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Serum Anti-Mullerian Hormone in Adolescents with PCOS- Correlation with Ultrasound Features

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Abstract

Study Objective: *Our study was undertaken to assess serum AMH levels in adolescents with PCOS and compare with controls and to evaluate the relationship between serum antimullerian hormone (AMH) levels and ovarian ultrasound features in adolescents with PCOS.*

Design: *This was a hospital based-Case control study.*

Setting: *Gynaecology outpatient department of a tertiary care hospital, May 2013 to July 2015.*

Participants: *169 Adolescent girls aged 13-19 years were included in the study.*

Interventions: *On day 3-5 of cycle, 5ml of venous blood was taken for serum AMH levels, transabdominal ultrasound was done and details of antral follicle count, distribution and ovarian volume were noted.*

Main outcome measures: *Serum AMH levels in adolescents with PCOS and controls. Evaluation of the relationship between serum antimullerian hormone (AMH) levels and ovarian ultrasound features in adolescents with PCOS.*

Results: *Serum Antimullerian hormone levels were elevated in cases [9.16(6.09-12.13)] when compared to the controls [5.8(3.67-7.57)] and this difference was statistically significant (p=0.0001). Serum AMH levels were not influenced by BMI or serum testosterone levels. Serum Anti Mullerian hormone levels had a positive relationship with the follicle count (p=0.0001) and peripheral follicle distribution (p=0.0001). There was a positive correlation between Serum AMH levels and mean ovarian volume(r=0.35, p=0.0001). Serum Anti Mullerian hormone levels for a derived cutoff value of $\geq$7.74 had a sensitivity of 58.82 %(95% CI, 47.62-69.39), specificity of 79.76 %(95% CI, 69.59-87.75), positive predictive value of 74.6%, negative predictive value of 65.6%, positive likelihood ratio being 2.91 and negative likelihood ratio of 0.52. Out of 45 cases with ultrasound features suggestive of PCOS, 27 had serum AMH levels $\geq$ 7.74. But, when we used AMH as a proxy marker for ultrasound features we were able to capture 27 of 45 cases contributing to approximately 60% yield.*

Conclusions: *Since, obtaining high-quality ovarian ultrasound is not possible transabominally and impaired due to obesity, serum AMH might serve as a surrogate marker for ovarian ultrasonographic features seen in PCOS. Hence serum AMH may therefore be a useful adjunct to assist in the diagnosis and follow-up of PCOS in adolescents.*

Keywords: *Serum AMH, Adolescents, PCOS*

1. INDRODUCTION

Polycystic ovary syndrome (PCOS) is one of the most prevalent endocrine-metabolic disorderafflicting females in the reproductive age group and often initially manifests inadolescence. In India,the prevalence of PCOS among the adolescents is 9.13% in the community.[1]Diagnosing PCOS in adolescents can be challenging as irregular menses is common following menarche, adolescents frequently suffer from acne and hirsutism may be significantly less prevalent in this age group. Therefore, the Rotterdam ultrasonographic (USG) criteria may lend particular credence to PCOS diagnosis in this population.

Unfortunately, obtaining optimal ultrasound is often impaired due toinability to perform transvaginal ultrasound in adolescents and obesity often prevents the attainment of high-quality transabominal USG.

So, a marker that correlates with the ovarian ultrasonographic features seen in PCOS would therefore be a particularly useful adjunct to assist in the diagnosis and/or follow-up of PCOS in adolescents.

In adult women it has already been demonstrated that serum antimullerian hormone (AMH) levels are raised in PCOS, it is closely associated with the follicle number and hence it has been proposed as a suitable biological marker.[2,3] Also in adolescents several studies [4-12] have shown serum AMH levels to be raised in girls with PCOS. Only one study[13] so far could not demonstrate any difference in AMH levels between PCOS girls and normal controls.

There are only two studies[4,9]in literature which tried to correlate the serum AMH levels with ovarian USG features of PCOS in adolescents. One study[9] showed positive correlation of AMH with all the three ultrasound features of PCOS namely follicle count, distribution and ovarian volume, whereas the other study[4] did not show positive correlation with follicle count. Apart from ovarian characteristics, studies have also demonstrated relationship of AMH with other two features used to define PCOS namely oligomenorrhea[6,7] and hirsutism.[4,9,10,12]

Due to the limited data available on relationship of AMH with ovarian characteristics in adolescents with PCOS, this study was proposed to evaluate the relationship between serum AMH levels and ovarian USG features in adolescents with polycystic ovary syndrome and compare it with controls.

2. Material And Methods

This study, a case control study was conducted in the Department of Obstetrics and Gynaecology in Jawaharlal Institute of Postgraduate Medical Education and Research, Puducherry from September 2013 to August 2015. The Ethics Committee of JIPMER, approved the study protocol. Adolescent girls aged 13-19 years were asked to participate. Girls fulfilling the inclusion criteria based on the menstrual history were recruited for the study if they were willing to participate.

2.1. Inclusion Criteria

2.1.1. Cases

Adolescent girls aged 13-19 years, postmenarchal, diagnosed as PCOS according to Rotterdam PCOS consensus criteria 2003[14] which require 2 out of the 3 following characteristics

1. Oligo/ anovulation

2. Clinical and/or biochemical signs of hyperandrogenism

3. Polycystic ovaries (12 or more follicles, 2 to 9 mm diameter and /or ovarian volume greater than 10 ml in at least one ovary)

2.1.2. Controls

Adolescent girls aged 13-19 years presenting to Gynaecology OPD with minor gynaecology complaints such as dysmenorrhea, white discharge per vaginum (WDPV), lower abdominal pain etc.

2.2. Exclusion Criteria

Girls with menstrual disorders, hyperandrogenism due to other endocrine causes such as Thyroid dysfunction, Hyperprolactinemia, Androgen secreting tumor, Cushing syndrome and Congenital adrenal hyperplasia.

3. Procedure

Since adolescent girls were the participants of this study, they and their parents were given brief information about this study and its purpose. After obtaining an informed consent from participants /their parents a detailed interview schedule containing socio-demographic details, medical/surgical/personal/ family history was administered. After the questionnaire was completed they were examined in the presence of a female staff nurse with privacy being maintained throughout. Physical examination including height, weight, BMI (body mass index), Acanthosis nigricans, thyromegaly, secondary sexual characteristics, hirsutism score and abdominal examination was carried out. The procedure followed was in accordance with the ethical standards of of the responsible committee on human experimenatation or with the Helsinki decalaration of 1975 as revised in 1983.

3.1. Parameters Studied

3.1.1. Serum AMH Levels

On day 3-5 of cycle, 5ml of venous blood was drawn from ante cubital vein of the subjects for

serum AMH levels, which was measured by ELISA (Enzyme linked Immmunosorbent assay) kits. Following collection, serum samples for AMH were stored at −20° C until hormone analyses were performed. Serum AMH was measured in duplicate using an ultrasensitive ELISA (Diagnostic Systems Laboratory, distributed by Beckman-Coulter) in accordance with the manufacturer's instructions. All serum AMH measurements were performed in the same lab using the same assay.

3.1.2. Antral Follicle Count, Follicle Distribution And Ovarian Volume On Transabdominal USG

Transabdominal USG was done using GE VOLUSON E8 with 2-5 MHz probes on day 3-5 of cycle. As transabdominal USG limits follicle visualization, the follicle count was described as'few' when less than 5, 'moderate' when between 5 and 12 and 'multiple'when >12 . Follicle distribution was described as central, peripheral or mixed. Maximal dimensions of the ovary in three planes (longitudinal, transverse and anteroposterior) was measured using calipers and ovarian volume was calculated using USG software. An ovarian volume of more than 10ml was considered enlarged. The ultrasound criteria for Polycystic ovaries were 12 or more subcaspular follicular cysts, 2-9 mm in diameter and /or increased ovarian volume >10 ml in at least one ovary.

3.1.3. Sample Size Calculation

We calculated sample size using OpenEpi, version 3 software. The standard deviation of serum AMH levels in PCOS and control groups was 3 and 1 ng/ml according to a study by Pawelczak et al 2012.[4] We estimated that seventy nine patients in each group would be required to achieve 80% power; α=0.05 and 2 sided 95% CI to detect a mean difference of 1ng/ml in serum AMH levels. Assuming 10% attrition/ wastage, 87 patients were studied in each group.

3.1.4. Statistical Analysis

Profile of participants are summarized in terms of percentages for categorical variables like education, menstrual problems, presence of clinical hyperandrogenism, follicle density, follicle distribution and continuous variables like age, BMI, menstrual flow and ovarian volumes are summarized in terms of mean (± SD). Since majority of the hormone profiles did not follow assumptions for normal distribution, hormonal levels are presented in terms of median and inter quartile range. Difference in Anti Mullerian hormone levels among cases and controls were compared using Wilcoxon Rank sum test. Statistical significance was considered at <0.05 level. Similarly other hormonal profiles were compared by Wilcoxon Ranksum test if the variables were not following normal distribution. In case of variables following normal distribution, they were compared using unpaired't' test.

Distribution of ovarian follicle and follicular density patterns with Anti Mullerian hormone levels were compared with either Pearson chi square or Fischer exact test.

Considering presence of any of the two following features as existing standards for diagnosis of PCOS (Clinical hyperandrogenism, oligomenorrhea and USG features of PCOS), true positive (sensitivity) and false positive (1-specificity) proportions classified at various AMH cut off levels were plotted. Area under the curve (AUC) for Anti Mullerian hormones were reported from the plotted values. The best cut off was selected by choosing the measure at which maximum diagnostic accuracy (proportions correctly classified) was found. For this selected cut off (AMH≥7.74) sensitivity, specificity and predictive values are presented as percentages. Analysis was done using SPSS version 16 software.

4. RESULTS

Majority of the girls belonged to the age group of 17 – 19 years in both the groups (65.8% among cases and 80.9% among controls), with a range of 13 – 19 years. As a whole, 21.3% of the study population had education upto high school, 42% upto higher secondary, and 36.6% were doing their graduation. Majority of the cases, 64.7% had either completed or completing higher secondary school education. Majority of the controls (64.2%) were graduates. There was a significant difference in the educational status between the two groups (p = 0.0001).

Most common complaint reported in PCOS girls was oligomenorrhea in 92.9% followed by secondary amenorrhea (3.5%) and hirsutism (3.5%). In the control group girls had presented with lower abdominal pain (38%) followed by white discharge per vaginum (35.7%), dysmenorrhea (25 %) and one had breast lump (1.1%).

Majority of girls in the cases group (44.71%) and control group (35.7%) had menarche at 12

years of age with minimum and maximum age being 10 years and 15 years respectively. In cases of PCOS Oligomenorrhea with PCOS changes on ultrasound was the most common phenotype seen in 32.9%, oligomenorrhea with hyperandrogenism & ultrasonographic changes of PCOS were seen in 24.7%, Oligomenorrhea + Hyperandrogenism/ Hyperandrogenism and PCOS was seen in 21.1% each. 22.3% of the cases had scanty flow, 72.9% had normal flow, and 4% of the cases had heavy flow. In the controls 2.3% had scanty flow and another 2.3% had heavy flow.

There was no significant difference between the mean heights between the cases when compared to the controls. But significance difference was noted in weight and BMI between cases and controls. A significantly higher proportion of cases were found to be overweight and obese when compared to the control group. (Table1) The prevalence of clinical hyperandrogenism was 54.1% among the cases. Subjects with clinical hyperandrogenism had Ferrimann Galleway score $\geq$ 7. None of the controls had clinical hyperandrogenism. 27% of the cases had acanthosis nigricans, when compared to 4.7% of the controls.

Table1. *Classification of participants according to BMI*

BMI	Cases n (%)	Controls n (%)	Total n (%)
Underweight (<18.5)	18 (21.2%)	25 (29.8%)	43 (25.4%)
Normal (18.5 – 22.99)	34 (40%)	46 (54.8%)	80 (47.3%)
Overweight &obese (≥23)	33 (38.8%)	13 (15.5%)	46 (27.2%)
Mean ± SD, kg/m2	22.7 ± 4.9	20.3 ± 3.6	0.0005

p value= 0.003

Serum testosterone level had a median value of 45.7 pg/ml with the inter quartile range, IQR (28.8 – 65.2) when compared to controls who had a median value of 35.4 pg/ml with the IQR (24 – 46). There was a statistically significant difference between the serum testosterone levels of both the groups (p=0.0021). There was no significant difference between the serum levels of prolactin (p=0.157), FSH (p=0.063), LH (p=0.133) and TSH (p=0.24).

Serum Anti Mullerian Hormone levels had a median of 9.165 ng/ml, IQR (6.09-12.13) in PCOS group, whereas in controls the median was 5.80 ng/ml, IQR (3.67-7.57). The serum AMH levels were significantly higher in the cases group when compared to controls (p=0.0001) .The mean value of Serum AMH levels among cases was 9.69 ± 5.15 and it ranged between 0.94 and 27.26 ng/ml. Among controls, mean value of serum AMH was 5.83 ± 3.4 and it ranged between 0.003 and 16.8 ng/ml.

Comparing the total number of follicles seen on ultrasound between the cases and controls, majority (94%) of the cases had multiple follicles (>12), whereas majority of the controls (78%) had only few (<5) follicles. 80% of the cases had follicles distributed peripherally, whereas 75 % of the controls had mixed distribution (both central & peripheral). The mean ovarian volume among the cases was 11.47 ± 4.06 ml, which was higher when compared to 4.60 ± 1.38 ml in controls.

4.1. Serum AMH Levels and Ovarian Ultrasound Characteristics in Adolescents with PCOS

Serum Anti Mullerian Hormone levels have a positive relationship with the follicle count and with peripherally distributed follicles. As seen in Figure1,

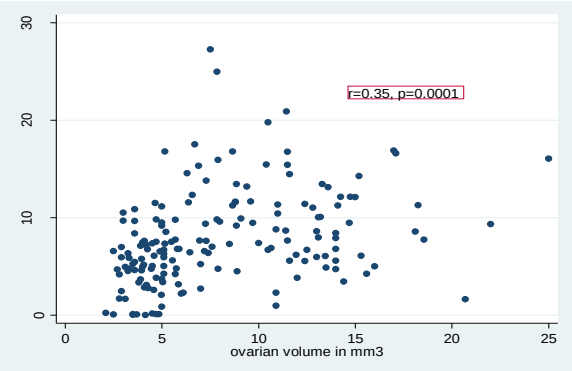

Figure1. *Correlation between AMH levels and mean ovarian volume*

Serum AMH levels positively correlate with mean ovarian volume (r=0.35) which is statistically significant (p = 0.0001).

Table2. *The relationship between serum AMH levels and follicle count / distribution in adolescents with PCOS*

	Serum AMH Levels ng/ml Median (IQR)	p value*
Follicle count		
Few (< 5)	5.19 (3.07 – 7.25)	0.0001
Moderate(5 – 12)	7.66 (6.67 – 11.16)	0.0001
Multiple(>12)	9.19 (6.05 – 12.13)	0.0001
Follicle distribution		
Central	2.44 (0.08 – 6.1)	0.0001
Mixed	6.55 (4.51 – 9.19)	0.0001
Peripheral	8.58 (5.58 – 13.13)	0.0001

4.2. Correlation Between AMH and BMI, Testosterone and Ovarian Volume among PCOS Subjects

As seen in Table3, ultrasound imaging demonstrated a positive correlation between Serum AMH levels and mean ovarian volume but Serum AMH levels were not influenced by BMI or serum testosterone levels.

Table3. *Correlation between Serum AMH and BMI, serum testosterone and mean ovarian volume in PCOS adolescents*

	Correlation coefficient, r	p value
BMI	**- 0.08**	**0.403**
Serum testosterone	**0.08**	**0.28**
Mean ovarian volume	**0.35**	**0.0001**

4.3. Diagnostic Utility of Serum AMH Levels in Adolescents with PCOS

The best cut off was selected by choosing the measure at which maximum diagnostic accuracy (proportions correctly classified) was found by ROC curve (Figure 2) and the distribution of the study population based on the derived cut off for Serum AMH level is shown in Table4.For the selected cut off (AMH≥7.74 ng/ml) sensitivity, specificity, positive and negative predictive values and likelihood ratios are depicted in Table5.

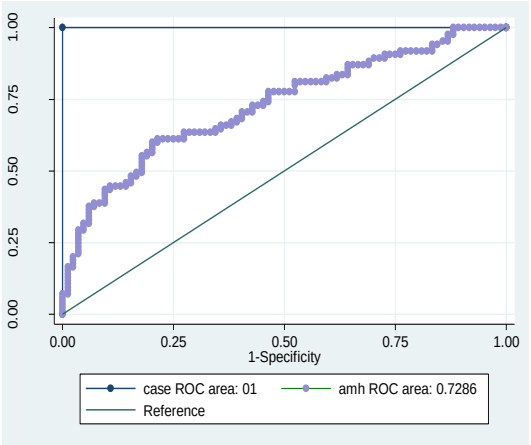

Figure2. *ROC curve of Serum AMH in PCOS subjects*

Table4. *Distribution of the study population based on the derived cut off for Serum AMH level*

AMH ng/ml	PCOS (n)	Normal subjects (n)	Total (n)
AMH (≥ 7.74)	50	17	67
AMH (<7.74)	35	67	102
Total	85	84	169

Table5. *Sensitivity, Specificity, Predictive values and Likelihood ratios for the derived cut off of Serum AMH Level (≥7.74 ng/ml)*

Characteristics	Values %	95% Confidence interval
1.Sensitivity	58.82	47.62 – 69.39
2.Specificity	79.76	69.59 – 87.75
3.Positive predictive value	74.63	62.51 – 84.42
4. Negative predictive value	65.69	55.63 – 74.81
5.Positive likelihood ratio	2.91	1.83 – 4.61
6.Negative likelihood ratio	0.52	0.39 – 0.68

The comparison of ultrasound based diagnosis and serum AMH based diagnosis of polycystic ovaries is shown in Table 6.In our study 85 subjects were diagnosed as PCOS based on Rotterdam's criteria. 45 cases had ultrasound features suggestive of PCOS and 50 cases had Serum AMH levels ≥ 7.74. Out of 45 cases with ultrasound features suggestive of PCOS, 27 had serum AMH levels ≥ 7.74. But, when we use AMH as a proxy marker for ultrasound features we were able to capture 27 of 45 cases contributing to approximately 60% yield.

Table6. *Comparison of diagnosis of PCOS based on ultrasound features /serum AMH levels*

Patterns * (Hirsutism, Oligomenorrhea , USG/AMH)	USG based diagnosis (n)	AMH based diagnosis (n)
000	63	78
001	41	26
010	2	7
011	10	5
101	35	22
111	18	14
100	0	13
110	0	4

**Pattern, 0-Absent, 1-Present*

5. DISCUSSION

In the past few years, serum AMH has been extensively studied as a surrogate marker for ovarian ultrasound characteristics in PCOS. In the present study we evaluated the relationship between AMH and ovarian ultrasound features in adolescents with PCOS and also compared the levels of Serum AMH in PCOS subjects and normal controls. This is one of the very few studies addressing the use of serum AMH levels in the diagnosis of PCOS in adolescent girls. The girls in our study were in the age group of 13 – 19 years which is comparable with other

studies.[4-13]Age distribution was similar in both the cases and controls in comparison to other studies.[4-8] In two studies comparing AMH levels in girls with PCOS [9,10] the age of controls was higher .

Menarche is attained at different ages in different populations. Nutritional status, geographical location, socioeconomic status etc. affect age at menarche.[15] The mean age of menarche in our study was 12 years which is similar to other studies. [4,5]A study done on 1051 Australian teenagers had found that 26% adolescents had menstrual disorders to an extent to interfere with their life or schooling.[16]In our study, majority of cases (93%) presented with the complaints of oligomenorrhea which was higher when compared to other studies in adolescents which have reported 50-60% prevalence.[4,10] 3.5% of the cases presented with secondary amenorrhea in contrast to a study[4] which reported a higher prevalence of 30% .

Clinical hyperandrogenism was seen in 54% of the cases in our study in comparison to 73% in another study .[4] Recent data [17]suggest that hirsutism and oligomenorrhea are appropriate screening criteria for polycystic ovary syndrome in adolescents.

The mean value of BMI varied significantly between the cases and controls in our study (p = 0.0005) similar to another study .[6] This is in contrast to a few other studies [4,5,7-10] which have shown no significant difference between PCOS girls and normal groups.

Acanthosis nigricans is considered as a marker of insulin resistance. In the present study 27% of PCOS cases had acanthosis nigricans. This is in contrast to a study[4] in 23 adolescents with PCOS which found it in 78% of cases. There are data suggesting increased insulin resistance in adults with PCOS. But data is limited in adolescents. A recent study[18] has found impaired glucose tolerance in adolescents with PCOS.

Testosterone levels in our study were significantly higher in our study which is in concurrence with other studies.[4,6,8-10] however another study[5] found no difference in androgens between PCOS cases and controls.

5.1. AMH Levels

Our data, similar to other studies [4-12] showed elevated serum levels of AMH in adolescents with PCOS when compared with controls. Increased serum AMH levels may represent an early manifestation of the disease. Only one study[13] found no significant difference in serum AMH levels in 58 adolescent girls with PCOS compared to controls and serum AMH levels did not differ between obese and non obese PCOS. They concluded that it was not a reliable predictor for the presence of PCOS. In our study the mean value of Serum AMH levels among PCOS girls was 9.69 $\pm$ 5.15 and it ranged between 0.94 and 27.26 ng/ml. Among controls, mean value of serum AMH was 5.83 $\pm$ 3.4 and it ranged between 0.003 and 16.8 ng/ml. The AMH levels are higher than the other studies which had AMH levels ranging from 4.1 $\pm$ 2.2 to 6.78 $\pm$ 3.55 in the cases and 2.4$\pm$ 1 to 3.38$\pm$1.48 ng/ml in the controls [4,6,7,9,10]

5.2. AMH And Oligo/Amenorrhea

The present study has found serum AMH levels to be higher in those with oligo-amenorrhea compared with normal cycles (p=0.0003) ,which is in agreement with other studies.[6,10] In contrast one study[5] demonstrated that AMH levels were higher even in girls with normal periods but with PCOM.

5.3. AMH and Clinical Hyperandrogenism

In a recent study[10] which prospectively studied 400 girls found that at age 16, AMH levels were higher in girls who developed hirsutism at age 26 compared with those without hirsutism. Our study also found a significant difference in AMH levels between those with and without hirsutism and this difference was found to be statistically significant (p<0.0001).

5.4. AMH and BMI

No significant correlation existed between AMH and BMI in our study which is in agreement with other studies. [4,7,13] Another study found a weak but significant correlation between serum AMH levels and BMI (r=0.124, p=0.013).[9] Negative correlation was seen in only two studies.[5,6] One study found AMH levels to be lower in obese adolescents when compared to lean controls[6], but our study could not find a significant difference in AMH levels between overweight/obese adolescents compared with non obese girls (p=0.6142).

5.5. AMH AND Testosterone

There are few studies demonstrating a positive correlation between serum testosterone levels and serum AMH [4,6,9,10]. One study showed a strong correlation of AMH with testosterone levels.[9] However a weak correlation between AMH and free testosterone which did not reach statistical significance has been demonstrated in

another study[4] similar to our study. In contrast no correlation was found in another study.[5]

5.6. AMH and Ovarian Ultrasound Characteristics

One study [5] reported a prevalence of polycystic ovarian morphology (PCOM) in 33.8% of seventy-four non-obese adolescents with regular menstrual cycles and AMH levels positively correlated with 2-5 mm follicle number.

Moreover, there are only two studies [4,9] which have tried to study the relationship between AMH and ovarian ultrasound characteristics in adolescents with PCOS.

The present study demonstrates that serum AMH levels have a positive relationship with follicle count, distribution and found to correlate well with the mean ovarian volume. One study[9] showed a positive correlation of ovarian size and PCOS appearance with serum AMH level in contrast to another study[4] which could not find any significant correlation with follicle number but there was a positive relationship with ovarian volume and peripheral distribution of follicles.

5.7. Diagnostic Utility of AMH in PCOS

The cut off value of AMH for diagnosis varies among studies due to variables such as AMH assay, PCOS diagnostic criteria and patient population. In the discriminant analysis, studies [9,10] have utilized a lower cut off of either 3.4 or 3.15 ng/ml when compared to our study where an AMH value of $\geq$7.74 best distinguished between PCOS and controls. One study[9] in 31 girls comparing PCOS with controls concluded that those with PCOS were 1.49 times more likely to have serum AMH levels >3.4ng/ml. Another was a prospective cohort study[10] which studied 400 girls with PCOS and the sensitivity and specificity of predicting PCOS at age 26 was 85.7% and 37.5% respectively.

This is in contrast to other studies which had used a higher cut off value of AMH of 8 [11]/8.6ng/ml [5]and the sensitivity and specificity is comparable to our study. Another recent study[12] also demonstrated a low diagnostic accuracy with AMH.

Our study and several others have demonstrated increased AMH levels in adolescents with PCOS. Moreover there seems to be a positive correlation with all ovarian ultrasound characteristics similar to one study [9] although another study[4] found positive correlation of AMH levels with ovarian volume and follicle distribution but not with follicle count.

This is one of the very few studies to provide information on the relationship between serum AMH and ovarian ultrasound features in adolescents with PCOS. Since the study population was recruited from a tertiary hospital, the relationship between serum AMH and ovarian ultrasound features may not reflect all adolescent girls with PCOS. Transabdominal ultrasound may have limited our ultrasound assessment of ovaries and the approximated AMH cut off value has not yet been validated in a sample population.

Results suggest that AMH can be used as a surrogate marker for ovarian ultrasound features of PCOS. However, future large prospective studies are needed to confirm these results.

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