Original Research Article

An observational analysis of clinical factors predicting ovulation induction among infertile women with Poly Cystic Ovarian Syndrome: At Tertiary care facility, Jaipur

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Abstract

Objective: To identify the predictive factors for ovulation with clomiphene citrate (CC) in polycystic ovarian syndrome (PCOS) patients with infertility. **Materials and Methods:** A prospective observational study was carried out at the IVF centre of a teaching hospital, Jaipur between January 2019 to December 2019. Total54 patients with PCOS as per Rotterdam criteria, who attended for treatment of fertility were enrolled in the study. Patients were given clomiphene citrate in incremental doses from 50 to 150 mg per day for five days. Clinical parameters (age, duration of infertility, waist circumference, body mass index(BMI), biochemical parameters (fasting blood sugar,fasting Serum insulin, serum total testosterone, serum luteinizing hormone(LH), serum follicle stimulating hormone(FSH), antimullerian hormone(AMH) and ultrasonographic parameters - antral follicle count(AFC), ovarian volume, uterine and stromal vessels pulsatility index between ovulating and non-ovulating patients were statistically analysed. **Results:** Total 40.74 % patients ovulated with increasing dose of clomiphene-citrate. A statistically significant difference was observed in waist circumference, serum testosterone, LH, AMH, AFC and Ovarian stromal artery PIbetween patients who ovulated and who did not. However, no significant difference was observed with regards to patient's age, BMI, fasting glucose, serum FSH, serum Insulin, ovarian volume and uterine artery PI. **Conclusion:** Factors like WC, Serum total testosterone, serum LH level, AMH, AFC and ovarian stromal artery PI could be used as tools to predict response to clomiphene-citrate in the treatment of PCOS patients. This can help clinicians in deciding more effective and patient tailored approach.

Key words- PCOS, Clomiphene citrate, Ovulation, AMH

Introduction

Polycystic ovarian syndrome (PCOS) is a common polygenic multi factorial condition with heterogeneous presentation affecting a wide population. The prevalence of PCOS has been reported to vary from 8% to 13% according to the different diagnostic criteria[1]. It is the most common cause of anovulatory infertility, accounting for as much as 91% of women in World Health Organisation (WHO) group II anovulation. PCOS patients can present with varied features like amenorrhea, oligomenorrhea, hirsutism and infertility. There can also be association of metabolic syndrome like insulin resistance, visceral obesity, hypertension and atherogenic dyslipidaemia putting patients at high risk for developing type 2 diabetes and cardiovascular diseases.Currently the most popular classification of PCOS in use is that of the Rotterdam criteria[2]. For the diagnosis of PCOS, two of the following are required:

1. Oligomenorrhoea and/ or Anovulation
2. Clinical and or biochemical signs of hyper-androgenism
3. PCO morphology on ultrasound

Other etiologies such as congenital adrenal hyperplasia, androgen secreting tumour and Cushing's syndrome must be excluded.

Ovulation induction in PCOS patients is a challenge for the clinician as ovarian response varies widely in different patients, some patients showing resistance while others experience an exaggerated response. Clomiphene Citrate is a widely used oral ovulogen for inducing ovulation in PCOS patients with proven efficacy and safety. The main action of PCOS is on hypothalamus where it blocks estrogen receptors.

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This leads to increase in gonadotropin-releasing hormone pulse thus increasing gonadotropin secretion from the anterior pituitary gland. In women responding to CC three to six cycles of ovarian stimulation are recommended. However, administration of drugs more than 12 cycles has the potential to increase the risk of ovarian malignancy and is therefore not recommended. Some patients do not respond to clomiphene citrate and are categorised as CC resistant. If such patients could be screened earlier by determining certain factors then they can be offered alternative treatments like metformin, letrozole, gonadotropins or ovarian drilling.

Aim of the present study was to find out if Clomiphene Citrate resistance could be predicted by initial screening with clinical, endocrinological or biochemical parameters.

Materials and methods

This was a prospective observational study carried out at IVF Centre, Sawai Man Singh Medical College, Jaipur, Rajasthan, which is a tertiary level care teaching hospital. The present study took place between January 2019 to December 2019.

The present study enrolled 54 patients attending the IVF centre for fertility treatment who were diagnosed with PCOS as per Rotterdam criteria. Total 54 infertile women with WHO Group II PCOS were required as sample size for present study at 80% study power and α error 0.05 assuming 10 independent study factors and 76% successful ovulation after treatment with CC. Patients were excluded if they were younger than 18 years and older than 35 years of age, BMI lower than 18.5 kilogram/meter² (kg/m²) or higher than 35 kg/m², having any endocrine or systemic disorder, using drugs like anti-androgens, oral contraceptives, ovulation inducing agents in the past three months or any history of adnexal surgery.

A detailed history was taken followed by clinical examination of all study participants. All patients were examined on day 2/3 of spontaneous or progesterone induced menstrual bleeding. Anthropometric measurements like Body mass index (BMI-calculated

as weight in kilograms divided by the square of height in meters) and waist circumference (WC- smallest measured circumference at the level of umbilicus) were taken. A morning fasting blood sample was taken on day 2 of the cycle to determine serum levels of total testosterone, luteinizinghormone (LH), follicle stimulating hormone (FSH), antimullerian hormone (AMH), blood sugar and insulin level.

Trans-vaginal ultrasonography (TVS) of all study participants was done on day two or three of menstrual cycle between 8 am to 11 am to avoid the effects of circadian rhythm on uterine blood flow. Patients were asked to void urine before TVS and assume semi recumbent position. TVS was performed using Sonoscape Digital Color Doppler Ultrasound System, Model: SSI-6000. Ovarian volume and Antral follicle count (AFC) of both the ovaries were measured. The average value of ovarian volume and AFC of both ovaries was calculated and used for the statistical analyses. Uterine and Stromal ovarian vessels doppler measurements were obtained. Pulsatility index (PI) of both uterine artery and ovarian stromal vessels was calculated. For doppler of ovarian stromal artery, colour signals were taken at the maximum distance from the surface of the ovary. For Doppler study of uterine artery, colour flow signals were taken from the ascending branches of the uterine arteries lateral to the cervix in the longitudinal plane.

The average PI of the right and left ovarian stromal arteries and uterine arteries was used for statistical analysis.

After recording patient characteristics, clomiphene citrate was started at an initial dose of 50 mg on day 2 or 3 of menstrual cycle or progestin induced bleed and continued for five days. Follicular growth was measured with serial USG till one or two follicles reached 18 mm. A trigger with Human Chorionic Gonadotropin (HCG) 5000 international unitintramuscular was given and ovulation was checked with ultrasound 36 hours later. If ovulation did not occur, the dose was increased by 50 mg/day up to 150 mg/day during the subsequent cycles. Patients who ovulated in doses from 50 mg to 150 mg were termed responsive. Patients were followed up for three cycles and if there was no ovulation, they were defined as clomiphene resistant. In this study, ovulation rate was taken as an end point and not pregnancy rate so as to decrease the effect of confounding factors such as tubal factor, male factor, and endometrial factor. Patient's data was analysed using Software SPSS 22.0 version.

Stastical analysis

The analysis of data was performed using descriptive statistical measures such as frequencies, percentages, mean and standard deviation and statistical package for social sciences (SPSS 22.0) was used, independent t-test was done. Receiver operating curve was plotted to compare the efficacy of variable to predict the ovulation. p-value of <0.05 was considered to be statistically significant.

Results

Present study enrolled 54 patients experiencing infertility with PCOS. Out of these, three (5.55%) patients ovulated with 50mg daily dose of clomiphene-citrate for five days, 19(35.18%) patients ovulated with 100 mg daily for five days and none of the patients ovulated with 150 mg Clomiphene citrate. The overall ovulation rate with increasing dose of clomiphene citrate therapy was found to be 40.75% (22/54) and 32 (59.25%) patients were classified as non-responders to Clomiphene Citrate. (Fig.1)

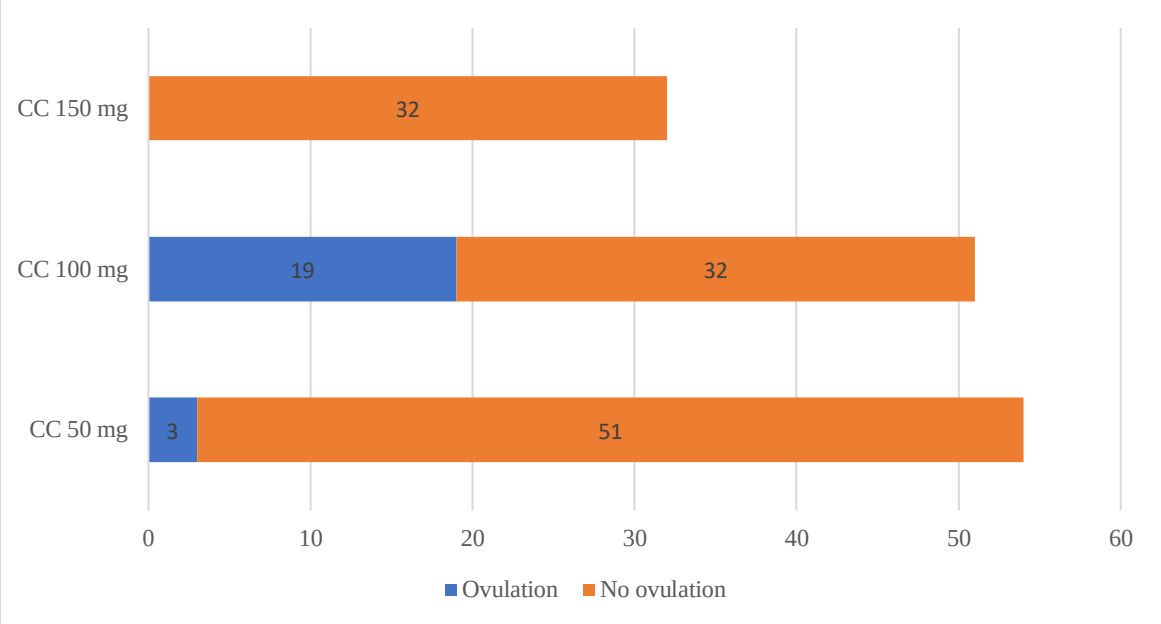

Figure 1- Patient response to ovulation induction with increasing dose of clomiphene citrate

Table 1 depicts a comparative analysis of Age, Duration of infertility, duration of amenorrhea, BMI and Waist circumference between ovulatory and anovulatory subgroups. There was no statistically significant difference in the mean age, duration of infertility, duration of amenorrhea and BMI (p value>0.05). There was significant difference in waist circumference between ovulatory and anovulatory patients (p value<0.05).

Table 1: Clinical characteristic of study participants

Variables	Ovulation	Mean±SD	Median	Min.	Max.	'p'value
Age (years)	Absent (n=32)	27.06±3.92	27	19	35	0.487
	Present (n=22)	26.27±4.29	25	21	35	
Duration of infertility (years)	Absent (n=32)	5.78±3.11	5	1	13	0.146
	Present (n=22)	4.64±2.24	4.5	1	11	
Amenorrhea (month)	Absent (n=32)	2.91±1.14	3	1.5	6	0.255
	Present (n=22)	2.52±1.34	2	0	6	

BMI (kg/m^2)	Absent (n=32)	26.44±5.14	24.5	20	35	0.171
	Present (n=22)	24.48±5.05	23.5	17	36	
WC (inches)	Absent (n=32)	35.08±4.66	34	28	45	0.034
	Present (n=22)	32.14±5.18	32	24	41	

(Table-2) Various Biochemical and Ultrasonographic factors were examined for the response of CC in terms of ovulation. A statistically significant difference was noted in serum luteinizing hormone, serum total testosterone, AMH, AFC and ovarian stromal artery pulsatility index between patients who experienced ovulation and those who did not (p value < 0.05). However, no significant difference was observed with regards to Serum FSH, Fasting glucose level, TSH, serum Insulin hormone level, ovarian volume and uterine artery pulsatility index (p value > 0.05).

Table 2- Comparison of Biochemical and Ultrasonographic characteristics of ovulatory and anovulatory participants

Variables	Ovulation	Number	Mean±SD	Median	Min.	Max.	'p' value*
FBS (mg/dl)	Absent	32	86.20±16.77	88	90	117	0.593
	Present	22	88.43±11.81	90	66	126	
LH (IU/L)	Absent	32	13.89±4.75	12.6	7.65	24.32	<0.001
	Present	22	6.74±2.40	6.43	2.83	12.2	
FSH (IU/L)	Absent	32	5.98±1.27	5.705	2.6	8.2	0.081
	Present	22	6.72±1.79	6.415	3.82	11	
Testosterone (ng/dl)	Absent	32	60.96±26.29	55.11	18.34	118	<0.001
	Present	22	36.15±12.24	35.54	15.06	66.8	
TSH (mIU/L)	Absent	32	3.10±1.96	2.7	0.6	9.37	0.886
	Present	22	3.18±2.07	2.655	0.66	8.39	
Insulin (mIU/L)	Absent	32	25.24±22.43	16.63	3.8	110.57	0.187
	Present	22	18.22±12.08	15.45	4.4	53.13	
AMH (ng/dl)	Absent	32	8.92±3.92	8.2	3.8	21	<0.001
	Present	22	5.38±1.86	5	4.1	13.34	
Average Ovarian Volume (cc)	Absent	32	11.27±2.54	11.92	5.5	17.5	0.275
	Present	22	10.50±2.49	10.25	6	17	
Total AFC	Absent	32	26.97±4.58	27	18	37	0.004
	Present	22	23.7±2.69	23.5	20	28	
Ovarian Stromal Artery PI	Absent	32	0.82±0.18	0.83	0.25	1.1	0.012
	Present	22	0.99±0.30	0.92	0.5	1.82	
Uterine Artery PI	Absent	32	2.44±0.53	2.74	1.2	3.28	0.724
	Present	22	2.38±0.66	2.25	1.2	4.17	

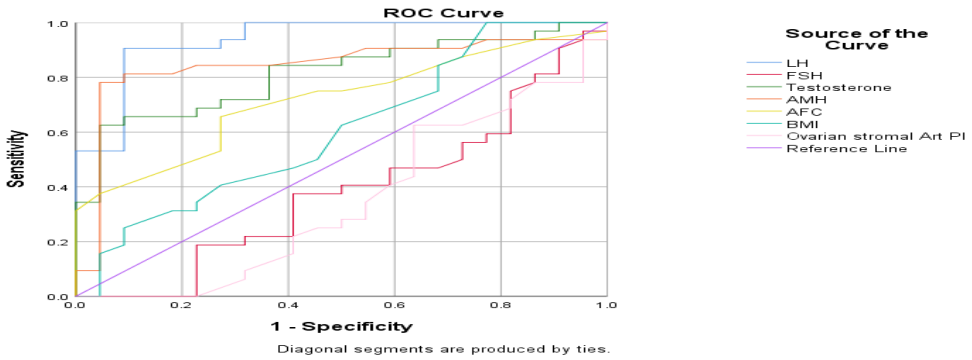

Figure 2- Receiver operating curve of prediction including Testosterone, AMH, Total AFC, LH, FSH, BMI and Ovarian stromal artery PI.

(Figure-2) Receiver operating curve was plotted to predict patient's non-response to clomiphene-citrate therapy. LH had maximum area under cure 0.937(0.872-1.00), followed by AMH 0.844 (0.725-0.962) and Testosterone 0.813 (0.699-0.926). So, the best predictor was found to be LH. Cut off value of LH was 9.21 (sensitivity-90.6% & specificity- 90.9%), AMH was 6.53 (sensitivity-78.1% & specificity- 95.5%) and Testosterone was 52.2 (sensitivity-62.5% & specificity- 95.5%).

Discussion

The present study was done to evaluate the factors predicting response to CC in infertile PCOS patient. According to the literature, approximately 75% of patients will ovulate, and about 50% of the total population will conceive after CC as the first line medication. Identifying patients having higher chances of nonresponse to CC can be beneficial as these patients can be offered alternative treatment earlier. This will be cost effective and at the same time reduce time to pregnancy.

In the present study patients who did not respond to CC therapy tended to have longer duration of amenorrhea, higher BMI and waist circumference, hyperandrogenism, increased testosterone and insulin levels, higher AMH level, larger ovarian volume, higher AFC and lower ovarian stromal artery pulsatility index. Out of the above-mentionedfactors waist circumference, Serum LH, Testosterone, AMH and AFC, ovarian stromal artery PI were found to be statistically significant. Rest of the parameters were not found to be statistically insignificant, which may be because of smaller sample size or selection bias as present study was a single center study. After

bivariate analysis weconcluded that obese patients with increased waist circumference, elevated AMH level, increased Luteinizing hormone levels, higher testosterone, higher AFC and lower ovarian stromal artery pulsatility index are most likely to remain anovulatory after CC therapy for ovulation.

In the present study, the ovulation rate following clomiphene Citrate induced ovulation was 40.74%, which is less than the figures previously reported in the literature including a study by Homburg[3] in which data of 5268 patients were analysed with respect to ovulation and pregnancy rates after giving clomiphene citrate, reported an ovulation rate of 73%. The lower ovulation rate with CC in present study could be because of the fact that maximum participants enrolled were referred from other hospitals after anovulation with standard therapy. In current study, out of total ovulated patients, 13.63% patients ovulated with CC 50 mg and 86.36% patients ovulated with CC 100 mg. None of them documented ovulation with CC 150 mg. With increasing dose of CC from 50mg to 100mg the probability of ovulation rises from approximately 45% to 90%. Therefore, a stair-step protocol to rapidly increase probability of ovulation has been suggested[4,5].

Present study has shown that patients with higher waist circumference were more likely to remain anovulatory. Weight reduction and lifestyle changes are considered important in overweight women with PCOS with anovulatory infertility[6]. As PCOS is often associated with central obesity, concomitant insulin resistance andhyperinsulinemia therefore increased physical activity and weight loss are recommended because they reduce hyperinsulinemia and insulin resistance, thereby increasing insulin sensitivity[7,8]. This improves the hormonal imbalance in the ovary and reduces androgen dominance[9]. The effect of weight reduction on AMH levels is, however, equivocal[10,11]. The effect of a modified low caloric diet on ovulation in women who were obese with anovulatoryPCOS was studied in a systematic review (17 studies, 533 patients). The conclusion was that in most studies, sporadic ovulation occurred after weight loss, and some patients even had regular ovulations[12].

In the current study, it was shown that PCOS patients with higher total testosterone were more likely to remain anovulatory with treatment with CC. These results are consistent with figures previously reported in the literature including a study by Ellakwa et al[13].

Another observation made in the present study was that baseline LH value and LH:FSH ratio were significantly higher in CC resistant group, which was similar to the study by Sachdeva et al[14].

In present study, we found significantly higher AFC in CC-resistant group than CC-sensitive group. Similar results were obtained in the study conducted by Sachdeva et al[14].

Doppler analyses of uterine and stromal vessels can help in predicting the response to CC.There are not many studies in literature regarding this. In a study by Mohamed Elsayed et al[15] a negative correlation of ovarian stromal artery PI and ovulation in PCOS patients was observed which was similar to findings of the present study. There are reports that elevated LH may be responsible for increased stromal vascularisation by causing neovascularisation.

The present study shows AMH as an important predicting factor for ovulation in PCOS. This finding was similar to a study by Andon at el[16] in which higher AMH level was the potential predictor of follicular growth failure in PCOS patients treated with CC. Similarly, Mahran et al[17] investigated 187 ovulation induction cycles with CC and revealed higher AMH levels in the anovulation group ($p<0.001$) than in the ovulation group. Wenyan Xi[18] also studied the relation of high AMH level with ovulation rate and found that patients with high AMH levels have less chance of responding to CC treatment.

Receiver operating curve was plotted to predict patient's non-response to clomiphene-citrate therapy. The best predictor was found to be LH, followed by AMH. Cut off value of LH was 9.21 (sensitivity-90.6% & specificity- 90.9%), AMH was 6.53 (sensitivity-78.1% & specificity- 95.5%) and Testosterone was 52.2 (sensitivity-62.5% & specificity- 95.5%). El-Halawaty et al. [19] evaluated the role of AMH in predicting clomiphene citrate-induced ovulation rates in patients with PCOS who were obese. In this study, an AUC of AMH of 0.71, a sensitivity of 71%, and a specificity of 65.7% were reported, which is similar to the present study.

Thus, screening the patients for these factors before starting ovulation induction treatment will help in providing appropriate counselling on the chances of success of treatment. Also patients less likely to respond can be offered alternative treatment after a short trial of CC.

Conclusion

Based on this study we concluded that differences in factors like waist circumference, serum LH, Testosterone, AMH, AFC and ovarian stromal artery PI were found to be statistically significant between responsive and non-responsivegroup, and a combination of these can be helpful in giving individualised and effective treatment for ovulation induction in PCOS patients.

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