

## **RELIABILITY OF ANTI-MULLERIAN HORMONE AND OTHER SEX HORMONES AS MARKERS OF PCOD**

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### **ABSTRACT**

Polycystic ovarian disorder (PCOD) is one of the leading endocrine disorder in women of reproductive age. It is a heterogenous complex syndrome affecting the metabolic, endocrine and reproductive functions. Anti-Mullerian hormone (AMH) is a dimeric glycoprotein belonging to the transforming growth factor-beta family. It is secreted by the pre-antral follicles of ovaries. In PCOD, the follicles fail to mature and there is increased number of pre and small antral follicles which leads to hormonal imbalance of AMH, androgens and other sex hormones. The present study was planned to assess the reliability of various endocrine markers viz. AMH, LH, FSH, Estradiol (E<sub>2</sub>) and Testosterone as markers of PCOD.

Based on the Rotterdam consensus, 57 infertile females were diagnosed for PCOD following initial screening, baseline hormone study and transvaginal sonography (TVS). The endocrine data of the above females was compared with that of normal females which constituted the control group (n = 50). The hormone levels in the PCOD group were compared with that of the control group by applying student's t-test. Women with PCOD showed higher serum AMH and LH/FSH ratio. A significant rise was also observed in serum testosterone levels, while serum FSH showed a decrease which further confirmed the increase in LH/FSH ratio. Women with PCOD have a significantly higher AMH level as compared to normal females of similar age. The study recommends inclusion of increased serum AMH levels and LH/FSH ratio in the inclusion criteria of PCOD.

**Keywords:** AMH, PCOD, Hyperandrogenism, LH/FSH, Sex Hormones

### **INTRODUCTION**

Polycystic ovarian disorder PCOD is the most common cause of infertility and accounts for 90-95% complaints of anovulation in women of reproductive age. PCOD is a syndrome of ovarian dysfunction mainly featuring hyperandrogenism and polycystic ovary (PCO) morphology (Laven *et al.*, 2002). The clinical manifestations include irregular menstrual cycle, signs of androgen excess and obesity. It is also associated with a risk of developing type 2 diabetes (Ehrmann *et al.*, 1999; Legro *et al.*, 1999).

However, in recent years it has been acknowledged that this syndrome exhibits a wide variety of signs and symptoms. Several studies have shown that women with regular menstrual cycle and/or PCO may also be suffering from this syndrome (Franks, 1989; Carmina and Lobo, 2001). As such PCOD is identified as a syndrome with no single diagnostic criterion. Moreover, PCOD remains a diagnosis of exclusion so that conditions like thyroid dysfunction, hyperprolactinemia etc. that mimic the PCOD phenotype should be excluded (Rotterdam ESHRE/ASRM-sponsored PCOD consensus workshop group, 2004). Recent studies suggest that during PCOD there may be imbalance of other hormones viz LH, FSH etc. besides androgen excess (Cook *et al.*, 2002; Laven *et al.*, 2004; Weenen *et al.*, 2004; Fiza *et al.*, 2014).

AMH is a peptide belonging to the transforming growth factor (TGF)  $\beta$  family. Chemically, it is a peptide homodimer of molecular weight 140 kDa and consists of two identical glycoprotein subunits, connected by disulphide bridges. The target organs for AMH in both sexes are gonads. In males, AMH is expressed in the Sertoli cells from the time of testicular differentiation during fetal development up to puberty. In females, on the other hand, AMH is produced by the ovarian granulosa cells from the 36<sup>th</sup> week of gestation to menopause (Lee *et al.*, 1996; Rajpert-De Meyts *et al.*, 1999). Since AMH is secreted by premature follicles and failure of follicular maturation is a distinct feature of PCOD, serum AMH level is

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expected to be elevated. Moreover, AMH induced aromatase activity affects secretion of other hormones (Pigny *et al.*, 2003).

The present study was, therefore, planned to assess the reliability of serum levels of AMH and other sex hormones as markers of PCOD.

### MATERIALS AND METHODS

311 female patients, age between 25-45 years, visited the infertility clinic (Jaipur Fertility Centre) of Mahatma Gandhi Medical College & Hospital. The females were subjected to standardized initial screening which included complete history, physical examination, routine biochemical, hematological and serology investigations and hormone assays viz. Thyroid stimulating hormone (TSH) and Prolactin. Following initial screening, the females were subjected to baseline study on day 3 of menstrual cycle that included estimation of serum LH, FSH, Estradiol (E2) and testosterone by immunofluorescence (ELFA) using VIDAS instrument and Biomerieux, France kits. Serum AMH was estimated by ELISA using Beckman Coulter gen II kits. LH/FSH ratio was also calculated for the above females. From the findings initial screening and baseline study, 57 females were diagnosed for PCOD based on the Rotterdam criterion. Another 50 females with no evidence of tubal blockage, normal ovulation and normal ovarian morphology were selected to constitute the control group. The baseline hormone study was performed in females of the control group also. The results obtained in the two groups were calculated as mean  $\pm$  SD and student's t-test was applied. A P-value of  $<0.05$  was considered as statistically significant.

### RESULT AND DISCUSSION

The endocrine data in the two groups was tabulated as mean  $\pm$  SD in table 1. A perusal of the data reveals that serum AMH levels were significantly higher for the PCOD group as compared to the control group. A highly significant rise was also observed in case of serum LH, whereas serum FSH showed a significant fall. Serum E2 levels did not show any significant variation in the two groups. Serum testosterone levels also showed a significant increase in the PCOD females than in the control group. The LH/FSH ratio was almost twice as high in the PCOD group as the control group.

**Table 1: Comparison of hormone levels in control and PCOD group**

	Control Group (n=50)	PCOD Group (n=57)	P- value
Age (years)	32.48 + 7.47	29.23 + 5.55	0.011
AMH (ng/ml)	1.69 + 0.89	8.40 + 4.17	0.000
LH (mIU/ml)	4.04 + 1.53	6.20 + 3.00	0.000
FSH (mIU/ml)	7.21 + 2.07	6.14 + 2.28	0.013
E <sub>2</sub> (pg/ml)	42.28 + 15.61	47.79 + 18.01	NS
Testosterone (ng/ml)	0.55 + 0.19	1.10 + 0.40	0.000
LH/FSH	0.59 + 0.23	1.10 + 0.63	0.000

*P-value as obtained on applying 't-test'.*

*NS - non significant*

PCOD encompasses a broad spectrum of signs and symptoms including both clinical and biochemical characteristics. Several studies have shown that women with regular menstrual cycle and/or PCO may also be suffering from this syndrome (Franks, 1989; Carmina and Lobo, 2001).

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The most distinct feature of PCOD is the failure of follicular maturation. The increase in production of AMH in patients of PCOD induces a decrease in the sensitivity of FSH receptors and thereby interrupts the maturation of follicles. This leads to an increase in the number of small antral follicles 2-5 mm in size. Such a situation is clinically characterized by anovulatory cycles and manifested as oligo- or amenorrhoea (Visser *et al.*, 2006). Since AMH is secreted by the premature follicles, higher levels of serum AMH can form the basis of diagnosing PCOD. The present study shows a significantly higher serum AMH concentration in females with PCOD. The finding is similar to that of Cook *et al.*, (2002); Laven *et al.*, (2004); La Marca *et al.*, (2004) and Fiza *et al.*, (2014).

Another typical feature of PCOD is hyperandrogenemia which may be attributed to the inhibition of peripheral aromatase activity under the influence of raised AMH levels. Rise in serum androgen levels is already included in the diagnostic criteria of PCOD and findings of the present study concord with the above fact.

Recent studies suggest that raised levels of AMH are involved in decreasing FSH-induced aromatase activity which in turn results in arrest of follicular maturation (Pigny *et al.*, 2003). Therefore, a negative correlation exists between serum AMH and FSH. This fact is being supported by the findings of present study. In the PCOD group serum AMH shows an increase whereas serum FSH exhibits a significant fall. The finding is similar to that of Fiza *et al.*, (2013).

Serum LH also shows a significant rise and hence a negative correlation with FSH in the PCOD group. The LH/FSH ratio was 0.59 in the control group whereas in the PCOD group it was as high as 1.10. In a previous study by Fiza *et al.*, (2013), a similar rise in the LH/FSH ratio was reported in females diagnosed for PCOD with raised serum testosterone levels. Previous studies have also reported a similar rise of LH/FSH ratio though the pattern was no specific and present in less than 50% cases only (Robinson *et al.*, 1992; Banezweska *et al.*, 2003).

### **Conclusion**

The findings of the present study confirm that serum AMH shows a significant rise in females suffering from PCOD as compared to females of similar age with normal ovarian morphology. Moreover, there is ample evidence to support that the rise in serum AMH leads to derangement of other hormones including LH, FSH and androgens.

The study, therefore strongly recommends inclusion of elevated serum AMH in the diagnostic criteria of PCOD. AMH is said to be a predictor of outcome of ovarian stimulation in females undergoing in-vitro fertilization. Additional studies are recommended to establish the correlation of serum AMH and other sex hormones with hyperandrogenism. In recent years AMH has gained recognition as a marker of ovarian reserve in in-vitro fertilization (IVF). Since PCOD is one of the most common cause of infertility, baseline study of AMH is especially important in females opting for IVF.

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