Free testosterone, luteinizing hormone/follicle stimulating hormone ratio and pelvic sonography in relation to skin manifestations in patients with polycystic ovary syndrome

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ABSTRACT

Objectives: To evaluate hormonal changes and pelvic sonography in polycystic ovary syndrome (PCOS) patients, especially free testosterone and their correlations with skin manifestations of this disease.

Methods: This prospective study was carried out in the Skin Clinic, Baghdad Teaching Hospital, Iraq in the period between April 2004-March 2005. We included 126 patients with PCOS in this study. Patients were divided into 3 groups according to the body mass index (BMI). Group I: normal weight (48 patients). Group II: overweight (n=29). Group III: obese (n=49). We included 75 healthy women as a control and divided into 3 groups according to their BMI: normal weight (n=35), overweight (n=25), and obese (n=15). All patients were evaluated for features of PCOS. Hormonal assay including serum and saliva free testosterone were determined with 3.2 pg/ml as the upper normal level for both. The luteinizing hormone/follicle stimulating hormone (LH/FSH) ratio was performed in serum of samples obtained on day 2 or 3 of the natural cycle in menstruated women, and any time in women with amenorrhea, and the ratio of LH/FSH ≥2 was regarded as the significant level. In the control group, the sonography and LH/FSH estimation were carried for all, saliva free testosterone was measured for only 30. All did not fulfill the criteria of PCOS, although some had one of the criteria.

Results: The age range was 15-39 (26.12±6.36) years with a mean BMI of 30.261±8.238. While the ages of 75 healthy control women ranged from 15-39 (28.82±6.45) years with a mean BMI of 26.99±4.41. Free saliva testosterone was abnormally elevated in 68, free serum testosterone elevated in 75, elevated LH/FSH ratio in 76, and positive U/S in 98 of patients. The free saliva testosterone and LH/FSH ratio were significantly increased in patients with PCOS in comparison with the control group. Frequency of skin manifestations was significantly increased in PCOS patients with abnormal saliva and serum free testosterone level in comparison with those of normal level hormones. There was a positive relationship between the increase in frequency of skin manifestations and increase in saliva and serum free testosterone levels, while their was no relation between LH/FSH ratio and frequency of skin manifestations.

Conclusion: Free testosterone level represents the most sensitive biochemical marker supporting the diagnosis of PCOS.

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Polycystic ovary syndrome (PCOS) is the most common endocrine disorder of women of reproductive age, affecting 5-10% of women in this age group.[1,2] The underlying defect in PCOS remains unknown, but there is growing consensus that key features include insulin resistance, androgen excess, and abnormal gonadotrophin dynamics, however, the main or principal underlying disorder is insulin resistance, with resulting hyperinsulinemia stimulating excess ovarian androgen production.[3] Current studies suggest that the ovary is the principle site of excess androgen, but some women with PCOS may have an adrenal contribution of the increased androgen production. The mechanism of this remains obscure and is almost certainly multifactorial.[4] The diagnosis of PCOS depends on 2003 criteria (2 out of 3) by Rotterdam European Society for Human Reproduction and Embryology/American Society for Reproductive Medicine (ESHRE/ASRM)-sponsored PCOS consensus workshop group which include: 1) oligo, anovulation, or both, 2) clinical, biochemical signs of hyperandrogenism, or both, 3) polycystic ovaries on ultrasound (U/S) examination. Also, exclusion of other etiologies such as congenital adrenal hyperplasia, androgen-secreting tumors and Cushing's syndrome.[5] Anovulation manifests itself as menstrual disturbance (amenorrhea, oligomenorrhea, or dysfunctional uterine bleeding) and infertility. Cutaneous manifestations

of androgen excess in PCOS include hirsutism, seborrhea, acne, male pattern baldness, acanthosis nigricans, skin tag, and obesity, which are common in women with PCOS, however, it is not a consistent finding as it occurs in 50% of affected women. Occasionally women have signs of more severe androgen excess (virilization) such as deepening of the voice, clitoromegaly, and musculinization.[6,7] There is no clear consensus on hormonal tests that can be expected to fully discriminate women with PCOS from normal cycling women. Dependent upon the androgen measured, between 50-90% of PCOS women have high serum androgen concentrations. The excess androgens can be derived from the ovary or the adrenal cortex. Testosterone primarily serves as a marker of ovarian production while dehydroepiandrosterone-sulfate (DHEA-sulfate) marks elevated adrenal production. Women with high serum androgen concentrations tend to have increases in both serum total and free testosterone concentrations. Testosterone-bound sex hormone binding globulin (SHBG) is considered a biologically inactive measurement, so the measurement of free testosterone is the most sensitive method of assessing hyperandrogenemia. Using salivary samples for the estimation of free testosterone level is an attractive concept because of the ease of sample collection; in general, steroid levels in saliva are thought to reflect the free level in the blood. Both the absolute level of circulating luteinizing hormone (LH) and its relationship to follicle stimulating hormone (FSH) levels are significantly elevated in PCOS women as compared with controls. This is due to an increased amplitude and frequency of LH pulses. However, the mean LH pulse amplitude is attenuated in obese women with PCOS. Thus, the LH value or LH/FSH ratio would not be helpful in establishing the diagnosis in such patients, so that measurement of serum LH levels should not be considered necessary for the clinical diagnosis of PCOS, and LH level could be useful as a secondary parameter especially in lean women with amenorrhoea, or in research.[5] Polycystic ovary appearance on U/S is consistent with, but not essential for, the diagnosis of PCOS. Most but not all PCOS subjects show characteristic U/S appearance. Previous study defines it by the presence of 10 or more follicular cysts 2-8 mm in diameter in one plane distributed evenly around the ovarian periphery in a chain like manner (pearl/necklace) with an echo dense ovarian stroma.[1] Therefore, the present work extensively investigates the relationship between frequency of skin manifestations and hormone levels.

Methods. This prospective study was conducted in the Department of Dermatology and Venereology in Baghdad Teaching Hospital, Baghdad, Iraq from April 2004 to March 2005. One hundred and twenty-six patients with PCOS, who fulfilled the Rotterdam 2003 criteria,[5] were included in this study. We divided the patients into 3 groups according to the body mass index (BMI): Group I (normal weight) whose BMI range between 18.5-24.9 (48 patients). Group II (overweight) whose BMI range between 25-29.9 (29 patients). Group III (obese) whose BMI >30 (49 patients). Seventy-five healthy females were also included in this study as a control group and divided according to BMI into 3 groups: Group I (normal) 35 patients, group II (overweight) 25 patients, and group III (obese) 15 patients. History from each patient was taken regarding all points related to the condition, a close clinical examination was performed searching for the presence of all skin manifestations associated with PCOS. The hormonal status for each patient was assessed including: LH/FSH - the concentrations of these hormones were determined by direct immunoenzymatic determination of LH and FSH in the serum of samples obtained on day 2 or 3 of the natural cycle in menstruating women, and at any time in women with amenorrhoea and the ratio of LH/FSH 2 or more was regarded as a significant level. Testosterone - the level of free testosterone was determined, 3.2 pg/dl as the upper normal level for both serum and saliva testosterone. Concentrations of these hormones were determined by direct immunoenzymatic determination of free testosterone in serum and saliva, and the kit was supplied by Diametra (Italy) for both. In the control group, the sonography and LH/FSH estimation where carried out for all, saliva free testosterone was measured for only 30, while serum free testosterone was not measured, as the kit was not available. They did not fulfill the criteria of PCOS, although some had one of the criteria. A trans-abdominal pelvic ultrasound was carried out for all patients using the stated criteria. Patients who have other endocrine abnormalities and patients who have taken contraceptive pill or hormonal therapy for at least the last 2 months were excluded from the study.

Results. This study showed increasing frequency of skin manifestations among PCOS patients with elevated levels of saliva free testosterone in comparison with the normal saliva free testosterone PCOS patients. There was a highly statistically significant difference between the 2 groups (**Table 1**). The *p*-value ranged between (0.027309–0.000008) such as in hirsutism, acne, androgenetic alopecia, acanthosis nigricans, skin tag, seborrhea, vaginal candidiasis, and bad odor. While other skin manifestations were also increased, but did not reach the statistically significant level, such as plantar hyperkeratosis, intertrigo, and stria distensae, $p>0.05$. Also, there was increasing frequency of skin

manifestations with increasing saliva free testosterone levels (**Table 2**). Regarding the differences in biochemical changes between PCOS patients and control group, there was statistically significance difference in mean saliva free testosterone between PCOS patients (n=126; mean±SD 3.43 ± 0.79) and control group (n=30; mean±SD; 1.29 ± 0.46), p=0.000000. The study showed also increasing frequency of skin manifestations among PCOS patients with elevated levels of serum free testosterone in comparison with the normal level serum free testosterone PCOS patients. There was a highly statistical significant difference between the 2 groups (**Table 3**). The p-value ranged from 0.044764-0.00003, such as hirsutism, acne, androgenetic alopecia, acanthosis nigricans, seborrhea, planter hyperkeratosis, vaginal candidiasis, and bad odor. While other skin manifestations were also increased but did not reach a statistically significant level, such as intertrigo and stria distensae. There was an increase in frequency of skin manifestations with an increase of serum free testosterone levels (**Table 4**). There was no statistically significant difference in frequency of skin manifestations among normal and abnormal LH/FSH ratio of PCOS patients (**Table 5**). There was a statistically significant difference in mean of LH/FSH ratio between PCOS patients and healthy controls, p=0.000001. The results show that the total number of PCOS patients with abnormal serum free testosterone was 75 (59.5%). While those with abnormal saliva free testosterone was 68 (54%). The number of PCOS patients with elevated LH/FSH ratio ≥2 was 76 (60.3%), and the number of PCOS with positive ultrasonography was 98 (77.8%). The comparison between normal weight, overweight and obese PCOS patients with regard to abnormal hormonal levels and positive U/S is shown in **Table 6**. There was an increase in serum and saliva free testosterone levels with the increase in BMI, although this did not reach the statistically significant level. Also, there was a statistically significant decrease in LH/FSH ratio with increasing weight in patients with PCOS. There was also a statistically significant increase in the positivity of U/S in obese PCOS patients in comparison with over weight and normal weight PCOS patients.

Table 1 - Skin manifestations among polycystic ovary syndrome with normal and abnormal saliva testosterone.

Skin manifestations	Saliva testosterone		*P*-value
	No. of patients (%)		
	Normal 58/126	Abnormal 68/126	
Hirsutism	36 (62)	64 (94.1)	0.000013
Acne	12 (20.7)	38 (55.9)	0.000061
Androgenetic alopecia	8 (13.7)	28 (41.2)	0.000674
Acanthosis nigricans	33 (56.9)	61 (89.7)	0.000030
Seborrhea	23 (39.7	46 (67.6)	0.001505
Plantar hyperkeratosis	18 (31)	31 (45.6)	0.068566
Skin tag	16 (27.6)	31 (45.6)	0.027309
Vaginal candidiasis	13 (22.4)	34 (50)	0.004248
Intertrigo	11 (19)	19 (27.94)	0.268314
Stria distensae	7 (12.1)	11 (16.2)	0.446922
Bad odor	37 (63.8)	65 (95.6)	0.000008

Table 2 - The frequency of skin manifestations and their percentages according to saliva free testosterone level.

Skin manifestations	Saliva free testosterone level (ng/dl)		
	No. of patients (%)		
	2.1 - 3.0 50/126	3.1 - 4.0 41/126	4.1 - 5.0 35/126
Hirsutism	31 (62)	34 (82.9)	35 (100)
Acne	11 (22)	20 (48.8)	19 (54.3)
Androgenetic alopecia	7 (14)	12 (29.3)	17 (48.6)
Acanthosis nigricans	30 (60)	31 (75.6)	33 (94.3)
Seborrhea	19 (38)	26 (63.4)	24 (68.6)
Plantar hyperkeratosis	17 (34)	31 (12.1)	19 (54.3)
Skin tag	14 (28)	15 (36.6)	18 (51.4)
Vaginal candidiasis	11 (22)	18 (43.9)	18 (51.4)
Bad odor	32 (64)	36 (87.8)	34 (97.1)
Stria distensae	3 (6)	9 (22)	6 (17.1)
Intertrigo	8 (16)	9 (22)	13 (37.6)

Discussion. There are different opinions on the question of what laboratory studies should be ordered

Table 3 - Skin manifestations among polycystic ovary syndrome with normal and abnormal serum testosterone.

Skin manifestations	Serum testosterone		*P*-value
	No. of patients (%)		
	Normal 51/126	Abnormal 75/126	
Hirsutism	33 (64.7)	67 (89.33)	0.000878
Acne	9 (17.6)	41 (54.66)	0.000034
Androgenetic alopecia	6 (11.8)	30 (40)	0.000591
Acanthosis nigricans	31 (60.8)	63 (84)	0.003161
Seborrhea	17 (33.3)	52 (69.3)	0.000072
Plantar hyperkeratosis	12 (23.5)	37 (49.3)	0.003157
Skin tag	14 (27.5)	33 (44)	0.044764
Vaginal candidiasis	10 (19.6)	37 (49.3)	0.000688
Intertrigo	9 (17.6)	21 (28)	0.129836
Stria distensae	6 (11.8)	12 (16)	0.341530
Bad odor	36 (70.6)	66 (88)	0.013449

Table 4 - The frequency of skin manifestations and their percentages according to serum free testosterone level.

Skin manifestations	Serum free testosterone level (ng/dl)		
	No. of patients (%)		
	2.1 - 3.0 43/126	3.1 - 4.0 44/126	4.1 - 5.0 39/126
Hirsutism	25 (58.1)	40 (90.9)	35 (89.7)
Acne	6 (32)	24 (54.5)	20 (51.3)
Androgenetic alopecia	4 (9.3)	16 (36.4)	16 (41)
Acanthosis nigricans	25 (58.2)	31 (70.5)	38 (97.4)
Seborrhea	14 (32.5)	25 (56.8)	30 (76.9)
Plantar hyperkeratosis	11 (25.6)	19 (43.2)	19 (48.7)
Skin tag	10 (23.6)	17 (38.6)	20 (51.3)
Vaginal candidiasis	7 (16.3)	18 (40.9)	22 (56.4)
Bad odor	27 (62.8)	39 (88.6)	36 (92.3)
Stria distensae	4 (9.3)	5 (11.4)	9 (23.1)
Intertrigo	5 (11.6)	12 (27.3)	13 (33.3)

Table 5 - Skin manifestations among polycystic ovary syndrome with normal and abnormal luteinizing hormone/ follicle stimulating hormone (LH/FSH) ratio.

Skin manifestations	LH/FSH ratio		*P*-value
	No. of patients (%)		
	Normal 50/126	Abnormal 76/126	
Hirsutism	43 (86)	57 (75)	0.102443
Acne	17 (34)	33 (43.4)	0.191796
Androgenetic alopecia	18 (36)	18 (23.7)	0.097460
Acanthosis nigricans	40 (80)	54 (71.1)	0.178790
Seborrhea	26 (52)	43 (56.6)	0.373990
Plantar hyperkeratosis	20 (40)	29 (38.2)	0.491367
Skin tag	23 (46)	24 (31.6)	0.073473
Vaginal candidiasis	19 (38)	28 (36.8)	0.477456
Intertrigo	12 (24)	18 (23.7)	0.431518
Stria distensae	11 (22)	19 (25)	0.431307
Bad odor	44 (88)	58 (76.3)	0.080320

Table 6 - Comparison between normal weight, overweight, and obese patients with polycystic ovary syndrome regarding abnormal hormone and positive ultrasound.

Variable	Normal (n=48)	Overweight (n=29)	Obese (n=49)	*P*-value
	No. of patients (%)			
Serum free testosterone	25 (52.1)	17 (58.6)	33 67.3	0.309819
Saliva free testosterone	22 (45.8)	16 (55.1)	30 61.2	0.366109
LH/FSH ratio	40 (83.3)	15 (51.7)	21 42.8	0.000139
U/S positive	32 (66.7)	21 (72.4)	45 91.8	0.008598

LH/FSH - luteinizing hormone/ follicle stimulating hormone, U/S - ultrasound

in evaluating a woman with PCOS. Although PCOS is primarily a clinical diagnosis (given a history of chronic anovulation and androgen excess) with the exclusion of the very uncommon condition of non-classical congenital adrenal hyperplasia,[8] however, elevated free testosterone level represents the most sensitive biochemical marker supporting the diagnosis. A raised LH concentration, although a useful marker of the syndrome, is now less favored as a diagnostic tool.[4] A ratio of LH/FSH equal or greater than 2:1 is certainly consistent with PCOS, as a diagnostic test, however, the LH/FSH ratio is often in the normal range and therefore is an insensitive test. This is because of the pulsatile nature of gonadotropins, resulting in a broad range of LH/FSH ratios in PCOS patients when a single blood sample is drawn in addition, the double-sandwich immunofluorometric assays currently used for LH determinations are associated with lower LH/FSH ratios than the immunoenzymatic determination used in the present study.[9] This study shows that the saliva free testosterone was elevated in 68 (54%) of PCOS patients and serum free testosterone was elevated in 75 (59.5%). Also, there was a highly statistically significant increase in mean saliva and serum free testosterone in PCOS patients in comparison with the controls. The distribution of skin manifestations was increased

significantly among PCOS patients with elevated saliva and serum free testosterone levels in comparison with those of normal level hormone patients. Also, there was a close positive relationship between increase in saliva and serum free testosterone level and increase in skin manifestations. However, the LH/FSH ratio showed no relationship to skin manifestations. Therefore, we should consider saliva and serum free testosterone as a very sensitive indicator of biochemical hyperandrogenemia, especially when there is no evidence of clinical hyperandrogenemia in an oligo or anovulatory women. Accordingly, measuring the free saliva testosterone is as useful as serum free testosterone, but it is easier to take saliva than a blood sample.

This study showed an increased saliva and serum free testosterone level with increased BMI, although this did not reach the significant level. There was an inverse relationship between elevated LH/FSH ratio and BMI, as the elevated LH/FSH ratio was more amplified in normal weight PCOS than obese, as had been reported,[10] in addition to the highly significant ratio of LH/FSH in PCOS patients in comparison with control. This study showed a highly statistically significant increase in mean saliva testosterone in PCOS in comparison with control. Finally, U/S was seen positive in 77.5% of PCOS patients and increased with the increase in BMI, as had been reported.[11]

In conclusion, skin diseases are important manifestations of PCOS, which are closely related to BMI and free saliva and serum testosterone.

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Ethical Consent

All manuscripts reporting the results of experimental investigations involving human subjects should include a statement confirming that informed consent was obtained from each subject or subject's guardian, after receiving approval of the experimental protocol by a local human ethics committee, or institutional review board. When reporting experiments on animals, authors should indicate whether the institutional and national guide for the care and use of laboratory animals was followed.