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Assessment of the Role of the Anti-Mullerian Hormone, Luteinizing Hormone/Follicle Stimulating Hormone Ratio in the Diagnosis of Polycystic Ovary Syndrome in Sudanese Women

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Abstract

BACKGROUND: The diagnosis of polycystic ovary syndrome (PCOS) is not an easy procedure, as the signs and symptoms are heterogeneous and of undefined aetiology.

AIM: This study is aimed to evaluate serum anti-Mullerian hormone (AMH) level and luteinizing hormone (LH)/folic stimulating hormone (FSH) ratio in women with PCOS in Sudan and to assess the diagnostic efficiency for the diagnosis of PCOS.

METHODS: In a case-control study, Serum AMH, LH, FSH was measured in the early follicular phase from Sudanese patients (N = 230) with PCOS and100 controls. The LH/FSH ratio was calculated, and its diagnostic power was evaluated by receiver operating characteristic curves.

RESULTS: The means of serum AMH, serum LH level and LH/FSH ratio of the test, were significantly increased in the test group compared to the control group (P-value < 0.000). The AMH sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) were found to be 83%, 99%, 99%, and 72% respectively. Serum AMH was considered adequate measures for the diagnosis of PCOS; its level showed an area under the ROC curve of 0.98 (95% confidence, P-value < 0.000). The best compromise between 98% specificity and 90% sensitivity was obtained with a cut-off value of 3.3 ng/mL for PCOS diagnosis. There was no correlation between age, body mass index (BMI) and AMH level in the test group.

CONCLUSIONS: The Serum AMH level and LH/FSH ratio were higher in patients than in control. However AMH level has better discriminative power and good diagnostic potency for the diagnosis of the PCOS among Sudanese women.

Introduction

Polycystic ovary syndrome (PCOS) is one of the most common female endocrine disorders that affects up to 10% of women worldwide. PCOS is accompanied by an imbalance of female sex hormones and increased androgen production leading to infrequent or prolonged menstrual periods, excess hair growth, acne and obesity [1]. The exact aetiology is not yet known. However many risk factors have been demonstrated such as genetic and epigenetic or environmental factor leading to intraovarian hyperandrogenism [1]. Lack of ovulation in PCOS results in continuous high levels of estrogen and insufficient progesterone which lead to increased serum luteinizing hormone (LH) levels as well as changes in Anti-Mullerian Hormone (AMH) secretion. The higher the antral follicles count, the higher AMH levels, and women with PCOS typically have high numbers of antral follicles [2].

The diagnosis of PCOS is not easy as signs and symptoms are heterogeneous, the lack of well-defined diagnostic criteria makes identification of this common disease confusing to many clinicians. The guideline made by the American Society of Reproductive Medicine (ASRM) and European Society for Human Reproduction Medicine (ESHR) delivered in 2003 (Rotterdam criteria) determined the diagnostic criteria for PCOS which include: irregular menstrual cycle, androgen excess symptoms, and ovary ultrasound. A diagnosis of PCOS requires the

presence of at least two of the three features after other androgen-excess disorders have been excluded [3].

There are a considerable number of reasons to believe that many women with PCOS have been missed diagnosed, because of the extensive heterogeneity in the clinical presentation of PCOS. Furthermore, with the rising epidemic of obesity, the prevalence of PCOS may increase, as obesity potentially worsens the endocrine and metabolic profile of PCOS [4]. To the best of our knowledge, this is the first study to assess the diagnostic efficiency of AMH level and LH/FSH ratio for the diagnosis of PCOS among Sudanese women.

Material and Methods

A total of 230 Sudanese women with PCOS diagnosed based on Rotterdam criteria were enrolled in this study. The control subjects were 100 women. The study was conducted at Dr Elsir Abu Alhassan Fertility Center, Khartoum, Sudan. Patients with a history of menstrual disturbances (hypothyroidism, congenital adrenal hyperplasia, Cushing's syndrome, hyperprolactinemia and hirsutism were excluded. Both groups were matched regarding age and BMI (BMI was given by = Weight(kg)/Height(m^2)). The study was ethically approved by the ethical approval committee for medical research of Alzaim Alazhari University, and informed consent was collected before the beginning of the study.

Blood sampling was performed in the early follicular phase, between day 2 and 5 after the last menstrual period both in PCOS patients and controls. Serum AMH levels were assessed by ELISA (Enzyme-Linked Immune Sorbet Assay) using BECKMAN COULTER Kit reagents. Serum LH and FSH were measured by mini VIDAS technology using BIOMERIEUX Kits reagent. The assay principle combines an enzyme immunoassay sandwich method with final fluorescent detection (ELFA). The method and the steps were followed as per company instructions.

Data were analysed using the statistical package for social sciences (SPSS ver.17) (IBM Corp., Armonk, NY, USA). Comparison of means of AMH, FSH, LH hormone and LH/FSH ratio was conducted using a t-test. Associations between hormonal levels between control and study groups were measured by using the Chi-square test. Correlation between age, BMI and AMH were tested using person correlation. The test was considered significant when the *P* value is less than 0.05. Receiver operating characteristic (ROC) curves were constructed to examine the diagnostic test performance, *i.e.* its capacity to discriminate between controls and patients with PCOS. Sensitivity (y-axis) against [1-specificity (x-axis)] was plotted at each threshold level, and the area under the curve (AUC) was computed by the nonparametric Wilcoxon test. AUC represents the probability of correctly identifying controls and patients with PCOS. A value of 0.5 means that the test result is no better than chance.

Results

The mean age of the test group and control group was (28.17 ± 5.12), (28.98 ± 5.52) respectively. BMI means of test and control was (25.72 ± 4.86), (25.16 ± 5.57) respectively.

The mean of serum AMH, LH level and serum LH/FSH ratio of study subjects was significantly increased ($P < 0.000$) compared to the control group. There was the insignificant difference ($P = 0.06$) between the mean of serum FSH level of test group and control group (6.30 ± 3.64) compared to (7.23 ± 5.24) respectively, as shown in Table1.

Table 1: Comparison between mean of AMH, LH, FSH, LH/FSH ratio for test and control group

Variable	Test group (n = 230)	Control group (n = 100)	*P*-value
AMH ng/ml	9.61 ± 5.82 (3.79-15.43)	1.80 ± 0.66 (1.14-2.46)	0.000
LH mIU/L	10.55 ± 7.82 (2.73-18.37)	7.77 ± 6.79 (0.98-14.56)	0.000
FSH mIU/L	6.30 ± 3.64 (2.66-9.94)	7.23 ± 5.24 (1.99-12.47)	0.06
LH/FSH ratio	1.80 ± 1.16	1.12 ± 1.22	0.000

The table shows the mean±standard deviation, range in brackets (Min-Max) and p-value. A t-test was used for comparison; *P* value less than 0.05 considered significant.

Table 2 showed the diagnostic power of AMH about sensitivity and specificity when 4.0 ng/ml was used as cut off point. The sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) were observed at 83%, 99%, 99% and 72% respectively. The diagnostic power of the LH/FSH ratio about sensitivity and specificity when 1:1 was used as cut off point were observed at 72%, 76%, 84% and 62% respectively.

Table 2: Shows the sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) of AMH and LH/FSH ratio to diagnose of PCOS

	AMH	LH/FSH
Sensitivity	0.834	0.729
Specificity	0.990	0.765
Positive Predictive Value (PPV)	0.995	0.843
Negative Predictive Value (NPV)	0.727	0.620
Likelihood Ratio+ (LR+)	85.07	2.399
Likelihood Ratio- (LR-)	5.967	2.571

The AUC for the different hormone serum measurements is shown in Table 3. The AUC for AMH was 0.98 (95% confidence interval, $P < 0.000$), the compromise between specificity and sensitivity was (96% and 92%), (96% and 91%), (98% and 90%) was

obtained with threshold values of AMH 3.1, 3.2, 3.3ng/ml respectively.

Table 3: AUC of serum hormone concentrations for PCOS detection in women

Hormone	AUC (95% CI) , *P* < 0.000	Compromise between specificity and sensitivity	Cut-off values
AMH	0.98	96%, 92% 96% and 91% 98% and 90%	3.1 ng/ml 3.2 ng/ml 3.3 ng/ml
LH/FSH ratio	0.74	76%, 72%	1:2

The AUC for LH/FSH ratio was 0.74 (95% confidence interval *P* < 0.000) and the best compromise between specificity and sensitivity (76%, 72%), was obtained with threshold values of LH/FSH ratio 1:1 as shown in Figure 1.

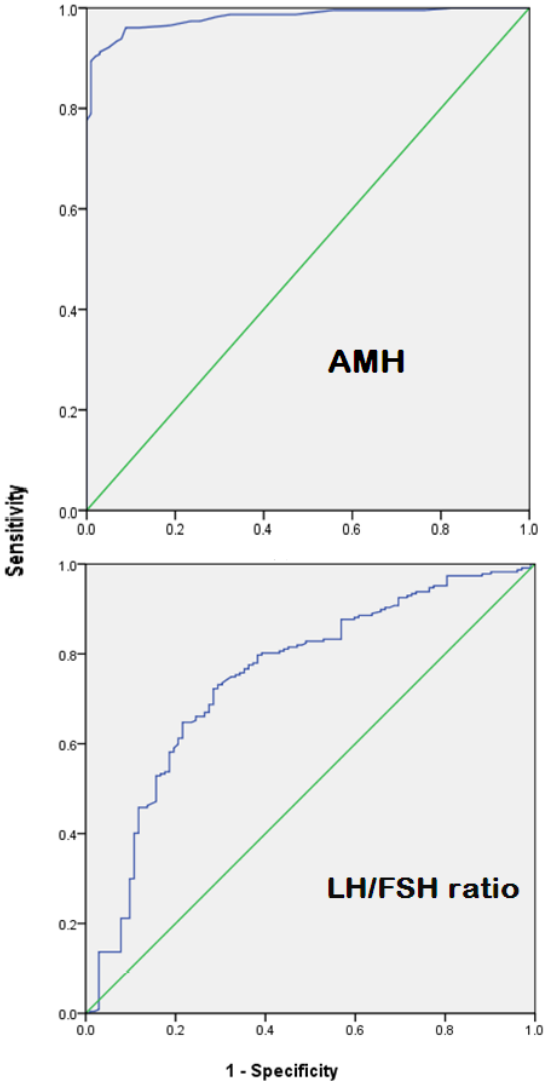

Figure 1: ROC curves for the detection of PCOS AMH and LH/FSH ratio

The results also showed that there was no correlation (*P* = 0.488) between age and AMH level in the test group (r = -0.046). Furthermore, there was no correlation (*P* = 0.492) between BMI and AMH level in the test group (r = 0.039), Figure 2 and 3 respectively.

Discussion

The present study is the first study to our knowledge to assess the role of the AMH level and LH/FSH ratio in the diagnosis of PCOS among Sudanese women.

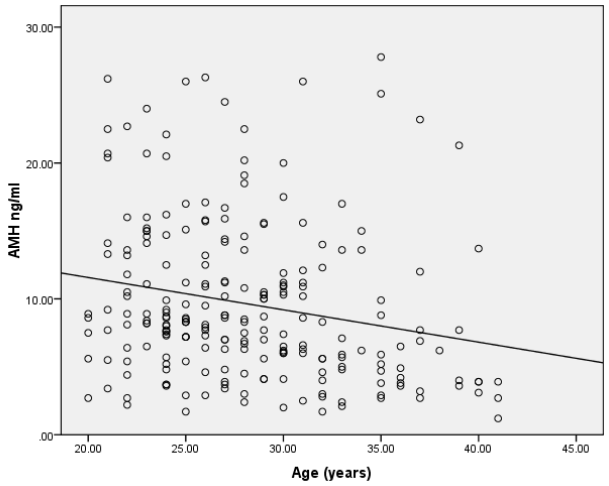

Figure 2: A scatter plot shows the correlation between the level of AMH and age in test (P-value = 0.488)

The significant increase of AMH level in PCOS women compared to healthy women in this study is by the study by Bungum L *et al.,* [5], in Sweden, they found a significant difference in mean AMH levels between the groups, the highest values being seen in the PCOS group. The result also agrees with results obtained by Pawelczak M *et al.,* [6], they noticed a positive relationship between serum AMH and ovarian volume as well as peripheral follicular distribution in adolescents with PCOS.

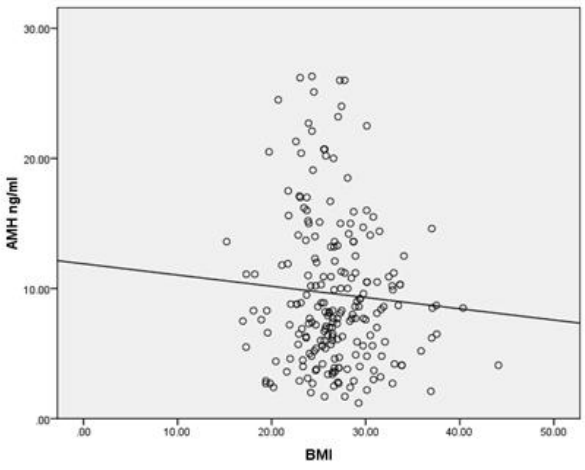

Figure 3: A scatter plot shows the correlation between the level of AMH and BMI in the test group (P-value = 0.492)

In the present study, the level of the LH and LH/FSH ratio was significantly higher in patients than in control group. In contrast, there was no significant

difference level of FSH level. This may be due to increased androgen level in PCOS women, which leads to abnormalities of ovaries hormone that contributing to a relative suppression of FSH production. Our finding is in harmony with the results reported by Siebert TI *et al.,* [7]. Sahmay S *et al.* also found the significant differences between the mean serum, FSH, LH and LH/FSH ratio of PCOS women and control groups in their study [8]. The specificity and NPV of AMH and LH/FSH ratio to diagnosis PCOS in the current study agrees with the results reported by Wetzka *et al.,* [9], AMH showed 90% specificity with 71.2% sensitivity for the diagnosis of PCOS. In the existing study Serum AMH, and LH/FSH ratio was considered adequate measures for the diagnosis of PCOS, they AURC levels showed 0.98 and 0.74 (95% confidence, *P*=0.000) respectively. Similarly, Pigny P *et al.* conveyed that AMH measurement has been found to offer a relatively high specificity and sensitivity (92 and 67%, respectively) for PCOS [10]. In contrast to our result, recently Cengiz H *et al.* did not find AMH to be a reliable predictor for the presence of PCOS [11]. Furthermore, Li et *a.,l* reported that the serum AMH measurements presented a relatively poor diagnostic power, with a sensitivity of 61.7% and a specificity of 70%. They attributed the causes to the lower prevalence of hyperandrogenism, obesity, and insulin resistance to racial differences in their study [12].

The cut-off value of AMH in this study (3.3 ng/ml) is compatible with the previous result obtained by Sahmay S *et al.,* [8] the large sample size was the common denominator between both studies, unlike cut-off values from other studies with small sample size [12] [13] [14]. The lack of correlation between age, BMI and AMH level observed in the current study is agreed again with result gotten by Sahmay S *et al.*, they concluded there was no relation of age and BMI between PCOS and non-PCOS subjects in their study [8].

In conclusion, AMH was shown to be a useful parameter for the diagnosis of PCOS in this study. Our results suggested that AMH is primarily a marker of ovarian function and not associated with other organ pathologies such as adrenal gland dysfunction or metabolic disturbances.

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