

Frequency of Phenotypes and their Clinical and Hormonal Characteristics of Polycystic Ovarian Syndrome

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ABSTRACT

Objective: To determine the frequency of phenotypes of polycystic ovarian syndrome (PCOS) in patients presenting with sub-fertility, and to compare the clinical and hormonal characteristics among them.

Study Design: Descriptive cross-sectional study.

Place and Duration of the Study: Department of Obstetrics and Gynaecology, Forest View Specialist Clinic, Peshawar, Pakistan, from August 2022 to January 2023.

Methodology: The study included 662 female patients presenting with menstrual irregularities, hyperandrogenism, and infertility to the clinic. PCOS was diagnosed on the basis of the Rotterdam criterion and clinical features and classified into different phenotypes on the basis of the National Institute of Health (NIH) panel criteria. Data were entered and analysed by IBM SPSS VERSION 23.0. The frequency of four phenotypes was calculated and phenotypes were compared for age, weight, hormonal profiles, and history of miscarriages. A $p < 0.05$ was considered statistically significant.

Results: Frequency of PCOS in patients with infertility was 59.76%. Phenotype A was seen in 58.2%, phenotype D in 23.3%, phenotype C in 16.9%, and phenotype B in 1.7% of cases. The LH/FSH ratio was statistically significant in phenotype A as compared to other phenotypes, while other parameters were non-significant.

Conclusion: The frequency of PCOS is high in patients with infertility. Phenotype A is the most common variant and is associated with significant impairment of the LH/FSH ratio.

Key Words: Polycystic ovarian syndrome, Subfertility, Phenotypes of PCOS, Hyperandrogenism, Anovulation, R-C1.

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INTRODUCTION

Polycystic ovarian syndrome (PCOS) is the most common endocrine disorder in reproductive-age women, with variable clinical presentation, associated long-term metabolic dysfunction, and lack of consensus on diagnostic criterion. Therefore, the definition of PCOS is still a matter of debate. The worldwide prevalence of PCOS has been reported as 4 to 20%¹ with higher prevalence in South Asian countries i.e., 3.7 to 22.5% in India² and 50 to 55.41% in Pakistan.^{3,4}

There have been numerous classification systems to diagnose and classify PCOS, based on the presence of hyperandrogenism, oligoanovulation (NIH 1990) criteria,⁵ and Rotterdam's criteria.⁶ Polycystic morphology on ultrasound is diagnosed by >12 follicles measuring 2-9mm in diameter or >10ml ovarian volume in at least one ovary.

AE-PCOS criteria considered that diagnosis of PCOS should be based on clinical or biochemical hyperandrogenism in combination with oligo or anovulation,⁷ thus, excluding non-hyper androgenic phenotype of PCOS. In 2012, NIH held an evidence-based methodology workshop which proposed a phenotypic approach to classify PCOS.⁸ The proposed phenotypic approach is highly convenient for clinical practice and epidemiologic research, as well as helpful in identifying those women with PCOS who are at the highest risk of metabolic dysfunction (Type A and B).⁹

Phenotype A includes clinical or biochemical hyperandrogenism (HA), oligoanovulation / ovulatory disorder (OA), and polycystic ovarian morphology (PCOM) on ultrasound. Phenotype B includes hyperandrogenism and ovulatory disorders (HA + OD). Phenotype C includes hyperandrogenism and polycystic ovarian morphology (HA + PCOM). Phenotype D includes ovulatory disorders and polycystic ovarian morphology (OD + PCOM).

Currently, patients of PCOS are being treated with uniform management without taking into consideration the different clinical phenotypes of PCOS, as some patients might have an element of hyperandrogenism more than that of ovulatory disorder and this might be over-treating or under-treating the different spectrum of disease. Patients with phenotype A are more prone to metabolic and reproductive dysfunction and resistant to ovarian stimulation as compared to the rest of the phenotypes and hence

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would need a timely referral. Patients with hyperandrogenism (B and C) would require endocrinology input to exclude other causes of hyperandrogenism. Patients with regular menstrual cycles and no ovulatory disorders may not require investigations such as the FSH/LH ratio. The aim of this study was to classify patients with PCOS into the phenotypes, evaluate their frequency, and compare their physical, biochemical, and hormonal parameters.

METHODOLOGY

This descriptive cross-sectional study was conducted in the Department of Obstetrics and Gynaecology, Forest View Specialist Clinic, Peshawar, from August 2022 to January 2023. Ethical approval was obtained from the Institutional Research and Ethical Board of Lady Reading Hospital. Sample size was calculated as 662 females based on the prevalence of PCOS as 52%, and confidence level of 99%, using the Epi info software calculator. Patients presenting with infertility, menstrual irregularities (oligo menorrhoea was defined as cycle length of >38 days as defined in the International Federation of Gynaecology and Obstetrics (FIGO) classification⁹ and secondary amenorrhoea as no menstrual bleeding for 3 consecutive cycles), and hirsutism (Ferriman Galway score of more than 8 and fulfilling the Rotterdam’s criteria of PCOM (polycystic morphology on ultrasound by >12 follicles measuring 2-9mm in diameter or >10ml ovarian volume in at least one ovary) were included in the study. Women with thyroid disorders, diabetes, hyperprolactinaemia, ovarian insufficiency, and use of oral contraceptive pills in the last 3 months were excluded. All the patients were provided with informed consent regarding inclusion in the study.

History was recorded regarding age, duration of subfertility, menstrual irregularities, hirsutism, and previous history of miscar-

riages. Patients were assessed for weight, and extent of hirsutism by Ferriman Galway scoring, acne, and acanthosis nigricans. Patients with a Ferriman-Galway score of >8 were considered as hirsute. Pelvic ultrasound (TVS) was done to look for antral follicular count (AFC), ovarian volume, and morphology on day 2-5 of the menstrual cycle. In order to minimise inter-observer variations, ultrasound was done by one radiologist using vaginal probe of 6MHz of ultrasound machine (TOSHIBA). Blood samples were drawn in early follicular phase of the cycle (day 2-3) for hormonal analysis (FSH, LH, AMH). Serum concentrations of hormones were determined by chemiluminescent immunoassays using Maglumi 800 and Mindray CL-900i with FSH and LH being measured in IU/L, and anti-mullerian hormone (AMH) in ng/ml. Patients were categorised into four phenotypes according to the National Institute of Health Consensus Panel as described earlier.

Data were entered and analysed using IBM SPSS version 23.0. Frequency and percentages were computed for qualitative variables such as polycystic phenotypes. Mean and SD values were calculated for quantitative variables such as age, weight, number of previous miscarriages, FSH, LH, AMH, and LH / FSH ratio. One-way ANOVA test was applied for comparison of the phenotypes regarding age, weight, number of previous miscarriages, FSH, LH, AMH levels, and LH / FSH ratios. A p-value was calculated by Chi-square test and a value of <0.05 was considered statistically significant.

RESULTS

Total number of patients presenting with subfertility was 8,400 in six months, and 5,020 patients were diagnosed with PCOS on the basis of Rotterdam criteria and clinical and biochemical parameters, thus giving the frequency of 59.76%.

Table I: Comparison of different phenotypes for physical and hormonal characteristics.

Variable	Phenotype	Mean	Std. deviation	Std. error	95% confidence interval for mean		df	F	p-value
					Lower-bound	Upper-bound			
Age	A (385)	25.16	4.792	0.244	24.68	25.64	3	2.247	0.082
	B (11)	27.64	5.679	1.712	23.82	31.45			
	C (112)	26.13	5.018	0.474	25.19	27.07			
	D (154)	25.05	4.571	0.368	24.32	25.77			
Weight	A (385)	72.14	14.221	0.725	70.71	73.56	3	2.468	0.061
	B (11)	64.91	11.022	3.323	57.50	72.31			
	C (112)	71.49	12.857	1.215	69.08	73.90			
	D (154)	69.26	12.436	1.002	67.28	71.24			
FSH	A (385)	6.149	2.2737	0.1159	5.921	6.377	3	2.587	0.052
	B (11)	6.455	2.7562	0.8310	4.603	8.306			
	C (112)	6.453	2.4422	0.2308	5.995	6.910			
	D (154)	7.133	6.7145	0.5411	6.064	8.202			
LH	A (385)	12.484	9.5541	0.4882	11.525	13.444	3	2.368	0.070
	B (11)	10.745	6.8918	2.0780	6.115	15.375			
	C (112)	9.964	9.0871	0.8586	8.263	11.666			
	D (154)	12.483	8.6801	0.6995	11.101	13.865			
AMH	A (385)	5.795	3.3703	0.1718	5.458	6.133	3	1.484	0.218
	B (11)	6.427	3.4053	1.0267	4.140	8.715			
	C (112)	5.265	3.4531	0.03263	4.619	5.912			
	D (154)	5.315	2.7813	0.2241	4.872	5.758			
Miscarriages	A (385)	0.37	0.857	0.044	0.29	0.46	3	2.551	0.055
	B (11)	0.36	0.505	0.152	0.02	0.70			
	C (112)	0.63	1.208	0.114	0.41	0.86			
	D (154)	0.40	0.736	0.059	0.28	0.51			
LH/FSH ratio	A (385)	2.1233	1.44780	0.7379	1.9783	2.2684	3	4.468	0.004
	B (11)	1.5698	0.82372	0.24836	1.0164	2.1232			
	C (112)	1.6055	1.23447	0.11665	1.3743	1.8366			
	D (154)	2.0404	1.33568	0.10763	1.8278	2.2530			

A p-value of <0.05 was considered statistically significant.

After applying the exclusion criteria, a total of 662 patients were included in the study. Among the included cases, 58.2% (n = 385) were phenotype A, 23.3% (n = 154) were phenotype D, 16.9% (n = 112) were phenotype C, and 1.7% (n = 11) were phenotype B.

Menstrual irregularities were observed in 83.08% (n = 550) of all included patients. The commonest menstrual irregularity was oligo amenorrhoea in 41.81% (n = 325), secondary amenorrhoea in 32.02% (n = 98), and irregular cycles in 23.09% (n = 127). Hirsutism was seen in 76.80% (n = 508) and PCOM was observed in 98.34% (n = 651).

Overall, the mean age was 25.34 years; mean weight was 71.24 kg; mean FSH level was 7.29 IU/L; mean LH was 12.12 IU/L; and mean AMH level was 5.605 ng/ml. Mean weight was greater in phenotype A (72.14 kg) as compared to other groups, mean FSH was higher in phenotype D (7.133 IU/L), mean LH was equal in Type A and D (12.48) as compared to B and C, mean AMH was higher in phenotype A (5.79), and mean number of miscarriages was more in phenotype C (1.208) as compared to other phenotypes. LH/FSH ratio mean was more in phenotype A followed by phenotype D. Non-significant differences were observed in four phenotypes regarding age, weight, number of previous miscarriages, FSH, LH, and AMH levels. However, significant difference was observed in the LH/FSH ratio which was significantly higher in phenotype A with a p-value of 0.004 (Table I).

DISCUSSION

In this study, the frequency of PCOS in patients presenting with infertility was found to be higher (59.76%) as compared to the rest of the studies in Pakistan (55% and 50%).^{3,4} The global prevalence of PCOS is estimated between 4% and 20%.¹ WHO estimates that 3.4% of women are affected by PCOS globally.¹⁰ The prevalence rate in India has been reported as 22.5%.¹¹ The wide variations in prevalence rates could be attributed to different diagnostic criterion, varying clinical features, the lack of awareness regarding disease by health professionals¹² and patients. Consequently, late presentation, lack of trained radiologists in low- or middle-income countries (LMIC) leading to low detection rates and genetic and environmental factors. The higher prevalence in this study could be due to the study centre being a referral clinic for women from all the provinces with menstrual irregularities and subfertility, hence reflecting a higher prevalence of PCOS in subfertile patients as compared to general population. Another contributory factor for higher prevalence could be the availability of experienced radiologists and hence higher detection rates.

Phenotype A was the most prevalent accounting for 58.2% of cases, phenotype D in 23.3%, phenotype C in 16.9%, and phenotype B was seen only in 1.7% of cases. These rates are in accordance with the study conducted in Turkiye by Senem

et al., reporting phenotype A as 34.83%, phenotype D as 25.84%, phenotype C as 24.15%, and phenotype B as 15.16%.¹² Phenotype A was the most prevalent phenotype (67.7%), with higher prevalence rates of phenotype B, and low prevalence of phenotype D (3.6%) in a study conducted in India.¹³ A study conducted in Iran concluded phenotype A as the most prevalent (58.6%), followed by phenotype D (31.7%), phenotype C (5.4%), and phenotype B (4.4%).¹⁴ A smaller sample size study by Savas *et al.* concluded phenotype C to be the most prevalent in the Turkish population.¹⁵ Studies from Iran by Mehrabian *et al.*¹⁶ and China by Zhang *et al.*¹⁷ found phenotype D as the most frequent phenotype.^{16,17}

Several other studies concluded that phenotype A as the most prevalent phenotype probably because it has all the three features of ovulatory disorders, hyperandrogenism, and PCOM which form the basis of the diagnosis of PCOS. All phenotypes require accurate evaluation of symptoms; diagnosis of PCOM by an experienced radiologist which if missed or not done can lead to inaccurate phenotypic classification and hence variations in prevalence rates. Phenotype D as the second prevalent phenotype (23.3%) including features of ovulatory disorders and PCOM on ultrasound.

Phenotype D does not include features of hyperandrogenism. As a result, there is a higher chance that these patients would seek help from a gynaecologist rather than an endocrinologist, and therefore, would undergo ultrasound for their menstrual irregularities. Endocrinologists treat many patients without getting radiology opinions and hence misclassifying or underreporting of phenotypes. Strict protocols need to be implemented uniformly in order to accurately identify and classify different phenotypes of PCOS, and hence additional studies are needed. The Androgen Excess and PCOS Society have suggested excluding patients with normal androgen levels among the PCOS phenotypes until more data becomes available.¹⁸

Menstrual irregularities due to ovulatory dysfunction were observed in 83.08% of all included patients. A study conducted in the Iranian population concluded menstrual irregularities in 94.6% of patients.¹⁶ Another study by Savas *et al.* conducted in Turkiye found menstrual irregularities in 68.2% of included patients.¹⁵ Clinical hirsutism was seen in 76.80% of all included patients in the study as compared to 95.2%¹⁵ and 68.2% in other studies. PCOM was observed in 98% of patients with phenotypes A, C, and D as compared to 80.8% reported by Savas *et al.*¹⁵ These variations in clinical presentations represent the heterogeneous natures, a variation of common syndrome, and presentation of patients with symptoms which are bothering them the most.

The study provided the mean differences for age, weight, history of miscarriages, LH, FSH, and AMH levels and LH / FSH ratio between the four phenotypes.

Mean weight was higher in phenotype A as compared to other phenotypes. Similar findings were also reported with higher BMI for phenotype A in other studies.^{19,20} Mean AMH representing ovarian reserve was also higher in phenotype A as compared to other phenotypes. Similar results were reported by a study conducted in India showing significantly higher AMH levels in phenotype A as compared to phenotype B and D.¹² Study conducted in Iran showed significantly higher AMH levels in phenotypes A and D.¹⁵ Higher AMH levels are reported in PCOS because of the recruitment of multiple antral follicles and increased production of AMH per antral follicle. The use of AMH as a diagnostic tool has been evaluated in many studies throughout the world. If proven to have diagnostic value, it could lead to early diagnosis of PCOS and in girls or adolescents who are not willing to undergo transvaginal ultrasound.

History of previous miscarriages was higher in phenotype C as compared to other phenotypes in this study. In another study conducted by Wang *et al.*, adverse pregnancy outcomes were reported to be higher in phenotypes A and D as independent risk factors.²¹ Recurrent miscarriages are associated with spontaneous and assisted conceptions in PCOS because of adverse endocrine (higher LH), metabolic, (insulin resistance), and endometrial *milieu*.

The study has shown a significantly high LH / FSH ratio in phenotype A than in other phenotypes.

This could be because phenotype A has all features of PCOS including anovulation and hyperandrogenism associated with higher FSH / LH ratios. Parveen *et al.* reported a higher LH / FSH ratio in phenotype A followed by phenotype B.²⁰ Another study conducted in Turkiye by Senem *et al.* concluded higher LH / FSH ratio in phenotype D than in other phenotypes.¹² The variation in LH / FSH ratio in women with PCOS as well as normal women is leading to speculation regarding the usefulness of the test. This could be because of changes in the diagnostic criteria of PCOS as it now includes more ovulatory patients as well. Higher LH / FSH ratio is associated with anovulation and hence poor ovulatory response after ovulation induction as well as adverse effects on a number of follicles and oocytes but better clinical progress of pregnancy and live birth.²⁰ If proven in other studies, the role of FSH / LH would be limited in patients with an ovulatory disorder in patients with PCOS.

Limitations of the study are that the sample was collected from a private clinic and hence is not a true representation of the general population but may reflect the higher prevalence of disease and its phenotypes due to selection bias as compared to a sample collected from general outpatient clinics. More studies need to be conducted in this area to know the true prevalence of the disease in the general population and in females with infertility.

CONCLUSION

PCOS is common in subfertile patients and should be considered in the initial evaluation of women presenting with subfertility. Phenotype A was the most prevalent phenotype and represents the severe form of PCOS due to the presence of all features. It is crucial to identify and classify the patients into different phenotypes and to evaluate their hormonal and physical characteristics, so as to identify the most severe and mild forms of the spectrum of disease for appropriate management and referrals. LH / FSH ratio although losing its importance according to recent ESHRE / ASRM consensus was significantly higher in phenotype A, representing the disease in its severe form along with all diagnostic features.

ETHICAL APPROVAL:

Ethical approval was obtained from the Institutional Research and Ethical Board of the Lady Reading Hospital before the initiation of research.

PATIENTS' CONSENT:

Informed consent was obtained from all participants regarding the inclusion of their anonymised data for publication.

COMPETING INTEREST:

The authors declared no conflict of interest.

AUTHORS' CONTRIBUTION:

RR: Concept and study design, drafting of discussion and literature review.

HU: Data collection, statistical analysis, and compilation of results.

HG: Data collection, statistical analysis, and collection of references.

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