Original Article

Comparison of Clinical, Metabolic, Hormonal, and Ultrasound Parameters among the Clomiphene Citrate-Resistant and Clomiphene Citrate-Sensitive Polycystic Ovary Syndrome Women

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Abstract

Objective: The objective of the study is to compare the clinical, metabolic, hormonal, and ultrasound parameters among the clomiphene citrate (CC)-sensitive and CC-resistant polycystic ovary syndrome (PCOS) women. **Materials and Methods:** This was a prospective observational study. **Setting:** The study was conducted at the infertility outpatient department in a government hospital. **Sample Size:** A total of 164 women with PCOS-related infertility were included. **Intervention:** Incremental dose of CC from 50 mg/day to 100 mg/day to 150 mg/day over three cycles was given. **Response:** Ovulation was the outcome. Those who failed to ovulate with 150 mg CC were CC resistant. **Results:** Of the total 164 PCOS women, 88 (53.7%) were CC resistant and 76 (46.3%) were CC sensitive. Of the 76 PCOS women who ovulated, maximum, i.e., 37 (22.6%) women ovulated with 100 mg CC. The most common diagnostic feature of PCOS in this study was hyperandrogenism (96.3%). CC-resistant PCOS women had significantly higher weight, waist circumference, waist-hip ratio, and body mass index (BMI). Significantly longer menstrual cycles and hyperandrogenism were significantly more common in CC-resistant group. CC-resistant group had a significantly higher ovarian reserve (ovarian volume, antral follicle count, and anti-Müllerian hormone values). Baseline luteinizing hormone (LH) values and LH-follicle stimulating hormone ratio were significantly higher in the CC-resistant group. **Conclusion:** Clomiphene-resistant PCOS women have significantly higher hyperandrogenism, longer cycles, more deranged metabolic profile, higher BMI, and ovarian reserve. These differences should be kept in mind while deciding the ovulation induction protocol.

Keywords: *Anti-Müllerian hormone, clomiphene, Ferriman–Gallwey score, metabolic syndrome, polycystic ovary syndrome*

Introduction

Polycystic ovary syndrome (PCOS) is a common endocrinological disorder seen in 6%–10% of the women.[1] It is characterized by polycystic ovaries, anovulatory cycles, and hyperandrogenism.

In nearly 20% of the infertile women, PCOS is said to be the key reason behind infertility.[2]

PCOS is a syndrome which manifests variably from adolescence as oligomenorrhea or hirsutism or obesity and goes on to affect the reproductive performance of the female by causing anovulation. Some may even be severely affected by metabolic syndrome, diabetes mellitus, or endometrial carcinoma. It also increases the risk of ovarian and breast carcinoma.[3]

PCOS falls in WHO type II anovulation (norm-gonadotropic norm-estrogenic anovulation) and is

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seen in 85% of anovulatory females. Although lifestyle modification is known to improve reproductive outcomes in females with PCOS, the gold standard treatment for norm-gonadotropic oligo/amenorrheic infertility (WHO Group II) was clomiphene citrate (CC)[4] until 2018, when ESHRE and ASRM have declared letrozole as the first-line treatment for ovulation induction (OI).

Those who fail to respond to CC are labeled as clomiphene resistant. It is common in approximately 15%–40% of women with PCOS.[5] Major factors postulated for CC resistance include obesity, insulin resistance, (seen in nearly 50%–70% of females with PCOS) and hyperandrogenemia.[6] Moreover, genetic predisposition is suggested to play a role in CC resistance.[7] However, still, the current data available on the causes of CC resistance are not sufficient enough to direct our treatment.

It is seen in various studies[8,9] that the females who initially failed to respond to CC develop better ovulation and pregnancy outcomes on treatment with insulin-sensitizing agents. This indicates that insulin resistance may be a cause of CC resistance in females with PCOS. In fact, insulin-sensitizing agents[10] decrease the dose of ovulation-inducing agent and time for follicular maturation in females with PCOS.

As of now, there have been no concrete studies to compare metabolic profile of females who respond to CC and those who do not. It is still an enigma as to why some women respond to clomiphene, while others do not. By identifying the various factors which affect the response of CC in patients with infertility, a lot of time can be saved by giving alternate options of treatment to these patients. This study was done with the aim to analyze various clinical, metabolic, hormonal, and ultrasound parameters which might affect the response to clomiphene.

Materials and Methods

This prospective observational study was conducted in the infertility outpatient department of a government hospital. The period of the study was 1 year and 164 patients with PCOS-related infertility were enrolled.

Approval was taken for this study from the ethical committee of the institution before starting the study.

The WHO estimates overall prevalence of primary infertility to be between 3.9% and 16.8%[11] (average 10.35%). In nearly 20% of these infertile women, PCOS is the cause behind infertility.[2] Based on this data, the overall prevalence of PCOS-related infertility is 3.36%. Using the prevalence as 3.36%, the sample size comes out to be 143. Hence, according to the formula, the estimated sample size should be at least 143. Thus, total of 312 patients were contacted in the study. However, 96 (30.77%) patients were excluded after primary evaluation (they fell in the exclusion criteria). Fifty-two (16.67%) patients lost to follow-up during the course of the study. Hence, the final sample size was 164 patients with PCOS-related infertility.

Inclusion criteria included women with PCOS (based on Rotterdam's criteria[1]) related infertility of age <40 years. Women on any insulin-sensitizing agent or lipid-lowering agent or having an endocrine disorder (such as thyroid dysfunction, insulin resistance, and adrenal disorders) or anorexia nervosa/bulimia nervosa or with hypothalamic or pituitary dysfunction were excluded.

All PCOS women desirous of pregnancy were evaluated after written informed consent. Relevant history was taken to rule out the exclusion criteria. The physical examination including her blood pressure, weight in kilograms using a beam balance, and height in upright posture without shoes using a stadiometer to the nearest 0.5 cm was recorded. Body mass index (BMI) was recorded from the above measurements. Owing to the differences in body fat distribution between Asian and Western population, the WHO expert committee in 2004[12] has proposed BMI cutoffs for Asian population which was used in this study.

Waist circumference (WC) was measured midway between lower rib margin and the iliac crest in the mid-axillary line at the end of normal expiration.[13] Hip circumference was measured with the measuring tape at the highest prominence of the buttocks and parallel to the floor.[13] WC and hip circumference were recorded after removing clothing from the area over waist and hip. The cutoff value of BMI was taken as <23 kg/m^2, for WC was 80 cm, and for waist-hip ratio (WHR) was 0.81 based on the study conducted in Asians.[13]

Thyroid was examined for any enlargement, nodules, or tenderness. The breast was examined for any enlargement or galactorrhea. Signs of androgen excess were looked for such as excessive hair growth, acne, or alopecia. Excessive hair growth was evaluated by modified Ferriman and Gallwey[14] (FG) score.

Transvaginal scan (TVS) was done by the same observer using a Philips ultrasound machine, model IU22 (TVS probe frequency range 5–7 MHZ). Ovarian volume of each ovary was assessed by ellipsoid formula, i.e., 0.52 × D1 (longitudinal) × D2 (oblique) × D3 (transverse) diameters. Mean ovarian volume was calculated by adding the volume of both ovaries and then dividing it by 2. A note of ovarian follicles in each

ovary was also made and total number of the follicles was counted by scanning each ovary from inner to the outer margin in longitudinal cross-section. Mean follicle number was calculated by adding the follicles of both ovaries and then dividing it by 2.

The patients enrolled in the study were called on day 2 of her next cycle for the investigations (follicle stimulating hormone [FSH], luteinizing hormone [LH], anti-Müllerian hormone [AMH], 17-hydroxyprogesterone levels [17 OHP], testosterone, androstenedione, Vitamin D, 75 g oral glucose tolerance test, fasting insulin, fasting triglycerides, high-density lipoprotein [HDL], low-density lipoprotein [LDL], and cholesterol levels). Ultrasonography abdomen was also done to rule out fatty changes in the liver. The homeostasis model assessment of insulin resistance (HOMA-IR),[15] a surrogate marker of insulin resistance, was used in this study. Patients with HOMA-IR >2 were defined as having insulin resistance.

All these patients were treated with CC starting with 50 mg/day on the day 2–5 of their cycle for 5 days. In case of failure of ovulation, the dose will be increased by 50 mg in subsequent cycles to a maximum dose of 150 mg over three cycles.

Response to CC was assessed by ovulation. TVS was done by the same observer using a Philips ultrasound machine, model IU22. A scan was done starting from day 10 of the cycle and until follicle size >18 mm or day 20 of the cycle. Patients were called after 2–3 days of development of dominant follicle to mature or presence of free fluid.

Based on the ovulation pattern, these patients were divided into two groups, one who ovulated with CC maximum 150 mg and others who did not ovulate considered as CC resistant. The patients who ovulated were further classified into three subgroups based on whether they ovulated with 50 mg or 100 mg or 150 mg of CC.

The various parameters were compared between the CC-resistant and CC-sensitive groups.

The various parameters of the patients were recorded as mean ± standard deviation (SD). Normality of quantitative data was checked by measures of Kolmogorov–Smirnov tests of normality. If data were normally distributed, independent *t*-test was applied for comparison of two groups (clomiphene-sensitive and clomiphene-resistant). Mann–Whitney U-test was used for statistical analysis of skewed continuous variables. Proportions were compared using Chi-square or Fisher's exact test, whichever applicable. All statistical tests were two-sided and performed at a significance level of α =0.05. The analysis of the data was done using an online software 'IBM, SPSS Statistics (version 24.0, Armonk, NY: IBM Corp)'.

Observations and Results

Of the total 164 PCOS women, 88 (53.7%) were CC resistant and 76 (46.3%) were CC sensitive. Of the 76 PCOS women who ovulated, maximum, i.e., 37 (22.6%) women ovulated with 100 mg CC. Ovulation with 50 mg and 150 mg CC was seen only in 19 (11.6%) and 20 (12.2%) women, respectively.

The most common feature of PCOS was hyperandrogenism (96.3%) followed by polycystic ovaries (89.1%) and then oligomenorrhea (82.3%).

Comparison of various clinical, biochemical, metabolic, and ultrasound features between CC-resistant and CC-sensitive group is given in Tables 1-3.

The mean weight was 27.98 ± 3.739 SD years and it was significantly different between the CC-resistant and CC-sensitive groups (64.17 ± 10.51 SD and 60.02 ± 10.51 SD kg, P = 0.127).

The mean BMI was 26.077 ± 4.306 kg/m^2 and a significant difference was noted between the CC-resistant and CC-sensitive groups (27.12 ± 4.16 SD and 24.88 ± 4.19 SD kg/m^2) (P = 0.001). Maximum proportion, i.e., 69 (42.1%), of the PCOS women enrolled in the study were overweight with the BMI between 23 and 27.5 kg/m^2.

The mean WC was 33.63 ± 3.72 inch and it was significantly different among the CC-resistant and CC-sensitive groups (34.28 ± 3.37 SD and 32.89 ± 3.98 SD inch, respectively, P = 0.001).

The mean WHR was 0.88 ± 0.044 and a significant difference noted in the mean WHR between CC-resistant and CC-sensitive groups (0.89 ± 0.04 SD and 0.87 ± 0.05 SD, respectively, P = 0.008).

Oligomenorrhea (menstrual cycle >35 days) was seen in as many as 135 out of 164 (82.3%) PCOS women. Cycle length >60 days was more common in CC-resistant PCOS women, seen in 73 (83%) women, as compared to CC sensitive seen in 31 (40.8%) wome n (χ2 (2) =31.268, P = 0. 000).

Normal FG score (<8) was seen in only 6 (4.7%) PCOS women. Mild hyperandrogenism (FG score 8–15) was present in 84 (50.2%) PCOS women and moderate–severe hyperandrogenism (FG > 15) was seen in 74 (45.1%) of PCOS women. Of the PCOS women with moderate–severe hyperandrogenism, 64 (72.7%) were CC-resistant PCOS women and 10 (13.2%) were

Table 1: Clinical and biochemical hyperandrogenism in clomiphene citrate-resistant and clomiphene citrate-sensitive groups

Parameter	Category	Overall distribution (*n*=164), *n* (%)	CC resistant (*n*=88), *n* (%)	CC sensitive (*n*=76), *n* (%)
BMI (kg/m^2)	<18.5	3 (1.8)	1 (1.13)	2 (2.63)
	18.5-23	36 (21.95)	22 (25)	14 (18.42)
	23-27.5	69 (42.07)	35 (39.77)	34 (44.74)
	>27.5	55 (33.54)	30 (34.10)	25 (32.89)
FG score	<8	6 (3.66)	2 (2.3)	4 (5.3)
	8-15	84 (51.22)	22 (25)	62 (81.5)
	>15	74 (45.12)	64 (72.7)	10 (13.2)
Testosterone levels (nmol/l)	Normal (<3.05)	115 (70.12)	45 (51.1)	70 (92.1)
	Increased (≥3.05)	49 (29.88)	43 (48.9)	6 (7.9)
Androstendione levels (ng/ml)	Normal (<3.3)	120 (73.17)	51 (58)	69 (90.8)
	Increased (≥3.3)	44 (26.83)	37 (42)	7 (9.2)

CC=Clomiphene citrate, BMI=Body mass index, FG=Ferriman-Gallwey

Table 2: Comparison of mean parameters in the two groups

Parameters	Mean±SD			*P*
	Overall (*n*=164)	CC resistant (*n*=88)	CC sensitive (*n*=76)	
Age (years)	27.98±3.739	27.99±3.97	27.97±3.48	0.980
Weight (kg)	62.24±10.68	64.16±10.51	60.02±10.50	0.013*
Height (m)	1.54±0.055	1.54±0.06	1.55±0.50	0.127
BMI (kg/m^2)	26.077±4.306	27.12±4.16	24.88±4.19	0.001*
WC (inches)	33.63±3.72	34.28±3.37	32.89±3.98	0.017*
WHR	0.88±0.044	0.89±0.04	0.87±0.05	0.008*
SBP (mmHg)	117.32±7.612	118±7.6	116±7.43	0.053
DBP (mmHg)	74.75±5.805	76±5.45	74±5.69	0.069
FG score	13.98±3.75	16.11±2.87	11.20±3.30	0.0001*
Testosterone (nmol/l)	2.74±1.28	3.30±1.39	2.08±0.735	0.0001#
Androstenedione (ng/ml)	2.97±1.36	3.54±1.53	2.31±0.723	0.0001#
Mean ovarian volume (cm^3)	12.52±3.07	13.65±3.26	11.21±2.23	0.001*
Mean AFC	11.81±3.17	13.19±3.07	10.21±2.47	0.000*
AMH (ng/ml)	10.58±5.00	12.22±5.62	8.69±3.30	0.0001*
OGTT1 (mg/dl)	90.14±13.07	92.65±14.79	87.24±10.08	0.008*
OGTT2 (mg/dl)	148.02±35.22	158.33±36.05	136.08±30.325	0.0001*
OGTT3 (mg/dl)	130.73±29.37	137.57±29.94	122.82±26.67	0.001*
Fasting insulin (mIU/L)	11.89±6.83	14.68±6.62	8.65±5.55	0.0001*
HOMA-IR	2.73±1.81	3.43±1.85	1.92±1.39	0.0001*
Serum triglycerides (mg/dl)	133.11±50.91	146.35±60.93	117.19±29.71	0.0001#
Serum cholesterol (mg/dl)	171.12±43.01	190.66±37.00	148.48±38.28	0.0001*
LDL (mg/dl)	110.55±0.06	118.82±25.26	100.97±18.31	0.0001*
HDL (mg/dl)	47.98±9.67	44.41±8.08	52.12±9.76	0.0001*
Baseline FSH (IU/l)	5.84±2.50	6.02±2.72	5.64±2.22	0.241
Baseline LH (IU/l)	13.53±7.42	14.81±8.05	12.04±6.34	0.007#
LH: FSH	2.48±1.18	2.66±1.22	2.27±1.11	0.035*
17 OHP (ng/dl)	1.38±0.77	1.49±0.8	1.26±0.7	0.062
Vitamin D (ng/ml)	16.87±9.55	16.01±10.62	17.86±8.10	0.01*

**t*-test, #Mann-Whitney U-test. BMI=Body mass index, SBP=Systolic blood pressure, DBP=Diastolic blood pressure, AFC=Antral follicle count, AMH=Anti-Mullerian hormone, OGTT1=First value of oral glucose tolerance test (fasting), OGTT2=Second value of oral glucose tolerance test (at 1 h after 75 g glucose), OGTT3=Third value of oral glucose tolerance test (at 2 h after 75 g glucose), HOMA-IR=Homeostatic model assessment for insulin resistance, LDL=Low-density lipoprotein, HDL=High-density lipoprotein, FSH=Follicle stimulating hormone, LH=Luteinizing hormone, 17 OHP=17-hydroxy progesterone, FG=Ferriman-Gallwey, CC=Clomiphene citrate, SD=Standard deviation, WHR=Waist-hip ratio, WC=Waist circumference

CC-sensitive women. Statistically significant differences were seen between the CC-sensitive and CC-resistant groups in the FG score, basal testosterone, and androstenedione levels (P = 0.0001).

Table 3: Comparison of clomiphene citrate-sensitive and clomiphene citrate-resistant groups

	CC resistant	CC sensitive	*P*
AFC (%)			
<6	0	1 (1.3)	0.0001#
6-10	11 (12.5)	39 (51.3)	
>10	77 (87.5)	36 (47.4)	
Total	88	76	
Fatty liver			
Absent	24	56	0.000*
Present	64	20	
Total	88	76	

*Chi-square test, #Fisher's exact test. CC=Clomiphene citrate, AFC=Antral follicle count

The mean ovarian volume of the women was 12.52 ± 3.07 and it was significantly different among the CC-resistant and CC-sensitive groups (13.65 ± 3.26 vs. 11.21 ± 2.23, $P = 0.000$).

The mean antral follicle count (AFC) of the women was 11.81 ± 3.07 and there was a significant difference between the CC-resistant and CC-sensitive groups (13.19 ± 3.07 vs. 10.21 ± 2.47, $P = 0.001$). Most of the CC-resistant PCOS women had AFC > 10 (87.5% vs. 47.4%).

The mean AMH of the women was 10.58 ± 5.00 and it was significantly higher in the CC-resistant group ($P = 0.001$).

The mean oral glucose tolerance test (OGTT, fasting, at 1 h, and at 2 h) values of the women were 90.14 ± 13.07, 148.02 ± 35.22, and 130.73 ± 29.37, respectively, and there were significant differences in the mean OGTT1 (92.65 ± 14.79 vs. 87.24 ± 10.08), OGTT2 (158.33 ± 36.05 vs. 136.08 ± 30.325), and OGTT3 (137.57 ± 29.94 vs. 122.82 ± 26.67) values between CC-resistant and CC-sensitive groups ($P = 0.008$, 0.0001, 0.001).

The mean fasting insulin was 11.89 ± 6.83 and there was a significant difference in the mean fasting insulin values between women in CC-resistant and CC-sensitive groups (14.68 ± 6.62 and 8.65 ± 5.55) respectively ($P = 0.001$).

The mean HOMA-IR of the women was 2.73 ± 1.81 and there was a significant difference in the mean HOMA-IR values between women in CC-resistant and CC-sensitive groups (3.43 ± 1.85 and 1.92 ± 1.39, respectively, $P = 0.001$).

The overall mean lipid profile (triglycerides, cholesterol, LDL, and HDL) values of the women were 133.11 ± 50.91, 171.12 ± 43.01, 110.55 ± 0.06 and 47.98 ± 9.67, respectively, and there was a significant difference in the mean triglycerides (146.35 ± 60.93 vs. 117.19 ± 29.71), cholesterol (190.66 ± 37.00 vs. 148.48 ± 38.28), LDL (118.82 ± 25.26 vs. 100.97 ± 18.31), and HDL (44.41 ± 8.08 vs. 52.12 ± 9.76) values between women in CC-resistant and CC-sensitive groups ($P = 0.0001$, 0.0001, 0.0001, 0.0001).

Fatty liver was present in 51.2% of the PCOS women. It was more common in CC-resistant (72.7%) as compared to CC-sensitive (26.3%) (χ^2 (1) =35.158, $P = 0.000$).

The mean baseline LH of the women was 13.53 ± 7.42, and there was a significant difference between the CC-resistant and CC-sensitive groups (14.81 ± 8.05 and 12.04 ± 6.34, $P = 0.007$) respectively.

The overall mean baseline LH: FSH of the women was 2.48 ± 1.18, and there was a significant difference between the CC-resistant and CC-sensitive groups (2.66 ± 1.22 and 2.27 ± 1.11, respectively, $P = 0.035$).

Of the total 164 PCOS women, 56 (34.14%) had LH/FSH of more than 3.

The mean Vitamin D levels of the women was 16.87 ± 9.55, and it was significantly different between the CC-resistant and CC-sensitive groups (16.01 ± 10.62 and 17.86 ± 8.10, respectively, $P = 0.01$).

Vitamin D deficiency was seen in 73.2% of PCOS women enrolled in the study had Vitamin D deficiency (Vitamin D <20 ng/ml).

Of the total 76 PCOS females who ovulated, only 26 (34.2%) actually conceived. Conception rates with 50/100/150 mg CC was 8 (10.5%), 12 (15.8%), and 6 (7.9%), respectively.

Discussion

The present study revealed that CC-resistant PCOS women had significantly higher weight, BMI, WC, and WHR. The cycles were significantly longer (oligomenorrhea more common) in CC-resistant women. Furthermore, those who did not respond to clomiphene had a higher FG score, total testosterone, and total androstenedione levels. The LH levels and the LH-FSH ratio were significantly higher in CC-resistant than CC-sensitive group. CC-resistant PCOS women had higher OGTT values, higher fasting insulin, and HOMA-IR values. Lipid profile (triglyceride levels, total cholesterol, LDL, and HDL levels) was more deranged in CC-resistant women than those who were CC-sensitive. In addition, fatty liver was significantly more common in CC-resistant women. CC-resistant women had significantly higher AMH, mean ovarian volume, and AFC. Furthermore, Vitamin D levels were significantly higher in the CC-resistant group.

However, there were no significant differences in the age, height, diastolic blood pressure, systolic blood pressure, FSH levels and 17OHP levels between the CC-sensitive and the CC-resistant groups.

In the CC-sensitive group, conception was seen in 15.85% of the PCOS women.

Several studies have proved a correlation between hyperandrogenism, insulin resistance, PCOS, and infertility.[16] In the present study, significantly higher FG score, testosterone, androstenedione, OGTT, fasting insulin, and HOMA-IR values were observed in CC-resistant women as compared to those who were CC-sensitive. These results were in concordance with the study conducted by Ellakwa *et al*.[17] [Table 4]. Hence, based on these results, it can be postulated that adding insulin-sensitizing agents such as metformin will improve insulin resistance, thus correcting hyperandrogenism resulting in better ovulation outcome with CC.

The high LH levels in PCOS females are known to suppress granulosa cells, leading to follicular arrest at the midantral stage.[18] Hence, this led us to hypothesize that CC-resistant PCOS women had higher LH levels and LH-FSH ratio as compared to the CC-sensitive PCOS women. In this study, significantly higher LH levels and LH-FSH ratio were seen in the CC-resistant than in CC-sensitive women. Similar results were noted in the study conducted by Akpinar *et al*.[19] [Table 4].

Important implications of obesity are that it increases the volume of distribution of various drugs.[19] Hence, higher dose of CC would be required in an obese patient to achieve equivalent action at the target organ as compared to nonobese. Furthermore, obesity decreases sex hormone binding globulin, resulting in increase in the levels of free estradiol and testosterone.[19] Moreover, fatty tissue converts testosterone into less potent estrogen, i.e., estriol. All these negative effects of obesity on OI led us to hypothesize that CC-resistant PCOS women had higher weight, BMI, WC, and WHR. The present study had significant differences in the weight (64.16 ± 10.51 vs. 60.02 ± 10.51, P = 0.013), BMI (27.12 ± 4.16 vs. 24.88 ± 4.19, P = 0.001), WC (34.28 ± 3.37 vs. 32.89 ± 3.98), and WHR (0.89 ± 0.04 vs. 0.87 ± 0.05). The study by Akpinar *et al*.[19] also showed higher BMI in CC-resistant group as compared to the CC-sensitive group (26.48 ± 3.3 vs. 24.3 ± 3.1).

As we are aware that high AMH values impair the action of FSH and also lead to the follicular arrest in PCOS women, so this led us to hypothesize that women who had higher AMH values are more likely to be CC-resistant. In this study, we found significantly higher AMH in CC-resistant group than CC-sensitive group (12.22 ± 5.62 vs. 8.069 ± 3.30, P = 0.0001).

Similar results were obtained in the study conducted by Wenyan *et al*.[20] (7.81 ± 3.49 vs. 5.34 ± 1.97, P < 0.001).

In this study, the prevalence of Vitamin D deficiency (Vitamin D levels <20 ng/ml) was 73.2%. There was a significant difference in the Vitamin D levels between the CC-resistant and CC-sensitive groups (16.01 ± 10.62 SD vs. 17.86 ± 8.10, P = 0.01). The literature reveals that the low levels of Vitamin D tend to exacerbate menstrual irregularities, hyperandrogenism, infertility, obesity, insulin resistance, metabolic syndrome, and cardiovascular disease in women with PCOS.[21] Hence, based on this study, we can postulate that Vitamin D supplementation in Vitamin D-deficient PCOS women will not only improve insulin resistance, metabolic syndrome preventing CVS diseases, and type II DM but also correct hyperandrogenism and menstrual irregularities, thus improving fertility. The results of this study were in contrast with study conducted by Kim *et al*.,[22] who showed no differences in the Vitamin D levels between PCOS women and the controls (19.6 ± 6.6 ng/ml vs. 20.1 ± 7.4 ng/ml). No

Table 4: Comparison with study by Ellakwa *et al*. and Akpinar *et al*.

	Present study (n=164)			Ellakwa *et al*. (n=150)			Akpinar *et al*. (n=426)		
	CC resistant	CC sensitive	P	CC resistant	CC sensitive	P	CC resistant	CC sensitive	P
Basal testosterone (nmol/l)	3.30±1.39	2.08±0.735	0.008	3.48±0.38	2.77±0.51	<0.001	-	-	-
Fating blood sugar (mg/dl)	92.65±14.79	87.24±10.08	0.0001	97.1±14.1	92.8±13.7	0.094	-	-	-
Fasting insulin (mIU/L)	14.68±6.62	8.65±5.55	0.0001	18.2±7.4	11.6±6.7	<0.001	-	-	-
HOMA-IR	3.43±1.85	1.92±1.39	0.0001	4.5±1.8	2.7±1.7	<0.001	-	-	-
LH (IU/l)	14.81±8.5	12.04±6.34	0.007	-	-	-	9.6±6.4	6.8±4.5	<0.001
LH: FSH	2.66±1.22	2.27±1.11	0.035	-	-	-	1.4±1.2	1±1.1	0.01

CC=Clomiphene citrate, HOMA-IR=Homeostatic model assessment for insulin resistance, FSH=Follicle-stimulating hormone, LH=Luteinizing hormone

study has yet been conducted to compare the Vitamin D levels in the CC-sensitive and CC-resistant PCOS women. Since the control population was not taken into account in the current study, so further research is demanded in this field.

The findings of this study have to be seen in light of potential limitations. Small sample size to study many factors was a significant limitation of this study. This was unavoidable as the study was done for a fixed period. Further, it was conducted at a tertiary care referral center. Hence, the majority of women were clomiphene resistant with multiple co-existant infertility factors such as endocrine disorders. Hence, the exclusion rate was high (30.77%). Another possible reason for the lower sample size was the high dropout rate (16.67%). Another limitation of this study was that the lifestyle modifications were not offered to these patients before starting clomiphene to avoid the confounding factors. Ideally, lifestyle modification and weight loss is the first-line management in women with PCOS related infertility.[23]

Conclusion

The differences in the clinical (weight, BMI, WC, WHR), metabolic (presence or absence of insulin resistance and metabolic syndrome, lipid profile), hormonal (LH, LH-FSH ratio, AMH), and ultrasound features (AFC, ovarian volume) should be kept in mind while deciding the OI protocol. We recommend lower thresholds for switching to alternate options such as gonadotropins in women with PCOS-related infertility, who are obese with more hyperandrogenism and irregular cycles, and those with deranged metabolic profile. This will make OI more patient-tailored and cost-effective. Moreover, this will help in prognosticating the patients and save a lot of time.

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Conflicts of interest

There are no conflicts of interest.

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