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Status of the homocysteine levels in polycystic ovary syndrome

Serum Homosistein düzeylerinin polikistik over sendromundaki yeri

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ÖZ

Amaç: Polikistik Over Sendromu (PKOS) olan hastalar, hiperhomosisteinemiye bağlı kardiyovasküler sistem ile ilişkili hastalıklar için uzun vadede risk grubundadır. Çalışmamız, hiperhomosisteineminin PKOS tanılı hastalarda önemi ve kardiyovasküler hastalıkların ön tanısında kullanılabilecek bir belirteç olup olmadığını araştırmayı amaçlamaktadır. Bu çalışmada, PKOS tanısı almış hastalar ile sağlıklı bireyler arasında serum homosistein düzeyleri karşılaştırılmıştır.

Gereç ve Yöntemler: Çalışmamız hastanemizin polikliniğine başvuran 20-49 yaş arası Rotterdam kriterlerine göre PKOS tanısı almış 24 hasta ve kontrol grubu olarak 26 sağlıklı gönüllü kadın ile yapılmış prospektif kesitsel bir çalışmadır. Çalışmaya dahil edilen tüm katılımcıların antropometrik ölçümleri kaydedildi. Çalışmaya katılan bireylerin kan örnekleri 12 saatlik açlığı takiben adetin 2. veya 3. gününde -80 derecede santrifüj edilerek serum homosistein düzeyleri ELISA (Enzyme-Linked ImmunoSorbent Assay) ile ölçüldü. Katılımcıların serum homosistein düzeyleri 30 µmol / L'nin altında ve 30 µmol / L'nin üzerinde olmak üzere iki gruba ayrıldı. Serum homosistein düzeylerine bakılmaksızın katılımcılar vücut kitle indeksi (VKİ) 25 kg (kilogram) / m2 (metrekare) üstü ve altı, bel çevresi 80 cm (santimetre) üstü ve altı olmak üzere gruplara ayrıldı. Oluşturulan yeni grupların serum homosistein seviyeleri karşılaştırıldı.

Bulgular: PKOS'lu kadınların serum homosistein düzeyleri, PKOS olmayan kadınlara göre daha yüksek bulundu (p = 0,001). PKOS hastaları, kontrol grubuna göre anlamlı derecede daha gençti (p = 0,017). VKİ, bel çevresi ve serum homosistein düzeyleri açısından istatistiksel olarak anlamlı bir fark bulunmadı (p> 0.5).

Sonuç: VKİ ve bel çevresi ne olursa olsun, fenotip A PKOS'lu hastalarda rutin serum homosistein düzeylerinin taranması ve hiperhomosisteinemili hastaların tedavi edilmesi, uzun vadede gelişebilecek kardiyovasküler sistem ilişki hastalıklarının öngörülmesinde ve önlenmesinde faydalı olabilir.

Anahtar Kelimeler: Kardiyovasküler Sistem Hastalıkları, Polikistik Over Sendromu, Serum Homosistein Düzeyi, Vücut kitle indeksi.

ABSTRACT

Aim: Patients with Polycystic Ovary Syndrome (PCOS) are in the risk group at long-term for Cardiovascular System (CVS) related diseases due to hyperhomocysteinemia. Our study aims to compare serum homocysteine levels between patients diagnosed with PCOS, and healthy individuals.

Materials and Methods: Our study was a prospective cross-sectional study of 20-49 years old patients who applied to Reproductive Endocrinology Outpatient Clinics, 24 patients diagnosed with PCOS using Rotterdam criteria, and 26 healthy volunteer women as a control group. Anthropometric measurements of all participants included in the study were recorded. The blood samples of the individuals participating in the study were taken on the 2nd or 3rd day of menstruation following 12-hour fasting, centrifuged at -80 degrees, and serum homocysteine levels were measured by ELISA (Enzyme-Linked ImmunoSorbent Assay). Participants' were divided into two groups according to their serum homocysteine levels (homocysteine level ≥ 30 µmol / L, <30 µmol / L). Regardless of serum homocysteine levels, the participants were divided into groups that body mass index (BMIs) were higher, and lower than 25 kg (kilogram) / m2 (square meter), a waist circumference above, and below 80 cm (centimeter). Serum homocysteine levels of the created new groups were compared.

Results: Serum homocysteine levels of women with PCOS were found higher than women without PCOS (p = 0.001). PCOS patients were significantly younger than the control group (p=0.017). No statistically significant difference was found between BMI, waist circumference, in terms of serum homocysteine levels (p > 0.5).

Conclusion: Regardless of the BMI, and waist circumference, screening routine serum homocysteine levels in young people with phenotype A PCOS, and treating patients with hyperhomocysteinemia may be useful in predicting, and preventing cardiovascular system relationship diseases that may develop in the long term.

Keywords: Body mass index, Cardiovascular System Diseases, Polycystic Ovary Syndrome, Serum Homocysteine Level.

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INTRODUCTION

Polycystic ovary syndrome is a heterogeneous disorder characterized by chronic anovulation, and hyperandrogenism. PCOS is affected by many genetic, and environmental factors (1). It affects 5-10% of women in the reproductive age (2). The diagnosis of PCOS is made by detecting two or more of Rotterdam Criteria (3). Rotterdam Criteria; chronic oligo / anovulation, clinical or biochemical hyperandrogenism, and polycystic ovarian appearance in TVUS (Transvaginal ultrasound).

Patients with PCOS are divided into subgroups. Patients encompassing all of hyperandrogenism, ovulatory dysfunction, and polycystic ovarian morphology form phenotype A, with hyperandrogenism, and ovulatory dysfunction form phenotype B, with hyperandrogenism, and polycystic ovarian morphology form Phenotype C, with polycystic ovarian morphology, and ovulatory dysfunction form phenotype D (4).Women in the PCOS (Phenotype A and B) classes have been shown to have higher values such as BMI, lipids, insulin, insulin resistance, metabolic syndrome than women in the ovulatory PCOS (phenotype C),and non-hyperandrogenic PCOS (phenotype D) class (5-7).

Homocysteine is a sulfur-containing amino acid formed during methionine metabolism. Hyperhomocysteinemia is a complex metabolic disorder. The atherogenic feature of hyperhomocysteinemia is thought to result from endothelial dysfunction, and it has been shown to be an independent risk factor for coronary artery disease (8, 9). Although a definite lower limit could not be determined for serum homocysteine level, 5-15 µmol / L is considered normal after 12 hours of fasting (10). The homocysteine level in mild form is between 15-30 µmol / L, 30-100 µmol / L in moderate form, and 100 µmol / L in severe form (11).

One of the long-term consequences of PCOS is the diseases associated with the cardiovascular system. It has not been understood which pathophysiological pathway of PCOS causes diseases associated with the cardiovascular system. In some studies, the presence of hyperhomocysteinemia in women with PCOS has raised a common pathophysiological pathway question (2).

Our study aims to reveal the relationship between PCOS, and serum homocysteine levels, an important risk factor for cardiovascular system related diseases, by looking at homocysteine levels in Phenotype A PCOS patients who do not have major risk factors for known cardiovascular system related diseases. Relationship between BMI, and waist circumference of the participants with serum homocysteine levels were investigated.

MATERIALS AND METHODS

Patients: Our study was a prospective cross-sectional study. The groups participating in the study were diagnosed with age between 20-49. Phenotype A PCOS, which is a group with high risk for cardiovascular system disease in the long term was included in our study. Phenotype A PCOS 24 patients covering all of hyperandrogenism, ovulatory dysfunction, and polycystic ovarian morphology that was followed for at least 5 years with the diagnosis of PCOS according to Rotterdam Criteria, which applied to the Reproductive Endocrinology Outpatient Clinic of our hospital between May-December 2019.The value of total testosterone above 0,75 ng / ml, and 1,4androstenedionevalue above 3 ng / ml were accepted as biochemical hyperandrogenemia. Clinical hyperandrogenemia criterias were hirsutism, and alopecia. The severity of hirsutism was evaluated with the Ferriman-Gallawey Scores (mFGS) system, which examined the distribution of hair in the body, and clinical hirsutism criteria was accepted in women with an mFG score above 6. Ovulatory disfunction criterias were oligo/anovulation. For menstrual irregularity; any cycle after the post-menarche lasts longer than 90 days; from post-menarche the 3rd year to the perimenaposal stage any cycle lasts shorter than 21 days or longer than 35 days, less than 8 menstrual cycles per year criterias were accepted. Menstrual cycles longer than 45 days were considered oligomenorrhea, and no menstruation in 3 consecutive cycles was accepted as amenorrhea. It was created with participants selected age between 20-49, 26 healthy volunteers who applied for other reasons. Healthy volunteers were composed of people with normal menstrual cycles, normal ovarian morphology, no hyperandrogenemia, and no drug use. Pregnancy, known hyperhomocysteinemia, and/or MTHFR gene mutation, systemic chronic disease, risk for cardiovascular system related diseases (Diabetes mellitus, hypertension, dyslipidemia, smoking, coronary artery disease at first-degree relatives under the age of 40), an age of under 20, and over 49 were the exclusion criterias. Individuals who met the inclusion, and exclusion criteria were included in the study according to their order of arrival at the outpatient clinic. Written consents were obtained from all volunteers participating in the study after their informed consent forms were read.

Age, gestational history, fasting periods, last menstrual periods, and additional diseases were questioned. Physical examination was done. Height lengths, body weights, and waist circumfe-

rences were measured using professionally calibrated instruments. BMI was calculated using the formula [weight (kg) / height length² (m²)]. Waist circumference was measured using a plastic tape measure around the navel, and was obtained by measuring the circumference of the midpoint of the combination of the tenth rib, and spina iliaca anterior superior. MFG scores of the participants were evaluated. For the diagnosis of PCOS, ultrasonographic (USG) evaluation was performed using 5megahertz (MHz) frequency transvaginal, and 3.5 MHz transabdominal transducer (Aplio 500; Toshiba, Japan-2015) by a 5year-old-experienced radiologist. The presence of 12 or more follicles with a diameter of 2-9 mm in one or both ovaries, and / or increased ovarian volume (> 10 ml) is considered PCOS.

Blood Samples: Antecubital venous blood samples (approximately 5 ml) from the individuals who met the inclusion criteria were taken on the 2nd or 3rd day of the menstrual period, following the 12-hour fasting period. The blood samples were placed in yellow capped, vacuumed, plastic gel tubes, and taken to the laboratory within 30 minutes. Blood samples for the measurement of homocysteine levels were centrifuged for ten minutes at 4000 cycle, after serum portion separated, until the day of assessment serum samples were placed into eppendorf tubes, which were kept at -80 ° C. When the determined number of participants were reached, the homocysteine level was evaluated. Serum samples were kept at room temperature for about half an hour before evaluation. Samples were assessed in the Algen Diagnostic Laboratory, CLOUD CLONE CORP. It was studied with Human Homocysteine ELISA kits produced by CHINA company.

Groups: Participants were divided into two groups as PCOS group, and healthy control group at the first stage. Serum homocysteine levels of PCOS group, and healthy control group were compared. Regardless of their serum homocysteine levels, participants were divided into two groups according to their BMI, those below 25 kg / m², and those above 25 kg / m², according to waist circumference below 80 cm, and over 80 cm. Serum homocysteine levels were compared.

In our study, participants' serum homocysteine levels were divided into two groups, those below 30 µmol / L (mild forms), and above 30 µmol / L (moderate,and severe forms).

Statistical analysis: Serum homocysteine levels, ages, BMIs, and waist circumference data of the study groups were uploaded to the computer environment via SPSS (Statistical Package for Social Sciences) for Windows 22.0 (SPSS Inc, Chicago, IL) for statistical analysis, and evaluated. Descriptive statistics were presented as mean ± standard deviation (minimum-maximum), frequency distribution, and percentage. In the evaluation of categorical variables, Pearson Chi-Square Test was applied. The suitability of variables to normal distribution was examined using visual (histogram, and probability plots), and analytical methods (Shapiro Wilk Test). Mann-Whitney U Test for variables that are found to be incompatible with normal distribution in statistical significance between two independent groups, and for variables that are found to conform to normal distribution; Student's T Test was used as a statistical method. The relationship between the variables was evaluated with the Spearman Correlation Test. The results were evaluated at 95% confidence interval, and significance was set at p <0.05.

RESULTS

A total of 50 individuals, 24 female patients diagnosed with PCOS, and 26 healthy control group were examined. The participants were divided into groups, the PCOS group, and the control group. The mean homocysteine level of the PCOS group was 63.7 ± 53.7 (minimum (min): 9-maximum (max): 170) µmol / L, and the mean homocysteine level of the control group was 38.7 ± 45.9 (min: 9-max: 184) µmol / L. The homocysteine level of the PCOS group was statistically significantly higher than control group (p = 0.028) (Table 1).

Table 1. Distribution of homocysteine level between PCOS, and control group.

	PCOS (n=24)	Control Group (n=26)	P
	mean±SD (min-max)	mean±SD (min-max)	
Homosistein (µmol/L)	63.7±53. 7 (9-170)	38.7±45. 9 (9-184)	**0.028[a]***

n: Number of individuals; PCOS: Polycystic ovary syndrome; a: Mann-Whitney U Test; * P <0.05

The homocysteine level of 58.0% (n = 29) of all individuals was below 30 µmol / L, and 42.0% (n = 21) of 30 µmol / L, and above 30 µmol / L. While the homocysteine level of 58.3% of patients in the PCOS group was 30 µmol / L, and above, 26.9% of the control group was 30 µmol / L,and above. The percentage of patients with PCOS diagnosed with homocysteine level of 30 µmol / L, and above was significantly higher than the control group (p=0.025) (Table 2).

Table 2. Distribution of homocysteine group in PCOS, and control group.

	PCOS (n=24)	Control Group (n=26)	p
	n (%)	n (%)	
Homocystein Group			
<30 µmol/L	10 (41.7)	19 (73.1)	**0.025[a]***
≥30 µmol/L	14 (58.3)	7 (26.9)	

n: Number of individuals; PCOS: Polycystic ovary syndrome; a: Pearson Chi-Square Test; * P <0.05

Mean age of PCOS group was 25.7 ± 4.7 (min: 21-max: 42) years, and 30.5 ± 7.4 (min: 20-max: 45) years in the control group. Patients with PCOS were significantly younger than the control group (p=0.017). There was no statistically significant difference between the study groups in terms of waist circumference, and BMI value (p>0.05) (Table 3).

Table 3. Distribution of age, BMI, waist circumference measurements between the PCOS group, and control groups.

	PCOS (n=24)	Control Group(n=26)	p
	mean±SD (min-max)	mean±SD (min-max)	
Age (year)	25.7±4.7 (21-42)	30.6±7.2 (20-45)	**0.011[a]***
Waist Circumference (cm)	88.0±13.3 (54-108)	84.6±13.3 (60-110)	0.373[b]
BMI (kg/m²)	25.2±5.1 (18.6-39.1)	24.4±4.4 (17.9-37.1)	0.557[b]

n: Number of individuals; BMI: Body mass index; PCOS: Polycystic ovary syndrome; a: Mann-Whitney U Test; b: Student's T Test; *

There was no statistically significant difference between the BMI groups, and waist circumference groups of individuals in terms of homocysteine level (p = 0.148), and (p = 0.905) (Table 4).

Table 4. Distribution of homocysteine levels between waist circumference, and BMI groups.

	n	Homocystein (µmol/L)	p[a]
		mean±SD (min-max)	
Waist Circumference Group			
<80 cm	14	52.1±49.1 (11-168)	0.905
≥80 cm	36	50.2±52.1 (9-184)	
BMI Group			
<25 kg/m²	29	61.2±56.5 (9-184)	0.148
≥25 kg/m²	21	36.1±38.4 (9-150)	

n: Number of individuals; SD: Standard deviation; BMI: Body mass index; a: Mann-Whitney U Test

with BMI, age and waist circumference (p> 0.05) (Table 5).

Table 5. Relationship of homocysteine level with age, waist circumference, and BMI values.

	Homocystein (µmol/L)			
	PCOS (n=24)		Control Group (n=26)	
	r	p	r	p
Age (year)	-0.288	0.172	-0.351	0.078
Waist Circumference (cm)	-0.088	0.681	-0.019	0.927
BMI (kg/m²)	-0.187	0.381	-0.126	0.539

r: Spearman Correlation Coefficient; PCOS: Polycystic ovary syndrome; BMI: Body mass index; *:P <0.05

DISCUSSION

In the current study, we investigated serum homocysteine levels of women with PCOS compared healthy individuals, and we found higher serum homocysteine levels in the PCOS patients regardless of age, BMI, and waist circumference.

Glucose intolerance, insulin resistance, abnormal lipid profile, and obesity observed in PCOS shows that PCOS is not only a reproductive endocrinopathy, but also a disease group with an increased risk for cardiovascular system related diseases (12). Since PCOS is very common, and affects women in the most productive age group in the society, cardiovascular system diseases secondary to PCOS are associated with a significant loss of labor, and serious increases in healthcare costs. Therefore, defining the cardiovascular system related disease development, pathophysiology, and risk factors associated with PCOS are essential for improving aforementioned costs.

Homocysteine was chosen as a marker in our study in order to determine whether it plays a role in the pathogenesis of PCOS. PCOS is associated a group of metabolic disorders which include CVS related diseases. In epidemiological studies, hyperhomocysteinemia has been reported as a cardiovascular risk factor, and has been shown to cause premature atherosclerosis (13,14). This risk increases proportionally with serum homocysteine level (15).

Current data in the literature show that serum homocysteine levels of women with PCOS are significantly higher than serum homocysteine levels of those without PCOS. In our study, serum homocysteine levels, and hyperhomocysteinemia frequency were found to be significantly higher in patients with PCOS group than the healthy control group. In a study by Eskandari

fertile 35 non PCOS participants were compared. Serum homocysteine levels of the group with PCOS were found significantly higher than non-PCOS group (16). In this study, comparisons were made between infertile patients whereas in our study, patients were included consecutively, regardless of fertilization status. In a study reported by Dunaif et al., hyperhomocysteinemia, which is an independent risk factor for obstetric vascular syndromes, has been shown to cause complications related to the cardiovascular system in the long-term, and short-term pregnancy outcomes in PCOS (17,18). Unlike this study, our study did not include short, and long-term follow-up of patients. In a study by Diwaker et al., The relationship between serum homocysteine level, and insulin resistance in PCOS patients was examined, and serum homocysteine levels were significantly higher in PCOS patients with insulin resistance, and hyperinsulinemia (19). In our study, no comparison was made in terms of insulin resistance, and insulin levels. In a study by Maleedhu et al., 142 patients with PCOS, and 65 healthy non-PCOS control groups were compared with serum homocysteine levels, BMI, and waist circumference. Serum homocysteine levels, BMI, and waist circumference were significantly higher in PCOS group than healthy non-PCOS control group (20). In our study, homocysteine levels were found to be high, but there was no difference between the two groups in terms of BMI, and waist circumference.

Serum homocysteine level varies depending on age. Serum homocysteine levels were found to be higher in older ages (21). In our study, the mean age of the group with PCOS was significantly younger than the mean age of the control group. Despite the mean age difference between the groups, the fact that the serum homocysteine levels of the young PCOS group was higher than the control group of homocysteine levels increase the strength of our study by showing that our study was not affected by the increase with age in serum homocysteine level. However, considering the number of samples in the PCOS, and control groups, more comprehensive, and large population studies are needed to show the relationship between age, and serum homocysteine levels.

Serum homocysteine level varies depending on BMI, and waist circumference. Hyperhomocysteinemia was found in patients with high BMI, and high waist circumference (22). In the PCOS group, and control group in our study, the absence of a statistically significant difference between participants' BMI, and waist circumference increases the reliability of our study in terms of homocysteine level.

One of the remarkable results of our study was the detection of hyperhomocysteinemia in some of the healthy individuals. Another interesting result was the normal level of homocysteine in some of the patients with PCOS. Homocysteine level is affected by genetic, and sociodemographic features (23,24). The fact that homocysteine level can be normal in a group at a high-risk group for cardiovascular diseases such as PCOS, or high levels of homocysteine in healthy individuals can be due to the other factors affecting homocysteine level.

In this study, with the detection of higher homocysteine levels in Phenotype A PCOS patients, these patients will be treated with folic acide (400 µG/per-day), and followed up for long-term complications. It will be understood whether CVS complications that may arise in long-term can be prevented by early diagnosis and treatment during follow-up.

The fact that the individuals in our study were not evaluated in terms of insulin resistance, genetic polymorphism, and nutritional status can be counted as the limitations. Measured homocysteine level was the total plasma homocysteine level can be considered as another limitation. When the literature is reviewed, it is seen that the majority of similar studies are planned in this way; however, the presence of many exclusion criteria increased the strength of the study by comparing homogeneous groups. The number of patients included in the study was limited, the mean age was young, and therefore the findings of cardiovascular disease have not been evident yet, may be considered among the deficiencies of our study.

CONCLUSION

Independent of BMI, and waist circumference, it can be useful in predicting, and preventing cardiovascular system related diseases that may develop in the long term by routine serum homocysteine screening in patients with phenotype A PCOS, and treatment of patients with hyperhomocysteinemia (homocysteine level ≥ 30 µmol / L). In this way, by treating hyperhomocysteinemia detected in early PCOS patients, larger patient population, and longer studies are needed to see if there is a decrease in the risk of developing cardiovascular system related disease.

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CONFLICT OF INTEREST:

We have no conflict of interest.

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