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Evaluation of Anti-Mullerian Hormone Levels as a Diagnostic Marker of Polycystic Ovarian Syndrome: A Case-Control Study in Reproductive Age Group Women

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ABSTRACT
Polycystic ovarian syndrome (PCOS) is the most common endocrine disorder affecting women of reproductive age. Presently the diagnosis of PCOS is based on clinical and ultrasound findings. But these findings are subjective. So, there is a need of a biochemical marker to diagnose PCOS accurately which doesn't get affected by the ovarian cycle. Anti-mullerian hormone (AMH) secreted by ovaries. In this project we studied the Anti-mullerian hormone levels in PCOS patients. To estimate the Anti-mullerian hormone levels in women with PCOS and establish the role of AMH as a diagnostic marker of PCOS. It was a case control study carried out in tertiary care centre. AMH levels were estimated by electrochemiluminescence method. Mean AMH level in the cases was 36.47±9.29 pmol L and the controls was 20.07±4.63 pmol L the difference was statistically significant ($p<0.001$). As values of AMH correlate well with patients diagnosed with the Polycystic ovarian morphology in USG and other biochemical parameters in PCOS patients, it can be concluded that elevated AMH is indicative of PCOS and can be a diagnostic marker of PCOS. It can be used along with other biochemical parameters. Polycystic ovarian syndrome, anti-mullerian hormone, diagnostic marker.

INTRODUCTION

Polycystic ovarian syndrome is a multifactorial spectrum of disorders affecting endocrine, reproductive as well as metabolic functions in a female. It is the most common endocrine disorder in women of a reproductive age[1]. It affects 5-10% of women in the reproductive age[2]. PCOS is considered as a heterogeneous disorder of uncertain cause. There have been various reports that it is due to a combination of genetic and environmental factors[3,4]. The risk factors for PCOS include obesity a lack of physical exercise and a family history of the condition. There is also some evidence that exposure to higher than typical levels of androgens and the anti-Müllerian hormone (AMH) in utero increases the risk of developing PCOS in later life[5]. The Rotterdam consensus guidelines for the diagnosis of polycystic ovarian syndrome consists three criteria: 1 Oligo or anovulation, 2 Hyperandrogenism (clinical/biochemical) 3 Evidence of polycystic ovaries on transvaginal ultrasound[6].

The diagnosis of PCOS is made if two of these three criteria are present. The diagnosis of PCOS remains a challenge for the clinician due to a wide spectrum of clinical presentation. Cysts may be detectable by ultrasound. However, transvaginal ultrasound criteria are often not feasible to be used in young women and adolescents. Also the variability in the interpretation is very common. Additionally, there are other conditions that produce similar symptoms these include adrenal hyperplasia, hypothyroidism and high blood levels of prolactin. As a result, various cases of PCOS may be missed or get misdiagnosed and hence are not appropriately treated [6].

Due to the limitation of diagnostic modalities, there arises a need of an objective biochemical marker which can diagnose PCOS accurately. Recently, studies are being conducted to evaluate the association between elevation in AMH levels and the clinical features of PCOS. Therefore, we conducted this case-control study to estimate the level of AMH in women with PCOS and to establish the role of AMH as a diagnostic marker of PCOS.

Aim and objectives: To estimate the level of Anti-mullerian hormone in women with PCOS and to establish the role of AMH as a diagnostic marker of PCOS.

MATERIALS AND METHODS

Study design: Case control study

Study population: Women attending Obstetrics and Gynaecology OPD and diagnosed with PCOS were screened as per the following eligibility criteria for recruitment.

Inclusion criteria

For cases:

- Newly diagnosed cases of PCOS according to Rotterdam criteria
- Age 20-40 years
- Willing to provide written informed consent

For controls:

- No signs or symptoms or USG findings suggestive of PCOS
- Age 20-40 years
- Willing to provide written informed consent

Exclusion Criteria (for both cases and controls):

- Pregnancy
- Lactation
- Hypothyroidism
- Hyperprolactinemia
- Any malignancy of uterus

Study procedure: The females found to be eligible for the study as per the above-mentioned inclusion and exclusion criteria were recruited (110 cases and 110 controls) and written informed consent was obtained from them. A proper history from all participants was taken. Weight and height were measured and BMI was calculated.

Three mL of blood sample was obtained from each participant in plain vials, centrifuged at 3000 rpm for 10 minutes and then stored at -20°C till processing.

Hormonal assay: AMH levels measured on the electrochemiluminescence analyzer of Roche diagnostics COBAS e 411.

RESULTS

After statistical analysis, following results were obtained. We observed that the mean age of the cases was 28.35±4.90 years and the controls was 28.18±4.86 years. There was no statistically significant difference between the means of the ages of the two groups, as evaluated by the independent t-test ($p = 0.986$) (Table 1). Mean height of the cases was 156.94±8.82 cm and the controls was 158.01±8.34 cm. There was no statistically significant difference between the means of the heights of the two groups, as evaluated by the independent t-test ($p = 0.879$) (Table 1). Mean weight of the cases was 71.25±13.88 kg and the controls was 51.45±5.30 kg. We observed a statistically significant difference between the means of the weights of the two groups, as evaluated by the independent t-test ($p<0.001$) indicating that cases with PCOS had body weights significantly greater than

Table 1: Comparison of demographics among the cases and controls

Parameter	Cases	Controls	p-value of comparison
Age (years)	28.35±4.90	28.18±4.86	0.986
Height (cm)	156.94±8.82	158.01±8.34	0.879
Weight (kg)	71.25±13.88	51.45±5.30	< 0.001*
BMI (kg m^2)	29.05±5.99	20.78±3.09	< 0.001*

(p<0.001 indicating a statistically significant difference between the cases and controls)

Table 2: Comparison of AMH values among the cases and controls

Parameter	Cases	Controls	p-value
Sample size	110	110	-
AMH levels (pmol/L)	36.47 ± 9.29	20.07 ± 4.63	< 0.001*

(p<0.001 indicating a statistically significant difference between the cases and controls)

Table 3: Categorization of AMH levels (elevated and non-elevated) in the cases and controls using cut off value

	AMH elevated n (%)	AMH not elevated n (%)	Total
Cases	92 (83.64)	18 (16.36)	110
Controls	11 (10)	99 (90)	110
Total	103 (46.82)	117 (53.18)	220

controls without the condition (Table 1). Mean BMI of the cases was 29.05±5.99 kg m^2 and the controls was 20.78±3.09 kg m^2. We observed a statistically significant difference between the means of the BMIs of the two groups, as evaluated by the independent t-test (p<0.001) indicating that cases with PCOS had body mass indices significantly greater than controls without the condition (Table 1). We compared the AMH levels between the cases and the controls by the independent t-test. We observed that the mean AMH level in the cases was 36.47±9.29 pmol L and the controls was 20.07±4.63 pmol L. This difference in the AMH means between the two groups was found to be statistically significant (p<0.001) (Table 2).

We also analyzed the number of samples in which the AMH values were elevated in the two groups, considering the normal range of AMH to be 10-25 pmol L. We observed that 92 out of 110 cases (83.64%) showed elevated AMH levels (≥25 pmol L) while the corresponding figure for controls was 11 out of 110 (10%). When evaluated by the Chi-squared test, this difference between the cases and controls was found to be statistically significant (p<0.001) (Table 3).

DISCUSSIONS

In this case-control study, we enrolled 110 cases (with PCOS) and 110 controls (without PCOS) to evaluate whether AMH serves as a marker for diagnosis of PCOS. For the cases and controls, there was no statistically significant difference in demographic characteristics like age and height (Table 1). However, there was statistically significant difference in the mean body weight and BMI of the cases and the controls. It indicated that cases with PCOS had body weights and BMI significantly greater than controls (Table 1).

Our findings are similar to those by Abbara *et al*., who also observed that there was no significant difference in age between cases and controls (p = 0.34) and that women with oligomenorrhea amenorrhea had higher BMI values than women with normal menstrual cycles (p = 0.02)[7]. In another study the authors have similarly reported similar age distribution among cases and controls (27.07±4.49 years vs 28.68±4.98 years p = 0.072). However, in this study the authors have also observed similar BMI values among cases and controls (29.02±6.53 kg m^2 v s 28.76±3.41 kg m^2 p = 0.389)[8]. We observed that difference in the AMH means between the two groups was statistically significant (p<0.001) (Table 2) which indicates that cases with PCOS had significantly elevated AMH levels as compared with the controls. We also observed that 92 out of 110 cases (83.64%) showed elevated AMH levels (≥25 pmol L) while the corresponding figure for controls was 11 out of 110 (10%). This difference between the cases and controls was also found to be statistically significant (p<0.001) (Table 3). Our findings support those observed with previously published literature. In their case control study, Zadehmodarres *et al*. have reported the mean of AMH levels in the cases (n = 60) to be significantly greater than the controls (n = 57) (7.14±6.53 ng mL vs 3.34±3.45 ng mL p = 0.001)[8].

Laven *et al*. in a larger cohort of PCOS (n = 128) women also replicated the same findings and suggested significantly higher AMH value (7.6 vs. 2.1 ng mL, p<0.001) compared to control (n = 41)[9]. This finding is also similar to that observed in our study.

Chao *et al*. recruited a group of 59 healthy, fertile, regularly cycling women and a second group of seven patients with premature ovarian failure or menopause and a third group of 45 PCOS patients in Taiwan. They also reported that AMH levels in PCOS patients were found to be significantly higher than those measured in healthy fertile controls[10]. This finding is also similar to what we have observed in our study. Thus, our study is in agreement with previously published literature on the elevation of AMH levels in PCOS patients compared with controls.

The elevation of AMH In PCOS can be deduced by the fact that AMH is secreted by granulosa cells of the preantral and small antral follicles in women, i.e. the immature follicles[11]. The expression of AMH tends to be greatest in the recruitment stage of folliculogenesis, in the preantral and small antral follicles. This expression diminishes as follicles develop and enter selection stage, upon which FSH expression increases [12]. Hence, as the number of immature follicles increases, which is observed in PCOS the expression of AMH also increases. The role of AMH in PCOS is yet to be explored fully. However the production of AMH seems to regulate folliculogenesis by inhibiting recruitment of follicles from the resting pool in order to select for the dominant follicle, after which the production of AMH diminishes[13]. Hence, an increase

in level of AMH may inhibit the maturation of follicles, leading to a greater number of immature follicles found in PCOS patients.

Summary: Based on the study conducted by us, we summarize the following. AMH values are significantly higher in patients with PCOS than controls. Patients with PCOS tend to have greater body weight and BMI values than controls.

CONCLUSION

Based on above mentioned findings, it can be suggested that AMH can be used as a marker for diagnosis of PCOS along with other parameters or with USG findings of PCO morphology. The cut off values need to be well defined through further studies. Studies are needed to be carried out in adolescent females for early diagnosis in whom it's difficult to diagnose PCOS due to the variable USG findings.

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