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ORIGINAL RESEARCH

Sensitive and specific markers for insulin resistance, hyperandrogenemia, and inappropriate gonadotrophin secretion in women with polycystic ovary syndrome: a case-control study from Bahrain

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Background: In women with polycystic ovary syndrome (PCOS), despite a high prevalence of insulin resistance, hyperandrogenemia, and disturbances in the secretion of gonadotrophin, the principal causes of biochemical abnormalities and the best endocrine markers for PCOS have not been fully identified.

Subjects and methods: Serum levels of insulin, glucose, follicle-stimulating hormone (FSH), luteinizing hormone (LH), total testosterone, estrogen, sex hormone-binding capacity (SHBG), and other related indices such as homeostasis model assessment, insulin glucose ratios, LH/FSH ratios, and the free androgen index (FAI) were determined and compared in women with PCOS (n = 50) and women without PCOS (n = 50).

Results: In multivariate logistic regression analyses, among all insulin resistance indices, only hyperinsulinemia (odds ratio [OR] = 2.6; confidence interval [CI]: 1.3–5.2; $P = 0.008$) was significantly and independently associated with PCOS when adjusted for body mass index (BMI), hyperandrogenemia, and LH/FSH ratios. The LH/FSH ratio (OR = 5.4; CI: 1.2–23.0, $P = 0.03$) was the only marker among those indices for inappropriate gonadotrophin secretion that significantly and independently associated with PCOS when adjusted for BMI and hyperinsulinemia. Among those indices for hyperandrogenemia, FAI (OR = 1.1; CI: 1.0–2.7; $P = 0.02$) and SHBG (OR = 1.2; CI: 1.2–3.4; $P = 0.03$) were significantly and independently associated with PCOS when adjusted for BMI and hyperinsulinemia. In addition, receiver operating characteristic analysis showed that the best predictive markers for PCOS were insulin (area under the curve [AUC] = 0.944; CI: 0.887–0.989), FAI (AUC = 0.932; CI: 0.895–0.993), SHBG (AUC = 0.924; CI: 0.87–0.978), and LH/FSH ratios (AUC = 0.906; CI: 0.821–0.965).

Conclusion: For insulin and LH/FSH ratios, FAI, and SHBG seemed the best predictors and markers for insulin resistance, inappropriate gonadotrophin secretion, and hyperandrogenemia, respectively, with high sensitivity and specificity for identifying Bahraini women with and without PCOS.

Keywords: polycystic ovary syndrome, insulin resistance, gonadotrophin, hyperandrogenemia, diagnostic markers

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Introduction

Polycystic ovary syndrome (PCOS) is one of the most common female endocrine disorders affecting women of reproductive age, with a prevalence of about 5%–10% in the general population.[1] It is a heterogeneous disorder of variable mild to severe

disturbances that affect reproductive, endocrine, and metabolic functions.[2] PCOS is characterized by menstrual disturbances due to hirsutism, chronic anovulation or oligo-ovulation, and acne due to excessive androgen production (hyperandrogenemia).[3] In addition, insulin resistance has been reported to be a major contributor in the pathogenesis of PCOS, although the mechanism of this association is poorly understood.[4,5] PCOS patients are often referred to endocrinologist, gynecologist, and dermatologist clinics; however, there is no information regarding the best indices for the diagnosis of insulin resistance, hyperandrogenemia, or inappropriate gonadotrophin secretion in Bahraini women with PCOS. Diagnosis and treatment of these conditions in PCOS women are costly and place a socioeconomic burden on government-supported health systems around the world.[6]

Obesity, particularly the abdominal phenotype, is one of the most often reported clinical features in PCOS women and about 50% of PCOS women are reported to be either overweight or obese. Indeed, it has been suggested that losing 5% body weight will result in an improvement in the regularity of the menstrual cycle and the correction of hirsutism in women with PCOS.[7] The association of obesity with the pathogenesis of PCOS has been extensively reported in the literature and there are more than 50 genes reported to be involved in the regulation of ovarian steroidogenesis, which may contribute to obesity and insulin/glucose homeostasis in PCOS women.[7–9]

There are conflicting reports regarding the best predictors of insulin resistance, hyperandrogenemia, and inappropriate gonadotrophin secretion in PCOS women in different populations. To find the best markers for insulin resistance in PCOS women, insulin, homeostasis model assessment (HOMA), and insulin/glucose ratio (IGR) have been investigated in a large number of studies.[10–13] In PCOS women, hyperandrogenemia and inappropriate gonadotrophin secretion can be assessed by measuring testosterone, sex hormone-binding capacity (SHBG), and the free androgen index (FAI), as well as luteinizing hormone (LH), follicle-stimulating hormone (FSH), and LH/FSH ratios.[14–23] It has been suggested that hormonal abnormalities such as hyperandrogenemia and inappropriate gonadotrophin secretion in PCOS women are not only related to the reproductive system but may also be associated with the features of metabolic syndrome and insulin resistance.[24] In classical PCOS, 30%–90% of patients have inappropriate gonadotrophin secretion, with a higher LH/FSH ratio (2–3:1) than normal women in the follicular phase.[25]

In this case-control study, the best indices for insulin resistance that discriminate between women with and without PCOS – hyperandrogenemia and inappropriate gonadotrophin secretion – were investigated.

Subjects and methods

Participants

Fifty PCOS women aged 16–38 years enrolled in the study after being recruited from the health centers in the Salmania Medical Complex, at the main hospital in Manama, Kingdom of Bahrain, from February to October 2008.

Inclusion criteria for PCOS cases were based on the Rotterdam 2003 criteria.[26] For diagnosis of PCOS, at least two of the following symptoms needed to be observed: oligoovulation or anovulation (usually manifested as oligomenorrhea or amenorrhea); elevated levels of circulating androgens (hyperandrogenemia) or their clinical manifestations (hyperandrogenemia); and polycystic ovaries, as defined by ultrasonography (ultrasonographic criteria: mean of 12 follicle numbers per ovary of both ovaries or ovarian volume of 10 mL, 0.5 × length × width × thickness).[26] Inclusion criteria for controls were a normal menstrual cycle with no history of insulin resistance, hyperandrogenemia, inappropriate gonadotrophin secretion, hirsutism, ovarian failure, or any other endocrine or major organ disorders. In this study, of the 65 control subjects, 15 were excluded because nine had insulin resistance, two had ovarian failure, two had hyperprolactinemia, and two were in the luteal phase. Hirsutism was assessed using modified Ferriman–Gallwey scoring, in which a score > 7 indicates hirsutism.[27] Fasting blood samples were collected in the follicular phase (cycle days 2–8) in all participants.

This study was approved by the Research and Ethics Committee of the College of Medicine and Medical Sciences, AGU, Bahrain, and written informed consent was obtained from all patients and controls.

Analytical methods

Serum insulin was measured by microparticle enzyme immunoassay and plasma glucose was measured using the hexokinase and glucose-6-phosphate dehydrogenase method. HOMA was calculated by multiplying fasting blood glucose (mmol/L) with fasting insulin (μU/mL) and dividing by 22.5.

Serum LH, FSH, total testosterone, and estrogen were determined by electrochemiluminescence immunoassay. SHBG was measured by noncompetitive immunoassays. Serum insulin was measured by microparticle enzyme immunoassay.

The FAI was calculated by dividing total testosterone by SHBG and multiplying by 100.

Statistical analysis

The normality of distribution of all the variables was assessed using the Kolmogorov–Smirnov test and any positively or negatively skewed variables were logarithmically transformed to reduce kurtosis before geometric means were calculated. Subsequently, the logarithmic transformations were used in further statistical analysis.

Logistic regression analysis was used to determine the association of obesity, insulin resistance, hyperandrogenemia, and inappropriate gonadotrophin secretion with PCOS. Multiple logistic regression analysis was used to determine the association of PCOS with hyperinsulinemia, adjusted for obesity, hyperandrogenemia, and LH/FSH ratios. It was also used to determine the association of hyperandrogenemia with PCOS, adjusted for obesity and hyperinsulinemia. Receiver operating characteristic (ROC) analysis was used to determine the best predictors for insulin resistance, hyperandrogenemia, and inappropriate gonadotrophin secretion in PCOS women. ROC curves were constructed by plotting the sensitivity (true-positive) on the ordinate as a function of the complement of specificity (false-positive) for all possible cutoff values of the diagnostic tests. Greater deviation toward the left upper corner of the curve indicated better detection of PCOS.

All reported *P* values were two tailed and *P* values <0.05 were considered statistically significant. The statistical analyses were performed using SPSS software (v 19; IBM Corp, Armonk, NY).

Results

The biometric and biochemical parameters determined in PCOS and healthy control women are illustrated in Table 1.

Table 1 Anthropometry and biochemical characteristics of PCOS patients and controls

Characteristics	Patients (n = 50)	Controls (n = 50)	*P* value
Age (years)	27.7 ± 5.3	28.3 ± 6.4	0.597
BMI (kg/m²)	34.1 ± 7.2	27.0 ± 6.2	<0.001
FSH (IU/L)	5.0 ± 1.2	6.0 ± 1.5	<0.001
LH (IU/L)*	6.20 ± 5.6	5.27 ± 2.4	0.130
LH/FSH Ratio	1.5 ± 0.82	0.97 ± 0.34	<0.001
E2 (pmol/L)*	212.1 ± 137	182.9 ± 98	0.139
Testosterone (nmol/L)*	1.42 ± 1.32	0.95 ± 0.75	0.004
SHBG (nmol/L)*	20.4 ± 17.1	35.53 ± 23.1	<0.001
FAI*	7.38 ± 3.1	2.77 ± 1.7	<0.001
Glucose (mmol/L)	5.1 ± 0.71	4.8 ± 0.45	0.017
Insulin (μU/mL)*	15.0 ± 3.0	6.5 ± 1.7	<0.001
HOMA-IR*	3.3 ± 1.4	1.4 ± 0.9	<0.001
IGR	4.54 ± 1.9	1.41 ± 0.50	<0.001

Note: *Data presented as geometric mean ± standard deviation.
Abbreviations: BMI, body mass index; E2, estradiol; FAI, free androgen index; FSH, follicle-stimulating hormone; HOMA-IR (Insulin Resistance), homeostasis model assessment-Insulin Resistance; IGR, insulin glucose ratio; LH, luteinizing hormone; SHBG, sex hormone-binding capacity.

Body mass index (BMI), HOMA, insulin, glucose, estradiol (E2), testosterone, estrogen, FAI, and LH/FSH ratios were significantly higher in women with PCOS than in controls, whereas FSH and SHBG were significantly lower in the cases than in the controls. Multivariate logistic regression analyses showed that high BMI, HOMA, insulin, LH/FSH ratios, testosterone, FAI, low FSH, and SHBG were significantly associated with PCOS (Table 2). However, when adjusted for high insulin, HOMA and FAI were independently associated with PCOS. Furthermore, hyperinsulinemia was determined to be independently associated with

Table 2 Associations of PCOS with obesity, insulin resistance, hyperandrogenemia, and inappropriate gonadotrophin secretion, determined by multiple logistic regression analyses

Variables	Crude OR (95% CI)	*P*	Adjusted for obesity OR (95% CI)	*P*	Adjusted for obesity and hyperinsulinemia OR (95% CI)	*P*	Adjusted for obesity, hyperandrogenemia and LH/FSH ratios OR (95% CI)	*P*
BMI	1.2 (1.1–3)	<0.0001						
LH	1.1 (3.7–18.5)	0.001	1.3 (1.1–1.3)	0.003	1.5 (0.9–6.2)	0.06		
FSH	1.7 (1.4–2.9)	0.001	2.6 (1.4–5.7)	0.01	0.66 (0.83–0.57)	0.31		
LH/FSH	5.0 (1.4–4.2)	0.001	4.5 (1.1–24.2)	0.03	5.4 (1.2–23.0)	0.03		
Testosterone	2.4 (0.31–1.91)	0.002	2.2 (1.1–4.0)	0.01	1.3 (0.25–6.6)	0.765		
SHBG	1.1 (1.0–1.1)	0.0001	1.4 (1.4–3.1)	0.01	1.2 (1.2–3.4)	0.03		
FAI	1.3 (1.1–1.5)	0.001	1.2 (1.1–1.4)	0.01	1.1 (1.0–2.7)	0.02		
E2	1.0 (0.99–1.1)	0.157	1.0 (0.99–1.0)	0.628	1.0 (0.99–1.0)	0.509		
HOMA	1.6 (2.8–25.6)	0.001	1.3 (2.4–42.1)	0.02			0.89 (2.9–760)	0.135
Insulin	2.3 (1.5–3.4)	0.0001	2.1 (1.2–3.8)	0.001			2.6 (1.3–5.2)	0.008
IGR	3.5 (3.7–26.4)	0.001	3.1 (4.9–32.6)	0.004			0.62 (0.87–45.0)	0.234

Abbreviations: BMI, body mass index; CI, confidence interval; E2, estradiol; FAI, free androgen index; FSH, follicle-stimulating hormone; HOMA, homeostasis model assessment; IGR, insulin glucose ratio; LH, luteinizing hormone; OR, odds ratio; SHBG, sex hormone-binding capacity.

PCOS when adjusted for obesity, hyperandrogenemia, and LH/FSH ratios.

ROC analyses using area under the curve revealed that the best predictive markers for insulin resistance, hyperandrogenemia, and inappropriate gonadotrophin in PCOS women were determined to be insulin, FAI, SHBG and LH/FSH ratios, respectively (Table 3).

Discussion

In this study, various predictors and discriminators for diagnosis of PCOS in Bahraini women were identified using multiple logistic regression and ROC analyses. The use of ROC curves to investigate the predictive power of endocrine parameters in the diagnosis of PCOS has been reported in a large number of studies.[14–23] The independent association of obesity and hyperinsulinemia with PCOS women observed in this study has also been extensively reported in different populations. In a recent study reported in this group of PCOS Bahraini women, insulin, leptin/adiponectin, and adiponectin/leptin ratios were reported to be the best marker to distinguish women without PCOS from those with PCOS.[28] However, consistent with recent reported studies,[10–13,29] among the markers indicating insulin resistance (insulin, HOMA, and IGR), insulin was independently associated with PCOS and it was the best marker for differentiating women with and without PCOS. This indicates that women with PCOS need to be evaluated for other related conditions associated with hyperinsulinemia, including type 2 diabetes mellitus, hypertension, dislipidemia, and atherosclerosis.[10] Medications that reduce circulating insulin have been suggested as effective therapies for PCOS and sufficient evidence has accumulated to justify the clinical use of insulin-sensitizing agents in the management of women with PCOS.[11]

Table 3 Analysis of receiver operating characteristics for the best predictors of PCOS

Test-result variables	AUC ROC	95% CI
Insulin	0.944	0.887–0.989
FAI	0.932	0.895–0.993
SHBG	0.924	0.871–0.978
LH/FSH	0.906	0.821–0.965
IGR	0.786	0.698–0.864
HOMA	0.693	0.582–0.805
Testosterone	0.666	0.553–0.779
FSH	0.674	0.564–0.784
FBS	0.615	0.506–0.725
E2	0.603	0.481–0.724
LH	0.593	0.472–0.714

Abbreviations: AUC, area under the curve; BMI, body mass index; CI, confidence interval; E2, estradiol; FAI, free androgen index; FBS, fasting blood sugar; FSH, follicle-stimulating hormone; HOMA, homeostasis model assessment; IGR, insulin glucose ratio; LH, luteinizing hormone; OR, odds ratio; ROC, receiver operating curve; SHBG, sex hormone-binding capacity.

One of the main aims of this study was to investigate whether total testosterone, estrogen, SHBG, and FAI were appropriate markers for assessing hyperandrogenemia in patients with PCOS. In this study, low SHBG was independently associated with PCOS when adjusted for obesity and hyperinsulinemia. In addition, ROC analysis showed that SHBG is one of the best markers of hyperandrogenemia and thus for the diagnosis of PCOS women. These results are consistent with recent studies suggesting the discriminative power of SHBG in PCOS women.[15,19] Hyperinsulinemic patients with PCOS were demonstrated to have decreased SHBG at the hepatic level. It has been suggested that a high insulin level in circulation may lead to the reduction of SHBG and that this consequently causes an increase in the concentration of free androgen in circulation.[19]

Androgen status in women has been assessed by FAI, which is also a measure for assessing the availability of circulating testosterone in females with hirsutism. In this study, FAI was also independently associated with PCOS and ROC analysis showed that it is one of the best markers for hyperandrogenemia in PCOS women, which is consistent with the results reported in previous studies of different populations.[14,18–21]

In this study, the independent association of high LH/FSH ratios with PCOS was observed when controlled for obesity and hyperinsulinemia. Several studies have suggested that the increased LH and LH/FSH ratios in PCOS women are not related to BMI or adiposity, and LH-pulse amplitude has been reported to be higher in lean PCOS women compared with obese PCOS women.[30] In this group of PCOS women, ROC analysis also showed that the predictive cover of LH/FSH ratio was better than that of LH/FSH ratio for diagnosing inappropriate gonadotrophin secretion in PCOS, a finding consistent those reported for some other studies of different populations.[14,17,18] The mechanisms of high LH/FSH ratios in PCOS women are not clear; however, it has been suggested that they might be related to primary defects in the hypothalamic and pituitary functions in PCOS women.[30]

One of the limitations of this study is that BMI was significantly higher in PCOS women than in the controls; therefore, it can be argued that due to the lack of matching

BMIs in the controls and cases, the hyperinsulinemia observed in this group of PCOS women could be due to obesity. Consequently, this study was inconclusive as to whether hyperinsulinemia can be considered a marker for distinguishing women with PCOS from those without it. However, results from this study's multiple logistic regression analysis showed that hyperinsulinemia was independently and significantly associated with PCOS when adjusted for obesity.

Conclusion

High insulin, FAI, LH/FSH ratios, and low SHBG can discriminate between women with and without PCOS, with both high sensitivity and high specificity. This is the first study to describe the best indices of insulin resistance, hyperandrogenemia, and inappropriate gonadotrophin secretion in Bahraini women with PCOS. Larger studies are required to investigate the significance of these findings for the routine clinical work-up of PCOS women. However, the results from this study may serve as a valuable adjunct to the diagnostic criteria used in scientific studies of this heterogeneous syndrome.

Disclosure

The authors report no conflicts of interest in this work.

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