

Association of Serum Anti-Müllerian Hormone and Free Testosterone with Different Phenotypes of Polycystic Ovary Syndrome

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Abstract

Background: Anti-Müllerian hormone (AMH) has lately been connected to polycystic ovary syndrome (PCOS) in a growing body of research, even though hyperandrogenism is one of the primary symptoms of PCOS.

Evaluate the association of serum anti-Müllerian hormone and free testosterone with different phenotypes of polycystic ovary syndrome.

Methods: This is cross-sectional study was carried out at Department of Biochemistry, College of Medicine, University of Baghdad, during the period from November 2023 to March 2024. It included 111 women, 91 of these women, age range (18-40 year) were diagnosed with polycystic ovary syndrome according to 2003 Rotterdam Consensus criteria, and 20 women were apparently healthy women. The PCOS women were sub-grouped into four phenotype groups (A, B, C and D). Investigations included serum measurements of free testosterone and anti-müllerian hormone by using enzyme linked immunosorbent assay (ELISA) technique in all included women.

Results: The results revealed that phenotype A is the predominant one of PCOS, while the B phenotype is the rare one. The mean (\pm SEM) values of free testosterone levels of phenotypes A, B, and C were significantly higher those of phenotype D and controls ($p=0.001$). The mean (\pm SEM) value of serum anti-Müllerian hormone levels was significantly increased in phenotypes A ($p=0.04$) and D ($p=0.01$) than C phenotype.

Conclusions: Phenotype A is the predominant one of PCOS phenotypes and is associated with highest serum AMH, free testosterone and obesity. Both free testosterone and AMH are helpful in differentiation of different phenotypes of PCOS.

Keywords: Anti-Müllerian hormone, Free testosterone, Phenotypes, Polycystic ovary syndrome.

Introduction

The most prevalent endocrine condition affecting women of reproductive age is polycystic ovarian syndrome (PCOS)(1). The quality of life and health of women are impacted by PCOS (2,3). Increase in hormone levels are higher than normal concentrations and these elevated levels can disturb the ovarian system and cause PCOS (4). Menstrual abnormalities, polycystic ovarian morphology, and hyperandrogenism are all

components of the diagnosis (5). Number of phenotypes are associated with the syndrome, such as oligo-ovulation in conjunction with polycystic ovarian morphology, hyperandrogenism with polycystic morphology, ovulatory dysfunction with hyperandrogenism, oligo-ovulation with polycystic ovarian morphology (6–8). One of the main causes of female infertility is polycystic ovarian syndrome (9–14). Since

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women with PCOS vary greatly from one another, it is impossible to pinpoint the precise effects of professional therapy (6). Furthermore, at this time, there are no pertinent particular serological markers to identify subgroups for targeted first treatment (6). Anti-Mullerian hormone (AMH), which is generated by granulosa cells in preantral and small antral ovarian follicles, belongs to the transforming growth factor- β family and may be used as an indicator of ovarian function and reserve (11,15).

Anti-mullerian hormone (AMH) and PCOS are known to be related, according to many research and general agreement, however this association is not yet used as diagnostic criteria. It is not evident how this relates to the PCOS subgroup's variability (6). One significant benefit of AMH as a biomarker is that its level does not significantly change with exogenous estrogen or throughout the stages of the menstrual cycle (15). Numerous investigations have shown elevated levels of AMH, especially in PCOS phenotypes A and D (15,16), and recommended include it in the diagnosis (17,18). Anti-mullerian hormone levels vary throughout PCOS phenotypes, with phenotypic A and D having greater levels than others (19). In cases with PCOS, the ovaries may produce excessive amounts of androgens, which may lead to hyperandrogenism and ovulation dysfunction (19).

Materials and Methods

The cross-sectional study was performed at the Department of Biochemistry, College of Medicine, University of Baghdad, and at Baghdad Teaching Hospital, Medical City, Baghdad, Iraq, during the period from November 2023 to March 2024. The study included 111 women, 91 of them have been previously diagnosed with polycystic ovarian syndrome (PCOS) by Consultant Gynecologist and 20 apparently healthy women as controls. The PCOS women were sub-classified into four groups according to their phenotypic characteristics: A, B, C and D. This study was approved to be carried out from the scientific and ethical committees of

Department of Biochemistry, College of Medicine, University of Baghdad. Ethical approval was also obtained from Baghdad Teaching Hospital, Medical City, Ministry of Health. Oral consent was obtained from all included women to be participate in this study.

Inclusion criteria of women with PCOS included the presence of at least two of the 2003 Rotterdam Consensus criteria and age ranged (18-40 year). In this consensus it was stated that for the diagnosis of PCOS, the patient should have at least two of the three major criteria: (1) Oligo/anovulation (2) hyperandrogenism (clinical or biochemical findings), and (3) and ultrasound evidence of polycystic ovaries (>12 follicles 2-9 mm in diameter or ovarian volume >10 mL in at least one ovary) and other androgen excess disorders should be excluded like congenital adrenal hyperplasia. After ruling out Cushing's disease, congenital adrenal hyperplasia, hyperprolactinemia, and androgen-secreting tumors, the diagnosis of PCOS is made if at least two of these three abnormalities are present (20). Polycystic ovarian morphology (PCOM) and hyperandrogenism (HA) are two characteristics of polycystic ovarian syndrome (21). Based on the Rotterdam criteria, there are four distinct phenotypes associated with this syndrome: Hyperandrogenism + PCO + oligomenorrhea (A), oligomenorrhea + hyperandrogenism (B), PCO + hyperandrogenism (C), and oligomenorrhea + PCO (D) (21).

Exclusion criteria included those women who taking oral contraceptive during blood draw and other diagnoses mimicking PCOS (i.e. prolactinoma, premature ovarian failure, congenital adrenal hyperplasia), thyroid gland dysfunctions, liver disease, kidney disease and cancers.

Five millimeters (ml). from peripheral vein was aspirated from each PCOS and control women, left to clot for 15 minutes, and then centrifuge for 10 minutes at 2500 rpm. The separated serum was stored at -45 °C till the day of measurements. Serum investigation

included measurements of free testosterone and anti-müllerian hormone (AMH) using semiautomatic ELISA Reader Huma, Reader by Human Diagnostics German company, Washer (COMBIWASH) by HUMAN Germany company. The principle of ELISA technique based on the Biotin double antibody sandwich technology to assay the Free testosterone and anti-müllerian hormone (AMH). Add hormones to the wells, which are pre-coated with its monoclonal antibody and then incubate. After that, add anti hormones antibodies labeled with biotin to unite with streptavidin-HRP, which forms immune complex. Remove unbound enzymes after incubation and washing. Add substrate A and B. Then the solution will turn blue and change into yellow with the effect of acid. The shades of solution and concentration of Human Free testosterone or AMH are positively correlated. LH FSH and PRL was measured using Tosoh AI-2000 Automated Immunoassay, Japan using Tosoh AI-2000 Automated Immunoassay, Japan which has principle that the ST AIA-PACK test is a two-site immunoenzymometric assay which is performed entirely in the ST AIA-PACK test cups serum present in the test sample is bound with monoclonal antibody immobilized on a magnetic solid phase and enzyme-labeled monoclonal antibody in the test cups. The magnetic beads are washed to remove unbound enzyme-labeled monoclonal antibody, and they are then incubated with a fluorogenic substrate 4-methylumbelliferyl phosphate (4MUP). The amount of enzyme-labeled monoclonal antibody that binds to the beads is directly proportional to the serum concentration in the test sample. A standard curve is constructed, and unknown sample concentration are calculated using this curve.

Statistical analysis SPSS version 25 software, mean and standard error of mean (SEM) were used to describe the obtained data used for all statistical analysis. ANOVA is used to evaluate the difference in the mean level of numeric data between more than 2 variables. Receiver operator characteristic (ROC) and Area under curve (AUC) was

studied and cutoff value, sensitivity and specificity of the parameters will be obtained in order to differentiate among and between the four phenotypes of PCOS and control women. Pearson correlation regression r was used to evaluate the correlation between numeric data. The significance level was chosen at p of less than 0.05.

Results

The results of study revealed that 42 out of 91 (46%) PCOS women were phenotype A, 3 out of 91 were phenotype B (3.2%), 15 out of 91 (16%) were phenotype C, and 31 out of 91 (34%) were phenotype D.

Table 1 reveals the mean (\pm SEM) values of age and BMI of the studied groups. The mean values of age of Phenotypes A ($p=0.01$), C ($p=0.02$) and D ($p=0.02$) were significantly higher than that of controls. However, there was insignificant differences in the mean value of age among the four phenotypes of PCOS women. The mean values of BMI of Phenotypes A ($p=0.001$), C ($p=0.01$), and D ($p=0.001$) were significantly higher that of control group along with non-significant differences among the phenotype groups.

Table 2 shows the mean (\pm SEM) values of serum free testosterone (FT) and AMH concentrations of the studied phenotypes of PCOS and control women. The mean values of FT levels of phenotypes A ($p=0.001$), B ($p=0.001$) and C ($p=0.001$) were significantly higher than that of control women. In addition, the mean values of FT levels were significantly increased in phenotypes A, B, and C in comparison with phenotype D (for all, $p=0.001$). The mean value of serum AMH levels were significantly elevated in phenotypes A ($p=0.04$) and D ($p=0.01$) when compared with phenotype C as well as on border line of significant level with control women ($p=0.06$, $p=0.05$, respectively). However, there was no other significant change in serum AMH among other studied phenotypes.

The study also found significant positive correlation between serum AMH and free testosterone levels in group C ($r= 0.76$,

p=0.001). However, there was no other significant correlation among the studied parameters in other groups. Also, the receiver operator characteristic (ROC) and area under curve (AUC) study revealed that serum FT at (cutoff = 0.47 ng/ml) was the best test in differentiation of group A phenotype from controls with AUC value 0.939 (sensitivity= 88.1 and specificity= 100). Similarly, in groups B and C phenotypes, FT measurements is the best one in differentiation of these PCOS phenotypes from controls. While, in group D, serum AMH is the best one in differentiation of this PCOS phenotype from controls (AUC=0.67). In differentiation of phenotype A from D, FT has the highest ROC and AUC was 0.933 with sensitivity 88.1% and specificity 100.0% at cutoff (FT > 0.48 ng/ml). However, in differentiation of phenotype A from C, AMH has the highest ROC, and the AUC was

0.732, with sensitivity 80.95% and specificity 66.67% at cutoff (AMH >2.9 ng/ml). Similarly, serum AMH was the best one in differentiation of phenotype A from B with AUC=0.762, sensitivity 71.43% and specificity 100.0% at cutoff (AMH > 3.16 ng/ml). In differentiation of phenotype B from C, also serum AMH has the highest AUC=0.667, sensitivity 100.0% and specificity 66.67%, at cutoff (AMH > 2.9 ng/ml). Serum FT has the highest AUC=0.874, sensitivity 66.67% and specificity 100.0% at cutoff (FT > 0.479 ng/ml) in differentiation of phenotypes B and D. Both serum FT (AUC=0.806, sensitivity 60% and specificity 100.0% at cutoff 0.479 ng/ml) and AMH (AUC=0.780, sensitivity 53.33% and specificity 90.32 at cutoff 2.6 ng/ml) has comparable differentiating ability between phenotypes C and D.

Table 1. Mean (\pm SEM) values of age and body mass index of polycystic ovarian syndrome Phenotypes and controls.

Parameter	Phenotype A (n=42)	Phenotype B (n=3)	Phenotype C (n=15)	Phenotype D (n=31)	controls (n=20)
Age (year)	25.76 \pm 0.65 •	29.33 \pm 1.86	24.80 \pm 1.46 •	25.94 \pm 0.97 •	29.95 \pm 1.42
BMI (Kg/m ²)	31.15 \pm 1.04 •	28.35 \pm 1.91	28.91 \pm 1.24 •	32.02 \pm 0.98•	25.05 \pm 0.64

ANOVA and t-test reveals: •significant decrease in mean values of age of phenotypes A (p=0.01), C (p=0.02) and D (p=0.02) and significant increase in BMI in phenotypes A (p=0.001), C (p=0.01) and D (p=0.001) than in controls.

Table 2. Mean (\pm SEM) values of Free testosterone and anti-müllerian hormone of polycystic ovarian syndrome groups and controls.

	Phenotype A (n=42)	Phenotype B (n=3)	Phenotype C (n=15)	Phenotype D (n=31)	Controls (n=20)
Free Testosterone (nmol/l)	0.79 \pm 0.10•	0.55 \pm 0.07•	0.52 \pm 0.04•	0.38 \pm 0.02	0.37 \pm 0.02
AMH (ng/ml)	5.52 \pm 0.73▲	3.02 \pm 0.07	2.91 \pm 0.25	4.35 \pm 0.33▲	3.46 \pm 0.22

ANOVA and t-test revealed: •significant increase in serum FT in groups A, B, and C than group D (p=0.001) and controls (p=0.001). ▲ significant increase in serum AMH in groups A (p=0.04) and D (p=0.01) than group C.

Discussion

The mean of age value of the entire group of PCOS women of the present study was found to be 25.5 year and that of BMI 30.69 Kg/m², which is in accordance with that reported by Mehra et al. who found the mean of age of their

PCOS women was 24.0 year (22), However, these authors found insignificant difference in age value among the PCOS phenotypes which agrees with our findings.

The present study found that phenotype A is the most common one and then phenotype D

of the included PCOS women, which is in agreement with previous study (23). In addition, Fraissinet *et al.* also found that phenotype A is the predominant one (54.3%), while phenotypic B is very uncommon (0.3%) which are in harmony with the results observed in the present study (24). Phenotype A, full-blown PCOS, was the most prevalent, accounting for 67.7% of cases (25). These results are supported by the study conducted by Gluzzak *et al.* (26) in which the prevalence of phenotype A is the predominant one of PCOS (60.2%). Amini *et al.* showed a higher prevalence of phenotypes A and D in comparison to phenotypes B and C (23) and ascribed it to the fact that PCOS patients are increasingly requesting that their condition be treated in accordance with their morphology and are aware of this. The phenotype A of PCO patients have the main three criteria and hence its highest prevalence in lots of studies can be due to this fact that it represents the bases to diagnose PCO. The present study found significant increase in serum free testosterone level in phenotypes A, B, and C compared with D (Table 2), which is in consistent with that reported by Mehra *et al.* who found the mean free and total testosterone levels were significantly higher in phenotype A, B and C as compared to phenotype D (22). These authors reported that increased serum testosterone levels in PCOS women are associated with excess of visceral fat (22). Gürsu *et al.* observed that obesity and hyperandrogenism were more common in phenotype A as compared to others suggesting a higher risk of adverse metabolic effect outcomes in this phenotypic group as compared to others (27).

The current study found that the mean value of serum AMH levels was highest in phenotype A and significantly increased in both phenotypes A and D compared to phenotype C (Table 2). These findings are in harmony with that reported by Amini *et al.* who found the mean of AMH in phenotypes A and D were significantly higher than B and C and patients in phenotype D were statistically younger than those of A and B Similarly,

Wiweko *et al.* and Jamil *et al.* also demonstrated that the mean value of AMH was higher in phenotype A (28,29). Wiweko *et al.* also reported that level of AMH has been shown to have correlation with oligo-anovulation (30) those PCOS women who were anovulatory had a 12-fold greater amount of AMH than ovulatory ones. Moreover, it has been revealed that serum AMH and total testosterone were significantly higher in phenotype A than in phenotypes C and D (24). Önal and Öztürk found that AMH levels were greatest in phenotypic A and lowest in the control group (32).

The results showed that phenotype A had the greatest AMH levels, especially those who have polycystic ovaries and hyperandrogenism (33,34). The levels of AMH, which is generated by small follicles in the ovaries, are a good indicator of follicular activity and ovarian reserve (32). Elevated AMH levels are the result of a decrease in the development and maturation of bigger follicles and an increase in the number of small follicles in PCOS individuals with polycystic ovaries and hyperandrogenism (20,34). However, Önal and Öztürk phenotype did not find any significant differences in AMH levels among different phenotypes of PCOS patients (A, B, C, D) suggesting that AMH may not be a useful biomarker for distinguishing between phenotypes. The inability of AMH to differentiate between PCOS phenotypes may be because AMH levels are influenced by various factors, such as age, body mass index, and ovarian reserve.

Phenotype A is the predominant one of PCOS phenotypes and is associated with highest serum AMH and free testosterone and obesity, while the B phenotype was the rare one. Phenotype D is also associated with high serum AMH, but low free testosterone. Free testosterone is the best differentiating test of phenotypes A, B, and C from controls, while AMH is the best one in discriminating phenotype D. Both free testosterone and AMH are helpful in differentiation of different phenotypes of PCOS.

Limitation

Inability to include of newly diagnosed woman with polycystic ovary syndrome because of limited cases that encountered during the time of study.

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Conflict of interest and ethical information

The entire work had permitted by ethical committees of local authorities. All participants provided an inscribed informed consent, and the research had conducted in line with the ethical morals identified in the 1975 treaty of Helsinki. The authors declare no potential conflicts of interest related to the present research.

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