Clinical and Biochemical Characteristics of Polycystic Ovary Syndrome in Croatian Population

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ABSTRACT

Polycystic ovary syndrome (PCOS) is a common endocrine condition affecting women of reproductive age. There are many typical signs and symptoms that allow for the diagnosis of PCOS depending on the criteria used. Interestingly, ethnicity influences the extent of these signs and symptoms; therefore, the frequency of symptoms varies between different countries and ethnic groups. The prevalence of this syndrome in Croatia is unknown, and it's clinical and biochemical characteristics have not yet been reported. During this study, we used the Rotterdam criteria to evaluate 365 Croatian women with PCOS, and compared them to 304 age matched controls to assess the clinical and biochemical abnormalities that occur in PCOS patients. The mean age of PCOS patients at presentation was 26.1±5.9 years and of controls were 28.0±4.2 years. Women with PCOS has significantly higher body mass index (BMI) than the control group, although in both groups most patients had normal weight (76.2% vs. *87.8%). Abdominal distribution of fat tissue was similar in both groups. Menstrual cycle abnormalities were observed in 90.7% of PCOS patients, and ultrasonographic appearance of polycystic ovaries was reported in 97.3% of PCOS cases. Nearly 75% of patients with PCOS had hirsutism and 49.6% had acne. We recorded significantly higher serum levels of luteinizing hormone (LH), total testosterone (TT), free testosterone (fT) and insulin, while the serum levels of sex hormone binding globuline (SHBG) and follicular stimulating hormone (FSH) were significantly lower than in the control group. Serum glucose values were not significantly different between the groups. In conclusion, chronic anovulation, hirsutism and ultrasound appearance of polycystic ovaries are the dominant features of PCOS in Croatian population. The majority of patients with PCOS had normal body weight. The incidence of insulin resistance in this group of patients is less than the previously described frequency in other populations of patients with PCOS and normal weight.*

Key words: *polycystic ovary syndrome, hirsutism, obesity*

Introduction

Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders in women of reproductive age. PCOS manifests itself with an array of clinical features and symptoms, the three most common being: disorders of ovulation, excessive production of androgens, and polycystic ovaries on ultrasound. PCOS is often associated with obesity and insulin resistance. For a number of years PCOS has been associated with skin and reproductive manifestations. However, this syndrome became very interesting to the medical community in the 1980's, when it was found that women with PCOS have a higher risk of obesity, insulin resistance, glucose intolerance, type 2 diabetes mellitus, dyslipidemia, hypertension, and metabolic syndrome[1]. The etiology of the syndrome is still unclear, however the existence of characteristic modes of inheritance within the family members, favors the

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theory that the syndrome is genetically determined[2–4]. The high prevalence and the great diversity of phenotypes is probably a result of the influence of environmental and genetic factor, including a small number of key genes involved in androgen biosynthesis, insulin genes, and proinflammatory genes. Until recently, there was no universally accepted clinical definition for PCOS. The 2003 Rotterdam ESHRE/ASRM-sponsored PCOS consensus workshop group concluded that two out of three criteria have to be met to fit the definition: chronic anovulation, clinical and/or biochemical hyperandrogenism, and polycystic ovaries, and after the exclusion of diseases that mimic PCOS[5]. Ovulation disorders usually become apparent in the form of oligomenorrhea, although about 20–30% of women with PCOS with oligomenorrhea have eumenorrhea (subclinical oligomenorrhea). Excessive production of androgens or hyperandrogenism (HA) is confirmed via laboratory analysis, characterized by elevated values of circulating androgens, or on the basis of clinical signs, primarily in the form of hirsutism. In 20–40% of women with hirsutism and polycystic ovaries, analysis of androgens in the serum fails to demonstrate elevated levels of these hormones. The reason is that the present available methods of measurement of serum androgens are not sufficiently reliable, or the lack of clear boundaries of normal values in the circulation of women. It is considered that normal circulating androgens in the presence of other clinical signs, does not exclude the diagnosis of PCOS. In the past, polycystic ovaries were determined on the basis of histopathological analysis, however, today evidence of polycystic ovaries on an ultrasound examination is sufficient. As PCOS is also a functional disorder of unknown etiology, the diagnosis is based on exclusion of diseases that manifests similarly. Diseases that mimic PCOS with increased androgens and ovulation disorders include: non-classic congenital adrenal hyperplasia (NCAH), tumors of the ovary and adrenal glands that secrete androgens, disorders of the adrenal cortex (e.g. Cushing's disease), and use of androgen or anabolic drugs. Endocrine disorders such as hyperprolactinemia and hypo-and hyperthyroidism should also be excluded in the work-up. The ethnicity of patients influences the extent of this signs and symptoms, especially with regard to hirsutism and obesity[6]. Therefore, the frequency of symptoms varies between different countries and ethnic groups. The prevalence of this syndrome in Croatia is unknown, and it's clinical and biochemical characteristics have not been reported so far.

Subjects and Methods

Subjects

The examined group included 365 women aged 26.1 ±5.9 years, whom had the diagnosis of PCOS according to the Rotterdam ESHRE/ASRM criteria[5]: 1) oligo- or anovulation, 2) clinical and/or biochemical signs of hyperandrogenism, and 3) polycystic ovaries on ultrasound (PCO). Oligo- or anovulation was determined according to menstrual cycle disturbances as: a) normal menstruation (menstrual interval of 21 to 35 days with normal progesterone levels >22.5 nmol/L on 21–24 day of the cycle, determined in two consecutive menstrual cycles; b) oligomenorrhea (an intermenstrual interval of 36 day-6 months) c) amenorrhea (an intermenstrual interval of 6 months or longer). Hyperandrogenism was evaluated on the basis of present hirsutism, and/or by elevated androgens (total testosterone (TT), free testosterone (fT), androstenedione (A) and dehidroepiandrosteron sulphate (DHEAS)). We defined hirsutism based a Ferriman-Gallwey index score (FG) of greater than 8, which were further divided into three categories: mild (FG 8–9), moderate (FG 10–14), and severe (FG>15)[7]. Severity of acne was divided into three groups: mild (acne comedonica), moderate (acne papulopustulosa), and severe (acne conglobata)[8]. Trans-vaginal ultrasound scanning (USS) was performed by only one expert ultrasonographer to avoid subjective influence on interpreting the results. PCO was defined as the presence of 12 or more follicles measuring 2–9 mm in diameter in each ovary and/or ovarian volume >10 mL. Other possible causes of the symptoms such as non-classical congenital adrenal hyperplasia (NCAH), androgen-secreting tumors, hyperprolactinemia, thyroid gland disturbances and Cushing's syndrome were excluded. If the patient met two of these criteria; she was included in the study after other etiologies were ruled out. In the control group, we included 304 women, aged 28.0±4.2 years, which were undergoing IVF treatment due to male infertility. This group included women who had no disorders of the menstrual cycle, without clinical biochemical signs of HA, and without ultrasound findings of polycystic ovaries. None of the patients in the control group had a hormonal disorder, autoimmune disease or a previous history of gynecological surgery. Prior to entrance into the study, each participant signed a consent form, had her Body Mass Index (BMI), and waist to hip ratio (WHR) calculated. Obesity and overweight were defined according to WHO criteria as a BMI ≥30 kg/m^2 and ≥25 kg/m^2 respectively. According to WHO STEPS, abdominal obesity is defined as a waist-hip ratio above 0.85 for females. All the patients were included in the study after a regular examination in the Division of Human Reproduction and Gynecologic Endocrinology of the Department of Obstetrics and Gynecology, University Medical Centre Zagreb, Croatia, during the period from October 2007 to June 2011. Any medications known to affect sex hormones were discontinued at least six months prior to enrolment. The study protocol was approved by the University of Zagreb Medical School Ethics Committee, under the number 04-1116-2006.

Biochemical analysis

Blood samples were withdrawn from all subjects in the early follicular phase of a spontaneous or progesterone induced menstrual cycle (day 3–5). The levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), total testosterone (TT), androstenedione, dehidroepiandrosterone sulphate (DHEAS), sex hormone binding globuline (SHBG), glucose and insulin were mea-

sured in the peripheral blood. Serum levels of LH, FSH, and total testosterone were measured by an immunodiagnostic procedure by VITROS that measures LH-VITROS, FSH-VITROS, and testosterone-VITROS (Ortho Clinical Diagnostics, Johnson & Johnson, Rochester, New York, USA). Serum levels of SHBG, DHEAS, and androstenedione were determined by a chemoluminescent immunometrical reaction, SHBG-Immulite, DHEAS--Immulite, and androstenedione-Immulite (Siemens Healthcare Diagnostics Inc., Deerfield, Illinois, USA). Coefficients of variation within and between the reaction rates were between 1.5% and 7.9%. The level of glucose in plasma was determined by an automated reference method using hexokinase (Siemens Dade Behring, USA), and serum insulin values were measured with the Immulite procedure as above (Siemens Healthcare Diagnostics Inc., Deerfield, Illinois, USA). Serum concentrations of free testosterone were calculated from TT and SHBG as previously described[9] using a web-based calculator (http://www.issam.ch/freetesto.htm). Insulin sensitivity calculated HOMA-IR according to the formula: (insulin (mU/L x glucose (mmol/L)) / 22.5)[10]. We defined insulin resistance as HOMA-IR ≥2.5[11]. The biochemical analyzes were performed at the Department of Clinical Biochemistry, Zagreb University Hospital Center, University of Zagreb, School of Medicine, Zagreb, Croatia.

Statistical analysis

The categorical variables were described by percentages, and the continuous as mean ± standard deviation. We used the independent Student's t-test to compare the values of the means between cases and controls. Differences in categorical characteristics between cases and controls were assessed using χ^2-test. All statistical analyses were done using the SPSS for Windows (version 15.0; SPSS Inc., Chicago, IL, USA). A p-value <0.05 was considered statistically significant.

Results

Clinical and biochemical characteristics of patients

Mean age at presentation was 26.1±5.9 years in PCOS group, and 28.0±4.2 in the control group. With comparison of clinical data, we have shown that women with PCOS had significantly higher BMI's than the control group, although in both groups most patients had normal weight (76.2% *vs*. 87.8%). Unexpectedly, the waist--to-hip ratio was similar between examined groups. Nearly 73% of patients with PCOS had hirsutism and 49.6% had acne. Oligomenorrhea was noted in 69.2%, amenorrhea in 21.5%, while 9.3% had normal menses. Most of the patients had ultrasonographic appearance of polycystic ovaries (97.3%). These findings were unilateral in three cases. The incidence of varying degrees of acne and hirsutism, as well as clinical features of PCOS and controls are listed in Table 1.

TABLE 1
COMPARISON OF CLINICAL CHARACTERISTICS BETWEEN PCOS PATIENTS AND CONTROL GROUP

	PCOS (N=365)	Control (N=304)	p value[1]
age (years)	26.1±5.9	28.0±4.2	<0.001
BMI (kg/m^2)	23.8±4.4	22.5±3.4	<0.001
BMI <25 (kg/m^2) (%)	76.2	87.8	0.001
WHR	0.78±0.08	0.79±0.07	0.863
Hirsutism (%)	73.2	12.8	<0.001
FG 8–9 (%)	21.8	5.8	<0.001
FG 10–14 (%)	38.1	7.0	<0.001
FG >15 (%)	13.3	0	<0.001
Acne (%)	49.6	18.2	<0.001
Mild (%)	28.1	10.2	<0.001
Moderate (%)	17.6	5.9	<0.001
Severe (%)	3.9	2.1	<0.001
Menstrual Cycle (%)			
Normal (%)	9.3	100	<0.001
Oligomenorrhea (%)	69.2	0	<0.001
Amenorrhea (%)	21.5	0	<0.001
US findings of PCO (%)	97.3	0	<0.001

BMI: body mass index; WHR: waist/hip ratio; US: ultrasound, PCO: polycystic ovaries
[1] Students' t-test was used for continuous and χ^2-test for categorical variables

As expected, we recorded significantly higher serum levels of LH, total testosterone (TT), freeT and insulin, while the serum levels of SHBG and FSH were significantly lower than in the control group. Serum glucose values were not significantly different between the groups. The results are shown in Table 2.

Elevated serum levels of TT (>2 nmol/L) were found in 60.8% of PCOS patients, compared to 4.6% in the control group. Values of other androgens (fT, A, DHEAS) were significantly elevated compared to the control. Insulin resistance (HOMA-IR >2.5) was found in 21.4% of patients and 3.0% of the control group, as shown in Table 2.

Discussion and Conclusion

In the present study, we evaluated the clinical and biochemical characteristics of women with polycystic ovary syndrome in Croatia. To our knowledge this study is the first report about clinical and biochemical features of PCOS in Croatia. Clinical evaluation of hyperandrogenism is subject to numerous criticisms. Assessment of hirsutism is relatively subjective; not all researchers use the standardized FG scale, and a large number of patients undergo esthetical treatments making it difficult to accurately assess the degree of hirsutism[5]. In this study, only one examiner evaluated the degree of hirsutism and acne, therefore avoiding possible research bias.

TABLE 2
DIFFERENCES BETWEEN CONCENTRATIONS OF VARIOUS HORMONES AND METABOLITES IN PCOS AND CONTROL GROUPS

	PCOS (N=365)	Controls (N=304)	p-value[1]
FSH (IU/L)	3.9±1.8	5.2±1.5	<0.001
LH (IU/L)	8.9±5.3	3.3±1.2	<0.001
TT (nmol/L)	2.6±0.9	1.2±0.3	<0.001
[2]TT >2.0 (nmol/L) (%)	60.8	4.6	<0.001
TT >3.0 (nmol/L) (%)	27.1	0	<0.001
fT (pmol/L)	43.9±24.2	14.6±4.3	<0.001
fT >26.0 (pmol/L) (%)	73.8	4.1	<0.001
A (nmol/L)	11.2±4.8	7.7±2.5	<0.001
DHEA-S (μmol/L)	6.7±2.7	5.0±2.0	<0.001
SHBG (nmol/L)	38.9±20.8	59.1±20.1	<0.001
glucose (mmol/L)	4.4±0.6	4.3±0.5	0.901
insulin (mIU/L)	12.1±8.2	6.9±2.4	<0.001
GIR	10.1±6.5	13.3±6.4	<0.001
HOMA-IR	2.4±2.7	1.4±0.6	<0.001
HOMA-IR >2.5 (%)	21.4	3.0	<0.001
HOMA-IR >2.5 and BMI <25 kg/m^2	16.5	3.4	<0.001
HOMA-IR >2.5 and BMI >25 kg/m^2	42.3	0	<0.001

BMI: body mass index; WHR: waist/hip ratio; FSH: follicle stimulating hormone; LH: luteinizing hormone; TT: total testosterone; FT: free testosterone; DHEAS: dehydroepiandrosterone sulphate; SHBG: sex hormone binding globulin; GIR: glucose to insulin ratio; HOMA-IR: homeostatic model assessment of insulin resistance

[1] Student t-test continuous variables and χ^2-test for categorical variables; [2] 95 percentile for the control group

In our cohort of patients with PCOS, 73.2% had hirsutism, and acne was found in 49.6% of patients, which is more that other researchers have found[12]. In a meta-analysis by Azziz et al., researchers calculated the cumulative incidence of hirsutism to be approximately 60% and acne 15–25%, in PCOS patients of all racial groups[12]. A described higher incidence of hirsutism and acne in our population of patients with PCOS correlates with studies that have shown a pronounced hyperandrogenic stigma that belongs to the Mediterranean ethnicity, in which Croatians belong[13]. Values of serum androgens were significantly higher in patients with PCOS compared to the controls. According to literature data, it is estimated that 60–80% of PCOS patients have elevated circulating androgens[14]. In our cohort of patients with PCOS elevated TT >3 nmol/L was noted in 27.1%. This value of total testosterone is the top reference value when using the manufacture's recommendations (Ortho Diagnostics, Johnson & Johnson's, Rochester, New York, USA). Today, more and more emphasis is placed on the problem of sensitivity and accuracy of determining total testosterone in women[15]. A serum concentration of TT is ten times lower, and serum concentration of fT is twenty times lower, in women compared to men[9,15]. In regards to this, we deem it necessary to set new limits on the values of testosterone in the female population. For our research population, elevated testosterone levels were defined in relation to the 95th percentile values for testosterone in the control group (2.0 nmol/L for TT, 26 nmol/L for fT). Sixty percent of patients with PCOS had elevated levels of TT above 2.0 nmol/L, as well as 73.8% had elevated levels of fT. Our findings are consistent with the findings of previous studies that have shown elevated levels of androgens in about 60–80% of patients with PCOS. Clinically evident changes in menstrual cycle were present in 90.7% of patients with PCOS in our study group compared to the previously reported 75% in studies whose results were summarized in a large meta-analysis of Androgen Excess Society (AES)[12]. One possible reason for the higher frequency of cycle disturbance is the fact that our patients with PCOS were recruited at the gynecology clinic. The main reason for referral to the clinic was due to cycle disturbances, which probably influenced our results. Ultrasound findings of polycystic ovaries were present in almost all of our patients with PCOS in the study. Only ten patients with PCOS (2.7%) had normal ultrasound findings, which is much lower than 25% of patients with PCOS in the already-mentioned meta-analysis of AES[12]. We believe that there is a limiting factor in the underlying studies included in the meta-analysis that influenced the huge difference in our results. In most American studies, ultrasound diagnostics were not performed by doctors but by ultrasound technicians, who do not possess clinical insight as compared to a physician. The advantage of our research is the fact that all ultrasound examinations were performed at one institution, with the same transvaginal ultrasound device, and by a physician who strictly kept to the Rotterdam criteria for the diagnosis of PCOS. Most of the patients included in this study had normal body weight in both groups surveyed (76.2% PCOS group vs. 87.8% control group). Values of serum insulin were within in normal limits in both groups of women, however values were significantly higher in the PCOS patient group. When we have a group of patients with PCOS and a BMI of <25 kg/m^2, insulin resistance occurs in 16.5% of the patients, whereas PCOS patients with a BMI equal to or above 25 kg/m^2, 42.3% will show insulin resistance. Increased incidence of insulin resistance in a group of women with a higher BMI may be expected, since it is considered that the increased body weight triggers a stronger manifestation of insulin resistance in patients with PCOS. According to literature, about 80% of patients with PCOS have an increased body weight, and 35% of patients with PCOS who have a normal body weight will have insulin resistance[16]. We studied a population of patients with PCOS, in which 21.4% of patients had insulin resistance, which is consistent with the findings of Gambineri et al., who investigated the presence of insulin resistance in a Mediterranean population of patients with PCOS[17]. The presence of insulin resistance

was 16.5% of patients with PCOS and BMI <25 kg/m^2, confirms that fact that its cause cannot be interpreted only by obesity[18]. SHBG serum levels in patients with PCOS were significantly lower than values measured in the control patient group. We have also expected to find the difference in body composition between PCOS group and control that may influence the sex hormone levels in PCOS patients[19] but this difference was not observed in our study. By analyzing the clinical and biochemical characteristics of our studied population of patients with PCOS, we can conclude that the majority of our patients expressed all three diagnostic features of PCOS (hyperandrogenism, menstrual abnormalities, and ultrasound findings of polycystic ovaries). The majority of patients with PCOS had a normal body weight. The incidence of insulin resistance in this group of patients with PCOS is less than the previously described frequency in other populations of patients with PCOS and normal weight[16,18,20,21].

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KLINIČKE I BIOKEMIJSKE KARAKTERISTIKE BOLESNICA SA SINDROMOM POLICISTIČNIH JAJNIKA U HRVATSKOJ POPULACIJI

SAŽETAK

Sindrom policističnih jajnika (PCOS) jedan je od najčešćih endokrinoloških poremećaja žena reprodukcijske dobi. Prezentira se, ovisno o kriterijima koji se koriste za postavljanje dijagnoze, s mnoštvom tipičnih znakova i simptoma. Pojavnost različitih znakova i simptoma razlikuje se među pojedinim etničkim skupinama. Prevalencija ovog sindroma u Hrvatskoj populaciji nije poznata, a njegove kliničke i biokemijske karakteristike do sada nisu istražene. U studiju smo uključili 365 žena s PCOS i usporedili ih s 304 zdrave žene koje su služile kao kontrole. Dijagnozu smo postavili na temelju Rotterdamskog konsezusa. Prosječna dob žena s PCOS iznosila je 26,1±5,9 godinu, a u kontrolnoj skupini 28,0±4,2 godinu. Žene s PCOS imale su značajno viši indeks tjelesne mase (ITM) u odnosu na kontrolnu skupinu, iako je većina žena uključenih u studiju imala normalnu tjelesnu težinu (76,2% naspram 87,8%). Učestalost abdominalne debljine bila je jednaka u obje ispitivane skupine. Abnormalnosti menstruacijskog ciklusa nađene su u 90,7% bolesnica s PCOS, a tipičan ultrazvučni nalaz policističnih jajnika u 97,3% slučajeva s PCOS. Gotovo 75% žena s PCOS imalo je hirzutizam, a 49,6% njih akne. Kod žena s PCOS nađene su značajno više vrijednosti luteinizirajućeg hormona (LH), ukupnog testosterone, slobodnog testosterone i inzulina, te snižene vrijednosti hormona koji veže spolne hormone (SHBG) i folikulostimulirajućeg hormona (FSH) u odnosu na kontrolnu skupinu. Vrijednosti glukoze, određene na tašte, nisu se

značajno razlikovale među ispitivanim skupinama. Zaključno, kronična anovulacija, hirzutizam i ultrazvučni nalaz policističnih jajnika najčešće su značajke PCOS u Hrvatskoj populaciji. Većina žena s PCOS normalne je tjelesne težine. Učestalost inzulinske rezistencije u žena s PCOS i normalnom tjelesnom težinom u Hrvatskoj populaciji značajno je manja u odnosu na druge etničke skupine.