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The value of the free androgen index depends on the phenotype of polycystic ovary syndrome — a single-centre experience

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Abstract

Introduction: The free androgen index (FAI) values differ among patients with polycystic ovarian syndrome; however, the differences are not fully understood or known.

The aim of the study was to evaluate FAI in women with polycystic ovary syndrome (PCOS) in regard to the phenotype of the PCOS and insulin resistance status.

Material and methods: Anthropometric, hormonal, and biochemical parameters were assessed in 312 recruited women with PCOS. The FAI values were calculated in the reproductive and metabolic phenotypes of PCOS in groups of insulin resistance status based on the homeostasis model assessment-insulin resistance (HOMA-IR) > 2.0 or fasting insulin (FI) > 10 mmol/L. To test the relationship between individual variables, Spearman's correlation analysis, the Kolmogorov-Smirnov test, and Student's t-test were used.

Results: The correlation between FAI values and HOMA-IR and FI was 0.42 and 0.47, respectively, in PCOS patients. A two fold higher FAI value was observed in metabolic PCOS phenotype when compared to the reproductive one (8.51 ± 5.56 *vs.* 4.40 ± 2.45 for HOMA-IR and 8.73 ± 6.09 *vs.* 4.31 ± 3.39 for FI, respectively; $p < 0.05$).

Conclusions: PCOS patients are not a homogenous group in terms of FAI value. Patients with metabolic PCOS phenotype are characterised by two-fold higher FAI values compared with reproductive PCOS phenotype. Further studies on the metabolic and androgenic status of different types of PCOS phenotypes should be carried out. **(Endokrynol Pol 2019; 70 (4): 330–335)**

Key words: *polycystic ovary syndrome; free androgen index; hyperandrogenism; insulin resistance*

Introduction

Polycystic ovary syndrome (PCOS) is an endocrine disorder affecting approximately 6–13% of women of reproductive age [1], characterised by reproductive and metabolic disorders. According to the Androgen Excess Society (AES), the diagnosis of PCOS is based on the fulfilment of criteria, including clinical or biochemical symptoms of hyperandrogenism, and the coexistence of one of the two following symptoms: oligo-ovulation/anovulation or polycystic ovarian picture in ultrasound examination [2]. In the diagnosis of PCOS it is also necessary to exclude other causes of hyperandrogenism, such as congenital adrenal hyperplasia, Cushing's syndrome, hypothyroidism, acromegaly, or hyperprolactinaemia [3].

PCOS is not a homogenic group of patients. There are several proposed phenotypes of the disorder. According to AES, three clinical PCOS phenotypes have been distinguished depending on the occurrence of polycystic ovaries, hyperandrogenisms, and oligo-ovulation. According to Dunaif and Diamanti-Kandarakis, the phenotypes are dependent on the metabolic status of patients, and there are reproductive and metabolic phenotypes [4].

The reproductive phenotype is characterised by hyperandrogenaemia [5], which is clinically manifested in 60% of patients as hirsutism and less often as acne or androgenic alopecia [6]. Ovarian dysfunction defined as oligo or anovulation may also be present. The manifestation of metabolic phenotype, besides hyperandrogenaemia and ovarian dysfunction, include insulin resistance (IR) status [7]. Insulin resistance is defined by a fasting insulin concentration over 10 mU/mL and a HOMA-IR value (homeostasis model assessment-insulin resistance) over 2 [8, 9].

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Clinically, hyperandrogenism is assessed using the Ferriman-Galwey hirsutism score, while the biochemical exponent of hyperandrogenism is the elevated level of free serum testosterone [10]; however, due to the cost and difficulty of this test [11], usually the total testosterone concentration is determined. Based on total testosterone and sex hormone-binding globulin (SHBG), the value of the free androgen index (FAI) is determined, which serves as the main measure of androgenisation [12].

Tissue resistance to insulin influences the equalising increase in insulin production and hyperinsulinaemia, and this in turn stimulates the production of adrenal androgens [13]. In addition, increased insulin levels inhibit SHBG synthesis in the liver, thereby increasing the concentration of free testosterone [14]. These mechanisms are responsible for increased intensity of androgenisation in women with insulin resistance, and thus higher FAI values.

The issue of the FAI cut-off point is not fully understood and can differ in individual populations. The aim of the study was to estimate the mean FAI value in the polycystic ovarian syndrome, depending on the phenotype of the disease, and to determine the correlation between the index of free androgens and the indicators of the state of insulin sensitivity.

Material and methods

The study included 312 women aged 18 to 40 years, mean age 25.4 ± 4.83 years (x ± SD), who were patients of the Clinical Department of Gynaecological Endocrinology and Gynaecology of the University Hospital in Krakow. PCOS was diagnosed using the AES criteria [2]. Oligo-ovulation was defined as menstrual periods that occur at intervals over 35 days, and anovulation as a complete absence of menstruation. Clinical hyperandrogenism was defined by the occurrence of acne or hirsutism, and biochemical hyperandrogenism by total serum testosterone levels above 1.67 nmol/L and elevated DHEAS concentration (depending on age: values above 5.42–11.1 μmol/l). The ovaries in the ultrasound picture were considered to be polycystic in the case of ovarian volume above 10 ml and the presence of at least 12 follicles 2–9 mm in diameter [2]. Exclusion criteria included: use of oral contraception in the last two months, congenital adrenal hyperplasia, Cushing's syndrome, uncontrolled hypothyroidism, acromegaly, hyperprolactinaemia, pregnancy, and lactation. Approval of the Ethics Committee of Jagiellonian University Medical College in Krakow was obtained. All patients involved in the study gave signed, informed consent (Resolution No. KBET/167/B/2013).

The measurement of body mass [kg] and height [cm] was performed, the body mass index (BMI [kg/m^2]) was calculated. Total testosterone [nmol/L], sex hormone binding globulin (SHBG [nmol/L]), dehydroepiandrosterone sulphate (DHEA-S [μmol/l]), prolactin in the diurnal profile (PRL [uIU/mL]), daily cortisol rhythm [nmol/L], 17-OH-progesterone [ng/mL], luteinising hormone [LH IU/L], follicle-stimulating hormone (FSH [IU/L]), oestradiol [pmol/L], and thyrotropin hormone (TSH uIU/mL) in serum were measured. Glucose [mmol/L] and insulin levels [mU/mL] were measured while fasting and 60 and 120 minutes after 75 g of oral glucose loading. To determine insulin sensitivity, the HOMA-IR value was determined according to the formula: HOMA-IR = (fasting insulin [mU/mL] x fasting glucose [mmol/L])/22.5. HOMA-IR > 2 and fasting insulin levels > 10 mU/mL were considered to be values corresponding to insulin resistance (IR) [15, 16].

The free androgen index (FAI) was determined on the basis of total testosterone and SHBG concentration in accordance with the formula: FAI = total testosterone [nmol/L]/SHBG [nmol/l]*100.

Ultrasonography of the ovaries was performed in all patients using transvaginal method (USG TV) or transabdominal method (USG TA) in virgins, and as preferred.

Taking into account the state of insulin sensitivity, patients participating in the study were divided into metabolic and reproductive phenotype according to the assumptions of Dunaif and Diamanti-Kandarakis [4]. In the first part of the study, the metabolic and reproductive groups were distinguished based on the HOMA-IR value, with a cut-off value of 2 (Fig. 1). In the second part of the study, the division was made based on fasting insulin concentration with a cut-off value of 10 mU/mL (Fig. 2).

Subsequently, the mean FAI values for each group were calculated and compared between groups, and thus the average FAI value for the reproductive and metabolic phenotype of PCOS was determined.

Physical examination

All participants of the study underwent physical examination, which included body mass and height measurement, on the basis of which the body mass index (BMI) was determined according to the formula: BMI = body mass [kg]/height [m]^2.

Clinical sings of hyperandrogenism were assessed using the Ferriman-Gallwey scale, in which a score above eight indicated hirsutism.

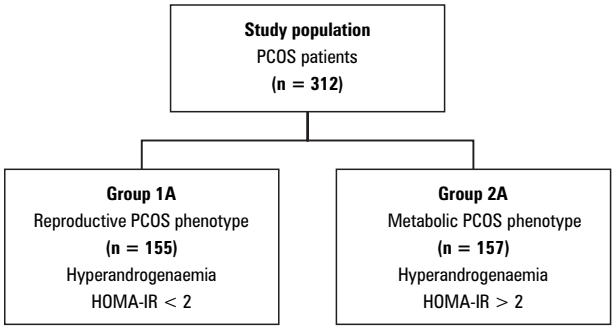

Figure 1. *Division into two groups based on HOMA-IR value. HOMA-IR — homeostasis model assessment-insulin resistance; PCOS — polycystic ovary syndrome*

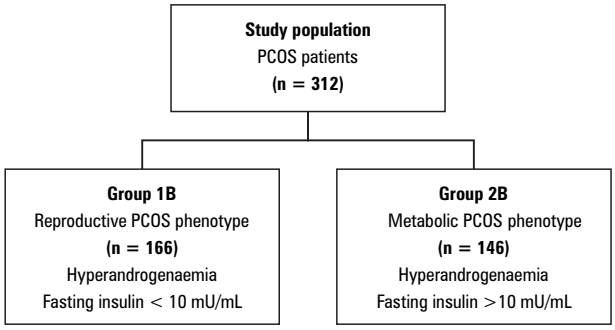

Figure 2. *Division into two study groups based on FI concentration. FI — fasting insulin; PCOS — polycystic ovary syndrome*

Table I. *Characteristic of study group — divided into two PCOS phenotypes depending on HOMA-IR*

	Group 1A (HA+ HOMA-IR < 2) (n = 155)	Group 2A (HA+ HOMA-IR > 2) (n = 157)	p-value
Age [yrs]	25.26923 ± 4.80	25.35 ± 5.95	0.89
Height [cm]	166.2961 ± 5.485488	166.41 ± 6.44	0.87
Weight [kg]	61.18 ± 11.22	76.01 ± 18.45	0.00
BMI [kg/m^2]	22.08 ± 3.86	27.35 ± 6.17	0.00
FAI	4.40 ± 2.45	8.51 ± 5.53	0.00
Testosterone [nmol/L]	1.92 ± 0.88	2.14 ± 1.01	0.043

PCOS — polycystic ovary syndrome; HA — hyperandrogenaemia; HOMA-IR — homeostasis model assessment-insulin resistance; BMI — body mass index; FAI — free androgen index

Table II. *Characteristic of study group — divided into two PCOS phenotypes depending on fasting insulin*

	Group 1B (HA+ FI < 10 mU/mL) (n = 166)	Group 2B (HA+ FI > 10 mU/mL) (n = 146)	p-value
Age [yrs]	25.28±4.78	25.35 ± 6.04	0.92
Height [cm]	166.27 ± 5.58	166.45 ± 6.39	0.79
Weight [kg]	61.35± 11.70	76.79 ± 18.24	0.00
BMI [kg/m^2]	22.10 ± 3.96	27.64 ± 6.08	0.00
FAI	4.37 ± 3.39	8.73 ± 6.09	0.00
Testosterone [nmol/L]	1.93 ± 0.89	2.18 ± 1.03	0.017

PCOS — polycystic ovary syndrome; HA — hyperandrogenaemia; FI — fasting insulin; BMI — body mass index; FAI — free androgen index

Laboratory tests

Participants of the study had blood samples collected in the morning, after an overnight fast, between the second and the sixth day of the menstrual cycle (follicular phase). The basic material was peripheral blood serum, which was centrifuged and stored at between –20°C and –70°C depending on the method. All parameters were determined with electrochemiluminescence immunoassay (ECLIA) methods using a Roche Cobas 6000 with a Cobas e601 module using automated commercial immunoassays (Roche Diagnostics International Ltd., Switzerland). The glucose concentration was determined by colorimetry using the automated Cobas8000 test. The FAI value and HOMA-IR were calculated using the formulas quoted above.

Statistical analysis

STATISTICA 13 was used to perform the statistical analysis. All parameters were given as mean ± standard deviation. Because the distribution of most variables was not normal, nonparametric statistics were used. Comparisons of individual variables were made using the Kolmogorov-Smirnov test and Student's t-test. To test the relationship between individual variables, Spearman's correlation analysis was used. For all analyses, the determinant of statistical significance was $p < 0.05$

Results

A total of 312 women took part in the study. The mean age of the respondents was 25.4 ± 4.83 years (x ± SD), mean BMI was 24.93 ± 6.05, mean HOMA-IR was 2.748 ± 2.951, mean testosterone was 2.042 ± 0.966 , and mean FAI was 6.454 ± 5.385. In total, 157 and 146 women were diagnosed as metabolic phenotype according to the HOMA-IR > 2.0 or FI > 10 used, and 155 and 165 women presented with reproductive phenotype. The characteristics of the groups are presented in Table I and II.

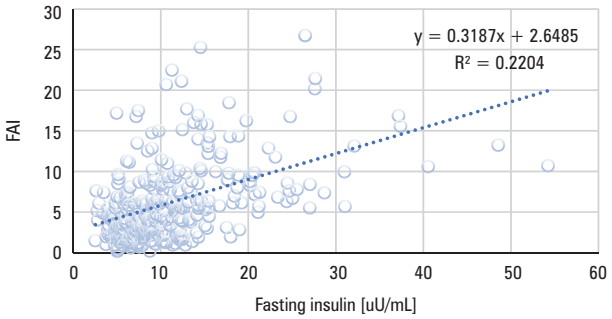

Figure 3. *Correlation between FAI and FI [mmol/L] concentration in all PCOS patients (n = 355). FAI — free androgen index; FI — fasting insulin; PCOS — polycystic ovary syndrome*

In all subjects with PCOS there was a correlation between HOMA-IR and FI and testosterone (respectively, 0.29 and 0.31) and between HOMA-IR and FI and FAI — 0.42 and 0.47, respectively (Fig. 3 and 4). The women with metabolic phenotype were heavier and had higher BMI compared to reproductive type irrespective of the insulin resistance indicator used ($p = 0.00$). The testosterone levels were higher in metabolic *vs.* reproductive

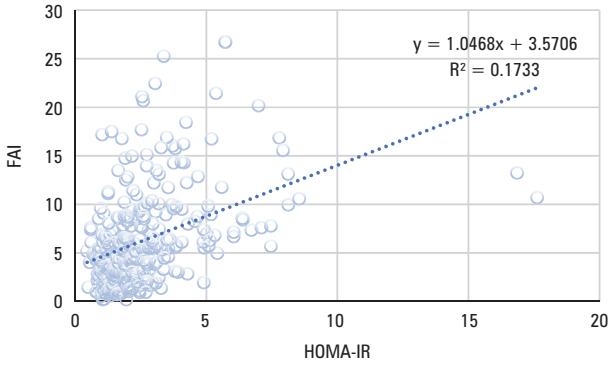

Figure 4. *Correlation between FAI and HOMA-IR in all PCOS patients (n = 355). FAI — free androgen index; HOMA-IR — homeostasis model assessment-insulin resistance; PCOS — polycystic ovary syndrome*

phenotype (2.14 ± 1.01 nmol/L *vs.* 1.92 ± 0.88 nmol/L for HOMA-IR, and 2.18 ± 1.03 nmol/L *vs.* 1.93 ± 0.89 nmol/L for FI). The FAI value was higher in metabolic *vs.* reproductive phenotype (8.51 ± 5.53 *vs.* 4.40 ± 2.45 for HOMA-IR, and 8.73 ± 6.09 *vs.* 4.37 ± 3.39 for FI) (Tab. I and II).

Discussion

In the study, the mean FAI value was estimated in two phenotypes of polycystic ovary syndrome, defined by Dunaif and Diamanti-Kandarakis [4], and the relationship between the state of insulin sensitivity and androgen concentration was determined. The study shows the relationship between FAI values and insulin resistance status in all PCOS patients; however, the index of free androgens is twofold higher in the metabolic PCOS phenotype as compared to the reproductive phenotype. Similarly, in this phenotype the level of total testosterone is significantly higher.

The obtained results are consistent with many other studies carried out in this area [17–21], but they are in contrast to the results obtained by Ganie et al. [22]. The reason for the differences may be the use of only one of the androgenisation exponents (e.g. androstenedione or total testosterone), which, in the case of PCOS, may cause underestimation [23]. Due to the fact that the free androgen index was considered the most objective assessment of androgenisation [24], it was used in our study.

In our study we also observed a correlation between insulin concentrations and HOMA-IR values with FAI, which is consistent with the results obtained by Kissebah et al. [25] and Burghen et al. [26]. There was also a correlation between total testosterone level and insulin concentration and HOMA-IR values, which is consistent with the Burghen study [26]. Significant positive correlation between HOMA-IR as well as BMI and FAI and testosterone level in PCOS patients was obtained also in the study conducted by Dickerson et al. [27]. However, some studies showed different results presenting negative correlation between testosterone level and insulin and HOMA-IR [28].

The mean FAI value, calculated in all PCOS patients, was 4.39. For the metabolic phenotype, the average FAI was much higher, at 8.6. The above results differ from the values of the mean FAI values obtained in other studies. Nadajara et al. [29], in a study conducted in a Malaysian population of women with PCOS, found a mean FAI value of 9.2. A study among Chinese women showed a mean FAI > 6.1 [31], while a Samoan population survey showed a higher value, i.e. 8.5 [30]. However, a European population study, conducted by Hahn et al., showed as FAI of 4.97 as the mean value in PCOS patients, which is closest to the value observed in our study [32]. The reason for the differences in FAI values may be the ethnic diversity of the studied populations, but also the different measurement methods used.

The androgen level in PCOS patients was analysed also in the studies, which divided patients according to their metabolic status. Brand et al. conducted a meta-analysis that showed the association between higher total and free testosterone levels and a prevalence of metabolic status in PCOS patients [33]. Positive correlation between androgen levels and metabolic status was observed also in other studies [34]. However, in the study conducted by Albu et al. the results were different — there were no significant differences between the estimated values of total testosterone in PCOS patients without and with metabolic syndrome [35], while the mean FAI value for PCOS patients without metabolic syndrome was 5.64 and in patients with co-occurring metabolic syndrome it was 10.26. The reason for that may be the division into phenotypes without using HOMA-IR or FI.

Some strengths and limitations of our study need to be discussed. This is one of the first studies investigating the androgen status in relation to phenotypes of PCOS in a Polish population. However, it was carried out only in one research centre, and thus concerned women living in a particular region of Poland. Also, the definition of insulin resistance was based on fasting insulin level and HOMA-IR values, while the "gold standard" remains the value of the metabolic clamp [36]. The study with the use of the metabolic clamp was performed on the subgroup of patients; therefore, it was not presented in the above manuscript. The cut-off values of HOMA-IR and fasting insulin level are variously defined by individual researchers; however, in our study the values used were close to those

found for the Polish population [10]. Despite the above limitations, the study allowed us to estimate the mean FAI value in particular PCOS phenotypes and thus to show the dependence between insulin resistance and the intensity of androgenisation.

Conclusions

Patients with metabolic PCOS phenotype are characterised by two-fold higher FAI values compared with reproductive PCOS phenotype, suggesting that PCOS patients are not a homogenous group in relation to androgen excess. Further studies investigating the relation between the metabolic and androgenisation status of women with PCOS should be carried out.

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