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Validity of Serum Testosterone, Free Androgen Index, and Calculated Free Testosterone in Women with Suspected Hyperandrogenism

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Abstract

Objectives: There are technical limitations for the currently available methods of measuring serum total and free testosterone in females. The study objectives were to evaluate the usefulness of serum total testosterone, sex hormone-binding globulin (SHBG), free androgen index (FAI), and calculated free testosterone (CFT) in the assessment of androgen status in women investigated for suspected hyperandrogenism.

Methods: This is a case control study that was conducted during the period from 1st May 2011 to 31st October 2011 on 122 patients aged (18-45 years) whom were referred to the Clinical Biochemistry Laboratory from the Endocrinology and Gynecology Clinics, Royal Hospital, Oman. Women with no clinical feature or laboratory data indicative of hormonal dysfunction and with midluteal progesterone >30 nmol/L were selected as controls (group 1; n=18). The patients were divided into subgroups based on the clinical/laboratory diagnosis of polycystic ovary syndrome (PCOS [group 2; n=19), hirsutism (group 3; n=18), menstrual disturbances (irregularities) or infertility (group 4; n=49), as well as combination of PCOS or hirsutism and menstrual disturbances or infertility (group 5; n=18). Serum total testosterone and SHBG were measured, FAI was calculated as percentage ratio of total testosterone to SHBG values, and CFT was calculated according to Vermeulen equation.

Results: There was a statistically significant difference in the mean levels of testosterone, FAI and CFT in each patient group compared with the control group. For diagnosing hyperandrogenism, each indicator was selected at the recommended cut-off: testosterone >3.0 nmol/L, SHBG <30 nmol/L, FAI >5%, and CFT >32 pmol/L. In group 2, 89.5% and 94.7% of the patients had increased FAI and CFT, respectively; compared with 36.4% for increased testosterone. In group 3, 88.9% and 88.9% of the patients had similarly increased FAI and CFT, respectively; compared with 66.7% for testosterone. In group 4, patients had 63.3% and 73.5% elevated FAI and CFT, respectively; compared with 53.1% for testosterone, while in group 5, patients had 83.3% and 88.9% elevated FAI and CFT, respectively, compared with 61.1% for testosterone.

Conclusion: The diagnosis of hyperandrogenism was most obvious when using CFT or FAI than testosterone alone. It is thus recommended to include these calculated parameters (CFT and/or FAI) in the routine investigation and assessment of women with disorders related to clinical or biochemical hyperandrogenism.

Keywords: Hyperandrogenism; Testosterone; SHBG; Free androgen index; Calculated free testosterone.

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Introduction

Testosterone is the principal male sex steroid hormone that is produced mainly in the testes and is responsible for the development of male sex characteristics including its effect on spermatogenesis. It is also produced in smaller amounts by the ovaries in women. In both sexes, there is contribution from the adrenal cortex in the synthesis of other androgens which are precursors for testosterone, the effect of which becomes evident in patients with adrenal disorders, particularly adrenal tumors and certain types of congenital adrenal hyperplasia. The levels of testosterone and its active metabolite, dihydrotestosterone during embryonic and fetal life have major contribution in the intrauterine sex development and sex organ differentiation.[1]

The majority (nearly 60%) of testosterone is bound to a specific high-affinity protein called sex hormone-binding globulin (SHBG), with albumin accounting for binding to the remaining nearly 38% of the hormone. A small fraction (nearly 2% males, 1% in females) represents the physiologically active free form that mediates the biological action of the hormone at the target tissues in both sexes.[1,2] SHBG is a plasma dimeric glycoprotein (MW 90,000 Da) comprising 373 amino acids. It is produced by the liver and its production is controlled by certain hormonal as well as physiological and pathological factors.[3] In the circulation, SHBG binds both testosterone and estrogens with higher affinity of binding to testosterone than to estrogens (affinity to testosterone is 5 times greater than to estrogens). In addition to binding steroids, SHBG binds to receptor sites on plasma membranes.[2,3] An increase in SHBG concentration decreases the bioavailability of testosterone and thus decreases the free hormone levels without noticeable change in total hormone levels, and vice versa. There is an inverse relationship between SHBG concentration and free testosterone status.[1,2] Conditions that are associated with decreased SHBG in females as in polycystic ovary syndrome (PCOS) are usually

accompanied with excess testosterone action or hyperandrogenism such as hirsutism, menstrual disturbance, or infertility.[4,5] In men, conditions associated with increased SHBG concentration (such as thyrotoxicosis, liver disease) lead to decreased free testosterone status and hypogonadism.[1,2]

Androgen excess is the cardinal underlying phenomenon in a variety of disorders in females particularly PCOS, idiopathic hirsutism, congenital adrenal hyperplasia, and ovarian/adrenal neoplasms, as well as insulin resistance.[6] Free or bioactive testosterone that is responsible for the pathogenesis of androgen excess status appears to be of more clinical relevance; however, this may not be easily elaborated due to its existence in small fraction within the total hormone concentration, its influence by the carrier proteins concentration as well as its metabolic status. Therefore, despite all advances in technology, direct measurement of free testosterone concentration is surrounded by many technical difficulties with many assays not easily feasible.[7,8] As alternatives, calculating the percentage ratio of total testosterone to SHBG concentration, called free androgen index (FAI), or calculating the free testosterone (CFT) from total testosterone levels and SHBG using a specified formula have increasing application as diagnostic tools for hyperandrogenism. This approach has improved the validity and efficiency of the test as a diagnostic tool, particularly in females.[9,10] From a clinical viewpoint and taking all these pitfalls into consideration, the core inclusion criteria for the diagnosis of PCOS according to the European Society for Human Reproduction (ESHR)/American Society of Reproductive Medicine (ASRM) [Rotterdam] criteria have based the PCOS diagnosis on clinical or biochemical evidence of hyperandrogenism, ovarian polycyst appearance on ultrasound and ovulatory dysfunction after exclusion of other similarly appearing disorders.[4,11]

In the Clinical Biochemistry Laboratory, Royal Hospital, Oman, the assessment of androgen status is usually achieved by measuring serum total testosterone and occasionally other androgens. The objectives of this study were to evaluate the usefulness of biochemical tests other than serum total testosterone namely, SHBG, FAI and CFT in the assessment of androgen status in women with suspected hyperandrogenism, based on laboratory and/or clinical symptoms.

Methods

This is a case control study that was conducted at the Clinical Biochemistry Laboratory, Royal Hospital, Oman, over a six-month period (from 1st May 2011 - 31st October 2011). Ethical approval for conducting this work was obtained from the Research and Ethical Review Committee, Directorate of Research and Studies, Royal Hospital, on 5/4/2011.

Serum specimens from 122 patients aged 18-45 years with the clinical/laboratory diagnosis or suspicious for PCOS, hirsutism, menstrual irregularities/disturbances, or/and infertility were identified from those who were referred to the Clinical Biochemistry Laboratory, from Endocrinology and Gynecology Clinics, Royal Hospital, Oman. A relatively small control group of 18 women aged 24-39 years was also selected from the women who were referred for mid-luteal progesterone levels (an index of ovulation) and proved to have progesterone levels >30 nmol/L and who did not have clinical features or laboratory data indicative of hormonal dysfunction. Data for serum testosterone and other hormonal results for all the women were taken from the laboratory computer system for data analysis and classification of patients. Measurement of SHBG and calculation of the FAI and CFT were done for all patients. The subjects were divided into the following subgroups (age presented as mean±SD):

1. Group 1: Control (n=18; aged 29±4.4 years)
2. Group 2: PCOS (n=19; aged 23.8±5.1 years)
3. Group 3: Hirsutism (n=18; aged 26.7±7.0 years)
4. Group 4: Menstrual disturbances (irregularities) or infertility (n=49; aged 27.6±5.4 years)
5. Group 5: PCOS or hirsutism and menstrual disturbances or infertility (n=18; aged 25.2±4.9 years)

Serum total testosterone and SHBG were measured by chemiluminescent microparticle immunoassay methods using Architect ci8200 Analyzer (Abbott, USA). The FAI was calculated as the percentage ratio of total testosterone to SHBG values (both in nmol/L, i.e. same unit), whereas CFT was calculated according to Vermeulen et al. equation.[12] The SSPS statistic software was used to calculate the mean±standard deviation (SD) and t-test was used to compare the differences in the means of each parameter (measured or calculated) between the different groups. Statistical significance was assigned at $p<0.05$. Also, the classification of the results for each parameter being normal or abnormal were based on the cut-offs recommended in the clinical practice and were not derived from the control group. Quality assurance was followed using both Internal QC and External QA scheme which are being followed by the laboratory for all concerned tests. However, for SHBG (a newly tested assay), the internal QC materials included in the kit were used in the evaluation.

Results

The medical records of the patient subsets (n=122) were reviewed regarding clinical hyperandrogenism and other components of ESHRE/ASRM (Rotterdam) Concensus criteria for the diagnosis of PCOS,[4] clinical symptoms, ultrasonographic findings.[12,13] Laboratory results were also reviewed and classified as previously stated.

The results of the different indicators of androgen status for the five groups are presented in Table 1. There was a statistically significant difference in the levels of testosterone, FAI and CFT between the controls and each patient group, with the significance being higher for FAI and CFT than for testosterone. Serum SHBG alone showed a significant difference only in group 2 and group 4 (each laboratory parameter was selected at the recommended cut-off; <30 nmol/L for hyperandrogenism SHBG and >5 for FAI.[12,13] For testosterone, a cut-off value of >3.0 nmol/L was followed as

being implemented in Clinical Biochemistry Laboratory at Royal Hospital. For CFT, there was no clear availability of recommended cut-off in the literature, it was also not provided by the manufacturer; however, a cut-off value of >32 pmol/L was used as it is the cut-off followed in other laboratories. Based on these cut-offs, an increase in the predictive value in the diagnosis of the hyperandrogenism state in the patients was noticed compared with testosterone alone, (Table 2). The degree of hyperandrogenism was most obvious when FAI or CFT were used rather than testosterone in the patient groups. In group 2, patients had increased FAI and CFT of 89.5% and 94.7%, respectively, compared with 36.4% for increased testosterone. While in group 3, patients had similarly increased FAI and CFT of 88.9% and 88.9%, respectively, compared with 66.7% for testosterone. Additionally in group 4, patients had elevated FAI and CFT of 63.3% and 73.5%, respectively, compared with 53.1% for testosterone. While in group 5, patients had elevated FAI and CFT of 83.3% and 88.9%, respectively, compared with 61.1% for testosterone. In each of these groups, the proportions of patients with low SHBG concentration alone at a cut-off of <30 nmol/L were lower than those having FAI, CFT, or testosterone.

Table 1: Laboratory parameters of androgen status in the different groups. Data presented as mean ± SD and statistical difference in each group compared with the control.

Groups	Total Testosterone (nmol/L)	SHBG (nmol/L)	FAI (%)	CFT (pmol/L)
Group 1 (n=18)	2.1±1.0	78.6±34.1	2.9±1.5	24.0±14.9
Group 2 (n=19)	3.6 ±1.5 (p<0.001)	38.4±36.6 (p<0.005)	15.6±17.8 (p<0.001)	62.8±26.3 (p<0.003)
Group 3 (n=18)	3.3 ±1.4 (p<0.05)	34.5±13.1 (NS)	10.4±4.7 (p<0.01)	59.7±24.6 (p<0.01)
Group 4 (n=49)	2.9 ±1.1 (p<0.01)	50.2±32.2 (p<0.04)	9.2±11.5 (p<0.03)	48.0±29.1 (p<0.02)
Group 5 (n=18)	3.4 ±1.3 (p<0.08)	32.9±21.5 (NS)	13.8±9.3 (p<0.05)	68.6±33.0 (p<0.07)

SHBG: sex hormone binding globulin; FAI; free androgen index; CFT: calculated free testosterone.

Table 2: Classification of patients based on the different cut-offs of laboratory parameters of androgen status in the different groups. Data presented as number and percentage of patients at the recommended cut-offs.

Groups	Test	Total Testosterone (nmol/l)		SHBG (nmol/l)		FAI (%)		CFT (pmol/l)	
	Cut-off	≤3.0	>3.0	<30	≥30	<5	≥5	≤32	>32
Group 1	n	18	0	0	18	17	1	17	1
(n=18)	%	100%	0 %	0 %	100 %	94.4 %	5.5 %	94.4 %	5.5 %
Group 2	n	12	7	9	10	2	17	1	18
(n=19	%	63.2 %	36.4 %	47.4 %	52.6 %	10.5 %	89.5 %	5.3 %	94.7%
Group 3	n	6	12	5	13	2	16	2	16
(n=18)	%	33.3 %	66.7 %	27.8 %	72.2 %	11.1 %	88.9 %	11.1 %	88.9 %
Group 4	n	23	26	13	36	18	31	13	36
(n=49)	%	46.9%	53.1%	26.5%	73.5%	36.7%	63.3%	26.5%	73.5%
Group 5	n	7	11	10	8	3	15	2	16
(n=18)	%	38.9%	61.1%	55.6%	44.4%	16.7%	83.3%	11.1%	88.9%

SHBG: Sex hormone binding globulin; FAI: free androgen index; CFT: calculated free testosterone.

Discussion

In the current study, there were significant differences in the mean values of all the three indicators for androgen status (FAI, CFT, and testosterone) in all the groups compared with the controls. The statistical significance was higher for FAI and CFT than for testosterone. In all groups, FAI and CFT appeared to be better predictors of hyperandrogenism than testosterone. When FAI and CFT were compared with testosterone for classifying androgen excess status, higher proportions of patients were identified using these two indicators than when testosterone alone was used. In group 2, the portion of women with PCOS had increased FAI and CFT of 89.5% and 94.7%, respectively, compared with 36.4% for increased testosterone. While the portion of group 2 with hirsutism had similarly increased FAI and CFT of 88.9% and 88.9%, respectively, compared with 66.7% for testosterone.

In group 4, the women with menstrual disturbances or infertility had elevated FAI and CFT of 63.3% and 73.5%, respectively, compared with 53.1% for testosterone. While in group 5 the women with either PCOS or hirsutism and menstrual disturbances or infertility had increased FAI and CFT levels of 83.3% and 88.9%, respectively, compared with 61.1% for testosterone. The cut-offs for these parameters was ≥5% for FAI, >32 pmol/L for CFT and >3.0 nmol/L for testosterone. In all these groups, the proportion of patients with low SHBG concentrations alone at the cut-off of <30 nmol/L were lower than those having elevated FAI, CFT or testosterone. Accordingly, it appears to be advantageous to use either or both indicators (CFT or FAI) for evaluating androgen status than using testosterone alone. These indicators require measurement of both testosterone and SHBG; hence these two tests represent an attractive profile for androgen status that will allow the calculation of the two derived indices, CFT and FAI.

In comparison with other studies, Cupisti et al. reported that for hirsute and PCOS, obese women are particularly associated with significantly increased CFT, FAI, and decreased SHBG,[14] an observation that was also confirmed by Meuller et al.[15] While Hahn et al.[13] found the highest area under the receiver operating characteristic curve (AUC-ROC) was for calculated bioavailable testosterone, followed by FAI and then CFT, with the lower AUC-ROC found to be for SHBG, total testosterone, and androstendione. Hahn et al.[13] also reported high sensitivity and specificity of 71.4% and 85.2%, respectively for FAI, and 75.9% and 83.3%, respectively for CFT in PCOS; confirming the significant correlation of these indices with hirsutism scores, ovarian volume and follicle count. Meuller et al.[16] also reported that the calculated FAI, CFT, and bioavailable testosterone were more appropriate markers for assessing hyperandrogenism than using the individual hormones that included total testosterone, free testosterone, dihydrotestosterone, dehydroepiandrostenedione and SHBG alone. The calculated indices (CFT and FAI) were also proven to be of high diagnostic accuracy by others.[17]

Conclusion

Taking into consideration the high prevalence of androgen excess status among women, the known technical limitations of the currently available methods for measuring total or free testosterone, and the diagnostic improvement when using FAI or CFT as indicators of androgen status, it is highly recommended to implement these calculated parameters in the routine investigation and assessment of women with disorders related to clinical or biochemical hyperandrogenism. For this approach, assessment of androgen status should include measurement of both total testosterone and SHBG, and based on their values, calculation of FAI and/or CFT can be obtained and reported as a profile. This will improve the diagnostic efficiency of testosterone in diagnosing hyperandrogenism.

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