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Selected markers of endothelial dysfunction in women with polycystic ovary syndrome

Ocena stężenia wybranych markerów uszkodzenia śródbłonka u kobiet z zespołem policystycznych jajników

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Abstract

Background: The increased incidence of cardiovascular disease in women with polycystic ovary syndrome (PCOS) has prompted researchers to look for indicators of early atherosclerotic changes in these patients. One of the earliest stages of atherogenesis is endothelial cell dysfunction. The aim of this study was to assess the levels of selected plasma markers of endothelial injury [E-selectin, endothelin-1 (ET-1) and von Willebrand Factor antigen (vWF:Ag)] in PCOS women before and after six months of treatment.
Material and methods: 32 patients with PCOS aged 18–36 years (mean age 25.16 ± 5.80) were included in the study. The control group consisted of 20 healthy women matched for age and body mass.
The levels of ET-1, vWF:Ag, E-selectin, fasting glucose, insulin, total cholesterol, HDL and LDL-cholesterol and triglycerides were assessed. In the PCOS group, all these tests were repeated after six months of treatment.
Results: The study showed higher levels of vWF:Ag ($p = 0.043$), E selectin ($p = 0.028$), insulin ($p = 0.044$), glucose ($p = 0.036$) and LDL ($p = 0.006$) in PCOS patients versus healthy women. A positive correlation was demonstrated between E selectin and glucose ($p = 0.0001$), triglycerides ($p = 0.014$) and uric acid ($p = 0.008$). vWF:Ag levels showed a positive correlation with glucose ($p = 0.04$) and triglycerides ($p = 0.036$). A positive correlation was also found between ET-1 and total cholesterol levels ($p = 0.012$) in PCOS women. After treatment, there was a significant reduction in E-selectin levels from baseline ($p = 0.002$) and an increase in the levels of HDL ($p = 0.0002$) and triglycerides ($p = 0.033$).
Conclusions: Elevated levels of vWF:Ag and E selectin in PCOS women suggest endothelial dysfunction in this group of patients. Glucose and triglyceride are significant factors affecting endothelial function in PCOS. **(Pol J Endocrinol 2011; 62 (3): 243–248)**

Key words: *polycystic ovary syndrome, endothelium, E-selectin, von Willebrand factor, endothelin-1*

Streszczenie

Wstęp. Zwiększona zapadalność na choroby układu sercowo-naczyniowego obserwowana u kobiet z zespołem policystycznych jajników (PCOS) skłania badaczy do poszukiwania wskaźników wczesnych zmian miażdżycowych u tych chorych. Jednym z najwcześniejszych etapów procesu aterogenezy jest dysfunkcja komórek endotelium.
Celem pracy była ocena stężenia wybranych osoczowych markerów uszkodzenia śródbłonka [rozpuszczalna forma selektyny E, endotelina 1 (ET-1) oraz czynnik von Willebranda (vWF:Ag)] u kobiet z zespołem policystycznych jajników przed i po 6-miesięcznej terapii hormonalnej.
Materiał i metody: Badanie przeprowadzono u 32 chorych z PCOS w wieku 18–36 lat (średnia wieku 25,16 ± 5,8). Grupę kontrolną stanowiło 20 zdrowych kobiet, odpowiednio dobranych pod względem wieku i masy ciała. U wszystkich badanych oznaczono stężenie endoteliny 1, czynnika von Willebrada i rozpuszczalnej formy selektyny E w osoczu oraz dokonano oceny gospodarki węglowodanowej (glikemia na czczo, stężenie insuliny) i lipidowej (cholesterol całkowity, cholesterol frakcji HDL, LDL, triglicerydy). W grupie chorych z PCOS badania te powtórzono po 6 miesiącach leczenia.
Wyniki: Stwierdzono znamiennie wyższe stężenie vWF:Ag ($p = 0,043$), selektyny E ($p = 0,028$), insuliny ($p = 0,044$), glukozy na czczo ($p = 0,036$) i cholesterolu frakcji LDL ($p = 0,006$) u kobiet z PCOS w porównaniu ze zdrowymi. Wykazano dodatnią korelację pomiędzy stężeniem selektyny E i stężeniem glukozy ($p = 0,0001$), triglicerydów ($p = 0,014$) i kwasu moczowego ($p = 0,008$) u chorych z PCOS. Stwierdzono także dodatnią korelację pomiędzy stężeniem vWF:Ag a stężeniem glukozy ($p = 0,04$) i triglicerydów ($p = 0,036$). Stężenie ET-1 dodatnio korelowało ze stężeniem cholesterolu całkowitego ($p = 0,012$) w grupie kobiet z PCOS. Po 6 miesiącach terapii hormonalnej średnie stężenie selektyny E było znamiennie niższe w porównaniu z okresem przed leczeniem ($p = 0,002$), zaobserwowano także zmiany w profilu lipidowym polegające na istotnym wzroście stężenia cholesterolu frakcji HDL ($p = 0,0002$) i triglicerydów ($p = 0,033$).
Wnioski: Podwyższone stężenie czynnika von Willebranda oraz selektyny E u kobiet z zespołem policystycznych jajników wskazuje na zaburzoną czynność śródbłonka w tej grupie chorych. Istotnymi czynnikami wpływającymi na funkcjonowanie enodtelium u kobiet z PCOS są glikemia i triglicerydemia. **(Endokrynol Pol 2011; 62 (3): 243–248)**

Słowa kluczowe: *zespół policystycznych jajników, śródbłonek, selektyna E, czynnik von Willebranda, endotelina 1*

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Introduction

The increased incidence of cardiovascular disease in women with polycystic ovary syndrome (PCOS) has prompted researchers to look for indicators of early atherosclerotic changes in these patients [1, 2]. One of the earliest stages of atherogenesis is endothelial cell dysfunction. Factors predisposing for endothelial injury include: hyperinsulinaemia, insulin resistance and dyslipidaemia, which often accompany PCOS. Androgen excess is an additional, independent factor that promotes atherosclerosis in this group of patients [3]. Injured endothelium is a source of excessive secretion of numerous adhesion molecules, cytokines and immunoglobulins responsible for initiation and progression of the atherosclerotic process [4]. Therefore there have been attempts to employ plasma markers of endothelial injury for early diagnosis of atherogenesis and for predicting the risk of late vascular complications [5, 6]. Endothelial cell dysfunction and the resulting adhesion and migration of leukocytes into the vascular wall are some of the earliest stages of atherogenesis. Interactions of vascular endothelial cells with activated leukocytes lead to abnormal vasodilation, increased permeability of the vascular wall and activation of the coagulation system. E-selectin is an adhesion molecule responsible for the binding of leukocytes to endothelial cells, and is therefore a recognised early marker of atherogenesis [7]. The soluble form is released to the circulation by endothelial cells that are activated by various pathogenetic factors. Endothelin-1 (ET-1) is an endothelium-derived contracting factor (EDCF) characterised by pressor and mitogen activities [8]. A recognised marker of functional or structural endothelial injury is plasma von Willebrand Factor (vWF), which acts as an adhesion molecule that facilitates platelet aggregation and adhesion to endothelial cells [9, 10].

Aim

The aims of the study were:

— to assess the levels of selected plasma markers of endothelial injury [soluble E-selectin, endothelin-1 and von Willebrand Factor antigen (vWF:Ag)] in young women with polycystic ovary syndrome before and after six months of treatment;

— to assess the correlation between plasma levels of endothelial dysfunction markers and the levels of the pituitary-ovarian axis hormones and the metabolic status markers.

Material and methods

A total of 32 patients with PCOS aged 18–36 years (mean age 25.16 ± 5.80) were included in the study. The control group consisted of 20 healthy women matched for age and body mass. Some exclusion criteria were: smoking, obesity, diabetes mellitus, infection, immune system disorders, cardiovascular disease and hormonal contraception for the past six months. Fasting blood samples were drawn between 8.00 and 9.00 in the morning from the ulnar vein at the beginning of the follicular phase (second or third day of the menstrual cycle).

All the patients underwent determination of the levels of pituitary-ovary axis hormones [follicle-stimulating hormone (FSH), luteinising hormone (LH), estradiol (E_2)] and androgens (free testosterone and androstenedione), an assessment of carbohydrate metabolism (fasting glucose, oral glucose tolerance test, insulin) and of lipid metabolism (total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides) and determination of plasma levels of ET-1, vWF:Ag and soluble E-selectin. In the PCOS group, all these tests were repeated after six months of treatment (contraceptive oral pills).

Plasma markers of endothelial dysfunction were tested via enzyme-linked immunosorbent assay (ELISA): vWF:Ag was determined using the von Willebrand Factor Antigen assay from Helena Biosciences Europe (UK); E-selectin was determined using the Human sE-Selectin assay from R&D Systems; and ET-1 was determined using the Human Endothelin-1 assay from R&D Systems.

Statistical analysis

The study variables were compared with the t-Student test and the correlation was assessed using the Pearson method. p values of < 0.05 were considered statistically significant.

Results

The study showed significantly higher levels of vWF:Ag ($p = 0.043$) and soluble E-selectin ($p = 0.028$) in PCOS patients versus healthy women. ET-1 levels did not differ significantly between the study groups. PCOS patients showed significantly higher levels of insulin ($p = 0.044$), fasting glucose ($p = 0.036$) and LDL-cholesterol ($p = 0.006$) than controls (Table I).

A positive correlation was demonstrated between soluble E-selectin levels in PCOS patients and the levels of: glucose ($p = 0.0001$), triglycerides ($p = 0.014$) and uric acid ($p = 0.008$) (Figures 1–3). A positive correlation was also found between the serum levels of vWF:Ag and those of glucose ($p = 0.04$) and triglycerides ($p = 0.036$) in PCOS patients. ET-1 levels showed a positive correlation with total cholesterol in PCOS patients ($p = 0.012$).

There was a significant reduction in mean soluble E-selectin levels in PCOS women after six months of treatment ($p = 0.002$). Changes in the lipid profile were

Table I. *Comparison of test results assessing endothelial function, hormonal and metabolic status in patients with PCOS before treatment and controls*

Tabela I. *Porównanie wyników badań oceniających czynność śródbłonka, stan hormonalny i metaboliczny u chorych z PCOS przed leczeniem i w grupie kontrolnej*

	PCOS before treatment (mean ± SD)	Control (mean ± SD)	p value
Soluble E-selectin [ng/ml]	37.52 ± 16.7	28.49 ± 7.08	0.028492
von Willebrand Factor antigen (%)	145.7 ± 38.5	132.0 ± 29.7	0.043328
Endothelin-1 [pg/ml]	11.77 ± 3.27	9.83 ± 2.91	0.343744
Insulin [μU/l]	7.37 ± 6.46	4.15 ± 3.15	0.044291
Free testosterone [pg/ml]	4.99 ± 2.64	1.44 ± 0.73	0.000001
Androstenedione [ng/ml]	5.70 ± 1.96	2.44 ± 0.85	0.000001
Follicle-stimulating hormone [mIU/ml]	5.19 ± 1.27	6.93 ± 2.98	0.005970
Luteinising hormone [mIU/ml]	6.19 ± 4.1	4.94 ± 2.03	0.212889
Fasting glucose [mg/dl]	94.52 ± 36.32	76.89 ± 5.15	0.036683
Cholesterol [mmol/l]	4.49 ± 1.05	4.54 ± 0.67	0.834976
HDL-cholesterol [mmol/l]	1.49 ± 0.39	1.50 ± 0.36	0.914510
LDL-cholesterol [mmol/l]	18.42 ± 3.67	2.80 ± 0.25	0.006494
Triglycerides [mmol/l]	1.18 ± -0.89	0.80 ± 0.24	0.066065
Uric acid [μmol/l]	280 ± 96.2	232.54 ± 54.74	0.052591

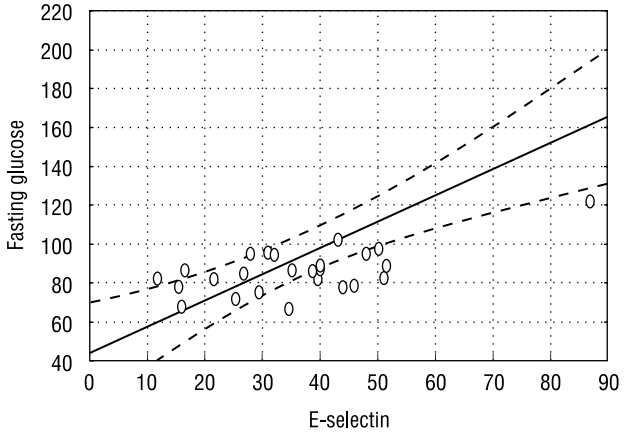

Figure 1. *Correlations between the levels of soluble E-selectin and glucose in women with PCOS before treatment*

Rycina 1. *Korelacja pomiędzy stężeniem selektyny E i stężeniem glukozy u kobiet z PCOS przed leczeniem*

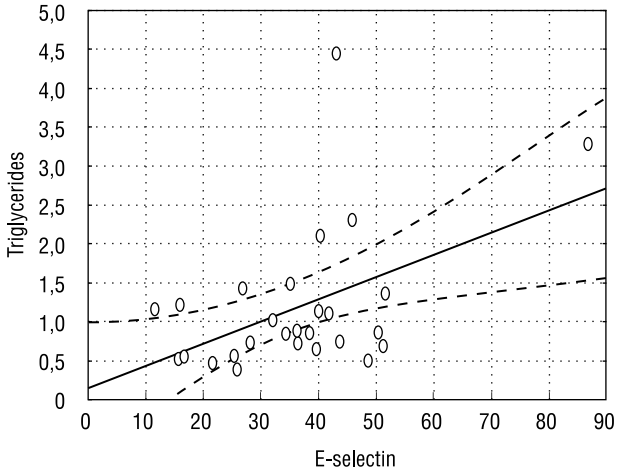

Figure 2. *Correlations between blood levels of soluble E-selectin and triglycerides in women with PCOS before treatment*

Rycina 2. *Korelacje pomiędzy stężeniem selektyny E i stężeniem triglicerydów we krwi u kobiet z PCOS przed leczeniem*

also observed and consisted of significant increases in the levels of HDL-cholesterol ($p = 0.0002$) and triglycerides ($p = 0.033$) (Table II). There were no significant differences in post-treatment soluble E-selectin levels between PCOS patients and controls. PCOS patients after hormone treatment showed significantly higher levels of insulin, total cholesterol, HDL-cholesterol and triglycerides compared to healthy controls (Table III). The positive correlation between the levels of soluble E-selectin and those of glucose, triglycerides and uric acid in PCOS patients was maintained after six months of treatment.

Discussion

Polycystic ovary syndrome (PCOS) is en endocrine disorder affecting 5–10% of females of reproductive

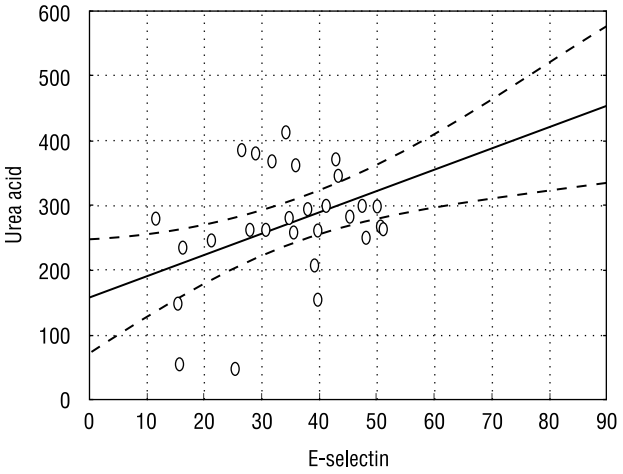

Figure 3. *Correlations between blood levels of soluble E-selectin and uric acid in women with PCOS before treatment*

Rycina 3. *Korelacja pomiędzy stężeniem selektyny E i stężeniem kwasu moczowego we krwi u kobiet z POCS przed leczeniem*

age [11]. Epidemiological studies have shown that the incidence of cardiovascular disease in PCOS patients exceeds that in women with normal ovarian function, and that the risk of myocardial infarction is seven-fold higher [12, 13]. Metabolic complications, such as hyperinsulinaemia, insulin resistance and dyslipidaemia, which develop in the course of PCOS, are recognised risk factors for atherosclerosis. Androgen excess is an additional, independent factor that promotes atherogenesis. Proinflammatory factors seem to play an important role in this process [14]. In women with androgen excess, genetic abnormalities have been shown in the form of polymorphism of the genes encoding inflammatory mediators, which may be included in the process of atherogenesis [15, 16]. However, the association between androgen excess and inflammation leading to endothelial injury remains unclear.

According to many authors, lipid abnormalities seen in PCOS patients put them at a particular risk of atherosclerosis [17, 18]. The profile of these abnormalities is very similar to those seen in metabolic syndrome [19]. Some authors even consider PCOS to be an early variant of this syndrome [20–22]. In our study, the lipid profile in PCOS patients and controls was similar, the only difference being the significantly higher level of the atherogenic LDL-cholesterol in PCOS patients before treatment compared to healthy women. As regards carbohydrate metabolism, PCOS patients had significantly higher mean levels of glucose and insulin compared to controls. Hence, in the group of women with PCOS, the presence of at least three metabolic status parameters affecting atherogenesis has been demonstrated.

Atherosclerosis is a complex disorder characterised by dyslipidaemia, inflammation and a propensity for

Table II. *Comparison of test results assessing endothelial function, hormonal and metabolic status in patients with PCOS before and after treatment*

Tabela II. *Porównanie wyników badań oceniających czynność śródbłonka, stan hormonalny i metaboliczny u kobiet z PCOS przed i po leczeniu*

	PCOS women before treatment (mean ± SD)	PCOS women after treatment (mean ± SD)	p value
Soluble E-selectin [ng/ml]	37.52 ± 16.7	25.56 ± 13.49	0.002947
von Willebrand Factor antigen (%)	145.7 ± 38.5	140.5 ± 33.45	0.09721
Endothelin-1 [pg/ml]	11.77 ± 3.37	12.57 ± 3.67	0.571478
Insulin [μU/l]	7.37 ± 6.46	6.85 ± 5.0	0.728807
Free testosterone [pg/ml]	4.99 ± 2.64	1.17 ± 0.9	0.000001
Androstenedione [ng/ml]	5.70 ± 1.96	3.61 ± 1.3	0.000023
Follicle-stimulating hormone [mIU/ml]	5.19 ± 1.27	5.08 ± 2.9	0.850277
Luteinising hormone [mIU/ml]	6.19 ± 4.1	2.97 ± 1.5	0.000971
Fasting glucose [mg/dl]	94.52 ± 36.32	84.65 ± 33.06	0.291537
Cholesterol [mmol/l]	4.49 ± 1.05	5.40 ± 0.91	0.001003
HDL-cholesterol [mmol/l]	1.49 ± 0.39	1.90 ± 0.35	0.000218
LDL-cholesterol [mmol/l]	18.42 ± 3.67	2.92 ± 1.06	0.060478
Triglycerides [mmol/l]	1.18 ± -0.89	2.18 ± 1.2	0.033417
Uric acid [μmol/l]	280 ± 96.2	268.12 ± 83.27	0.629279

Table III. *Comparison of test results assessing endothelial function, hormonal and metabolic status in patients with PCOS after treatment and controls*

Tabela III. *Porównanie wyników badań oceniających stan hormonalny i metaboliczny u chorych z PCOS po leczeniu i w grupie kontrolnej*

	PCOS women after treatment (mean ± SD)	Controls (mean ± SD)	p value
Soluble E-selectin [ng/ml]	25.56 ± 13.49	28.49 ± 7.08	0.384045
von Willebrand Factor antigen (%)	139.5 ± 33.45	132.0 ± 29.7	0.11573
Endothelin-1 [pg/ml]	12.57 ± 3.67	9.83 ± 2.91	0.139570
Insulin [μU/l]	6.85 ± 5.0	4.15 ± 3.15	0.037240
Free testosterone [pg/ml]	1.17 ± 0.9	1.44 ± 0.73	0.287389
Androstenedione [ng/ml]	3.61 ± 1.3	2.44 ± 0.85	0.001162
Follicle-stimulating hormone [mIU/ml]	5.08 ± 2.9	6.93 ± 2.98	0.040228
Luteinising hormone [mIU/ml]	2.97 ± 1.5	4.94 ± 2.03	0.006576
Fasting glucose [mg/dl]	84.65 ± 33.06	76.89 ± 5.15	0.305869
Cholesterol [mmol/l]	5.40 ± 0.91	4.54 ± 0.67	0.000986
HDL-cholesterol [mmol/l]	1.90 ± 0.35	1.50 ± 0.36	0.000604
LDL-cholesterol [mmol/l]	2.92 ± 1.06	2.80 ± 0.25	0.665386
Triglycerides [mmol/l]	2.18 ± 1.2	0.80 ± 0.24	0.008513
Uric acid [μmol/l]	268.12 ± 83.27	232.54 ± 54.74	0.112738

thrombosis [23]. The initiation and progression of atherosclerotic processes are associated with vascular endothelial dysfunction and the activation of adhesion molecules [24, 25]. Vascular endothelial dysfunction may be an early marker of cardiovascular disease and predict coronary artery disease even before atheromatous changes develop in the arteries. Endothelial dysfunction in our patients with PCOS is evidenced by the significantly higher levels of soluble E-selectin and vWF:Ag compared to healthy women. Other authors have obtained similar results [26]. The positive correlation between the level of soluble E-selectin and the levels of glucose, triglycerides and uric acid and between the level of vWF:Ag and the levels of glucose and triglycerides suggests an association between metabolic status and endothelial function in women with PCOS.

In contrast to other authors, we found no significant difference in ET-1 levels between PCOS patients and healthy individuals [27, 28]. On the other hand, a positive correlation was observed between ET-1 level and cholesterol level in PCOS patients.

The six months of hormone therapy resulted in a significant reduction of soluble E-selectin levels. Hormone treatment did not affect the levels of the other markers of endothelial injury or the levels of glucose and insulin. A significant increase in HDL-cholesterol and triglyceride levels was, however, observed, which is probably related to the side effects of the contraceptives. Similar results have been obtained by other authors [29, 30]. The levels of soluble E-selectin, vWF:Ag and ET-1 in women with PCOS post-treatment did not differ from the levels of these markers in the control group. However, compared to controls, PCOS patients had persistently and significantly higher levels of insulin and significantly higher levels of total cholesterol, HDL-cholesterol and triglycerides. The positive correlation between the level of soluble E-selectin and the levels of glucose, triglycerides and uric acid was maintained. The results confirm the importance of metabolic status in modifying endothelial function in women with PCOS.

Determining the actual contribution of androgen excess to the elevated cardiovascular risk in women with PCOS is not easy, as the manifestations of coronary artery disease rarely develop before the menopause [31]. Retrospective studies suggest a higher prevalence of coronary artery occlusion and cerebrovascular events in patients with a history of PCOS [32, 33]. Considering the young age of patients with PCOS and the long duration of the disease, detection of early vascular changes in this group of patients is of particular practical significance.

Conclusions

Elevated levels of vWF:Ag and soluble E-selectin in young women with PCOS suggest endothelial dysfunction in this group of patients.

Glucose and triglyceride levels are significant factors affecting endothelial function in PCOS.

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