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Anti-müllerian hormone is found raised in polycystic ovarian syndrome

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Abstract:

Background: Polycystic ovarian syndrome (PCOS) is a common problem causing menstrual irregularity and infertility among women of fertile age. Increased level of anti-müllerian hormone (AMH) is currently thought to be an important marker for PCOS.

Objective: Determine the AMH levels in PCOS.

Methods: This cross-sectional study included 80 PCOS patients [age: 23.7±4.8, years; body mass index, BMI: 26.7±4.5 kg/m2; (mean ±SD)] diagnosed on the basis of Rotterdam 2003 criteria and 80 healthy women of fertile age as controls [age: 26.3±2.9, years; BMI: 21.7±2.8 kg/m2; (mean ±SD)]. AMH (ng/ml) was measured by enzyme linked immunosorbant assay (ELISA) whereas other hormones (follicle stimulating hormone, FSH; luteinizing hormone, LH; Testosterone) by immunochemiluminometric assay.

Results: AMH was significantly higher in PCOS (9.21 ± 0.50 vs. 4.40 ± 0.41, ng/ml, M ± SE; $p<0.001$) than that of healthy controls. Though not statistically significant, AMH showed inverse relationship with FSH (mIU/ml, r = - 0.129; p = 0.253), and BMI (kg/m2, r = - 0.046; p = 0.686) whereas positive relationship with testosterone (ng/dl, r = 0.146; p = 0.197) and LH (mIU/ml, r = 0.102; p = 0.368). Holding cut-off value at 3.5 ng/ml for AMH, sensitivity and specificity of AMH was found to be 67% and 78.33% respectively.

Conclusions: PCOS women of fertile age have higher AMH level than that of healthy control subjects. It can be considered as an important seromarker for the diagnosis of PCOS.

Key words: AMH, PCOS.

Introduction

Polycystic ovarian syndrome (PCOS) is the most common form of chronic anovulation associated with androgen excess. Large scale studies have observed a prevalence rate of PCOS 5% to 10% among reproductive-age women.[1,2] Currently, there are 3 widely accepted sets of criteria for diagnosis of PCOS.[3,4] After excluding other causes of hyperandrogenism, National Institute of Health (NIH) criteria (1990) requires - I) evidence of hyperandrogenism (clinical or biochemical) and II) evidence of anovulation or oligo-ovulation. Rotterdam criteria (2003) added the presence of polycystic ovarian morphology (2 out of 3 need to be present). The Androgen Excess and PCOS society criteria (2006) considered polycystic ovarian morphology as an alternative evidence of ovarian dysfunction. However, the diagnosis of PCOS is often a diagnostic dilemma, as because not all patients express hyperandrogenism. A substantial number of women may have polycystic appearing ovaries

owing to causes other than PCOS. About 75% of all anovulatory women may have polycystic ovaries.[3] Therefore, a surrogate serum marker has long been looked for that could precisely be suggestive of PCOS.

Anti-mullerian hormone (AMH) is a 140 k-Da dimeric glycoprotein hormone of the transforming growth factor- β family, secreted solely by granulosa cells of small pre-antral and antral ovarian follicles upto 9 mm in diameter. Its level in serum shows little fluctuations throughout the menstrual cycle. Serum AMH level is considered to represent a reliable marker of ovarian follicular pool.[5] Women with PCOS have a two to six- fold greater number of follicles (primary, secondary and antral) in ovaries. Possibly it is secondary to hyperandrogenism.[6,7] Circulating AMH levels in PCOS women are found to be higher than in healthy controls.[8] Many factors reduce serum AMH levels, but, it is only PCOS that increases its serum level.[7] Since AMH level reflect the number of growing follicles, their measurement may be used as a marker of ovarian follicle impairment in PCOS. In anovulatory women with PCOS, the follicular development is halted at 6-9 mm diameter. These increased follicles contribute to the increased AMH, as well as AMH productions per granulosa cells are also increased.

Therefore, it is tempting to assume that, increased AMH levels observed in oligomenorrheic girls without evidence of hyperandrogenism may reflect numbers of growing and antral follicles and possibly, polycystic ovaries.[9] By considering circulating AMH as a surrogate marker for antral follicle number, as proposed by Pigney et al.[10] elevated AMH levels in oligomenorrheic girls without evidence of hyperandrogenism may fulfill the diagnostic criteria of PCOS as designated by Rotterdam consensus 2003. Serum AMH levels are stable throughout menstrual cycle, and are unmodified by pregnancy, gonadotropin releasing hormone treatment and administration of short-term oral contraceptives. These make AMH an ideal marker of ovarian reserve.

Some hormonal and metabolic profile in PCOS patients in Bangladeshi women have been studied by other investigators.[11,12] This study was aimed to see the AMH and its relevance to PCOS patients in our country

Materials and methods

Subjects

This was an observational cross-sectional study comprised of 80 PCOS patients and 80 healthy controls. Inclusion criteria were: age between 18 and 35 years, PCOS diagnosed on the basis of Revised Rotterdam Consensus 2003 criteria. Oligo-ovulation defined as length of menstrual cycle > 35 days or <10 periods per year. Clinical hyperandrogenemia as evidenced by hirsutism. Normal level of serum testosterone was taken as <58 ng/dl. Exclusion criteria included primary amenorrhea (age ≥16 years), hyperandrogenemia and infertility due to other known causes (primary hypothyroid, CAH, hyperprolactinemia). Serum testosterone level for women > 58 ng/ml was considered as elevated. On the basis of different fertility literatures serum AMH > 3.0 ng/ml considered as high. Obesity defined as BMI ≥ 28 kg/m2 and overweight defined as BMI ≥ 23 kg/m2. Healthy controls met the following criteria: 1) regular menstrual cycle with an interval of 21-35 days; 2) at least one natural pregnancy(if married); 3) no medical history of hirsutism or severe acne; 4) no evidence of endocrine disease; 5) no history of ovarian abnormalities; and 6) no history of ovarian or uterine surgery. Informed written consent was taken from all participants.

Assays

AMH was estimated by single measurements by an enzyme-linked immunosorbent assay, AMH GEN II ELISA kit (Beckman Coulter, Inc. USA) whereas other hormones (follicle stimulating hormone, FSH; luteinizing hormone, LH; Testosterone) by immunochemiluminometric assay. Values of AMH were presented as nanograms per milliliters (conversion factor to pmol/l = ng/ml × 7.1). AMH was calculated using kc3 biographs with help of the standard supplied with the kit. QC (quality control) was used in each assay run to assess precisions of the assay. Intraassay CV (co-efficient of variance) was 3.4 to 5.4% and interassay CV 4.0 to 5.6% for AMH assay

Statistical analysis

AMH levels were expressed as the mean ± (SE). Student's t-test for continuous variables and Chi-Square test for discrete variables were used. Correlation among variables was assessed by using Pearson's correlation test. Multiple regressions were done to see the impact of independent factors over AMH. P values ≤ 0.05 were considered statistically significant.

Results

Baseline characteristics of subjects (as seen in Table-I) revealed statistically higher BMI (26.7 ± 4.5 vs. 21.7 ± 2.8, kg/m2; p <0.001) in PCOS than that of control. Menstrual irregularity (86.3% vs. 1.3%; p<0.001) and infertility rate (p<0.001) were also significantly higher in the PCOS. As shown in Table-II, 83.8% PCOS subjects were overweight with hirsutism. Acne was 16.3%, acanthosis nigricans 38.8% and hypertension 18.8%, (defined as >130/85 mmHg, any or both) in PCOS; these were absent in healthy controls.

Table1. *Characteristics of the studied PCOS patients and control*

Variables	PCOS n = 80	Controls n = 80	p
Age (mean ±SD, year)	23.7±4.8	26.3±2.9	<0.001
BMI (mean ±SD, kg/m^2)	26.7±4.5	21.7±2.8	<0.001
S. testosterone (mean ±SE,ng/dl)	42.39±5.40	-	-
S. FSH (mean ±SE, mIU/ml)	5.68±0.29	-	-
S. LH (mean ±SE, mIU/ml)	8.76±1.64	-	-
PCO by USG (frequency)	67 (77)	-	-
Menstrual disturbance	69 (86.3)	1 (1.3)	<0.001
Family history of PCOS	6 (7.5)	2 (2.5)	0.276
*Infertility Primary	14/43 (32.6)	0/34	<0.001
Secondary	8/43 (18.6)	0/34	
MR/Abortion	12/43 (27.9)	10/34 (29.4)	NS

**analyzed over the married ladies only. Within parenthesis are percentages over column total*

(34 control subjects and 43 PCOS patients were married)

PCOS: *polycystic ovarian syndrome*

BMI: *body mass index*

FSH: *follicle stimulating hormone*

LH: *luteinizing hormone*

USG: *ultrasonography*

MR: *menstrual regulation*

SD: *standard deviation*

SE: *standard error*

Table2. *Physical examination findings in PCOS subjects (n = 80)*

Variable (s)	Frequency (n, %)*
BMI (> 23 kg/m2)	67 (83.8)
Hirsutism	67 (83.8)
Acne	13 (16.3)
Acanthosis nigricans	31 (38.8)
Hypertension (>130/85 mmHg, any or both)	15 (18.8)

**None of these characteristics were present in the healthy control subjects*

PCOS: *polycystic ovarian syndrome*

BMI:*body mass index*

As depicted in Table-III, AMH level was significantly higher (9.21 ± 0.50 vs. 4.40 ± 0.41, ng/ml; p<0.001) in the PCOS patients than that of controls. However, when compared according to age-group, this was significantly different between PCOS and controls in the age group 23-27 years (9.91 ± 0.71 vs. 4.52 ± 0.54, ng/ml; p<0.001) and age-group 28-31 years (8.28 ± 1.51 vs. 4.22 ± 0.68, ng/ml; p<0.011).

Table3. *Basal serum AMH levels in control women and in women with PCOS*

Group of subjects	Controls (n = 80) AMH (ng/ml)±(SE)	PCOS (n = 80) AMH (ng/ml)±(SE)	p-value
Whole group	4.40 ± 0.41	9.21 ± 0.50	<0.001
Age group(years) n(control, PCOS)			
23 – 27 (49,32)	4.52 ± 0.54	9.91 ± 0.71	<0.001
28 – 31 (25,7)	4.22 ± 0.68	8.28 ± 1.51	<0.011

AMH: *anti-mullerian hormone*

SE: *standard error*

PCOS: *polycystic ovarian syndrome*

Relationship of AMH with FSH, testosterone, BMI and LH is shown in Table-IV. Correlation co-efficient for none of the relationships were significant statistically. But FSH (r =- 0.129; p = 0.253) and BMI (r = - 0.046; p = 0.686) showed inverse relationship, whereas, testosterone (r = 0.146; p = 0.197) and LH (r = 0.102; p = 0.368) showed positive relationship. AMH level was dichotomized holding cut-off value at 3.5 ng/ml. By multiple regression analysis as shown in Table-V, none of the factors such as – age (p = 0.123), LH (p = 0.397), FSH (p = 0.299),testosterone (p = 0.796) and BMI (p = 0.545) showed any statistical significance for independent relationship.

Table4. *Relationship between the AMH plasma level and BMI or other biological data (serum levels) in patients with PCOS*

Variables	PCOS (n=8o) Correlation co-efficient (r)	p
AMH, FSH	- 0.129	0.253
AMH, Testosterone	0.146	0.197
AMH, BMI	- 0.046	0.686
AMH, LH	0.102	0.368

PCOS: *polycystic ovarian syndrome*

AMH: *anti-mullerian hormone*

FSH: *follicle stimulating hormone*

LH: *luteinizing hormone*

BMI: *body mass index*

Table5. *Regression statistics of variables that influenced plasma AMH (≥3.5 ng/ml) concentrations in PCOS patients*

Independent variables	B	SE	P
Constant	1.392	0.337	0.001
Age	- 0.014	0.009	0.123
LH (mIU/mL)	0.002	0.003	0.397
FSH (mIU/mL)	- 0.017	0.016	0.299
Testosterone (ng/dl)	0.000	0.001	0.796
BMI (kg/m^2)	- 0.006	0.010	0.545

PCOS: *polycystic ovarian syndrome*

AMH: *anti-mullerian hormone*

LH: *luteinizing hormone*

FSH: *follicle stimulating hormone*

BMI: *body mass inde*

Table-VI shows the subgroups of PCOS and control subjects divided on the basis of cut-off value of AMH at 3.5ng/ml. AMH ≥ 3.5ng/ml was considered as positive in the diagnosis of PCOS. Thus 67 out of 80 in the PCOS patients and 33 out of 80 controls could be labeled as positive. The calculated sensitivity was found to be 67% and specificity 78.33%.

Table6. *Sensitivity and specificity of AMH for the diagnosis of PCOS holding cut-off as 3.5 ng/ml (Chao et al., 2011)*

Group(s)	Anti-mullerian hormone (ng/ml)		Total
	≥ 3.5 ng/ml	<3.5 ng/ml	
PCOS	67	13	80
Control	33	47	80
Total	100	60	160

PCOS: *polycystic ovarian syndrome*

Sensitivity = true positive / (all positive) × 100

= 67/ (67+33) × 100

= 67 %

Specificity= true negative / (all negative) × 100

= 47/ (47+13) × 100

= 78.33%

Discussion

Present study was conducted to see the AMH level and its impact on PCOS patients and control subjects among women of fertile age group. It was clearly observed that AMH was higher in PCOS patients than that of control subjects which was statistically significant. This result supports the findings in other studies.6, 9, 13, 14 PCOS patients were more overweight than controls and infertility was significantly higher among them. The sensitivity and specificity of AMH for detecting PCOS in patients aged 18-35 years were calculated to be 67% and 78.33% respectively, using an AMH cut-off value of 3.5 ng/ml as followed in another study.15

Mean AMH differed significantly between PCOS subjects and healthy controls. Increased serum AMH concentrations in PCOS patients have been explained by the increased number of small ovarian follicles responsible for AMH secretion.16 In the ovary AMH is produced from granulosa cells of pre-antral and small antral follicles. From experimental data, mainly obtained in rodents, the proposed functions of AMH are: 1) inhibition of the initial recruitment of primordial follicles, through a paracrine effect and 2) inhibition of aromatase activity in granulosa cells, thus reducing the production of estradiol (E2).17 This last effect combined with the fact that AMH could reduce the follicle sensitivity to FSH in the mouse ovary both in vivo and in vitro18 make it possible that an excessive production of AMH may be the cause of follicular arrest in PCOS. Observed higher frequency of menstrual irregularity and infertility in PCOS in this study may be explained in this way.

Pathophysiology of PCOS has been known to be multifactorial. Anovulation and/or oligo-ovulation are the main underlying cause for infertility. Altered LH: FSH ratio, hyperandrogenemia, and hyperinsulinemia as well as insulin resistance – all had been thought to be linked to the probable cause of anovulatory cycles. But in the past decade much attention had been concentrated on AMH in context of PCOS. Several factors have been reported to be associated with AMH secretion. A negative correlation was observed between FSH and AMH levels in some studies.6,19 Low dose recombinant FSH therapy in PCOS patients decreased serum AMH levels20, suggesting the negative role of AMH in aromatase expression during dominant follicle selection. Increasing serum FSH will cause a shift of small antral follicles to larger ones, expressing less AMH, thus a decline in AMH and allowing dominance of follicle to occur. It has been observed that AMH serum levels significantly and inversely correlate to FSH levels in healthy women.21 Apropos with the above facts, in the present study a negative relationship was observed between FSH and AMH though not significant statistically. Follicles from AMH knockout mice have been shown to be more sensitive to FSH than those from the wild type.18 This further suggests that the inhibiting effect of AMH on aromatase activity acts through a decrease in granulosa cell sensitivity to FSH. The balance between the opposite effects of AMH and FSH on aromatase activity might be crucial for the cohort at the time of the selection process for dominant follicle.

Since pronounced androgen secretion is one of the typical features of PCOS, it was of interest to see whether AMH levels correlate to serum androgen levels, thus reflecting the degree of hyperandrogenemia. Positive relationship was found between AMH and testosterone serum levels among PCOS women but failed to reach any significant level. Previous studies6,9,13,22 observed significant positive correlation between AMH and androgens. Failure to detect significant correlation in present study might be explained in the way that it did not see other androgenic components than only serum testosterone.

Chao et al. 15 used a cut-off value of 3.5 ng/ml of AMH in discrimination of PCOS from control and observed sensitivity and specificity on its basis as 74% and 79%. Holding the same cut-off value, we observed a sensitivity of 67% and specificity of 78% which seems alike. Negative relationship was seen between age and AMH level by regression analysis but found to be non significant. The age related decline in AMH level among control women is supported by other studies whereupon negative correlation between age and AMH has been reported.14 As because AMH levels correlate with the number of early antral follicles which might represent the size of the resting follicle pool, AMH may constitute a marker for ovarian aging.19

It was interesting to note that in the present study mean AMH level in healthy control group was higher than normal conventional values. The highest level was observed in the 18-22 years age-group (early reproductive period). As AMH is regarded as a marker of fertility reserve, this finding may indicate young fertile women of our population may have higher fecundability in comparison to other ethnic groups. There is a growing body of medical literature which indicates that female reproductive function may differ by race. Racial/ethnic difference in ovarian reserve may influence reproductive outcome. This area needs further research and exploration.

It may be concluded that PCOS women in Bangladesh have significantly higher serum AMH than healthy women during reproductive period. Age related decline of AMH occurs in healthy women as well as in PCOS women. This is indicative of ovarian aging. Observed relatively higher AMH level in healthy control group may reflect ethnic variation. Sensitivity and specificity of AMH for diagnosing PCOS was calculated to be 67% and 78% respectively holding a cut-off value of AMH at 3.5 ng/ml. Thus, AMH seems to be an important seromarker in the diagnosis of PCOS irrespective of other characteristics of PCOS.

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