

REVIEWS

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The Roles of Inflammatory Markers, Hemostasis Factors, and Oxidative Stress in the Pathogenesis of Polycystic Ovary Syndrome

Rola czynników stanu zapalnego, czynników hemostazy i stresu oksydacyjnego w patogenezie zespołu wielotorbielowatych jajników

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Abstract

Polycystic ovary syndrome (PCOS) affects 5 to 10% of women in reproductive age. In the pathogenesis of PCOS, hormonal disorders (ovarian and pituitary), insulin resistance and, recently, chronic inflammation are taken into account. The markers of low-grade chronic inflammation which are considered with regard to the inflammatory theory of PCOS are C-reactive protein, interleukin-6, tumor necrosis factor- α , fibrinogen, tissue plasminogen activator, plasminogen activator inhibitor-1, and as well as homocysteine, paraoxonase 1, interleukin-18, procalcitonin, and white blood cell count. Results of the latest studies concerning serum inflammatory markers and their relationships in women with PCOS are presented here (*Adv Clin Exp Med* 2007, 16, 2, 317–322).

Key words: PCOS, inflammation, C-reactive protein, interleukin-6, tumor necrosis factor- α .

Streszczenie

Zespół wielotorbielowatych jajników (PCOS – *polycystic ovary syndrome*) dotyczy 5–10% kobiet w wieku rozrodczym. W patogenezie tego zespołu są brane pod uwagę zaburzenia hormonalne (jajnikowe i przysadkowe), insulinooporność, a ostatnio również przewlekły proces zapalny. Do wykładników stanu zapalnego, rozpatrywanych w kontekście teorii zapalnej PCOS, należą: białko C-reaktywne, interleukina-6, czynnik martwicy nowotworu- α , fibrynogen, tkankowy aktywator plazminogenu, inhibitor pierwszy tkankowego aktywatora plazminogenu, a także homocysteina, paraoksonaza 1, interleukina-18, prokalcytonina i liczba leukocytów we krwi. W pracy przedstawiono najnowsze badania kliniczne, w których oceniano stężenia we krwi oraz wzajemne korelacje markerów stanu zapalnego u kobiet z PCOS (*Adv Clin Exp Med* 2007, 16, 2, 317–322).

Słowa kluczowe: PCOS, zapalenie, białko C-reaktywne, interleukina-6, czynnik martwicy nowotworu- α .

Polycystic ovary syndrome (PCOS) was described in 1935 by Stein and Leventhal [1]. Since then, many studies have been carried out, but the pathogenesis of PCOS remains insufficiently explained. PCOS is a very common disease, affecting 5–10% of women in reproductive age. Hormonal abnormalities, i.e. excessive androgen production by the ovaries and adrenal glands as well as disturbances in gonadotropin secretion by the pituitary gland, are the best described manifestations. Whether the primary defect involves

the regulation of gonadotropin secretion or steroidogenesis in the gonads is not at all clear. Insulin resistance plays an important role in the pathogenesis of PCOS. Kahn in 1976 [2] and Burghen in 1980 [3] were the first to draw attention to the relationship between PCOS and hyperinsulinism. Although there are numerous reports showing that insulin resistance commonly exists among women with PCOS, neither the Rotterdam nor the NICHD PCOS criteria mentions hyperinsulinism or insulin resistance [4].

Nowadays, low-grade chronic inflammation is considered to be at the basis of PCOS. The question whether chronic inflammation is an independent factor of PCOS development or rather secondary to obesity and insulin resistance, which are common in women with this syndrome, is still open. The most known factors examined in women with PCOS within the context of the inflammatory theory are C-reactive protein (CRP), interleukin-6 (IL-6), and tumor necrosis factor- α (TNF- α), with their receptors. More and more is being written about fibrinogen, tissue plasminogen activator (t-PA), and plasminogen activator inhibitor-1 (PAI-1) as coagulation and fibrinolysis parameters, but also chronic inflammatory factors. The latest markers of inflammation in PCOS are procalcitonin, white blood cell count, and interleukin-18. Two other substances connected indirectly (with chronic inflammation) and directly (with oxidative stress) are homocysteine and paraoxonase 1 (PON-1), which are also considered to be factors playing a role in the pathogenesis of PCOS (Tab. 1).

Table 1. Inflammatory markers in PCOS

Tabela 1. Czynniki stanu zapalnego w PCOS

Inflammatory marker (Czynnik stanu zapalnego)	Position in references (Literatura)
C-reactive protein (Białko C-reaktywne)	5, 9, 10, 11, 12
Tumor necrosis factor- α (Czynnik martwicy nowotworu α)	21, 22, 23, 24, 25, 26
Interleukin-6 (Interleukina 6)	16, 17, 18, 19
Interleukin-18 (Interleukina 18)	7
Tissue plasminogen activator (Tkankowy aktywator plazminogenu)	27
Plasminogen activator inhibitor-1 (Inhibitor pierwszy tkankowego aktywatora plazminogenu)	28, 29, 30
Homocysteine (Homocysteina)	35, 36, 37, 38
Paraoxonase 1 (Paraoksonaza 1)	39
Procalcitonin (Prokalcytonina)	5
White blood cell count (Liczba leukocytów we krwi)	6

C-reactive Protein

C-reactive protein is a classic acute-phase protein synthesized in the liver. The concentration of this protein increases in response to infection, hypoxia, trauma, burn, and inflammation [8].

A moderate increase in CRP level is observed in liver ailments and autoimmune and neoplastic diseases. Cigarette smoking, obesity, and aging are connected with small CRP increases as well. CRP is a valuable marker of chronic inflammation and a prognostic cardiovascular risk factor independent of other atheromatous risk factors. It is interesting that IL-6 is an essential stimulator of CRP synthesis, and TNF- α together with IL-1 stimulate IL-6 production [8].

Many studies reveal elevated serum CRP levels in women with PCOS. Kelly et al. observed substantially increased CRP concentrations in PCOS women compared with a control group appropriately matched regarding age and body mass index (BMI) [9]. CRP level correlated positively with BMI in the PCOS and the control groups, but negatively with insulin sensitivity in both groups. The authors demonstrated that CRP concentration is dependent on BMI, but is higher in PCOS women independently of BMI. Talbott et al. measured CRP levels in women with PCOS and the relationship between CRP and carotid intima-media wall thickness [10]. They observed elevated CRP concentrations in PCOS women compared with a control group. BMI was the most important factor which correlated positively with carotid intima-media wall thickness. Boulman and Fenkci also reported increased CRP levels in lean as well as obese women with PCOS [11, 12]. In 2005, Purder et al. observed elevated CRP concentrations in women with PCOS [5]. They indicated that higher CRP level, as a marker of chronic inflammation, is related more with visceral obesity and central fat distribution than with PCOS per se. This is not surprising because fat issue, especially visceral, is a site of expression of pro-inflammatory factors such as IL-6 and TNF- α . An excess of visceral fat may play a key role in linking chronic inflammation with metabolic disorders, including PCOS.

Interleukin-6

IL-6 is a cytokine belonging to the acute-phase proteins and is engaged in inflammatory processes. It is produced by numerous cells: monocytes, macrophages, fibroblasts, endothelium cells, T and B lymphocytes, fat cells, and muscle cells. IL-6 stimulates the proliferation and differentiation of hematopoietic cells and activates the expressions of acute-phase-protein genes (e.g. CRP) in hepatocytes. IL-6 inhibits the activity of lipoprotein lipase and leptin production, intensifies liver synthesis of triglycerides and, consequently, influences fat and carbohydrate metabolism [13].

Serum IL-6 concentration is positively correlated with obesity and insulin resistance and IL-6 expression in adipose tissue with obesity [14–17]. In this context, the role of IL-6 in women with PCOS was examined. In 2002 and 2003, Escobar-Morreale et al. demonstrated a relationship between hyperandrogenism in women with PCOS and the C174G polymorphism in the promotor region of the IL-6 gene and as well as the Gly148Arg polymorphism of the gp 130 gene, which decodes a subunit of the IL-6 receptor [18, 19]. In another study, Mohling et al. measured IL-6 and CRP levels as markers of chronic inflammation and parameters of hyperandrogenism, insulin resistance, and obesity in women with PCOS and an appropriate control group [20]. There was no difference in IL-6 and CRP levels between the two groups. When the women were divided into two groups, i.e. those with BMI > 25 kg/m² and those with BMI < 25 kg/m², IL-6 and CRP concentrations were significantly higher in the women with BMI > 25 kg/m² in both the PCOS and the control groups. The authors indicated a significant relationship between IL-6 and CRP levels and parameters of obesity and insulin resistance (BMI, waist-to-hip ratio, the HOMA homeostasis model, and the quantity of fat tissue measured by the DEXA method). They did not observe correlation between IL-6 and CRP levels and hormonal parameters of PCOS, i.e. levels of testosterone, androstenedione, and dehydroepiandrosterone sulfate and the luteotrophin-to-folliculin ratio. The authors concluded that obesity and metabolic disorders activate chronic inflammation in women with PCOS, whereas there is no direct relationship between low-grade chronic inflammation and PCOS per se.

Tumor Necrosis Factor- α

TNF- α is another inflammatory marker examined in women with PCOS. It is a cytokine which plays a role in the defense of the organism in inflammatory processes and neoplastic disease. TNF- α is produced, among others, by macrophages, monocytes, T and B lymphocytes, and fibroblasts, but it is also synthesized in adipocytes and smooth and skeletal muscle cells, where it influences lipid and carbohydrate metabolism in autocrine and paracrine ways. TNF- α plays an important role in insulin resistance by insulin receptor substrate-1 (IRS-1) inactivation and then by serine phosphorylation induction. Inactive IRS-1 is an inhibitor of insulin receptor tyrosine kinase. TNF- α may also worsen insulin sensitivity by stimulating lipolysis and increasing free fatty-acid release [21]. TNF- α expression in fat tissue corre-

lates with obesity and insulin resistance [22]. Some studies found elevated serum TNF- α levels in obese as well as in non-obese women with PCOS. Gonzalez et al. found significantly higher TNF- α concentrations in PCOS women of normal weight than women from an appropriately matched control group [23]. TNF- α levels in the obese PCOS and the control women were similar and statistically higher than in the non-obese women with PCOS. Sayin et al. also demonstrated elevated serum TNF- α levels in non-obese women with PCOS [24]. In both studies the authors observed no significant correlation between TNF- α concentration and levels of testosterone, dehydroepiandrosterone sulfate, luteotrophin, and fasting insulin. The results of these reports show that there are other factors besides obesity and insulin resistance which are responsible for elevated TNF- α levels in non-obese women with PCOS.

The role of TNF- α in the pathogenesis of PCOS is interesting. In animals, TNF- α influences increased secretion of ovarian steroids, ovulation disorders, and ovary cell apoptosis. In the opinion of some researchers, the disorders observed in animals may be similar to those in hyperandrogenic women with PCOS [25]. As shown in the report by Escobar-Morreale et al. carried out in hyperandrogenic (including PCOS) women, TNF- α plays a role in the pathogenesis of hyperandrogenism independently of obesity and insulin resistance [26].

Tissue Plasminogen Activator and Plasminogen Activator Inhibitor-1

The levels of other inflammatory markers, i.e. the hemostasis parameters fibrinogen, t-PA and PAI-1, were also determined in women with PCOS. Kelly et al. showed increased t-PA levels in women with PCOS compared with an appropriately matched control group [27]. T-PA level correlated positively with BMI and negatively with insulin sensitivity in both the PCOS and the control group. When comparing women of the PCOS and of the control group with similar BMI and insulin sensitivity, the t-PA level was significantly higher in the women with PCOS. The authors concluded that elevated t-PA level and dysfibrinolysis may be cardiovascular risk factors in women with PCOS. PAI-1 was a more precisely examined marker of coagulation and fibrinolysis in women with PCOS. Atiomo et al. demonstrated in 1998 increased fibrinogen and PAI-1 levels in women with PCOS in comparison with a control group [28]. But in other study in 2000 the same authors

did not demonstrate elevated PAI-1 activity in women with PCOS compared with an appropriately matched control group [29]. Very interesting were the results of Glueck et al., who revealed that increased PAI-1 level was an independent risk factor of recurrent miscarriages in women with PCOS [30]. Higher PAI-1 concentration leads to fibrinolytic disorders and microthrombus formation in placenta, which results in miscarriages and infertility.

The Role of Oxidative Stress

Oxidative stress plays a role in the development of insulin resistance and hyperandrogenism in polycystic ovary syndrome. This state is described as an increased activity of oxygen free radicals which develops as a result of disturbances in the balance between the production and removal of toxic oxygen derivatives. Imbalance disorders between the oxidant and antioxidant systems in favor of oxidative stress have been observed in women with PCOS. In 2005, Gonzalez et al. reported increased activity of reactive oxygen species in obese and non-obese women with PCOS compared with a control group [31]. Oxygen free-radical levels correlated positively with serum testosterone and androstendione concentrations, which strongly speaks for a stimulation of hyperandrogenism by oxidative stress. In *in vitro* studies it was demonstrated that ovarian androgen production was stimulated by oxidative stress and inhibited by antioxidant compounds, such as statins. It cannot be excluded that in inhibiting androgen synthesis, statins act not only by an antioxidant mechanism, but also or primarily by diminishing the global cholesterol pool, which is the substrate for androgen synthesis.

TNF- α links oxidative stress with hyperandrogenism and insulin resistance. Reactive oxygen compounds stimulate mononuclear cells and fat cells to increased TNF- α synthesis and release. Moreover, oxygen free radicals lead to activation of the pro-inflammatory transcription factor nuclear factor κ B, which promotes TNF- α transcription [32]. At the basis of the insulin resistance induced by oxidative stress lies serine kinase activation and phosphorylation of, among others, the insulin receptor and IRS-1 [33, 34].

Homocysteine

Elevated serum homocysteine level is a common marker of chronic inflammation and oxidative stress and an independent risk factor of atherosclerosis in women with PCOS. Hyperhomo-

cystinemia directly leads to increased risk of cardiovascular diseases by damage to endothelial cells, disturbances in platelet function, and mobilization of the oxidative stress cascade. In three independent studies, Yarali [35], Vrbikova [36], and Loverno [37] found elevated homocysteine levels in women with PCOS. They did not observe a relationship between homocysteine level and insulin sensitivity. In another report, Bayraktar et al. demonstrated not only increased serum homocysteine concentrations in women with PCOS, but also significantly higher homocysteine levels in women with insulin resistance compared with women without [38]. The question about the role of homocysteine in PCOS pathogenesis is still open.

Paraoxonase 1

PON-1 is a serum antioxidative enzyme. Diminished PON-1 level leads to increased risk of atherosclerosis and, consequently, to cardiovascular disease [39]. Pro-inflammatory factors as well as androgen excess lead to lower PON-1 expression, which results in increased oxidative stress. Taking into account that oxidative stress disturbs insulin function and its role in the development of insulin resistance, diminished serum PON-1 activity has an essential relationship with the disorders in carbohydrate metabolism observed in women with PCOS [39].

The authors conclude that low-grade chronic inflammation is connected with the development of metabolic syndrome and cardiovascular diseases. Positive correlations exist between inflammatory markers and insulin resistance [9, 15] and obesity [16, 21]. Fat tissue plays a key role in this relationship (Fig. 1). Increased expressions of pro-inflammatory cytokines are observed in the fat tis-

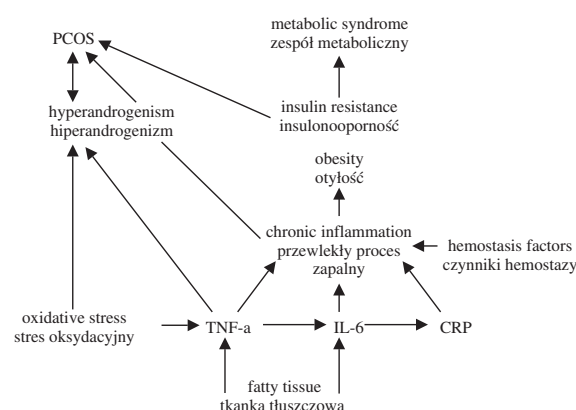


Fig. 1. Relationships between adipose tissue, inflammatory markers, hemostasis factors, metabolic syndrome and PCOS

Ryc. 1. Wzajemne powiązania między tkanką tłuszczową, wykładnikami stanu zapalnego, czynnikami hemostazy, zespołem metabolicznym i PCOS

sue of obese people [15, 20], directly inducing insulin resistance by influencing the pathway of changes released by insulin binding with its receptor. On the other hand, inflammatory factors activating the hypothalamo-pituitary-adrenal axis favor central obesity and secondary insulin resistance [17, 34]. In recent reports, authors have revealed elevated levels of low-grade chronic inflammatory factors and substances related to

hemostasis and oxidative stress in women with polycystic ovary syndrome. In some of these reports, increased levels of inflammatory markers were not related with obesity or insulin resistance. To now it is difficult to answer the question whether chronic inflammation is an independent cause of PCOS or plays a role in the pathogenesis of this syndrome only through its relationship with obesity and insulin resistance.

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