# Markers of hyperandrogenism in South Asians with polycystic ovary syndrome

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## Abstract

***Background:*** Many clinical and biochemical criteria are used to assess hyperandrogenism in subjects with polycystic ovary syndrome (PCOS). Standard indicators used to confirm hyperandrogenism are based predominantly on western data. Whilst the phenotype of PCOS has ethnic specific variation, specific cutoffs for hyperandrogenism in South Asians have not been defined.

***Objectives:*** To evaluate the effectiveness of modified Ferriman-Gallwey score (FG Score), serum total testosterone and free androgen index (FAI) in the assessment of hyperandrogenism in PCOS.

***Materials and methods:*** A case control study was conducted on 100 women aged 20-45 years (mean age=30) attending a specialized endocrine clinic in Colombo, Sri Lanka from 1st January 2010 to 1st June 2013. Confirmed cases with PCOS (Rotterdam criteria 2003) were age matched for controls from healthy volunteers.

Recommended cut-offs for hyperandrogenism: FG score ≥8, testosterone (T) >3.5 nmol/L and FAI >5 were applied and receiver operating characteristics (ROC) curves were drawn to compare the diagnostic power of each parameter.

***Results:*** 50 cases with PCOS and 50 controls were studied. Cases versus controls had significantly greater FG score, testosterone and FAI:median FG=10 vs 3, mean testosterone 2.762±1.78 vs 1.045± 0.40 (p=0.0001), mean FAI 7.31±7.55 vs 3.64±4.87 (p= 0.01);76% cases and 4% controls had FG score ≥8 (p=0.0001), 30% cases had elevated (T) with none among controls (p=0.00001), 43.3% cases and 14.7% controls had FAI ≥5 (p=0.002). The diagnostic power of serum testosterone was greater than that of FAI in subjects with FG score ≥8. Area under the curve (AUC) for T and FAI were 0.832 and 0.766 respectively.

***Conclusion:*** Clinical assessment by FG score detects hyperandrogenism in PCOS patients more frequently compared to serum testosterone and free androgen index. A higher detection rate was observed in controls when FAI was used as the indicator, suggesting a possible influence from changes in SHBG concentration. Hence, total testosterone having greater diagnostic power than FAI in confirming hyperandrogenism, is the recommended biochemical test in the diagnostic work up of PCOS.

## Introduction

The polycystic ovary syndrome (PCOS) is the commonest endocrine disorder of women of reproductive age worldwide (1). A study conducted among a semi-urban population in Sri Lanka has demonstrated that the prevalence of PCOS is 6.3% (2).

According to an editorial published in the *British Medical Journal* (3), the definition of PCOS by The Androgen Excess and PCOS Society revolves around the presence of hyperandrogenism (clinical and/or biochemical) and ovarian dysfunction (oligo-anovulation) and/or polycystic ovaries with the exclusion of related disorders (4).

Meanwhile the Rotterdam 2003 consensus diagnostic criteria require any 2 out of the 3 criteria to be positive and the criteria being oligo-anovulation, clinical and/or biochemical signs of hyperandrogenism, polycystic ovaries and the exclusion of other aetiologies (congenital adrenal hyperplasia, androgen-secreting tumour, Cushing's syndrome) (5).

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The commonest cutaneous manifestations of hyperandrogenemia in PCOS include hirsutism, acne and male pattern of hair loss (androgenic alopecia) (6).

In the determination of hyperandrogenism, the true androgen status can be assessed either by measuring free testosterone or by calculating the free androgen index (FAI). The FAI is the ratio of the total serum testosterone (TT) concentration to the concentration of sex hormone binding globulin (SHBG). This is also referred to as the testosterone free index (TFI) and is typically calculated on a molar/molar basis and re-scaled by a factor of ten, one hundred or one thousand, as shown below (7).

FAI = (Total Testosterone (TT) nmol/L / SHBG nmol/L) × 10, or × 100, or × 1000

The modified Ferriman Gallwey score, serum total testosterone level and FAI are accepted as the markers of hyperandrogenism worldwide. Although there are studies that evaluate the effectiveness of these markers, there is paucity of data in the South Asian context.

Therefore we aimed to evaluate the effectiveness of modified Ferriman-Gallwey score (m-FG Score), serum total testosterone and free androgen index (FAI) in the assessment of hyperandrogenism among South Asians with PCOS.

## Methodology

### Study design

A case control study was conducted on 100 women aged 20-45 years (mean age = 30 years) attending a specialized endocrine clinic at De Soysa Hospital for Women, Colombo, Sri Lanka from 1st January 2010 to 1st June 2013. Treatment naïve women with PCOS diagnosed by Rotterdam consensus criteria were selected on a convenient sampling basis whilst attending their initial clinic visit at the diagnosis of PCOS. Age matched, unmedicated women among healthy volunteers with regular cycles and no complaints of hyperandrogenism were recruited during the same period as controls. Exclusion of PCOS in controls was confirmed by detailed clinical evaluation, biochemical testing of serum gonadotrophins and testosterone and ovarian ultrasound by a trained radiologist. The control group was also subjected to testing for confounding endocrine disorders such as glucose intolerance and thyroid disease by fasting blood glucose and serum TSH testing.

### Subject recruitment

The study subjects were recruited to the two cohorts from the women attending a specialist endocrine clinic at De Soysa Hospital for Women, Colombo, Sri Lanka from 1st January 2010 to 1st June 2013. They were invited to join the study by clinical research associates (MB, SADU, KGHDeS). Volunteers among female staff members from the same institution were recruited after evaluating them and excluding PCOS. Written informed consent was obtained from all cases and controls.

### Ethical approval

The project was approved by the Ethics Committee of the Research and Development Divisions of the Faculty of Medicine, University of Colombo, Sri Lanka.

### Inclusion criteria

*Anovular PCOS:* Anovular cycles are defined when the cycle length is more than 35 days, and the lack of demonstrable ovulation by midcycle and luteal phase ultrasound scans, and midluteal serum progesterone.

*Polycystic ovaries on ultrasound:* defined by transvaginal ultrasound scan of ovaries, performed within the first 5 days from the onset of menstruation, and finding 12 or more follicles, measuring between 2 and 9 mm and/or an ovarian volume >10 $cm^3$ (5).

*Hyperandrogenism:* Clinical evidence of hirsutism FG ≥8, serum testosterone (T) >3.5 nmol/L and/or FAI >5.

### Exclusion criteria

Pregnancy, Cushing's syndrome, hypothyroidism, hyperprolactinaemia, late onset CAH, androgen secreting ovarian/ adrenal tumour and those on hormonal contraceptives, anti-psychotics, anti-epileptics.

### Main outcome measures

Modified Ferriman-Gallwey score (m-FG) was used in women with and without PCOS to quantitatively measure the degree of hirsutism. Serum total testosterone levels and sex hormone binding globulin (SHBG) levels were measured in order to calculate the FAI (FAI = Testosterone (nmol/l) 100/ SHBG (nmol/l)).

### Data collection

Each subject was interviewed by the research associates based on a standard questionnaire that was completed at interview. Menstrual dating and irregularity, hirsutism and acne were recorded.

The presence of hirsutism at baseline clinical evaluation was scored in every woman by the same investigator using the modified FG score and recorded on two occasions with the mean value taken as the final score to quantify the presence of terminal hair over nine body

areas (i.e., upper lip, chin, chest, upper and lower abdomen, upper and lower back, upper arms and thighs).

Blood samples were collected between 8.00 and 9.00 am within the first 5 days of a spontaneous period. Samples were analyzed at the Reproductive Biology and Endocrinology Laboratory, Department of Obstetrics and Gynaecology, Faculty of Medicine, Colombo, using the following techniques: total testosterone and SHBG – immunometric assay (IMMULITE Diagnostic Products Corporation, USA). Laboratory controls were used to monitor accuracy and precision of the analyzer, reagents and assay results. Inter and intra assay precision checks demonstrated coefficient of variation of 5.4% and 4.3% for testosterone and 3.8% and 3.1% for SHBG.

## Data analysis

### Calculation and data analysis

Free androgen index = (Testosterone/SHBG) × 100. Statistical analysis was performed using the computer program Statistical Package for Social Sciences (SPSS version 21.0). The Kolmogorov-Smirnov test was used to determine the normality of distribution for all variables. Continuous data were compared by performing Mann-Whitney test for two medians and categorical data were analyzed by performing the Chi square test.

Diagnostic cut-offs for hyperandrogenism applied for cases and confirmed controls were: FG ≥8, serum testosterone (T) >3.5 nmol/L and FAI >5. The sensitivity and specificity of each of these parameters were determined by using receiver operating characteristics (ROC) curves drawn to compare diagnostic power of each parameter using SPSS. ROC curves were constructed by plotting the sensitivity on the ordinate as a function of the complement of specificity for all the possible cut off values.

## Results

50 women with PCOS (mean age 29.4 years) and 50 age matched controls were studied. Table 1 depicts the comparison of clinical and biochemical markers of hyperandrogenism of cases and controls.

Women with PCOS had significant hirsutism when compared with age matched controls. Their mean serum testosterone and FAI mirror this difference. FG score >8 was significantly higher in cases than among age matched controls (p = 0.001); with 76% of women with PCOS vs 4% of controls fulfilling this criterion. Serum testosterone exceeded 3.5 nmol/L in 30% of cases compared to none among controls (p = 0.001). FAI exceeded 5 in 43.3% of cases vs 14.7% controls (p = 0.02).

The ROCs of FAI and testosterone levels are represented in Figure 1. According to the defined cut off the best combination of sensitivity for testosterone is 27% with specificity of 97.5%. The sensitivity at defined cut-off for FAI is 50% with specificity of 87.5%.

The AURCs for FAI and serum testosterone are indicated in Table 2, with a higher value for serum testosterone (0.823) than that for FAI (0.766), which confirms that serum testosterone testing yields a greater probability than FAI in the detection of PCOS.

**Table 1. Indicators of hyperandrogenism among south Asian women with PCOS Vs controls**

| | *Cases* | *Controls* | *P value* |
|---|---|---|---|
| Number of subjects | 50 | 50 | |
| Mean age (years) | 29.4±5.6 | 29.6±5.9 | |
| Median FG | 10 | 3 | |
| Mean serum testosterone (nmol/L) | 2.762±1.78 | 1.045±0.40 | 0.001 |
| Mean FAI | 7.31±7.55 | 3.64±4.87 | 0.01 |
| FG score > 8 | 76% | 4% | 0.0001 |
| Elevated testosterone (>3.5 nmol/L) | 30% | 0 | 0.0001 |
| FAI > 5 | 43.3% | 14.7% | 0.002 |

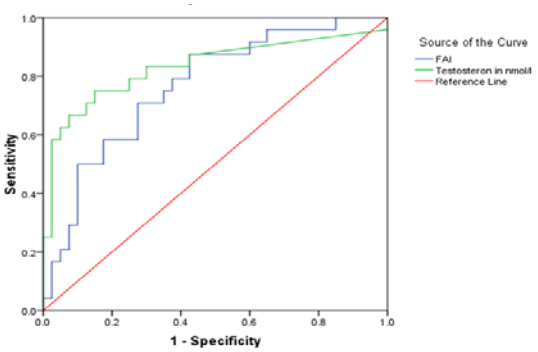


**Figure 1. Receiver operating characteristis curves for testosterone and FAI in subjects with Fg>8.**

**Table 2. Data obtained by ROC curves comparing testosterone and FAI in PCOS**

| | *Testosterone* | *FAI* |
|---|---|---|
| Area under the curve | 0.832 | 0.766 |
| Sensitivity (at defined cut off) | 27% | 50% |
| Specificity (at defined cut off) | 97.5% | 87.5% |

## Discussion

In 1961, Ferriman and Gallwey described a scoring system to determine the degree of hirsutism using 11 different body sites and this was subsequently subjected to several modifications resulting in a more sensitive, precise version. The modified Ferriman-Gallwey (m-FG) scoring system accepts a total score of equal to or more than 8 as hirsutism from 9 sites (8). This continues to be the most widely used method for visually scoring excess terminal body or facial hair growth for the clinical or investigational assessment of hirsutism (upper lip, chin, chest, upper back, lower back, upper abdomen, lower abdomen, upper arms, and thighs).

Many studies have previously evaluated the effectiveness of the m-FG Score, serum total testosterone and the FAI in determining hyperandrogenism. Ethnic specific differences in the phenotype of PCOS are well known. To the best of our knowledge there has been no report on this aspect of study in the diagnosis of PCOS among indigenous South Asian women.

In vivo and in vitro studies using cultured theca cells consistently showed that ovarian theca cells in affected women with PCOS are more efficient at converting androgenic precursors to testosterone than are normal theca cells (9). Insulin plays both direct and indirect roles in the pathogenesis of hyperandrogenemia in PCOS (9). Nearly a half of the circulating testosterone in normal adult women is derived from the peripheral conversion of androstenedione, and the remainder derived from the ovary and adrenal cortex. The important tissues in which this conversion takes place are the lung, liver, adipose tissue and skin. Plasma dihydrotestosterone is produced virtually entirely by 5 alpha reductase activity in the periphery, with plasma androstenedione being its major precursor. Insulin stimulates testosterone biosynthesis by human theca cells from women with PCOS by activating its own receptor and using inositol glycan mediators as the signal transduction system as well as influencing the FAI through inhibition of hepatic production of SHBG (10).

The Endocrine Society's Clinical Guideline recommends measuring serum testosterone as a good initial test for hyperandrogenism in the hirsute woman, provided it is less costly and more widely available (11). A case control study evaluated the effectiveness of serum testosterone in the diagnosis of hyperandrogenism among clinically diagnosed patients (n=133; mean age 28 years) with PCOS and healthy volunteers (n=54; mean age 28 years). This study measured total testosterone, SHBG, luteinizing hormone (LH), follicle-stimulating hormone (FSH), androstendione, dehydroepiandrosterone sulfate (DHEAS) and calculated the bioavailable testosterone by FAI. They found bioavailable testosterone to have a greater accuracy than the FAI, followed by free testosterone (12).

Yet another case control study conducted in the United Kingdom utilized liquid chromatography and tandem mass spectrometry methods for analyzing testosterone and androstenedione in PCOS. The incidence of PCOS being 13.9%, the reference interval for testosterone was 1.8 nmol/l with the conclusion that early follicular phase serum testosterone measured using tandem mass spectrometry and FAI are valuable in the laboratory diagnosis of PCOS (13).

Similar studies performed in Turkey (14) and Oman (15), have concluded that total testosterone and AFI are effective in diagnosing hyperandrogenism, although they reported the AFI being the superior test.

Sri Lanka has major resource limitations with biochemical tests in endocrinology, particularly hyperandrogenism, with prohibitive costs when performing tests such as serum SHBG. However, in order to maintain quality of care in our standard practice when managing the commonest endocrine disorder of young women, there must be an evidence based approach to clinical practice recommendations.

Hence this study that aims to determine the predictive capability of markers of hyperandrogenism will be useful. The more deviated towards the left upper corner the ROC curve is, the higher the sensitivity and specificity of the diagnostic test for the detection of PCOS. This study shows that total testosterone level has a higher accuracy in the detection of PCOS than the FAI among Sri Lankan women (Figure 1).

The area under ROC curve (AURC) represents the probability of correctly distinguishing between affected and unaffected subjects. Therefore the perfect diagnostic test, not having false positive or false negative results would have an AURC of 1 and on the contrary, a test with AURC $\leq$ 0.5 would not discriminate affected from non-affected individuals.

This study provides evidence to prove the effectiveness of total testosterone and FAI in South Asians to diagnose hyperandrogenism.

Most studies have found the FAI as a better indicator than serum testosterone to detect hyperandrogenism, while others proved equal effectiveness of both methods, but favouring total testosterone over FAI. Another study conducted in a similar manner has concluded that bioavailable testosterone is more reliable than the FAI (16).

A literature review conducted in 2010 has concluded that the m-FG scoring method is a useful visual instrument for assessing excess terminal hair growth, and the presence of hirsutism, in women (17). This study also provided us with statistically significant evidence for the effectiveness of m-FG to assess hyperandrogenism.

The identified major limitation of the m-FG as an assessment tool is inter-observer variability (18). In the current study the particular bias was avoided by the same investigator performing the physical examination on two separate occasions to determine the m-FG score. Modified FG score is recognized as a clinically useful assessment tool and many studies have suggested that there should be a population specific cut-off value to use in the clinical context (18). The results of this study illustrates that the cut-off point of 8 is appropriate for the South Asian population as well.

## Conclusion

Modified Ferriman-Gallwey score (m-FG Score), serum total testosterone and free androgen index (FAI) are effective in the assessment of hyperandrogenism in South Asians. The clinical assessment by FG score detects hyperandrogenism in PCOS patients more frequently compared to serum testosterone and free androgen index. A higher detection rate was observed in controls when FAI was used as the indicator, suggesting a possible influence from changes in SHBG concentration.Our data provides clear evidence to support the measurement of serum total testosterone as the first test when determining hyperandrogenism to confirm the diagnosis of PCOS.

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