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ORIGINAL ARTICLE

Is Idiopathic Hirsutism Truly Idiopathic?

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Abstract

Objective To determine whether *other* androgens [androstenedione (A4), 17-hydroxy progesterone (17OHP) and dehydroepiandrosterone (DHEA)] were elevated in women with classically defined idiopathic hirsutism (IH)/patient-important hirsutism (PIH).

Study Design Retrospective analysis.

Setting Outpatient endocrine department of a tertiary care hospital.

Patients In total, 30 consecutive women with IH/PIH were included. IH/PIH was defined as presentation with hirsutism with normal menstrual cycles (25–35 days), normal total (< 45 ng/dL) and free T (fT) (< 0.6 ng/dL) and normal ovaries sonologically (transabdominal ultrasonogram ovarian volume < 10 cm^3) without any other signs of virilization. Clinical and biochemical details were collected and analyzed. Androgens were measured by LC–MS/MS. A4 ≥ 2.5 ng/mL, DHEA ≥ 15 (age < 18) or ≥ 11.8 (age ≥ 18) ng/mL, DHEAS ≥ 2847 ng/mL or 17OHP ≥ 2 ng/mL were considered high.

Results With the mean age of 22 years and mean BMI of 25 kg/m^2, 12/30 (40%) had IH and remaining PIH. DHEA alone was elevated in 60% and A4 alone in 33%. Overall, 23/30 (73%) had any one elevated androgen with normal total and free testosterone. There was no correlation with modified Ferriman–Gallwey score, and there was no significant difference in androgens between IH and PIH.

Conclusion A high proportion of women with classically defined IH/PIH have elevated DHEA and/or A4. Though on pharmacotherapy basis, there would be no change in management, the role of hyperandrogenemia detected by sensitive assays on metabolic functions and cardiovascular risk has to be studied.

Keywords Patient-important hirsutism · Dehydroepiandrosterone · Androstenedione · Liquid chromatography–mass spectroscopy

Introduction

Ascertaining hyperandrogenism in women, either clinical or biochemical, has inherent problems. Tissue sensitivity to androgens varying within an individual at different sites of body and between individuals; the modified Ferriman–Gallwey score (mFGS) varying with each ethnicity and the lack of objective measures to check other hyperandrogenic signs (acne, female pattern hair loss, etc.)—all these factors impede accurate diagnosis of clinical hyperandrogenism. Cross-reactivity in the steroid immunoassays that are widely available; total testosterone being falsely low due to low SHBG & unreliability in free testosterone calculations have paved way for recommendation that testosterone should be measured preferably by liquid chromatography–tandem mass spectrometry (LC–MS/MS). Hence, we chose to

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measure by gold standard technique so that biochemical hyperandrogenism could be accurately diagnosed.

In premenopausal PCOS women, elevated dehydroepiandrosterone sulfate (DHEAS) with normal androstenedione (A4) and total testosterone (TT) was observed in up to 40% of them [1]. However, routine screening with A4 and DHEAS is not recommended [2]. For several years now, 11-oxygenated C19 steroids (keto and hydroxyl forms) have been identified in humans and hypothesized to play a role in activating androgen receptor [3]. 17-hydroxyprogesterone is known to produce androgens through 'backdoor pathway' and dehydroepiandrosterone (DHEA), and A4 contributes as a substrate to 11-oxygenated C19 steroids that would result in hyperandrogenism. Hence, we measured these *other* androgens to see if they are elevated, contributing to hirsutism when TT is normal.

Idiopathic hirsutism (IH) is defined as hirsutism without hyperandrogenemia (normal total and free T) or other signs/symptoms of hyperandrogenic endocrine disorder [4]. Also, there is another definition that includes DHEAS along with testosterone [5]. The premise is that these *other* androgens might not be significantly elevated if T is normal. However, the same guideline acknowledges the fact that utilizing better techniques to measure androgens may detect occult hyperandrogenemia [4]. It is well known that currently LC–MS/MS is the gold standard for measuring steroids. With the advent of LC–MS/MS, the cutoff of TT in normal women has been lowered. We wanted to know which of these *other* androgens is/are elevated and in what proportion are they elevated in women with IH/PIH.

Materials and Methods

This was a retrospective analysis from a tertiary care hospital in the state of Kerala, India. In the conduct of this clinical study, Declaration of Helsinki for Medical Research involving human subjects was followed. After obtaining institutional review board clearance (Giridhar Eye Institute IEC/IRB protocol no SL 03/08), we conducted a search in the database for the diagnosis of 'idiopathic hirsutism' or 'patient-important hirsutism.' Since we started analyzing the androgens by LC–MS/MS from an accredited laboratory since December 2018, we excluded search entries prior to this date.

IH was defined as hirsutism (mFGS ≥ 3) with normal menstrual cycles (25 to 35 days), normal total (< 70 ng/dL) and free T (fT) (< 0.8 ng/dL) and normal ovaries sonologically (transabdominal ultrasonogram (USG) ovarian volume < 10 cm^3) without any other signs of virilization [6]. In all women, other causes of hirsutism such as hypothyroidism, hyperprolactinemia, and medications such as anabolic steroids, androgenic steroids and valproate were ruled out. When mFGS < 3 in the above said criteria, it was defined as PIH. The criterion for defining hirsutism is known to be variable, but our cutoff of mFGS ≥ 3 was defined from these references [7–9]. Of the retrieved records, when the presenting compliant was infertility, acne or alopecia, they were excluded. In all, we retrieved 35 patient entries of which 5 had elevated fT and hence were relabeled them as idiopathic hyperandrogenism and we excluded them.

For all 30 IH women, clinical, anthropometric and details of investigations were collected. Acne was graded with global acne scoring system [10]. A score of > 4 was considered positive. mFGS was assessed in all women by a single endocrinologist. Blood samples were taken, and USG was performed in early follicular phase (< 7 days from last menstrual period). Serum was separated, frozen to -20 °C and shipped to laboratory for analysis. All women underwent transabdominal USG by a single blinded radiologist, and only the ovarian volume was calculated (by traditional formula 0.5 x length x width x height in centimeters) for ascertaining polycystic ovary morphology [11].

For steroid analysis, the steroid standards were obtained from IsoSciences (Ambler, USA). The measurements of 5 steroids-A4, TT, 17OHP, DHEA and DHEA-S were done using an internally developed and validated (unpublished) LC–MS/MS assay using Waters™ XEVO™ TQD system (Waters corporation, Milford, USA). The steps included preparing serum aliquots, analyte extraction, elution, chromatographic separation, MS analysis and interpretation—all of which were done in the LC–MS center at Department of Biochemistry, Neuberg Anand Reference Laboratory, Bangalore. The limits of quantification for the analytes were A4—0.1 ng/mL, TT—0.02 ng/dL, 17-OHP—0.1 ng/mL, DHEA-0.98 ng/mL and DHEA-S-100 ng/mL. The calibration and QC were done in accordance with NABL ISO-15189, and CV % was between 5 and 8 for all steroids. Free testosterone was calculated using published and validated method [12]. Either of A4 ≥ 2.5 ng/mL, DHEA ≥ 15 (age < 18) or ≥ 11.8 (age ≥ 18) ng/mL, DHEAS ≥ 2847 ng/mL or 17OHP ≥ 2 ng/mL were considered high [11, 13].

Statistics

Descriptive variables were expressed as percentages. Continuous variables were expressed as mean $\pm$ standard deviation or median with interquartile ranges for parametric and nonparametric variables. Correlation between various clinical and biochemical values was determined with Spearman's correlation. With hirsutism ≥ 3 as binary response variable, all androgens were evaluated with ROC curve for a cutoff with best trade-off between sensitivity and specificity. Level of significance to reject null hypothesis was 5%. Data were analyzed with IBM® SPSS® version 23 (IBM Corp, released 2015; Armonk, NY, USA).

Results

Mean age of this cohort was 22 years; mean BMI was 25 kg/m^2 (Table 1). Acne was present in 7 (23%), but none had female pattern hair loss. IH was present in 12/30 (40%) and PIH in 18/30 (60%). While all the details were available for the whole cohort, USG was not performed in 3 women due to logistic reasons. A4 alone was elevated in 10/30 (33%) women and DHEA alone in 20/30 (60%) of women. Four women had 17OHP levels > 2 ng/mL, and they underwent ACTH stimulation to rule out non-classical CAH. All 4 had normal stimulated 17OHP values and they also had either elevated A4 or DHEA. Of 4 women with elevated DHEAS, 2 had elevated A4 and DHEA and another 2 had elevated DHEA alone. In all, 22/30 (73%) had at least one elevated androgen in spite of having normal TT and fT measured by LC–MS/MS (Fig. 1). Even when we exclude women with elevated DHEAS (which is the stricter IH criteria), 18/26 (69%) still had elevated DHEA and/or A4.

Spearman's correlation was performed between androgens and between androgens and mFGS. A4 had strong correlation with TT (r = 0.72, p < 0.001) and 17OHP (r = 0.63, p < 0.001). TT also correlated with fT (r = 0.69, p < 0.001). While fT did not correlate with other androgens after Bonferroni correction, DHEA did not correlate with any in first place itself. None of the androgens correlated with mFGS. There were no significant differences in androgen levels between IH and PIH (Table 2).

In receiver operating characteristic curve analysis, with binary outcome of hyperandrogenism as defined by mFGS ≥ 3, there were no significant cutoffs that could be determined for any of the androgens.

Table 1 Clinical and biochemical characteristics of idiopathic hirsutism women

Parameter	Value (N = 30)
Age (years)	22 ± 5
BMI (kg/m^2)	25.3 ± 6.2
Acne (%)	7 (23)
Modified Ferriman–Gallwey score	2 (0.3)
Total testosterone (ng/dL)	36 ± 14
Free testosterone (ng/dL)	0.6 (0.5,0.8)
Androstenedione (ng/mL)	2.3 (1.3,2.5)
Dehydroepiandrosterone (ng/mL)	19.1 (11.1,30.3)
17-hydroxy progesterone (ng/mL)	0.5 (0.4,1.3)
Dehydroepiandrosterone sulfate (ng/mL)	1833 ± 925

Values are expressed as number (percentage), median (interquartile range) or mean ± SD

Discussion

The most important implication of this retrospective study is that the notion 'TT and fT are sufficient enough to screen for hyperandrogenemia' has to be changed. This is because of the fact that about 73% of hirsute/PIH women had elevated DHEA and/or A4. Hence, we suggest to incorporate DHEA and/or A4 into the evaluation of the androgens in women with IH/PIH. Whether such determination will benefit those women when anti-androgens are added should be evaluated prospectively.

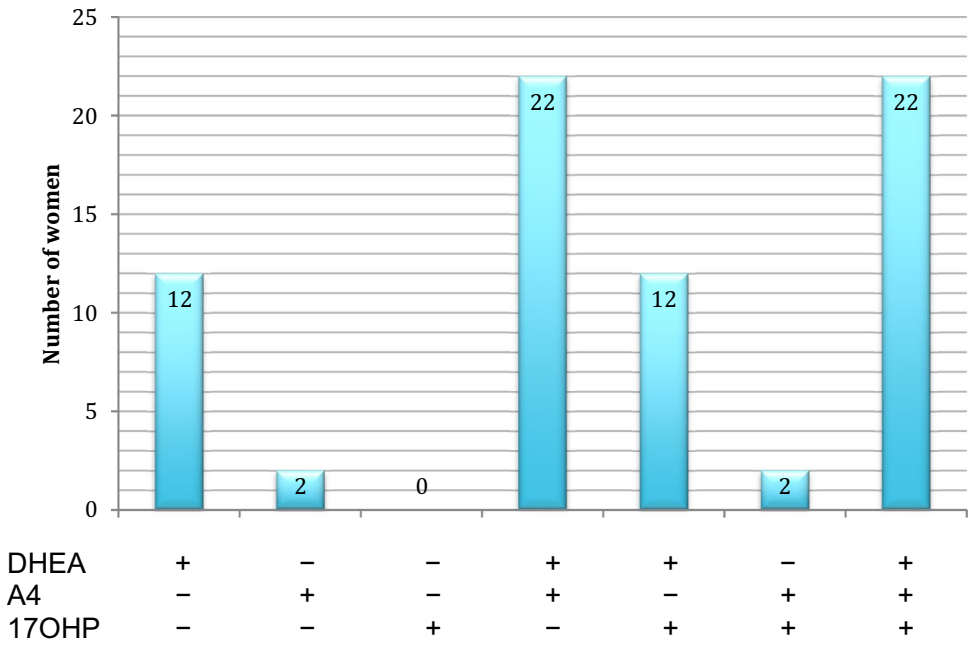

Fig. 1 Distribution of various androgens in women with idiopathic hirsutism. Total N = 30. + sign indicates elevated values, while − sign indicates normal values. In case of 2 or more + signs, it means either of the steroids can be elevated. *DHEA* dehydroepiandrosterone, *A4* androstenedione, *17OHP* 17-hydroxy progesterone

Table 2 Androgen levels according to degree of hirsutism

Androgen	mFGS < 3 (*N* = 18)	mFGS ≥ 3 (*N* = 12)	*P*
Total testosterone (ng/dL)	35 ± 16	36 ± 11	0.8
Free testosterone (ng/dL)	0.6 (0.5,0.8)	0.7 (0.4,0.8)	0.8
Androstenedione (ng/mL)	2.3 (1,2.5)	2.2 (1.3,2.7)	0.6
Dehydroepiandrosterone (ng/mL)	13.5 (9.5,30.5)	25 (15,31)	0.3
17-hydroxy progesterone (ng/mL)	0.5 (0.4,1.3)	0.5 (0.3,1.7)	0.8
Dehydroepiandrosterone sulfate (ng/mL)	1657 ± 830	2099 ± 1031	0.2

Values are expressed as median (interquartile range) or mean ± SD

While total testosterone was compared with Student's t test, other parameters were compared with Mann–Whitney *U* test

mFGS modified Ferriman–Gallwey score

This seems to be the only study, as far as we know, to study hyperandrogenemia by LC–MS/MS in patients with only IH. There have been studies to determine the prevalence of IH in women with hirsutism [14, 15]. The prevalence varied from 6 to 17% in both studies wherein IH was defined based on ovulatory cycles (luteal phase progesterone measurements) and normal androgens (TT, fT, DHEAS) measured by immunoassay. As discussed in our results, even when elevated DHEAS is included into criteria for diagnosing IH, still 69% of remaining women had either elevated DHEA and/or A4. In another previous large study concerning women who had undergone androgen measurements (DHEAS, T, A4), it was shown that only 49% of premenopausal PCOS would have been diagnosed with isolated measurement of T [1]. Though androgen patterns in PCOS must not be compared with IH, still it gives us the idea that evaluating all the androgens would substantially change the diagnosis. Another fact to be appreciated is that previously, androgens used to be measured by immunoassays due to which the cutoffs to define abnormalities would be higher because of high cross-reactivity. The cut-off for testoterone measured by LC-MS/MS was modified from 70 to 45 ng/dL recently [6]. With this cut-off, 2/30 women would have been relabelled as idiopathic hyperandrogenism. If the cutoffs are lowered according to a newly published study, TT ≥ 25 ng/dL; A4 ≥ 1.5 ng/mL and DHEA ≥ 6.1 ng/mL would be considered abnormal [16]. Then, in our cohort, 25/30 (83%) would have high TT; 20/30 (66%) would have high A4; 29/30 (96%) would have high DHEA levels. Then, almost all patients with IH would be labeled as idiopathic hyperandrogenism.

A4, 17OHP and DHEA are androgenic as they get converted to 11-oxygenated steroids—both hydroxyl and keto forms. Of late, these steroids have been shown to stimulate androgen receptors almost with equal efficacy [17]. Hence, we try to impress upon the fact that *other* androgens might actually explain the pathophysiology of IH and that IH might not be truly 'idiopathic.' Recently, a study from China had shown that 8% of women with IH, acne or both, had heterozygous CYP21A2 mutations [18]. We recognize that determining *other* androgens in IH/PIH group does not alter the pharmacotherapy and only results in the change in diagnosis from IH to idiopathic hyperandrogenism. Whether the detected hyperandrogenemia in this group has the same implications as in PCOS (in increasing the cardiovascular risk) is the larger question.

There are several limitations in our study. Firstly, referral bias of relatively severe hirsute women does exist in a tertiary care center. Secondly, anovulation does occur in up to 60% of women with regular menses and normal androgens as evidenced by basal body temperature monitoring and mid-luteal progesterone [14]. But we did not measure mid-luteal progesterone in our women. Such measurement and determination of anovulation would have led to change in diagnosis from IH to PCOS, but the more important discussion of this paper is to highlight that there exists a high proportion of women who have elevated unconventional androgens.

Conclusion

This study reiterates that apart from TT and fT, DHEA and A4 needs to be measured to rule out hyperandrogenism in patients suspected of IH/PIH. Though it seems that additional testing would detect occult hyperandrogenism and explain the actual cause of hirsutism in IH, the diagnostic and therapeutic changes due to such additional testing warrant further research.

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Compilance with Ethical Standards

Conflict of interest Authors disclose that there are no conflicts of interests.

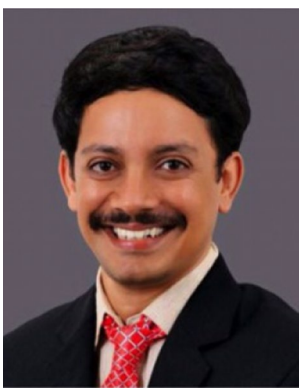
Ethical statement This study was in accordance with the ethical standards of the institution and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent Informed written consent was obtained from all participants.

References

1. Elhassan YS, Idkowiak J, Smith K, Asia M, Gleeson H, Webster R, et al. Causes, patterns, and severity of androgen excess in 1205 consecutively recruited women. J Clin Endocrinol Metab. 2018;103(3):1214–23. https://doi.org/10.1210/jc.2017-02426.
2. Teede HJ, Misso ML, Costello MF, Dokras A, Laven J, Moran L, et al. Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome. Hum Reprod. 2018;33(9):1602–18. https://doi.org/10.1093/humrep/dey256.
3. Turcu AF, Nanba AT, Auchus RJ. The rise, fall, and resurrection of 11-oxygenated androgens in human physiology and disease. Horm Res Paediatr. 2018;89(5):284–91. https://doi.org/10.1159/000486036.
4. Martin KA, Anderson RR, Chang RJ, Ehrmann DA, Lobo RA, Murad MH, et al. Evaluation and treatment of hirsutism in premenopausal women: an endocrine society clinical practice guideline. J Clin Endocrinol Metab. 2018;103(4):1233–57. https://doi.org/10.1210/jc.2018-00241.
5. Azziz R, Carmina E, Sawaya ME. Idiopathic hirsutism. Endocr Rev. 2000;21(4):347–62. https://doi.org/10.1210/edrv.21.4.0401.
6. Robert D, Nerenz EJ, Ann M. Reproductive endocrinology and related disorders. In: Nader Rifai ARH, Wittwer C, editors. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. London: Elsevier Health Sciences; 2017. p. 1617–1654.
7. Escobar-Morreale HF, Carmina E, Dewailly D, Gambineri A, Kelestimur F, Moghetti P, et al. Epidemiology, diagnosis and management of hirsutism: a consensus statement by the Androgen Excess and Polycystic Ovary Syndrome Society. Hum Reprod Update. 2012;18(2):146–70. https://doi.org/10.1093/humupd/dmr042.
8. DeUgarte CM, Woods KS, Bartolucci AA, Azziz R. Degree of facial and body terminal hair growth in unselected black and white women: toward a populational definition of hirsutism. J Clin Endocrinol Metab. 2006;91(4):1345–50. https://doi.org/10.1210/jc.2004-2301.
9. Souter I, Sanchez LA, Perez M, Bartolucci AA, Azziz R. The prevalence of androgen excess among patients with minimal unwanted hair growth. Am J Obstet Gynecol. 2004;191(6):1914–20. https://doi.org/10.1016/j.ajog.2004.06.064.
10. Doshi A, Zaheer A, Stiller MJ. A comparison of current acne grading systems and proposal of a novel system. Int J Dermatol. 1997;36(6):416–8. https://doi.org/10.1046/j.1365-4362.1997.00099.x.
11. Lee TT, Rausch ME. Polycystic ovarian syndrome: role of imaging in diagnosis. Radiographics. 2012;32(6):1643–57. https://doi.org/10.1148/rg.326125503.
12. Vermeulen A, Verdonck L, Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. J Clin Endocrinol Metab. 1999;84(10):3666–72. https://doi.org/10.1210/jcem.84.10.6079.
13. Eisenhofer G, Peitzsch M, Kaden D, Langton K, Pamporaki C, Masjkur J, et al. Reference intervals for plasma concentrations of adrenal steroids measured by LC-MS/MS: impact of gender, age, oral contraceptives, body mass index and blood pressure status. Clin Chim Acta. 2017;470:115–24. https://doi.org/10.1016/j.cca.2017.05.002.
14. Azziz R, Waggoner WT, Ochoa T, Knochenhauer ES, Boots LR. Idiopathic hirsutism: an uncommon cause of hirsutism in Alabama. Fertil Steril. 1998;70(2):274–8. https://doi.org/10.1016/s0015-0282(98)00141-1.
15. Carmina E. Prevalence of idiopathic hirsutism. Eur J Endocrinol. 1998;139(4):421–3. https://doi.org/10.1530/eje.0.1390421.
16. Skiba MA, Bell RJ, Islam RM, Handelsman DJ, Desai R, Davis SR. Androgens during the reproductive years: what is normal for women? J Clin Endocrinol Metab. 2019;104(11):5382–92. https://doi.org/10.1210/jc.2019-01357.
17. Pretorius E, Africander DJ, Vlok M, Perkins MS, Quanson J, Storbeck KH. 11-ketotestosterone and 11-ketodihydrotestosterone in castration resistant prostate cancer: potent androgens which can no longer be ignored. PLoS ONE. 2016;11(7):e0159867. https://doi.org/10.1371/journal.pone.0159867.
18. Gao Y, Yu B, Mao J, Wang X, Nie M, Wu X. The prevalence of heterozygous CYP21A2 deficiency in patients with idiopathic acne, hirsutism, or both. Endocrine. 2019. https://doi.org/10.1007/s12020-019-02104-7.

About the Author



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