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Anti-Mullerian Hormone in Normoglycemic Obese and Non-Obese Women with Polycystic Ovary Syndrome from North India

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Abstract

Objective: Anti-Mullerian Hormone (AMH) is associated with various pathological conditions of ovary including Polycystic Ovary Syndrome (PCOS). Obesity, BMI and hyperandrogenism in these patients' triggers increase in small antral follicles, which affects AMH secretion.

Method: In the present study AMH, levels were compared with clinical, biochemical, and hormonal profiles in obese and non-obese PCOS women from North India.

Results: One hundred and eight cases Normoglycemic PCOS women were enrolled for this study. Forty-six patients (42.6%) were obese with BMI ≥ 25kg/m^2 and 62 (57.4%) had BMI < 25kg/m^2. Forty-eight (44.4%) patients had AMH <5 ng/ml, 50 (46.2%) had AMH between 5 to 10 ng/ml and 10 (9.2%) patients had AMH > 10 ng/ml. Mean fasting insulin was significantly higher in obese PCOS patients (P=0.01) and with AMH < 5ng/ml. Mean serum LDL-c was significantly elevated (P=0.01) in non-obese PCOS patients with AMH levels less than 5ng/ml and mean serum LH was significantly non-obese patients with AMH < 10ng/ml.

Conclusion: Low AMH may serve as an important marker for PCOS irrespective of BMI and should be routinely measured for diagnosis as well as for the management of PCOS.

Introduction

Anti-Mullerian Hormone (AMH) also known as Mullerian inhibiting factor and Mullerian-inhibiting hormone, is a glycoprotein (140kDa) encoded by AMH gene located on chromosome 19p13.3 [1]. In females, it is produced by granulosa cells that surround the egg sac within the ovary and in fetal males by sertoli cells during embryogenesis [2]. The differentiation of sex into male and female begins with the secretion of AMH by male fetal sertoli cells and its absence in females. AMH is expressed at all steps of folliculogenesis, starting from the time when primordial follicles grow into small preantral follicles [3].

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Plasma levels of AMH in women serves as an important biomarker of ovarian reserves and indicates the small follicular growth. The reproductive span in healthy women is predicted by progressive age-related decline of plasma AMH levels. Recent studies have also shown the importance of this hormone in various pathological conditions of ovary including Polycystic Ovary Syndrome (PCOS). PCOS is the most common endocrine disorder-affecting women in their reproductive years and is characterized by irregular menstrual cycles, chronic anovulation and hyperandrogenism [4]. They exhibit wide range of metabolic disorders that include obesity, metabolic syndrome and hyperandrogenism [5].

In women with PCOS, the small ovarian antral follicles increase and AMH secreted by these developing follicles can be used as an important marker to detect follicular impairment. It is suggested that the increased secretion of LH and/or testosterone may have a positive effect on the secretion of AMH by the ovarian follicles [6,7]. A positive correlation has also been found between high AMH levels and androgen over-production due to intrinsic defects of thecal cells. High AMH levels have been predicted as a response to various treatments of PCOS, while improvement in various clinical and biochemical parameters have been associated with decline in AMH, thus supporting a very important role of AMH in diagnosis and treatment of PCOS [3,7]. The reason for high AMH in PCOS from antral follicles remains unknown, but there is evidence of obesity, increased Body Mass Index (BMI) and hyperandrogenism that influence its over-production [9].

As obesity has been suggested to be an important factor that mediates the association between AMH and various parameters of PCOS. We conducted the current study that compared AMH levels with clinical, biochemical, and hormonal parameters in obese and non-obese PCOS women from North India.

Methods

Subjects and study design: Women with PCOS attending the OPD/IPD department of Endocrinology at tertiary care hospital Srinagar, Kashmir were recruited. The diagnosis of PCOS was based on the criteria formulated by Rotterdam 2003 PCOS consensus [10]. Women with abnormal glucose tolerance, history of chronic illness, pregnancy, lactation, intake of drug such as steroids, androgens, oral contraceptives, anti-epileptics or drugs known to interfere in glucose or lipid metabolism were excluded from the study.

Clinical measurements: All patients underwent detailed medical history and physical examination. Oligomenorrhea was defined as an inter-menstrual interval of >35 days or a total of <8 menses per year. Amenorrhea was defined as absence of menstruation during last 6 or more months. All women were subjected to anthropometric assessment such as measurement of height, weight, BMI, Waist/Hip Ratio (WHR), blood pressure, and detailed systemic examination. The waist circumference was measured midway between the superior border of iliac crest and the lowermost margin of ribs at the end of normal expiration. The hip circumference was measured at the greatest posterior protuberance of buttocks, with the subject standing erect, feet together, without volitionally contracting gluteus muscles. Both measurements were taken to the nearest centimeter. Height and weight were also measured. BMI was calculated by the formula: body weight (kg)/ height (m^2). Obesity was defined as BMI ≥ 25 kg/m^2. Hirsutism assessment was done using modified Ferriman-Gallwey score by counting nine specified body areas by a single observer with a good reproducibility. A score of > 9 out of a total of 36 was taken as significant [11]. Acne was assessed in all subjects and moderate to severe acne (cystic acne) was taken as a clinical feature of hyperandrogenemia [12].

Biochemical and hormone measurements: Fasting blood samples were collected for glucose, biochemical parameters, Luteinising Hormone (LH), Follicle Stimulating Hormone (FSH), Testosterone (T), TSH, T3, T4, Prolactin (PRL), Insulin and AMH after overnight fast (10-12 hours). The samples were collected in serum activated vacutainers and Heparin Coated Vacutainers (for AMH) under cold conditions during early follicular phase of spontaneous or medroxy-progesterone induced (in patients with irregular periods) menstrual cycle. All the patients were subjected to trans-abdominal ultrasonography for ovaries and adrenal glands.

All biochemical parameters including serum glucose were estimated by using auto-analysers Abbott C4000/ Siemens Dimensions RXL Max. Hormones including insulin was analysed using auto-analysers Abbott i1000SR/Siemens Advia Centaur XP.

The blood samples for AMH assay were centrifuged immediately and plasma was stored at -80˚C until analysed. AMH levels were quantitatively measured by using AMH Gen II enzyme linked immunosorbent assay (ELISA) commercially available kits (Beckman Coulter; USA, ref A79765/66) as per the manufacturer`s protocol. The inter-assay CV was 4.5 % to 5.6% and the intra-assay CV was 3.6% to 5.4%. The available range of measurement using this kit was 0.01 to 22.5 ng/ml.

Statistical analysis: Statistical analysis was done using SPSS version 25 software (Lead Technologies, Lead, US). The continuous data were expressed as mean ± SD. Student's unpaired t-test was used to compare two groups. Analysis of Variance (ANOVA) was used to compare quantitative variables. The correlation between plasma AMH and other variables was estimated by Pearson's correlation method. Tests were considered significant at $P \leq 0.05$.

Results

One hundred and eight cases were enrolled for this study. These subjects were Normoglycemic and confirmed PCOS as per the Rotterdam 2003 criteria. The mean age of patients was 22.19 ±4.4 years (14 to 38 years) and the mean age of menarche was at 13.3±1.4 years (9 to 17 years). Irregular cycles were present in 87/108 (81%) patients. Acne was present in 60 out of 108 cases (56%). FG score ≥9 was present in 86/108 (80%) patients.

We subdivided our cases into two subgroups taking BMI 25kg/m^2 as cut off: Group I as non-obese/lean PCOS with BMI < 25kg/m^2 and Group II as obese/overweight PCOS with BMI ≥ 25kg/m^2. Forty six out of 108 patients (42.6%) were obese with BMI ≥ 25kg/m^2 and 62/108 (57.4%) had BMI < 25kg/m^2 and were non-obese/lean. Also, plasma AMH levels were subdivided into three groups: Group 1 (< 5 ng/ml), Group 2 (5 to 10 ng/ml) and Group 3 (> 10 ng/ml) [24]. Forty eight out of 108 (44.4%) patients had AMH <5 ng/ml, 50/108 (46.2%) had AMH between 5 to 10 ng/ml and 10 (9.2%) patients had AMH > 10 ng/ml. The clinical, biochemical, and hormonal parameters of the subjects are summarized in Table 1, 2 and 3. Ultra-sonographic findings showed the presence of multiple follicles with increased bilateral ovarian echogenic stroma in 27 patients within Group 1, 21 patients within Group 2 and 4 subjects within Group 3.

Table 1: Clinical parameters in obese vs lean patients with PCOS according to the plasma AMH levels.

Clinical parameters	Group 1 (AMH<5 ng/ml)			Group 2 (AMH 5 to 10 ng/ml)			Group 3 (AMH > 10 ng/ml)		
	Group I	Group II	*P* value	Group I	Group II	*P* value	Group I	Group II	*P* value
Age (years)	21.09±3.9	22.34±4.5	0.32	21.95±3.6	22.67±5.2	0.58	28.33±0.6	25.2±2.8	0.1
Age of menarche (years)	13.7±1.5	13.5±1.3	0.62	13.0±1.4	13.1±1.5	0.81	12.6±1.2	13.4±0.8	0.24
Weight (kgs)	51.73±6.2	69.2±7.9	<0.001	52.8±7.07	66.6±6.6	<0.001	55.0±5.0	72.8±11.9	0.04
Height (cms)	153.6±2.7	154.1±3.5	0.60	155.3±6.7	151.4±10.2	0.13	158.3±8.5	154.4±1.8	0.24
Waist (cms)	79.19±8.6	95.4±9.2	<0.001	80.08±7.2	92.0±7.5	<0.001	81.6±8.7	96.8±6.9	0.01
Hip (cms)	88.23±6.8	97.6±7.2	<0.001	89.19±4.9	100.07±19.8	0.01	91.3±3.05	98.1±3.48	0.01
BMI	21.89±2.3	29.17±3.3	<0.001	21.85±2.4	29.4±5.1	<0.001	22.03±2.76	30.5±4.7	0.02
FG score	12.09±3.5	13.9±5.9	0.22	12.85±3.9	13.6±4.9	0.56	13.6±4.04	14.6±5.9	0.79

Table 2: Biochemical parameters in obese vs lean patients with PCOS according to the plasma AMH levels.

Biochemical parameters	Group 1 (AMH<5 ng/ml)			Group 2 (AMH 5 to 10 ng/ml)			Group 3 (AMH > 10 ng/ml)		
	Group I	Group II	*P* value	Group I	Group II	*P* value	Group I	Group II	*P* value
FBG(mg/dl)	91.4±7.03	88.9±8.5	0.28	90.2±7.8	87.4±8.08	0.22	92.6±5.0	97.14±21.4	0.73
Cholesterol (mg/dl)	173.2±30.8	186.2±36.7	0.2	181.9±44.3	190.2±33.1	0.45	202.6±65.4	171.5±36.4	0.35
Triglycerides (mg/dl)	150.7±59.9	163.3±52.5	0.44	155.4±49.1	163.0±49.7	0.59	172.0±68.4	157.0±86.3	0.79
HDL-c (mg/dl)	46.4±7.5	46.3±11.6	0.97	44.8±7.04	45.0±8.1	0.92	48.0±6.2	49.2±8.2	0.82
LDL-c (mg/dl)	112.9±23.6	97.1±17.2	0.01	112.2±23.5	102.9±24.6	0.18	131.6±19.3	107.28±19.7	0.1
Urea (mg/dl)	19.2±3.8	17.7±3.6	0.17	18.6±5.8	19.9±4.1	0.36	15.3±4.5	17.2±6.1	0.64
Creatinine (mg/dl)	0.7±0.1	0.7±0.2	1.0	0.7±0.1	0.7±0.2	1.0	0.8±0.1	0.7±0.1	0.18
Total Bilirubin (mg/dl)	0.5±0.2	0.6±0.2	0.09	0.7±0.3	0.6±0.3	0.25	0.9±0.3	0.7±0.2	0.24

Table 3: Hormonal parameters in obese vs lean patients with PCOS according to the plasma AMH levels.

Hormonal parameters	Group 1 (AMH<5 ng/ml)			Group 2 (AMH 5 to 10 ng/ml)			Group 3 (AMH > 10 ng/ml)		
	Group I	Group II	*P* value	Group I	Group II	*P* value	Group I	Group II	*P* value
LH (µIU/ml)	5.3±2.5	3.5±1.4	0.003	5.2±2.7	3.6±1.7	0.01	4.8±0.4	4.6±2.4	0.89
FSH (µIU/ml)	5.4±1.6	5.3±1.6	0.83	5.5±1.5	5.3±1.7	0.67	6.2±1.7	5.5±1.4	0.51
Testosterone (ng/ml)	0.6±0.3	0.5±0.2	0.17	0.5±0.2	0.4±0.2	0.08	0.4±0.2	0.8±0.2	0.51
Prolactin (ng/dl)	13.0±4.2	14.7±4.7	0.2	16.1±4.9	13.5±3.9	0.04	17.3±7.4	15.5±4.4	0.63
Fasting Insulin (µIU/ml)	5.80±3.4	7.9±2.3	0.01	7.2±3.9	5.2±4.8	0.12	4.8±3.3	6.2±7.07	0.75

Discussion

AMH belongs to the family of TGF-β family and has been proposed as a marker of ovarian reserve and ageing [13]. Various mechanisms have been proposed that suggest the relationship between AMH and PCOS. Metabolic syndrome, hyperandrogenism and reproductive dysfunction have been suggested to cause elevated AMH in PCOS patients. Studies have concluded that obesity, Insulin Resistance (IR), and hyperandrogenism play major roles in the increasing level of AMH. An inverse relationship between AMH and obesity has been described in women with and without PCOS [14], but there is limited data that examines the relationship between AMH and other cardiometabolic markers, including glucose and lipids, in women with PCOS.

IR plays has a significant role in the pathophysiology of PCOS. It is estimated that around 50–80% of patients with PCOS have elevated mean insulin levels. Insulin being a reproductive and metabolic hormone is positively correlated with BMI. AMH has been shown to have negative correlation with fasting glucose, HOMA IR and BMI. In this study, we found that mean fasting insulin was significantly higher in obese PCOS patients (P= 0.01) and with AMH < 5ng/ml. This suggests that decrease AMH levels may have a significant role in metabolic syndrome and obesity in PCOS women, which would augment androgen production in them [15,16]. Low AMH levels have been predicted as a marker for greater risk of metabolic syndrome, single unit decrease in AMH was associated with 11 % increase in metabolic syndrome [17]. We found no significant difference in mean fasting insulin between obese and non-obese cases in groups with elevated AMH levels (P=0.12 & P=0.75). A recent study by Sahmay et al., also found that serum AMH levels between women with IR and without IR in PCOS were not significantly different and their study revealed no significant correlation between serum AMH levels and IR in women with PCOS [18]. We did not find any significant difference in fasting blood glucose between obese and non-obese PCOS subjects in any of our AMH groups. Since FBG has been shown to have a significant influence on AMH, it

was for this reason only normoglycemic PCOS subjects were enrolled for the present study and elevated serum glucose would have given false positive results.

The role of ovarian hormones in deranged lipid levels and developing coronary artery disease has been well established in PCOS females. A longitudinal study including 1,015 premenopausal PCOS women showed lower AMH levels were associated with high LDL-C and triglycerides. We also found that the mean LDL-c was significantly elevated (P=0.01) in non-obese PCOS patients with AMH levels less than 5ng/ml. This difference was not observed in other AMH groups. Positive correlation between AMH and LDL-c has also been described in a cross-section study of 252 women aged 18-46 years with PCOS [17]. Mean HDL-c were statistically non-significant and were comparable in all the AMH groups, this is in contrast to a cross-sectional study of 951 healthy reproductive-age women that showed low AMH levels associated with decreased HDL-C and higher waist circumference [19].

The mean serum LH was significantly elevated in our non-obese patients with AMH < 10ng/ml when compared to the same group obese patients. The LH levels were comparable between obese and lean groups where AMH was elevated i.e. > 10ng/ml. AMH and LH have been shown to be positively correlated, higher LH levels have been noted in normal weight PCOS patients than in overweight or obese PCOS patients [20]. In obese PCOS, women LH concentrations result in increased aromatization of androgens to estrogens in the peripheral fat tissue, resulting in LH suppression [21]. Also, obesity may reduce ovarian potential and affect the AMH catabolism.

Most women with PCOS exhibit higher levels of circulating luteinizing hormone, suggesting increase in frequency of Gonadotropin-Releasing Hormone (GnRH) release and AMH as compared to healthy women [22,23]. It is unclear whether this defect is primary or secondary to other parameters of PCOS, but recent study has shown that GnRH-positive neurons express AMH receptors and that exogenous AMH potently increases GnRH neuron firing and GnRH release in murine living tissue explants [22].

Conclusion

Our study suggests that low AMH may be an important marker for PCOS irrespective of BMI and should be routinely measured for diagnosis as well as for the management of PCOS. However, further studies on larger sample size are required to explain the effects of AMH levels on various parameters in PCOS females. Strength of our study was that we included normoglycemic PCOS subjects thus eliminating the potential influence of elevated glucose on AMH levels and to the best of our knowledge, this is the first study from our region that compared AMH levels in obese and non-obese PCOS women.

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