ORIGINAL ARTICLE

UDC: 618.11:616-008.9]:616.12-037
https://doi.org/10.2298/VSP170504158B

Association of vascular and inflammatory markers with metabolic disorders in women with polycystic ovary syndrome

Udruženost vaskularnih i inflamatornih markera metaboličkih poremećaja kod žena sa sindromom policističnih jajnika

Aleksandra Atanasova Boshku*, Daniela Ivanova Panova*, Beti Zafirova Ivanovska†

Ss Cyril and Methodius University of Skopje, Faculty of Medicine, *University Clinic of Obstetrics and Gynecology, †Institute of Epidemiology and Biostatistics with Medical Informatics, Skopje, Republic of North Macedonia

Abstract

Background/Aim. The prevalence of metabolic disorders, obesity and insulin resistance in women with polycystic ovary syndrome (PCOS) occur early in life and places this group at risk of cardiovascular disease. Hyperhomocysteinemia and increased C-reactive protein (CRP) activity have an effect on promoting atherosclerosis. This study was designed to evaluate whether high sensitivity (hs-CRP) and homocysteine (Hcy) are elevated in PCOS and to elucidate their possible relation to obesity, insulin resistance, or metabolic changes usually present in women suffering from PCOS. **Methods.** Serum concentration of hs-CRP and plasma levels of Hcy were evaluated in 73 PCOS women and 43 healthy women, together with clinical, anthropometric and hormonal parameters. **Results.** The mean of body mass index (BMI), waist circumference (WC), waist to hip ratio and mean concentration of luteinizing hormone (LH), testosterone, androstenedione, free androgen index, fasting insulin, homeostatic model assessment of insulin resistance (HOMA- IR), hs-CRP and Hcy were significantly higher in PCOS women compared to age-matched healthy women. There was a positive correlation between hs-CRP and BMI, WC, insulin, triglycerides ($p < 0.001$) and significant negative correlation with LH, sex hormone binding protein (SHGB), HOMA-IR, high density lipoprotein cholesterol (HDL-C) ($p < 0.001$). The Hcy concentration had a significant negative correlation with HDL-C level ($p < 0.05$). The present study demonstrated increased mean concentration of Hcy in hs-CRP women with PCOS. **Conclusion.** Our results support the use of these biomarkers in the evaluation of potential risk for cardiovascular diseases and early prognosis and treatment implications.

Key words:
polycystic ovary syndrome; cardiovascular diseases; risk factors; homocysteine; c-reactive protein.

Apstrakt

Uvod/Cilj. Prevalencija metaboličkih poremećaja, gojaznosti i insulinske rezistencije kod žena sa sindromom policističnih jajnika (PCOS) prisutna je u ranim fazama života i postavlja ovu grupu u rizik za rani razvoj kardiovaskularnih bolesti. Hiperhomocisteinemija i povecana aktivnost C-reaktivnog protein (CRP) imaju uticaj na promociju ateroskleroze. Cilj ove studije je bio da se proceni da li su vrednosti visoko senzitivnog (hs-CRP) i homocisteina (Hcy) povišene kod PCOS i da se razjasne njihove moguće veze sa gojaznošću, insulinskom rezistencijom i metaboličkim promenama. **Metode.** Serumski nivoi hs-CRP i plazma nivoi Hcy su bili analizirani kod 73 žene sa PCOS i 43 zdrave žene, zajedno sa kliničkim, antropometrijskim i hormonskim parametrima. **Rezultati.** Srednje vrednosti indeksa telesne mase (BMI), obima struka, odnosa kukova i struka kao i koncentracija luteinizirajuceg hormona, testosterona, androstenediona, indeksa slobodnog androgena, insulina na gladno, homeostatskog modela za procenu insulinske rezistencije (HOMA-IR), i Hcy su bili značajno povećane kod žena sa PCOS u poređenju sa zdravim ženama istih godina. Ustanovljena je pozitivna korelacija između hs-CRP i BMI, holesterola, insulina, triglicerida ($p < 0,001$) i značajna negativna korelacija sa luteinirajućim hormonom (LH), vezujućim globulinom polnih hormona (SHGB), HOMA-IR, HDL-C ($p < 0,001$). Koncentracija Hcy imala je značajnu negativnu korelaciju sa HDL-C ($p < 0,05$). **Zaključak.** Istraživanjem je ustanovljena povećana srednja koncentracija Hcy i hs-CRP kod žena sa PCOS. Naši rezultati ukazuju na opravdanost upotrebe ovih biomarkera u proceni potencijalnog rizika za kardiovaskularne bolesti i rane prognoze i implikacija lečenja.

Ključne reči:
jajnik, policistični, sindrom; kardiovaskularne bolesti; faktori rizika; homocistein; c-reaktivni protein.

Correspondence to: Aleksandra Atanasova Boshku, University Clinic of Obstetrics and Gynecology, Goce Delčeva 9, Skopje, Republic of North Macedonia. E-mail: aleksandraatanasova@yahoo.com

Introduction

One of the most frequent condition frequently seen in women of reproductive age is the polycystic ovary syndrome (PCOS). This condition affects 12%–19% of the female population and this usually depends on ethnicity and criteria used for diagnosing PCOS [1, 2]. The diagnosis for PCOS is defined by the Rotterdam classification from 2003 where at least 2 out of 3 criteria must be present: oligo and/or anovulation, clinical and/or biochemical hyperandrogenism and polycystic ovaries on ultrasound [3]. This heterogeneous endocrine disorder is presented by different clinical sub-phenotypes and the phenotype prevalence generally depends whether it is diagnosed in the tertiary health care setting or detected randomly in the unselected population. This may be due to several reasons like race/ethnicity predominance, severity of clinical manifestation, access to medical care, degree of obesity and severity of hirsutism, which are the complaints most likely to affect quality of life and reason to seek medical assistance [4]. Apart from the well-known characteristics of polycystic ovary syndrome, these women are facing many other endocrine disturbances including fertility problems, insulin resistance (IR), impaired glucose tolerance, hyperinsulinemia, dyslipidemia and assemblage of metabolic syndrome profile. Data showed an increased diabetes risk, hypertension all of which are cardiovascular risk factors [5, 6]. These risks can be aggravated by the PCOS management strategies and by the presence of high body mass index (BMI). Some important therapies, like taking oral contraceptive pill (OCP) affecting the reproductive features such as menstrual cycle regulation and hirsutism management, may also increase cardiovascular risk [7, 8]. Obesity and excess weight are major chronic diseases in Western world countries. In general, the obesity and insulin resistance increase type 2 diabetes (T2DM) and cardiovascular disease (CVD). Similarly, in the PCOS obesity stimulates IR and promotes reproductive and metabolic disarrangements [9]. The metabolic features, combined with excess weight often seen in PCOS, make these young women a high-risk group. Chronic inflammation may be one of the factors contributing to obesity and obesity-related disorders, including atherosclerosis and endothelial dysfunction, diabetes, and steatosis [10]. These changes are present at the adipose tissue level but also liver, muscles and macrophages usually because of altered homeostasis of inflammatory cytokines. Adipose tissue, is a potent endocrine organ which synthetizes and releases cytokines, acute-phase proteins, and inflammatory mediators. These molecules have paracrine, autocrine or systemic function influencing glucose metabolism, energy balance, proinflammatory and anti-inflammatory activities [11]. Serum markers of low-grade chronic inflammation are being increasingly recognised as predictors of cardiovascular disease over the past years. Homocysteine (Hcy), a thiol-containing amino acid, is produced by the intracellular demethylation of methionine. Total Hcy (tHcy) represents the sum of all forms of Hcy including oxidised, protein-bound and free Hcy. Accumulation of Hcy is usually seen as a result of the defect in enzymatic pathway [12]. Several studies indicate that non-enzymatic factors also can influence Hcy levels including age, gender, nutrition and smoking [13]. Wald et al. [14] found a significant associations between Hcy concentration and the risk of ischemic heart disease and deep vein thrombosis. According to the authors, the results of the meta-analysis provide strong evidence for a causal relationship between elevated blood Hcy and cardiovascular disease. High sensitive C-reacitve protein (Hs-CRP) is an acute phase protein produced by the liver, directly secreted by adipose tissue, but also there are suggestions that it is produced in the atherosclerotic lesion, smooth muscle cells and macrophages [15].

There was an increased interest of use of Hcy and hs-CRP as early markers for early subclinical inflammation and atherosclerosis to improve a risk stratification in the asymptomatic individuals [13, 15].

The present study aimed to evaluate the relationships of plasma Hcy and hs-CRP levels with the anthropometric and biochemical parameters and their correlation with the metabolic profile of women with PCOS.

Methods

A cross-sectional study was conducted at the Department of Clinical Chemistry, University Clinic for Gynecology and Obstetrics, the Republic of North Macedonia. The study and all procedures were approved by the Ethics Committee for Research on People and Animals, at the Medical Faculty in Skopje, University St.” Cyril and North Methodius”, Macedonia. All participants in the study signed the informed consent before their participation in the study.

Seventy-three premenopausal, 18 to 40-year old women with POCS, based on the diagnostic criteria from the Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, were involved in this study [3]. The control group consisted of 43-age-matched healthy females who volunteered for this study. All participants completed a questionnaire that included age, marital status, lifestyle habits and use of vitamin supplementation. The participants’ anthropometric assessment included the body weight, height, waist and hip circumference. The patients with abnormal levels of prolactin, thyroid hormones, renal or hepatic dysfunction, diabetes type 1 or 2 and congenital adrenal hyperplasia were excluded. The patients using any medication like hormonal supplementation and insulin sensitizers, or any vitamins in a period of 6 months were excluded.

Biochemical measurements

All blood samples were taken from the subjects after 12–14 hours overnight fasting between 3rd–7th day of the menstrual cycle, or at any given day for women with absent menstrual cycles in previous two or more months for the evaluation of hormonal parameters. The blood samples were withdrawn at 8–10 am from an antecubital vein after 5 min rest in the supine position, allowed clot for at least 30 minutes and then centrifuged. The resulting serum and plasma were aliquoted, frozen and maintained at -40˚C. Serum follicle stimulating hormone (FSH), luteinizing hormone (LH),

estradiol (E2), testosterone (T), androstenedione (A), dehydroepiandrostenedione-sulphate (DHEA-s), prolactin (PRL), Sex hormone binding globulin (SHBG) and the insulin levels were measured by the chemiluminescent immunometrical assay (Immulite 2000 HP, Diagnostics Products Corp). Plasma glucose was performed by the glucose oxidase method (Cobas Integra 400 plus analyser, Roche Diagnostic). For the homocysteine analysis, blood was collected in ethylenediaminetetracetic acid (EDTA) containing tubes. Hcy was measured on the Cobas Integra plus analyser, using enzyme-cycling method. Homocysteine enzymatic assay has higher specificity due to very low interference from cystathionine (an intermediate product in homocysteine metabolism). The principle of this method is a reduction of the oxidised form of homocysteine into free homocysteine. Using Hcy S-methyl transferase (HMT-use) and co-substrate, S-adenosyl methionine (SAM), free Hcy transforms to form methionine (Met) and S-adenosyl homocysteine (SAH). In the presence of S-adenosyl homocysteine- hydrolase, SAH is hydrolyzed into adenosine and Hcy. The created Hcy re-enters into reaction in which is transformed into methionine and SAH. In this cyclic transformation of Hcy, adenosine is accumulated and it is transformed into inosine and ammonia in the presence of adenosine hydrolase. The enzyme glutamate dehydrogenase (GLDH) catalyses the reaction of ammonia with 2-ketoglutarate and nicotinamide adenine dinucleotide (NADH) to form NAD+, which results in a decline of the absorbency at 340 nm. The concentration of Hcy present in the sample is directly proportional to the amount of NADH converted to NAD+. Quantitative determination of hs-CRP was determined using particle enhanced turbidimetric assay on the Cobas Integra 400 plus analyser. The measuring range of hs-CRP was 0.1–20 mg/L, with a lower detection limit of 0.1 mg/L. This method was standardised by the method comparison to the Tina-Quant CRP high sensitive assay. The height and weight measurements were taken twice and the mean of two measurements was used to calculate the BMI. The waist circumference (WC) was measured midway between the superior border of the iliac crest and the lowermost margin of the ribs, using the waistline measure employed with subjects standing without clothing covering the waist area. The hip circumference was measured at the point with the maximum circumference over the buttocks. The waist to hip ratio (WHR) was calculated as a ratio between the waist and hip circumference. The presence of insulin resistance was determined by basal insulin concentrations, fasting glucose concentrations and homeostasis model assessment HOMA-IR was calculated as fasting insulin (mIU/L) x fasting glucose (mmol/L) / 22,5 [16]. The free androgen index (FAI) was calculated by the standard formula: testosterone/SHBG x 100.

Statistical analysis

All statistical procedures were done using the SPSS 17 software for Windows. The Kolmogorov – Smirnov test was performed for the normality of distribution of all variables. The data were expressed as the mean ± standard deviation. The comparisons between the groups were performed using the independent *t*-test. The correlation analyses between Hcy and hs-CRP and other variables was obtained using the Spearman's rank coefficient. The differences between groups were considered to be statistically significant for the $p \leq 0.05$.

Results

From 116 women included into the study, 73 were with PCOS and 43 were healthy women. All participants were Caucasian, representative of nationalities that live at the territory of the Republic North Macedonia. The anthropometric and hormonal characteristics of women with PCOS and the control group were summarised in Table 1.

There was no statistical differences between the mean age of two groups: the women with PCOS were 23.9 ± 3.9 years of age and the women without PCOS were 24.67 ± 4.8 years of age. The PCOS patients showed the significantly higher BMI (27.6 ± 6.2 kg/m^2) vs. control (25.2 ± 6.0 cm, $p < 0.05$), WC (96.3 ± 14.8 cm) vs. control (87.6 ± 16.2 cm), ($p < 0.001$) and WHR (0.87 ± 0.07) vs. control (0.81 ± 0.05 cm), ($p < 0.001$). The concentrations of LH, LH/FSH ratio, total testosterone, DHEA-S, and FAI were significantly higher in the PCOS group ($p < 0.001$). The serum levels of FSH (5.61 ± 1.7 mIU/L vs. 6.3 ± 1.34 mIU/L), ($p < 0.01$), E2 (56.55 ± 22.7 pg/mL vs. 44.7 ± 10.76 pg/mL), ($p < 0.05$) and SHBG (34.2 ± 22.2 pg/mL vs. 52.35 ± 20.35 nmol/L), ($p < 0.001$) were significantly lower in the PCOS group. We observed no significant difference between patients for TSH and PRL concentration (Table 1).

The PCOS patients had significantly higher concentration of Hcy (11.98 ± 2.88 mmol/L) compared with the control group (8.5 ± 3.0 mmol/L), ($p < 0.001$), and hs-CRP (3.02 ± 4.7 mg/L) vs. (2.5 ± 4.1 mg/L), ($p < 0.05$) (Table 2).

The mean concentration of fasting glucose was slightly higher in the PCOS group (5.2 ± 0.4 mmol/L) vs. the control group (5.2 ± 0.4 mmol/L), ($p < 0.05$). The markers of insulin resistance, fasting insulin (15.35 ± 14.0 mIU/L vs. 6.7 ± 3.6 mIU/L), ($p < 0.001$) and HOMA- IR (7.37 ± 5.5 vs. 1.5 ± 0.89), ($p < 0.001$) were significantly higher in the PCOS group compared to the control group.

We observed changes in the lipid parameters between two studied groups. The PCOS group had a statistically significantly higher concentration of cholesterol (4.9 ± 1.0 mmol/L) vs. the controls (4.4 ± 0.7 mmol/L), ($p < 0.01$), LDL-C (3.0 ± 0.9 mmol/L) vs. control (2.63 ± 0.69 mmol/L), ($p < 0.01$), triglycerides (1.24 ± 0.78 mmol/L) vs. the controls (0.78 ± 0.23 mmol/L), ($p < 0.01$). A significantly lower HDL-C was found in the PCOS women (1.22 ± 0.34 mmol/L) vs. the controls (1.43 ± 0.28 mmol/L), ($p < 0.05$) (Table 2).

In Table 3, the correlation parameters between Hcy and hs-CRP with the selected parameters in the PCOS group are presented.

The Hcy values showed a statistically significant inverse correlation with HDL-C ($p < 0.05$). There was no significant correlation between Hcy and the parameters of PCOS such as testosterone, the LH/FSH ratio or the FAI.

Table 1

The anthropometric and hormonal characteristics of woman with polycystic ovary syndrome (PCOS) and the control group

Variables	PCOS group (n = 73) mean ± SD	Control group (n = 43) mean ± SD	*p*
Age (years)	23.9 ± 3.9	24.67 ± 4.8	0.11
BMI (kg/m$^{2)}$	27.6 ± 6.2	25.2 ± 6.0	0.03
WC (cm)	96.3 ± 14.8	87.6 ± 16.2	0.001
WHR	0.87 ± 0.07	0.81 ± 0.05	0.001
FSH (mIU/L)	5.61 ± 1.7	6.3 ± 1.34	0.01
LH (mIU/L)	9.49 ± 4.6	4.6 ± 3.2	0.001
LH / FSH	1.75 ± 0.9	0.73 ± 0.54	0.001
PRL (ng/mL)	11.5 ± 5.3	12.7 ± 5.6	n.s
E2 (pg/mL)	56.55 ± 22.7	44.7 ± 10.76	0.05
TSH (mIU/L)	2.3 ± 1.6	2.3 ± 0.74	n.s
DHEA-S (ng/mL)	3.83 ± 2.6	3.1 ± 4.2	0.001
Testosterone (nmol/L)	2.26 ± 0.88	0.98 ± 0.38	0.001
FAI	9.2 ± 7.3	2.27 ± 1.54	0.001
SHBG (nmol/L)	34.2 ± 22.2	52.35 ± 20.35	0.001

SD – standard devation; FSH – follicle-stimulating hormone; LH – luteinizing hormone; PRL – prolactin; E2 – estradiol, INS – insulin; TSH – thyroid-stimulating hormone; DHEA-S – dehydroepiandrostenedione-sulphate; FAI – free androgen index; SHGB – sex hormone binding globulin; HOMA-IR – homeostasis model assessment for insulin resistance.

Table 2

The metabolic characteristics of woman with polycystic ovary syndrome (PCOS) and the control group

Variables	PCOS group	Control group	*p*-value
Fasting glucose (mmol/L)	5.2 ± 0.4	5.2 ± 0.4	0.05
Fasting insulin (mIU/L)	15.35 ± 14.0	6.7 ± 3.6	< 0.001
HOMA-IR	7.37 ± 5.5	1.5 ± 0.89	< 0.001
Cholesterol (mmol/L)	4.9 ± 1.0	4.4 ± 0.7	0.012
Trigliceride (mmol/L)	1.24 ± 0.78	0.78 ± 0.23	< 0.01
HDL-C (mmol/L)	1.22 ± 0.34	1.43 ± 0.28	< 0.05
LDL-C (mmol/L)	3.0 ± 0.9	2.63 ± 0.69	0.01
Homocysteine (mmol/L)	11.98 ± 2.88	8.5 ± 3.0	< 0.001
hs-CRP (mg/L)	3.02 ± 4.7	2.5 ± 4.1	< 0.05

HOMA-IR – homeostatic assessment model for insulin resistance; HDL-C – high-denisity lipoprotein cholesterol; LDL-C – low-density lipoprotein cholesterol; hs-CRP – high sensitive C-reactive protein.

Table 3

The correlation coefficients (r) between homocysteine (Hcy), highsensitivity-C-reactive protein (hs-CRP) and other clinical parameters

Correlation coefficients	Homocysteine		hs- CRP	
	r	*p*	r	*p*
Age (years)	0.064	0.540	-0.052	0.683
BMI (kg/m^2)	-0.013	0.911	0.669	0.0001
WC (cm)	-0.003	0.980	0.576	0.0001
WHR	-0.108	0.380	0.209	0.098
FSH (mIU/L)	-0.021	0.865	-0.097	0.455
LH (mIU/L)	0.126	0.311	-0.369	0.003
LH/FSH ratio	0.094	0.448	-0.270	0.035
DHEA-S (μg/mL)	0.022	0.867	0.51	0.710
Testosterone (nmol/L)	-0.006	0.964	-0.083	0.516
FAI	-0.032	0.810	0.335	0.012
SHBG (nmol/L)	0.034	0.800	-0.464	0.001
Glucose (mmol/L)	0.085	0.475	0.025	0.842
Insulin (mIU/L) -	-0.290	0.811	0.534	0.001
HOMA-IR	-0.053	0.657	-0.318	0.010
Cholesterol (mmol/L)	0.046	0.705	0.242	0.054
Triglycerides(mmol/L)	0.090	0.461	0.370	0.002
HDL-C (mmol/L)	-0.278	0.034	-0.512	0.001
LDL –C (mmol/L)	-0.025	0.860	0.276	0.038

FSH – follicle-stimulating hormone; LH – luteinizing hormone; FAI – free androgen index; SHGB – sex hormone binding globulin; HOMA-IR – homeostasis model assessment for insulin resistance; BMI – body mass index; WC – waist circumference; WHR – waist to hip ratio; DHEA-S – dehydroepiandrostenedione-sulphate; HDL-C-high density lipoprotein cholesterol; LDL-C – low density lipoprotein cholesterol.

The serum hs-CRP values showed a significantly strong positive correlation with BMI, WC, insulin, and triglycerides ($p < 0.001$). A significant inverse correlation was observed among hs-CRP and LH, SHGB, HOMA-IR, HDL-C.

Discussion

Our study results demonstrated that the patients with PCOS had the increased inflammatory markers compared to the age and BMI-matched control group, indicating that the inflammation seen in PCOS might be related to the presence of disorder rather than obesity.

PCOS is a proinflammatory disorder and the increased levels of circulatory inflammatory markers such as tumor necrosis factor alpha (TNF-alpha), interleukin-6 (IL-6) have were found in several studies [10, 16]. The reasons of presence of low level chronic inflammation in PCOS has not been clarified yet, and it remains uncertain whether it is connected with PCOS itself, or it is associated with obesity.

It was shown that both obesity and inflammation contribute to the metabolic complications for women of reproductive age. The state of the cardiovascular system in women with PCOS might be due to different factors, including the insulin resistance, androgen status and BMI [6]. However, excess androgen should be the most important component of PCOS [17].

The women with polycystic ovarian syndrome face a variety of metabolic disorders, among which dysfunction of insulin activity has the most significant effect on the disorders presented later in life. Obesity, hyperinsulinemia, hyperandrogenaemia and insulin resistance are some of the metabolic disorders which shadow this syndrome and rise a question of possible increased cardiovascular risk among these women. High-sensitivity CRP is an acute phase protein that is mainly produced by the liver as a response to multiple reasons mainly to the increased levels of systemic inflammatory cytokines TNF-α and IL-6 [15]. Hs-CRP is considered to be a sensitive marker of low-grade inflammation, Met-Sy and as a predictor of future cardiovascular diseases (CVD) [18]. During the past years, conflicting results were published regarding the presence of endothelial dysfunction and the low-grade chronic inflammation in PCOS. A number of reports indicated that the levels of hs-CRP are significantly elevated in the women with PCOS pointing at the strong relationship between BMI and visceral fat as a reason for the elevated CRP levels [19]. Escobar-Morreale et al. [20] found that hs-CRP is the most reliable circulating marker of low-grade chronic inflammation in the women with PCOS and elevated levels of CRP are a reflection of presence of chronic inflammation in this condition. In his study, González [21] revealed that hs-CRP concentration was higher in obese women than in normal-weight women, regardless of PCOS. Thus, hs-CRP elevations attributed to PCOS were concealed by the presence of obesity and were below the range to predict a metabolic or cardiovascular risk. On contrary, some researchers believe that CRP elevation is associated with endocrine disorders [22]. Cho et al. [23] in their study showed that the mean concentration of CRP in the women with PCOS was higher than in healthy individuals, but inter-individual biological variations between the patients and healthy controls were similar. The inverse correlation between CRP concentration and insulin cannot solely explain insulin resistance, but it is more likely that CRP can be a marker of metabolic changes that contribute to the syndrome. In this study, we were challenged, for the first time to our knowledge, to evaluate hs-CRP in a sample of adolescent woman with PCOS living in North Macedonia. Presented data are in agreement with results demonstrated in previous studies that have found the higher hs-CRP serum concentration in the women with PCOS [19, 24]. We found a significant strong correlation between the hs-CRP concentration and BMI and WC. Kurt et al. [25] and Güdücü et al. [26] reported that both, obese and lean women with PCOS had increased CRP concentrations when compared with the BMI match control groups [25, 26]. These authors suggest that increased body weight and central fat are major basis of metabolic aberrations associated with CVD in PCOS while hs-CRP is a marker indicating existence of low-grade chronic inflammation and increased CVD risk. A significant correlation was stated previously between CRP and insulin resistance. We found a strong correlation of hs-CRP and fasting insulin concentrations and HOMA-IR. In the PCOS patients, correlation of hs-CRP with HOMA-IR was related to the presence of abdominal obesity, but independent of WHR. This finding suggests the increased risk of early atherosclerosis and cardiovascular events in the PCOS women, which at the same time is more pronounced and dependent on accumulated central fat deposit. In a study with a Croatian cohort of PCOS women, with a low prevalence of obesity, a significant association was found between SHBG and CRP, independent of insulin resistance, measured by HOMA-IR, supporting that low SHBG concentrations can be an independent marker of enhanced cardiovascular risk in the females with PCOS [27]. In our study, we observed a significant strong negative correlation between hs-CRP and SHGB and a weak positive correlation with FAI. We did not find any correlation with the androgen levels. However, the hs-CRP levels correlated with LH and LH/FSH ratio, triglycerides and LDL-C. This emphasises that obesity and metabolic alterations have influence in low-grade chronic inflammation and elevated CRP in the PCOS woman.

Moderately increased Hcy has a cytotoxic effect on vascular endothelium where oxidative stress leads to endothelial dysfunction causing vascular remodelling [28]. The elevated plasma concentrations of Hcy are an independent risk factor for early atherosclerosis and other vascular diseases [14]. Celik et al. [29] found higher levels of Hcy in the PCOS women. Contrary, Morgante et al. [30] did not find the significant elevation of Hcy concentration in PCOS woman. The present study is the first one in relevant literature to demonstrate increased Hcy in the cohort of PCOS women living in North Macedonia. The relationship between Hcy and PCOS may be explained by the presence of the increased low-grade chronic inflammation which is one of the main pathophysiological mechanisms in PCOS. Schachter et al. [31] indicate in their studies that insulin resistance is the major determinant of Hcy in the PCOS woman. In the present study, no association was found between Hcy, fasting insulin, IR, body mass, or other variables of PCOS. Supporting

the present study, Kilic-Okman et al. [32] indicated that the age, BMI and insulin resistance were not a predictor for Hcy levels. We did not observe any correlation between Hcy and BMI in the PCOS women. However, our study demonstrated a negative correlation between Hcy and serum HDL-C concentrations. The metabolite of Hcy combined with the lower HDL-C levels can be an initiation of development or disruption of endothelial cells and vascular remodelling. Homocysteine concentrations are dependent on few parameters, such as tobacco smoking and B-vitamins and folic acid intake [33]. The strong side of this study was that all involved subject were non-smokers, no vegetarians, and none of them was using any vitamin supplements, so the factor that can influence Hcy concentrations were eliminated.

Conclusion

The results of this study demonstrated presence of the low-grade chronic inflammation and increased inflammatory markers among the North Macedonian adolescent woman with PCOS compared to the corresponding BMI-matched control group. There is a growing data of evidence indicating disturbed Hcy metabolism as well as confirmation of the presence of chronic inflammation in the PCOS women, leading to the increased CVR risk. Accordingly, the presence of insulin resistance was shown, accessed through the indices of insulin action as it was basal insulin and HOMA-IR model. The markers of low-grade chronic inflammation, such as hs-CRP, were associated with BMI and accumulated central fat as well as with the presence of insulin resistance and disturbed lipid metabolism, while the elevation of Hcy was related with lipid metabolism. This finding further confirmed the existence of a proinflammatory state in a woman with PCOS. Nonetheless, these results suggest that screening for Hcy and CRP-Hs status may be beneficial and used for early identification CVD in PCOS in term of taking early preventive measures. Screening for hyperhomocysteinemia can be valuable before the use of oral contraceptive pills at a young age. Lifestyle changes with diet and exercise interventions particularly in overweight category can be advised in terms of limiting factors which are associated with increased cardiovascular risk, insulin resistance, hypertension, and dyslipidemia.

REFERENCES

1. *March WA, Moore VM, Willson KJ, Phillips DI, Norman RJ, Davies MJ.* The prevalence of polycystic ovary syndrome in a community sample assessed under contrasting diagnostic criteria. Hum Reprod 2010; 25(2): 544–51.
2. *Wijeyaratne CN, Dilini US, Balen AH.* Ethnic-specific polycystic ovary syndrome: Epidemiology, significance and implications. Expert Rev Endocrinol Metab 2013; 8(1): 71–9.
3. *Conway G, Dewailly D, Diamanti-Kandarakis E, Escobar-Morreale HF, Franks S, Gambineri A,* et al. ESE PCOS Special Interest Group: The polycystic ovary syndrome: A position statement from the European Society of Endocrinology. Eur J Endocrinol 2014; 171(4): P1–29.
4. *Ezeh U, Yildiz BO, Azziz R.* Referral bias in defining the phenotype and prevalence of obesity in polycystic ovary syndrome. J Clin Endocrinol Metab 2013; 98(6): E1088–96.
5. *Bickerton AS, Clark N, Meeking D, Shaw KM, Crook M, Lumb P,* et al. Cardiovascular risk in women with polycystic ovarian syndrome (PCOS). J Clin Pathol 2005; 58(2): 151–4.
6. *Guleria AK, Syal SK, Kapoor A, Kumar S, Tiwari P, Dabadghao P.* Cardiovascular disease risk in young Indian women with polycystic ovary syndrome. Gynecol Endocrinol 2014; 30(1): 26–9.
7. *Teede H, Deeks A, Moran L.* Polycystic ovary syndrome: A complex condition with psychological, reproductive and metabolic manifestations that impacts on health across the lifespan. BMC Med 2010; 8: 41.
8. *Hart R, Doherty DA.* The potential implications of a PCOS diagnosis on a woman's long-term health using data linkage. J Clin Endocrinol Metab 2015; 100(3): 911–9.
9. *Diamanti-Kandarakis E, Dunaif A.* Insulin resistance and the polycystic ovary syndrome revisited: An update on mechanisms and implications. Endocr Rev 2012; 33(6): 981–1030.
10. *Sathyapalan T, Atkin SL.* Mediators of Inflammation in Polycystic Ovary Syndrome in Relation to Adiposity. Mediators Inflamm 2010; 2010: 758656.
11. *Coelho M, Oliveira T, Fernandes R.* Biochemistry of adipose tissue: An endocrine organ. Arch Med Sci 2013; 9(2): 191–200.
12. *Jacques PF, Bostom AG, Wilson PW, Rich S, Rosenberg IH, Selhub J.* Determinants of plasma total homocysteine concentration in the Framingham Offspring cohort. Am J Clin Nutr 2001; 73(3): 613–21.
13. *Obersby D, Chappell DC, Dunnett A, Tsiami AA.* Plasma total homocysteine status of vegetarians compared with omnivores: A systematic review and meta-analysis. Br J Nutr 2013; 109(5): 785–94.
14. *Wald DS, Law M, Morris JK.* Homocysteine and cardiovascular disease: evidence on causality from a meta-analysis. BMJ 2002; 325(7374): 1202.
15. *Koenig W.* High-sensitivity C-reactive protein and atherosclerotic disease: From improved risk prediction to risk-guided therapy. Int J Cardiol 2013; 168(6): 5126–34.
16. *Deligeoroglou E, Vrachnis N, Athanasopoulos N, Iliodromiti Z, Sifakis S, Iliodromiti S*, et al. Mediators of chronic inflammation in polycystic ovarian syndrome. Gynecol Endocrinol 2012; 28(12): 974–8.
17. *Wake DJ, Strand M, Rask E, Westerbacka J, Livingstone DE, Soderberg S,* et al. Intra-adipose sex steroid metabolism and body fat distribution in idiopathic human obesity. Clin Endocrinol (Oxford) 2007; 66(3): 440–6.
18. *DeBoer MD.* Obesity, systemic inflammation, and increased risk for cardiovascular disease and diabetes among adolescents: A need for screening tools to target interventions. Nutrition 2013; 29(2): 379–86.
19. *Toulis KA, Goulis DG, Mintziori G, Kintiraki E, Eukarpidis E, Mouratoglou SA*, et al. Meta-analysis of cardiovascular disease risk markers in women with polycystic ovary syndrome. Hum Reprod Update 2011; 17(6): 741–60.
20. *Escobar-Morreale HF, Luque-Ramírez M, González F.* Circulating inflammatory markers in polycystic ovary syndrome: A systematic review and meta analysis. Fertil Steril 2011; 95(3): 1048–58.e1–2.
21. *González F.* Inflammation in Polycystic Ovary Syndrome: Underpinning of insulin resistance and ovarian dysfunction. Steroids 2012; 77(4): 300–5.
22. *Wu Y, Zhang J, Wen Y, Wang H, Zhang M, Cianflone K.* Increased acylation-stimulating protein, C-reactive protein, and lipid levels in young women with polycystic ovary syndrome. Fertil Steril 2009; 91(1): 213–9.

23. *Cho LW, Jayagopal V, Kilpatrick ES, Atkin SL*. The Biological Variation of C-Reactive Protein in Polycystic Ovarian Syndrome. Clin Chem 2005; 51(10): 1905–7.
24. *Guzelmeric K, Alkan N, Pirimoglu M, Unal O, Turan C*. Chronic inflammation and elevated homocysteine levels are associated with increased body mass index in women with polycystic ovary syndrome. Gynecol Endocrinol 2007; 23(9): 505–10.
25. *Kurt RK, Okyay AG, Hakverdi AU, Gungoren A, Dolapcioglu KS, Karateke A*, et al. The effect of obesity on inflammatory markers in patients with PCOS: A BMI-matched case-control study. Arch Gynecol Obstet 2014; 2(290): 315–9.
26. *Güdücü N, Işçi H, Yiğiter AB, Dünder I*. C-reactive protein and lipoprotein-a as markers of coronary heart disease in polycystic ovary syndrome. J Turk Ger Gynecol Assoc 2012; 13(4): 227–32.
27. *Baldani DP, Skrgatic L, Cerne JZ, Oguic SK, Gersak BM, Gersak K*. Association between serum levels and pentanucleotide polymorphism in the sex hormone binding globulin gene and cardiovascular risk factors in females with polycystic ovary syndrome. Mol Med Rep 2015; 11(5): 3941–7.
28. *Steed MM, Tyagi SC*. Mechanisms of cardiovascular remodeling in hyperhomocysteinemia. Antioxid Redox Signal 2011; 15(7): 1927–43.
29. *Celik C, Bastu E, Abali R, Alpsoy S, Guzel EC, Aydemir B*, et al. The relationship between copper, homocysteine and early vascular disease in lean women with polycystic ovary syndrome. Gynecol Endocrinol 2013; 29(5): 488–91.
30. *Morgante G, La MA, Setacci F, Setacci C, Petraglia F, De Leo V*. The cardiovascular risk factor homocysteine is not elevated in young women with hyperandrogenism or hypoestrogenism. Gynecol Obstet Invest 2002; 53(4): 200–3.
31. *Schachter M, Raziel A, Friedler S, Strassburger D, Bern O, Ron-El R*. Insulin resistance in patients with polycystic ovary syndrome is associated with elevated plasma homocysteine. Hum Reprod 2003; 18(4): 721–7.
32. *Kilic-Okman T, Guldiken S, Kucuk M*. Relationship between homocysteine and insulin resistance in women with polycystic ovary syndrome. Endocr J 2004; 51(5): 505–8.
33. *Chen S, Wu P, Zhou L, Shen Y, Li Y, Song H*. Relationship between increase of serum homocysteine caused by smoking and oxidative damage in elderly patients with cardiovascular disease. Int J Clin Exp Med 2015; 8(3): 4446–54.

Received on May 4, 2017.
Revised on October 4, 2017.
Accepted on October 9, 2017.
Online First November, 2017.