

Anti-Mullerian Hormone (AMH) Levels in Women with and without Polycystic Ovary Syndrome (PCOS)

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ABSTRACT

Background: The incidence of polycystic ovarian syndrome (PCOS) has reached almost epidemic proportions in women of reproductive age worldwide. Current evidence indicates that onset of the disease may occur at a much younger age than hitherto believed.

Aim: We evaluate if serum anti-mullerian hormone levels could serve as a specific indicator of the syndrome.

Methodology: This prospective, cross-sectional study is based on 101 infertile women, 51 diagnosed with PCOS whereas 50 without PCOS, 20-40 years of age. All subjects underwent detailed clinical examination, anthropomorphic measurements and transvaginal ultrasonography. Serum AMH, luteinizing hormone (LH), follicle stimulating hormone (FSH), prolactin and thyroid stimulating hormone (TSH) levels were measured by ELISA.

Results: Mean serum AMH levels were significantly higher in PCOS group as compared to subjects without PCOS but infertile (9.9 ± 1.1 vs 2.1 ± 0.3 ng/ml, respectively; $P < 0.001$). Mean FSH levels were markedly lower in women with PCOS (6.4 ± 0.3 ng/ml) relative to those without PCOS (10.1 ± 0.9 ng/ml; $P < 0.001$). However, LH:FSH ratio was not consistently higher in PCOS group and no significant differences were found in mean LH, TSH and prolactin levels of the two groups.

Conclusion: The study provides evidence that raised levels of AMH are associated with PCOS and can serve as a specific and useful biomarker of the disease. This investigation further suggests that increased LH levels and/or an increase in the LH:FSH ratio have only marginal value as diagnostic criteria of PCOS in our population.

Keywords: Anti-Mullerian Hormone, Polycystic ovarian syndrome, Gonadotropins, Thyroid stimulating hormone

INTRODUCTION

Polycystic ovarian syndrome (PCOS) is a common disease in women presenting infertility and/or disorders of the menstrual cycle. Recent surveys show that incidence of PCOS has reached almost epidemic proportions in women of reproductive age in many parts of the globe and its rate has been on increase worldwide. Recent evidence also indicates that onset of the disease may occur at a much younger age than hitherto believed. Being a heterogeneous disease there has been considerable debate and discussion as to what set of symptoms or diagnostic criteria could be used to nominate the condition as PCOS. Presently this syndrome is generally defined in accordance with the Rotterdam recommendations¹, as presenting simultaneously at least two of the following conditions: polycystic ovaries; hirsutism; and amenorrhoea. The ovarian changes in the polycystic ovaries have been ascribed due to an imbalance of luteinizing hormone (LH) and follicle stimulating hormone (FSH) secretion by the pituitary². The LH/FSH ratio in women with PCOS is higher in the initial stages of menstrual cycle and in most cases LH levels are higher than those of FSH as compared to normal LH concentrations³. Increased serum LH concentrations in association with PCOS have been ascribed to a higher frequency and amplitude of LH pulses in patients with PCOS⁴. As a consequence, the developing follicles are arrested during their growth and there is an increase in

number of pre-antral and small antral follicles. It has further been suggested that increased LH is also responsible for stimulating androgen production by the follicular theca cells manifested by excessive hirsutism in PCOS patients⁵. The androgen levels specifically of free Testosterone have been reported to be raised 2-3-fold in PCOS patients compared to those of normal women with regular ovulatory cycles⁶.

So Apart from raised androgen levels indicating hyperandrogenism, few specific biochemical markers have been used for diagnosis of the disease. Elevated LH levels and assessment of LH:FSH ratio have not proved to be reliable for diagnosis of the syndrome in the past, But now Some recent reports indicate that anti-mullerian hormone (AMH) levels may be associated with the presence of PCOS⁷. In the female, AMH levels have been shown to reflect the follicular reserve in the ovaries. In the adult ovary, AMH is also known to limit the formation of primary follicles by inhibiting excessive follicular recruitment by FSH⁸. Furthermore, in some investigations serum levels of AMH have been positively associated with androgen levels⁵. In the present study we have systemically examined serum concentrations of AMH in patients already diagnosed with PCOS and age-matched women without PCOS, to evaluate if presence of raised levels of this hormone could serve as a specific indicator of the syndrome in our patients.

METHODOLOGY

This prospective, cross-sectional study was conducted at the Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore and the Australian Concept Infertility Medical Centre (ACIMC), Lahore. A total of 111 female subjects 20-40 years of age, presented with primary or secondary fertility. Out Of these 51 women were diagnosed with PCOS whereas 50 were negative for this

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disease. The PCOS patients were identified by the following standard: Revised RC (2003) criteria (2 out of 3) 1.Oligo- or anovulation (OA) 2.Clinical and/or biochemical signs of hyperandrogenism (HA) 3.Polycystic ovaries on ultrasonography.

After taking informed consent, all subjects underwent detailed medical examination, and anthropomorphic measurements and patient history were obtained. Ovarian morphology and presence or absence of cystic follicles was assessed by transvaginal ultrasonography. Serum AMH, LH, FSH, prolactin and thyroid stimulating hormone (TSH) levels were measured by commercially available ELISA kits. Additionally, AMH levels were determined in 10 healthy, fertile and normally cycling females to provide reference values of this hormone in our population. The significance of differences between the two groups were analyzed by Student's t-test. P value < 0.05 was considered statistically significant. All calculations were made by Statistical Program for Social Sciences(version 20 SPSS, Inc, Chicago, IL, USA). Qualitative variables were presented in the form of percentage and frequency.

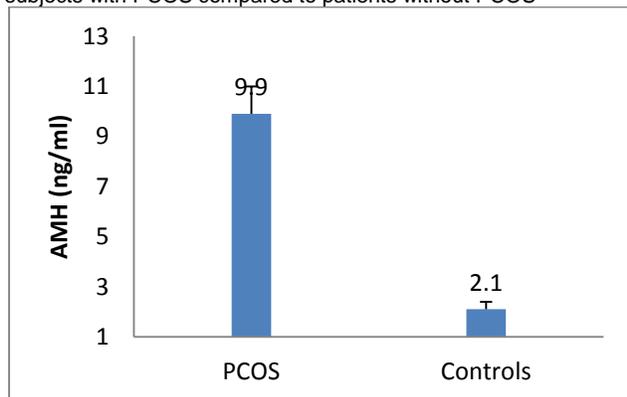
RESULTS

Physical characteristics: The physical characteristics of subjects with PCOS and control subjects are given in Table 1. The number of subjects with primary infertility in each group was not markedly different from each other. Regular menstrual cycle was reported in 76% women with PCOS and 88% subjects without PCOS. However marked difference between the two groups was observed in the incidence of heavy and painful bleeding during menstruation.

Clinical characteristics: Hyperandrogenism indicators in the two groups of patients are shown in Table 2. Clinical signs of hyperandrogenism were markedly pronounced in PCOS group in comparison to the control group (80% vs 32%, respectively). Hirsutism was more prevalent in PCOS subjects as compared to non-PCOS (80% vs 10%, respectively).

Biochemical characteristics: Biochemical parameters used in this study are listed in Table 3. Mean serum concentrations of AMH were significantly higher (P <0.001) and 2-3-fold greater in PCOS patients compared to non-PCOS subjects (Fig 1).

Fig.1: Serum anti-mullerian hormone (AMH) (mean±SEM) in subjects with PCOS compared to patients without PCOS



AMH levels in healthy normal cycling women with the same ethnic and social background were 1.8±1.0ng/ml and comparable to those of subjects without PCOS. Higher than 10ng/ml were observed in 62% of patients compared to 12% in subjects without PCOS. Mean AMH levels in subjects without PCOS were comparable to those of healthy women. On the other hand FSH levels were significantly lower in subjects with PCOS compared with those of non-PCOS subjects, but were within the normal range in both groups. However, LH and prolactin concentrations were comparable in both the groups. Mean TSH levels tended to be higher in subjects with PCOS but were not significantly higher than those in subjects without PCOS.

Table 1: Physical characteristics and fertility status of patients with and without PCOS

Characteristic	Subjects with PCOS (n=51)	Subjects without PCOS (n=50)
Age (years)	33.3 ± 0.4	33.4 ± 0.4
BMI	26.6 ± 0.6	27.0 ± 0.6
Age at menarche (years)	13.4±0.1	13.1 ±0.2
Primary infertility (%)	78	68
Irregular menstrual cycle (%)	24	12
Dysmenorrhoea(%)	20	4
Heavy bleeding (%)	26	6

Values of age, BMI and age of menarche represent mean ± SEM

Table 2: Hyperandrogenism indicators of patients with and without PCOS

Indications	Subjects with PCOS (n=51)	Subjects without PCOS (n=50)
Hirsutism*	40	05
Facial acne	28	16
Alopecia	33	16

*Based on Ferriman-Gallwey score > 7

Table 3: Biochemical characteristics of patients with and without PCOS

Biochemical markers	Subjects with PCOS (n=51)	Subjects without PCOS (n=50)	P value
AMH ^a (ng/ml)	9.9±1.1	2.1±0.4	<0.001
FSH (mIU/ml)	6.4±0.3	10.1±0.9	<0.001
LH (mIU/ml)	6.9±0.6	7.8±1.2	<0.18
Prolactin (ng/ml)	15.6±0.9	17.1±1.0	<0.63
TSH (µIU/ml)	3.5±1.5	1.4±0.1	<0.095

^aMean AMH level in healthy fertile women (n=10); 1.8±1.0 ng/ml

DISCUSSION

The present study was carried out to evaluate whether serum AMH levels could serve as a biochemical marker of PCOS in our patients. Previous studies show that AMH secretion by granulosa cells in polycystic ovaries increases several-fold compared to normal ovaries^{9, 10}. In the present study we demonstrate significantly higher serum AMH levels in patients with PCOS than those in subjects without polycystic ovaries (9.9 ng/ml vs 2.1ng/ml; P<0.001). In another study concentrations of AMH have been found to be five times higher in follicular fluid from un-stimulated follicles from women with anovulatory PCOS compared to

women who were ovulatory⁵. That increased AMH production is an intrinsic property of granulosa cells in PCOS has been confirmed by raised levels of AMH messenger RNA in polycystic ovarian granulosa cells even following stimulation for IVF^{5, 11}. Together these studies demonstrate that increase in AMH concentration is largely due to an increase in production of AMH by each follicle and not merely due to a consequence of an increase in follicle number. However, it has been suggested that elevated serum AMH levels in patients with PCOS may also be due to disturbances in folliculogenesis, resulting in an accumulation of excessive pre-antral and small antral follicles¹². Furthermore, some evidence has been presented showing that a cessation of antral follicle development towards the dominant follicle could be due to a suppression of aromatase activity by AMH and a lower follicle sensitivity to FSH^{13, 14}. In the present study in spite of raised levels of AMH levels in patients with PCOS, majority of the subjects had regular cycles and apparently normal ovulatory function. It is, therefore, suggested that AMH may not directly influence the development of the preovulatory follicle.

Presently there is no consensus in literature regarding cut-off values of serum AMH levels in normal women. Circulating concentrations and cut-off values of the hormone, therefore, remain method- and laboratory-dependent¹⁵. As observed in our study AMH levels in women with PCOS were 2-fold of the average values in subjects without PCOS, A previous study also reports a 2-3-fold increase in AMH levels in serum from women with PCOS compared to that of subjects with normal ovaries¹⁶. In another investigation a cut-off value of 7.3 ng/ml with a 76% specificity and 70% sensitivity to predict PCOS has been documented¹⁷. Also, Pigny and co-workers reported that specificity and sensitivity of serum AMH measurement were 92% and 67%, respectively¹⁸. In the present study we have found a lower cut-off value of 4.2ng/ml of serum AMH levels associated with presence of PCOS.

CONCLUSION

Serum AMH levels can serve as a fairly reliable biochemical marker of the presence of polycystic ovaries and consequently can be used as one of the routine tests for the diagnosis of suspected cases of PCOS. In this investigation serum AMH levels in PCOS patients were 2-3 fold of the concentrations observed in age-matched subjects without PCOS. Increased levels of AMH in women with PCOS appear to be independent of BMI and hyperandrogenism as evidenced by hirsutism in women of reproductive age appears to be a more regular feature of the disease than abnormal levels or ratio of gonadotropins (LH/FSH).

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