 4)	(0 patient)* (0%) (Total 6)	3 patients (75%) (Total 4)	16 patients (57.1%) (total 28)	8,495	< 0.05

NS: Not significant,
*: Indicates the group with the difference.
Group 1 (HA+OA): Hyperandrogenism and/or hyperandrogenemia + oligo and/or amenorrhea,
Group 2 (HA+PCO): Hyperandrogenism and/or hyperandrogenemia + polycystic ovary,
Group 3 (PCO+OA): Polycystic ovary + oligo and/or amenorrhea,
Group 4 (HA+OA+PCO): Hyperandrogenism and/or hyperandrogenemia + oligo and/or amenorrhea + Polycystic ovary.

There was no significant difference in median age between the patient groups. When BMI and waist/hip ratio were evaluated, no significant difference was observed between the groups. mFG score was found as 14 (9 - 22) in group 1, 13 (6 - 24) in group 2, 5 (0 - 7) in group 3, and 13 (4 - 24) in group 4. Accordingly, mFG score was significantly lower in group 3 than in the other groups ($p< 0.01$). Prolactin levels and thyroid function tests of the patients were evaluated as normal. A 1 mg dexamethasone suppression test was performed to exclude Cushing's disease; the cortisol level was below 1.8µg/dL in all the patients excluding 7 of them. A 2 mg dexamethasone suppression test was performed in 7 patients with cortisol levels above 1.8µg/dL and all of them had cortisol levels below 1 µg/dL. Synthetic ACTH (Synacthen depot ampoule) and 0.5 mg intramuscular ACTH stimulation test performed in all patients in the early follicular period did not reveal any adrenal enzyme defect.

The TG level was the lowest in group 2 and the highest in group 4. There was a significant difference between group 2 and group 4 ($p< 0.01$), while there was no difference between the other groups in terms of TG and other lipid parameters. When the hormonal characteristics of the patient groups were evaluated; free T and A levels were statistically significantly lower in group 3 than in group 4 ($p< 0.05$). No significant difference was observed between other groups (Table 2).

Basal LH levels were statistically significantly lower in group 2 than in groups 1 and 4 ($p< 0.05$), while no difference was observed between group 3 and the other groups. LH/FSH values were also significantly lower in group 2 than in groups 1 and 4 ($p< 0.05$). In group 3, there was no difference between the other groups in terms of LH/FSH ratio. Mean basal cortisol, E_2, 17-OHP, and FSH levels were similar in all groups (Table 2).

Peak insulin and AUC insulin values were significantly lower in group 2 than in the other groups ($p=0.015$, $p=0.040$, respectively). Impaired fasting glucose was

detected in two patients (both from group 4) and IGT in 13 patients (9 patients from group 4, 2 patients from group 3, one patient from group 1, and one patient from group 2). Basal insulin, fasting glucose, glucose/insulin ratio, OGTT 2nd hour glucose, HOMA-IR and AUC glucose values were similar in all groups and there was no significant difference in terms of impaired fasting glucose and IGT (Table 3).

Buserelin test was performed for the patients in order to evaluate ovarian function. The AUC hormone levels obtained as a result of the test were similar between the groups (Table 4).

Table 2. Distribution of hormone values of PCOS groups

	Group 1 (n=14) OD(min-max)	Group 2 (n=13) OD(min-max)	Group 3 (n=11) OD(min-max)	Group 4 (n=57) OD(min-max)	p
DHEAS ng/mL	2438.36±1566.68	2467.62±1673.67	2783.09±1274.79	2517.60±1222.32	NS
SHBG nmol/L	41,50(6.00-382.00)	57,00(18.00-121.00)	31,00(22.00-139.00)	33,00(6.00-154.00)	NS
tT ng/dL	68,50(32.00-131.00)	56,00(26.00-130.00)	69,00(27.00-81.00)	68,00(13.00-231.00)	NS
sT pg/mL	2.61(1.46-8.09)^{ab}	2.38(1.25-3.43)^{ab}	1.89(1.03-3.09)^{a}	2.91(1.13-8.09)^{b}	< 0.05
SAI	5,65(0.55-69.98)	3,64(1.69-18.04)	5,26(1.97-9.94)	7,89(0.63-72.87)	NS
A ng/mL	2.45(1.05-5.83)^{ab}	2.61(1.30-9.90)^{ab}	2.34(1.18-3.07)^{a}	3.48(1.29-9.05)^{b}	< 0.05
LH mIU/mL	5.97(3.24-24.39)^{a}	3.93(1.26-5.33)^{b}	5.24(0.77-15.26)^{ab}	6.06(1.69-20.81)^{a}	< 0.05
FSH mIU/mL	5,31(3.52-7.21)	5,39(3.10-54.75)	4,52(2.06-7.80)	5,44(1.43-10.64)	NS
LH/FSH	1.09(0.53-4.62)^{a}	0.66(0.33-1.20)^{b}	1.07(0.37-1.97)^{ab}	1.17(0.30-5.42)^{a}	< 0.05
E_2 pg/mL	52,18(21.77-145.88)	60.73(40.20-240.78)	61,45(11.85-106.00)	53,81(9.54-169.96)	NS
17-OHP ng/mL	1,45(0.55-5.78)	1,46(0.36-5.59)	1,08(0.50-1.61)	1,49(0.11-6.20)	NS
Cortisol µg/dL	14,03(6.22-31.53)	14,52(3.92-39.43)	12,26(9.01-30.20)	12,98(3.61-65.50)	NS

Values are given as median.

NS: Not significant.

Alphabetical superscripts: According to multiple comparison tests, the same letters represent the similarity of the groups and different letters represent statistically significant differences between the groups.

Table 3. Insulin resistance parameters of PCOS subgroups

	Group 1 (n=14) OD(min-max)	Group 2 (n=13) OD(min-max)	Group 3 (n=11) OD(min-max)	Group 4 (n=57) OD(min-max)	p
Basal Insulin µIU/mL	12,30(1.74-24.90)	11,70(2.34-43.26)	15,10(9.37-26.00)	14,20(2.33-59.25)	NS
Fasting Glucose mg/dL	79,50(64.00-101.00)	83,00(70.00-96.00)	79,00(65.00-98.00)	79,00(54.00-123.00)	NS
Glucose/Insulin Ratio	5,89(2.84-45.40)	6,80(1.98-35.47)	5,03(3.30-8.65)	4,99(1.23-36.90)	NS
Peak Insulin µIU/mL	122.45(28.04-263.20)^{a}	49.12(11.17-237.52)^{b}	110.92(49.06-306.00)^{a}	100.57(13.22-425.10)^{a}	< 0.05
HOMA-IR	2,20(0.34-4.87)	2,09(0.47-9.09)	2,88(1.81-5.47)	2,87(0.49-12.82)	NS
AUC Insulin µIU/mLx2 hrs	7032.23(2703.60-13000.50)^{a}	3389.40(873.60-15206.70)^{b}	6694.65(3076.20-23850.00)^{a}	6948.60(1021.95-32138.40)^{a}	< 0.05
OGTT 2nd hr Glucose mg/dL	104.64±26.83	104.54±22.53	112.73±37.64	111.96±27.70	NS
AUC Glucose mg/dLx2 hrs	13798.93±3241.49	13110.00±2690.54	14290.91±2830.97	14320.53±3275.87	NS

Values are given as median.

NS: Not significant.

Alphabetical superscripts: According to multiple comparison tests, the same letters represent the similarity of the groups and different letters represent statistically significant differences between the groups.

Table 4. Responses of PCOS subgroups to buserelin test

	Group 1 (n=14) OD(min-max)	Group 2 (n=13) OD(min-max)	Group 3 (n=11) OD(min-max)	Group 4 (n=57) OD(min-max)	p
AUC LH mIU/mLx24 hrs	1010,49(497.28-5009.52)	726,09(298.98-1213.62)	836,34(63.45-1280.70)	922,14(239.04-1997.85)	NS
AUC E_2 pg/mLx24 hrs	3575,19(2159.40-2831.90)	3748,68(2272.62-6036.30)	4194,60(1854.87-6800.76)	4099,50(1114.20-4037.45)	NS
AUC 17-OHP ng/mLx24 hrs	55,91(30.81-149.82)	38,70(23.04-133.98)	46,08(23.31-302.40)	59,49(16.59-528.36)	NS

Values are given as median.
NS: Not significant.

Discussion

PCOS is the most common endocrine disorder affecting women of reproductive age and affects 4- 21% of the general population [6,7]. The pathogenesis of PCOS is complex and still unclear and is associated with various heterogeneous diseases. Since it was first described by Stein and Leventhal in 1935, the diagnostic criteria have been reevaluated over the years and polycystic ovary morphology was added to the diagnostic criteria in the Rotterdam ASRM/ESHRE 2003 consensus.

In a study by Hsu et al., androgen increase, and ovulatory dysfunction were detected in 103 (61%) of 170 patients diagnosed with PCOS, while the remaining 67 (39%) patients had either androgen increase or ovulatory dysfunction with PCOS confirmed by US [8]. In the study of Belosi et al. [9], the ratio of women with classic PCOS and mild PCOS was 79.13%, 21.46%, and 20.87%, respectively.

Clinical and biochemical hyperandrogenism is considered as an indispensable criterion for PCOS. PCO+OA phenotype, known as non-hyperandrogenism phenotype, was found to be different from other sub-phenotypes with many features. In one study, PCO+OA phenotype was evaluated as significantly different from other phenotypes and similar to the control group, especially in terms of metabolic syndrome and insulin resistance [10]. In the present study, although hyperandrogenism and insulin resistance were more common in classic PCOS groups, significant insulin resistance was also found in PCO+OA phenotype. The high mean BMI in this group may have contributed to these findings. In addition, it can be stated that HA+PCO group was associated with lower insulin resistance, lipid levels and BMI and lower metabolic risk.

Other very important criteria in PCOS patients are BMI and waist/hip ratio. Especially central obesity is recognized as an important part of metabolic syndrome [11]. In the study of Hsu et al; significantly increased BMI and waist/hip ratio were observed in classic PCOS phenotypes compared to non-classic phenotypes. In addition, when compared with the control group, no significant difference was observed in terms of BMI, mFG score and acne in the PCOS group without androgen increase (PCO+OA) [8]. In the current study, no significant difference was observed between the groups in terms of BMI and waist/hip ratio indicating central obesity, but the highest BMI was in the HA+OA+PCO and PCO+OA groups. mFG score was significantly lower in the group without hyperandrogenism (PCO+OA) compared to the other groups as expected. Our results also show that the classic PCOS phenotype is prone to obesity.

In the studies conducted, LH levels were found to be higher in all sub-phenotypes compared to the control group, while FSH levels were found to be similar in all groups [12-14]. In the present study, LH level was significantly higher in HA+OA and HA+OA+PCO group compared to HA+PCO group. LH/FSH levels were also significantly higher in HA+OA and HA+OA+PCO groups compared to HA+PCO group. In PCO+OA group, LH levels and LH/FSH ratios were assessed as similar to the other groups. Mean LH/FSH ratios were below 2 in all groups. Increased LH level and LH/FSH ratio above 2 have high sensitivity in the diagnosis of PCOS, however, are not essential for the diagnosis. In addition, these findings support the theory that gonadotropins do not play a primary role in the pathophysiology of PCOS, and that the increase in single androgens is at the forefront.

In a study of 166 patients with PCOS and 277 controls, 17-OHP levels were found to be higher in the PCOS group but within normal limits. [12]. In another study, 17-OHP levels were found to be at the lowest level in the control group and highest in the HA+PCO phenotype. [13]. In this study, 17-OHP levels were highest in the HA+PCO group, although not statistically significant, and 17-OHP levels were at or above the upper limit of normal in all groups on average. This may be an indication of increased activity of the cytochrome P450c17α enzyme in both adrenal and ovarian theca cells. Moreover, PCO morphology seems to be directly proportional to this hyperactivity.

In a study by Welt et al. the highest T levels were detected in HA+OA+PCO, HA+PCO and HA+OA phenotypes, while they were found to be low and similar to each other in PCO+OA and control group. [13]. In addition, SAI levels were found to be highest in the HA+OA phenotype, similar in the PCO+OA and control groups, and moderately high in the HA+PCO phenotype [13]. In this

study, tT, free T and SAI levels were highest in the HA+OA and HA+OA+PCO group, but free T levels were statistically lower in the PCO+OA group, although there was no statistically significant difference between the groups in terms of tT and SAI. The highest free T level was in the HA+OA+PCO phenotype. It is very difficult to define hyperandrogenemia in PCOS as it is influenced by the factors such as ethnicity, age, and genetics. These results support that SAI and free T appear to be more sensitive in the assessment of hyperandrogenemia. Increased androgen levels are a typical laboratory finding in PCOS. It is expected that free T levels are higher in classical PCOS when compared to other groups.

In the current study, the lowest SHBG levels were found in HA+OA+PCO and PCO+OA phenotypes. The low SHBG levels expected in PCOS in PCO+OA phenotype may be significant in terms of showing that this phenotype represents a subgroup of PCOS. The fact that BMI was higher in this group than in the other groups, although not significantly, may indicate a positive correlation between low SHBG and increased insulin resistance, and between BMI and insulin resistance and decreased SHBG levels.

Insulin resistance and consequent compensatory hyperinsulinemia is a common finding in both lean and obese PCOS patients and is known to be independent of BMI. In the evaluation of insulin resistance in PCOS, the characteristics of the patient population studied, ethnicity and the insulin resistance measurement methods used have a significant effect on the results. The mechanism of insulin action abnormalities in PCOS is not clearly known. It can be seen that insulin resistance and related compensatory hyperinsulinemia seem to play an important role in the pathophysiology of metabolic syndrome [15]. HOMA-IR and glucose/insulin ratio are commonly used tests to assess insulin resistance.

Studies have shown that the groups with biochemical hyperandrogenemia have the highest insulin resistance [16,17]. This suggests that factors such as hyperandrogenemia contribute to insulin resistance and that there is a positive correlation between these factors and insulin resistance in the PCOS population. In the same study, when ovulatory and anovulatory PCOS women with the same degree of obesity were evaluated, it was shown that insulin resistance was higher in the anovulatory group, and a positive correlation was found between insulin resistance index and androgen levels and PCOS morphologic parameters (ovarian volume and ovarian follicle number).

In a study conducted by Chae et al. in Korean women, it was shown that the HOMA-IR value did not differ in PCOS subgroups but was significantly higher compared to the control group [12]. In the same study, no difference was observed in all PCOS subgroups in terms of 2nd hour glucose values in OGTT, and fasting insulin was found to be lower in PCO+OA group than in HA+OA+PCO group. OGTT 2nd hour insulin levels were significantly higher in HA+OA+PCO and HA+OA groups when compared to PCO+OA group [12]. This suggests that postprandial hyperinsulinemia has a significant effect on hyperandrogenism and ovarian function in women with PCOS. In the study, fasting glucose and insulin values and 2nd hour glucose values after OGTT were similar between PCOS phenotypes in all subgroups. However, 2nd hour AUC insulin and peak insulin levels were significantly lower in HA+PCO phenotype compared to other phenotypes. The fact that significant insulin resistance was observed in the PCO+OA group, which was evaluated as mild PCOS, may indicate that this group represents PCOS. In addition, the lowest levels of insulin resistance parameters in the HA+PCO phenotype may indicate that PCO morphology is more closely associated with insulin resistance than hyperandrogenemia.

Defects in the function of the hypothalamo-pituitary-ovarian axis have been identified in the etiology of PCOS. The pulse frequency and amplitude of GnRH were altered to cause an increase in LH. The finding of increased 17-OHP and A secretion in Theka cells with the use of GnRH agonists in the patients with PCOS indicates a *de novo* steroidogenesis difference (overexpression of cytochrome P450c17α gene) in these cells [18]. GnRH agonists are potent, specific stimulators of the pituitary gonadal axis and with a single dose of GnRH agonists, gonadotropin secretion occurs within 4 hours and gonadal secretion within 24-72 hours in women [19].

In a study in which 17-OHP concentrations after buserelin stimulation were found to be significantly higher in patients with PCOS than in the control group, it was suggested that GnRH stimulation with buserelin may be a reliable method for the investigation of cytochrome P450c17α enzyme activity in women with PCOS. In the current study, a positive correlation was observed between 17-OHP, which increased in response to busereline, and increased ovarian volume [20]. In the current study, responses to the buserelin test to assess ovarian function were similar between the groups. In conclusion, the fact that increased ovarian 17-hydroxylase and 17-20 lyase activity was observed in all phenotypes in patients with PCOS may indicate that the GnRH stimulation test can be used safely in the diagnosis. This reinforces the view that an increase in single androgen plays a major role in the pathogenesis.

In the current study, PCOS sub-phenotypes according to the 2003 Rotterdam diagnostic criteria were evaluated metabolically. Many previous studies have investigated whether the newly formed phenotypes HA+PCO and PCO+OA represent PCOS in terms of metabolic profile, and in particular, the PCO+OA group, which is a phenotype without hyperandrogenism, has been considered as mild PCOS. The fact that PCOS, whose diagnosis is still unclear, is related to genetic and ethnic origin and is associated with many diseases with heterogeneous etiology such as insulin resistance, hyperlipidemia, type 2 DM, and CVD has affected the results of the studies. As a result of our evaluation in this study, it can be said that PCO+OA phenotype represents PCOS in metabolic terms, but larger studies with a larger number of patients are needed.

Declarations

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Conflict of interests: The authors declare that they have no conflict of interest.

Availability of data and material: The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Author's contribution: All authors contributed to the study conception, design, material preparation, data collection and analysis. The first draft of the manuscript was written by [MY], and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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