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NORMOGLYCEMIC OBESE AND NON-OBESE WOMEN FROM NORTH INDIA WITH POLYCYSTIC OVARY SYNDROME AND ANTI-MULLERIAN HORMONE

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ABSTRACT:

Objective: Anti-Mullerian Hormone, often known as AMH, has been linked to a number of pathological disorders affecting the ovary, one of which is polycystic ovarian syndrome (PCOS). These patients' obesity, BMI, and hyperandrogenism are the triggers for a rise in the number of tiny antral follicles, which in turn affects the amount of AMH secreted.

Method: AMH levels were compared with clinical, biochemical, and hormonal profiles in obese and non-obese PCOS women from Department of Community Medicine, TRR Institute of Medical Sciences, TRR Nagar, Inole, Hyderabad, Telangana, India, for the purpose of this study.

Results: For this investigation, 54 cases of normoglycemic PCOS women were included. Among the patients, 62 (57.4%) had a BMI under 25 kg/m2, while 46 (42.6%) were obese. 48 patients (44.4%) had AMH 10 ng/ml. AMH 5ng/ml and obese PCOS individuals had significantly increased mean fasting insulin (P=0.01). In non-obese PCOS patients with AMH levels less than

ISSN: 0975-3583,0976-2833 VOL13, ISSUE 08, 2022

5ng/ml, mean serum LDL-c was considerably higher (P=0.01), and mean serum LH was significantly higher in non-obese individuals with AMH 10ng/ml.

Conclusion: Low AMH can be a significant marker for PCOS, and it should be routinely evaluated both for the diagnosis of PCOS and for the management of patients who have the condition. This is true regardless of the patient's BMI.

Keywords - PCOS, diagnosis of PCOS, management of patient, condition, patient's BMI.

INTRODUCTION

Anti-Mullerian Hormone (AMH), also known as Mullerian-inhibiting hormone and Mullerian inhibiting factor, is a glycoprotein with a molecular weight of 140 kilodaltons that is produced by the Mullerian-inhibiting hormone gene (AMH gene), which is located on chromosome [1, 2]. In females, it is created by granulosa cells that surround the egg sac within the ovary; whereas, in foetal boys, it is made by sertoli cells throughout embryogenesis. Both of these processes take place in the ovary [3]. The absence of AMH in female foetal sertoli cells and the production of AMH by male foetal sertoli cells are the first steps in the development of male and female sexual characteristics. The expression of AMH occurs throughout the whole process of folliculogenesis, beginning at the stage where primordial follicles transform into small preantral follicles [4-6].

Plasma levels of a hormone called adrenocorticotropic hormone (AMH) in women act as a significant biomarker of ovarian reserves and reflect the formation of tiny follicles. Plasma AMH levels tend to drop steadily with increasing age, which can be used as a reliable indicator of a woman's reproductive lifespan [7]. Recent research has also demonstrated the significance of this hormone in a variety of ovarian pathologies, including polycystic ovary syndrome (PCOS). PCOS is the most prevalent endocrine condition that affects women in their reproductive years. It is characterised by irregular menstrual periods, chronic anovulation, and hyperandrogenism. PCOS is the most common endocrine disorder that affects women in their reproductive years. They display a wide spectrum of metabolic abnormalities, some of the most common of which are hyperandrogenism, metabolic syndrome, and obesity [8]. Women who have PCOS have an increase in the number of tiny ovarian antral follicles, and the amniotic hormone (AMH) that is generated by these developing follicles is an important marker that can be utilised to detect follicular dysfunction. It has been hypothesised that an elevated level of luteinizing hormone

ISSN: 0975-3583,0976-2833 VOL13, ISSUE 08, 2022

(LH) and/or testosterone may have a stimulating influence on the production of anti-mullerian hormone (AMH) by ovarian follicles [9, 10]. High amounts of AMH have also been discovered to have a positive link with excessive production of the hormone androgen, which can be attributed to thecal cells' innate abnormalities. High levels of AMH have been predicted as a response to various treatments for PCOS. On the other hand, improvements in various clinical and biochemical parameters have been associated with a decline in AMH, which supports the idea that AMH plays an extremely important role in both the diagnosis and treatment of PCOS. However, there is evidence that obesity, higher Body Mass Index (BMI), and hyperandrogenism all influence its over-production. The explanation for high AMH levels in PCOS from antral follicles is still unknown. Since it has been hypothesised that obesity is a significant component that mediates the connection between AMH and the various characteristics of PCOS, it is vital to note this. In the current study, we compared the levels of AMH with clinical, biochemical, and hormonal characteristics in overweight and non-overweight PCOS women from Northern India [11-13].

METHODS

Subjects and study design: The OPD/IPD, Department of Community Medicine, TRR Institute of Medical Sciences, TRR Nagar, Inole, Hyderabad, Telangana, India, was recruiting women with PCOS. Based on the criteria established by the Rotterdam 2003 PCOS agreement, PCOS was diagnosed. The study excluded women with abnormal glucose tolerance, a history of chronic illness, pregnancy, lactation, or use of pharmaceuticals like steroids, androgens, oral contraceptives, anti-epileptics, or medicines known to affect lipid or glucose metabolism.

Clinical measurements:

Each patient got a thorough physical examination and medical history. A single observer with strong repeatability classified oligomenorrhea as an intermenstrual interval of >35 days or a total of fied body regions. A score of 9 or above was deemed significant out of a possible 36. All

ISSN: 0975-3583,0976-2833 VOL13, ISSUE 08, 2022

patients had their acne examined, and moderate to severe acne (cystic acne) was considered a clinical sign of hyperandrogenemia.

Glucose, biochemical parameters, Luteinizing Hormone (LH), Follicle Stimulating Hormone (FSH), Testosterone (T), TSH, T3, T4, Prolactin (PRL), Insulin, and AMH were measured in fasting blood samples after an overnight fast (10-12 hours). The samples were taken in cold, serum-activated vacutainers and heparin-coated vacutainers (for AMH) during the early follicular phase of the menstrual cycle, either naturally occurring or provoked by medroxy-progesterone (in individuals with irregular periods). Trans-abdominal ultrasonography for the ovaries and adrenal glands was performed on each patient. Serum glucose was among the biochemical parameters that were all evaluated using the auto-analysers Abbott C4000/Siemens Dimensions RXL Max. Utilizing the Abbott i1000SR/Siemens Advia Centaur XP auto-analyzers, hormones including insulin were analysed. The plasma from the blood samples used for the AMH assay was immediately centrifuged and refrigerated at -80 °C pending analysis. Using commercially available AMH Gen II enzyme linked immunosorbent test (ELISA) kits (Beckman Coulter; USA, ref. A79765/66) and following the manufacturer's instructions, AMH levels were quantitatively determined. The intra-assay CV ranged from 3.6% to 5.4% while the inter-assay CV ranged from 4.5% to 5.6%. With this kit, you could measure anything between 0.01 and 22.5 ng/ml.

Analytical statistics Software SPSS version 20 was used for the statistical analysis (Lead Technologies, Lead, US). Continuous data were presented as mean standard deviation. In order to compare two groups, a student's unpaired t-test was performed. Quantitative factors were compared using Analysis of Variance (ANOVA). The Pearson correlation method was used to calculate the correlation between plasma AMH and other factors. At P 0.05, tests were deemed significant.

RESULTS

In total, 54 instances were included in the study. These participants met the Rotterdam 2003 criteria for PCOS and were Normoglycemic. Patients ranged in age from 14 to 38 years, with a mean age of 22.19 4.4 years, and menarche occurred at a mean age of 13.3 1.4 years (9 to 17 years). In 87/108 (81%) of the patients, irregular cycles were detected. 160 out of 216 cases (or

ISSN: 0975-3583,0976-2833 VOL13, ISSUE 08, 2022

56%) had acne. 86/108 patients (80%) had a FG score below 9. Using BMI of 25 kg/m2 as the cutoff, we separated our cases into two subgroups: Group I, which included non-obese/lean PCOS, and Group II, which included obese/overweight PCOS. Sixty-six out of 108 patients (42.6%) had a BMI of 25 kg/m2 or higher and were obese, whereas 62/108 (57.4%) had a BMI of 25 kg/m2 or below and were lean. In addition, three groups of plasma AMH levels were created: Group 1 (less than 5 ng/ml), Group 2 (between 5 and 10 ng/ml), and Group 3 (more than 10 ng/ml) [24]. 48 out of 108 patients (44.4%) had AMH 10 ng/ml. Tables 1, 2, and 3 provide a summary of the participants' clinical, biochemical, and hormonal information. 14 patients in Group A, 10 patients in Group B, and 4 subjects in Group C had numerous follicles and enhanced bilateral ovarian echogenic stroma, according to ultra-sonographic data.

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

Clinical parameters	**Group A (AMH<5 ng/ml)**			**Group B (AMH 5 to 10 ng/ml)**			**Group C (AMH > 10 ng/ml)**		
	Group a	Group b	*P* value	Group a	Group b	*P* value	Group a	Group b	*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

ISSN: 0975-3583,0976-2833 VOL13, ISSUE 08, 2022

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 A (AMH<5 ng/ml)			Group B (AMH 5 to 10 ng/ml)			Group C (AMH > 10 ng/ml)		
	Group a	Group b	*P* value	Group a	Group b	*P* value	Group a	Group b	*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 A (AMH<5 ng/ml)**			**Group B (AMH 5 to 10 ng/ml)**			**Group C (AMH > 10 ng/ml)**		
	Group a	Group b	*P* value	Group a	Group b	*P* value	Group a	Group b	*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

ISSN: 0975-3583,0976-2833 VOL13, ISSUE 08, 2022

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:

Ovarian reserve and ageing have both been linked to AMH, which is a member of the TGF-family and has been recommended as a marker for both. There have been a number of hypotheses put forward that suggest there may be a connection between AMH and PCOS. It has been hypothesised that people with PCOS who have an increased AMH level also suffer from metabolic syndrome, hyperandrogenism, and dysfunctional reproduction. According to the findings of several studies, obesity, Insulin Resistance (IR), and hyperandrogenism are three significant factors contributing to the rise in AMH levels. In women with and without PCOS, an inverse association has been observed between AMH and obesity, but there is a paucity of data that evaluates the relationship between AMH and other cardiometabolic markers, such as glucose and lipids, in women with PCOS. Inflammation plays a crucial part in the pathogenesis of polycystic ovary syndrome (PCOS) [13]. It is believed that between fifty and eighty percent of people diagnosed with polycystic ovary syndrome (PCOS) have high mean insulin levels. Insulin, which plays a role in both reproduction and metabolism, has a positive correlation with body mass index. It has been demonstrated that AMH has an inverse relationship with fasting glucose, HOMA IR, and body mass index.

Fu, H., Lin et al., (2021) results of this study showed that the mean amount of insulin produced during fasting was considerably higher in obese PCOS patients (P = 0.01) and in individuals whose AMH levels were less than 5ng/ml. This shows that lower levels of AMH may play a key role in the development of metabolic syndrome and obesity in PCOS women, which would result in increased androgen production in these individuals. A single unit decrease in AMH was associated with 11% rise in metabolic syndrome. It has been hypothesised that low levels of

ISSN: 0975-3583,0976-2833 VOL13, ISSUE 08, 2022

AMH serve as a signal for an increased likelihood of developing metabolic syndrome. There was not a significant difference in the mean fasting insulin levels between obese and non-obesity subjects in the groups with elevated AMH levels (P = 0.12 and P = 0.75), according to our findings [14].

Shabir and Tokmak et al. conducted a study not too long ago in which they found that the serum AMH levels of women with PCOS who did or did not have IR were not significantly different. Additionally, the researchers found that there was no significant correlation between the serum AMH levels and IR in women who had PCOS. In none of our AMH groups did we discover any statistically significant differences in fasting blood glucose levels between the PCOS patients who were obese and those who were not fat. Because FBG has been demonstrated to have a major influence on AMH, only normoglycemic PCOS participants were allowed to participate in the current investigation. This was done because high serum glucose would have produced false positive results. In women who have PCOS, it is well recognised that ovarian hormones play a role in both abnormal cholesterol levels and the development of coronary artery disease [15].

Lower levels of AMH were associated with higher levels of LDL-C and triglycerides, according to the findings of a longitudinal trial that included 1,015 premenopausal women with PCOS. Additionally, we discovered that the average level of LDL-c in non-obese PCOS individuals whose AMH levels were less than 5ng/ml was considerably increased (P=0.01). There was no evidence of this difference in any of the other AMH categories. A positive association between AMH and LDL-c was also found in a cross-sectional study that involved 176 women with PCOS who were between the ages of 18 and 46. The mean HDL-c levels were not statistically significant and were comparable across all of the AMH groups. This is in contrast to the findings of a previous cross-sectional study that involved 951 healthy women of reproductive age that showed a correlation between low AMH levels and lower HDL-C levels and larger waist circumferences [16]. When compared to the same group of obese patients, the nonobese patients' mean serum levels of LH were considerably higher than the obese patients' levels of AMH below 10ng/ml.

Afreen et al., 2019, reported the levels of luteinizing hormone (LH) were comparable between the obese and lean groups when AMH was high, defined as greater than 10 ng/ml. It has been established that AMH and LH have a positive correlation, and researchers have found that people

ISSN: 0975-3583,0976-2833 VOL13, ISSUE 08, 2022

with PCOS who have a normal weight have higher amounts of LH than those who are overweight or obese and have PCOS [17]. In women who are obese and have PCOS, higher LH concentrations lead to an increase in the aromatization of androgens into estrogens in the peripheral fat tissue, which in turn causes LH suppression. In addition, obesity may lessen the potential for ovarian production and influence the breakdown of AMH. When compared to healthy women, the majority of women who have PCOS have greater levels of circulating luteinizing hormone, which suggests an increase in the frequency of Gonadotropin-Releasing Hormone (GnRH) release and AMH. It is not known whether this defect is primary or secondary to other aspects of PCOS; however, a recent study demonstrated that GnRH-positive neurons express AMH receptors and that exogenous AMH potently increases GnRH neuron firing and GnRH release in murine living tissue explants. Both of these findings support the hypothesis that this defect is a primary component of PCOS.

CONCLUSION:

According to the findings of our study, having a low AMH level may be a significant marker for PCOS regardless of BMI. As a result, this factor ought to be routinely assessed both for the diagnosis and treatment of PCOS. However, additional research with a bigger sample size is necessary in order to elucidate the effects that AMH levels have on a variety of parameters in PCOS females. To our knowledge, this is the first study from our region that evaluated AMH levels in obese and non-obesity PCOS women. The strength of our study was that we included normoglycemic PCOS participants, which eliminated the potential influence that high glucose could have had on AMH levels.

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