METHYLENETETRAHYDROFOLATE REDUCTASE POLYMORPHISM IN POLYCYSTIC OVARY SYNDROME AND THE EFFECT OF ORAL CONTRACEPTIVE USE

Faika Ceylan ÇIFTÇI

Department of Obstetrics and Gynecology, Koru Hospital, Ankara

Nevin SAĞSÖZ

Department of Obstetrics and Gynecology, Kırıkkale University, Faculty of Medicine, Ankara

Corresponding Author: faikaceylanciftci@gmail.com

ABSTRACT

Background: Elevated plasma homocysteine level is an independent risk factor for cardiovascular disease in women with polycystic ovary syndrome (PCOS). In the presence of methylnetetrahydrofolate reductase (MHTFR) mutation, elevated homocysteine levels are observed.

The purpose of this study was to detect the existence of MHTFR polymorphism in patients diagnosed as having PCOS in the reproductive period and to determine whether the levels of serum homocysteine and folate changed in patients with different MHTFR genotypes after the administration of an oral contraceptive agent.

Methods: The study included 50 PCOS patients and 50 healthy controls aged between 17 and 35. The patients diagnosed with PCOS were divided randomly into two groups. While the first group of 25 patients was administered *30 µg ethynyl estradiol+150 µg desogestrel* the other group of 25 patients was monitored without administering medication.

Results: It was found moreover that the MTHFR enzyme led to an increase in the serum homocysteine levels of both the MTHFR and the control group. MTHFR polymorphism was found to be of similar ratios in both the PCOS patients and the controls in this study. Decreased levels of serum homocysteine, fasting insulin and homeostasis model assessment insulin resistance (HOMA-IR) levels were observed in CC and CT genotypes, but not in the TT group after treatment.

Conclusion: It was observed in this study that the levels of cardiovascular risk factors like homocysteine, insulin, and HOMA-IR dropped in the patients who were administered oral contraceptives for 6 months.

Keywords: Polycystic ovary syndrome, methylenetetrahydrofolate reductase polymorphism, Oral contraceptives, Homocysteine

INTRODUCTION

Polycystic Ovary Syndrome (PCOS) is a common endocrine disorder that affects 15% of the women in the reproductive period [1]. It is characterized by chronic anovulation, hyperandrogenism and oligo/amenorrhea [2]. As a consequence of hyperandrogenism, insulin resistance and dyslipidemia, women with PCOS have an increased risk of cardiovascular disease [3, 4, 5], and high cardiovascular mortality and morbidity are observed in these patients [6]. Elevated plasma homocysteine levels constitute an independent risk factor for cardiovascular disease [7]. A strong association has been detected between cardiovascular disease and plasma homocysteine levels. However, the exact mechanism of this association has not yet been fully determined. Genetic factors are important for the metabolic cycle of homocysteine synthesis. The enzymatic defects caused by genetic mutations lead to a significant increase in homocysteine concentrations [8]. It is suggested that the most frequently encountered cause of abnormal serum homocysteine levels in the population is the reduced efficiency of methylenetetrahydrofolate reductase enzyme (MHTFR) [9]. MTHFR enzyme participates in the remethylation of homocysteine to methionine. Folate also participates in this process as a co-factor. C677T mutation leads to an impairment in the enzymatic activity of the MTHFR gene. This mutation develops as a result of the replacement of alanine by valinine in nucleotide 677 [10]. The C677T mutation develops in the homozygote form. This polymorphism develops as a variant of MTHFR, which is called thermolabile MTHFR, and in the presence of this mutation elevated homocysteine levels are observed as well [11]. Although it has not yet been fully explained through which mechanism elevated homocysteine levels constitute an independent risk factor for cardiovascular disease, it is thought to exert this influence by causing endothelial dysfunction or by impacting on the coagulation cascade [12].Thermolabile MTHFR had been implied as a conceivable risk factor for stroke, psychiatric disorders, heart disease, diabetes, hypertension, and cancer, but its true effect on PCOS has not yet been fully explained[4]. Based on these findings, it has been suggested that the increased risk of cardiovascular disease in PCOS stems from the elevated homocysteine and reduced folate levels that develop in MTHFR polymorphism. Some studies on the subject have found MTHFR polymorphism, increased homocysteine and reduced folate levels in PCOS cases [13]. There are few studies in the literature investigating the impact of PCOS treatment on the homocysteine level. In particular, there is no previous study on the influence of the widely applied OC treatment of PCOS on plasma homocysteine levels. This study, which was performed on patients diagnosed with PCOS in reproductive period according to the criteria of 1990 *National Institutes of Health & Child Health & Human Development* (NICHD), aimed to detect the existence of C677T polymorphism and to determine whether the levels of serum homocysteine and folate in these patients changed in different MTHFR genotypes after the administration of an oral contraceptive agent.

MATERIAL AND METHODS

Patients:

This study included 50 patients admitted to the Outpatient Clinics of Gynecology and Obstetrics in Kirikkale University School of Medicine, Kirikkale, Turkey, where they were diagnosed with PCOS

and 50 healthy subjects for controls during January 1 2004 and December 30 2005. Healthy women were selected for control group from those applied for contraception. The approval of the Chair of the Ethical Committee of Kirikkale University Faculty of Medicine (No 2004/100) was received for the study. The subjects, as well as the healthy controls, were duly informed about the study before volunteering to participate in it. Written approvals were received from the patients and healthy controls who had agreed to take part in the study.

Sample size was determined on the basis of a pilot study (3.9 µmol/L), which indicated that, with 25 patients in each group, a power of 94% would be required to detect a difference of 4 µmol/L in the mean ***Homocysteine*** *between two groups at a significance level of 0.05.*

The study included women patients between the ages of 17-35 who are in the reproductive period, who had chronic anovulation as well as clinical and/or laboratory hyperandrogenism but did not have any other hormonal or systemic pathology. The exclusion criteria were as follows: collagen tissue disease, thyroid dysfunction, prolactinoma or other endocrinopathies (Cushing syndrome, congenital adrenal hyperplasia with late-onset etc.), kidney or liver dysfunction, a history of neoplasia, a history of thromboembolic event, use of medicines affecting the sex hormone, carbohydrate metabolism, atherosclerosis, systemic diseases like diabetes mellitus and hypertension, depression or psychological complaints, unwillingness to fill the test forms, unwillingness to undergo additional examinations, refusal to receive treatment, and smoking. Other criteria of exclusion from the study were as follows: request for an alternative treatment, development of serious complications during the treatment, lack of compliance with the study, pregnancy, and inability to receive the treatment.

The patients and healthy control group participating in the study were evaluated in three distinct groups. Group I included 25 patients with PCOS who were monitored for six months without administering any treatment. Group II included 25 other patients with PCOS who were administered an oral contraceptive(OC) (30 µg *Ethinyl estradiol*+150 µg *Desogestrel*) Group III included 50 healthy control subjects who were not administered any kind of treatment and were not called six months later for checks. On the first admission of the patients and healthy controls to the outpatient clinics, their age, height, weight, waist/hip ratio, and BMI information were recorded. Their anamneses were received and physical examinations were performed. Hirsutism was assessed by the Ferriman-Gallewey scoring system. Pelvic or transvaginal ultrasonography was performed.

The following parameters were examined in the groups: the patients and healthy control subjects who had applied to our polyclinic were called on the third day of their menstrual cycle, told to fast in the night before. For homocysteine 1,5 cc of blood and for MTHFR polymorphism 2,5 cc of blood were received into two separate tubes with EDTA. Then the plasma in the blood samples was separated for measuring the homocysteine. The plasma and blood placed into tubes with EDTA for investigating MTHFR polymorphism were preserved for six months at -70 ºC, to be examined together at the end of

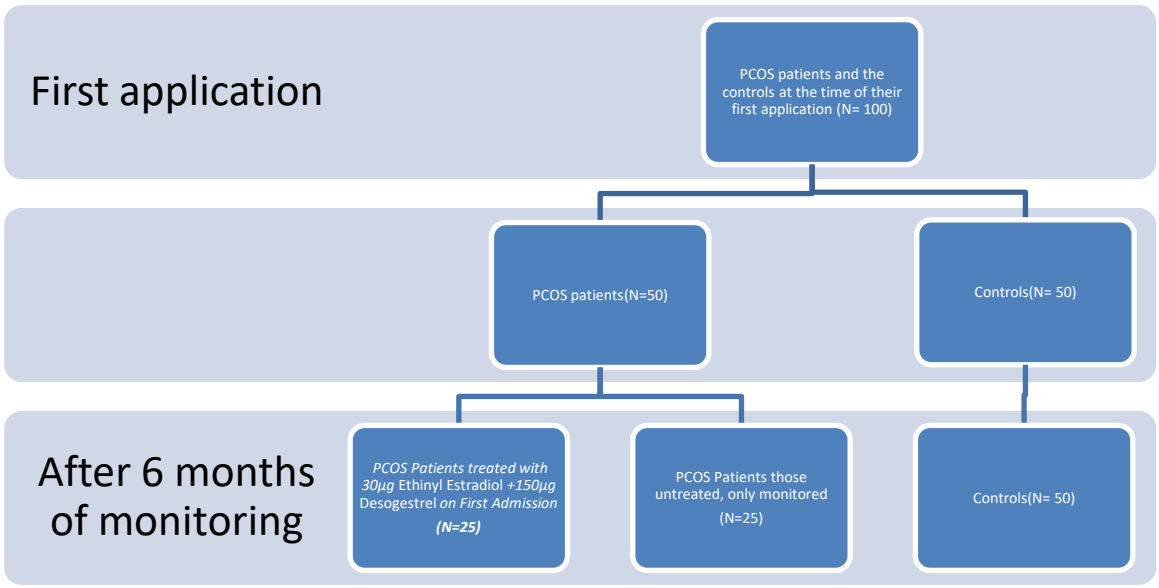
the study. By the end of the study, on the third day of the menstrual cycle of patients with PCOS, they were called again to the polyclinic after a night of fasting, and 1.5 cc of blood was received into tubes with EDTA for measuring homocysteine. The separated plasmas were examined together with the plasmas that had been received before. Again, on the third day of the menstrual cycles of patients and healthy controls, they were called to the polyclinic after a night of fasting to obtain blood samples. The samples were analyzed for the following values (Intraassay sensitivity and specificity; Interassay sensitivity and specificity values are given in order *according to the "CV" values which were written in kits' prospectuses*.) : fT3(%1.1-3;<%0.1;%1.9-8.2;0.998), fT4(%1.7-5.7;<%0.1;%3-10.7;0.999), TSH(%1.8-8.6;%0.008-0.038;%3.6-8.7; 0.993), PRL(%2-4;<%0.1;%3.7-5;0.998), DHEAS(%1.7-2.8;%0.06-10.8;%2.4-3.6;0.952), , total testosterone(%1.2-4.7;%2-6;%3.4-8.4;0.972), 17OH progesterone(%1.2-11.8 %0.001-0.682 %2.4-11.9 0.993), SHBG(%2.1-2.7;<%0.1;%2.7-5.6;0.981), FSH(%1.5-1.8; <%0.1;%3.8-5.3;0.998),LH(%0.8-1.8;<%0.1;%2-5.2;0.993), estradiol(%2.4-8.5;%0.001-0.757;%2.8-11.9;0.999), androstenedione, free testosterone and as biochemical parameters AST(%1.8-2.6;<%0.1;%2-2.9;0.998), ALT(%0.5-1;<%0.1;%1.5-1.9;0.998), vitamin B_{12}(%1.6-3.3;%0.0003;%2.2-5.2;0.999), and folate(%2.5-8;%0.7-2.5;%3.7-10.9;0.994). Moreover, the serum progesterone level on the 21st day of their cycles was assessed as well. In the period following 12 hours of fasting, which was preceded by a three-day diet of 300 g of carbohydrate, the basal blood glucose and insulin were measured first, and then the level of glucose was measured on the 30th, 60th, 90th and 120th minutes after the administration of 75 g of glucose.

The diagnoses of PCOS were made according to the criteria of 1990 NICHD [14,15]. The medical histories of all the subjects participating in the study were recorded, and their systemic checks and physical examinations were duly performed. Hirsutism was assessed in 9 different regions, according to the Ferriman–Gallwey (FG) scoring system (upper lip, lower jaw, back, chest, umbilicus, pubis, arm, thigh, sacrum). Those subjects with a FG score of 8 and above were considered to be hirsute [15]. Bodyweight (kg), waist and hip circumference and the waist-to-hip ratio were measured. The Body Mass Index (BMI) was calculated as kg/m^2.

OGTT and ADA were assessed according to the diagnosis criteria [16]. Insulin resistance was determined by the *HOMA* (*Homeostasis Model Assesment*) method, according to the formula *HOMA* = fasting blood glucose (mmol/l) X fasting glucose (µU/ml)/ 22.5 [17]. The same procedures described above were applied to the subjects in the control group.

Study Protocol and Treatment:

The medicine selected for use in the study was the oral contraceptive 3*0 µg Ethinyl estradiol+150 µg Desogestrel (Desolett®, Organon,* Istanbul, Turkey), and the period of treatment was 6 months. Beginning from the first case, the subjects were randomly divided into two groups. *Desolett* treatment was administered to the first subject, while no treatment was administered to the following subject. After the first two cases, the patients were divided respectively into two groups as those who began to receive treatment and those who did not. At the end of six months, the procedures described above were applied again.



DNA Analysis; MTHFR C677T Gene Polymorphism Method:

After genomic DNA was obtained from the peripheral blood of the patients and controls, Polymerase Chain Reaction (PCR) was applied with the appropriate primaries. PCR was performed in the Department of Infectious Diseases in Kırıkkale University Faculty of Medicine. The primaries (MWG) were as follows: MTHFR P1 N: 5’ AAG GTG TCTGCGGGA CC 3’; MTHFR P1 Mut. (ARMS) : 5’ AAG GTG TCTGCGGGA CT 3’; MTHFR P2 5’ TCA AGTGGTTCT GAT GAC AG 3’ These primaries were used to amplify the gene region that contained the MTHFR mutation (C 677 T) and was 453 bp in size. The amplification was performed according to the last nucleotide at the 3’ end of p1,

which contains the modifications peculiar to alien and mutant types. This procedure, known as the ARMS method, was successfully applied to analyze MTHFR polymorphism containing C677T mutation.

The Thermal Cycler Program:

The "Thermal Cycler" program was applied. Following PCR, the analysis was performed with 2% agarose (*SERVA*, cat #11380) gel electrophoresis. The results were evaluated with the gel documentation system *(SYNGENE*, *GEGEGENIUS*). In this assessment: CC (Homozygote Normal) → MTHFR p1: N (+);MTHFR p1 ARMS : (-)/ CT (Heterozygote) → MTHFR p1: N (+);MTHFR p1 ARMS : (+)/ TT (Homozygote Patient) → MTHFR p1 : N (-)/ MTHFR p1 ARMS : (+)

Folate and B_{12}vitamin assays:

The vitamin B12 and folic acid levels were measured in the laboratory of the Department of Biochemistry and Clinical Biochemistry at Kırıkkale University, Faculty of Medicine by a researcher blinded to the differences between the groups. They employed the B_{12} *elecsys* ve *folate elecsys* kits in the apparatus *Elecsys* 2010 (*Roche*), and used the chemiluminescence method. Since the linearity of the *folate elecsys* kit was not suitable for the purpose, the specimens were diluted six-fold with a special diluent before they were examined. The results were multiplied by the dilution factor. Vitamin B_{12} was calculated as pg/ml and folic acid as ng/ml.

Homocysteine assay:

In the laboratory of the Department of Biochemistry in Gazi University, Faculty of Pharmacy, by using high-performance liquid chromatography (HPLC) and a method previously described by Goddijin-Wessel and Wouters, a researcher blinded to the differences between the groups measured the homocysteine levels in the blood plasma of the patients and controls [18, 19]. The results were expressed as μmol/L. All the measurements were repeated on two different days. A difference of at most 10% between two measurements and a 5% difference from the mean value was considered as acceptable. In cases when the difference turned out to be greater, the analysis was repeated for the third time. The mean values of these analysis results were calculated.

Statistical Analysis:

Ferriman Galleway scoring system (FG) and BMI with waist-to-hip ratio was examined for the PCOS and control group; both before and after the treatment, with *paired t* according to clinical findings in terms of hirsutism. Statistical analysis of the difference for the measured biochemical parameters and hormones was done by *student t* test. These parameters were evaluated before and after the treatment with the *paired t* test. Homocysteine, Insulin, HOMA-IR and AUC*glucose* were examined between PCOS and the control group by *student t*, and non-parametric Wilcoxon test was used both before and after the treatment in the groups treated with and without PCOS.

Kruskal Wallis was used for statistical analysis for CC, CT, TT genotypes in MTHFR Polymorphism. Patients with PCOS according to their MTHFR genotype and the control group were analyzed among

themselves according to their genotypes using the Mann Whitney U test. Serum Vitamin B_{12} and folate levels of women with PCOS according to their genotypes and the women in the control group were evaluated using *Kruskal Wallis* analysis according to their CC, CT, TT genotypes. 25 patients with PCOS according to their MTHFR Genotype and 25 patients who received 30µg *Ethinyl estradiol* + 150µg *Desogestrel* treatment and 25 patients who were followed up without medical treatment were examined by Wilcoxon test to determine their serum Vitamin B_{12} and folate levels at the time of first application and after 6 months according to their CC, CT, TT genotypes.

25 patients who had PCOS according to their MTHFR Genotype who received 30µg of *Ethinyl estradiol* + 150µg *Desogestrel* treatment and 25 other patients who were followed up without medical treatment were examined with Wilcoxon nonparametric test to determine the changes in their levels of AUC_{glukoz} and homocysteine after taking insulin, *HOMA-IR* and OGTT after the first application and after 6 months according to their CC, CT, TT genotypes..

Pearson correlation analysis was applied to examine the correlation between variables. Spearman test was applied for non-normal distributions. In all examinations $p<0,05$ value was accepted as statistically significant.

RESULTS

All the 50 PCOS patients included in the study remained until the end. The 50 PCOS patients and the 50 controls were of comparable age. In comparison with the controls, the PCOS group had higher BMI, waist/hip ratio and Ferriman-Gallewey score (FG) ($p<0,05$). Moreover, the PCOS group had higher Lp a ($p=0,001$), Apo B ($p=0,005$), serum homocysteine ($p=0,001$), fasting insulin ($p=0,001$) and *HOMA-IR* ($p=0,001$) levels. The mean age of the 50 subjects with PCOS was 24,74±4,93, while their mean FG score was 10,27±4,70 (Table 1).

Six months after treatment with 30µg*Ethinyl estradiol*+150µg *Desogestrel*, a significant drop was detected in the serum homocysteine level of the 25 PCOS patients treated in this way, from 13,27±4,50 µmol/L to 10,24±4,54 µmol/L ($p=0,001$). In these patients, moreover, the *HOMA-IR* level dropped from 4,18±2,56 to 2,48 ± 1,50 ($p=0,001$), and fasting serum insulin from 18,01±8,56 µIU/ml to 12,90±6,10 µIU/ml ($p=0,006$). On the other hand, while the $AUC_{glucose}$ level was 14719±4536 after OGTT, it was found to be 14520±3367 after ($p=0,909$). Following six months of treatment, a significant decrease was observed in the serum free-testosterone and androstenedione levels ($p=0,030$, $p=0,005$ respectively), while the decrease in the DHEA-S level was not statistically significant ($p=0,504$)(Table 2).

In the other 25 PCOS patients who were simply monitored without administering treatment, there was a drop in the serum homocysteine level from 12,42±3,24 µmol/L 12,13±2,95 µmol/L ($p=0,187$). These changes observed in the untreated, monitored patients by the end of six months were not found to be statistically significant. No difference was detected in these patients between the initial and sixth-month levels of *HOMA-IR* ($p=0,320$) and fasting serum insulin ($p=0,124$). On the other hand, a significant increase was observed in the $AUC_{glucose}$ levels after OGTT. After six months of monitoring without treatment, a statistically significant decrease was observed in this group in the serum free testosterone

level (p=0,038), while no difference was detected in the serum DHEA-S (p=0,413) and androstenedione (p=0,632) levels (Table 2).

Among the 25 PCOS patients administered 30µg*Ethinyl estradiol*+150µg *Desogestrel*, 10 had MTHFR gene polymorphism of subtype CC, 11 of subtype CT, and 4 of subtype TT. Among the other 25 untreated, monitored PCOS patients, 7 had MTHFR gene polymorphism of subtype CC, 17 of subtype CT and 1 of subtype TT. Among the 50 controls, 18 had MTHFR gene polymorphism of subtype CC, 27 of subtype CT, and 5 of subtype TT. When the PCOS patients and healthy controls were compared as regards MTHFR gene polymorphism, the distribution of gene polymorphism subtypes was found to be similar between the two groups (p=0,3).

When the PCOS patients and controls were compared with respect to the three genotypes, in all three genotypes the insulin, *HOMA-IR* and homocysteine levels of the PCOS patients appeared to be significantly higher than the values of the controls. In genotype CC, insulin was 20,90±10,35µIU/ml in the PCOS patients and 6,98±4,44µIU/ml (p=0,001) in the controls; in genotype CT, it was 17,85±7,30µIU/ml in the PCOS patients and 8,81±8,72µIU/ml (p=0,001) in the controls; and in genotype TT it was 18,85±6,70µIU/ml in the PCOS patients and 5,08±2,66µIU/ml (p=0,009) in the controls. Thus the insulin levels were found to be significantly higher in the PCOS group in all three genotypes. In genotype CC, *HOMA-IR* was 4,77±2,70 in the PCOS patients and 1,39±0,84 (p=0,001) in the controls; in genotype CT, it was 3,93±2,00 in the PCOS patients and 1,44±0,61 (p=0,001) in the controls; and in genotype TT it was 3,95±1,81 in the PCOS patients and 0,97±0,57 (p=0,008) in the controls. Thus the *HOMA-IR* levels were found to be significantly higher in the PCOS group in all three genotypes. In genotype CC, homocysteine was 11,86±2,11 µmol/L in the PCOS patients and 6,66±1,83µmol/L (p=0,001) in the controls; in genotype CT it was 12,22±3,34 µmol/L in the PCOS patients and 7,26±1,83µmol/L (p=0,001) in the controls; in genotype TT it was 19,66±5,48 µmol/L in the PCOS patients and 10,70±1,86µmol/L (p=0,009) in the controls. Thus the homocysteine levels were found to be significantly higher in the PCOS group in all three genotypes.In folate levels, on the other hand, no such difference was observed (in genotype CC p=0,468, in genotype CT p=0,259, in genotype TT p=0,295).In genotype CC, Vitamin B_{12} was 351,91±154ng/ml in the controls and 281,86±128,43ng/ml (p=0,032) in the PCOS patients. In genotype TT it was 378,24±161,77ng/ml in the controls and 207,48±49,15ng/ml (p=0,028) in the PCOS patients. Thus in genotypes, CC and TT vitamin B_{12}werefound to be higher in the controls than in the PCOS patients, while no such difference was detected in genotype CT. When the three genotypes CC, CT, and TT were compared with each other, a significant difference was detected as regards homocysteine. While no difference in homocysteine levels was detected between genotypes CC and CT in the PCOS patients (p=0,261), a statistically significant difference was detected between CC and TT (p=0,003) and CT and TT (p=0,005). The homocysteine level was 11,86±2,11µmol/L in type CC and 12,22±3,34µmol/L in type CT.In the PCOS group, in genotype TT, the serum homocysteine level was found to be 19,66±5,48µmol/L, higher than in the other genotypes (p=0,011). Also in the control group, the homocysteine level in genotype TT, 10,70±1,86µmol/L, was higher than the values in genotypes CC (6,66±1,83µmol/L) and CT (7,26±1,83µmol/L). In the control group as well, while no difference was found between CC and CT (p=0,358), a significant difference in homocysteine was found between CC and CT (p=0,003) and CT and TT (p=0,001)(Table 3).

The serum vitamin B_{12}(p=0,784) and folate (p=0,965) levels in the controls were found to be similar in all three MTHFR genotypes. In the PCOS patients, no significant difference was detected in serum vitamin B_{12}(p=0,193) and folate (p=0,078 levels between genotypes CC, CT and TT.

In the PCOS patients with genotype CC, after six months of treatment, there was a drop from 11,21±2,01 μmol/L to 9,24±2,41 μmol/L (p=0,037) in the homocysteine level; from 20,10±7,50 μIU/ml to 12,24±5,49μIU/ml (p=0,013) in the insulin level; and from 4,69±2,47 to 2,82±1,85 (p=0,005) in the *HOMA-IR* value. No significant difference was found between the levels of vitamin B_{12}andfolate at the time of application and after six months of treatment (p=0,059, p=0,114 respectively). In the PCOS patients with genotype CT, at the end of six months' treatment, there was a drop from 12,25±2,76 μmol/L to 8,66±1,97 μmol/L (p=0,004) in the serum homocysteine level and from,74±2,92 to 2,05±1,20 (p=0,003) in the *HOMA-IR* value. No significant difference was detected in the vitamin B_{12} (p=0,328) and folate (p=0,575) levels. In the case of PCOS patients with genotype TT, no significant difference was observed in the serum homocysteine, insulin, *HOMA-IR*, vitamin B_{12} (p=0,068) and folate (p=0,465) levels (Table 4).

In the untreated, monitored PCOS patients with genotype CC, by the end of six months, there was a drop from 12,78±2,03μmol/L to 12,33±2,00μmol/L (p=0,018) in the homocysteine level; from 22,04±14,10μIU/ml to 17,76±9,73 μIU/ml (p=0,612) in the insulin level; and from 4,90±3,20 to 4,61±2,48 (p=0,499) in the *HOMA-IR* value. Nevertheless, these changes were not statistically significant. No significant difference was found either between the levels of vitamin B_{12} and folate at the time of the first admission and after six months of monitoring (p=0,091, p=0,612). In the PCOS patients with genotype CT, after six months of monitoring, there was found a drop from 12,20±3,75μmol/L to 12,00±3,39 μmol/L (p=0,193) in the serum homocysteine level and from 4,06±1,16 to 4,05±1,16 (p=0,619) in the *HOMA-IR* value. However, these changes were not statistically insignificant. Similarly, no significant difference was detected in genotype CC in the B_{12} (p=0,619) and folate (p=0,068) levels. By the end of six months, no difference was found in the B_{12} and folate levels of the treated and untreated patients with respect to their MTHFR genotypes (p=0,310).

In the PCOS patients, no correlation was found between the parameters of age (p=0,234, r=-0,171), BMI (p=0,964, r=0,007), fasting blood glucose (p=0,232, r=-0,172), fasting insulin (p=0,602, r=-0,076) and *HOMA-IR* (p=0,250, r=-0,166).

Discussion

Although the exact etiopathogenesis of PCOS has not been clarified yet, it is known that genetic predisposition, impaired gonadotropin secretion, and ovary steroid production, as well as hyperinsulinemia or insulin resistance, play a role in the development of this syndrome [20]. In studies on the genetic basis of PCOS, more than 30 genes have been examined [21]. MTHFR C677T gene polymorphism is an enzymatic defect that causes increased homocysteine levels [4]. Reduced 5,10 MTHFR enzyme activity leads to elevated plasma homocysteine values. Although the frequency of thermolabile MTHFR enzyme differs from one society to another, it has been detected in around %17 of patients with cardiovascular disease [22] and 28% of patients with hyperhomocysteinemia who are diagnosed early in life with vascular disease [23].

Reduced, MTHFR enzyme activity is one of the most common reasons for elevated plasma homocysteine levels. In C677T mutation, while MTHFR activity decreases in homozygote mutant TT genotype in comparison with heterozygote CT and homozygote normal CC genotypes, a significant increase is observed in the homocysteine level [24]. It is not fully clear yet whether homozygosity is a real risk factor for the development of cardiovascular diseases in the presence of MTHFR C677T mutation. There are studies supporting this finding [25, 26] as well as those that do not [27, 28, 29]. The homozygote MTHFR C677T mutation causes an increased risk for the early development of coronary artery disease [4, 30]. It is argued that there is an increased risk of coronary heart disease in people with MTHFR C677T polymorphism of genotype TT when compared with genotype CC. In other studies, it is reported that people with hereditary thrombophilia and C677T MTHFR polymorphism of the homozygote genotype do not have a high risk of thrombosis [31]. In this study, the ratio of those with the MTHFR genotype was found to be 10% in PCOS patients and 10% in the controls. In both groups, five subjects were found to have MTHFR C677T polymorphism of genotype TT. The prevalence of MTHFR polymorphism differs between ethnic groups. For the T allele, the frequency of homozygotesis 10% among the people of the white race in Europe [32]. In a study conducted on young women with a risk of myocardial infarction, MTHFR of homozygote genotype TT was found in 12% of the patients and 10% of the controls [33]. The polymorphism ratios we found agreed with these values. In this study, we found the serum homocysteine level to be higher in those PCOS patients with a C677T MTHFR mutation of the homozygote genotype than in the patients with the other genotypes. This finding agreed with the results of previous studies. Nevertheless, a number of replication studies on MTHFR have failed to supply evidence for this association [1, 34, 35].

In recent years, the view of PCOS as a systemic disease has been gaining ground. The fact that PCOS patients also have insulin resistance, central obesity, dyslipidemia, and impaired fibrinolysis heightens the risk of Type 2 DM and cardiovascular disease [5]. While retrospective studies support that DM is encountered frequently in these patients, they do not corroborate the presence of a high risk of death from ischemic heart disease. However, *cross-sectional* studies do show that there is a clear association between PCOS and ischemic heart disease [6,36].

Recently the most stressed cause of PCOS etiopathogenesis is peripheral insulin resistance and the hyperinsulinemia that develops as a consequence. Previous studies have demonstrated the presence of insulin resistance in PCOS and its association with hyperandrogenism [36]. Insulin resistance, hyperinsulinemia, dyslipidemia, hyperhomocysteinemia, increased systolic blood pressure, endothelial dysfunction, and coagulation impairments all increase the risk of cardiovascular disease in PCOS [4, 6]. Insulin resistance was assessed by the *HOMA-IR* method in this study. In agreement with the existing literature, we found higher *HOMA-IR* and fasting insulin levels in the PCOS patients in this study. Homocysteine is an independent risk factor for cardiovascular, cerebrovascular and peripheral vascular diseases [1, 7, 37]. It is known that the prevalence of cardiovascular disease increases early in life in PCOS. Based on the fact that homocysteine is an independent risk factor in the development of various vascular diseases, it is thought that elevated homocysteine levels may be associated with PCOS [34]. It has been shown that homocysteine levels increase in patients with PCOS [38, 39] Loverro et al. have demonstrated that the homocysteine level increases in direct proportion to the insulin level [38]. We

determined in this study that patients with PCOS had elevated homocysteine values but found no association between insulin level, insulin resistance, and BMI.

Considering the metabolic consequences of PCOS and its role in the development of cardiovascular events, the approach to treatment must not only focus on eliminating clinical symptoms, but also on protecting the patients from the risks of cardiovascular disease, DM, and cancer. Oral contraceptives containing *Desogestrel*and *Gestoden* are less androgenic with respect to the other, low-dose OCs; they also have a less suppressive influence on both carbohydrate and lipoprotein metabolisms and a more suppressive effect on ovarian activity [40]. Moreover, it has been observed that this kind of OC treatment does not induce any significant changes in homocysteine levels [41, 42]. Accordingly, an oral OC treatment containing 30µg Ethinyl*estradiol*+150µg*desogestrel* was administered to the patients in this study.

There are few studies in the literature investigating the impact of PCOS treatment on the homocysteine level. In particular, there is no previous study on the influence of the widely applied OC treatment of PCOS on plasma homocysteine levels. In PCOS, a significant increase has been detected in serum homocysteine levels 27±4 weeks after starting *metformin* treatment [43]. In our own study, we observed a significant drop in the serum homocysteine levels of the subjects using OC. We also detected a statistically significant drop in *HOMA-IR* and fasting serum insulin levels after six months of OC treatment. Nader et al. administered to hyperandrogenic women OC containing desogestrel, low-efficacy androgenic progesterone, for six months. By the end of the treatment period, they did not observe a significant change in serum insulin concentrations but detected a moderate impairment in glucose tolerance [44]. Previous studies have shown that OCs, depending on the kind of progesterone they contain (androgenic progesterone in particular), increase insulin resistance and lead to impairment in glucose tolerance [45]. While no change was observed in our study in the serum insulin and *HOMA-IR* levels of the untreated, monitored PCOS patients after six months, $AUC_{glucose}$ levels were observed to increase. In the patients administered OC for six months, in contrast, *HOMA-IR* levels decreased while $AUC_{glucose}$ levels did not change. A significant decrease was observed on the other hand in serum free testosterone and androstenedione levels after six months of medical treatment. This finding was in agreement with the literature as well [46]. In the untreated, monitored PCOS patients, no significant difference was detected in the homocysteine, *HOMA-IR* and fasting insulin levels after six months.

Among the MTHFR genotypes in PCOS patients treated with 30µg*Ethinyl estradiol*+150µg*Desogestrel* for six months, a reduction was observed in the serum homocysteine and insulin levels and the *HOMA-IR* value in genotype CC. In PCOS patients with genotype CC, a drop was observed in the serum homocysteine level and the *HOMA-IR* value. In PCOS patients with genotype TT, in contrast, no difference was observed in the serum homocysteine and insulin levels and the *HOMA-IR* value before and after treatment. Among the MTHFR genotypes in untreated, monitored PCOS patients, a significant decrease occurred in the homocysteine level in genotype CC. In PCOS patients with genotypes CT and TT, on the other hand, no statistically significant difference was observed. In their study on MTHFR polymorphism and its association with homocysteine levels in PCOS etiopathogenesis, Orio et al. investigated MTHFR C677T polymorphism and homocysteine levels in 70 PCOS patients and 70 healthy controls with low folate intake. They found no difference between the PCOS patients and the controls as regards serum homocysteine levels. In the MTHFR polymorphism, no difference was found in the serum

homocysteine level in genotypes CC and CT, while elevated homocysteine levels were observed in genotype TT. Their study showed that the thermolabile MTHFR enzyme affected serum homocysteine concentrations in only healthy controls with low folate intake [34].

On the other hand, Tanis et al. investigated the links of myocardial infarction with MTHFR C677T mutation, low folate levels, and hyperhomocysteinemia, as well as the influence of OCs on homocysteine levels. In genotype TT, they found a twofold increase in the risk of myocardial infarction in the presence of low folate levels. Moreover, they determined that the use of OCs had no effect on serum homocysteine levels [33]. In conclusion, the MTHFR polymorphism was found in similar ratios among the healthy controls and PCOS patients in this study. Compared with the controls, an increase was observed among the PCOS patients in such cardiovascular risk factors as serum homocysteine, *HOMA-IR* and fasting insulin. The thermolabile MTHFR enzyme caused the homocysteine levels to increase in both the PCOS patients and the healthy controls. In the patients who used 30µg*Ethinyl estradiol*+150µg*Desogestrel* for six months in this study, a reduction was observed in the cardiovascular risk factors of homocysteine, insulin, and *HOMA-IR*. No difference was observed on the other hand in the folate levels. Among the PCOS patients with MTHFR polymorphism, after six months' treatment with 30µg*Ethinyl estradiol*+150µg*Desogestrel*, a drop was observed in the fasting insulin, *HOMA-IR* and serum homocysteine values of the subjects with genotype CC, while a drop in *HOMA-IR* and serum homocysteine values was observed in the subjects with genotype CT. No difference was observed in the subjects with genotype TT after treatment. No change was observed in any of the three groups in the folate levels. In light of these findings, it was concluded that after the use of OC no change occurred in the cardiovascular risk factors of PCOS patients with genotype TT.

Conclusion

In this study, we found the serum homocysteine level to be higher in PCOS patients with a C677T MTHFR mutation of the homozygote genotype compared to the patients with the other genotypes. Nevertheless, a number of replication studies on MTHFR have failed to supply evidence for this association.

There is no previous study on the influence of the widely applied OC treatment of PCOS on plasma homocysteine levels. In our own study, we observed a significant drop in the serum homocysteine levels of the subjects using OC. We also detected a statistically significant drop in *HOMA-IR* and fasting serum insulin levels after six months of OC treatment. In the PCOS patients with MTHFR polymorphism, after six months' treatment with 30µg*Ethinyl estradiol*+150µg*Desogestrel*, a reduction was found in the fasting insulin, *HOMA-IR* and serum homocysteine values of the subjects with genotype CC, while a decrease in *HOMA-IR* and serum homocysteine values was detected in the subjects with genotype CT.

This study showed that the levels of cardiovascular risk factors like homocysteine, insulin, and HOMA-IR dropped in the patients who were administered oral contraceptives for 6 months. New, large and detailed studies are needed on this subject, conducted with a greater number of patients in order to enlighten a new approach.

Limitations of the study:

The present study excluded subjects with the confounding factors for cardiovascular risk generally found in the normal population, so as to examine the independent effects of OC on PCOS and MTHFR Polymorphism. Therefore, the study does not suggest any information about the effects of OC on the patients who have the risk factors of coronary heart disease, and its results are not valid for the population at large.

REFERENCES

1- Karadeniz M, Erdogan M, Zengi A, Eroglu Z, etal. Methylenetetrahydrofolate reductase C677T gene polymorphism in Turkish patients with polycystic ovary syndrome. Endocrine. 2010 Aug;38(1):127-33. DOI: 10.1007/s12020-010-9370-0.

2- Franks S.; Polycystic ovary syndrome: a changing perspective. Clin Endocrinol (Oxf). 1989 Jul;31(1):87120.

3- Talbott E, Clerici A, Berga SL, Kuller L, etal. Adverse lipid and coronary heart disease risk profiles in young women with polycystic ovary syndrome: results of a case-control study. J Clin Epidemiol. 1998 May;51(5):415-422.

4- Fu LY, Dai LM, Li XG, Zhang K, etal. Association of methylenetetrahydrofolate reductase gene C677T polymorphism with polycystic ovary syndrome risk: a systematic review and meta-analysis update. Eur J Obstet Gynecol Reprod Biol. 2014 Jan;(172):56-61. DOI: 10.1016/j.ejogrb.2013.10.001.

5- Toulis KA, Goulis DG, Mintziori G, Kintiraki E, etal. Meta-analysis of cardiovascular disease risk markers in women with polycystic ovary syndrome. Hum Reprod Update. 2011 Nov-Dec;17(6):741-60. DOI: 10.1093/humupd/dmr025.

6- Wild S, Pierpoint T, McKeigue P, Jacobs H. Cardiovascular disease in women with polycystic ovary syndrome at long-term follow-up: a retrospective cohort study. Clin Endocrinol (Oxf). 2000 May;52(5):595-600

7- Clarke R, Daly L, Robinson K, Naughten E, etal. Hyperhomocysteinemia: an independent risk factor for vascular disease. N Engl J Med. 1991 Apr 25;324(17):1149-55.

8- Jacobsen DW. Homocysteine and vitamins in cardiovascular disease. Clin Chem. 1998 Aug;44(8):1833-43.

9- Frosst P, BlomHJ, Milos R, Goyette P, etal. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nat Genet. 1995 May;10(1):111-3.

10- McQuillan BM, Beilby JP, Nidorf M, Thompson PL, etal. Hyperhomocysteinemia but not the C677T mutation of methylenetetrahydrofolate reductase is an independent risk determinant of carotid wall thickening. The Perth Carotid Ultrasound Disease Assessment Study (CUDAS). Circulation. 1999 May 11;99(18):2383-8.

11- Tawakol A, Omland T, Gerhard M, Wu JT, etal. Hyperhomocyst(e)inemia is associated with impaired endothelium-dependent vasodilation in humans. Circulation. 1997 Mar 4;95(5):1119-21. Young IS., Woodside JV., Folate, and homocysteine. CurrOpin Clin NutrMetab Care, 2000 Nov.;3(6):427-432.

12- Tsanadis G, Vartholomatos G, Korkontzelos I, Avgoustatos F, etal. Polycystic ovarian syndrome and thrombophilia. Hum Reprod.,2002, 17(2):314-319.

13- Berek JS, Hershlag A, Peterson CM. Endocrine Disorders Novak Gynecology 13th Edition. USA, Lippincott Williams And Wilkins 2002: 871-930.

14- Ferriman D, Gallwey, JD. 1961. Clinical assessment of body hair growth in women J. Clin. Endocrinol. Metab., (21): 1440-47

15- Matthews DR, Hosker JP, Rudenski AS, Naylor BA, etal. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985 Jul;28(7):412-9.

16- Harris MI, Eastman RC, Cowie CC, Flegal KM, etal. Comparison of diabetes diagnostic categories in the U.S. population according to the 1997 American Diabetes Association and 1980-1985 World Health Organization diagnostic criteria. Diabetes Care. 1997 Dec;20(12):1859-1862.

17- Matthews DR, Hosker JP, Rudenski AS, Naylor BA, etal. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985 Jul;28(7):412-9.

18- Goddijn-Wessel TA, Wouters MG, van de Molen EF, Spuijbroek MD, etal. Hyperhomocysteinemia: a risk factor for placental abruption or infarction. Eur J Obstet Gynecol Reprod Biol. 1996 May;66(1):23-9.

19- Wouters MG, Boers GH, Blom HJ, Trijbels FJ, etal. Hyperhomocysteinemia: a risk factor in women with unexplained recurrent early pregnancy loss. Fertil Steril. 1993 Nov;60(5):820-5.

20- Ożegowska K, Bogacz A, Bartkowiak-Wieczorek J, Seremak-Mrozikiewicz A, etal. Is there an association between the development of metabolic syndrome in PCOS patients and the C677T MTHFR gene polymorphism? Ginekol Pol. 2016;87(4):246-53. DOI: 10.17772/gp/61751.

21- Urbanek M, Legro RS, Driscoll DA, Azziz R, etal. Thirty-seven candidate genes for polycystic ovary syndrome: strongest evidence for linkage is with follistatin. Proc Natl Acad Sci U S A. 1999 Jul 20;96(15):8573-8.

22- Kang SS, Wong PW, Susmano A, Sora J, etal. Thermolabile methylenetetrahydrofolate reductase: an inherited risk factor for coronary artery disease. Am J Hum Genet. 1991 Mar;48(3):536-45.

23- Engbersen AM, Franken DG, Boers GH, Stevens EM, etal. Thermolabile 5,10-methylenetetrahydrofolate reductase as a cause of mild hyperhomocysteinemia. Am J Hum Genet. 1995 Jan;56(1):142-50.

24- Lievers KJ, Boers GH, Verhoef P, den Heijer M, etal. A second common variant in the methylenetetrahydrofolate reductase (MTHFR) gene and its relationship to MTHFR enzyme activity, homocysteine, and cardiovascular disease risk. J Mol Med (Berl). 2001 Sep;79(9):522-28.

25- Kang SS, Zhou J, Wong PW, Kowalisyn J, etal. Intermediate homocysteinemia: a thermolabile variant of methylenetetrahydrofolate reductase. Am J Hum Genet. 1988 Oct;43(4):414-21.

26- Kang SS, Wong PW, Susmano A, Sora J, etal. Thermolabile methylenetetrahydrofolate reductase: an inherited risk factor for coronary artery disease. Am J Hum Genet. 1991 Mar;48(3):536-45.

27- Verhoef P, Kok FJ, Kluijtmans LA, Blom HJ, etal. The 677C-- >T mutation in the methylenetetrahydrofolate reductase gene: associations with plasma total homocysteine levels and risk of coronary atherosclerotic disease. Atherosclerosis. 1997 Jul 11;132(1):105-13.

28- Brattström L, Wilcken DE, Ohrvik J, Brudin L. Common methylenetetrahydrofolate reductase gene mutation leads to hyperhomocysteinemia but not to vascular disease: the result of a meta-analysis. Circulation. 1998 Dec 8;98(23):2520-6.

29- Verhoef P, Stampfer MJ, Buring JE, Gaziano JM, etal. Homocysteine metabolism and risk of myocardial infarction: relation with vitamins B6, B12, and folate. Am J Epidemiol. 1996 May 1;143(9):845-59.

30- Mager A, Lalezari S, Shohat T, Birnbaum Y, etal. Methylenetetrahydrofolate reductase genotypes and early-onset coronary artery disease. Circulation. 1999 Dec 14;100(24):2406-10.

31- Wijeyaratne CN, Nirantharakumar K, Balen AH, Barth JH, etal. Plasma homocysteine in polycystic ovary syndrome: does it correlate with insulin resistance and ethnicity? Clin Endocrinol (Oxf). 2004 May;60(5):560-7.

32- Botto LD, Yang Q. 5,10-Methylenetetrahydrofolate reductase gene variants and congenital anomalies: a HuGE review. Am J Epidemiol. 2000 May 1;151(9):862-77.

33- Tanis BC, Blom HJ, Bloemenkamp DG, van den Bosch MA, etal. Folate, homocysteine levels, methylenetetrahydrofolate reductase (MTHFR) 677C --> T variant, and the risk of myocardial infarction in young women: effect of female hormones on homocysteine levels. J Thromb Haemost. 2004 Jan;2(1):35-41.

34- Orio Jr. F, Palomba S., Di Biase S., Colao A., et al.: Homocysteine levels and C677T Polymorphism of methylenetetrahydrofolate reductase in women with polycystic ovary syndrome. The Journal of Clin. Endocrinol. And Metabolism; 2003; Feb, 88(2);673-679.

35- Choi SW, Gu BH, Ramakrishna S, Park JM, etal. Association between a single nucleotide polymorphism in MTHFR gene and polycystic ovary syndrome. Eur J ObstetGynecolReprod Biol. 2009 Jul;145(1):85-8.

36- Legro RS. Polycystic Ovary Syndrome and Cardiovascular Disease: a premature association? Endocrine Reviews2003;24(3):302-312.

37- Pezzini A, Grassi M, Iacoviello L, Del Zotto E, etal. Inherited thrombophilia and stratification of ischaemic stroke risk among users of oral contraceptives. J Neurol Neurosurg Psychiatry. 2007 Mar;78(3):271-6.

38- Loverro G, Lorusso F, Mei L, Depalo R, etal. The plasma homocysteine levels are increased in polycystic ovary syndrome. Gynecol Obstet Invest. 2002;53(3):157-62.

39- Karadeniz M, Erdogan M, Zengi A, Eroglu Z, etal. Methylenetetrahydrofolate reductase C677T gene polymorphism in Turkish patients with polycystic ovary syndrome. Endocrine. 2010 Aug;38(1):127-33. DOI: 10.1007/s12020-010-9370 0.

40- Speroff L, DeCherney A. Evaluation of a new generation of oral contraceptives. The Advisory Board for the New Progestins. Obstet Gynecol. 1993 Jun;81(6):1034-47.

41- Lussana F, Zighetti ML, Bucciarelli P, Cugno M, etal. Blood levels of homocysteine, folate, vitamin B6 and B12 in women using oral contraceptives compared to non-users. Thromb Res. 2003;112(1-2):37-41.

42- Merki-Feld GS, Imthurn B, Keller PJ. Effects of two oral contraceptives on plasma levels of nitric oxide, homocysteine, and lipid metabolism. Metabolism. 2002 Sep;51(9):1216-1221.

43- Vrbíková J, Bicíková M, Tallová J, Hill M, etal. Homocysteine and steroids levels in metformin treated women with polycystic ovary syndrome. Exp Clin Endocrinol Diabetes. 2002 Apr;110(2):74-6.

44- Nader S, Riad-Gabriel MG, Saad MF. The effect of a desogestrel-containing oral contraceptive on glucose tolerance and leptin concentrations in hyperandrogenic women. J Clin Endoc Met. 1997;82(9):3074-3077.

45- Godsland IF, Crook D, Simpson R, Proudler T, etal. The effects of different formulations of oral contraceptive agents on lipid and carbohydrate metabolism. N Engl J Med. 1990 Nov 15;323(20):1375-81.

46- Mastorakos G, Koliopoulos C, Creatsa G. Androgen and lipid profiles in adolescents with polycystic ovary syndrome who were treated with two forms of combined oral contraceptives. FertilSteril 2002;77(5):919-927.

Table 1. The clinical and laboratory features of the PCOS patients (those treated with 30µg *Ethinylestradiol*+ 150µg *Desogestrel* and those untreated, only monitored) and the controls at the time of their first admission.

	PCOS	*CONTROL*	***P***	*PCOS TREATED*	*PCOS UNTREATED*	*P*
N	50	50		25	25	
Age (year)	22,74 ± 4,93	24,73 ± 4,66	N.S.	23,72 ± 5,62	23,12 ± 5,56	N.S.
BMİ (kg/m2)	27,02 ± 6,83	24,21 ± 3,95*	P=0,013	28,20 ± 7,85	25,87 ± 5,53	N.S.
Waist/Hip Ratio	0,84 ± 0,06	0,76 ± 0,01**	p<0,001	10,48 ± 5,63	10,12 ± 3,85	N.S.
FG Score	10,27 ± 4,70	0,40 ± 1,40**	p<0,001	0,84 ± 0,08	0,85 ± 0,04	N.S.
LH(µıu/ml)	8,21 ± 2,75	5,45 ± 2,19**	p<0,001	4,23 ± 1,20	3,63 ± 1,44	N.S.
FSH (mIU/ml)	3,98 ± 1,37	7,72 ±2,27**	p<0,001	8,32 ± 2,14	7,69 ± 2,74	N.S.
PRL	18,21 ± 7,91	16,72 ± 7,23	N.S.	18,18 ± 8,63	18,25 ± 7,30	N.S.
Androstenedione (ng/ml)	3,45 ± 1,32	2,20 ± 0,64**	p<0,001	3,32 ± 0,94	3,58 ± 1,63	N.S.
Free Testesterone (pg/ml)	8,29± 3,66	3,85 ± 1,40**	p<0,001	6,06 ± 3,42	8,29 ± 3,90	N.S.
DHEAS (µg/dl)	278,39 ± 98,26	202,18 ± 70,04**	p<0,001	263,45 ± 100,13	293,33 ± 96,03	N.S.
D XXI Progesterone	1,83 ± 1,52	20,94 ± 6,40**	p<0,001	2,06 ± 1,52	1,59 ± 1,51	N.S.
Folate (ng/ml)	10,21 ± 2,59	11,27 ± 3,47	N.S.	10,13 ± 2,83	10,30 ± 2,38	N.S.
B_{12} Vitamin (pg/ml)	299,94 ± 133,83	357,70 ± 159,43	N.S.	294,01 ± 132,71	291,06 ± 128,91	N.S.
Homocysteine (µmol/L)	12,84 ± 3,92	7,39 ± 2,16**	p<0,001	13,27 ± 4,50	12,42 ± 3,24	N.S.
Insulin µIU/ml	18,99 ± 8,38	7,79 ± 7,03**	p<0,001	18,01 ± 8,56	19,96 ± 8,23	N.S.
HOMA-IR	4,22 ± 2,24	1,38 ± 0,70**	p<0,001	4,18 ± 2,56	4,26 ± 1,91	N.S.
$AUC_{glucose}$	13881 ± 3702	13363 ± 2214	N.S.	14719 ± 4536	13042 ± 2436	N.S.

* p<0,05 **p<0,001. The data are expressed in the form Mean+SD (Standard Deviation). HOMA-IR: homeostasis model assessment insulin resistance, LH: luteinizing hormone, FSH: follicle-stimulating hormone, DHEAS: dehydroepiandrosteronesulfate, E2: estradiol, BMI: Body mass index, FG: Ferriman–Gallwey,

AUC: area under curve, PRL: prolactin, N.S.: Not Significant (p>0,05). The data are expressed in the form Mean± SD (Standard Deviation)

Table 2: The clinical and laboratory features of the subjects treated with 30µg *Ethinyl estradiol*+150µg *Desogestrel* and the controls at the time of their first application and after 6 months of monitoring

	30µg of Ethinyl estradiol+150µgD esogestrel		*CONTROL GROUP*	
	BEFORE TREATMENT	**AFTER 6 MONTHS OF TREATMENT**	**FIRST APPLICATION**	**AFTER SIX MONTHS**
BMI (kg/m2)	28,20 ± 7,85	26,50 ± 7,26**	25,87 ± 5,53	25,55 ± 5,57
FG Score	10,48 ± 5,63	7,56 ± 5,31**	10,12 ± 3,85	9,15 ±4,16*
Waist/Hip Ratio	0,84 ± 0,08	0,78 ± 0,07**	0,85 ± 0,04	0,82 ± 0,06*
FSH (mIU/ml)	4,22 ± 1,20	6,13 ± 1,49**	3,63 ± 1,44	4,74 ± 2,39*
LH(mIU/ml)	8,32 ± 2,14	6,16 ± 2,79*	7,69 ± 2,74	6,67 ± 2,36
PRL	18,18 ± 8,63	17,48 ± 5,87	18,25 ± 7,30	17,83 ± 5,29
Androstenedione (ng/ml)	3,32 ± 0,94	2,70 ± 0,48*	3,58 ± 1,63	3,42 ± 0,63
Free Testesterone (pg/ml)	3,42 ± 1,16	2,83 ± 0,55*	3,90 ± 1,55	3,35 ± 0,72*
DHEAS (µg/dl)	263,45 ± 100,13	248,33 ± 87,45	293,33 ± 96,03	306,06 ± 77,52
D XXI Progesterone	2,06 ± 1,52	10,31 ± 3,68**	1,59 ± 1,51	4,53 ± 5,32*
Folate (ng/ml)	10,13 ± 2,83	9,49 ± 2,91	10,30 ± 2,38	9,45±2,84
B_{12} Vitamin (pg/ml)	294,01 ± 132,71	254,90 ±78,40	291,06 ± 128,91	279,46 ± 54,99
Homocysteine (µmol/L)	13,27 ± 4,50	10,24 ± 4,54**	12,42± 3,24	12,13 ± 2,95
Insulin (µIU/ml)	18,01 ± 8,56	12,90 ± 6,10*	19,96 ± 8,23	17,05 ± 8,64
HOMA-IR	4,18 ± 2,56	2,48 ±1,50**	4,26 ± 1,91	4,17 ± 1,19
$AUC_{glucose}$	14719 ± 4536	14520 ± 3367	13042 ± 2436	15163 ± 2184**

* p<0,05 **p<0,001. The data are expressed in the form Mean+SD (Standard Deviation).

HOMA-IR: homeostasis model assessment insulin resistance, LH: luteinizing hormone, FSH: follicle-stimulating hormone, DHEAS: dehydroepiandrosteronesulfate, E2: estradiol, BMI: Body mass index, FG: Ferriman–Gallwey, AUC: area under the curve, PRL: prolactin.

Table 3: According to the MTHFR genotype, the *HOMA-IR*, post-OGTT $AUC_{glucose,}$ homocysteine, serum B_{12}vitamin and folate levels of the PCOS patients and controls

	PCOS (N=50)			*CONTROL (N=50)*		
Genotype	**CC**	**CT**	**TT**	**CC**	**CT**	**TT**
Insulin (µIU/ml)	20,90±10,35*	17,85±7,30*	18,85±6,70*	6,98±4,44	8,81±8,72	5,08±2,66
HOMA-IR	4,77±2,70*	3,93±2,00*	3,95±1,81*	1,39±0,84	1,44±0,61	0,97±0,57
$AUC_{glucose}$	13410±4760	13793±2914	15972±3670	13191±1852	13638±2520	12498±622
Homocysteine (µmol/L)	11,86±2,11*	12,22±3,34*	19,66±5,48 *	6,66±1,83	7,26±1,83	10,70±1,86
B_{12} Vitamin (pg/ml)	281,86±128,43	314,2±135,7*	207,5 ± 49,2*	351,9 ±154,0	357,8 ±168,1	378,2 ±161,8
Folate (ng/ml)	10,88±2,86	10,19±2,46	8,07±0,95	11,39±3,15	11,14 ±3,49	11,57±5,09

* p<0,05 **p<0,001. The data are expressed in the form Mean+SD (Standard Deviation).

HOMA-IR: homeostasis model assessment insulin resistance, AUC: area under the curve. The data are expressed in the form Mean□SD (Standard Deviation)

Table 4: According to the MTHFR genotype, the insulin, HOMA-IR, post-OGTT $AUC_{glucose}$, homocysteine, serum B_{12} vitamin and folate levels of the PCOS patients treated with 30µg *Ethinyl estradiol +150µg Desogestrel* on their first admission and six months after treatment.

	The Values of the PCOS Patients treated with 30µg Ethinyl Estradiol *+150µg* Desogestrel *on First Admission (N=25)*			*The Values of the PCOS Patients treated with 30µg* Ethinyl Estradiol *+150µg* Desogestrel *after Six Months of Treatment (N=25)*		
Genotype	**CC**	**CT**	**TT**	**CC**	**CT**	**TT**
Insulin	20,10	15,68	19,21	12,24	11,66	17,96
(µIU/ml)	±	±	±	±	±	±
	7,50	9,84	7,68	5,49 *	6,82	3,35
HOMA-IR	4,69	3,74	4,13	2,82	2,05	2,84
	±	±	±	±	±	±
	2,47	2,92	2,04	1,85 *	1,20 *	1,33
$AUC_{glucose}$	13560	15036	16747	13522	15019	15645
	±	±	±	±	±	±
	5669	3631	3735	5095	1197	1459
Homocysteine	11,21	12,25	21,19	9,24	8,66	17,08
	±	±	±	±	±	±
(µmol/L)	2,01	2,76	4,94	2,41 *	1,97 *	7,73
B_{12} Vitamin	325,69	300,89	195,85	244,82	267,20	246,32
(pg/ml)	±	±	±	±	±	±
	151,45	126,57	48,17	58,13	104,07	44,13
Folate	11,18	9,97	7,94	10,28	9,53	7,37
(ng/ml)	±	±	±	±	±	±
	3,55	2,12	1,05	3,13	2,42	1,67

* p<0,05 **p<0,001. The data are expressed in the form Mean+SD (Standard Deviation).

HOMA-IR: homeostasis model assessment insulin resistance, AUC: area under the curve. The data are expressed in the form Mean±SD (Standard Deviation)