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Original Article

Albumin and Sex Hormone Binding Globulin based Testosterone Indices in Polycystic Ovarian Syndrome

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ABSTRACT

***Objective*:** To evaluate total testosterone, "Free Androgen Index", calculated free testosterone, % free testosterone, calculated bioavailable testosterone, and % testosterone as a possible biomarker to predict presence of Polycystic ovarian syndrome as per Rotterdam criteria.

***Study Design*:** Comparative cross-sectional study.

***Place and Duration of Study*:** Pakistan Naval Hospital, Islamabad Pakistan, from Jan 2018 to Jul 2020.

***Methodology*:** A total of 328 female subjects were included in the study, which included 166 subjects with polycystic ovarian syndrome and 162 without polycystic ovarian syndrome as per Rotterdam criteria. Various albumin and sex hormone binding globulin-based androgen measures, including calculated free testosterone and calculated bioavailable testosterone, and only the included measure Free Androgen Index were calculated. These various measures were evaluated between polycystic ovarian syndrome and non-polycystic ovarian syndrome. Following that, receiver operating curve analysis was carried out to see area under curve for various androgen excess parameters to measure the diagnostic performance of each measure for diagnosing polycystic ovarian syndrome.

***Results*:** Almost all androgen excess measures demonstrated significant differences between subjects with or without polycystic ovarian syndrome. Area under curve as measured by receiver operator curve analysis shows highest area under curve for as: Bioavailable testosterone =0.792[95% CI: 0.743-0.842], Free testosterone =0.791[95% CI: 0.743-0.842], Free Androgen Index =0.782[95% CI: 0.731-0.833], Total testosterone =0.748[95% CI: 0.696-0.800], %Bioavailable testosterone =0.675[95% CI: 0.619-0.734], % Free testosterone =0.671[95% CI: 0.612-0.729], and Sex Hormone Binding Globulin =0.337[95% CI: 0.0.78-395].

***Conclusion*:** Free testosterone followed by Bioavailable testosterone and Free Androgen Index, have demonstrated higher Area Under Curve (diagnostic performance) in identifying Polycystic ovarian syndrome-related androgen excess, which can not only allow diagnostic help but therapeutic monitoring may be another utility.

Keywords: % Bioavailable testosterone**,** % calculated free testosterone (cFT), Calculated Bioavailable testosterone (cBT), Free Androgen Index (FAI), Homeostasis Model Assessment for Insulin Resistance (HOMA-IR), Polycystic ovarian syndrome (PCOS), Sex Hormone Binding Globulin (SHBG), Total Testosterone.

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INTRODUCTION

Sometimes termed the "Thief of Womanhood", the disease "Polycystic Ovarian Syndrome (PCOS)" is still in need of a clear definition, with much ambiguity surrounding its modalities for diagnosis.[1] Since the earlier days of Irving F. Stein and Michael Leo Leventhal gave their account of this syndrome, much progress has been made in exploring various aspects of this pathology, diagnostic modalities and treatment strategies.[2] As we dig deeper down into the clinical presentations of this pathology, we learnt more and more about its heterogeneous nature, multi-system involvements and long-term atherosclerotic cardio-vascular diseases (ASCVD) associations.[3]

More studies have related androgenization of ovaries to be a key pathological factor in the causation of multiple cystic ovaries leading to reproductive cycle defect and hirsutism.[4] Attempting to identify PCOS using testosterone alone has not been found useful, leading to the use of various calculated measures using binding proteins like albumin and Sex Hormone binding globulins (SHBG).[5,6] These measures include the Free Androgen Index (FAI), calculated Free Testosterone (cFT), % free testosterone, Bioavailable testosterone (cBT), and % Bioavailable testosterone.

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However, the available data in terms of comparison for diagnosing underlying androgen excess associated with PCOS is scarce and minimal.[7] Furthermore, emerging data from literature highlights variation in phenotypes like lean-PCOS and obese-PCOS and effects of body weight, which have been associated with the plethora of genetic changes between races.[8,9] The available data on the subject is still evolving, and especially there is a real need to address the ever-increasing frequency of diagnosis of PCOS in young and middle-aged female populations requiring a more mathematical parameter to diagnose and monitor the disease in real-time clinics.[10] There is, thus, a need for more local data on the subject. We conducted a study to evaluate testosterone levels and various calculated SHBG and albumin-derived indices among subjects diagnosed to have PCOS and otherwise, both by Rotterdam criteria and ultrasound diagnosis of PCOS.

METHODOLOGY

The cross-sectional study was conducted from January 2018 to December 2020 at the Pakistan Naval Hospital, Islamabad. The Hospital Ethical Review Committee formally approved the study (vide HAF ERB – 21). Patients were recruited at Gynaecology and General Family Outpatient Departments (OPDs) as per "non-probability convenience sampling" from the target population visiting the hospital for routine evaluation and complaints of reproductive cycle defects and/or hirsutism. We iused a sample size calculator for cross-sectional studies with population prevalence (0.05) based upon previous studies identifying a prevalence rate between 3% to 7% and estimated effect size=1.[11]

Inclusion Criteria: Female patients with PCOS and 162 without PCOS as per Rotterdam criteria were included.

Exclusion Criteria: We excluded patients who had known gynaecological, medical, autoimmune or psychiatric disorders and were not included.

Patients showing a willingness to participate were formally included in the research program and were formally interviewed as per a questionnaire after they signed a written consent form. Patients were explained about the sampling and examination requirements, data confidentiality, use of patient-provided information, and research work leading to publication. Finally, patients were requested to visit the Pathology Department between 08:00 and 09:00 hrs during the follicular phase, i.e., day 2±1. On the day of presentation, patients underwent assessment for anthropometric parameters, blood pressure, followed by clinical recording of reproductive cycle issues, if any, followed by clinical examination by a gynaecologist for measurement of hirsutism as per modified Ferriman-Galleway (FG) score.[12] Patients attending sampling day with infectious disorders or using any medication, including supplements, were also excluded from the study on the sampling day. Approximately 10ml of blood was collected for measuring Fasting plasma glucose, glycated haemoglobin, testosterone, estradiol, SHBG, Albumin, lipid profile, ALT, Estradiol, and quantitative CRP. The consultant radiologist carried out a radiological examination. Radiological diagnosis of PCOS was made once there were more than 12 follicles with a size range between 2-9 mm in diameter and ovarian volume. However, ovarian volume was considered mandatory for the diagnosis of PCOS.[13,14] We analyzed Fasting glucose, total cholesterol, triglyceride by GPO-PAP enzymatic method, total cholesterol by CHOD-PAP method, and triglycerides by GOD-PAP method, qCRP, LDLc and HDLc using detergent-based direct enzymatic method on clinical chemistry auto-analyzer on Selectra ProM platform. Chemiluminescence Microparticle Immunoassay (CMIA) methodology was measured on random access immunoassay analyzer ARCHITECT iSystem) provided by Abbot Diagnostics. Insulin was measured using serum insulin and analyzed using the chemiluminescence method on an immunoassay analyzer. Insulin resistance was measured (Immulite® 1000). The diagnosis of Polycystic Ovarian Syndrome (POCS) in our data set was diagnosed as per the "Rotterdam criteria".[15,16] Surrogate insulin resistance calculated index "Homeostasis Model Assessment for Insulin resistance (HOMA-IR)" was used as per the formula given in litrature.[17] One patient was excluded due to very high testosterone and requested a reanalysis along with a few others who were asked for resampling which were lost to follow up. Various testosterone indices were calculated per the methods shown in literature[11], and a total of 328 subjects were finally included in the analysis.

The data was initially entered into Excel software and later transferred into Statistical Package for the Social Sciences (SPSS) version 24:00. The Independent sample t-test was used to measure the differences in age, anthropometric indices, glycemia, ALT and lipid indices among subjects with and without Polycystic Ovarian Syndrome (PCOS). Direct and indirect calculated indices for androgen excess were evaluated among subjects with and without Rotterdam-defined

PCOS criteria and ultrasound presence of PCOS by independent sample test. Receiver Operating Curve (ROC) analysis was used to calculate the "Area Under Curve (AUC)" for various testosterone indices, keeping Rotterdam-defined PCOS criteria as a diagnostic entity. Pearson's correlation was measured for various androgen indices with insulin resistance.

RESULTS

Main outcome measures include androgen indices, including total testosterone (nmol/L), Sex Hormone Binding Globulin (SHBG) in nmol/L, Prolactin (mIU/L), Free Androgen Index (FAI), Free testosterone (cFT) in nmol/L, % Free testosterone, Bioavailable testosterone (cBT) in nmol/L and % Bio-available testosterone. The mean age among the evaluated population was 27.92±7.62 years. Differences between various anthropometric, demographic, and biochemical parameters between subjects with (n=166) and without PCOS(n=162) were evaluated. There was no difference for BMI between groups segregated as per presence or absence of PCOS as per Rotterdam criteria as BMI (PCOS:29.23±5.76 vs non-PCOS:28.51± 5.61, p=0.252), fasting plasma glucose (PCOS:5.25±1.13 vs non-PCOS:5.31±1.63, p=0.688), total cholesterol (PCOS:4.40±0.92 vs non-PCOS: 4.22±0.81, p=0.076), (PCOS:4.40±0.92 vs non-PCOS: 4.22±0.81, p=0.076), Serum triglycerides (PCOS:1.33±0.847 vs non-PCOS: 1.39±0.799, p=0.487), HDLc (PCOS:1.04±0.0.35 vs non-PCOS: 1.06±0.689, p=0.487) except LDLc (PCOS:2.70±0.835 vs non-PCOS: 2.43±0.735, p=0.0.003).

Table-I: Differences in Various Direct and Indirect Measures of Androgen Indices in subjects with (n=166) and without Polycystic Ovarian Syndrome (n=162) as per Rotterdam Criteria

Parameters	PCOS diagnosis as per Rotterdam criteria	Mean±SD	p-value
Total testosterone (nmol/L)	PCOS diagnosed	1.78±0.85	<0.001
	PCOS not diagnosed	1.17±0.45	
Sex Hormone Binding Globulin (SHBG) in nmol/L	PCOS diagnosed	41.38±31.86	<0.001
	PCOS not diagnosed	55.27±34.61	
Prolactin (mIU/L)	PCOS diagnosed	412±286.01	<0.001
	PCOS not diagnosed	390±368.86	
Free Androgen Index (FAI)	PCOS diagnosed	6.35±4.85	<0.001
	PCOS not diagnosed	2.78±1.79	
Free testosterone (cFT) in nmol/L	PCOS diagnosed	0.038±0.059	<0.001
	PCOS not diagnosed	0.017±0.008	
% Free testosterone	PCOS diagnosed	1.86±0.701	<0.001
	PCOS not diagnosed	1.47±0.502	
Bioavailable testosterone (cBT) in nmol/L	PCOS diagnosed	0.84±1.25	<0.001
	PCOS not diagnosed	0.40±0.19	
% Bioavailable testosterone	PCOS diagnosed	43.12±15.77	<0.001
	PCOS not diagnosed	33.72±12.10	

Table-I shows direct and indirect measures of androgen indices in subjects with and without Rotterdam-defined PCOS, with almost all direct or indirect indices and formula-driven measures showing significant androgen excess among subjects with PCOS. As measured by ROC analysis, the diagnostic efficacy of these direct and indirect androgen indices indicated the highest AUC for Bioavailable testosterone and the lowest for %Free testosterone [Figure]. Ultrasound presence or absence of polycystic ovaries in comparison to Rotterdam-defined PCOS criteria indicated higher FAI (p<0.05), total testosterone (p<0.05) and % Bioavailable Testosterone (p=0.079). At the same time, SHBG levels were slightly lower in PCOS (p=0.089) [Table-II].

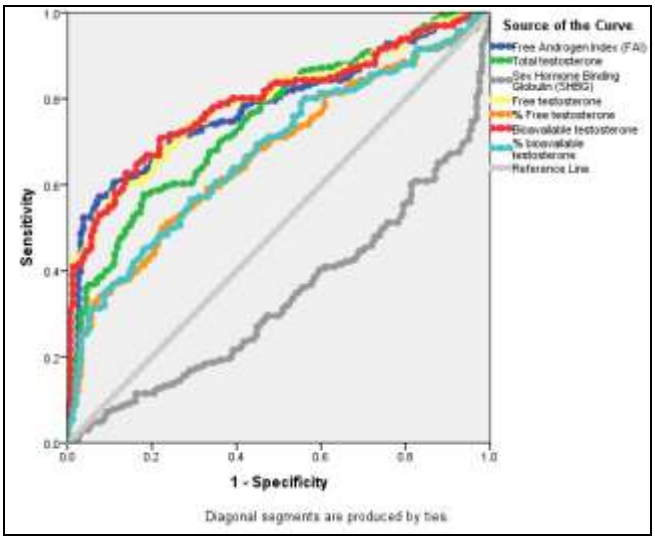

Figure: Area Under Curve (AUC) as calculated by Receiver Operator Curve (ROC), Analysis shows highest to lowest AUC as: Bioavailable testosterone (cBT)=0.792[95% CI: 0.743-0.842], Free testosterone (cFT)=0.791[95% CI: 0.743-0.842], Free Androgen Index (FAI)=0.782[95% CI: 0.731-0.833], Total testosterone =0.748[95% CI: 0.696-0.800], %Bioavailable testosterone =0.675[95% CI: 0.619-0.734], % Free testosterone =0.671[95% CI: 0.612-0.729], and Sex Hormone Binding Globulin (SHBG)=0.337[95% CI: 0.0.78-395]

DISCUSSION

Both SGBG and SHBG with albumin-derived indices demonstrated higher androgen levels than total testosterone in PCOS in comparison to subjects not diagnosed to have PCOS as per Rotterdam-defined criteria. This finding is in concordance with previous results with some data variation. However, PCOS, once established through radiological methods, was not found to reach statistical significance for albumin-added androgen indices. However, equations incorporating Albumin and SHBG, especially cBT,

showed higher AUC than total testosterone and FAI, albeit minimally. However, it is also important to mention that % of total testosterone showed the highest positive correlation with insulin resistance, followed by FAI. However, these Albumin with SHBG equations did not show significant differences between subjects with or without PCOS as per ultrasound diagnosis in comparison to FAI and total testosterone. Other studies have also shown these research-related variabilities.[12-14]

Table-II: Differences for Testosterone, SHBG and SHBG with Albumin Derived Androgen Indices between having PCOS (n=87) and no PCOS (n=241) as per Radiological Findings

Parameters	PCOS solely based upon radiological criteria	Mean±SD	*p*-value
Free Androgen Index (FAI)	PCOS diagnosed	5.75±5.02	0.008
	PCOS not diagnosed	4.17±3.61	
Total testosterone (nmol/L)	PCOS diagnosed	1.70±0.80	0.003
	PCOS not diagnosed	1.40±0.71	
Sex Hormone Binding Globulin (SHBG) in nmol/L	PCOS diagnosed	43.59±27.03	0.089
	PCOS not diagnosed	49.92±35.98	
Prolactin (mIU/L)	PCOS diagnosed	423.77±316.26	0.456
	PCOS not diagnosed	392.99±333.88	
Free testosterone (cFT) in nmol/L	PCOS diagnosed	0.033±0.040	0.160
	PCOS not diagnosed	0.026±0.045	
% Free testosterone	PCOS diagnosed	1.76±0.66	0.104
	PCOS not diagnosed	1.63±0.63	
Bioavailable testosterone (cBT) in nmol/L	PCOS diagnosed	0.69±0.40	0.412
	PCOS not diagnosed	0.59±1.05	
% bioavailable testosterone	PCOS diagnosed	40.88±15.22	0.079
	PCOS not diagnosed	37.62±14.62	

Our data, though suggesting total testosterone showing inferior performance in terms of diagnosing PCOS, still become a part of a proper diagnostic strategy where testosterone binders in plasma are used in equation form. FAI and cBT have emerged as more competitive markers to predict the presence or absence of PCOS than total testosterone. So why and how evidence-based are these findings? Firstly, Albumin, the most abundant and common transporter of all proteins, can affect testosterone levels in the blood. On the contrary, SHBG was tailor-made to specifically bind testosterone, and thus, the information from both parameters, once coined together, provides more valuable information. Our study showed that bioavailable and free testosterone depicted higher diagnostic efficiency than indices only employing SHBG-based equations. Secondly, androgen excess affects not just ovaries. However, there are also dermatological manifestations covered only by Rotterdam criteria, which could be one reason that Rotterdam criteria for PCOS demonstrated a better association with markers of androgen excess. Finally, data review on the subject has also shown variable associations between androgen excess, androgenized ovaries and hirsutism. Al Kindi *et al*. evaluated different components included in the PCOS definition with total testosterone and FAI and calculated Free testosterone (cFT) to learn that the latter was the most raised parameter, followed by FAI and total testosterone. At the same time, the isolated presence of menstrual abnormalities or infertility also demonstrated a similar pattern, with cFT being more predictable.[18] Bioavailable testosterone was not calculated in this study. However, Nadaraja *et al*. showed that cBT showed superior AUC values followed by cFT and FAI, thus augmenting our results.[19] Another study by Chanukvadze *et al*. has demonstrated sequentially cFT, cBT, and FAI to be even better related to PCOS components than free testosterone measurements.[12] In summary, it appears that calculated and bioavailable testosterone and, to some extent, FAI show better association with PCOS and its various components, as has also been demonstrated in our study.

Various androgen indices showed a weak positive correlation with insulin resistance, except SHBG, showing a negative correlation. Still, the highest correlation was seen between FAI and % Bioavailable Testosterone. The possible higher FAI correlation may be due to the underlying effect of SHBG. However, studies indicate multiple factors like thyroid ailment, higher prolactin levels, and others affecting the FAI equation. They thus may only be somewhat reliable.[20] Literature review suggests that prolactin and raised TSH levels can reduce key associations with PCOS.[21,22] Though our study did not evaluate the TSH pro,lactin was lower in PCOS subjects, so that this finding could be one possible attribute leading to high FAI due to SHBG.

Despite certain limitations, we, as authors, feel the research work is clinically significant. It highlights the significance of cFT and cBT as slightly superior markers in evaluating androgen assessment than previously relied upon FAI. Secondly, the local dynamics of the current study and its cross-sectional nature merit further work at the epidemiological level to strengthen our findings to reach a consensus on the singular calculated index to work within endocrine,

dermatology and gynaecological clinics. Not much data in this specific area related to PCOS has been done in our region, and our data may be the baseline for future researchers to move forward to elucidate the phenotypic variations of this syndrome.

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LIMITATION OF STUDY

Firstly, the lean and obese phenotypes of PCOS have been in literature and possibly insulin resistance being higher in obese PCOS compared to lean PCOS may downsize the role of insulin resistance in PCOS. Secondly, we also interpret a small sample size study, where we could manage close to 85% of the targeted sample due to the start of the ongoing COVID-19 pandemic leading to the closure of OPDs, which can introduce type-2 statistical error. Finally, the heterogeneous nature of PCOS has been well-appreciated and is currently under multi-dimensional research, especially with the growing molecular dimension of pathology.

CONCLUSION

Free testosterone followed by Bioavailable testosterone and Free Androgen Index, have demonstrated higher AUC (diagnostic performance) in identifying PCOS-related androgen excess, which can not only allow diagnostic help but therapeutic monitoring may be another utility.

Conflict of Interest: None.

Funding Source: None.

Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

SHK & UU: Study design, data interpretation, drafting the manuscript, critical review, approval of the final version to be published.

ZAQ & US: Conception, data analysis, drafting the manuscript, approval of the final version to be published.

RS & CQUHN: Data acquisition, critical review, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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