

Increased Ratio of Total Testosterone to Dihydrotestosterone May Predict an Adverse Metabolic Outcome in Polycystic Ovary Syndrome

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Abstract

Background: Androgen excess may correlate with metabolic risk in polycystic ovary syndrome (PCOS). The aim of the study was to determine the role of total testosterone to dihydrotestosterone (TT/DHT) ratio in assessing the adverse metabolic outcome in PCOS.

Methods: This cross-sectional study encompassed 40 PCOS women recruited on the basis of revised Rotterdam criteria 2003, and 40 age-matched control subjects. TT, sex hormone binding globulin (SHBG) and insulin levels were measured by chemiluminescent microparticle immunoassay (CMIA) while DHT by enzyme-linked immunosorbent assay (ELISA). In addition, TT/DHT ratio, free androgen index (FAI), and insulin resistance (IR) by homeostatic model of assessment of insulin resistance (HOMA-IR) were calculated.

Results: TT/DHT ratio was significantly higher in PCOS group than control group ($P < 0.001$). No significant difference was found for DHT ($P = 0.261$). PCOS patients had significantly higher TT (0.69 ± 0.26 vs. 0.30 ± 0.13 ng/mL; $P < 0.001$), FAI ($P < 0.001$) and low SHBG ($P = 0.004$) compared to controls. TT/DHT ratio was significantly higher in PCOS with impaired glucose tolerance (IGT) ($P = 0.037$) and metabolic syndrome (MetS) ($P = 0.041$). The best cutoff value for TT/DHT ratio to diagnose PCOS was observed to be 2.38 (sensitivity: 70%, specificity: 32.5% and area under the curve (AUC): 0.753). TT/DHT ratio also showed positive correlation with weight ($r = 0.323$, $P = 0.042$), waist circumference (WC) ($r = 0.372$, $P = 0.018$), HOMA-IR ($r = 0.385$, $P = 0.014$), 2-h post 75-g glucose (2h-PG), ($r = 0.413$, $P = 0.008$) and triglyceride (TG) level ($r = 0.402$, $P = 0.010$) in PCOS.

Conclusions: There is a close relation between the TT/DHT ratio and adverse metabolic outcome in PCOS. Therefore, TT/DHT ratio may be considered as a predictor of adverse metabolic findings in PCOS.

Keywords: PCOS; TT/DHT ratio; IGT; Metabolic syndrome; Insulin resistance; Obesity

Introduction

Based on the clinical picture and laboratory findings, three main phenotypes of polycystic ovary syndrome (PCOS) can be defined: metabolic, hyperandrogenic and reproductive [1]. Prevalence of PCOS varies from 6-18% depending on the diagnostic criteria used [2]. The etiology of PCOS is still far from clear. PCOS is not the consequence of a single gene defect, but rather the result of a polygenic interaction together with environmental factors [3]. It appears that insulin resistance (IR) and hyperinsulinemia play a permissive role. Hyperinsulinemia stimulates both ovarian (mainly) and adrenal androgen secretion and suppresses sex hormone binding globulin (SHBG) synthesis from the liver, thereby resulting in increase in free, biologically active androgens [4, 5]. In addition to hyperandrogenemia, women with PCOS may present with typical metabolic abnormalities such as IR and obesity at a young age. Long-term exposure to these abnormalities throughout fertile life may exacerbate the adverse effects and expose these women to higher risks of metabolic syndrome (MetS), cardiovascular diseases (CVDs) and type 2 diabetes mellitus (T2DM) [6-8].

There are several hypotheses for the association of hyperandrogenism (HA) with poor metabolic profile. PCOS women with androgen excess are at a higher risk of IR, liver disease, and subclinical atherosclerosis compared to PCOS patients with normal androgen level [9]. There is a lack of clarity on which androgen to be measured and how often should they be measured to determine the metabolic risk factors [10]. The metabolic complications in PCOS should not be quantified on single hormone measurement, but should comprise the quantification of other steroid hormones and a further analysis of the relationship of these hormones to each other. At present, testosterone is the most common measurement in routine clinical

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cal practice [11]. Increased peripheral conversion of testosterone to the potent androgen (dihydrotestosterone (DHT)) by 5 α -reductase activity has been linked to an adverse metabolic profile in PCOS [12]. It is estimated that more than 60% DHT in women is produced from the skin and originates from androstenedione. Recent studies have shown that a high total testosterone to dihydrotestosterone (TT/DHT) ratio was useful to see an adverse metabolic profile not only in PCOS patients but also useful to predict development of PCOS in “theoretically healthy” obese women [13, 14].

The present study was undertaken to analyze whether the TT/DHT ratio may be helpful in predicting metabolic risk in PCOS women.

Materials and Methods

Subjects

This study was conducted in the Department of Endocrinology, Bangabandhu Sheikh Mujib Medical University (BSM-MU), Dhaka. Sample size was estimated according to a previous study [15]. Forty Bangladeshi women aged 16 - 35 years with PCOS diagnosed on the basis of revised Rotterdam consensus 2003 criteria [1, 14] were recruited. Control group included 40 age-matched healthy women having regular menstrual cycle, no history of clinical and biochemical HA, no polycystic ovary morphology on ultrasonography (USG) and without clinical evidence of any endocrine diseases. Patients were not included if they had: primary amenorrhea, hyperprolactinemia (serum prolactin values greater than 25 ng/mL), hypothyroidism (serum thyroid stimulating hormone (TSH) greater than 5 μ IU/mL) and non-classical congenital adrenal hyperplasia (CAH) diagnosed in case of basal or adrenocorticotropic hormone (ACTH) stimulated 17-OH progesterone greater than 10 ng/mL [16]. Women on medication for < 6 months prior to the study (including oral contraceptives, glucocorticoids, metformin, ovulation induction agents, and estrogenic or anti-androgenic drugs or any medication for dyslipidemia or anti-obesity drugs) or suffering from other systemic diseases (e.g. chronic kidney disease, liver diseases etc.) were also excluded from the study. Prior to commencement, the research protocol was approved by the Institutional Review Board (IRB). Informed written consent was taken from all subjects.

Protocol

Anthropometric measurements were taken by the same investigator and hirsutism was assessed using a modified Ferriman-Gallwey (FG) score [17]. For TT, SHBG, DHT, follicle-stimulating hormone (FSH), luteinising hormone (LH), TSH and prolactin, samples were collected on any day between the second and the seventh day of a spontaneous bleeding episode or randomly in the case of amenorrhea. Samples for fasting plasma glucose (FPG) and 2-h post 75-g glucose (2h-PG) as well as fasting insulin (FI) and lipid profile were taken. For

serum levels of DHT and SHBG, blood was centrifuged; about 1 mL of serum was separated in an Eppendorf tube and stored at a temperature of -20 °C. Oligomenorrhea was defined as delayed menses > 35 days or < 8 spontaneous hemorrhagic episodes/year, clinical HA (hirsutism using modified FG-score of > 8) or biochemical HA (TT \geq 53 ng/dL) [18], polycystic ovarian morphology on ultrasonography (USG) (12 or more follicles in each ovary measuring 2 - 9 mm in diameter, and/or increased ovarian volume > 10 cm³).

Analytic methods and calculated values

The circulating levels of TT, FSH, LH, TSH, and prolactin levels were measured by CMIA in the Department of Microbiology and Immunology. DHT samples were run by enzyme-linked immunosorbent assay (ELISA) and SHBG by Beckman Coulter at once in the Department of Microbiology and Immunology. Transabdominal (in unmarried) or transvaginal USG was performed preferably in early follicular phase. FPG was analyzed by glucose oxidase method whereas FI by CMIA, Architect plus ci4100 and lipid profile was assessed by automated analyzer, Architect Plus ci8200. TT/DHT ratio was calculated by dividing TT by DHT (ng/mL) and free androgen index (FAI) was calculated as TT/SHBG \times 100 (nmol/L), a cutoff value of \geq 5 was used to define HA [19]. To define IR, homeostatic model of assessment of insulin resistance (HOMA-IR) with a cutoff of \geq 2.6 was used as evidenced by a previous study in Bangladeshi population [20]. HOMA-IR was calculated using the formula: HOMA-IR = FPG (mmol/L) \times FI (μ IU/mL)/22.5 [21]. MetS was defined following the definition provided by the revised Rotterdam consensus 2003 criteria [1]. Prediabetes was defined as follows: impaired fasting glucose (IFG) when FPG was between 5.6 - 6.9 mmol/L and impaired glucose tolerance (IGT) when the 2h-PG value during a 75 g oral glucose tolerance test (OGTT) was between 7.8 - 11.0 mmol/L. Diabetes mellitus (DM) was confirmed by FPG \geq 7.0 mmol/L, a 2h-PG value during a 75 g OGTT of \geq 11.1 mmol/L [22]. Obesity was defined as per the Western Pacific Region Office of World Health Organization [23], where body mass index (BMI) of 23 - 24.9 kg/m² was considered overweight and BMI \geq 25 kg/m² as obese.

Statistical analysis

All data were expressed either as frequency or mean \pm standard deviation (SD) as applicable, Student's independent *t*-test and Chi-square tests were used to compare variables between the two groups. When more than 20% of the expected counts were less than 5, Fisher's exact test was applied. Receiver operating characteristics (ROC) curve was constructed to determine the optimal cutoff point of TT/DHT ratio to diagnose PCOS and adverse metabolic parameters. Pearson's correlation was done to see the correlation of TT/DHT ratio with clinical and biochemical parameters in PCOS and control. P value of \leq 0.05 was considered to be statistically significant.

Table 1. Clinical and Metabolic Characteristics of Studied Subjects

Parameters	PCOS (n = 40)	Control (n = 40)	P value
Age (years)	23.18 ± 4.66	23.00 ± 4.32	0.862
Body mass index (kg/m ²)	26.14 ± 4.16	20.84 ± 3.48	< 0.001
Waist circumference (cm)	84.05 ± 10.85	71.15 ± 5.82	< 0.001
Hip circumference (cm)	100.98 ± 10.17	88.38 ± 7.40	< 0.001
Waist/hip ratio	0.83 ± 0.06	0.80 ± 0.04	0.046
Systolic blood pressure (mm Hg)	111.5 ± 14.24	104.50 ± 10.85	0.055
Diastolic blood pressure (mm Hg)	72.50 ± 10.56	69.38 ± 9.35	0.097
Menstrual disturbance	36 (90%)	0	-
Hirsutism	22 (55%)	0	-
Acanthosis nigricans	23 (57.5%)	0	-
PCO in ultrasonogram	31 (77.5%)	0	-
Fasting insulin (μU/mL)	23.01 ± 21.57	8.54 ± 3.80	< 0.001
Fasting plasma glucose (mmol/L)	4.50 ± 0.67	3.67 ± 0.40	< 0.001
2-h post 75-g glucose (mmol/L)	7.40 ± 1.95	6.10 ± 1.23	< 0.001
HOMA-IR	4.44 ± 3.70	1.40 ± 0.70	< 0.001
Total cholesterol (mg/dL)	165.50 ± 24.34	160.78 ± 24.12	0.386
Triglyceride (mg/dL)	134.90 ± 76.30	91.88 ± 28.87	0.002
High-density lipoprotein (mg/dL)	37.39 ± 7.24	49.65 ± 9.54	< 0.001
Low-density lipoprotein (mg/dL)	101.38 ± 24.79	92.73 ± 18.90	0.083

Data were expressed as frequency, percentage, and mean ± SD. Comparison between PCOS and control was done by Student's *t*-test and Chi-square test. PCOS: polycystic ovary syndrome; PCO: polycystic ovaries; HOMA-IR: homeostatic model assessment of insulin resistance; SD: standard deviation.

Results

Clinical and metabolic profile

PCOS women (n = 40, age (mean ± SD): 23.18 ± 4.66 years) were compared with 40 age-matched healthy control subjects (age: 23.00 ± 4.32 years). Comparison of the clinical and metabolic parameters among the PCOS and control groups are shown in Table 1. BMI was statistically and significantly higher in PCOS women (26.14 ± 4.16 vs. 20.84 ± 3.48 kg/m², P < 0.001) than control. PCOS group revealed higher level of waist circumference (WC) (84.05 ± 10.85 vs. 71.15 ± 5.82 cm; P < 0.001), hip circumference (HC) (100.98 ± 10.17 vs. 88.38 ± 7.40 cm; P < 0.001), waist hip ratio (WHR) (0.83 ± 0.06 vs. 0.80 ± 0.04; P = 0.046), FI (23.01 ± 21.57 vs. 8.54 ± 3.80 μU/mL; P < 0.001), FPG (4.50 ± 0.67 vs. 3.67 ± 0.40 mmol/L; P < 0.001), 2h-PG (7.40 ± 1.95 vs. 6.10 ± 1.23 mmol/L; P < 0.001), HOMA-IR (4.44 ± 3.70 vs. 1.40 ± 0.70; P < 0.001), triglyceride (TG) (134.90 ± 76.30 vs. 91.88 ± 28.87 mg/dL; P = 0.002) and high-density lipoprotein cholesterol (HDL-C) (37.39 ± 7.24 vs. 49.65 ± 9.54 mg/dL; P < 0.001).

Androgenic profile

Table 2 depicts the hormone levels between the two groups.

TT (0.69 ± 0.26 vs. 0.30 ± 0.13 ng/mL; P < 0.001), TT/DHT ratio (3.51 ± 1.95 vs. 2.02 ± 1.03; P < 0.001) and FAI (10.93 ± 8.52 vs. 2.93 ± 2.17; P < 0.001) were significantly higher in PCOS than those of control whereas DHT level (0.252 ± 0.180 vs. 0.207 ± 0.180 ng/mL; P = 0.261) did not show any significant difference. Serum level of SHBG (32.16 ± 21.33 vs. 47.66 ± 24.70 nmol/L; P = 0.004) was significantly low in PCOS.

Glycemic status

Table 3 illustrates that 32.5% of PCOS patients had prediabetes (32.5% vs. 5%; P = 0.002) and 5% had DM; 65% had IR (65% vs. 7.5%; P < 0.001) and 42.5% had MetS (42.5% vs. 5%; P < 0.001).

Comparison of TT/DHT ratio with metabolic profiles

Table 4 shows the level of TT/DHT ratio in PCOS and control subjects comparing the presence or absence of adverse metabolic parameters within the PCOS group and in PCOS and control. TT/DHT ratio was raised in PCOS patients with MetS (with MetS: 4.33 ± 2.54 vs. without MetS: 2.90 ± 1.06; P = 0.041) and in those who had IGT (with IGT: 4.73 ± 2.73 vs. without IGT: 2.92 ± 1.08; P = 0.037) but

Table 2. Hormone Profile of PCOS and Control Groups

Parameters	PCOS (n = 40)	Control (n = 40)	P value
TT (ng/mL)	0.69 ± 0.26	0.30 ± 0.13	< 0.001
DHT (ng/mL)	0.252 ± 0.180	0.207 ± 0.180	0.261
TT/DHT ratio	3.51 ± 1.95	2.02 ± 1.03	< 0.001
SHBG (nmol/L)	32.16 ± 21.33	47.66 ± 24.70	0.004
FAI	10.93 ± 8.52	2.93 ± 2.17	< 0.001
TSH (μIU/mL)	2.55 ± 1.43	1.67 ± 0.93	0.002
Prolactin (ng/mL)	12.67 ± 11.40	15.43 ± 6.19	0.183

Data were expressed as mean ± SD. P values were calculated using Student's *t*-test. PCOS: polycystic ovary syndrome; TT: total testosterone; DHT: dihydrotestosterone; TT/DHT: total testosterone/dihydrotestosterone ratio; SHBG: sex hormone binding globulin; FAI: free androgen index; TSH: thyroid stimulating hormone; SD: standard deviation.

Table 3. Glycemic Status and Insulin Resistance in PCOS and Control Groups

Parameters	PCOS (n = 40)	Control (n = 40)	P value
Prediabetes	13 (32.5)	2 (5)	0.002
Diabetes mellitus	2 (5)	0	-
Insulin resistance	26 (65)	3 (7.5)	< 0.001
Metabolic syndrome	17 (42.5)	2 (5)	< 0.001

Data were expressed as frequency, percentage. P values were calculated using Chi-square test. PCOS: polycystic ovary syndrome.

not in control group. However, the ratio was not raised in patients with IR and obesity neither among the PCOS nor in control group. In addition to the above findings, when comparison was done within PCOS and control group for obesity, TT/DHT ratio was found to be significantly higher in PCOS (PCOS: 3.71 ± 2.16 vs. control: 2.10 ± 1.09; P = 0.032). Comparison between PCOS and control for MetS, IGT and IR could not be done due to very few numbers of

controls.

ROC curve analysis for diagnosis of PCOS and adverse metabolic profile by TT/DHT ratio

Figure 1 showing the ROC curve analyses revealed that the best cutoff value for the TT/DHT ratio to diagnose PCOS was

Table 4. Level of TT/DHT Ratio in PCOS and Control Groups With Metabolic Parameters

Metabolic parameters		TT/DHT ratio		P ^a
		PCOS (n = 40)	Control (n = 40)	
Metabolic syndrome	Yes (n = 17, 2)	4.33 ± 2.54	3.06 ± 0.41	N/D
	No	2.90 ± 1.06	1.96 ± 1.03	
	P	0.041	0.146	
Impaired glucose tolerance	Yes (n = 13, 2)	4.73 ± 2.73	1.55 ± 1.79	N/D
	No	2.92 ± 1.08	2.04 ± 1.01	
	P	0.037	0.521	
Insulin resistance	Yes (n = 26, 3)	3.74 ± 2.10	2.10 ± 1.18	N/D
	No	3.07 ± 1.61	2.00 ± 1.03	
	P	0.304	0.876	
Obesity	Yes (n = 28,10)	3.71 ± 2.16	2.10 ± 1.09	0.032
	No	3.05 ± 1.32	1.99 ± 1.03	
	P	0.339	0.764	

Data were expressed as mean ± SD, compared using Student's *t*-test. P: comparison within PCOS group; P^a: comparison between PCOS vs. control; N/D: not done because this category was not sufficient in control group. PCOS: polycystic ovary syndrome; TT/DHT ratio: testosterone/dihydrotestosterone ratio.

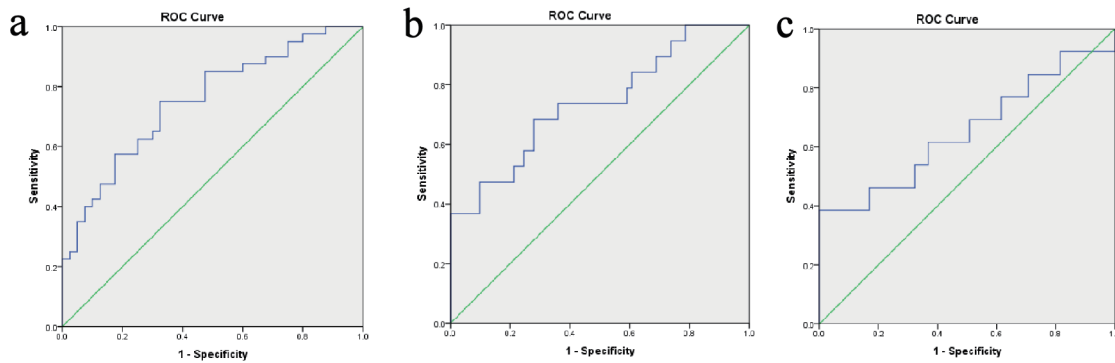


Figure 1. (a) ROC curves analyses revealed the best cutoff value of TT/DHT ratio of 2.38 for the diagnosis of PCOS (sensitivity: 70%, specificity: 32.5%, and AUC: 0.753). In PCOS patients, ROC curves analyses were conducted for best cutoff values of TT/DHT ratio for the diagnosis of (b) metabolic syndrome (2.53, sensitivity: 73.7%, specificity: 39.3%, and AUC: 0.738) and (c) impaired glucose tolerance (2.78, sensitivity: 61.5%, specificity: 38.5%, and AUC: 0.653). PCOS: polycystic ovary syndrome; AUC: area under the curve; ROC: receiver operating characteristics; TT/DHT: total testosterone/dihydrotestosterone ratio.

2.38 (sensitivity: 70%, specificity: 32.5%, and AUC: 0.753). In PCOS patients, the cutoff value for the TT/DHT ratio for MetS calculated via ROC curve analyses was 2.53 (sensitivity: 73.7%, specificity: 39.3%, and AUC: 0.738) and for IGT 2.78 (sensitivity: 61.5%, specificity: 38.5%, and AUC: 0.653). Figure 2 showed when a TT/DHT ratio of ≥ 2.38 was used as a cutoff value of TT/DHT ratio to diagnose PCOS, 70% of PCOS and 32.5% of control subjects had a high ratio ($P = 0.001$).

Correlations

Table 5 showing the Pearson’s correlation analysis in PCOS patients depicted that the TT/DHT ratio significantly correlated with weight ($r = 0.323$, $P = 0.042$), WC ($r = 0.372$, $P = 0.018$), 2h-PG ($r = 0.413$, $P = 0.008$), HOMA-IR ($r = 0.385$, $P = 0.014$) and TG ($r = 0.402$, $P = 0.010$) but none in the control

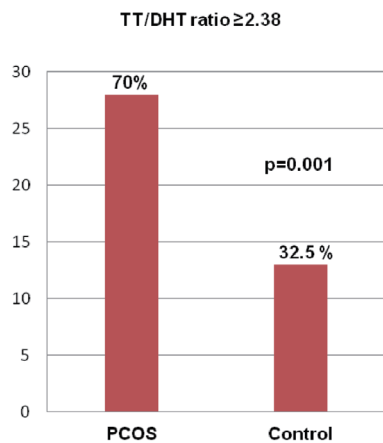


Figure 2. Frequency of a raised TT/DHT ratio (≥ 2.38) in the PCOS and control groups. (Data were expressed as frequency and percentage; comparison between PCOS and control groups was done by Chi-square test. PCOS: polycystic ovary syndrome; TT/DHT ratio: total testosterone/dihydrotestosterone ratio.

group ($P =$ non significant (NS) for all).

Discussion

PCOS is a heterogeneous disease with several metabolic characteristics, clinical phenotypes and HA as a key factor. The present study observed that the TT/DHT ratio was higher in PCOS women with adverse metabolic parameters, and a higher TT/DHT ratio was observed within PCOS patients who had MetS and IGT; similar observations were made in recent studies [13, 14, 24]. These findings were also supported by significant correlations with parameters of glucose intolerance (i.e. higher level of FPG, 2h-PG and HOMA-IR) and other adverse metabolic factors, like weight, WC and TG level in PCOS group. The ROC curve analysis predicted the nearest cutoff value of TT/DHT ratio to diagnose PCOS was 2.38; Munzker et al (2015) revealed ROC curve value of 4.37 as the cutoff value [13]. The lower cutoff value found in the present study may be explained by the use of CMIA and ELISA rather than mass spectrometry for hormonal analysis. However, ROC curve value is more appropriate than arbitrary cutoff values of other investigators in international studies for our studied population. Subsequently, a number of studies have been conducted in order to identify potential predictors of the metabolic risk in PCOS patients. Investigators have also observed that a worse metabolic profile in PCOS patients was associated with higher testosterone concentrations [25]; and among the obese women not meeting PCOS criteria, those with hyperandrogenemia have a worse metabolic phenotype [26].

In addition to the above findings, when comparing the PCOS and control group, it was observed that PCOS women with obesity had a higher ratio than those of control with obesity. However, MetS, IGT and IR could not be compared between the two groups because the numbers in these variables were not mutually exclusive in the control group.

When TT/DHT ratio cutoff of ≥ 2.38 was used to diagnose PCOS, it was interestingly observed that 32.5% of control women also had a high ratio. These observations of high ratio

Table 5. Correlations of TT/DHT Ratio With Clinical and Biochemical Parameters in PCOS and Control Groups

Determinants of “r”	PCOS (n = 40)		Control (n = 40)	
	r	P	r	P
Weight (kg)	0.323	0.042*	0.007	0.966
Body mass index (kg/m ²)	0.165	0.253	-0.002	0.991
Waist circumference (cm)	0.372	0.018*	0.013	0.939
Fasting plasma glucose (mmol/L)	0.072	0.660	0.056	0.733
2-h post 75-g glucose (mmol/L)	0.413	0.008*	0.241	0.134
Fasting insulin (μIU/L)	0.305	0.056	0.188	0.246
HOMA-IR	0.385	0.014*	0.162	0.317
Triglyceride (mg/dL)	0.402	0.010*	0.307	0.054
High-density lipoprotein (mg/dL)	-0.187	0.247	-0.124	0.445

Correlation was done between the two groups by Pearson correlation. PCOS: polycystic ovary syndrome; TT/DHT ratio: total testosterone/dihydrotestosterone ratio, HOMA-IR: homeostatic measurement of assessment of insulin resistance. *P < 0.05.

may also be associated with adverse metabolic profile not only in PCOS but also in control women with metabolic derangements; Ambroziak et al (2017) also made similar observations [14]. Metabolic disturbances observed in patients with PCOS result from the unfavorable influence of androgens on glucose and lipid metabolism as well as on insulin action. In turn, hyperinsulinemia stimulates androgens synthesis in ovaries, continuing the vicious cycle [27]. The findings of the present study focused on the importance of measuring androgen concentrations in order to look for relation between androgen levels and poor metabolic constellations.

In the present study, serum TT and DHT could not be assessed by standard liquid chromatography and tandem mass spectrometry method. In addition, for assessing IR we used surrogate marker instead of gold standard hyperinsulinemic euglycemic clamp method. The strength of the study is the inclusion of an age-matched equal number of control group, and PCOS subjects were not preselected to have biochemical HA or any other specific characteristics. However, due to the relatively small size of the investigated groups, further studies and larger trials are needed to verify our results.

Conclusions

The present study demonstrated that the TT/DHT ratio may be important as a predictor for adverse metabolic outcome in PCOS women. This component may be considered for the assessment of metabolic abnormalities in PCOS.

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Conflict of Interest

The authors declare that they have no conflict of interest concerning this article.

Informed Consent

All patients had informed written consent prior to participation in the study.

Author Contributions

MA Hasanat, Hurjahan Banu, Sukanti Shah and Nazma Akhtar designed the research; Sukanti Shah, Begum Moriom Zamila and Afroza Begum collected the data; Sukanti Shah and Tania Sultana did the statistical analysis; Hurjahan Banu and Sukanti Shah prepared the manuscript. MA Hasanat supervised and coordinated the study. Md Fariduddin went through the manuscript and provided moral support. All authors read and approved the final paper.

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