DOI: 10.17772/gp/61751 Ginekol Pol. 2016, 87, 246-253

PRACE ORYGINALNE

ginekologia

Is there an association between the development of metabolic syndrome in PCOS patients and the C677T MTHFR gene polymorphism?

Czy istnieje związek między rozwojem zespołu metabolicznego u kobiet z zespołem policystycznych jajników, a polimorfizmem C677T genu reduktazy metylenotetrahydrofolianowej?

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Abstract

Introduction: *Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women of reproductive age. PCOS is characterized by anovulation, polycystic ovaries, hyperandrogenism leading to infertility, dermatological and psychological problems, as well as the risk of developing Metabolic Syndrome (MetS) and cardiovascular disease (CVD). The exact cause of PCOS remains unclear. Various biochemical and genetic markers have been implicated in predisposition to PCOS, but no single variant has been associated with the syndrome. Some authors connect hyperhomocysteinemia (HHcy) with MetS and its components. The MTHFR gene C677T polymorphism is a common genetic abnormality leading to hyperhomocysteinemia.*

Objectives: *The aim of the study was to confirm the existence of a possible correlation between metabolic disturbances in PCOS and the MTHFR C677T polymorphism.*

Material and methods: *A total of 98 patients diagnosed with PCOS according to the Rotterdam criteria and 101 age-matched healthy controls were included in the study. Genotyping of MTHFR C677T was performed by the real time PCR method.*

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Otrzymano: **28.12.2015**
Zaakceptowano do druku: **10.02.2015**

Results: *Statistically significant differences were observed between those two groups with regard to body mass index (BMI), waist circumference (WC), hip circumference (HC), fasting insulin, total cholesterol (TC), and triglycerides (TG). No significant differences in the prevalence of the genotypes of the MTHFR C677T gene polymorphism were found between the PCOS group and controls. Despite the lack of significant differences, we observed a tendency for a higher prevalence of the TT genotype in the PCOS group (p=0.06). No statistically significant differences were observed between the PCOS group and the control group in terms of the presence of the MetS components and the predisposition to develop MetS.*

Conclusions: *Our study did not confirm an association between the MTHFR C677T gene polymorphism and the development of MetS in PCOS. Further studies with larger sample size might be useful to determine this association.*

Key words: **metabolic syndrome** / **gene polimorphism** / **polycystic ovary syndrome** / / **metylenetetrahydrofolate reductase** /

Streszczenie

Wstęp: *Zespól policystycznych jajników (PCOS) jest najczęstszym zaburzeniem endokrynologicznym u kobiet w wieku rozrodczym, charakteryzującym się brakiem owulacji, jajnikami policystycznymi oraz hiperandrogenizmem, które prowadzą do niepłodności, problemów dermatologicznych i psychologicznych oraz zwiększają ryzyko rozwoju zespołu metabolicznego i choroby sercowo-naczyniowej. Przebadano wiele biochemicznych i genetycznych markerów, które mogłyby mieć wpływ na rozwój PCOS, jednak jak do tej pory nie udało się wskazać jednego pewnego czynnika. Istnieją badania łączące hiperhomocysteinemię z zespołem metabolicznym i jego składowymi. Częstym zaburzeniem genetycznym prowadzącym do hiperhomocysteinemi jest polimorfizm C677T genu reduktazy metylenotetrahydrofolianowej (MTHFR).*

Cel: *Celem badania było zweryfikowanie związku zaburzeń metabolicznych w zespole policystycznych jajników z polimorfizmem genu C677T MTHFR.*

Materiał i metody: *98 pacjentek ze zdiagnozowanym PCOS na podstawie kryteriów Rotterdamskich zostało porównane ze 101 pacjentkami z grupy kontrolnej dobranej zgodnie wiekowo. Metoda real-time PCR została użyta do wykonania oznaczeń genetycznych.*

Wyniki: *Istotne statystycznie różnice między grupami zauważono w Body Mass Index (BMI), obwodzie talii, obwodzie bioder, poziomie insuliny na czczo, cholesterolu całkowitego, trójglicerydów. Nie zauważono istotnych statystycznie różnic w występowaniu genotypów polimorfizmu C677T genu MTHFR między grupą badaną, a kontrolną. Zaobserwowano jedynie tendencję do częstszego występowania genotypu TT w grupie kobiet z PCOS (p=0,06). Nie zauważono różnic statystycznych między częstością występowania składowych zespołu metabolicznego oraz w rozwoju pełnoobjawowego zespołu metabolicznego między tymi grupami.*

Wnioski: *Nasze badanie nie potwierdziło związku polimorfizmu genu C677T MTHFR z rozwojem zespołu metabolicznego w PCOS. Dalsze badania na większej grupie pacjentek mogłyby być pomocne w ocenie tego związku.*

Słowa kluczowe: **poliformizm genetyczny** / **zespól metaboliczny** / **zespół policystycznych jajników** / **redukcja metylenotetrahydrofolianowa** /

Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women of reproductive age [1, 2]. Currently, insulin resistance and abdominal obesity appear to be key factors in the pathogenesis of PCOS [3-4]. This group of patients is thus more likely to develop components of Metabolic Syndrome (MetS) such as hypertension, dyslipidemia, obesity and type 2 diabetes mellitus (DM) leading to premature cardiovascular disease (CVD) [5–8]. The exact cause of PCOS remains unclear and it is believed to be multifactorial – a combination of genetic background with the environmental factors and an individual capacity, leading to a spectrum of reproductive and metabolic disorders presented in PCOS, however no single variant has conclusively and repeatedly been associated with the syndrome [9-12]. MetS is defined as a cluster of metabolic disturbances, including central obesity, dyslipidemia, elevated blood pressure (BP) and high glucose concentrations [13]. MetS itself and its components are associated with increased risk of DM and CVD, as well as with higher mortality rate[14,15]. The reported prevalence of metabolic syndrome in women with PCOS varies depending on the criteria used to define both, PCOS and metabolic syndrome, and ranges from 30% to 47% [16–20].

Homocysteine (Hcy) is a sulfur-containing amino acid derived from methionine metabolism [21]. There are studies connecting hyperhomocysteinemia (HHcy) to MetS and its components, because it has been associated with hyperinsulinemia and insulin resistance in a number of studies [22–29]. The most common cause of hyperhomocysteinemia is reduced activity of methylenetetrahydrofolate reductase (MTHFR) - an enzyme responsible for the folate-dependent re-methylation of homocysteine to methionine [30-31]. The deficiency in MTHFR activity can lead to impaired DNA methylation [32, 33] and

DOI: 10.17772/gp/61751

activation of repair mechanism that may result in chromosomal breakage, decreased nitric oxide formation, elevated production of reactive oxygen species, and the production of proinflammatory cytokines [34-39]. Hyperhomocysteinemia may be involved in oocyte maturation, ovulation, proliferation, and differentiation of granulosa cells, as well as steroidogenesis [36, 37]. The above stated facts may lead to the connection of MTHFR C677T gene polymorphism and metabolic disturbances in PCOS women. This association has been investigated in various populations and from different angles, with both positive and negative results [38–42].

Objectives

The aim of the study was to investigate a possible correlation between metabolic disturbances in PCOS patients and the MTHFR C677T polymorphism.

1. Material and methods

1.1. Study subjects

The patients were recruited from the Department of Infertility and Reproductive Medicine, Poznan University of Medical Sciences, between July 2012 and December 2013. We studied 164 patients with PCOS and 108 age-matched healthy controls.

Inclusion criteria for selection of PCOS cases and controls:

The diagnosis of PCOS was confirmed according to the Rotterdam consensus criteria [43].

All patients with diabetes mellitus, hypertension, hyperprolactinemia, thyroid disorders, Cushing's syndrome, acromegaly, premature ovarian failure, virilising adrenal or ovarian tumors, and oral contraceptive pill use within the last 6 months were excluded from the study. Other endocrinopathies and associated disorders were excluded by measuring basal prolactin, thyroid stimulating hormone, and 17-hydroxyprogesterone levels. Hyperandrogenism was identified based on the presence of hirsutism as reflected by a Ferriman-Gallwey score (FG) [44] and/or presence of acne and/or elevated androgen levels.

Controls were defined as healthy age-matched women, free of menstrual cycle irregularities, clinical or biochemical hyperandrogenism, lack of medication intake and PCOS symptoms on ultrasound, with no history of endocrine or autoimmune disorders, and surgery in the pelvic region.

1.2. Patient evaluation

Medical and family history were investigated for all patients, and clinical examination was performed, which included the measurement of the body weight, height, waist circumference (WC) at the midpoint between the lateral iliac crest and the lowest rib margin at the end of normal expiration, waist to hip ratio (WHR), and hip circumference (HC) measured at the widest level of the greater trochanters. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters (kg/m^2). According to the World Health Organization categories, overweight was defined as BMI between 25.0 and 29.9 kg/m^2, and obesity was defined as BMI of ≥30.0 kg/m^2 [45]. All the patients enrolled in the study were evaluated during the early follicular phase of the menstrual cycle (days 3–5) after antidiabetic and contraceptive drugs had been discontinued for at least 6 months.

1.3. Biochemical and hormonal analysis

Biochemical parameters were measured at the Central Laboratory of the University Hospital, which is a certified facility meeting the criteria of ISO 9001. Blood samples for biochemical and hormonal analysis were drawn from the antecubital vein between 8 and 10 AM after a 12-hour overnight fast. Samples which were not analyzed on the same day were centrifuged and the plasma was aliquoted and stored in -70ºC until assayed.

The 75-g oral glucose tolerance test (OGTT) was performed in all patients to measure glucose and insulin levels. Blood samples were obtained at baseline and at 30-min. intervals for 2 hours. Glucose level in venous blood was determined by means of the enzymatic (heksokinase) method with Roche Diagnostics laboratory reagents on a Hitachi 912 analyzer. Insulin level was measured with the AxSYM Insulin microparticle immunodiagnostic test (Microparticle Enzyme Immunoassay, MEIA) from Abbott.

Total serum cholesterol, HDL cholesterol, and triglycerides (TG) levels were measured with appropriate Roche Diagnostics reagents (Cholesterol CHOD-PAP, HDL-C plus, and Triglycerides GPO-PAP, respectively) on a Hitachi 912 analyzer, and LDL cholesterol level was calculated using the following formula: LDL cholesterol = total cholesterol – HDL cholesterol – TG/5. The following definitions of normal levels were used: total cholesterol, 50.0–200.0 mg/dL; HDL cholesterol, 35.0–70.0 mg/dL; triglycerides, 50.0–150.0 mg/dL; and LDL cholesterol, 35.0–130.0 mg/dL.

1.4. Molecular analysis

Genotyping of *MTHFR C677T* (*rs*1801133) and MTHFR A1298C (rs1801131) was performed by real time PCR method with the use of LightCycler® 480 system. DNA was isolated from white blood cells using QIAamp Blood Mini Kit (QIAGEN, USA). Analysis of *rs*1801133 and rs1801131 polymorphisms of the MTHFR gene was determined using HybProbe probes (TiBMolbiol, Poland). Genotyping of MTHFR polymorphisms was based on melting curve analysis. LightSNiP set contained proper concentration of starters and probes specific for the amplified fragment. Preparation of the LightSNiP set used for real-time PCR was performed according to the manufacturer's instructions. PCR cycling reactions consisted of initial denaturation at 95°C (5 min), and 40 cycles with denaturation (15 s at 95°C), annealing (15 s, 60°C), and extension (15 s at 72°C). Each 96-well plate contained a mixture of case and negative control DNA samples. The molecular analysis was performed at the Laboratory of Experimental Pharmacogenetics, Department of Clinical Pharmacy and Biopharmacy, Poznan University of Medical Sciences, Poland.

1.5. Statistical Analysis

All statistical analyses were performed using the Statistica software. The distribution of the variables was investigated using the Shapiro-Wilk test and nonparametric tests. In particular, the Mann-Whitney U test and the Kruskal-Wallis test were used for non-normal distributions. Continuous variables are expressed as medians (interquartile range (IQR), 25th–75th percentile), unless otherwise indicated. Between-group differences and differences among genotype groups were assessed by the Mann-Whitney U test. Chi-square analysis was used to compare the distribution of genotypes and alleles for the MTHFR C677T gene polymorphism

Table I. Demographic, anthropometric and biochemical parameters of the studied groups (PCOS cases and controls).

Parameter	PCOS	Control	P value
Age (yrs)	27.2 ±4.5	28.1± 3,5	0.08
BMI (kg/m^2)	23.7±5.4	21.7±2,8	0.00001*
WC (cm)	80.7±12.8	71.6±12.8	0.00001*
HC (cm)	101.1±11.3	97.5±7.4	0.02*
WHR	0.79±0.1	0.73±0.1	0.06
Fasting glucose (mg/dl)	88.3±9.9	89.58±9.8	0.13
Fasting insulin (μU/ml)	9.4±7.8	6.5±3.3	0.00001*
TC(mg/dl)	189.2±32.5	169.7±16.0	0.00005*
TG (mg/dl)	89.8±52.5	87.9±15.5	0.00015*
LDL-C (mg/dl)	86.7±13.9	79.4±9.5	0.43
HDL-C (mg/dl)	62.4±17.8	59.2±6.6	0.44
Chol/HDL	3.2±1.1	3.2±0.8	0.86
SBP	114.0±15.0	112.0±10.2	0.2
DBP	70.2±11.1	71.3±8.9	0.34

Abbreviations:
BMI – body mass index; WC – waist circumference; HC – hip circumference; WHR – waist to hip ratio; TC – total cholesterol; TG – triglycerides; LDL-C – low density lipoprotein cholesterol; HDL-C – high density lipoprotein cholesterol; VAI – visceral adiposity index; SBP – systolic blood pressure; DBP – diastolic blood pressure
** P<0,05 was considered statistically significant +Mann Whitney U test was used*

between the groups and to assess between-group differences with respect to allelic and genotypic frequencies. Unconditional logistic regression analysis was performed to estimate the effects of the MTHFR C677T gene polymorphisms. The OR with 95% CI was calculated to estimate the risk of the different genotypes and alleles. For all analyses, a two-tailed p value of <0.05 was considered as statistically significant.

Local Ethics Committee approved of the study. Written consent was obtained from all the subjects enrolled in this study.

2. Results

2.1. Demographic and clinical characteristics of study subjects

Demographic, biochemical and clinical parameters of the PCOS patients and controls are summarized in Table I. Age distribution was not significantly different between the two groups. Significant differences were observed between those two groups in body mass index (BMI), waist circumference (WC), hip circumference (HC), fasting insulin, total cholesterol (TC) and triglycerides (TG) concentrations (TG).

We also investigated whether there was a difference between the genotype distribution and the presence of metabolic syndrome components, but no statistically significant difference was found (Table II).

2.2. Genotypic and allelic frequencies

Genotypic and allelic frequencies for the MTHFR C677T gene polymorphisms are presented in Table III. No significant differences in the prevalence of the MTHFR C677T gene polymorphism between PCOS group and controls were found. Despite the lack of significant differences, we did observe the tendency for a higher prevalence of the TT genotype in the PCOS group (p=0.06), which in some studies was claimed to be associated with the development of metabolic and cardiovascular disturbances.

2.3. Association between C667T gene polymorphism and metabolic syndrome

There were only 3 patients in the PCOS group and 1 in the control group who presented MetS according to the IDF criteria. No statistically significant differences were observed between the PCOS group and control group in the genetic predisposition to develop MetS.

2.4. Association between the C667T gene polymorphism with individual components of metabolic syndrome

The associations of individual components of MetS with the MTHFR C677T gene polymorphism were also investigated. Multivariate logistic regression analysis showed no significant association between the MTHFR C677T gene polymorphism and any of the MetS components (Table IV).

Also, we found no positive association between the presence of more MetS components and the MTHFR C677T polymorphism (Table V), but a tendency for the occurrence of the TT genotype and the T allele and the bigger number of MetS components was observed.

Discussion

Apart from hormonal disturbances, PCOS patients frequently present features of the metabolic syndrome, including insulin resistance, obesity, and dyslipidemia, suggesting an increased risk

DOI: 10.17772/gp/61751

Table II. Patients with metabolic parameters fullfilling IDF MetS criteria

Parameter	IDF criteria	PCOS N=164	Controls N=108	p
NO		50	27	<0,09
↑BP	≥130 or ≥80 mmHg	63	42	=0,47
↑WC	≥80 mg/dl	82	17	<0,00001
↑↑BMI	>25 kg/m^2	82	12	<0,00001
↑TG	≥150 mg/dl	8	0	<0,02
↓↓HDL-C	<50 mg/dl	39	38	=0,4
↑FG	≥100 mg/dl	15	11	=0,8

Abbreviations: *BMI – body mass index; WC – waist circumference; WHR – waist to hip ratio; TC – total cholesterol; TG – triglycerides; HDL-C – high density lipoprotein cholesterol; BP – blood pressure; * P<0,05 was considered statistically significant Chi^2*

for cardiovascular disease. Contemporary research is focused on the emerging epidemic of type 2 diabetes and obesity. Thus, more energy is being directed toward their earlier detection, improved therapy, and potential prevention in PCOS patients as a potential risk group for these disturbances.

Multiple genetic and environmental factors influence the development of MetS [14]. Data on the connection between MetS, together with its components, and hyperhomocysteinemia (HHcy), continue to accumulate [22, 23]. The link between those two disturbances remains unclear but numerous authors suggest that endothelial dysfunction may promote the development of insulin resistance and affect DNA methylation [46–48]. The MTHFR C677T polymorphism has been known to be one of the most important genetic risk factors leading to HHcy [49, 50]. The C677T polymorphism of the MTHFR gene causes a decreased activity of the MTHFR enzyme, resulting in HHcy, which is associated with hyperlipidemia, hypertension, obesity, and diabetic components of MetS [30, 33, 57]. Choi et al., indicated that studies on the genetic association between the MTHFR gene polymorphism and PCOS may provide insights into the role of the MTHFR gene in the pathological milieu of PCOS and, consequently, change the diagnostic approach [53]. To the best of our knowledge, our study has been the first to investigate the possible relationships of the MTHFR C677T polymorphisms with MetS among the Polish population.

In our study, we found no significant correlation between the presence of any of the MTHFR gene polymorphism genotypes and the incidence of PCOS. Regardless, we observed a tendency for a higher percentage of recessive TT genotype in the PCOS population (p=0.07). The same results were confirmed by Karadeniz et al. [54] and Tsanadis et al. [55]. The study of Madhu et al. [35], showed a relative risk of 1.32 for the presence of the CT genotype in women with PCOS. In our study, the risk was defined at 0.75 for the CT genotype in the PCOS group. In the study of Yang et al., the 677TT genotype was associated with a significant correlation with MetS components such as high FBG, high WC. Generally, those patients had more MetS components than patients with other genotypes [46,56]. It is generally believed that the TT genotype may place individuals at greater risk for insulin resistance and central adiposity. Thus, higher levels of insulin resistance pose a higher threat of developing diabetes mellitus, metabolic syndrome, and endothelial dysfunction, which are significant risk factors for the development of CVD. As stated previously, the relationship between MTHFR and cardiovascular disease is not unique to patients with PCOS, but within this investigation, further studies with sufficiently large sample size should evaluate whether the risk associated with the T allele is higher than expected in the general population.

The C677T gene polymorphism not only did not increase the risk of PCOS in our study, but also we found no relationship between the MTHFR gene polymorphism and MetS. The same results were presented by Yang et al. [46]. On the other hand, the study of Vasilopoulos et al., revealed a 4.02-fold higher risk of MetS among the PCOS population [57].

The association between the MTHFR C677T gene polymorphism and MetS was also confirmed in the study of Kim et al. [58] and Elligrond et al. [56]. As those studies were conducted in different populations, different factors have confirmed the differences among their results, including different dietary and lifestyle habits of the studied populations, mean patient age, as well as various specific diseases examined in the studies and different associations between the environmental effect on gene expression (epigenetic).

The analysis of the individual components of MetS and MTHFR polymorphisms did not reveal any significant differences which would allow to use them as genetic predictors of MetS. Contrary to our findings, the study of Yang et al. [46] exhibited a significant association of the TT genotype with high fasting blood glucose (FBG), which is also consistent with the results of previous studies [57, 59]. These differences may have occurred

Table III. Genotype distribution and allele frequency of MTHFR C677T polymorphism in the studied population (PCOS women and Control Group).

Polymorphism	PCOS (N=164) (%)*	Control (N=108) (%)	OR (95% CI)	P value
CC	87 (51.8)	53(53.5)	0.93 (0.5668 - 1.5332)	0.7822
CT	52 (30,9)	37(37.4)	0.75 (0.4456-1.2664)	0.2829
TT	**29 (17.3)**	**9 (9.1)**	**2.09 (0.9436- 4.6131)**	**0.0693**
C	226 (67.3)	143 (72.2)	0.93 (0.7086-1.2240)	0.6099
T	110 (32.7)	55(27.8)	1.18 (0.8155-1.7033)	0.3818

** Values are given as number (%)*

Table IV. Association of MTHFR C677T gene polymorphism with single component of MetS.

Genetic Model	High WC*	High BP	High FBG	High TG	Low HDL-C
Homozygous codominant (TT/CC)	1.0 (0.15-6.53)	0.33 (0.02-7.14)	0.8 (0.04-17.2)	1.0 (0.01-255.6)	9.5 (0.83-109.2)
Heterozygous codominant (CT/CC)	1.67 (0.29-9.45)	0.04 (0.01-0.62)	0.4 (0.05-3.42)	3.0 (0.09-473.1)	1.22 (0.31-4.84)
Dominant (TT+CT/CC)	1.33 (0.32-5.64)	0.09 (0.01-1.00)	0.5 (0.07-3.35)	3.0 (0.09-473.1)	1.81 (0.51-6.4)
Recessive (TT/ CT+CC)	0.85 (0.14-5.07)	1.64 (0.14-19.4)	1.14 (0.06-21.9)	0.33 (0.01-52.6)	8.73 (0.82-92.9)
Allelic (T/C)	1.11 (0.38-3.25)	0.4 (0.11-1.51)	0.67 (0.15-2.98)	1.0 (0.01-92.4)	2.20 (0.85-5.70)

Abbreviations: *MTHFR – methylenetetrahydrofolate reductase; MTRR – methionine synthase reductase; OR – odds ratio; CI – confidence interval; BP – blood pressure; FBG – fasting blood pressure; TG – triglycerides; HDL-C – high density lipoprotein cholesterol; WC – waist circumference. Chi² test was used.*

Table. V. MTHFR C677T polymorphisms and the number of MetS components.

Genetic Model	1 Components	≥2 Components
Homozygous codominant (TT/CC)	0.7 (0.1-4.2)	1.3 (0.5-3.3)
Heterozygous codominant (CT/CC)	1.2 (0.4-3.5)	0.7 (0.4-1.4)
Dominant (TT+CT / CC)	1.1 (0.4-3.0)	0.8 (0.5-1.5)
Recessive (TT/ CT+CC)	0.6 (0.1-3.6)	1.5 (0.6-3.6)
Allelic (T/C)	0.9 (0.4-2.1)	0.9 (0.6-1.3)

Chi² test was used.

due to the relatively young age of our studied group, but they manifested already a significantly higher fasting insulin level, one of the predictive factors of at least prediabetes.

Contrary to the study of Yang et al. [46], and Vasilopoulos et al. [57], we found no correlation between high WC and the MTHFR C677T gene. Our results are consistent with the study of Teruzzi et al. [60]. In light of the discrepancies and the conflicting reports, further studies with larger sample size are needed.

The study of Jain et al. [35], demonstrated an increased risk of hyperlipidemia in women with PCOS and CT genotype of the MTHFR C677T polymorphism, probably leading to the development of CAD later in life. Our study showed that, regardless of the fact that PCOS patients presented higher TC and TG concentrations, no association between any of the genotypes and elevated risk of hyperlipidemia in the PCOS group was found. No association was also found between the studied polymorphisms and the number of MetS components.

More long-term, prospective case-control studies on a larger group of patients need to be carried out in the Polish population to seek correlation between MTHFR C677T polymorphism and PCOS, as well as the clinical and biochemical findings of PCOS to further verify our results.

DOI: 10.17772/gp/61751

Our study is not without limitations, mainly a relatively small study population and the fact that it included residents of the same city, which may not fully represent the general population. We did not evaluate environmental, dietary, and behavioral factors in this study, which might also play a role in the development of those disturbances Also, the gene-environment interactions could not be fully investigated.

The strengths of the study include comparable patient age and sample size between study and control groups. To the best of our knowledge, this has been the first study in the Polish population to evaluate the relationship between MetS in PCOS and the MTHFR C677T polymorphism. Summarizing, we confirm that young PCOS women tend to have more abnormal anthropometric and biochemical parameters, predisposing them to the development of metabolic syndrome. The MTHFR C677T gene polymorphism seems to not elevate the risk of the development of metabolic disturbances in the PCOS population.

Conclusions

1. Our research did not detect any significant associations between the C677T MTHFR gene polymorphism and the presence of MetS components in the group of PCOS women.
2. A more frequent occurrence of the TT genotype in the PCOS group indicates that research on bigger groups of patients might evaluate the impact of the TT genotype on the development of PCOS.
3. Our study population was relatively young, so research on older groups might generate conflicting results.

Oświadczenie autorów:

1. Katarzyna Ożegowska – autor koncepcji i założeń pracy, zebranie materiału, analiza staytystyczna wyników, opracowanie wyników badań, przechowywanie dokumentacji, przygotowanie manuskryptu i piśmiennictwa, uzyskanie funduszy na realizację badań laboratoryjnych – autor zgłaszający i odpowiedzialny za manuskrypt.
2. Agnieszka Seremak-Mrozikiewicz – pomoc przy koncepcji manuskryptu, korekta manuskryptu.
3. Anna Bogacz – wykonanie oznaczeń laboratoryjnych, przygotowanie części manuskryptu dotyczącej opisu metody.
4. Joanna Bartkowiak-Wieczorek - wykonanie oznaczeń laboratoryjnych, przygotowanie części manuskryptu dotyczącej opisu metody.
5. Leszek Pawelczyk – współautor protokołu, korekta i aktualizacja literatury, korekta i akceptacja ostatecznego kształtu manuskryptu.

Źródło finansowania:

Grant UMP w Poznaniu dla Młodych Naukowców nr: 502-14-01110139-10200.

Konflikt interesów:

Autorzy nie zgłaszają konfliktu interesów oraz nie otrzymali żadnego wynagrodzenia związanego z powstawaniem pracy.

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