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Prevalence of Hyperandrogenemia in the Polycystic Ovary Syndrome Diagnosed by the NIH 1990 Criteria

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Abstract

Objective—To determine the prevalence of elevated total (TT) and free testosterone (FT), and DHEAS, alone and in combination, in polycystic ovary syndrome (PCOS) patients.

Design—Cross-sectional analysis

Setting—Tertiary care academic medical center

Patients—Seven hundred and twenty patients diagnosed with PCOS according to the NIH 1990 criteria.

Interventions—History and physical exam, and blood sampling.

Main Outcome Measure(s)—Hyperandrogenemia, defined as at least one androgen value above the 95th percentile of 98 healthy control women, i.e. TT >88 ng/dl, FT >0.75 ng/dl, and DHEAS >2750 ng/ml.

Result(s)—A total of 716 PCOS subjects were included. The overall, prevalence of hyperandrogenemia in PCOS was 75.3%. Supranormal levels of FT were present in 57.6%, of TT in 33.0%, and of DHEAS in 32.7% of PCOS patients. When assessing the prevalence of two abnormal values, the prevalence of simultaneously elevated androgens was lowest with TT and DHEAS (1.7%) and highest with TT and FT (20.4%). Altogether, simultaneous elevations in all three markers were found in 8.7% of PCOS subjects.

Conclusion—Approximately three-fourths of patients with PCOS diagnosed by the NIH 1990 criteria have evidence of hyperandrogenemia; the single most predictive assay was the measurement of FT with ~60% of patients demonstrating supranormal levels.

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Keywords

Polycystic ovary syndrome; hyperandrogenemia; total testosterone; free testosterone; dehydroepiandrosterone sulfate

Introduction

As one of the most common endocrinopathies, androgen excess affects approximately 7% of reproductive-aged women (1,2,3). Among this large cohort of women, the majority carry the diagnosis of polycystic ovary syndrome (PCOS) (4). This is based on the presence of ovulatory dysfunction or polycystic ovaries, in association with hirsutism and/or elevated androgen levels, after the exclusion of specific identifiable androgen excess disorders i.e. androgen-secreting neoplasms, hyperandrogenic insulin-resistant acanthosis nigricans syndrome, and non-classic adrenal hyperplasia (NCAH).

As a result of its diagnosis by exclusion, PCOS has been a disorder that has been difficult to standardize. Most women with PCOS have both clinical and biochemical evidence of hyperandrogenism. The presence of multiple phenotypes that can exist also complicates the diagnosis (8). The major clinical manifestations of hyperandrogenism are acne, male-pattern hair loss, and hirsutism, defined as excess terminal body hair in a male distribution. The accuracy and evaluation of signs of hirsutism and clinical hyperandrogenism may be subject to individual observer biases (9). Substantial ethnic variability further complicates the diagnosis of PCOS by clinical evaluation (10).

All major diagnostic criteria for PCOS used today include hyperandrogenemia as one of the diagnostic features (5-7,16). While assessing the presence of clinical hyperandrogenism may be subjective, the assessment of hyperandrogenemia need not be. However, unlike the recent trends toward establishing clinical standards for the diagnosis of PCOS, the hormonal evaluation of PCOS and hyperandrogenemia remains to be standardized (11,12). Past studies have relied on normative standards based on small sample size of control patients (1,13). Furthermore, many investigators studies have relied only on the measurement of serum total testosterone (TT) levels for detecting biochemical hyperandrogenism in PCOS (14,15). In the present study we propose to determine the prevalence of elevated TT, free testosterone (FT), and DHEAS, alone and in combination, in a large cohort of patients with PCOS. Using this information, we will be able to determine the sensitivity of serum hormone evaluation and assess the utility of current nonspecific standards in the evaluation of hyperandrogenemia in PCOS.

Materials and Methods

Subjects

All patients presenting to our reproductive endocrinology clinic for the evaluation of symptoms potentially related to androgen excess between October 1987 and June 2002 were prospectively evaluated and recorded, and the data were maintained in a computerized database (Alpha Four v. 6.0; Alpha Software, Burlington, MA). The study was approved by the Institutional Review Board for Human Use at the University of Alabama at Birmingham. Patients evaluated included those presenting with oligo/amenorrhea, ovulatory dysfunction, excess hair growth, virilization, alopecia, or acne. Of the 873 patients who presented for evaluation, 720 were retrospectively analyzed and diagnosed with PCOS as described by the NIH 1990 criteria (5). None of the subjects were premenarchal or postmenopausal, had undergone prior hysterectomy, oophorectomy or natural menopause, or had been previously

diagnosed or were receiving hormonal treatment for at least three months prior to their evaluation.

Clinical Evaluation

All patients underwent a complete history and physical examination. The following parameters were recorded prospectively: height, weight, race, age, gravidity, parity, degree of ovulatory dysfunction, presence of acne, and hirsutism score. PCOS was defined by 1) the presence of hyperandrogenemia or clinical hyperandrogenism, 2) oligo-ovulation, and 3) the exclusion of other disorders. Ovulatory dysfunction was defined as a history of intermenstrual intervals greater than 35 days or by a day 22-24 progesterone levels less than 4 ng/mL in patients with vaginal bleeding intervals of 27-34 days. Clinical hyperandrogenism was defined by hirsutism with a modified Ferriman-Gallwey (mFG) score of greater than 6 (16). Hyperandrogenemia was defined by an androgen value exceeding the 95th percentile of 98 race-matched eumenorrheic control women from the same population as reported by Knochenhauer *et al* (1), with TT >88 ng/dL, FT > 0.66 ng/dL, or DHEAS >2750 ng/mL. Patients were excluded if they had received hormonal therapy for 3 months prior to their initial visit. Blood sampling for androgens were performed without regard to the time or day of the cycle.

PCOS was diagnosed only after other disorders had been excluded including hyperprolactinemia, thyroid disorders, 21-hydroxylase-deficient NCAH, Cushing's syndrome and virilizing androgen-secreting neoplasms. On physical exam, the waist was measured at the narrowest portion of the torso approximately midway between the lower costal margin and the iliac crest, and the hip circumference was measured over the widest portion of the gluteal and greater trochancteric region. Additionally, each patiënt was weighed and had her height measured.

Laboratory Analysis

A 30-cc sample of bleed was drawn for subsequent hormonal analysis, and was stored at -70°C until the time of assay. Serum samples were analyzed for FT, TT, sex hormone binding globulin (SHBG), and DHEAS. Total testosterone was measured by an in-house radioimmunoassay (RIA) method after serum extraction with ether and using dextran-coated charcoal for separation of bound and FT as described by Azziz *et al.* (17). SHBG activity was measured by competitive binding, using Sephadex G-25 and [^{3}H]T as the ligand, and the FT was calculated as described by Pearlman *et al.* (18). The levels of DHEAS were measured by a direct radioimmunoassay (RIA) using a commercially available kit (Diagnostic System Laboratories, Webster, TX).

Statistical Analysis

Summary statistics, including minimum, maximum, mean, median, Quartile 1 (25th percentile), Quartile 3 (75th percentile), and Standard deviation are given for the following variables: age, body mass index (BMI), waist-to-hip ratio (WHR), TT, FT, SHBG, DHEAS, and the mFG score. In addition, summary statistics for the Base 10 log of TT, FT, and DHEAS are presented.

Results

Seven hundred and sixteen PCOS subjects were included in the study; four patients were excluded because of missing or incomplete laboratory values. The characteristics of the study population are depicted in Table 1. The base 10 log of TT, FT, and DHEAS were analyzed to assess for more normal distribution of data. The overall prevalence of

hyperandrogenemia in PCOS was 75.3%. Supranormal levels of FT were present in 57.5%, TT in 33.0%, and DHEAS in 32.7% of PCOS patients.

The specific prevalence of elevated serum androgen profiles are depicted in Table 2. The single most predictive assay was the measurement of FT which was supranormal in nearly 60% of all patients. Elevated DHEAS levels as the sole abnormality were present in nearly 14% of patients. Alternatively, supranormal levels of TT as the sole abnormality were found in only 2% of patients. When studying the combination of supranormal androgen levels, the combination of TT and FT had the highest prevalence (20.4%) compared with TT and DHEAS (1.7%), and FT and DHEAS (8.5%). All three androgens were elevated in only 8.7% of the subjects studied.

Discussion

The diagnosis of PCOS follows that of the diagnosis of any syndrome, and as such requires the evaluation of a constellation of signs and symptoms. Assessing for evidence of hyperandrogenemia is only an adjuvant tool for the diagnosis of hyperandrogenic disorders such as PCOS. The criteria for diagnosis must be complemented with a thorough clinical assessment for hyperandrogenism and menstrual dysfunction. In order to be effectively utilized, however, it is important that the proper diagnostic ranges or limits of hyperandrogenemia be established. Establishing these ranges can be made by measuring androgens in a large population of well-characterized normal women, in whom PCOS has been excluded. Alternatively, normative ranges can be established by assessing androgen levels in an unselected population.

In 2006, the Androgen Excess Society charged a task force to review all available data and recommend an evidence-based definition for polycystic ovary syndrome (PCOS), whether already in use or not, to guide clinical diagnosis and future research (19). Their recommendation for the androgen evaluation in PCOS was to measure FT concentration using high quality assays, principally either directly by equilibrium dialysis, or to calculate FT based on TT and SHBG levels measured accurately (i.e. TT by mass spectrometry, or after extraction and chromatography; and SHBG by competitive binding or specific ELISA assays). Free T was much more sensitive than either TT or DHEAS in detecting hyperandrogenemia. A similar conclusion was reached by a task force of the Endocrine Society (12).

Our study of over 700 PCOS patients supports these recommendations indicating that the most useful test to detect hyperandrogenemia in PCOS is the FT level, when assessed using a high quality and sensitive assay, perhaps because this measure is the product of both ovarian androgen excess and inhibited hepatic production of SHBG. An elevated DHEAS level increases the number of patients detected as hyperandrogenemic by an additional 14%. Alternatively, the sole measure of TT, as proposed by some investigators (14,15) has limited value, with only one-third of subjects deemed hyperandrogenemic by the test, even when using a highly specific assay. Combined, however, the measurement of FT, TT, and DHEAS combined provides a reasonable test in the evaluation of PCOS with a sensitivity of 75.3%.

Prior studies attempting to assess the utility of androgen assays to evaluate their potential as single diagnostic tests for screening PCOS have been limited by their smaller study populations. Escobar-Morreale's analysis of receiver operating characteristics to determine the diagnostic performance of several serum parameters found that decreased SHBG levels, and increased free androgen index, FT, and DHEAS concentrations, though coincident with some of our findings, utilized only 8 PCOS study patients (20). Turhan *et al.* (21) evaluated prospectively the performance of serum LH, FSH, testosterone, FT, SHBG, and insulin

concentration in a group of 32 PCOS patients and 25 healthy controls. These investigators observed that LH/FSH and insulin levels best predicted PCOS status. In another recent study, Koskinen *et al.* (22) found that LH, LH/FSH ratio, testosterone, and androstenedione serum concentrations were useful in the detection of PCOS. There may be potential bias in this study as women with PCOS were recruited retrospectively. Additionally, the diagnosis of PCOS was based on the presence of oligomenorrhea and sonographic polycystic ovaries rather than the more Standard NIH 1990 criteria. Additionally, a recent study evaluated the value of free androgen index in identifying patients with PCOS and found that it to be a good diagnostic marker to distinguish hyperandrogenism in 12 patients with PCOS (23). We could not calculate this in our population since sex hormone binding globulin was not measured directly, but primarily as sex hormone binding globulin activity using a competitive binding modeling approach.

For this study we used normative values developed by assessing 98 healthy women (45 White and 53 Black) ages 18-45 years, who were non-hirsute (*i.e.* F-G score #5), non-acneic, eumenorrheic (regular cycles at, 35-day intervals), and were taking no hormonal therapy, selected from the general a population of women undergoing a pre-employment physical exam(1). These women were recruited from a population similar to that of our PCOS subjects (i.e. from women residing in Birmingham, Alabama or its surroundings). The normative limit was established using the upper control 95th percentile to account for the inherent skewedness inherent to the distribution of androgen values in the population. We should note that this estimate assumes that 5% of this population may have an occult disorder (e.g. PCOS). Although we did not assess ovulatory function in these eumenorrheic women, in view of the low probability of such an occult disorder we feel comfortable in using the relatively strict criteria of upper 95th percentile of this population to define the 'normal' limit.

In conclusion, approximately three-fourths of patients with PCOS diagnosed by the NIH 1990 criteria have evidence of hyperandrogenemia; the single most predictive assay was the measurement of FT, with approximately 60% of patients demonstrating supranormal levels. If we are limited to only one androgen test, then FT would be the best choice. Alternatively, the sole measure of TT may have a limited value, with only one-third of subjects deemed hyperandrogenemic by this test alone, and the addition of DHEAS measurement increased the number of patients detected as hyperandrogenemic by 15.7%. All assays should be performed using high quality, high sensitivity techniques. In the case where FT is normal, but TT and/or DHEAS are elevated, we still feel that these patients fulfill the NIH criteria for PCOS. Given the relative insensitivity of androgen levels in the circulation assessed at one point in time for the detection of overall hyperandrogenism and the fact that up to 30% of PCOS individuals will have a significant adrenal component, any abnormality in androgen levels, including isolated elevations in TT and DHEAS, should be considered as evidence of hyperandrogenemia.

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Table 1
Characteristics of PCOS patients (n=716)

Age (yr)	27.67 ± 7.26
BMI (kg/m^2)	33.39 ± 9.26
WHR	0.83 + 0.09
TT (ng/dl)	90.28 + 55.67
SHBG (nmol/liter)	182 + 71
FT (ng/dl)	0.92 + .57
DHEAS (ng/ml)	2350.8 + 1289.6
mF-G score	8.2 + 4.9
Race	
White (%)	83.2
Black (%)	14.4
Other (%)	2.4
Acne (%)	14.5
Oligo-ovulatory (%)	100.0
Infertile (%)	32.7
Hirsute (%)	72.2
Obesity (%)	60.0

Body mass index (BMI), Waist-to-hip ratio (WHR), Total Testosterone (TT), Sex hormone binding globulin (SHBG), Free Testosterone (FT), dehydroepiandrosterone sulfate (DHEAS), modified Ferriman-Gallwey (mFG) score.

The base 10 log of TT, FT, and DHEAS were analyzed to assess for more normal distribution of data.

Table 2
Prevalence of Specific Combinations of Androgen Levels in PCOS

TT	FT	DHEAS	Percent PCOS patients
normal	normal	normal	24.7%
↑	↑	normal	20.4%
normal	↑	normal	20.0%
normal	normal	↑	13.8%
↑	↑	↑	8.7%
normal	↑	↑	8.5%
↑	normal	normal	2.2%
↑	normal	↑	1.7%

TT is total testosterone, FT is free testosterone, and DHEAS is dehydroepiandrosterone sulfate.