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RESEARCH ARTICLE

STUDY OF DIFFERENT PHENOTYPES IN POLYCYSTIC OVARIAN SYNDROME AND THEIR CORRELATION WITH AMH

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ABSTRACT

Objectives: To study different phenotypes in PCOS and their correlation with AMH
Methods: This prospective case-control study included 90 patients attending Gynaecology out patient Department of Dr RML Hospital, New Delhi from 1st November 2015 to 31st March 2017. Clinical history and examination including Ferriman Gallwey scoring, BMI, investigations including pelvic ultrasonography and blood serum FSH, LH, estradiol, TSH, prolactin, testosterone (total) and AMH was done for all the women. The patients were divided into two equal study groups of 45 each – PCOS diagnosed using Rotterdam criteria and Controls, using inclusion and exclusion criteria.
Results: The mean age and BMI of cases and control were similar with no statistical difference. Mean FG score of 10.13 in PCOS case was statistically higher than in control. Mean AMH levels of 6.08ng/ml in cases was almost twice that of 2.98 ng/ml in control ($p<0.0001$). In PCOS, positive correlation of AMH to FG score and negative correlation with oligomenorrhea was observed. Phenotype A (HA+OA+PCOM) was most prevalent (42.22%) with highest AMH level of 7.96 ng/ml. Prevalence of phenotype D (OA+PCOM), phenotype B (OA+HA) and phenotype C (HA+PCOM) were 28.88%, 15.55%, and 13.33% respectively.
Conclusion: AMH levels were significantly higher in PCOS than control. Phenotype A was the commonest, with highest AMH levels. AMH had a positive correlation to FG score and negative correlation with oligomenorrhea.

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INTRODUCTION

Polycystic ovarian syndrome (PCOS) is a perplexing, ill defined, heterogeneous endocrine disorder common in females of reproductive age group. PCOS female presents with a spectrum of symptoms varying in severity. Clustering of cases in families strongly suggests the role of genetic factors in the development of PCOS. There is lack of a standardized diagnostic modality, limited case-control population and incomplete knowledge regarding the exact etiology and pathogenesis of PCOS. (Sekar *et al*., 2015) Diagnosis of PCOS can be made using following :1) National institute of Child Health and Human Development criteria or NIH criteria (1990) 2) Rotterdam criteria (2003) 3) Androgen Excess and Polycystic Ovary Syndrome (AE-PCOS) criteria (2009). Rotterdam criteria is most widely accepted worldwide and a women is diagnosed with PCOS if she has two out of the three following features: 1) oligomenorrhea or amenorrhea 2) clinical and/or biochemical hyperandrogenism 3) polycystic ovaries on ultrasound with a cut off of presence of more than 12 follicles with a diameter of 2-9 mm or when ovarian volume is more than 10cucm. Oligomenorrhea) was taken as fewer than eight menstrual cycles during the previous 12 months or menstrual interval of more than 35 days. Hyperandrogenism was defined either clinically as Ferriman-Gallwey(FG) score of >8 or biochemically as serum testosterone level of >2.67nmol/l.Polycystic ovarian morphology (PCOM) was considered when in either ovary on ultrasound there was presence of more than 12 follicles with a diameter of 2-9 mm or when ovarian volume was more than 10cucm. Four phenotypes of PCOS have been described based on the presence of oligomenorrhea (OA), hyperandrogenism (HA) and polycystic ovarian morphology on ultrasound (PCOM). These groups were: Phenotype A = OA+HA+PCOM; Phenotype B = HA+OA; Phenotype C = HA+PCOM; Phenotype D = OA+PCOM. Anti Mullerian hormone (AMH) is a glycoprotein expressed by ovarian follicle <8mm and is not affected by menstrual cycle and COC use, making it a potential diagnostic and prognostic marker of PCOS. (Jeppesen *et al*., 2013)

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MATERIALS AND METHODS

The present study was a prospective case-control study conducted on 90 women attending Out Patient Department of Obstetrics and Gynaecology; PGIMER &Dr RML hospital, New Delhi from 1st November 2015 to 31st March 2017. After taking informed written consent they were divided equally into 45 cases and 45 controls based on inclusion and exclusion criteria. PCOS cases were diagnosed according to Rotterdam criteria with atleast two of the three criteria present. Control consisted of women having regular menstrual cycle, normal ovarian morphology on ultrasound and no abnormality in hormonal profile . Women taking COC in past three months and history of previous ovarian surgery were excluded from the study. Clinical history included complaint of oligomenorrhea, hirsutism, infertility and acne and examination included FG score and BMI. A total of 8 ml was withdrawn in 2 plain vials on day 2-3 of menses or withdrawal bleeding. Samples were then centrifuged at 3000 rpm in centrifugation machine at the biochemistry for serum analysis. One vial of centrifuged sample was stored at -80 degrees in deep freezer for batch analysis of Anti Mullerian hormone by ELISA assay which used a competitive enzyme immunoassay technique utilizing a monoclonal anti-AMH antibody and an AMH-HRP conjugate in an anti-AMH coated plate. The minimum detection level for the kit was 0.025 ng/ml. On the other sample hormonal assay for T3, T4 and TSH, FSH, LH, estradiol, prolactin, testosterone(total) was performed using chemiluminiscence immunoassay on the ECiQvitros from Johnson's and Johnson's. TAS was performed for all the women. Results of clinical history, investigations and imaging studies were recorded along in a proforma. Cases were further subdivided into 4 phenotypes A, B, C and D depending upon the presence of oligomenorrhea (OA), hyperandrogenism (HA) and polycystic ovarian morphology (PCOM) on ultrasonography.

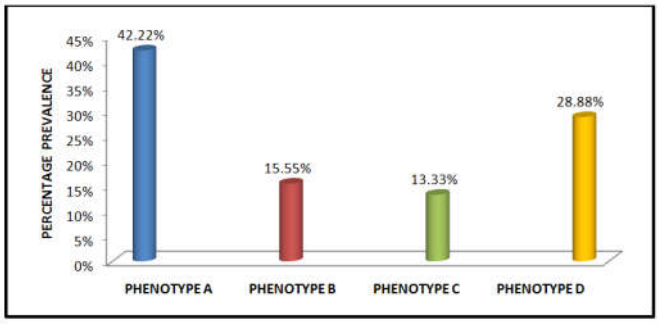

Figure 1. Distribution of PCOS according to phenotypes

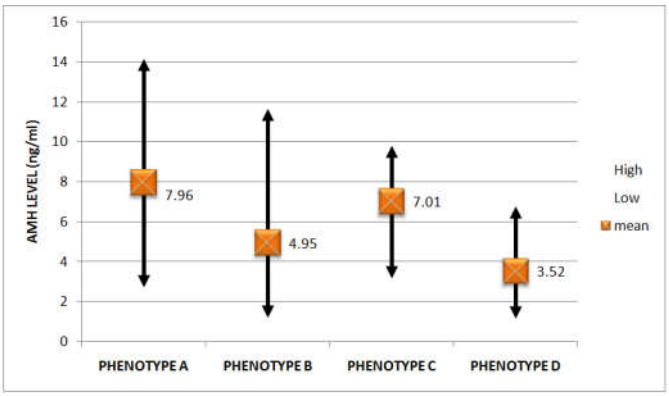

Figure 2. Mean AMH levels in each phenotype

commonest (62.22%) clinical presentation in PCOS cases followed by complaint of hirsutism (20.0%), infertility (13.33%) and acne (4.44%).However after a detailed history, 86.67% were found to be having oligomenorrhea. and similarly 71.11% females were found to be having clinical hyperandrogemia on FG Score. Mean FG score for PCOS and controls was 10.13 and 4.8 respectively and was statistically

Table 1. Prevalence and AMH levels in different PCOS phenotypes

Study	Year		Phenotype a (oa+ha+pcom)	Phenotype b (ha+oa)	Phenotype c (ha+pcom)	Phenotype d (oa+pcom)
Sahmay et al	2013	Prevalence	47.4%	10.3%	17.9%	24.3%
		AMH levels (ng/ml)	9.5±6.1	3.06±2.4	6.12±3.6	8.02±6.2
Wiweko et al	2014	Prevalence	29.6%	2.8%	4.2%	63.4%
		AMH levels (ng/ml)	11.1±5.6	11.5(6.0-17.1)	8.72±2.4	6.1(3-16.9)
Present study	2017	Prevalence	42.22%	15.55%	13.33%	28.88%
		AMH levels (ng/ml)	7.96 ± 3.01	4.95 ± 3.82	7.01 ± 2.77	3.52 ± 1.84

Statistical analysis

The data was entered in MS EXCEL spreadsheet and analysis was done using Statistical Package for Social Sciences (SPSS) version 21.0. Qualitative variables were correlated using Chi-Square test /Fisher's exact test. Quantitative variables were compared using Unpaired t-test/Mann-Whitney Test between the two groups and ANOVA/Kruskal Wallis test between more than two groups. Pearson correlation coefficient/Spearman rank correlation coefficient was used to assess the association of AMH with various parameters. A p value of <0.05 was considered statistically significant.

RESULTS

The mean age and mean BMI of PCOS cases and control was 24.49 years and 25.47 years and 24.56 kg/m^2 and 24.13 kg/m^2 respectively with no statistical difference (p<.05) making them statistically similar. Oligomenorrhea/amenorrhea was the significant. (p<0.0001).Total testosterone levels in cases and controls was 1.22±0.67 nmol/L and 1.19±0.7 nmol/L respectively and were not statistically significant. Polycystic ovarian morphology was reported in 84.44% (38) of PCOS cases. Mean AMH level in PCOS and controls was 6.08 ± 3.36 ng/ml and 2.98 ± 2.02 ng/ml respectively and it was statistically higher compared to controls (p <0.0001). AMH levels was positively correlated to FG score (r = 0.686; p=0.00) and negative correlated to oligomenorrhea (r = -0.63; p<0.0001) in PCOS. Majority (42.22%) were suffering from severe form of PCOS i.e. Phenotype A (HA+OA+PCOM). This was followed by phenotype D (OA+PCOM), phenotype B (OA+HA) and phenotype C (HA+PCOM) in 28.88%, 15.55%, and 13.33% respectively. [Fig-1] Highest AMH levels were found in phenotype A with a mean of 7.96± 3.01 ng/ml ranging from 2.73-14.2 ng/ml. Least AMH levels were seen in phenotype D with a mean average of 3.52 ± 1.84 ng/ml. Hence least AMH level was in phenotype D in which there is no hyperandrogemia .Difference in AMH levels in all 4

phenotypes were statistically significant with p value = 0.001 (Fig-2).

DISCUSSION

Age profile observed in present study was similar to that observed in previous studies. (Begawy *et al*., 2010; Woo *et al*., 2012; Sahmay *et al*., 2013) In the present study mean BMI of cases and controls was statistically similar and this was in agreement to previous studies. (Begawy *et al*., 2010; Homburg *et al*., 2013; Wiweko *et al*., 2014) However, Pigny *et al* stated that mean BMI in PCOS patients was significantly higher than controls. (Pigny *et al*., 2006) Oligomenorrhea was the most common symptom in our study with prevalence of 86.67%. which was similar to 95.7% reported by Li *et al*. (2010) Ramanand *et al*, in an Indian study reported oligomenorrhea in 65% newly diagnosed PCOS females. (Ramanand *et al*., 2013) On the contrary, Azziz *et al* (2004) and Jalilian *et al*. (2015) reported a lower frequency of in 22.8% and 28% respectively. (Azziz *et al*., 2004; Jalilian *et al*., 2015) In the present study, hirsutism, was present in 20% females, but on evaluation 71.11% were found to have hyperandrogenism based on FG scorie. Ramanand *et al* in a study from India also stated that only 12.5% females presented with hirsutism but on evaluation 44.16% were found to have hyperandrogenism. (Ramanand *et al*., 2013) Similarly Azziz *et al* also reported 76% prevalence of hirsutism in PCOS females. (Azziz *et al*., 2004) Studies by Naderi in 2011 and Fauser *et al*. (2012) also had similar observations. (Naderi *et al*., 2011; Fauser *et al*., 2012) This could be because Indian population per say is more hirsute and it is only when the facial hair appear, does a patient complaint it to the medical facility. Jalilian *et al* in 2015 reported hirsutism in only 13% of Iranian PCOS females. (Jalilian *et al*., 2015) Hence there is difference in presence of hirsutism due to ethnic variation. Ferriman Gallwey scores in PCOS was twice that in control but none of the female in our study was found to be have biochemical hyperandrogenism as serum testosterone was normal in both cases as well as control. This could be because serum total testosterone levels is not an ideal marker for demonstrating hyperandrogenism in PCOS. Free testosterone levels or FAI index are considered better indicator of androgen excess. Hyperandrogenism in previous studies was diagnosed on basis of free testosterone levels. (Begawy *et al*., 2010) However, Woo and Li *et al* found a significant difference between PCOS and control using total serum testosterone. (Woo *et al*., 2012; Li *et al*., 2010) Fifteen percent of females suffering from polycystic ovarian syndrome in the present study had normal ultrasound finding which was in congruence with observations made by Azziz *et al*. (2006) and Mortensen *et al*. (2006). (Azziz *et al*., 2006; Mortensen *et al*., 2006) In present study, phenotype A (OA+HA+PCOM) was most prevalent (42.22%) and phenotype C (HA+PCOM) was least prevalent (13.33%). This was similar to Sahmay *et al* who also reported phenotype A as the most prevalent type of PCOS. However in their study least common was phenotype B(OA+HA). (Sahmay *et al*., 2013) In contrast to this Wiweko *et al* reported phenotype D (OA+PCOM) as the most common with prevalence of 63.4% and Phenotype A as second most common. (Wiweko *et al*., 2014) (Table 1) The study population in above studies were different, with Wiweko *et al* study on Indonesian women, Sahmay *et al* conducted their study in Istanbul and ours being on Indian population. Ethnic background may effect prevalence of phenotypic variation in PCOS females. In the present study, women with Phenotype A had highest AMH level and the most severe form of PCOS. Similar had been reported by Coney *et al*. (2008)

In our study, AMH level of 6.08 ng/ml in PCOS was twice that in control. Sahmay *et al* also stated that AMH levels are 2-3 times higher in women with PCOS than those without it. (Sahmay *et al*., 2013) Tehrani *et al* in 2010 and Villarroel *et al* in 2011 found significantly higher values of AMH in PCOS group as compared tocontrol. (Ramezani Tehrani *et al*., 2010; Villarroel *et al*., 2011) In the present study, in PCOS females a negative correlation was observed between AMH and number of menstrual cycles per year or oligomenorrhea and this was in congruence to previous studies. (Pigny *et al*., 2006; Mahran, 2015) In the present study there was a positive correlation of AMH levels with FG score in PCOS and this was in congruence with Mahran. (Mahran, 2015) Sahmay *et al* also found that AMH levels were higher in females with hyperandrogenism. (Sahmay *et al*., 2014) No correlation of AMH with testosterone levels could be demonstrated in the present study. However Carlsen *et al* in 2009, Woo *et al* in 2012 and Sopher *et al* (2015) found a positive correlation of AMH with androstenedione. (Woo *et al*., 2012; Carlsen *et al*., 2009; Sopher *et al*., 2014)

Conclusion

Phenotype A was the most prevalent phenotype in PCOS with highest AMH level and with maximum severity. Phenotype D was the secondt commonest with lowest AMH level which could be because there is neither clinical and/or biochemical hyperandrogenism in this phenotype. There is positive correlation of AMH with hyperandrogenism and severity of PCOS and negative correlation with oligomenorrhe .Hence phenotype will give an idea about severity of PCOS.

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