ANTI-MÜLLERIAN HORMONE AS A DIAGNOSTIC MARKER IN EGYPTIAN INFERTILE POLYCYSTIC OVARY SYNDROME FEMALES: CORRELATIONS WITH VITAMIN D, TOTAL TESTOSTERONE, DYSLIPIDEMIA AND ANTHROPOMETRIC PARAMETERS

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Summary

Background: Recent studies have highlighted the role of anti-Müllerian hormone (AMH) in numerous ovarian disorders. Polycystic Ovary Syndrome (PCOS) is one of the major causes of infertility in Egypt. Several reports have linked PCOS with vitamin D deficiency. This investigation illustrates the possibility of using serum AMH for PCOS diagnosis in infertile Egyptian females, determines the variables affecting it and correlates it with serum 25(OH)D, testosterone, dyslipidemia and anthropometric parameters.

Methods: All parameters were assessed either with ELISA or colorimetrically in 53 infertile PCOS women and 17 age matched apparently healthy controls diagnosed according to Rotterdam consensus.

Results: Serum AMH, total testosterone, triacylglycerol (TG) levels and BMI were significantly higher in PCOS group compared to healthy controls (p=0.0239, p=0.0381, p=0.0457, and p=0.0067, respectively), while serum 25(OH)D levels and HDL-cholesterol (HDL-C) were significantly lower (p=0.0397 and p=0.0443, respectively). No significant correlation existed between AMH and 25(OH)D.

List of abbreviations: AMH, Anti-Müllerian hormone; SHBG, sex hormone binding globulin; IR, Insulin resistance; LDL-C, LDL-Cholesterol; PCOS, Polycystic Ovary Syndrome; ROC, Receiver Operating Characteristic; S.D., Standard Deviation; TC, Total cholesterol; TG, Triacylglycerols; VDR, Vitamin D receptor.
D, BMI and dyslipidemia markers. AMH was found to have a significant negative correlation with age and a highly significant positive one with total testosterone in PCOS group \((r=-0.303, p=0.027\) and \(r=0.370, p=0.008\), respectively). In the receiver operating characteristic curve of AMH, the cut-off value was 42.63 pmol/L with a specificity of 59% and a sensitivity of 82%. Multivariate regression analysis showed total testosterone to be the only determinant for AMH \((\beta=0.381\) and \(p=0.038\)).

**Conclusions:** There should be a future trend of using AMH as a diagnostic marker for PCOS in Egyptian females. The variation in serum AMH levels is determined by total testosterone.

**Keywords:** anti-Müllerian hormone, polycystic ovary syndrome, vitamin D, fertility, hyperandrogenemia

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**Introduction**

Polycystic Ovary Syndrome (PCOS) is considered a dangerous threat to women of reproductive age with a prevalence of 6–16% all over the world. PCOS is a complex endocrine disease that could be diagnosed according to the Rotterdam consensus; by the presence of two of the following criteria: oligomenorrhea or amenorrhea, clinical and/or biochemical features of hyperandrogenism or polycystic ovaries on performing ultrasound investigations. Nevertheless, there are other common features of PCOS such as obesity, insulin resistance (IR) and dyslipidemia (1).

Anti-Müllerian hormone (AMH) is a dimeric glycoprotein in nature. It originates from transforming growth factor \(\beta\)-superfamily (2). Serum AMH is generally produced from the granulosa cells of preantral and small antral follicles. This starts from birth up till menopause. Thus, serum AMH level reflects the ovarian reserve (3).

Recent studies have highlighted that vitamin D deficiency is a pandemic phenomenon affecting not only countries of high latitude, having shorter hours of sunlight, but also sunny countries of low latitude such as Egypt (4, 5). Serum vitamin D levels are evaluated by measuring serum 25(OH)D due to its longer half-life (6). Vitamin D receptor (VDR), mediating vitamin D actions, is mainly expressed in ovarian tissues, endometrium, fallopian epithelial cells, decidua and placenta (7–9). Therefore, vitamin D deficiency is found to contribute to the pathogenesis of female reproductive system disorders. Furthermore, it has a negative impact on female fertility as in PCOS (10).

Regarding dyslipidemia and PCOS, several investigations have revealed that in non-diabetic and non-hypertensive PCOS females, the levels of triacylglycerols (TG) and non-HDL-cholesterol (HDLC) were twice as high compared to control group (11). Moreover, a recent meta-analysis expressed the same pattern of dyslipidemia in PCOS females (12). However, few studies have focused on the female Egyptian population. Therefore, the aim of this present study is to illustrate the possibility of using serum AMH for PCOS diagnosis in infertile Egyptian females, determine the variables affecting its levels and to evaluate its correlation with 25(OH)D, total testosterone, dyslipidemia and anthropometric markers.

**Materials and Methods**

**Study population**

This current investigation is a cross-sectional study which included 53 PCOS females aged from 17 to 39 with primary or secondary infertility. The diagnosis of PCOS was made according to Rotterdam criteria (13). Two out of the three following conditions are required to confirm a diagnosis of PCOS: oligo- and/or anovulation (defined by the presence of oligomenorrhea or amenorrhea), clinical and/or biochemical features of hyperandrogenism (defined by having clinical hirsutism (Ferriman-Gallwey score \(\geq 6\)), acne or alopecia and/or elevated androgens) or polycystic ovaries on ultrasound (having \(> 12\) follicles in each ovary, measuring from 2–9 mm and/or ovarian volume \(> 10\) mL). Patients and control subjects were recruited from the outpatient clinics of different hospitals all over the governorates of Egypt. Patients diagnosed with Cushing syndrome, androgen secreting tumors, congenital adrenal hyperplasia and hyperprolactinemia were excluded from the study.

The control group comprised 17 apparently healthy females aged from 19 to 35. All control females had regular cycles ranging from 25 to 35 days and had no ovarian gynecological disorders or endocrine abnormalities.

The present study was performed after obtaining informed consent from all subjects and the approval of the ethics committee and the Review Board at the German University in Cairo and in accordance with The Code of Ethics of the Declaration of Helsinki.
Laboratory procedures

All blood samples were taken on a random day of the menstrual cycle. Weight and height were measured on the day of blood sampling and were used to calculate BMI. Serum was separated and samples were stored at –80 °C until time of analysis.

Serum AMH and 25(OH)D were measured using AMH Gen II ELISA (Beckman Coulter, Inc., USA) and DRG 25-OH Vitamin D (total) ELISA (DRG Instruments GmbH, Germany), respectively. Serum total testosterone was measured by immunoassay (Architect 2nd Generation, Abbott Diagnostics, USA). TG, HDL-C and total cholesterol (TC) were determined using enzymatic colorimetric assay (Diamond Diagnostics, D-P international, Egypt). LDL-Cholesterol (LDL-C) was calculated from TG and HDL-C values using the Friedewald formula (14).

Statistical analysis

The subjects’ characteristics were compared using the Student’s t-test and the Mann-Whitney U test, as appropriate. Spearman Coefficient was used to determine the correlation between AMH and the various parameters. Multiple backward regression analysis was performed to evaluate the preferential effect of the different studied variables on AMH level. Cut-off values of serum AMH levels to predict its use for PCOS diagnosis, were analyzed using Receiver Operating Characteristic (ROC) curve. The area under curve (AUC) was calculated. The yield values were from 0.5 (no predictive power) to 1.0 (perfect prediction). The optimal cut-off was determined using Yodden’s Index: maximum [sensitivity – (1-specificity)] (15). Statistical significance was set at p ≤ 0.05. All data were expressed as mean ± standard deviation (S.D.). Statistical analysis was performed using SPSS Statistics for Windows, version 24.0 (IBM Corp, Armonk, New York, USA) and Graph Pad Prism for Windows, version 5.00 (Graph Pad Software, San Diego, California, USA).

Results

Results of different variables measured in both the PCOS and control group

There was no significant difference in age between the PCOS and control groups, nor was there a difference in serum levels of LDL-cholesterol or TC. In contrast, BMI, AMH, total testosterone and TG were significantly higher in PCOS females in comparison to control group. On the other hand, 25(OH)D and HDL-cholesterol were significantly lower in PCOS when compared to control as shown in Table I.

Distribution of Vitamin D across different BMI cut-offs in PCOS and control group

In this present study, it was observed that in the normal BMI category, the mean vitamin D level was 39.05 nmol/L in PCOS group while in control, it was 52.38 nmol/L. Moreover, in the overweight category, the mean vitamin D was 33.85 nmol/L in PCOS group versus 32.70 nmol/L in control group. Finally, in the obese group the mean vitamin D level was 26.50 nmol/L in PCOS group, in contrast to 30.33 nmol/L in controls. Hence, it was observed that within the PCOS group, as BMI increased, the mean serum vitamin D decreased. A similar trend was observed in the control group, as shown in Table II.

Correlation between anthropometric parameters, total testosterone, 25(OH)D and dyslipidemia markers

In the current investigation, by using Spearman correlation coefficient, a significant negative correlation was found between serum AMH and age in PCOS group (r = -0.305, p = 0.027). Furthermore, serum AMH showed highly significant positive correlation with serum total testosterone in PCOS group (r = 0.37, p = 0.008). Finally, no correlation existed between AMH and 25(OH)D and dyslipidemia markers, as shown in Table III.

Table I Comparison between age, BMI, serum AMH, serum 25(OH)D, serum total testosterone and dyslipidemia markers in PCOS and control groups.

| Variable                | PCOS (n = 53) | Control (n = 17) | p-value |
|-------------------------|---------------|-----------------|---------|
| Age (years)             | 25.96 ± 5.70  | 26.24 ± 4.90    | 0.8580  |
| BMI (kg/m²)             | 29.94 ± 5.20  | 25 ± 4.7        | 0.0067**|
| AMH (pmol/L)            | 51.27 ± 32.5  | 30.42 ± 19.21   | 0.0239* |
| Total testosterone (nmol/L) | 1.35 ± 0.45 | 1.04 ± 0.31     | 0.0381* |
| 25(OH)D (nmol/L)        | 31.32 ± 14.85 | 48.65 ± 27.30   | 0.0397* |
| TG (mmol/L)             | 1.51 ± 0.77   | 1.13 ± 0.39     | 0.0457* |
| HDL-C (mmol/L)          | 1.09 ± 0.44   | 1.32 ± 0.51     | 0.0443* |
| LDL-C (mmol/L)          | 3.56 ± 1.63   | 3.53 ± 1.32     | 0.8800  |
| Total cholesterol (mmol/L) | 5.56 ± 1.62 | 5.37 ± 1.19     | 0.8210  |
Regression analysis models

Multivariate backward regression model was performed in the PCOS group to determine the preferential effect of the different studied variables on AMH level. AMH was considered the dependent variable. On the other hand, age, BMI, total testosterone, 25(OH)D, TG, HDL-C and LDL-C were considered as independent variables. It was deduced that total testosterone was the only determinant for AMH variation. It was shown that 14.5% variability in serum AMH levels could be explained by total testosterone ($b = 0.381$, $p = 0.038$). Other parameters were not significantly related.

ROC Curve

An ROC curve was constructed to test the ability of AMH to be used as a diagnostic tool for PCOS diagnosis. The AUC was 0.634 with 95% CI (0.5 – 0.798). The optimal cut-off point was found to be 42.63 pmol/L (Sensitivity 59%, Specificity 82%) as shown in Figure 1.
**Discussion**

PCOS is a prominent underlying cause of infertility in females. Moreover, women with PCOS are at high risk of type 2 diabetes mellitus, metabolic syndrome, dyslipidemia, cardiovascular diseases, depression and sleep apnea, so the search for new biomarkers is crucial to counteract these huge complications. Hence, our study aims to examine the potential of using serum AMH as a biomarker of PCOS, to explore its determinants and to test its correlation with serum 25(OH)D, total testosterone, dyslipidemia and anthropometric parameters in Egyptian infertile females with PCOS. In the present investigation, it was clear that BMI was significantly higher in PCOS group. These results are consistent with the study of Lego et al. (16). However, these results alone are not adequate to establish a causal relationship between high BMI and PCOS diagnosis (17). In addition, PCOS is found in lean individuals as well as obese in different populations (18). Nevertheless, obesity contributes to the pathogenesis of PCOS, and could lead to the exacerbation of PCOS symptoms. It causes IR and hyperinsulinemia, which in turn leads to a decrease in hepatic sex hormone binding globulin (SHBG) production and subsequent hyperandrogenism (19). Moreover, insulin stimulates ovarian androgen production in theca cells, by binding to its own receptor (20). Furthermore, this excessive androgen production could lead to acne, alopecia and hirsutism which are major features of PCOS (21).

Previous studies have well correlated dyslipidemia with PCOS. In the present study, serum HDL-C was significantly lower in PCOS group compared to control, whereas serum TG were significantly higher. These results agree with Brunzell et al. (22) who observed the same pattern of dyslipidemia in PCOS. Moreover, they indicated that this pattern was similar to that observed with IR. On the other hand, several studies have shown that dyslipidemia in PCOS is independent of BMI. This lipid profile persisted when comparing subjects of same age and BMI (23–25). In this study, no significant difference was found between PCOS and controls concerning serum LDL-C and serum TC. These results are similar to those observed by Pirwany et al. (26).

In the present investigation, serum AMH was found to be significantly higher in PCOS group. This increase in PCOS has been previously reported in several studies done by Pigny et al. (27), Lie et al. (28) and Laven et al. (29). The increase in AMH could be attributed to the increase in the number of preantral and antral follicles. This is caused either by the slow growth of follicles mediated by the increase in AMH (30) or due to signaling abnormalities in the follicles (31). Moreover, the level of AMH in serum was found to reflect the severity of PCOS symptoms such as hirsutism, anovulation and hyperandrogenism (29, 32, 33). Furthermore, on correlating age and AMH in different study groups, it was found that serum AMH was negatively correlated with age. This negative correlation reflects the decrease in ovarian reserve observed with increase in age. This was also confirmed in the AMH dynamics model constructed by Kelsey et al. (34). The model showed that serum AMH starts to increase at birth, and reaches a peak at 25 years, then decreases gradually till reaching undetectable levels at time of menopause at the age 45–50 years.

In the present study, it was observed that total testosterone serum levels were significantly higher in PCOS compared to controls. These results are consistent with those of Tehrani et al. (35) and Skalba et al. (36). Hyperandrogenism observed in PCOS has more than one etiology. First, increased thickness of thecal layers observed in PCOS leads to excessive steroidogenic expression and activity (37). Second, elevated AMH has an inhibitory effect on FSH-induced aromatase activity. This leads to a decrease in the conversion of testosterone to estrogen, and an increase in testosterone levels (38). This effect of AMH explains the correlation found in our study between AMH and total testosterone in PCOS group. Furthermore, in the regression model, total testosterone explained the variability in serum AMH levels by 14.5% and was the only determinant of AMH. This agrees with the study of Begway et al. (39). They proposed that hyperandrogenism may contribute to the increase of small antral follicles and hence increasing AMH secretion in PCOS.

Several studies have reported that 25(OH)D is essential to maintain normal reproductive function in females. In the present study, serum 25(OH)D was significantly lower in the PCOS group compared to control. This is consistent with the fact that vitamin D deficiency affects sunny countries such as Egypt (4). Moreover, this significant decrease in vitamin D in the PCOS group agrees well with the study conducted by Hahn et al. (40). They correlated the decrease in 25(OH)D with features of PCOS such as obesity, dyslipidemia and levels of AMH and total testosterone (40). Furthermore, within the PCOS group, as BMI increased, the mean vitamin D decreased and a similar trend was observed in control group. This observed 25(OH)D deficiency trend in PCOS could be attributed to the higher BMI observed in PCOS group, rather than to PCOS itself. This inverse correlation could be explained by the fact that vitamin D is fat soluble and gets sequestered in adipose tissue, lowering its serum levels (6).

On the other hand, no statistically significant correlation was found between serum 25(OH)D and serum AMH in either of the study groups. These results are consistent with those of Cappy et al. (41) and Mumford et al. (42). Cappy et al. (41) demonstrated no significant difference in serum AMH levels before and after 25(OH)D supplementation in the
PCOS group or in normal ovarian reserve group. Furthermore, Mumford et al. (42) found that vitamin D was not associated with fertility or AMH in women with proved fertility. These results suggest that vitamin D supplementation might be of little benefit in improving AMH which is considered the clinical marker of ovarian reserve. Moreover, in a large retrospective study by Pearce et al. (43), including 340 subjects of which 58 PCOS patients and 282 norm-o-vulatory controls, there was no association between seasonal fluctuations in vitamin D and AMH levels in either of the studied groups. Nevertheless, in an interventional study, Dennis et al. (44) observed that the seasonal variation of 25(OH)D serum levels was comparable to a seasonal variation in serum AMH. In another prospective study (n = 49), vitamin D supplementation lead to 36% decrease of AMH into normal levels in women with PCOS. This could be due to the fact that 25(OH)D improves the PCOS diagnosis, rather than altering the expression of AMH (45).

In order to investigate the use of AMH as a potential diagnostic tool of PCOS, the ROC curve was constructed. The AUC was found to be 0.634 with 95% CI (0.5–0.798). The cut-off was determined to be 42.63 pmol/L (sensitivity = 59%, specificity = 82%). Comparable values for AMH cut-offs were reported by other studies. Wiweko et al. (46) reported an AUC of 0.87 and cutoff of 31.77 pmol/L (sensitivity = 76.1%, specificity = 74.6%). This study included 71 PCOS women and 71 controls and the results were obtained using the Beckman Coulter Gen II ELISA. Furthermore, Dewailly et al. (47) defined a threshold of 35.7 pmol/L (AUC = 0.949, sensitivity = 81%, specificity = 92%) in a study including 240 patients, using 2nd generation AMH-EIA ELISA from Beckman Coulter. The major differences from the present study are either the number of subjects recruited, their race and ethnicity and the different assays used to measure serum AMH. These results collectively emphasize the strength of AMH as a possible biomarker for PCOS diagnosis (32, 33, 48). Moreover, serum AMH could also be superior to transvaginal ultrasonography, as it doesn’t depend on neither the operator’s skills, nor the technological advancement of the specific device used (49). Thus, serum AMH measurement represents a more standardized method for diagnosing PCOS.

In Egyptian PCOS females,

1. There should be a future trend of using AMH as a potential tool for PCOS diagnosis instead of it being abandoned for this long time.
2. Serum AMH and serum total testosterone are positively correlated.
3. Furthermore, the variation in AMH could be attributed to total testosterone levels as demonstrated by regression analysis.
4. AMH and 25(OH)D are not significantly correlated in either PCOS or control groups.

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Conflict of interest statement

The authors stated that they have no conflicts of interest regarding the publication of this article.

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