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## Original Research Article

# Clinical, hormonal and ovarian morphological correlation in women with polycystic ovary syndrome

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## ABSTRACT

**Background:** Aim of the study was to study the correlation between clinical, ultrasonographical and hormonal features in women diagnosed as polycystic ovary syndrome (PCOS) and association with vitamin D levels.

**Methods:** This prospective study was conducted among women attending gynecological outpatient department (OPD) of Subharti Medical College, Meerut over a period of two years among 100 patients with clinical diagnosis of PCOS/PCOD according to Rotterdam criteria (2013) were included in this study. All biochemical investigations to be carried out for levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), vitamin D levels, lipid profile to understand the endocrinal and metabolic derangements if any in the patient. Ultrasound pelvis for ovarian study was conducted to know the ovarian morphology, no of follicles if any and their size, which were helpful in the diagnosis of PCOS.

**Results:** Nulliparity and multiparity was reported among 32% and 68% of the subjects respectively. Most common complaint was hirsutism (43%). According to ultrasonography (USG), PCOS was found to be positive and negative among 87% and 13% of the subjects respectively. Most of the subjects had vitamin D level of 20-50 while <20 vitamin D level was found among 27% of the subjects. Vitamin D deficiency was found to be more in subjects having morphological presence of PCOS as compared to subjects with morphological absence of PCOS with statistically significant difference.

**Conclusions:** On correlating ultrasonological findings with clinic hormonal changes in PCOS women we found that hirsutism and vitamin d deficiency was significantly more common in women with sonological findings suggestive of PCOS.

**Keywords:** PCOS, Vitamin D, USG, Hirsutism, LH, FSH

## INTRODUCTION

Polycystic ovary syndrome (PCOS) is considered to be a multifaceted disease with a spectrum of manifestations affecting not only women of childbearing age, but also adolescents and postmenopausal women.<sup>1</sup> PCOS, by the nature of the disease, adversely influences the fertility and reproductive health of the affected women; moreover, with its association with other lifestyle diseases, it is also the cause of significant cardiovascular and metabolic morbidity.<sup>2,3</sup>

The clinical manifestations of PCOS include oligomenorrhoea, hirsutism, excessive acne and hair loss. In adolescence, it causes significant psychiatric disturbances such as anxiety and depression. PCOS is the leading cause of anovulatory infertility in women. The metabolic consequences include impaired glucose tolerance, type 2 diabetes, obesity and increased risk of cardiovascular diseases. Metabolic complications and increased cardiovascular morbidity were found to be more in the classic PCOS compared to other phenotypes, even after adjustment for obesity.<sup>4</sup>

Women with PCOS usually suffer from metabolic disturbances and insulin resistance (IR), which might be associated with vitamin D metabolism. Vitamin D influences glucose and insulin metabolism, and low vitamin D status is a risk factor for impaired glucose tolerance, IR and T2DM.<sup>5-7</sup> Serum 25-hydroxyvitamin D (25-OHD) concentration is an effective indicator of vitamin D status in humans and vitamin D metabolism affects glucose and insulin metabolism and plays a significant role in T2DM.<sup>8-10</sup> The mechanisms by which serum vitamin D levels influence IR or T2DM are not yet clear, but previous studies found some important clues. First, low vitamin D concentration leads to an increased serum parathyroid hormone (PTH) level and elevated concentrations of PTH alter glucose metabolism and reduce insulin sensitivity.<sup>11,12</sup> Vitamin D may increase insulin receptor expression to improve insulin responsiveness in cells for glucose transport.<sup>13</sup> Further, vitamin D and the vitamin D receptor (VDR) regulate the expression of more than 300 genes, including the genes associated with glucose metabolism.<sup>14</sup> In view of the above correlations between vitamin D and insulin or glucose metabolism, several previous studies have examined the role of vitamin D in PCOS.<sup>15,16</sup> Although there is still no definite consensus on the significance of serum vitamin D levels in patients with PCOS and those without PCOS, an inverse correlation between serum 25-OHD concentrations and metabolic disturbances was reported in PCOS patients.<sup>17</sup>

The diagnosis of polycystic ovarian disease is sometimes elusive and requires visualization of both ovaries. The first ultrasonic observation of enlarged ovaries in patients with the disease were those of Kratochwil et al and Zemlyn. Several reports have recently appeared on the sonographic morphology of polycystic ovaries. The ultrasonic diagnosis of polycystic ovaries in many of these studies was not confirmed by hormonal assay and most of these reports did not correlate the ultrasonic appearance of PCOS to the clinical and hormonal findings.<sup>18</sup>

Notwithstanding the significant reproductive, endocrine and metabolic morbidity of PCOS, little is known of its different modes of presentation in Indian population. The aim was to study the correlation between clinical, ultrasonographical and hormonal features in women diagnosed as PCOS and association with vitamin D levels.

## METHODS

This prospective study was conducted among women attending gynecological OPD of Subharti Medical College, Meerut over a period of two years (November 2019 to August 2021). The study protocol for all procedures was approved by the institutional review board for ethical clearance of the institution and was performed in accordance with the code of ethics of the World Medical Association according to the declaration of Helsinki of 1975, as revised in 2000. All patients were asked to sign a written consent form prior to commencement of the study.

Patients with clinical diagnosis of PCOS/PCOD according to Rotterdam criteria (2013) were included in this study. A total 100 cases, in age group of 18-45 years, were included in the study after obtaining a written informed consent.

Women of age group between 18 years to 45 years, patients with clinical diagnosis of PCOS/PCOD according to Rotterdam criteria (2013) by European society for human reproduction and embryology, which includes two of the three criteria's (Oligo ovulation or chronic anovulation, clinical or biochemical signs of hyperandrogenism and polycystic ovarian appearance on ultrasonography) were included in the study. Subjects were excluded, if they had age <18 years or >45 years, had currently received vitamin D supplementation in last 6 months, with some known systemic diseases (cardiac, hepatic, endocrinal, and gastrointestinal diseases) and taken medication in the last 6 months which is known to interact with vitamin D metabolism (steroids, thiazide, anti-tubercular drugs, phenobarbitone, and phenytoin).

## Definition

Ovulatory dysfunction was defined as menstrual cycles  $\geq 35$  days in length or menstrual cycles of <8 cycles/year.

Obesity was defined as body mass index (BMI) of 30.

Presence of stigmata of insulin resistance like, acanthosis nigricans were noted. Presence of stigmata of hyperandrogenism were noted.

Hirsutism was defined as excessive facial and or body terminal hair showing a male pattern distribution.

Ferriman and Gallwey scoring system was used to assess hirsutism. A score of  $\geq 8$  was taken as hirsute.

Acne was defined as presence of comedones on the face, neck, chest, upper back or arms.

The criteria to define PCOS by ultrasound were: presence of 12 or more follicles in each ovary measuring 2-9 mm in diameter and/or increased ovarian volume (>10 ml). The follicle distribution and stromal echogenicity were omitted. Only one ovary fitting this definition was sufficient to define PCOS.

## Method

Detailed history about various general and clinical aspects was taken from the patients attending the faculty of department of obstetrics and gynecology and who are willing to take part in the study.

Detailed menstrual history and obstetric history including history of married life, gravida status, parity, and number of abortions was asked from the patients to rule out any exclusion criteria of the study. History of any past disease or drug intake was taken to satisfy the exclusion criteria's.

Clinical and general examination was done which include measurement of pulse, blood pressure, respiratory rate and temperature. This will help to know the general health condition of the patient. Details of any dermatological complications such as hirsutism, acne, and patters of alopecia will be asked from the patient.

Anthropometric examination including measurement of weight (in kg) and height (in m) was done to calculate the BMI of the patient, and other anthropometric indices like waist circumference, hip circumference, waist/hip ratio to be calculated. These helped to measure the level of overweight and obesity in the subjects. Gynecological examination including per abdominal examination, per speculum examination and per vaginal examination was done to rule out any other gynecological complication which may affect the study.

Blood samples were collected in fasting state at 0 hour and 2 hours after 75 gm glucose infusion to calculate the glucose tolerance test (GTT) value. All biochemical investigations to be carried out for levels of LH, FSH, vitamin D levels, lipid profile to understand the endocrinal and metabolic derangements if any in the patient. Ultrasound pelvis for ovarian study was conducted to know the ovarian morphology, no of follicles if any and their size, which were helpful in the diagnosis of PCOS.

#### Statistical analysis

Data so collected was tabulated in an excel sheet, under the guidance of statistician. The means and standard deviations of the measurements per group were used for statistical analysis (SPSS 22.00 for windows; SPSS Inc., Chicago, USA). Difference between two groups was determined using student t-test as well as chi square test and the level of significance was set at  $p < 0.05$ .

## RESULTS

Out of 100 subjects, maximum subjects were from the age group of 27-35 years (52%) followed by 36-45 years (29%) and 18-26 years (19%). Nulliparity and multiparity was reported among 32% and 68% of the subjects respectively. Most common complaint was hirsutism (43%). Other complaints viz. menstrual disturbance, dermatological, infertility and weight gain was found among 15%, 19%, 3% and 31% of the subjects respectively (Figure 1). BMI ( $\text{kg/m}^2$ ) 25-29.9 was revealed among 28 subjects while 65 subjects were having BMI between 18.5-24.9. Nineteen women had increased waist/hip ratio of  $>0.86$ . 48 women had low waist/hip ratio of  $<0.8$  (Table 1).

43% of the subjects had LH value of  $>20$  while 38% and 18% of the subjects had LH ratio of 20 and  $<5$  respectively. FSH value of  $<5$ , 5-20 and  $>20$  was found among 44%, 29% and 27% of the subjects respectively. LH/FSH value of  $<2/1$  and  $>2/1$  was revealed in 59% and 41% of the subjects respectively (Table 2).

**Table 1: Distribution of BMI and waist/hip ratio among the study subjects.**

BMI ( $\text{kg/m}^2$ )	N=100	%
$>35$	1	1
30-34.9	5	5
25-29.9	28	28
18.5-24.9	65	65
$<18.5$	1	1
<b>Waist/hip ratio</b>		
$<0.8$	48	48
0.8-0.86	33	33
$>0.86$	19	19

**Table 2: LH, FSH and LH/FSH value among the study subjects.**

LH	N=100	%
$<5$	19	19
5-20	38	38
$>20$	43	43
<b>FSH</b>		
$<5$	44	44
5-20	29	29
$>20$	27	27
<b>LH/FSH</b>		
$<2$	59	59
$>2$	41	41

Deranged lipid profile and GTT was found among 37% and 39% of the subjects respectively (Table 3).

**Table 3: Lipid profile and GTT among the study subjects.**

Lipid profile	N=100	%
Normal	63	63
Deranged	37	37
<b>GTT</b>		
Normal	61	61
Deranged	39	39

According to USG, PCOS was found to be positive and negative among 87% and 13% of the subjects respectively. Most of the subjects had vitamin D level of 20-50 while  $<20$  vitamin D level was found among 27% of the subjects.

**Table 4: PCOS findings according to USG and vitamin D level among the study subjects.**

PCOS	N=100	%
Morphology present	87	87
Morphology absent	13	13
<b>Vitamin D</b>		
$<12$	27	27
12-20	61	61
$>20$	12	12

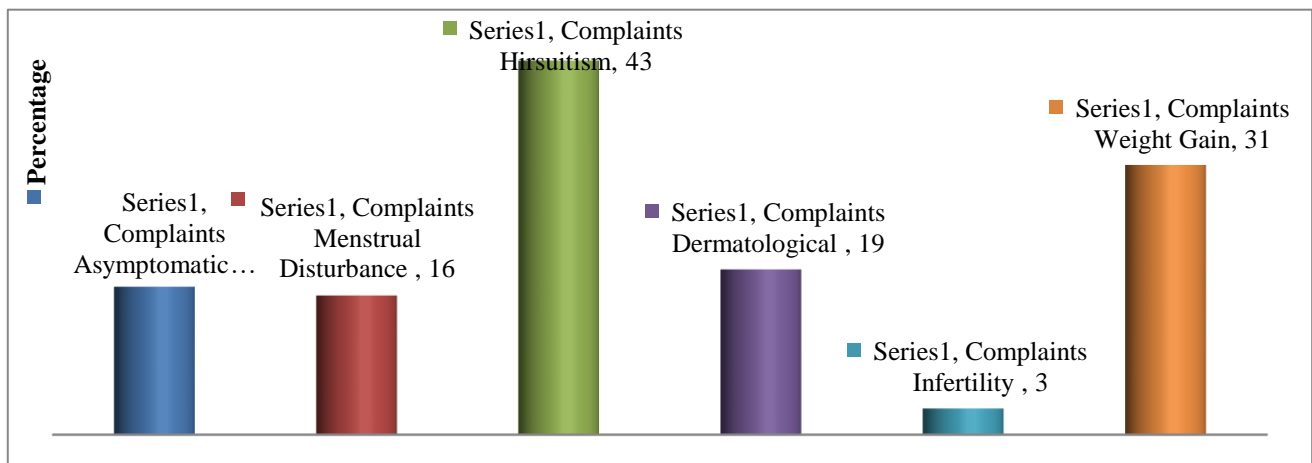
Menstrual disturbance and hirsutism was revealed more in subjects having morphological presence of PCOS as compared to subjects with morphological absence of PCOS with statistically significant difference. Vitamin D

deficiency was found to be more in subjects having morphological presence of PCOS as compared to subjects with morphological absence of PCOS with statistically significant difference (Table 5).

**Table 5: Correlation of USG findings with clinic-hormonal changes in PCOS.**

Variables	PCOS				P value	
	Present (N=87)		Absent (N=13)			
	N	%	N	%		
Asymptomatic	15	17.24	2	15.38	0.81	
Menstrual disturbance	15	17.24	1	7.69	0.037*	
Hirsutism	41	47.13	2	15.38	<0.01*	
Infertility	3	3.45	0	0.00	0.68	
LH/FSH						
<2	52	59.77	7	53.85	0.76	
>2	35	40.23	6	46.15		
GTT (deranged)	35	40.23	4	30.77	0.18	
BMI, mean (SD)	25.61	3.94	25.01	3.58	0.59	
>30	5	5.75	1	7.69	0.09	
25-29.9	26	29.89	2	15.39		
18.5-24.9	55	63.22	10	76.92		
<18.5	1	1.15	0	0	0.52	
Waist/hip ratio, mean (SD)	0.82	0.21	0.81	0.17		
<0.8	42	48.28	6	46.15		0.07
0.8-0.86	27	31.03	6	46.15		
>0.86	18	20.69	1	7.69		
Vitamin D						
<12	26	29.89	1	7.69	<0.01*	
12-20	58	66.67	3	23.08		
>20	3	3.45	9	69.23		

\*statistically significant



**Figure 1: Presenting complaints among the study subjects.**

## DISCUSSION

In the pathophysiology of PCOS, the pulsatile release of gonadotropic releasing hormone (GnRH) is disturbed, and this has a negative impact on the follicle development in ovaries. Some follicles do not fully mature, some follicles go into atresia, and some stay in the ovarian periphery in

the form of small cysts. Polycystic-looking ovaries are not definite for diagnosing PCOS. Studies have reported considerably different rates in terms of the incidence of polycystic ovary (PCO) morphology in patients with PCOS and there are inconsistent results regarding the effects of PCO morphology in PCOS patients.<sup>19</sup> This prospective study was conducted among women attending

gynecological OPD of Subharti Medical College, Meerut over a period of two years among 100 patients with clinical diagnosis of PCOS/PCOD according to Rotterdam criteria (2013).

Out of 100 subjects, maximum subjects were from the age group of 27-35 years (52%) in our study. PCOS is reported to be more prevalent in younger ages (<35) than among older women, proposing that due to a physiological decline of the follicular cohort leading to a normalized ovarian ultrasonographic appearance with advancing age. Muralidhara et al and Spandana et al revealed similar age distribution.<sup>20,21</sup> In contrast Gowri et al and Sidhmalswamy et al reported slightly younger age as compared to our study.<sup>22,23</sup>

In our study, nulliparity and multiparity was reported among 32% and 68% of the subjects respectively. Spandana et al revealed that 68.9% of the subjects were nulliparous and 31.1% of the subjects were parous.<sup>21</sup>

Most common complaint was hirsutism (43%). Other complaints viz. menstrual disturbance, dermatological, infertility and weight gain was found among 15%, 19%, 3% and 31% of the subjects respectively in our study. Similarly Sidhmalswamy et al revealed that the most common presentation was hirsutism (39.8%), next common was hirsutism with acne which accounted for 11%.<sup>23</sup> In a study done by Mandrelleet al, hirsutism was present in 28.3%, acne in 9.2% and acanthosis was seen in 15.8%.<sup>24</sup> Gowri et al in their study showed that patients with PCOS acne vulgaris topped the list with 27 patients (67.5%) showing acne, followed closely by hirsutism in 25 (62.5%) patients, seborrhea was seen in 21 (52.5%) patients, AGA in 12 (30%) patients, acanthosis nigricans in 9 (22.5%) patients and acrochordons in 4 (10%) of patients.<sup>22</sup>

BMI ( $\text{kg/m}^2$ ) 25-29.9 was revealed among 28 subjects while 65 subjects were having BMI between 18.5-24.9. Only one subject had BMI <18.5. 52% of the women had waist/hip ratio of >0.80 in this study, highlighting that Indians have more central obesity. Spandana et al similarly revealed that the mean BMI was  $25.95 \pm 4.96$ .<sup>18</sup> >50% of the subjects had BMI >25. WHR >0.85 was seen in 38% of the patients. Gowri et al in their study showed that prevalence of obesity was about 32.5%.<sup>22</sup> In a study done by Mandrelleet al, WHR was more in 45.8% of the patients.<sup>24</sup> Thangaveluet al in their study found that BMI and WHR was elevated in polycystic ovary syndrome patients compared to the control subjects.<sup>25</sup>

43% of the subjects had LH value of >20 while 38% and 18% of the subjects had LH ratio of 20 and <5 respectively. FSH value of <5, 5-20 and >20 was found among 44%, 29% and 27% of the subjects respectively. LH/FSH value of <2/1 and >2/1 was revealed in 59% and 41% of the subjects respectively. Abnormality of the hypothalamic-pituitary ovarian or adrenal axis has been implicated in PCOS. Disturbance in the pulsatility of GnRH results in

the relative increase in LH to FSH release. An abnormal feedback mechanism by ovarian estrogen is blamed to play role in this discriminated increase in LH release. Many researchers consider elevated LH: FSH (>2) diagnostic for PCOS. Nizem et al in their study showed that 16% had elevated LH/FSH ratio.<sup>26</sup> Spandana et al similarly revealed that 6% had increased FSH, 27% had elevated LH and LH: FSH (>2) was elevated in 35% of patients.<sup>21</sup> Inanet al in their study found that serum mean level of LH was significantly higher in PCOS group with PCO morphology was significantly lower.<sup>19</sup> The LH/FSH levels was found to be higher too.

Deranged lipid profile was found among 37% of the subjects in this study. Dyslipidemia is common among patients with PCOS. It is possible to have increased low density lipoprotein (LDL), triglycerides (TG) and decreased high density lipoprotein (HDL) along with PCOS. Dyslipidemia is related to unhealthy diets, obesity, metabolic syndrome, hyperandrogenism, physically inactive lifestyles, and genetic factors.<sup>1</sup> Inanet al in their study found that the total cholesterol and TG levels were significantly lower.<sup>19</sup>

Deranged GTT was reported among 39% of the subjects. Most of the subjects had vitamin D level of 20-50 while <20 vitamin D level was found among 27% of the subjects in our study. The 25-OHD concentration is an indicator of vitamin D status in the human body and vitamin D deficiency is a major problem in PCOS because it relates to metabolic syndrome, which includes obesity, IR, and glucose intolerance. Jia et al in their meta-analysis found that the levels of 25-OHD and the quantitative insulin-sensitivity check index in the PCOS group were remarkably lower than in the controls, whereas the homeostasis model assessment of insulin resistance in the PCOS group was markedly higher than in the controls.<sup>21</sup> The 25-OHD levels were significantly elevated after PCOS patients received vitamin D3 treatment.

According to USG, PCOS was found to be positive among 87% of the subjects. Menstrual disturbance and hirsutism was revealed more in subjects having morphological presence of PCOS as compared to subjects with morphological absence of PCOS with statistically significant difference. Vitamin d deficiency was found to be more in subjects having morphological presence of PCOS as compared to subjects with morphological absence of PCOS with statistically significant difference in this study. Spandana et al in 2017 found that 97% were detected to have PCOS in USG.<sup>21</sup>

One limitation of the study is the sample size, which included a small group of participants. It is a cross sectional observational study, so long term complications of PCOS could not be studied. Future research should focus on early detection of the predisposing risk factors in PCOS development, including long-term studies with the goal of modifying environmental factors so that risk may be significantly reduced.



## CONCLUSION

We found that hirsutism and weight gain were the most common presentations in women with PCOS followed by dermatological and menstrual complaints. In our study, vitamin D levels were significantly lower in women with PCOS as compared to general population. On correlating ultrasonological findings with clinic hormonal changes in PCOS women we found that hirsutism and vitamin D deficiency was significantly more common in women with sonogological findings suggestive of PCOS. For future research large randomized controlled clinical trials are necessary to explore whether this association has a causal linkage.

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