Original Article

Anti-Mullerian Hormone as A Diagnostic Tool for PCOS Patient : Study in a Tertiary Level Hospital

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Abstract:

Polycystic ovary syndrome (PCOS) is a frequently encountered problem affecting 6-11% women of reproductive age.[1] Purpose of the study was to determine whether serum AMH level can be used to diagnose PCOS.

Methods: It was a cross sectional study conducted among 55 sub-fertile women of reproductive age (18-35 year) in a tertiary level hospital during the period of January 2018 to December 2018. The study subjects were divided into group I (PCOS patient with subfertility by Rotterdam Criteria 2003) and group II (non PCOS subfertile patients of reproductive age). Menstrual history, obstetrical history, physical examination, clinical assessment of androgenesis, ovarian ultrasound assessment and level of AMH, FSH, LH were collected.

Result: Twenty five PCOS & 30 non PCOS sub-fertile patients were recruited. Mean age in PCOS & non PCOS were 25.24±4.03 years and 27.8±5.01 years respectively. The mean serum AMH in PCOS was 11.03±3.78 ng/ml and in non PCOS was 3.93±1.92 ng/ml, their difference was statistically significant.

Conclusion: AMH can be used as a diagnostic aid for PCOS.

Introduction:

Subfertility defined as an inability of couple to conceive after one year of unprotected intercourse. The most common cause of treatable subfertility is polycystic ovarian syndrome (PCO) affecting approximately 6-11% of women in reproductive age.[1] Abnormality of reproductive hormones can trigger anovulatory cycle resulting in infertility and menstrual disorders.[2] Based on the 2003 Rotterdam consensus, the three diagnostic criteria of PCOS are (i) oligomenorrhea/anovulatory cycle (ii) clinical or biochemical hyperandrogenism (iii) ultrasound feature of PCOS [3] a diagnosis is made in the presence of at least 2 out of these 3 criteria. Based on these criteria we were able to categorize patients into two groups, group I (PCOS by Rotterdam criteria 2003) and

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group II (non PCOS patient of reproductive age group). Anti-Mullerian hormone (AMH) has a glycoprotein dimer structure and is a member of transforming growth factor-b (TGF.b) family. AMH is produced by the granulosa cells surrounding preantral and antral follicles and has an important role in the development and maturation of follicles.[2] Several studies have suggested that AMH serum levels may be marker for PCOS. As the diagnostic criteria for ultrasound findings is the presence of more than 12 follicles with a diameter of (2-9) mm or when the ovarian volume is more than 10 cm³, it may correlate with AMH.

The level of AMH circulating in the blood is not affected by the menstrual cycle nor altered during use of oral contraceptives therefore it can be used as a potential biological marker for PCOS.[4] AMH expression occurs after development of the follicle and continues through the preantral and small antral follicles. AMH expression then decreases with the selection of follicles for dominance and is no longer expressed during the FSH dependent stages of follicular growth or in atretic follicle.[5] AMH has an inhibitory effect on cyclic follicular recruitment by reducing the follicular sensitivity to FSH.[6] AMH also reduce the number of LH receptor in granulosa cells.[7]

PCOS patients have barrier that keep follicles in preantral and small antral phase (those produce AMH) preventing them to become dominant follicle. Thus serum AMA

become (2-4) fold higher in PCOS women than healthy women of same age and begin to decline five years later than non PCOS.[8] Weerakiet's et al. stated that AMH plasma level can be a marker of the degree to which follicular genesis is impaired in PCOS.[9] AMH also inhibit the activity of aromatase enzyme, suggesting that AMH can contribute to severity of PCOS.[10]

A study by Dewailly et al. indicated that AMH may also be used as a surrogate marker of classical hyper-androgenism.[11] Skalba et al. found significant differences in AMH and LH in PCOS patient.

Currently there are no studies in Bangladesh focusing association between PCOS Phenotype and AMH level so the purpose of this study was to assess the AMH level in subfertile PCOS patient and non PCOS subfertile patient to determine the relationship of serum AMH level with clinical parameter, hormonal level and ultrasound feature of PCOS patients.

Materials and method:

This cross sectional observational study was performed among Outpatient Department and admitted patients of Gynae and Obs. department of Kurmitola General Hospital from January 2018 to December 2018 over a period of one year. Study included 55 subfertile patients of 18-35 year of age and was divided into 2 groups. Group I (subfertile PCOS patient according to Rotterdam criteria 2003) and group II (subfertile non PCOS, normo ovulating patients). Rotterdam criteria required at least 2 out of the 3 following characteristics: (i) oligomenorrhea/ anovulution (ii) clinical Hyperandrogenism (defining by hirsutism, acne, alopecia acanthosisnigrican and obesity) (iii) TVS findings of PCOS as (AFC ³ 12, ovarian volume ³ 10cm). Clinical evaluation and ultrasound examination were performed and findings of laboratory examination of FSH, LH, AMH were collected.

Group II non PCOS patients were women without endometriosis, cyst or other gynaecological disorders. They had regular menstrual cycle (26-35) days, did not have endocrine abnormality, (Prolactin, TSH, FSH) at normal level and not in a state of clinical Hyperandrogenism and morphologically normal ovaries. Secondary data from medical records were used to obtain data on the subject's menstrual cycle, physical examination and laboratory. AMH level measured by enzyme-linked immunosorbent assay (ELISA) with units of ng/ml. Normal weight was defined as BMI value (18.50-24.9) (kg/m²) and over-weight BMI> 25 kg/m².[12] Others components measured were FSH, LH, PRL. AMH level. Data were analysed using SPSS 12.0.

Result:

Fifty five sub-fertile women of reproductive age 18 – 35 years were recruited. Among them 25 were PCOS (group I) and 30 were non PCOS (group II). The mean age of PCOS and non PCOS group were 25.24±4.03 years versus 27.8±5.01 years respectively (p = 0.0445). Forty percent women in PCOS group were aged 21-25 year while non PCOS group most (54.25%) were in 26-30 year group. Duration of sub-fertility was 7.04 ± 3.11 year among PCOS patients and 5.33 ± 2.66 years among non PCOS (p = 0.0323) patients. The body mass index (BMI) of PCOS patient were significantly higher 25.40±3.48 kg/m² compared to non PCOS patient 22.63±2.77 kg/m² (p= 0.002). Oligomenorrhea, clinical Hyperandrogenism and obesity were found significantly more among PCOS patient, 68 %, 28%, 32% respectively; whereas in non PCOS group the rates were 23.33%, 6.67% and 6.67% respectively (p = 0.0018, 0.033, 0.015 respectively).

Mean Elevated LH level was significantly higher in PCOS group than non PCOS group; 10.15 ± 3.32 versus 4.45 ± 1.28 (p <0.0001). There was no statistically significant difference between two groups in terms of LH/FSH ratio (2.07 ± 1.06 versus 1.74 ± 0.67). Serum AMH was significantly higher in PCOS than non PCOS (11.03 ± 3.78 ng/ml vs 3.93 ± 1.92 ng/ml)

Table I

Distribution of the study subject according to age (n=55)

Age	PCOS (n=25)	Non PCOS (n=30)	p-value
18 – 20	5 (20.0)	3 (10.0)	
21 – 25	10 (40.0)	6 (20.0)	
26 – 30	8 (32.0)	14 (46.7)	
31 - 35	2 (8.0)	7 (23.3)	
Mean±SD	25.24 ± 4.03	27.80 ± 5.01	0.0445

Table II

Oligomenorrhea, Hyperandrogenism and obesity of the study subjects (n=55)

	PCOS (n=25)	Non PCOS (n=30)	p-value
Oligomenorrhea	17 (68.0)	7 (23.3)	0.001
Hyperandrogenism	7 (28.0)	2 (6.7)	0.033
Obesity	8 (32.0)	2 (6.7)	0.015

Table III

BMI, duration of sub-fertility, AMH, LH, FSH and LH/FSH ratio in PCOS and Non-PCOS study subjects (n=55)

	PCOS (n=25)	Non PCOS (n=30)	p-value
BMI (Kg/m^2)	25.40 ± 3.48	22.63 ± 2.77	0.002
Duration of sub-fertility (years)	7.04 ± 3.11	5.33 ± 2.66	0.0323
AMH (ng/ml)	11.03 ± 3.78	3.93 ± 1.92	<0.0001
LH	10.15 ± 3.32	4.45 ± 1.28	<0.0001
FSH	5.53 ± 2.51	5.27 ± 1.86	0.6612
LH/FSH	2.07 ± 1.06	1.74 ± 0.67	0.1665

Unpaired t test was done

Here, AUC of AMH is 0.928. Optimal sensitivity and specificity were achieved at a cutoff level 4.85.
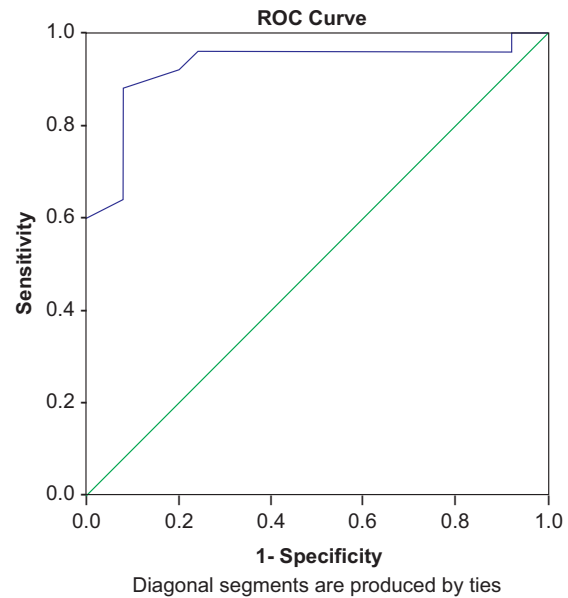

Diagonal segments are produced by ties

Fig.-1: *ROC curve of AMH in predicting PCOS. AUC=0.928.*

Table IV

Level of AMH in predicting PCOS (n=55)

AMH	PCOS(n=25)	Non PCOS(n=30)	Total	OR	p-value
≥4.85	21 (84.0)	10 (33.3)	31 (56.4)	10.5	<0.001
< 4.85	4 (16.0)	20 (66.7)	24 (43.6)	(2.82-38.96)	
Total	25 (100.0)	30 (100.0)	55 (100.0)		

Chi-square test was done

Discussion:

In this study the average age of PCOS patients was significantly lower than non PCOS patient (p=0.0445). This finding is consistent with studies of Rousseau et al.[13] and John et al. [14] who both reported that the proportion of women with PCOS decrease with age. This can be caused by decrease in number of AFC throughout the reproductive years that occurs in normal women, a phenomenon that also applies to patients with PCOS. In our study a significant increase in mean BMI (25.4±3.48) was found in women with PCOS when compared to non PCOS patient (22.63±2.77) (p=0.002). This finding is consistent with the study that found higher BMI in women with PCOS than in women without PCOS.[15]

Obesity and overweight have negative impact on consequence of PCOS[16] some studies evaluated between weight loss and serum AMH level. Nybacka et al.[17] described a significant decrease in serum AMH level after

diet in obese women with PCOS. Moran et al. showed a better menstrual improvement after weight loss in women with lower baseline serum AMH[18]. So, serum AMH could be used as a potential predictive factor of menstrual improvement with weight loss in PCOS.

In our study LH level was significantly higher in PCOS patient (10.15±3.32) than non PCOS patients (4.45±1.28) (p=0.0001). Piltonen et al. stated that there was an apparent correlation between AMH and LH because they found that LH was elevated in patients with PCOS who also and very high AMA level. [19, 20, 21]

We found a significantly higher serum AMH level (11.03±3.78 ng/ml) in PCOS women compared to non PCOS women (3.93±1.92 ng/ml). The OR of serum AMH level was 10.5 meaning that patients with higher AMH level (4.85 ng/ml) have 10.5 times higher possibility to suffer from PCOS compared for patients with low AMH. This finding has consistently been reported in numerous

studies.[12,19,20] This increase is due to increased synthesis and secretion of AMH by polycystic ovaries.[12] Pellat et al. reported that AMH production increase approximately 75 times higher in each polycystic ovarian granulosa cell.[21] Elevated serum AMH level in PCOS patients may also be caused by disturbances in folliclegenesis resulting in accumulation of excessive preantral and small antral follicle[22] cessation of antral follicle development toward the dominant follicle is due to suppression of aromatase activity by AMH and by lower follicle sensitivity to FSH.[23,24] When USG cannot provide accurate data, the level of AMH many be used to replace the number of follicles as a diagnostic criterion.[25] In addition measurement of serum AMH level may also be used as an indicator of PCOS patients' response to therapeutic approaches,[26] including ovarian response to clomiphene citrate,[27] evaluation after treatment with insulin sensitizers[20] and monitoring after laparoscopic ovarian drilling.[28]

Conclusion:

This study shows that serum AMH levels can be used as diagnostic and prognostic modalities in PCOS patients. However our sample size was relatively small for each group. So study to be continued with large sample size to evaluate AMH as a tool for diagnosis of PCOS.

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