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Anti-Mullarian Hormone As A Diagnostic Marker of Polycystic Ovary Syndrome

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ABSTRACT

Objective: To determine the utility of Anti-mullerian hormone (AMH) as a diagnostic marker of Polycystic ovarian syndrome (PCOS).

Study Design: Comparative cross-sectional study

Place and Duration of the Study: Department of Chemical Pathology and Endocrinology, Army Medical College Rawalpindi, Pakistan in collaboration with the Department of Gynaecology and Obstetrics, Pak-Emirates Military Hospital, Rawalpindi Pakistan from Jan 2019 to Jan 2020.

Methodology: The study recruited 30 cases of PCOS and 30 age-matched Controls using Rotterdam criteria. Anti-mullerian hormone (AMH) and testosterone were measured for both the study groups.

Results: Receiver operator curve (ROC) analysis confirmed the utility of anti-mullerian hormone in diagnosing patients with PCOS. The AUC was 0.754 with 95% CI (0.629-0.880). The optimal cut-off point was 3.22ng/mL (Sensitivity 80%, Specificity 66.7%).

Conclusion: The AMH is appraised as an independent screening marker for PCOS patients. Using this single parameter can decrease the patient's inconvenience and financial burden on the health care system and the patient.

Keywords: Anti-mullerian hormone, Infertility, Obesity, Polycystic ovary syndrome.

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INTRODUCTION

Polycystic Ovarian Syndrome (PCOS) is the major cause of disturbed ovulation in the fertile age group of women.[1] It is considered when significant clinical features point out towards this disorder. High levels of androgens and muddled ovulation are not the only features. Other salient features are obesity, hirsutism and metabolic contortion.[2] interestingly, we cannot single out one characteristic as a prerequisite for this ailment. Therefore, morphology, laboratory investigations and metabolic profiles all support each other in diagnosing this malady.[3]

Rotterdam criteria is the most comprehensive and understood criteria to diagnose it. Among the various criteria components, two or more should be present to make the diagnosis.[4] The salient features of this criterion are oligomenorrhoea, raised androgen levels and ultrasound examination consistent with PCOS morphology.[5] Amongst all these, the ultrasonographic findings suggesting a central stroma and its periphery have a follicular distribution are considered the most definitive.[6] Moreover, this follicular distribution is further described in the number of follicles per ovary; at least 20 FNPOs are mandatory to diagnose PCOS.[7]

The beneficial aspect of using AMH is that its levels are not disturbed, irrespective of the stage of menstruation. It is a non-invasive method of diagnosing a diseased condition.[8] AMH also helps in understanding the pathophysiology of this disease.[9] It is not a mere marker of follicle count but also an indicator of hormonal disturbance. The higher the levels of AMH, the worse the disease symptoms.With ageing, AMH levels fall as the patient's symptoms and quality of life increase.[10]

Considering the points mentioned above, there is a need to establish a simple way to diagnose PCOs using laboratory investigations, making it easier for patients and healthcare workers and reducing the burden on the healthcare system.

METHODOLOGY

The comparative cross-sectional study was conducted at the Department of Chemical and Endocrinology, Army Medical College, Pakistan in collaboration with the Department of Gynaecology and Obstetrics, Pak-Emirate Military Hospital,

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Rawalpindi, Pakistan after Institutional Ethical Review committee approval (Certificate No ERC/ID/85). Sample size was calculated by using the WHO sample size calculator, keeping the reported prevalence of PCOS to be 3%.[11]

Inclusion Criteria: Cases were diagnosed based on Rotterdam criteria that included: Oligomenorrhoea or amenorrhea, clinically and lab-proven hyperandrogenism, PCOS morphology on ultrasound and confirmation by Gynaecologist. Age-matched controls were included in the study, only healthy females with normal menstrual history and no other obstetric or gynaecological complaint.

Exclusion Criteria: All women who have endometriosis, a cause of infertility other than PCOS, females with infertility treatment and less than 20 years or more than 45 years were excluded.

The non-probability convenient sampling technique was adopted for sample collection. Additionally, informed consent was taken from each patient, and then 5ml of venous blood was drawn in a Gel tube for hormone analysis. The analysis of hormones was done on the same day with a routine hormonal profile except for AMH. The sample for AMH was stored at minus 30 degrees Celsius to run in a batch. Testosterone levels were estimated as it is a part of the Rotterdam Criteria.

Statistical Package for Social Sciences (SPSS) version 21:00 was used for data analysis. Quantitative variables were expressed as mean±SD and qualitative variables were expressed as frequency and percentages, taking a *p*-value of ≤ 0.05 to be significant. The ROC curve was used to assess the diagnostic accuracy of AMH by calculating sensitivity and specificity using a two-by-two table.

RESULTS

Sixty subjects were recruited in the study, equally distributed cases and controls. Oligomenorrhoea was found in 48(80%) of patients. Hirsutism was observed in 29(48%) of patients, while 43(88%) of patients revealed polycystic ovaries on ultrasound. Amongst cases, all subjects had irregular periods, while 9(14%) had irregular cycles in controls. Table-I shows the mean age and mean of the two hormones tested. The ROC curve was used to assess the diagnostic utility of AMH for PCOs, which showed an AUC of 0.754 with 95% CI (0.629-0.880). The optimal cut-off point was 3.22ng/mL with a Sensitivity of 80% and Specificity of 66.7%, as shown in the Figure. The 2X2 table was made to check the sensitivity and specificity of AMH in PCO. The positive predictive value was 70.6%, and a negative predictive value of 23.1% indicated that AMH can be reliably used to diagnose PCOs (Table-II).

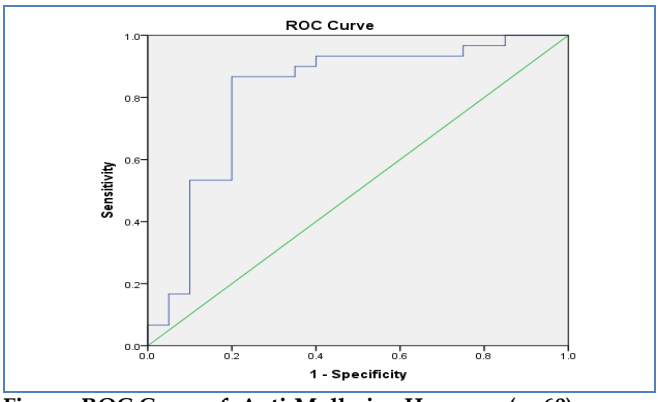

Figure: ROC Curve of Anti-Mullarian Hormone (n=60)

DISCUSSION

Diagnosing PCOS on ultrasonography can sometimes be confusing or problematic.[10] The subjective judgment is necessary for interpreting ultrasound results, and interobserver variability is always present. Moreover, transvaginal ultrasound is mostly recommended to visualise cysts in the ovary. This technique involves expertise, patient willingness and, in some cases, social hindrance. In addition, the use of multiple diagnostic markers and different phenotypes of PCOS further complicates the diagnosis.[11]

In our study, 46% of women in the control group had regular menstrual cycles, and 14% had irregular cycles, though they did not have PCOS. On the other hand, 100% menstrual irregularity was noted in the case group, most likely due to hormonal imbalance or patients taking treatment for infertility treatment.

Different hormone levels analysed in study lab investigations revealed a significant difference between cases and controls. Luteinising hormone, Follicular stimulating hormone LH: FSH ratio, Progesterone, 17-hydroxyprogesterone, SHBG and AMH levels were significantly deranged in our study. However, levels of Prolactin, Testosterone and Estrogen were non-significant. Progesterone levels were low in the Case Group because most of the cycles in PCOS are ovulatory. Elevated LH levels were found in ~50% of women with PCOS, but the majority of the women were without metabolic impairment. This rise in LH is secondary to the acceleration of the frequency

Table–I: Comparison Between Cases and Controls (n=60)

	Case (n=30)	Control (n=30)	*p*-value	Normal Reference Values
Age(years)	30.5±4.44	30.5±6.5		
Testosterone (nmol/L)	1.47±0.46	1.28±0.87	0.276	0–1.83
LH(mIU/ml)	17.79±6.63	4.57±2.00	<0.001	1-12
FSH(mIU/ml)	6.59±2.62	3.26±1.67	<0.001	1-11
LH:FSH	2.80±0.59	1.52±0.53	<0.001	1-2
AMH (ng/ml)	5.83±2.99	3.1±2.84	0.001	0.147-8.13

Table-II: Two by two Contingency Table for Anti-Mullarian Hormone Cut-off Values (n=60)

	Clinically diagnosed PCO	
Positive AMH Result	True Positive (24)	False Positive (10)
Negative AMH Result	False Negative (6)	True Negative (20)
Sensitivity=24/(24+6)=80%		
Specificity=20/(20+10)=66.7%		
PPV=(TP/TP+FP)=70.6%		
NPV=(TN/FN+TN)=23.1%		
Diagnostic Accuracy==(TP+TN)/All patients*100=73.33%		

of GnRH secretion, which, as per some authors, is thought to be the consequence of a negative feedback failure due to prenatal hypothalamic exposure to androgens. Conversely, mean FSH levels are lower than controls in many published series, with no current explanations.[9,11] Both phenomena lead to an increase in the LH/FSH ratio, which was used as diagnostic criteria in the past but was rejected because of its low sensitivity. In women with PCOS, serum levels of AMH and LH are positively correlated. This correlation is independent of serum androgen and FSH levels.[12] The cause may be a high level of LH that could stimulate AMH secretion. Although testosterone levels increase in PCOS, they were insignificant in our study. The reason behind this was that the majority of cases were on infertility treatment, taking oral hormones (antiandrogens), and topical creams.

17 OHP and SHBG are important biomarkers in the diagnosis of PCOS, and both parameters were found to be significantly correlated in our study. The level of 17 OHP and SHBG is raised in PCOS patients because when LH stimulation approaches maximum level, 17 OHP level also increases, yet normally androgen production shows mild or insignificant increases.13, 14 This rise in SHBG level was also documented by other studies.[15]

Many studies have reported raised levels of serum AMH in women with PCOS as compared to the controls, and it can be stated that serum AMH levels can be used as a diagnostic marker of PCOS. However, the reported mean serum AMH level and the suggested cut-off value of AMH for PCOS differed in multiple studies, probably because of differences in the sample size, sample selection criteria, and PCOS phenotypes among the studies.[2]

The raised AMH levels found in cases in our study are comparable with a few other studies, and the difference between the two study groups was reportedly significant in these studies. The positive correlation between AMH and LH was also documented by other researchers.[11]

The cut-off determined by patient data through the ROC curve was found to be 3.22ng/ml with a sensitivity of 80% and specificity of 66.7%. These results are comparable to the study conducted by Wiweko *et al*.[15] Hence, this cut-off can be used for diagnosing cases of PCOS.

In the study of Peigne et al., the AMH level was elevated with a specificity of 92% and sensitivity of 67% for the diagnosis of PCOS with a cut-off of 8.4ng/mL. This cut-off value of Peigne et al. is higher than the value determined by data in our study. The reason behind this is the large number of patients they took (104 women, 59 cases and 45 controls). Their study shows high specificity but low sensitivity.[16] Calzada et al. also reported elevated serum AMH levels in young adolescent Chinese women with PCOS. However, according to them, serum AMH has poor diagnostic value with a sensitivity of 61.7% and specificity of 70%, and they found a cut-off of 8 ng/mL.[17] They suggested that the reason for low specificity and sensitivity can be the low prevalence of hyperandrogenism, obesity and insulin resistance.

The cut-off value closest to our study was found by Gupta et al, which was 4.45ng/mL (they took 71

cases and 71 controls) in Indonesia with 71.1% sensitivity and 74.6 specificity,18 comparable to our study. Our specificity is slightly smaller because our sample size was smaller.

CONCLUSION

Our study showed that elevated levels of AMH have a substantial association with PCOS and can be used as a single, specific and useful diagnostic marker of PCOS. Using this single parameter can decrease the patient inconvenience and final burden on the health care system. As a single diagnostic marker, AMH enables physicians to set a particular management protocol for PCOS with infertility, improving the quality of life.

Conflict of Interest: None.

Authors Contribution

Following authors have made substantial contributions to the manuscript as under:

SA & AH: Data acquisition, data analysis, data interpretation, critical review, approval of the final version to be published.

RS & MMS: Study design, data interpretation, drafting the manuscript, critical review, approval of the final version to be published.

AI & RA: Conception, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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