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Normal cut-off values for hyperandrogenaemia in Iranian women of reproductive age

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ABSTRACT

Objective: The normal cut-off values for hyperandrogenaemia differ between populations. Although there are various ways to determine the normal range of hormones in a population, use of a gold standard and the 95th percentile approach are not appropriate to diagnose hyperandrogenism. This study aimed to determine normative cut-off androgen levels for Iranian women of reproductive age using *k*-means cluster analysis.
Study design: The subjects were 1126 women, aged 18–45 years, initially selected at random from women of reproductive age from different geographic regions of Iran. Serum levels of hormones were measured, and complete data were available for 923 women following the implementation of exclusion criteria. The reference group comprised 423 eumenorrhoeic non-hirsute women selected from the total population. Normative cut-off levels were computed using *k*-means cluster analysis ($k = 3$). Analyses were also conducted based on age and body mass index (BMI) groups for the 923 subjects.
Results: Cluster cut-off values for the total study population were similar to the 95th percentile values, whereas the percentiles for the free androgen index, total testosterone, dehydroepiandrostenedione sulphate and androstenedione were 79.3%, 74.1%, 96.7% and 71%, respectively, in the reference group.
Conclusions: Determination of cut-off values for hyperandrogenism based on age and BMI categories provides the most objective way of determining normal upper limits of androgen levels among Iranian women of reproductive age.

1. Introduction

Hyperandrogenism is defined as an abnormally high level of androgen produced by the ovaries or the adrenal glands. It is mainly a result of polycystic ovary syndrome (PCOS) [1], a common androgen-related disorder that affects approximately 7–12% of women of reproductive age [2]. Women with PCOS are at increased risk for infertility, endometrial carcinoma, type 2 diabetes mellitus, hypertension and cardiovascular disease [3–7]. PCOS is diagnosed in 82% of cases of hyperandrogenaemia, and the most common clinical manifestations of hyperandrogenaemia are hirsutism, persistent acne and alopecia [8,9]. Laboratory evaluations for the diagnosis of hyperandrogenaemia should include measurement of total testosterone (TT), free testosterone, androstenedione (A4) and dehydroepiandrostenedione sulphate (DHEAS) [1].

It is well known that normal cut-off values for hyperandrogenaemia differ between populations [10]. Eighty to ninety percent of patients with hirsutism have diagnostic hyperandrogenaemia [11], and as the degree of hair growth and consequently the cut-off values for hirsutism vary between ethnicities and races, the thresholds of hyperandrogenism are affected by these factors [12]. As mean androgen levels vary widely between women of various races, it has been suggested that the normal ranges or limits of androgen levels should be determined specifically for well-characterized, normal women of each population [10]. As such, the present study aimed to determine normal cut-off levels for hyperandrogenaemia in Iranian women of reproductive age.

There are various ways to determine normal ranges of hormones in a population. The 95th percentile is commonly used to define the upper range of normality [13]. However, as an earlier study by the present authors found high prevalence rates for hirsutism and PCOS [14], this method was not used in the current study in order to reduce the risk of bias. Use of a gold standard is common, but there is no gold standard for diagnosis of hyperandrogenism. Cluster analysis appears to be a useful approach, and *k*-means cluster analysis is one of the simplest

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and most popular clustering techniques [15], used previously by Zhao et al. [16] and DeUgarte et al. [13]. This procedure provides a simple and easy way to classify a given data set through a certain number of non-overlapping clusters (k clusters). In general, the k-means method produces k different clusters of greatest possible distinction [17,18]. In this method, the algorithm finds k clusters and their centroids, and assigns each point to its nearest centroid. There is no standard, accepted 'best' way to determine the number of clusters (k). Typically, the Ek curve is calculated to determine the most appropriate number of clusters [19]. The value of k at the curve's knee determines the final number of clusters.

2. Materials and methods

2.1. Subjects

For this population-based cross-sectional study conducted between 2009 and 2010, 1126 women aged 18–45 years from different geographic regions of Iran were selected from the participants of the Iranian PCOS prevalence study [14]. Eligible women were invited for comprehensive interviews and to obtain blood pressure, anthropometric, hormonal and metabolic measurements. These data were collected for all women, except 97 women who did not attend the clinics and 10 women whose hormonal and metabolic profiles were not available. This study was performed on two groups of women: the total group and the reference group. Women who were pregnant at the time of the study ($n = 43$) and menopausal women ($n = 37$) were excluded from the study; as such, the total group comprised 923 women. For selection of the reference group, women with PCOS (using the Rotterdam definition) ($n = 136$), anovulation ($n = 77$), polycystic ovaries ($n = 74$), hirsutism ($n = 205$), hypothyroid ($n = 10$) and hyperprolactinaemia ($n = 5$) were selected from the total group; of these, 423 eumenorrhoeic non-hirsute women were enrolled as the reference group.

Using the Rotterdam criteria, PCOS was defined by the presence of two or more of the following: (1) oligo-/anovulation; (2) hyperandrogenaemia and/or hyperandrogenism; and (3) polycystic ovaries [20]. Anovulation was defined as vaginal bleeding episodes at intervals $\geq$35 days [21,22].

All participants underwent clinical examination, and body weight, height, waist and hip circumferences were measured by trained staff. Height and weight were measured with subjects in light clothes and without shoes, using standard apparatus. Weight was measured to the nearest 0.1 kg on a calibrated beam scale. Height and waist circumference were measured to the nearest 0.5 cm with a measuring tape. Body mass index (BMI) was calculated as weight divided by height squared (kg/m^2).

A blood sample for biochemical measurements was taken from each subject on the second or third day of the menstrual cycle, after a 12-h overnight fast. Blood samples were collected in EDTA-treated test tubes. Written informed consent was obtained from all participants.

2.2. Laboratory measurements

Plasma was separated in a refrigerated centrifuge at 3000 revolutions/min for 10 min, and serum was stored at −80 °C until tested. Fasting plasma glucose was measured using an enzymatic colorimetric method with glucose oxidase. A4 was measured by enzyme immunoassay (Diagnostic Biochem Canada Co., Ontario, Canada), and sex hormone binding globulin (SHBG) was measured by immunoenzymometric assay (Mercodia, Uppsala, Sweden). All enzyme-linked immunosorbent assays (ELISAs) were performed using a Sunrise ELISA reader (Tecan Co., Salzburg, Austria). The intra- and interassay coefficients of variation were: DHEAS, 2.0% and 5.1%; SHBG, 1.2% and 5.7%; and A4, 2.2% and 3.5%, respectively. The free androgen index (FAI) was calculated as the ratio of TT divided by SHBG (both expressed in the same units) and multiplied by 100.

2.3. Statistical analysis

Continuous variables were checked for normality using the one-sample Kolmogorov–Smirnoff test, and expressed as mean ± standard deviation (SD) and/or median (interquartile range), as appropriate. The FAI was calculated according to the following equation [23]:

$$\mathrm{FAI} = 100 \times \frac{\mathrm{TT\,(nmol/l)}}{\mathrm{SHBG\,(nmol/l)}}$$

Serum levels of FAI, TT, DHEA and A4 were reported for the total population and the reference group as mean, median, SD and percentiles (5th, 10th, 90th and 95th); and by age and BMI groups for the total study population. Normative cut-off levels were computed using k-means cluster analysis ($k = 3$). Data analysis was performed using Statistical Package for the Social Sciences Version 15.0 (SPSS Inc., Chicago, IL, USA). Statistical significance was set at $p < 0.05$.

3. Results

In total, 923 women completed the study. Of these, 423 women fulfilled the criteria for the reference group. Characteristics of the total study population and the reference group are presented in Table 1. The mean (SD) age of the total study population was 34.3 (7.6) years and the mean (SD) BMI was 26.8 (5) kg/m^2. For BMI, parity and age at menarche, the reference group reflected the total study population from which they were drawn.

The mean, median and percentiles of FAI, TT, DHEAS and A4 in the total study population and reference groups are presented in Table 2. K-means cluster analysis was used to determine the cut-off values of FAI, TT, DHEAS and A4 in both groups. In the total study population, cluster cut-off values were similar to 95th percentile values, but in the reference group, percentiles for FAI, TT, DHEAS and A4 were 79.3%, 74.1%, 96.7% and 71%, respectively.

All markers decreased with age. Levels of FAI, TT, DHEAS and A4 differed significantly between the two age groups (<35 and $\geq$35 years) of the total study population (Table 3).

Comparison between the three BMI groups demonstrated a decrease in mean DHEAS and A4 (Table 4).

Table 5 illustrates the cut-off values for all parameters identified in age and BMI groups. Although cut-off values for FAI varied in normal-weight and obese women of both age groups, they did not appear to vary in overweight women.

Table 1
Characteristics of the total study population and the reference group.

Variables	Total study population		Reference group	
	N	Mean ± SD	N	Mean ± SD
Age	915	34.3 ± 7.6	423	35.3 ± 7.4
BMI (kg/m^2)	915	26.8 ± 5	423	26.7 ± 5
Parity	731	2.5 ± 1.4	359	2.55 ± 1.3
Menarch age	889	13.4 ± 1.5	405	13.3 ± 1.4
FBS (mg/dl)	908	88.9 ± 26	418	88.4 ± 21.3
WC (cm)	915	85.02 ± 12.1	423	84.6 ± 11.9
FAI	908	3.6 ± 2.6	420	2.6 ± 1.4
DHEAS	923	164.3 ± 105.1	422	138.2 ± 80.4
TT (nmol/l)	913	0.63 ± 0.6	421	0.5 ± 0.2
A4	913	1.5 ± 0.6	421	1.2 ± 0.5

BMI, body mass index; FBA, fasting blood sugar; 2HPG, 2-h post-load glucose; DHEAS, dehydroepiandrostenedione sulfate; T, testosterone; A4, androstendion; WC, waist circumference.