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Evaluation of physical attributes, clinical symptoms, and biochemical markers in women presenting with polycystic ovarian syndrome

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Abstract : Polycystic Ovarian Syndrome (PCOS) is the most common cause of secondary amenorrhea, but yet is mostly misdiagnosed. The current recommendation for its diagnosis requires the measurement of free testosterone, which is rather challenging and resource-consuming. The present study aimed at assessing the relationship between various biochemical and clinical signs and symptoms and PCOS as an attempt to predict its diagnosis.
Methods: Patients were screened for secondary amenorrhea before being invited to participate in this study. Once the diagnosis is confirmed, clinical and biochemical assessments were undertaken and blood samples were collected for this purpose. Several previously validated scales were utilized to assess acne, hirsutism, and the psychological wellbeing of the patients. Bivariate and multivariate statistical analyses were conducted to assess the relationship between different variables and PCOS outcome.
Results: One hundred and seventeen patients were enrolled in this study. Out of the total, sixty five (47.86%) were confirmed to have PCOS. Women diagnosed with PCOS had a significantly higher average of BMI, WHR, blood glucose, lipid profile, LH/FSH ratio, blood pressure and depression scores, as compared to women with other causes of secondary amenorrhea. Multivariate logistic regression showed that women with higher BMI and WHR, dyslipidemia, acne, hirsutism, higher LH/FSH ratio, depression, hypertension and hyperglycemia are more likely to have PCOS as a diagnosis.
Conclusions: These clinical and biochemical parameters could predict the possibility of having PCOS among Iraqi women. These findings offer a simple and practical guideline to raise the suspicion about PCOS diagnosis among Iraqi women.

Introduction:

Polycystic ovarian syndrome (PCOS) is now considered one of the most common endocrinopathy which affects 4-8% of women of reproductive age [1,2]. The PCOS is characterized by a range of symptoms as a result of hormonal imbalance. It is the commonest cause of secondary amenorrhea, and mostly associated with reproductive symptoms such as infertility, irregular menstrual periods, and anovulation, or symptoms resulting from high free androgen levels; such as hirsutism, male pattern of hair distribution, and acne [3].Women with PCOS are at increased risk of insulin resistance and developing type 2 diabetes [4]. The diagnosis of PCOS is based on several criteria that require the measurement of free testosterone, clinical assessment of the symptoms of PCOS, and the detection of ovarian cysts by ultrasonography [5]. Despite its profound effect on women causing significant distress, up to 70% of women with PCOS remain undiagnosed [6]. This is because the

measurement of free testosterone is rather challenging and costly[4], and the ultrasound assessment is not reliable as almost 70% of adolescent women have polycystic ovaries on ultrasound [7]. This seems particularly important in low-resource settings, where the measurement of free testosterone may not be possible.

Therefore, the present study aimed at assessing the relationship between the physical attributes, clinical symptoms and various biochemical markers as an attempt to define predictors of PCOS diagnosis among Iraqi women.

Methods:

Study population

Because amenorrhea may result from various medical conditions, we followed the American Family Physician publication guidelines on approaching this syndrome[8]. Briefly, a thorough medical history, physical examination, laboratory assessment of selected serum hormones level, and ultrasonography imaging were undertaken to ensure the stringency of the diagnosis. Patients diagnosed with secondary amenorrhea were invited to participate in this study. An information sheet explaining the purpose of the study was provided to the patients. Patient's written consent was obtained in line with the ethical principles expressed in the Declaration of Helsinki. Patients were recruited from Al Imamayin Al Kadhymain Medical City, Baghdad, during a period from December 2015 to October 2016.

Clinical assessment

Patients were clinically examined for evidence of hyperandrogenemia, metabolic syndrome, thyroid disease, adrenal disease, neurological diseases, psychiatric disorders, and other systemic illnesses. Full medical and gynecological history, vital signs, and physical examination were performed to identify any clinical signs or symptoms associated with other causes of amenorrhea. The anthropometric measurements (body mass index, or BMI, and Waist-hip ratio, or WHR) were also calculated. The modified, Ferriman and Gallway (mFG) method [9], by visually grading hair growth in women in nine body areas, was utilized to assess the presence and severity of hirsutism and male-pattern of hair distribution. In addition, the previously validated comprehensive acne severity scale (CASS) [10] was employed to assess the severity of acne when present. The infertility was assessed according to the World Health Organization definition (WHO), which entailed the "inability of a sexually-active, non-contracepting couple to achieve pregnancy in one year" [11].

The assessment of the psychological wellbeing

Information on thepsychological wellbeing of the patients were collected by utilizing a previously published and psychometrically-validated Depression Anxiety Stress Scale (DASS) (the 21-item version). This is a widely used, self-reported, screening tool for anxiety and depression, which was first described by Lovibond SH and Lovibond PF [12]. In detail, this tool solicited information on three constructs; Depression, Anxiety and Stress. The self-reported 21 questions assess the presence and severity of symptoms on a scale from zero to three; with a score of "zero" indicates that the question does not apply to the patient, and a score of "three" indicates that the symptom applied to the patient very much for most of the time. The final score reflects the presence and the severity of the condition; with either "normal" (between 0-9 for depression, 0-7 for anxiety, and 0-14 for stress), "mild" (10-13 for depression, 8-9 for anxiety, and 15-18 for stress), "moderate" (14-20 for depression, 10-14 for anxiety, and 19-25 for stress), "severe" (21-27 for depression, 15-19 for anxiety, and 26-33 for stress), or "extremely severe" (28+ for depression, 20+ for anxiety, and 34+ for stress) [13].

Biochemical assessment

At first, serum human chorionic gonadotropin (hCG) level (abcam #ab108636) was measured to exclude the possibility of pregnancy. Serum hormones assessment included the measurement of thyroid stimulating hormone (TSH) (abcam #ab108660) and free thyroxine level (abcam #ab108662) (to rule out thyroid dysfunction), serum prolactin level (abcam #108655), free androgen index (FAI), which is defined as the total testosterone level (abcam #108666) divided by sex hormone binding globulin (SHBG) (Sigma-Aldrich #RAB0734-1KT) multiplied by 100. The FAI represents an indicator of the free testosterone level. The Follicle-

Stimulating Hormone (FSH) (abcam #ab108678) and Luteinizing Hormone (LH) (abcam #ab178658) levels were also estimated and the FSH/LH ratio was calculated.

Whenever there was a clinical suspicion of Cushing syndrome, an overnight dexamethasone suppression test was undertaken. Similarly, when acromegaly was suspected, insulin-like growth factor (IGF)-1 (abcam #ab108873) was assessed to rule out growth hormone access. Fasting blood sugar was estimated, then two hours after ingestion of 75 gm of glucose, post-load blood sugar level was calculated. Lipid profile was assessed by measuring total cholesterol (Cell Biolabs #STA-384), low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C) (abcam #65390), and triglycerides (calculated by subtracting LDL and HDL from total cholesterol then multiplied by 5). Blood samples were taken early in the morning when the patients were in fasting state, except for the glucose tolerance test as blood samples were drawn two hours after the ingestion of glucose.

Statistical analyses

Data were encoded and entered into SPSS Statistical software package (IBM version 22) for further analysis. Descriptive analyses, such as the mean value, standard deviation (SD), frequency and percentage, were employed to describe the study population. Independent samples t-test was performed to assess the statistical difference between groups. Multi-variate logistic regression analysis was performed to assess the correlation between clinical symptoms, biochemical markers, and psychological characteristics and their predictive value of hyperandrogenism status. For this purpose and based on FAI, data were dichotomously categorized into either patients with high androgen level (hyperandrogenism group; total testosterone > 3nmol/L, SHBG < 30 nmol/L, and FAI >5%) or normal androgen level (other causes of amenorrhea). The adjusted odds ratio (AOR) was calculated and presented with 95% confidence interval (CI). Graphpad Prism statistical software (version 7.02) was used to produce the study figure. Statistical significance was defined as p-value equal or less than 0.05.

Results

Study population

This study enrolled 117 patients whom were diagnosed with secondary amenorrhea. The mean age of these patients is 25.95 years (±5.98). The clinical, biochemical and psychological characteristics of these patients are presented in table 1. Out of the total, fifty six patients (47.86%) were confirmed to have PCOS according to Rotterdam diagnostic criteria of polycystic ovarian syndrome [5]. The other causes of secondary amenorrhea involved hypothalamic amenorrhea (14.5%), hypothyroidism (9.4%), medications (9.4%),primary ovarian insufficiency (5.98%), intrauterine adhesions and cervical stenosis (5.12%), hyperprolactinemia (4.27%), andeating disorders (3.4%). The bivariate analysis (table 1) showed a statistically significant difference between these groups in WHR ($p<0.01$), BMI, acne, hirsutism ($p<0.001$), blood pressure, fasting blood glucose ($p<0.01$), random blood glucose, LH/FSH ratio, and lipid profile ($p <0.001$). Interestingly, there was no significant difference between the two groups in the anxiety or stress scores. However, patients diagnosed with PCOS had a significantly higher depression score when compared to other patients ($p <0.01$).

Table 1: Clinical, biochemical and psychological characteristics of study population.

Variables	**Patients diagnosed with PCOS (N= 56)**	**Patients diagnosed with other causes of amenorrhea (N=61)**	**p-value**
Age (years)	24.72 (± 5.51)	27.18 (±6.44)	NS
WHR	0.907 (±0.041)	0.821 (± 0.024)	<0.01
BMI	33.72 (± 1.78)	25.41 (± 1.44)	<0.001
Acne	104.92 (±23.72)	21.13 (±9.22)	<0.001
Hirsutism	3.47 (±0.68)	1.17 (±0.43)	<0.001
Infertility (percentage of total)	62.5%	57.38%	NS
Blood pressure (systolic/diastolic) (mmHg)	153.78 (±16.62)/98.91 (±8.11)	111.32 (±9.91)/71.17 (±10.23)	<0.01

Fasting blood glucose (mg/dl)	123.17 (±13.14)	104.44 (± 8.50)	<0.01
Random blood glucose (mg/dl)	211.47 (±23.11)	135.18 (±17.98)	<0.001
Total cholesterol (mgs/dl)	277.88 (±29.34)	162.89 (±19.01)	<0.001
High density lipoprotein cholesterol (mgs/dl)	31.69 (±8.09)	54.31 (±11.12)	<0.01
Low density lipoprotein cholesterol (mgs/dl)	199.73 (±17.73)	84.52 (±11.99)	<0.001
Triglycerides (mgs/dl)	232.30 (±21.01)	120.32 (±13.14)	<0.001
LH/FSH ratio	4.78 (±1.01)	1.27 (±0.19)	<0.001
Prolactin	17.37 (±5.08)	18.19 (±2.91)	NS
Free androgen index (%)	14.79 (±2.66)	1.98 (±0.20)	<0.001
Total testosterone (nmol/L)	4.10 (±1.04)	0.88 (±0.09)	<0.001
Sex hormone binding globulin (nmol/L)	27.71 (±4.14)	44.38 (±6.16)	<0.001
Anxiety score	12.11 (±7.09)	10.98 (±6.67)	NS
Depression score	18.19 (±4.81)	11.27 (±2.27)	<0.01
Stress score	28.48 (±8.89)	24.47 (±7.39)	NS

Data are presented as the mean value (± standard deviation). BMI: body mass index; FSH: follicle-stimulating hormone; LH: luteinizing hormone; NS: not significant; PCOS: polycystic ovarian syndrome; WHR: waist-hip ratio.

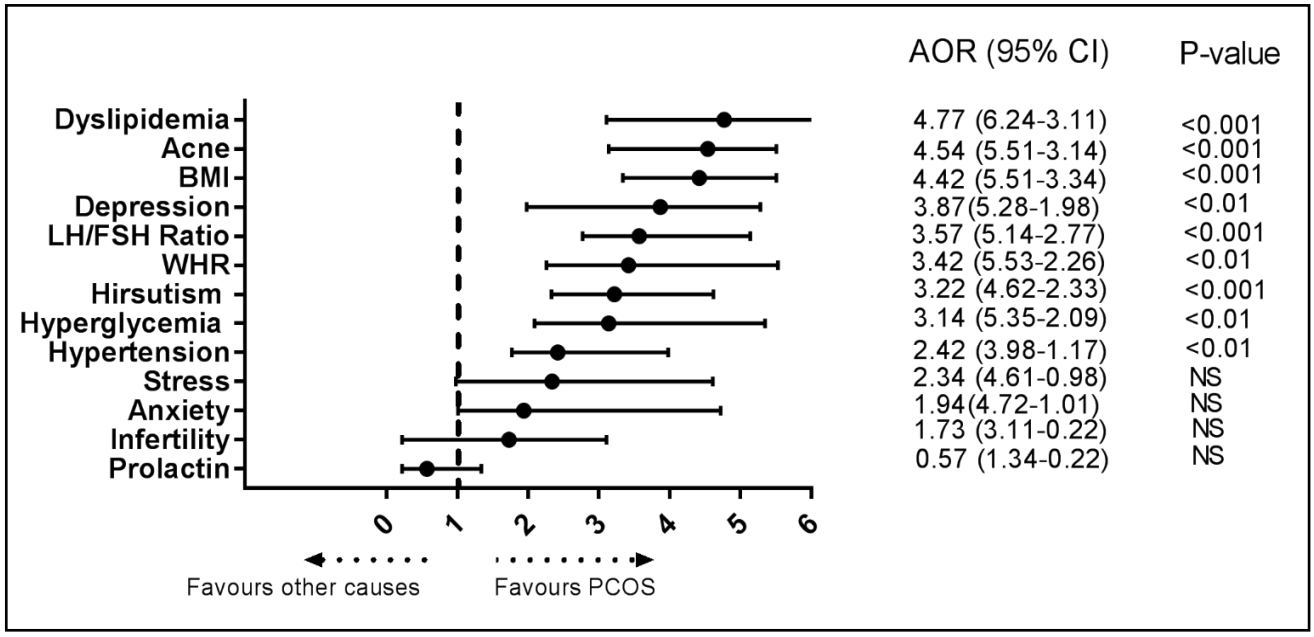

Figure 1: The relationship between various clinical, biochemical and psychological characteristics and their predictive value of PCOS diagnosis. AOR: Adjusted Odds Ratio; BMI: body mass index; FSH: follicle-stimulating hormone; LH: luteinizing hormone; NS: not significant; PCOS: polycystic ovarian syndrome; WHR: waist-hip ratio.

The predictive value of clinical, biochemical and psychological parameters in PCOS

A stepwiselogistic regression analysis was undertaken to adjust for any potential confounding effect of other variables and to describe the predictive value of each variable to the diagnosis of PCOS (figure 1). In detail, biochemical markers, such as dyslipidemia (AOR= 4.77, 95% CI=6.24, 3.11, p <0.001), LH/FSH ratio (AOR= 3.57, 95% CI=5.14, 2.77, p <0.001), and hyperglycemia (AOR= 3.14, 95% CI=5.34, 2.09, p <0.01) could significantly predict the diagnosis of PCOS. On the other hand, serum prolactin level failed to do so (p> 0.05). Clinically, patients presented with acne (AOR= 4.54, 95% CI=5.51, 3.14, p <0.001), hirsutism (AOR= 3.22, 95% CI=4.62, 2.33, p <0.001), hypertension (AOR= 2.42, 95% CI=3.98, 1.17, p <0.01), higher BMI (AOR= 4.42, 95% CI=5.51, 3.34, p <0.001), or high risk WHR (AOR= 3.42, 95% CI=5.53, 2.26, p <0.01) were

several folds more likely to have PCOS. In contrast, having infertility as the chief complaint could not predict the likelihood of PCOS.

Psychologically, patients presented with clinical depression (AOR= 3.87, 95% CI=5.28, 1.98, p <0.01) were more likely to have PCOS as compared to patients who didn't suffer from it. This does not seem the case with other psychiatric disorders, such as anxiety or general stress (p>0.05).

Discussion:

The present study described the relationship between several clinical, biochemical and psychological factors, and their role in predicting the possibility of having PCOS. While PCOS is associated with a surge in free androgens, and so it is expected to observe its side-effects such as acne, hirsutism and male-pattern of hair distribution, however, after adjusting for other potentially confounding variables, our study confirmed that these clinical signs are accurate indicators of androgen rise. Nevertheless, previously published literature showed that the relationship between the severity of acne and free androgens is not linear, and hence it does not reflect the severity of PCOS [14]. In addition, hirsutism and acne can be associated with other illnesses, apart from PCOS, and therefore they should not be solely interpreted as diagnostic to PCOS [15]. Therefore, other clinical, biochemical and psychiatric indicators should be considered in order to raise the suspicion of PCOS.

In addition, the findings of the present study revealed that obesity and high-risk WHR are predictors of PCOS. One possible explanation is the relationship between insulin resistance and PCOS. Previous evidence described the relationship between PCOS and hepatic insulin resistance [16], characterized by increased post-absorptive glucose production coupled with reduced sensitivity to insulin, resulting in a synergistic deleterious effect on glucose tolerance and obesity [17]. The latter evidence also speculated that common genetic defects might predispose to both insulin resistance and the reproductive abnormalities observed in PCOS. This may explain why, in the present study, hyperglycemia significantly predicted the PCOS diagnosis. Normally, a decrease in the insulin sensitivity should result in a reciprocal increase in insulin secretion to maintain a normal blood glucose level [18]. However, in PCOS, oxidative stress and the subsequent systemic inflammation were implicated in impairing β-cell function, regardless of body weight and the degree of insulin resistance, and thus may be the leading cause of hyperglycemia [19]. In addition, the present study also showed that dyslipidemia is a significant predictor of PCOS. Previous evidence implicates the role of insulin resistance and increased free androgens in increasing catecholamine-induced lipolysis,which leads to the release of free fatty acids into the circulation [20]. As a result, the flux of free fatty acids stimulates the liver to produce more LDL, and less of HDL cholesterol, and hence causing dyslipidemias. It is noteworthy that although dyslipidemia is possibly the most common abnormality associated with PCOS, some studies reported a substantial percentage of women with PCOS and still have a normal lipid profile [21].

Moreover, the present study reported that the LH/FSH ratio significantly predicted the PCOS outcome. This biochemical abnormality seems unique to PCOS, however, if this ratio is normal, this should not exclude the diagnosis of PCOS. One study assessed the LH/FSH ratio in a large cohort of PCOS patients, and reported that less than 50% of these patients had a significant increase in this ratio [22]. On the other hand, another study reported a significant correlation between the LH/FSH ratio and the degree of insulin resistance, and therefore may reflect the severity of PCOS [23]. This ratio seems also helpful to provide prognostic information on the fertility status. One study showed that having high LH/FSH ratio inversely correlated with the number of follicles and oocytes developed, the percentage of mature oocytes, and hence pregnancy rate [24]. This was also supported by another study which described a similar finding, and argued that high LH level may have a deleterious effect on folliculogenesis and endometrial receptivity, and thus negatively affects pregnancy rate[25].

Surprisingly, the present study described that being stressed or anxious does not predict the diagnosis of PCOS. In contrast, depressed patients were almost four times more likely to have PCOS. This is consistent with a published meta-analysis which described a higher rate of depression among women with PCOS, however, this difference was also determined by BMI [26]. The latter evidence described that women with lower BMI tended to report lower scores of depression when compared to the control group. The association between BMI and depression is controversial, with some data describing a positive correlation[27], while others described a negative trend[28], or no association at all[29]. However, recent publication described a U-shaped relationship between BMI and depression; with overweight and obese patients tended to be more depressed [30]. The latter study may better explain the findings of the present study, as the PCOS group had a significantly higher BMI, averaging in the

obese category. In addition, insulin resistance was also implicated as another reason for depression among these patients [31]. Insulin modulates the central serotonin (5-HT) level, a monoamine neurotransmitter which plays a major part in mood, anxiety and happiness, and thus its dysregulation may cause depression possibly via behavioral and/or neuroendocrinologic pathways [32]. However, the mechanism of depression in PCOS patients remains unclear, and whether if the hormonal imbalance could possibly influence neuronal pathways responsible for this presentation.

Conclusions:

The present study offers the conclusion that PCOS diagnosis can be predicted based on several biochemical markers, clinical and psychiatric signs and symptoms. Biochemical markers, such as dyslipidemia, hyperglycemia, and LH/FSH ratio could predict the possibility of PCOS diagnosis. Clinically, higher BMI and WHR, acne, hirsutism, and hypertension are useful indicators which favor PCOS diagnosis. Psychiatric disorders, such as depression, could also predict PCOS. However, these findings should be interpreted with caution owing to several limitations. First, the data were collected from a convenient sample from Iraqi patients attending one hospital in Baghdad, and therefore the results cannot be generalized to the larger population. Second, clinical and psychiatric assessment were conducted by either self-reported questionnaire, or clinical assessment by a qualified physician. Therefore, reporting bias cannot be ruled out. However, this study offers a simple and a practical guideline to recognize and diagnose PCOS patients among Iraqi women. These findings may help to improve the health practice in low-resource settings, especially in rural and regional hospitals in Iraq.

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Conflict of interest:

The authors declare no conflict of interest.

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