European Journal of Obstetrics & Gynecology and Reproductive Biology 242 (2019) 166–169

Contents lists available at ScienceDirect

European Journal of Obstetrics & Gynecology and Reproductive Biology

journal homepage: www.elsevier.com/locate/ejogrb

Full length article

Estradiol/testosterone and estradiol/androstenedione indexes and nutritional status in PCOS women – A pilot study

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ARTICLE INFO

Article history:
Received 25 June 2018
Accepted 10 May 2019

Keywords:
Estradiol/testosterone index
Estradiol/androstendione index
Nutritional status
PCOS

ABSTRACT

Objective: The aim of the study was to analyze interrelations between estradiol/testosterone (E2/T) and estradiol/androstenedione (E2/A) indexes and nutritional status, insulin resistance in PCOS.
Study design: A cross-sectional study involved 76 PCOS (41 obese) and 67 Non-PCOS (40 obese) women. Anthropometric parameters and body composition were assessed. In fasting state of serum glucose, androgens, estradiol, FSH, LH, SHBG and insulin were measured. E2/T and E2/A indexes and the homeostasis model assessment of insulin resistance (HOMA-IR) were calculated.
Results: The values of E2/T and E2/A indexes were significantly lower in the PCOS than Non-PCOS subjects, but did not differ significantly between the obese and normal weight groups. The lowest E2/T and E2/A values were observed in the normal weight PCOS group. Multivariable regression analyses revealed that the presence of PCOS was the major factor affecting both the $\log_{10}$ E2/T ($\beta = -0.16$) and $\log_{10}$ E2/A ($\beta = -0.15$) indexes. In addition, $\log_{10}$ E2/A index variability was explained by percentage of body fat ($\beta = 0.57$). HOMA-IR was not among the explanatory factors in all above models.
Comment: The E2/A index is more affected by nutritional status than E2/T index. The lower value of both indices in PCOS women with normal body mass suggest that aromatase activity in PCOS are related to nutritional status.

Introduction

Hyperandrogenism, especially elevated circulating testosterone levels is a key disturbance observed in polycystic ovary syndrome (PCOS) [1]. In women, testosterone, in the circulation, is strongly binding with sex hormone binding globulin (66–78%), and weekly with albumin (20–32%). Only 1–2% of the total pool of testosterone is not protein-bound (free fraction) and therefore biologically active [2].

The markers of hyperandrogenism in women with PCOS are free testosterone and free androgen index (FAI) [3,4]. The total testosterone levels in PCOS can be normal or slightly increased (maximal 5.2 nmol/L) [5]. Higher total testosterone levels are characteristic for tumors that synthetize androgens [4,5]. It was shown that increased total testosterone levels in PCOS women, participates in the pathogenesis of atherosclerosis [6].

Subcutaneous, visceral and liver adipose tissue are the places of extragonadal aromatization of sex hormones due to activity of aromatase (encoded by *CYP19*) [7,8]. This enzyme catalyzes the conversion of androgens (both androstenedione and testosterone) to estrogens (both estrone and estradiol) during steroidogenesis [9]. Thus, estradiol (E2) to testosterone (T) index has been used to evaluate aromatase activity [10,11]. Potentially, similar role may play E2 to androstenedione (A) index.

It has been shown that aromatase activity is decreased in PCOS [12,13]. Moreover, the lower values of E2/T index were observed in PCOS women, regardless of nutritional status [14]. In addition, an association between lower E2/T index and atherogenic lipid profile as well as oligo-ovulation in PCOS were shown [15]. Furthermore, higher E2/T index were associated with better response to treatment with metformin [16]. Thus, it seems that this index may be useful to distinguish the group of PCOS women with good prognosis to response to metformin therapy [17].

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https://doi.org/10.1016/j.ejogrb.2019.05.045
0301-2115/

The aim of the study is to analyze interrelations between E2/T and E2/A indexes and nutritional status and insulin resistance in PCOS.

Material and methods

The cross-sectional study involved 76 PCOS women (35 normal weight and 41 obese) with stable body mass during last 3-month period hospitalized in Department of Endocrinological Gynecology from 2010 to 2011. The diagnosis of PCOS was based on Rotterdam ESHRE/ASRM criteria from 2003 [3]. 67 healthy women without concomitant diseases (27 normal weight and 40 obese) serve as the control group. In the control group PCOS was excluded on the basis of medical history – regular menstrual cycles, the lack of biochemical and clinical features of hyperandrogenism and normal ovarian image in ultrasound. Patients suffering from Cushing's syndrome, thyroid dysfunctions, androgen secreting tumor, and enzyme deficiency (21-hydroxylase in particular), decreased ovary reserves, type 1 and 2 diabetes were not enrolled. Any pharmacological therapy, smoking and alcohol abuse were among the exclusion criteria. The study was conducted after obtaining of the informed consent from each participant. Study protocol was approved by the Ethical Committee of Medical University of Silesia.

Normal weight was defined as body mass index (BMI) from 18.5 to 24.9 kg/m^2 and obesity as > 30.0 kg/m^2. The characteristics of the study groups are presented in Table 1.

All of the study women were tested within 3 and 5 days of menstrual cycle. Anthropometric measurements (body mass and height) were performed, and BMI was calculated according to the standard formula. Body composition was assessed by bioimpedance method using Bodystat 1500 (Douglas, Isle of Man). 15 ml samples of venous blood were withdrawn in the morning between 8.00–9.00 a.m., after an overnight fast (16 h). The blood samples were collected according to recommendation of manufacturer of the kits. Serum and plasma samples were stored frozen in −70 °C.

Laboratory procedures

Plasma glucose and lipids was estimated by colorimetric methods using the commercially available test kits (Roche, Switzerland). Serum insulin concentration was determined by enzyme-linked immunosorbent assay (ELISA) (DRG Instruments GmbH, Marburg, Germany) with a limit of quantification (LoQ) of 1.76μIU/ml and intra– and inter-assay coefficients of variations of 2.2% and 4.4%, respectively. HOMA-IR index was calculated with the standard formula: HOMA-IR = fasting concentration of insulin (μIU/ml) x fasting concentration of glucose (mmol/l)/ 22.5.

Serum follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E_2) testosterone, free testosterone, androstenedione, DHEA-S and SHBG were determined by ELISA (DRG Instruments GmbH, Marburg, Germany) with LoQ of 0.86 mIU/mL, 1.27 mIU/mL, 9.7 pg/mL, 0.083 ng/mL, 0.002 pg/mL, 0.019 ng/mL, 0.044 μg/mL and 0.2 nmol/l, respectively; the respective intra- and inter-assay coefficients of variations were 5.5% and 6.1% for FSH, 5.6% and 6.2% for LH, 4.7% and 7.8% for E2, 3.6% and 7.1% for testosterone, 6.4% and 8.0% for free testosterone, 6.5% and 10.2% for androstenedione, 4.8% and 7.5% for DHEA-S and 5.3% and 9.0% for SHBG.

The E2/T and E2/A indexes were calculated.

Statistical analysis

Statistical analyses were performed using STATISTICA 13.0 PL (TIBCO Software Inc., Palo Alto, California, USA) software and R (3.4.2) software environment. There was no missing data in the database. The results are presented as mean values ± standard deviation in case of normal data distribution or as median with

Table 1
Serum concentrations of hormones and estradiol/testosterone index values in analyzed groups of PCOS and non-PCOS.

	PCOS		Non-PCOS	
	Normal weight (N = 35)	Obese (N = 41)	Normal weight (N = 27)	Obese (N = 40)
FSH (mIU/mL)	5.5 (4.4–6.6)	5.9 (4.4–8.5)	5.3 (3.5–6.5)	5.5 (4.3–7.1)
LH (mIU/mL)	8.2* (5.2–11.4)	11.5$^{\&\&}$ (8.2–15.3)	7.7 (6.2–9.7)	9.1 (3.2–15.4)
LH/FSH	1.6 (1.0–2.4)	1.7 (1.2–2.6)	1.5 (1.1–2.5)	1.5 (0.6–2.5)
Androstenedione (ng/mL)	2.8$^{**\#++}$ (2.1–3.6)	1.9 (1.4–2.8)	2.1 (1.5–3.3)	1.8 (1.3–2.6)
DHEA-S (μg/mL)	2.7 (2.2–3.5)	2.8 (2.1–3.5)	2.7 (2.1–3.9)	2.4 (1.7–3.3)
Total testosterone (ng/mL)	0.7 (0.6–1.0)	0.6 (0.5–0.8)	0.6 (0.4–0.8)	0.6 (0.4–0.8)
Estradiol (pg/mL)	43.6 (32.7–72.6)	44.0 (31.6–66.0)	57.9 (35.2–82.7)	58.4 (24.3–112.9)
SHBG (nmol/L)	33.4*** (16.2–49.9)	18.8$^{\&\&\&\wedge\wedge}$ (11.6–27.4)	38.6$^{\$}$ (28.2–80.4)	22.8 (16.3–47.4)
FAI	3.0$^{*\#}$ (1.2–4.8)	3.4$^{\&\&\&\wedge\wedge}$ (2.1–4.9)	1.5$^{\$}$ (0.9–2.2)	2.1 (1.3–4.4)
Estradiol/testosterone index	58.3$^{\#\#\#+++}$ (45.7–86.9)	71.0$^{\wedge\wedge\wedge}$ (48.0–92.2)	96.5$^{\$\$\$}$ (53.0–153.0)	79.9 (51.0–162.8)
Estradiol/androstenedione index	20.7^{+++} (10.2 – 32.2)	21.5$^{\wedge\wedge\wedge}$ (15.8 – 34.3)	24.2 (14.8 – 45.2)	31.0 (15.9 – 66.7)

median (lower quartile – upper quartile).
$^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ normal weight PCOS vs. obese PCOS.
$^{\#}p < 0.05$; $^{\#\#}p < 0.01$; $^{\#\#\#}p < 0.001$ normal weight PCOS vs. normal weight non-PCOS.
$^{+}p < 0.05$; $^{++}p < 0.01$; $^{+++}p < 0.001$ normal weight PCOS vs. obese non-PCOS.
$^{\&\&}p < 0.01$; $^{\&\&\&}p < 0.001$ obese PCOS vs. normal weight non-PCOS.
$^{\wedge}p < 0.05$; $^{\wedge\wedge}p < 0.01$; $^{\wedge\wedge\wedge}p < 0.001$ obese PCOS vs. obese non-PCOS.
$^{\$}p < 0.05$; $^{\$\$}p < 0.01$; $^{\$\$\$}p < 0.001$ normal weight non- PCOS vs. obese non-PCOS.

lower-upper quartile in case of skewed data distribution. Distribution of variables was evaluated by the D'Agostino-Pearson test. Homogeneity of variances was assessed by the Levene test. Quantitative variables were compared with the two-way multivariable analysis of variances ANOVA with Duncan post-hoc test (in case of normal data distribution or after logarithmic normalization). The assessment of association between variables was done with the multivariable linear regression and the backward stepwise procedure. Three models were proposed with independent variables such as: BMI index, body fat percentage and mass and the following variables: PCOS occurrence, age, HOMA-IR values. Variables with skewed distribution were logarithmic normalized. Outliers were identified based on Cook's distance values. The Cook-Weisberg test was used to test the residuals for heteroskedasticity. Models calculation was performed including evaluation of multicollinearity, which was assessed with the variance inflation factor (VIF). The VIF should not exceed more than 5. Goodness of fit of obtained model was assessed with the F test and determination coefficient R^2. All the results were considered as statistically significant with a p value of <0.05.

Results

The age of both obese subgroups (PCOS and Non-PCOS) as well as of normal weight subgroups (PCOS and Non-PCOS) was similar. However, the obese subgroups were older than corresponding normal weight subgroups (Table 1). Body mass and BMI did not differ between analogous subgroups of PCOS and Non-PCOS.

As expected, in obese PCOS subgroup serum concentrations of insulin, LDL-cholesterol, triglycerides and HOMA-IR value were significantly higher, while HDL-cholesterol level was significantly lower than in normal weight PCOS subgroup (Table 1).

Serum LH levels and LH/FSH index were significantly higher in obese PCOS than Non-PCOS and not differ between normal weight PCOS and Non-PCOS groups. Serum androstenedione and free testosterone levels were highest in normal weight PCOS group. While, serum testosterone and estradiol levels did not differ between study groups. Serum SHBG levels were lowest and FAI index the highest in obese PCOS group.

Estradiol/testosterone (E2/T) and estradiol/androstenedione (E2/A) indexes

The E2/T index value were lower in normal weight and obese PCOS groups than in both Non-PCOS groups. In addition, in PCOS group, E2/T was lower in normal weight group, while in Non-PCOS in obese group (Table 1).

The E2/A index was lower in PCOS than Non-PCOS subjects [20.8 (11.6–33.6) vs 26.7 (15.3–57.7); $p < 0.001$], however no difference between normal weight and obese Non-POCS and PCOS was found (Table 2).

Multivariable regression analyses

A multivariable, stepwise regression analysis for all three models revealed that the factor explaining variability of the $\log_{10}$ E2/T index was the occurrence of PCOS, only ($\beta = -0.16$; SE $(\beta) = 0.048$; $p < 0.01$; $R^2 = 0.074$).

Similar three models for $\log_{10}$ E2/A index revealed that the factor significantly explaining variability of this index were occurrence of PCOS ($\beta = -0.15$; $SE(\beta) = 0.06$; $p < 0.05$) and body fat percentage ($\beta = 0.57$; $SE(\beta) = 0.29$; $p < 0.05$), $R^2 = 0.078$.

HOMA-IR were not among explanatory factors in all above models.

Discussion

The results presented in this study demonstrate the lower values of E2/T and E2/A indexes in PCOS women, and an independent effect of body fat percentage on E2/A index, only.

Our results, concerning values of E2/T index are partially in accordance with a study recently published by Chen et al. [14]. Contrary to our results of Chen et al. [14], we did not observe a significant difference in values of E2/T index between normal weight and obese PCOS as well as Non-PCOS women. We also did not show an independent effect of nutritional status on the values of E2/T index in multivariate, stepwise regression analysis. It might be caused by insufficient number of the study group. Despite of this, the differences observed between normal weight and obese PCOS and Non-PCOS subgroups suggested that in obese PCOS women higher aromatase activity in adipose tissue partially compensate its decrease activity characteristic for PCOS. This hypothesis is supported by a negative effect of PCOS and positive of body fat percentage on the values of E2/A index, shown in multivariate regression analysis.

It has been suggested that insulin is one of factors inducing aromatase expression in visceral adipose tissue, that was independent of gender [18]. We were confused seeing the lack of independent association between values of E2/T and E2/A and HOMA-IR indexes as well as insulin levels in multivariate regression analysis. Probably the effect of body fat percentage on the values of E2/A index was stronger, and weakened the effect of HOMA-IR. In addition, we should have in mind that adipose tissue is not the unique place showing aromatase activity. Furthermore, it seems that E2/A index may better, then E2/T, reflects aromatase activity in adipose tissue. This supposition is supported by studies showing thatmetformin is more effective in PCOS women resistant to clomiphene therapy with higher values of E2/T index [12,16]. It has also been found that metformin diminishes aromatase activity stimulated by FSH in ovary, independently from AMP-kinase pathway [16].

Numerous studies revealed that risk of ovulation disturbances increases with BMI [19], and the effectiveness of in vitro is worse in obese women [20]. In addition, weight loss, especially decrease of visceral obesity, results in the return of ovulation in PCOS women [21]. One study showed higher values of E2/T index in normal ovulatory than chronic oligo-ovulatory PCOS women, independently of nutritional status [15]. However, our study does not confirm this observation, as all PCOS women enrolled had chronic ovulation disturbances, and higher values of both E2/T and E2/A indexes were shown in obese than normal weight subgroup. Therefore, it seems that the role of E2/T and E2/A indexes in the assessment of ovulation disturbances needs further studies taking into account also other confounders that influence ovulation. Moreover, studies assessing the impact of weight loss on values of E2/T and E2/A indexes, as well as the association between these changes and improvement in ovulation, should be performed.

The limitation of our study is the size of study subgroups and the lack of stratification of PCOS with normal weight for subgroups with and without metabolic obesity. Moreover, the distribution of body fat and its visceral deposits were not directly assessed using DEXA or CT scanner. Furthermore, the aromatase expression in adipose tissue and in ovary was not assessed.

Comment

The E2/A index is more affected by nutritional status than E2/T index. The lower values of both indices in PCOS women with normal body mass suggest that aromatase activity in PCOS are related to nutritional status.

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