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Thyroid disorders in polycystic ovary syndrome

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Abstract. – OBJECTIVE: Thyroid disorders, especially Hashimoto's thyroiditis (HT), are observed significantly more often in patients with polycystic ovary syndrome (PCOS) than in the general population – approximately 27% and 8%, respectively. This is extremely important in young women, because both disorders are connected with fertility problems. As HT and PCOS occur together, fertility problems may become a serious clinical issue in these patients.

MATERIALS AND METHODS: A systematic literature review in PubMed of PCOS- and HT-related articles in English, published until December 2015 was conducted.

RESULTS: The reasons for joint prevalence still remain unclear. Genetic and autoimmune backgrounds are recognized to be possible common etiological factors. Three genetic polymorphisms have been described to play a role in PCOS as well as in HT. They are polymorphism of the gene for fibrillin 3 (FBN3) regulating the activity of transforming growth factor-b (TGF-β) and regulatory T cell levels, gonadotropin-releasing hormone receptor (GnRHR) polymorphism and CYP1B1 polymorphism standing for estradiol hydroxylation. High estrogen-to-progesterone ratios owing to anovulatory cycles, as well as high estrogen levels during prenatal life, disrupt development of the thymus and its function in maintaining immune tolerance, and are suspected to enhance autoimmune response in PCOS. Vitamin D deficiency could be also involved in the pathogenesis of HT and PCOS.

CONCLUSIONS: The above-mentioned common etiological factors associated with fertility problems in HT and PCOS require further research.

Key Words:
Hypothyroidism, Hashimoto's disease, Polycystic ovary syndrome, Fertility disorders.

Introduction

Polycystic ovary syndrome (PCOS) is the most common female endocrinopathy in women of reproductive age. It is characterized by a wide range of symptoms, which can occur in different combinations and with different intensity. First of all, they are: ovarian dysfunction, which can cause rare menstruation or absence of it, hyperandrogenism and/or hyperandrogenemia, polycystic ovary/ovaries on gynaecological ultrasound[1] and, in many cases, disorders such as insulin resistance, hyperinsulinemia, obesity (especially the central type of obesity), hypertension, nonalcoholic steatohepatitis (NASH) and, finally, fully developed metabolic syndrome.

The prevalence of PCOS is noted in 8-12% of women of reproductive age[1]; however, in the most current Danish research[2] from 2014, it was set at up to 16.6%. These numbers vary because of the different diagnostic criteria. Nowadays, the most widely accepted criteria are the Rotterdam criteria from 2003[1]. According to them, the diagnosis is set if any two out of three criteria are met, in the absence of other entities that might cause these findings:

1) Oligoovulation and/or anovulation.
2) Signs of androgen excess (clinical or biochemical).
3) Polycystic ovaries (one or two) on gynecological ultrasound.

Following these criteria, four phenotypes of PCOS have been marked out: phenotype A, when all three criteria are fulfilled and phenotypes with two out of three fulfilled criteria: menstrual disorders and hyperandrogenism, hyperandrogenism

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and polycystic ovaries on ultrasound and finally menstrual disorders and polycystic ovaries on ultrasound. The heterogeneity of these phenotypes is the main cause of difficulties in determining clear pathogenesis, as well as causal treatment of PCOS.

Thyroid disorders may occur with thyroid hormone deficiency (hypothyroidism), thyroid hormone excess (hyperthyroidism) or with thyroid hormones within the normal range (euthyreosis). Hashimoto's thyroiditis (HT) also called chronic lymphocytic thyroiditis is the most frequent cause of hypothyroidism and also the most frequent autoimmune disorder. The diagnosis is made by detecting elevated levels of anti-thyroid peroxidase antibodies (anti-TPO) and/or antithyroglobulin (anti-Tg) in the serum and a characteristic thyroid image on ultrasound – hypoechoic or heterogeneous echotexture. The gold standard in the diagnosis of HT is lymphocytic infiltration and fibrosis found on histological examination; however, nowadays it is not performed routinely. HT may occur with clinical hypothyroidism (most often), euthyreosis or hyperthyroidism.

According to the National Health and Nutrition Survey (NHANES) – the biggest epidemiological research conducted in the USA on a group of 17 000 subjects – the frequency of hypothyroidism with a thyroid-stimulating hormone (TSH) level above 4.5 mIU/L in women of reproductive age is approximately 4%[3]. In research on the incidence of thyroid disorders, conducted in Colorado on 25 000 individuals, the percentage of hypothyroidism increased with age[4]. Therefore, the most probable thyroid disorder among young women is HT with normal thyroid function and elevated levels of anti-TPO and/or anti-Tg. The prevalence of anti-TPO and anti-Tg in the age group of 20-29 years is 11.3% and 9.2% respectively, while in the age group of 30-39 years it is 14.2% and 14.5%, respectively.

NHANES revealed that the prevalence of hyperthyroidism in the eastern population is low[3]. TSH below 0.4 mIU/L was observed in 3% of subjects of reproductive age.

Hypothyroidism has an important impact on women's reproductive health. In the prepubertal period, it can lead to delayed puberty. In adult women hypothyroidism, as well as hyperthyroidism, is considered to be the cause of menstrual disorders and decreased fertility as a consequence. Women with undiagnosed PCOS are often referred for thyroid examination. On the other hand, overt hypo- or hyperthyroidism, accompanied by menstrual disorders, may suggest the symptoms of PCOS.

The presence of receptors for signalling pathways (thyrotropin-releasing hormone – TRH, TSH) and thyroid hormones has been demonstrated in animal studies in monkey uterus. Moreover, the impact of long-term estrogen and estrogen-progestogen treatment on their expression has been evidenced[5]. The expression of TSH, TRα1 and TRβ1 receptors has been stated in the human endometrium as well[6]. The highest TRα1 and TRβ1 receptor expression were found in receptive endometrium before ovulation. Thyroid hormones adjusted endometrial synthesis of protein mRNA, among others leukemia inhibitory factor (LIF), which is important during the implantation process, and glucose transporter 1 (GLUT1)[7]. Free triiodothyronine (fT_3) has been detected in follicular fluid[8]. TSH and its receptors' transcripts have been revealed not only in ovarian structures such as oocytes, cumulus oophorus cells, granulosa cells and ovarian epithelium, but also in syncytiotrophoblast villi[8]. Moreover, in the placenta there are present membrane transporters for triiodothyronine (T_3) and thyroxine (T_4), deiodinase enzymes type 2 and 3, which regulate thyroid hormone activity in the placenta, and transthyretin – a protein binding thyroid hormones and retinol. *In vitro* research on bovine oocytes and embryos suggests that thyroid hormones play a role in the implantation process. Oocytes and embryos mentioned above, cultured in medium enriched with thyroid hormones, presented better outcomes regarding blastocyst formation, implantation capacity, decreased apoptosis rate and vitality after cryopreservation[9].

Overt hypothyroidism is accompanied with TRH increase, which causes hyperprolactinemia, luteinizing hormone (LH) pulsatile secretion disruption, sex hormone-binding globulin (SHBG) synthesis decrease, estrogen peripheral metabolism disruption and increase of ovarian androgen production. In research on female pigs, hypothyroidism resulted in increased gonadotropin receptor sensitivity in the ovary, which finally promoted ovarian hypertrophy, as well as multiple ovarian cyst formation[10]. Hypothyroidism may contribute to heavy, irregular menstrual bleeding, spotting during the menstrual cycle, insufficient endometrial thickness, ovulation disorders and, in consequence, endometrial proliferative phase disorders.

Sex hormone imbalance has likewise been described in hyperthyroidism. It covers: SHBG

synthesis increase, estrogen metabolism disruption, increased estrogen to androgen conversion, higher basal gonadotropin-releasing hormone (GnRH) concentration and stronger pituitary to GnRH response. Menstrual disorders have been also observed, while in women in euthyreosis ovulation remains regular.

Taking into consideration the high prevalence of both PCOS and HT in women of reproductive age, common possible etiological and clinical associations between them are discussed in the following article. A systematic literature review in PubMed of PCOS- and HT-related articles in English, published until June 2016 was conducted.

PCOS, HT and Female Fertility Disorders

Decreased fertility is associated with HT and PCOS, though it seems that in patients with these two diseases, fertility disorders appear more frequently and are be more pronounced.

Antithyroid antibodies are observed in 5-10% of women of reproductive age[3]. The presence of antibodies alone does not automatically influence thyroid function. Although overt hypothyroidism may manifest with serious reproductive disorders, they should retreat after introducing an appropriate treatment. However, patients with HT in euthyreosis may still have difficulties conceiving or delivering at term[11,12].

Antithyroid antibodies are the most frequent autoimmune findings in infertile couples with at least two consecutive failed *in vitro* fertilization (IVF) attempts[13]. The only research estimating ovarian reserve in HT published so far involved women in the premenopausal period treated with levothyroxine (LT_4). No differences between study and control groups with regard to antral follicle count (AFC), follicle-stimulating hormone (FSH) level and menstrual cycle length were noted[14]. Anti-mullerian hormone (AMH) was another parameter analyzed in this research. AMH is a protein produced by granulosa cells in the ovarian follicle during the reproductive period. The established ranges of AMH allow prediction of the ovarian reserve. Levels below the normal range indicate a decreased ovarian reserve; on the contrary, levels exceeding the norm are characteristic for PCOS. In the research mentioned above, the AMH level was, surprisingly, significantly higher in the Hashimoto's group treated with LT_4 than in controls, whereas in patients with HT in euthyreosis without treatment it was lower than in the control group[15]. It is really interesting, considering the fact that analogical research conducted on patients with autoimmune diseases such as rheumatoid arthritis, spondyloarthritis or Behçet disease revealed a decreased ovarian reserve[16]. Significantly, a lower fT_3, but normal TSH, the level was marked in infertile women with HT treated with LT_4 compared to fertile women with HT during treatment[17]. The researchers elevated the LT_4 dose in the study group in order to obtain a similar fT_3 level in both groups. All 21 women from the study group conceived and delivered at term. This study indicated that the role of fT_3 in infertility requires further investigation and, what is more, that TSH within normal limits does not always express the thyroid hormone state adequately.

In some researches[18,19], autoimmune thyroid diseases were connected with a three- to fivefold increased miscarriage rate. On the other hand, no difference in anti-TPO prevalence was found among women with unexplained recurrent pregnancy loss and the general population. Anti-TPO did not have any predictive value concerning the course of the next pregnancy[20]. In another study[21], preventive LT_4 treatment in women with anti-TPO and normal thyroid function decreased the miscarriage rate from 13.8% to 3.5% and preterm delivery rate from 22.4% to 7%.

The pregnancy rate among infertile women with and without HT who underwent treatment using assisted reproductive technologies (ART) was comparable; however, the miscarriage rate was significantly higher among women with HT – 53% *vs.* 23%[22]. In the Italian research, the pregnancy rate in women who had undergone ART procedures, the group with TSH $\leq$ 2.5 μIU/mL achieved 22.3%, while in the group with TSH > 2.5 μIU/mL it was 8.9%[21]. On the other hand, factors other than autoimmune thyroid disease seemed to be more important in delivering at term after IVF in women older than 38 years, as the prevalence of thyroid disorders in patients who delivered and miscarried did not differ significantly[23]. Similarly, no correlation was found between anti-TPO presence before pregnancy and miscarriage in a small group of patients undergoing IVF. Anti-TPO presence did not decrease the chance of conceiving[24]. The meta-analysis by Thangartinam et al[25] revealed that antithyroid antibodies increase the miscarriage risk three times and preterm delivery risk twice. Finally, LT_4 treatment can reduce these risks by 50%[25]. The precise mechanism which explains how an-

tithyroid antibodies impact fertility has not been elucidated yet. Few hypotheses for antibody presence impact on miscarriage have been suggested. Possibly, women with increased antithyroid antibodies, despite euthyreosis in laboratory findings, may suffer from mild hypothyreosis, or these antibodies are a secondary manifestation of a general immune imbalance. Another explanation could be the fact that women with HT get pregnant at an older age, which is an independent risk factor[13]. Lately, Plowden et al[26] have presented very interesting and strong evidence in the discussion. In a well-designed, prospective cohort study conducted on 1228 healthy women with a history of pregnancy loss, no association between TSH levels ≥ 2.5 mIU/L (free T_4, fT_4, within normal parameters) or the presence of antithyroid antibodies with fecundity, pregnancy loss or live birth was shown.

In 2012, the Endocrine Society published Guidelines for Thyroid Dysfunction in Pregnancy[27]. According to them, universal screening of healthy women for thyroid dysfunction, as well as the presence of anti-TPO antibodies, before and during pregnancy is not recommended. In the case of screening for thyroid disorders in newly pregnant women, the committee is incoherent. Some members recommend screening of all pregnant women by the ninth week or at the time of their first visit. Others recommend targeted screening, among others, in women: over age of 30 years, with thyroid antibodies, with other autoimmune diseases, with a prior history of miscarriage or preterm delivery and, finally, with infertility. If serum TSH is greater than 2.5 mIU/L in the first trimester or above 3 mIU/L in the second and third trimesters, LT_4 therapy should be instituted. If the hypothyroidism has been diagnosed before pregnancy, adjustment of LT_4 dose to reach serum TSH not higher than 2.5 mIU/L before pregnancy is recommended. However, in the case of nothing except antithyroid antibody presence in pregnancy, LT_4 treatment is not advised. During pregnancy and breast feeding, suggested iodine intake should reach 250 μg per day[27].

Fertility disorders are a characteristic feature of PCOS. Ovulation disorders are diagnosed in 75-85% of PCOS patients. It is estimated that only 30% of cycles are ovulatory in this group of patients[26]. PCOS constitutes the most frequent cause of female infertility due to the lack of ovulation. In women with PCOS time to conceive is significantly longer than in the general population and 40-70% of them are diagnosed with infertility (defined as lack of conception after one year of regular unprotected sexual intercourse). Decreased fertility is a consequence of ovarian dysfunction, which is influenced by obesity, metabolic disorders, chronic inflammation and hormonal imbalance[28]. Excessive androgen concentration in the ovary may contribute to premature luteinization of granulosa cells; further discrepancy in paracrine secretion of growth factors in the ovary may inhibit oocyte maturation[28]. An abnormally high LH concentration during the whole menstrual cycle has a very negative impact. It is supposed to cause incomplete ovarian follicle maturation during the first phase of the cycle and poor endometrial receptiveness in the luteal phase[29]. Metabolic disorders at a cellular level during oocyte maturation are also underlined as a possible cause contributing to anovulation. *In vitro* research revealed that granulosa cells of PCOS patients without ovulation showed poor glucose uptake, which can aggravate their energetic supply and worsen oocyte quality in consequence.

Women with PCOS undergo treatment using ART techniques more frequently compared to the general population (13.7% *vs.* 1.5%)[30]. Most of the research on miscarriage risk and recurrent pregnancy loss in PCOS was conducted before setting the Rotterdam criteria in 2003. The data consistent with actual criteria are scarce – according to the only research, PCOS patients constitute 10% of women with recurrent pregnancy loss[31]. On the contrary, a Japanese team[32] stated that PCOS is not a predictive factor of subsequent miscarriage in cases of recurrent pregnancy loss. Factors promoting miscarriages in PCOS are responsible for an increase of prothrombotic activity: insulin resistance, hyperinsulinemia, hyperandrogenemia, hyperhomocysteinemia and genetic polymorphisms: plasminogen activator inhibitor 1 (PAI-1) gene locus 4G/5G polymorphism, responsible for higher PAI-1 concentration in serum, and angiotensin-converting enzyme (ACE D/I) polymorphism[33]. A significantly lower miscarriage rate has been observed in a group of 120 pregnant women receiving metformin during the whole pregnancy (11.6% compared to 36.3% in the control group)[34]. A lower miscarriage rate was also marked in a small group of pregnant PCOS patients with thrombotic risks, who received low-molecular-weight heparin and metformin[35]. Nonetheless, it is essential to conclude that it is not a recommended treatment according to current management in PCOS[36]. Women with PCOS present a higher risk of developing ges-

tational diabetes, pregnancy-induced hypertension and preterm delivery, both in spontaneous pregnancies and after ART technique use[37]. Fetal growth abnormalities: small for gestational age (SGA) and large for gestational age (LGA), most likely due to insulin resistance, are also diagnosed in these patients more frequently[30]. The risk of preterm delivery is strongly correlated with the presence of pregnancy-induced hypertension. Obesity extends the average time to conceive as well as preterm delivery risk[38]. The impact of hyperandrogenism on the course of pregnancy was studied in two Danish researches. A higher risk of preterm delivery and preeclampsia in PCOS patients with hyperandrogenism was revealed in the first one[39], whereas no difference between studied and control groups was observed in the second study[40].

Despite the fact that both endocrinopathies discussed in this review contribute to reproductive health disorders, their combined influence on fertility has not been investigated so far. The only case-control study revealed that patients with PCOS and increased anti-TPO level are at risk of clomifene citrate resistance with an odds ratio equal to 7.7[41].

Metabolic Risks

Metabolic changes observed in HT and PCOS are heterogeneous; however, they are often related. They generally cover higher BMI, glucose and lipid abnormalities.

Metabolic disorders in Hashimoto's disease are more pronounced in patients with overt or subclinical hypothyreosis than in euthyroid patients on T_4 replacement therapy[42]. It seems then that they are primarily connected with thyroid dysfunction and improve with normal hormone levels[42]. It is confirmed that total cholesterol level, triglycerides, LDL and non-LDL cholesterol rise with TSH concentrations, within TSH normal ranges as well[43]. Moreover, a cross-sectional study unveiled that BMI correlated positively with TSH serum concentration and negatively with fT_4 level. Weight gain in a 5-year period, which corresponded with a BMI increase of 1%, also correlated with thyroid function. The impact of thyroid function on BMI was equal to smoking and physical activity[44].

Metabolic changes in PCOS are well known and widely reported[45]. In HT metabolic changes result from thyroid dysfunction degree, in the same way, insulin resistance degree and the presence of other cardiovascular risks depend on PCOS phenotype. Women with PCOS with regular cycles present notably better metabolic parameters (including BMI, glucose fasting level, HOMA-IR index) than women with oligo- and amenorrhoea[46].

The latest published[47] prevalence of metabolic syndrome in PCOS was estimated at 11.9%. In the Swedish study[48], in a group of 43-year-old women with PCOS with average BMI 28.3 kg/m^2, during 14 years of follow-up, only 23% of them developed metabolic syndrome. Possibly this is associated with a usually lower BMI in the female population in Sweden. TSH concentration in PCOS patients correlates with BMI, waist circumference, diastolic blood pressure, LDL cholesterol and triglycerides level corresponding with the general population[49]. The differences concerning BMI and HOMA-IR were recognized in PCOS patients with TSH between 2 and 4.5 mIU/L and below 2 mIU/L. HOMA-IR in both groups was independent of BMI and age[50].

HT and PCOS coexistence may exacerbate metabolic changes in comparison to disturbances present in the single disease. In fact, girls suffering from both PCOS and HT had higher values of: BMI, fasting glucose level, HOMA-IR index and cholesterol compared to girls with HT only or a control group[51]. PCOS features correlated negatively with fT_4 quartiles[51]. Also, women with PCOS and subclinical hypothyreosis had higher levels of triglycerides, fasting insulin and HOMA-IR than women with PCOS alone. Both groups did not differ with regard to total cholesterol, HDL and LDL cholesterol levels[52].

An interesting report concerning the use of metformin in obese patients with PCOS and hypothyreosis has appeared. These patients were prescribed 1500 mg of metformin daily or placebo for 6 months[53]. A significant decrease in TSH levels was observed in the metformin group, but not in the placebo group, after 6 months of metformin treatment. No significant change in fT_3 and fT_4 was observed throughout the study in any group[53]. The authors speculate that metformin could have a possible central mechanism of action.

Joint Prevalence

The first systematic, prospective study[54] on the prevalence of autoimmune thyroid diseases in PCOS, published in 2004, involved 175 patients with PCOS and 168 healthy women, with an average age of 28 years; 26.9% of patients with PCOS and 8.3% of women in the control

group had elevated concentrations of anti-TPO and/or anti-Tg. The patients in the PCOS group presented significantly higher antibody and TSH levels as well. The characteristic features of HT on ultrasound were observed in 42.3% of PCOS patients and only in 6.5% of subjects in the control group. Finally, HT, meeting both diagnostic criteria, was three times more likely to occur in PCOS women (in 20.6% and 6.5%, respectively)[54]. In the most recent study[55], the higher prevalence of HT in PCOS was confirmed – HT was diagnosed in 27% of PCOS patients (with an average age of 24 years) compared to 8% of controls. An interesting aspect of HT risk in PCOS patients was underlined in the Brazilian study. The prevalence of autoimmune thyroid disease in 65 women with PCOS and 65 without PCOS was estimated as 43.1% and 26.2%. However, after excluding the impact of weight and insulin resistance, no greater risk in the PCOS group was found. Thus, weight and insulin resistance have been recognized to be independent risk factors for developing HT[56]. Eventually, the authors of the meta-analysis from 2013, on the relationship between thyroiditis and PCOS, which involved six studies, stated that the prevalence of AIT, serum TSH, anti-TPO and anti-Tg positive rate in PCOS patients were all significantly higher than those in control groups[57].

The issue of PCOS prevalence in HT patients has been considered in only one research so far. The PCOS incidence in girls of 13-18 years of age with HT was significantly higher than in a control group without anti-TPO (46.8% and 4.3%, respectively)[51].

Joint Etiology and Pathogenesis

The etiology of HT and PCOS is complex and not yet fully clarified. In HT etiology the role of genetic, immune and environmental factors as well as iodine insufficiency/excess is underlined. It is believed that PCOS has a polygenic background. Genetic factors are responsible for susceptibility to the disease and subsequent environmental influence, mainly high-calorific diet and scarce physical activity, stimulate the development of the disease. Regarding PCOS, the role of autoimmune factors is also discussed.

The possible common genetic and autoimmune factors, including the influence of genetic polymorphisms and other factors like tumor growth factor-β (TGF-β), regulatory T cells (Tregs), the thymus, vitamin D deficiency and sex hormone disorders are discussed in the following section.

Genetic Susceptibility

Studies on the families and siblings of patients with HT revealed strong hereditary susceptibility to the disease. The HT risk in children and siblings of a patient with HT increases 32 and 21 times, respectively, with predominance in women[58]. Several genes related to development, progression and symptom aggravation have been found. They are: human leukocyte antigens (HLA-DR), cytotoxic T-lymphocyte-associated protein 4 (CTLA4), CD40, protein tyrosine phosphatase 22 (PTPN22), interleukin 2 receptor alpha (IL2RA) and vitamin D receptor (VDR)[59,60]. The gene for Tg is the only thyroid-specific gene among them[59].

Familial occurrence of PCOS is widely recognized. In first-degree relatives, a more frequent presence of PCOS features has been documented[61]. Among genes suspected to influence PCOS development are: fibrillin 3 (FBN3), insulin, insulin receptor, insulin receptor substrate 1, transcription factor 7-like 2, calpain 10, the fat mass- and obesity-associated protein SHBG and VDR[61]. However, the results of research on most of them are inconclusive. Lately, in genome-wide association studies (GWAS), the DENND1A gene, coding a protein participating in endosomal membrane transport, was regarded as a susceptibility gene for PCOS for the Asian population[61].

The role of Genetic Polymorphisms

The FBN3 polymorphisms, by affecting TGFß activity, are taken into consideration in both PCOS and HT pathogenesis. TGFß is secreted by numerous cells, including macrophages, and influences cell recognition, proliferation and apoptosis[62]. FBN1, FBN2 and FBN3 are genes coding for fibrillins – components of the extracellular matrix which enable TGF-β binding[63]. As long as TGF-β is bound by fibrillins, it remains inactive, until its release allows proper TGF-β activity. Despite the fact that, according to GWAS, none of the TGF-β signalling pathway proteins are crucial in PCOS, the role of TGF-β activity is considered in PCOS pathogenesis, especially in prenatal programming of PCOS[62]. High FBN3 expression was observed in fetal tissues, comprising ovarian stroma as well[62]. After the first trimester of pregnancy FBN3 expression in ovarian stroma disappears. In this manner, FBN3 regulates the activity of TGF-β, which takes part in ovarian development and function during prenatal life[64,65].

Higher TGF-β1 levels were shown in women with PCOS, apart from women with PCOS carry-

ing allele 8 (A8) of D19S884 in the FBN3 gene. The TGFß1 level in these women did not differ from findings in healthy women without the A8 mutation[63]. Thus, according to previous findings the FBN3 polymorphism determines whether women with PCOS present normal or increased TGF-β1 levels. Additionally, A8 carriers with PCOS had higher aldosterone concentrations compared with A8-negative women with PCOS[63]. As the TGF family influences cell proliferation and apoptosis, a higher TGFß1 concentration during fetal life would explain collagen accumulation and fibrosis in the ovarian stroma of a polycystic ovary. TGF-β is one of the molecular mediators playing a role in vessel remodelling, leading to atherosclerosis and renin-angiotensin-aldosterone system activation contributing to hypertension. Both disorders are observed in the course of PCOS[66]. The results of vitamin D3 supplementation on TGF-β1 bioavailability in PCOS have been shown. In the group supplemented with vitamin D, TGF-β1 bioavailability decreased and so did triglycerides, the Ferriman-Gallwey score and menstrual cycle length[67]. This research unveils the possibility of a new therapeutic approach for PCOS patients with increased TGF-β1 concentrations (A8-negative). They could benefit from anti-TGF drug implementation, which certainly will be the issue of coming studies.

In HT lower TGFß1 serum concentration has been found than in controls. Moreover, there was no increase after LT_4 treatment, which indicates a rather closer relationship with HT than with hypothyreosis per se[64,68]. TGFß takes part in immune tolerance development – it enforces FOXP3 protein expression and subsequent Treg formation[58]. Therefore, TGF-β is supposed to be involved in the onset autoimmune diseases, for example, HT. A hypothesis that women with PCOS carrying A8 of D19S884 in the FBN3 gene have lower TGFß1 levels, and thereby are more prone to HT development than women with A8-negative PCOS, deserves consideration and further investigation[64].

A relationship between the 3'-UTR variant rs1038426 of gonadotropin-releasing hormone receptor (GnRHR) and insulin secretion, insulin sensitivity, insulin and TSH serum concentrations in PCOS has been evidenced. Following this hypothesis insulin secretion, insulin resistance and thyroid function in PCOS could be regulated by GnRHR polymorphisms[69].

The CYP1B1 gene is related to PCOS and the enzyme which oxidizes estradiol to 4-hydroxyestradiol. CYP1B1 L432V (rs1056836) is associated with T_4, fT_3 and fT_4[70], which may represent a third link between genetic polymorphisms in PCOS and thyroid function.

The Role of the Thymus

Self-tolerance, which maintains and counteracts autoimmunity, is enabled by two mechanisms. The first one is based on the central immune tolerance and involves the destruction of autoreactive T cells in the thymus during prenatal life. The second one is connected with peripheral immune tolerance, in which Tregs play the key role[71]. Tregs originate from the thymus and naïve peripheral T cells. Their role is to suppress the immune system and to prevent an excessive immune response[71]. An experimental mouse model of autoimmune thyroid disease revealed that CD4+ CD25+ FOXP3+ Tregs take part in breaking the excessive autoimmune reaction[72]. TGFß induces FOXP3 expression, which afterwards stimulates Treg formation[72]. According to the TGFß1 role discussed above, lower Treg levels should be expected in HT than in healthy subjects.

Animal models have shown the influence of estrogens on immune factors taking part in PCOS pathogenesis. Estrogen injections in female mice before the tenth day of gestation, a period when the thymus matures, resulted in anovulation and polycystic ovaries[73]. In mice which were deprived of the thymus before the injection and given mature thymocytes afterwards, no polycystic ovary structure was observed[74]. When high doses of estrogens did not affect the maturing thymus, and developed thymocytes were supplied later ovulation, as well as proper ovary structure, was preserved. After estrogen injection, mice with an intact thymus had significantly lower thymocyte levels than controls[74]. It is believed that estrogens affecting the maturing thymus lead to cessation of Treg production, which among other things results in the formation of numerous ovarian follicles. This thesis supports the theory of autoimmune factors in the etiology of PCOS[64,74].

A second important conclusion from the study mentioned above is that estrogens must act during a proper time window in order to cause irreversible PCOS symptoms[75]. At the time, they induce steroidogenic enzyme activity in the ovary[76]. In the following research, rats were given a prenatal estrogen, testosterone or dihydrotestosterone injection. Interestingly, polycystic ovaries and similar hypothalamic neurotransmitter secretion were observed after both estrogen and testosterone injections, but not after dihydrotestosterone

ones[77]. Whether it can be simply attributed to the aromatization reaction still remains a question.

In women who were prescribed diethylstilbestrol (DES) in the USA in the years 1940-1970, the highest infertility rate was observed in women exposed between the ninth and twelfth week of prenatal life[78], the period of the most intense thymus development. Higher prevalence of autoimmune diseases was also noted[79].

Considering the issue of fetal PCOS programming, a different hypothesis – hyperandrogenization – cannot be omitted. In patients with congenital adrenal hyperplasia, polycystic ovaries are observed more frequently[80]. In sheep and rhesus monkeys submitted to androgenization in prenatal life, disorders characteristic for PCOS were recognized in adult life[81]. Moreover, rats exposed to androgens prenatally had higher values of insulin, leptin and fasting glucose level as well as glucose tolerance test[82]. The impact of prenatal androgenization on DNA epigenetic modifications was assessed in a primate model[83]. The observed changes were, among others, in the TGFß signalling pathway.

The Role of Sex Hormones

Sex hormone participation in the pathogenesis of autoimmunity seems to be reliable, as women suffer from autoimmune diseases significantly more often than men. Five percent of the general population has an autoimmune disease and 78% of them are females. The onset of autoimmune diseases in women is observed earlier, and it often correlates with sex hormone rise[84]. The female to male ratio among subjects with autoimmune thyroiditis is significantly lower in the pre-puberty period in comparison with puberty and adulthood (1.6; 6.7 and 10.3, respectively)[85].

In animal models, estrogens contribute to an increase of B cell activity and to a decline of T cell activity[86]. The production of autoantibodies turned out to be greater in females than males[87]. Estrogens were described to decrease T suppressor cell activity, increase B cell activity, enhance secretion of interleukin 6 (IL6) – a cytokine related to Th2 – and, finally, to shift the immune response to Th2 and antibody secretion[84]. Women present a higher CD4+/CD8+ ratio compared to men[84]. On the other hand, supraphysiological concentrations of estrogens (for example, during pregnancy or oral contraception therapy) turn out to cause immunosuppression. Oral contraception therapy prevented multiple sclerosis exacerbations before menstruation and in the follicular phase[88]. Likewise, an additional estrogen supply correlated negatively with anti-TPO presence[42]. The symptoms of both multiple sclerosis and rheumatoid arthritis tend to be relieved during pregnancy, especially in the third trimester, when estrogen and progesterone levels are the highest[89].

Apart from T suppressor cells, Th1 response and activation of CD8+, androgens reduce the activity of the remaining components of the immune system[90]. Progesterone inhibits macrophage proliferation, IL6 synthesis and peripheral antibody production[90]. It is supposed that progesterone fluctuations during the menstrual cycle correspond to reversible functional changes in the immune system[91].

In the course of the menstrual cycle, higher levels of estrogens during the luteal phase and menstruation and lower levels during the follicular phase lead to a shift of the immunity mediated by T helper cells – from Th1 to Th2 response[92]. In young women during the menstrual cycle the IL6 level (related to Th2) correlated negatively with progesterone level – it was highest in the follicular phase and lowest in the luteal phase[93]. It has been evidenced that IL6 inhibits FOXP3 induction and, as a consequence, Treg formation. The authors concluded that estrogens inhibit and progesterone promotes Treg production[93]. However, other researchers have come to different conclusions – according to them, estrogens promote Tregs, because their number rose in the late follicular phase and declined in the luteal phase[92]. According to most studies dealing with the influence of female sex hormones on Tregs *in vitro*, in turn, both estrogens and progesterone promote Treg synthesis and enhance their function[94]. Additionally, in a mouse model progesterone induced Tregs characterized by increased stability and greater efficiency in suppressing inflammation than Tregs induced by TGFß1[95]. It turned out that androgens also stimulate Treg differentiation, by modulating the expression of FOXP3[89]. A statistically significant correlation occurred around the ovulation period.

Pregnancy is connected with a few immunity regulations in order to ensure immune tolerance to fetal antigens, but mainly with the Th1 to Th2 shift[96]. It is probably a result of the estrogen-induced production of Tregs, with both Th1 and Th2. However, Th2 seem to be less prone to this suppression and tend to prevail in pregnancy[97]. After delivery, the level of Tregs turns down and shifts the immune response to the Th1 type, which often results in autoimmune disease

aggravation[97]. A few large retrospective studies revealed the association between parity and the risk of autoimmune thyroid disease[98].

Women with PCOS usually have similar estrogen levels, higher testosterone and lower progesterone levels compared to healthy women[54]. In PCOS patients with oligomenorrhoea or anovulatory cycles, periods of low progesterone concentration and high estrogen-to-progesterone ratio occur frequently. Considering the previously discussed impact of estrogens and progesterone on autoimmunity, it is justified that this situation may lead to increased susceptibility to autoimmune disorders[54,65]. Androgens, on the contrary, suppress immune system activity; however, it appears that they are not potent enough to overcome estrogen-induced action. Therefore, the imbalance between estrogens, progesterone and androgens in PCOS may constitute a predisposing factor to HT development. Referring this hypothesis to the different PCOS phenotypes, the highest risk of autoimmune disease would be expected in patients with anovulation and without hyperandrogenism. Patients presenting the classic phenotype with oligomenorrhoea and hyperandrogenism would have an intermediate risk, and the lowest susceptibility would be expected in patients with ovulatory cycles and hyperandrogenism[65]. A study comparing PCOS phenotypes in the context of HT has not been conducted yet, although it has been evidenced that the estrogen-to-progesterone ratio correlates with the anti-TPO concentration in women with PCOS. No dependence regarding androgen levels has been shown[99]. The results of a study comparing infertile women with PCOS and infertile women with regular cycles are also closely related. Subclinical hypothyroidism with TSH > 2.5 μIU/mL has been found significantly more often in PCOS patients than in regularly menstruating patients. No correlation between TSH and androgens has been unveiled, yet TSH level correlated positively with insulin resistance and BMI[100].

The (Controversial) Role of Vitamin D

The positive impact of vitamin D on the immune system has been shown and it is believed that it can prevent autoimmunity development[101]. Inadequate vitamin D intake may be connected with a higher incidence of autoimmune diseases. Vitamin D binds with VDR, which has been found in many tissues including lymphocytes, monocytes and dendritic cells[101].

Two polymorphisms connected with vitamin D – VDR gene and CYP27B1, hydroxylase converting hydroxyvitamin D_3 (25(OH)D) into an active form – are related to HT[102]. The vitamin D level itself affects polymorphism of the VDR gene and other genes related to vitamin D[103]. Genetic variants connected with a lower vitamin D range may play a role in autoimmune disease onset. Some research has displayed that vitamin D deficiency is connected with autoimmune thyroid disease risk and thyroid dysfunction severity as well as with anti-TPO presence[104]. The degree of vitamin D deficiency is correlated with hypothyreosis severity. The probable explanation would be vitamin D's effect on VDR expression in dendritic cells. VDR stimulation induces self-tolerance, acting through CD4+ CD25+ FOXP3+ Treg differentiation, which is responsible for the peripheral action. In fact, vitamin D supplementation in healthy subjects significantly increased the Treg percentage in relation to starting levels[105]. Nonetheless, the following researchers did not confirm the association with thyroid dysfunction severity[106]. In a recent study no differences regarding vitamin D level in patients with HT, Graves' disease and control groups were observed[107]. A statistically significant correlation between anti-TPO and vitamin D deficiency in women, but only in a premenopausal group, has been found in a large Korean population study. It may indicate a joint estrogen and vitamin D contribution to thyroid autoimmunity[108].

Vitamin D deficiency is also believed to play a role in women with PCOS and could be the missing link in the explanation of metabolic disorders. The relationship between low vitamin D level and the components of metabolic syndrome has been shown[109]. VDR gene polymorphism and genes related to vitamin D correlated with hormonal and metabolic parameters in PCOS; however, their direct effect on predisposition to develop PCOS has not been confirmed[105]. Only in the Iranian study were VDR ApaI polymorphisms found to predispose to PCOS.

Vitamin D range correlates negatively with insulin resistance, hyperinsulinemia and hyperandrogenism[109]. However, it is worth mentioning that this correlation was not confirmed in lean women with PCOS[110]. Vitamin D supplementation is supposed to adjust menstrual regularity and normalize the AMH level[111].

The effect of vitamin D supply was also studied with regard to factors lately associated with PCOS: advanced glycation end products (AGEs), markers of inflammation and oxidative stress. Supplementation of vitamin D increased the level of soluble receptors of AGEs (which binds

and deactivates them)[112]. In the Iranian research vitamin D and calcium supplementation led to a decrease of inflammation and oxidative stress markers[113].

However, in systematic reviews on the role of vitamin D in metabolic and hormonal disorders in PCOS, published in 2013 and 2015, it was pointed out that the results of the research included were inconclusive, mainly due to methodological reasons. The decision considering routine screening of vitamin D level in PCOS patients and eventual supplementation can be made after conducting double-blind randomized trials[114,115].

Conclusions

Thyroid disorders, especially HT, in the course of PCOS, are observed significantly more frequently than in the general population. However, their concomitance in the context of fertility disorders has not been investigated extensively.

The data concerning joint etiology, pathogenesis and clinical consequences are scarce. This is probably caused by complex etiology of both diseases and changes of PCOS diagnostic criteria over time.

Genetic susceptibility contributes to the development of both diseases; however, a common genetic background has not been discovered. Three genetic polymorphisms: FBN3, GnRHR and CYP1B1, have been described to play a role in PCOS as well as in HT. Polymorphism of the FBN3 gene seems to be most suitable, due to the relationship with TGFß activity, and Treg level in consequence. The role of TGFß in the pathogenesis of both disorders has been understood. Among patients with PCOS only A8 D19S884 carriers had a normal TGFß1 level; others have been found to have increased values compared with the general population. The first ones would be more prone to develop autoimmune diseases.

High estrogen levels during prenatal life may disrupt development of the thymus and its role in creating immune tolerance – by that means it could contribute to both HT and PCOS development.

Women with PCOS, compared to healthy women, may be more susceptible to the development of HT because of imbalance between estrogens, progesterone and androgens. Probably, the estrogen level is not opposed adequately by progesterone, which can be low due to anovulation. Apparently, even a relatively high androgen level is not able to reduce the stimulating impact of estrogens on autoimmunity.

Vitamin D deficiency is related to the pathogenesis of autoimmune thyroid disorders as well as PCOS. However, from the research available at present, we cannot draw many certain conclusions. In relation to the latest publications, we can also discuss whether vitamin D deficiency is a consequence of PCOS or just coexists with it. To elucidate this issue and the differences caused by seasons of the year, various definitions of deficiency and diverse analytical methods in a randomized population study should be performed.

The mechanism of more pronounced metabolic disorders in cases of coexistence of both disorders compared to single occurrence requires further explanation.

To sum up, it seems that HT and PCOS may be closely related, which reflects frequent common prevalence. The data concerning fertility issues, joint immune background, the role of sex hormones, vitamin D and the cause of metabolic disorder exacerbation still remain ambiguous. Further, detailed research is needed to be carried out in these fields.

Conflict of Interest

The authors declare no conflicts of interest.

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