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A randomised double blind clinical study on effect between clomiphene citrate plus metformin and clomiphene citrate alone on induction of ovulation in women with polycystic ovarian syndrome

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ABSTRACT

Background: Polycystic ovarian syndrome (PCOS) is the most common endocrine disorder of women of reproductive age-group, characterised by a combination of hyperandrogenism, chronic anovulation, and polycystic ovaries. It is one of the most common cause of hyperandrogenism, hirsutism and anovulatory infertility. The aim of the study is to determine & compare the rate of ovulation, regularisation of menstrual cycle and rate of pregnancy by use of combination of Clomiphene citrate and Metformin & with use of Clomiphene citrate alone in patients with PCOS.

Materials and Methods: A total 110 patients (55 in each group) having primary subfertility and diagnosed as PCOS in age group of 18 to 40 years of age, selected between April 2017 – March 2018. The primary outcome i.e, incidence of ovulation was detected by folliculometry. The secondary outcomes, i.e, regularisation of menstrual cycle & Pregnancy rates were taken into account and compared in both the groups.

Results: Overall ovulation was found highest at the end of 3rd cycle of ovulation induction, 48.8% and 41% respectively in each group. The Primary outcome was significantly higher in group a given combination of clomiphene citrate and metformin (83.3%) with respect to group B given clomiphene citrate only (65.9%). The secondary outcomes were significantly higher in group given CC plus metformin (89.6%). Pregnancy rates were higher in 1st group 33.3% than 2nd group (20.5%).

Conclusion: In the present study one group was given combination of Clomiphene citrate with metformin and another group was given clomiphene citrate only for ovulation induction. It was found that combination of clomiphene citrate and metformin was more effective in induction of ovulation in women suffering from PCOS when compared to induction of ovulation with clomiphene citrate alone.

1. Introduction

Polycystic ovarian syndrome (PCOS) is the most common endocrine disorder of reproductive aged women and affects approximately 4 to 12 percent in general population. This familial disorder appears to be inherited as a complex genetic trait.[1] It is characterised by a combination of hyperandrogenism (either clinical or biochemical), chronic anovulation, and polycystic ovaries. It is frequently associated with insulin resistance and obesity.[2] It is the most common cause of hyperandrogenism, hirsutism and anovulatory infertility in developed countries.[3,4]

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The prevalence of PCOS varies widely among different countries and has been reported to be lower in Asian countries than in Western countries, with a prevalence of 9.1% in India, 6.3% in Srilanka,5.6% in China.[5]

1.1. PCOS is diagnosed by Rotterdam diagnostic criteria[6]

1. Oligoovulation or anovulation.
2. Clinical and/or biochemical signs of hyperandrogenism.
3. Polycystic ovaries and exclusion of other etiologies (congenital adrenal hyperplasia, androgen secreting tumours, Cushing's syndrome).

Courtesy: Novak and berek gynaecology 15^{th} ed page 1079

The presence of two of the three criteria is sufficient for diagnosis of PCOS.

The sonographic criteria for PCO requires the presence of 12 or more follicles in either ovary measuring 2 to 9 mm in diameter and/or increased ovarian volume (>10ml).[6] Obesity occurs in more than 50% of patients with PCOS and associated with higher waist to hip ratio and insulin resistance indicating an increased risk of diabetes mellitus and cardiovascular disease.[7] Insulin resistance causes compensatory hyperinsulinemia.[8,9] Both insulin and LH stimulate androgen production by the ovarian theca cells.[10] As a result,affected ovaries secrete elevated level of testosterone and androstenedione resulting in hyperandrogenic state. Anovulation in women with PCOS is characterised by inappropriate gonadotropin secretion and insulin resistance, as a substantial number of anovulatory patients with PCOS may resume ovulatory cycles when treated with metformin, an insulin sensitizer.

Infertility or subfertility is a frequent complaint in women with PCOS and results from anovulatory cycles. More over, in women with infertility secondary to anovulation, PCOS is the most common cause.[11]

The first line therapy for ovulation induction in patients of PCOS suffering from infertility or subfertility due to anovulation is Clomiphene citrate. CC is non-steroidal triphenylethylene derivative that demonstrates both estrogen agonist and antagonist properties.[12]

Negative feedback that is normally produced by estrogen in hypothalamus is reduced. GnRH secretion is altered and stimulates pituitary gonadotropin release. The resulting increase in FSH levels, in turn drives ovarian follicular activity. The effective dose of CC ranges from 50mg/dl to 250mg/dl, although doses in excess of 100mg/dl are not approved by the FDA. CC is easy to use and leads to ovulation in most patients.[13]

However, pregnancy rates are <=50%.[14] Patients who donot ovulate on maximum dose of CC are considered CC resistant.[15]

Insulin sensitising agents shows promise in the treatment of PCOS, when administered to insulin resistant patients. These compounds act to increase target tissue responsiveness to insulin thereby reducing the need for compensatory hyperinsulinemia,[16] and promotes weight loss, thus causing resumption of ovulatory function in overweight women with PCOS. Current insulin sensitizing agents include the biguanides and thiazolidinediones. Of these, Metformin administered to women with PCOS, increase the frequency of sponataneous ovulation, menstrual cyclicity and ovulatory response to CC.[17]

This has led to the recommendation to use Metformin alone or in combination with Clomiphene Citrate as first line treatment in infertile women with PCOS.[18]

Different studies have been performed in this topic,some stated combination of CC with Metformin is more efficacious than CC alone in patients of PCOS. Some studies have no definite conclusion.

The best treatment for ovulation induction is still not known, so this study is chosen to help in establishing the role of combination therapy of clomiphene citrate and metformin for ovulation induction in patients of PCOS. Our aim in this study is to compare the efficacy of use of Clomiphene citrate and Metformin and Clomiphene citrate alone for ovulation induction in PCOS patients suffering from infertility or subfertility.

The hypothesis of this study is that combination of metformin and clomiphene citrate is more efficacious in ovulation induction in patients of PCOS as compared to clomiphene citrate only.

2. Aims and Objectives

1. To determine the rate of ovulation, regularisation of menstrual cycle and rate of pregnancy by use of combination of Clomiphene citrate and Metformin in patients with PCOS.
2. To determine the rate of ovulation, regularisation of menstrual cycle and rate of pregnancy use of Clomiphene citrate in patients with PCOS.
3. To compare the rates of ovulation, cycle regularisation and pregnancy in the two groups.

2.1. Specific objectives

1. Here the main aim is to compare the efficacy of clomiphene citrate used in combination with metformin vs clomiphene citrate alone, in patients of PCOS for ovulation induction.
2. To estimate the pregnancy rates in both groups.
3. To assess whether there is any statistical significance in efficacy in two groups.

3. Materials and Methods

3.1. Study area

The study was conducted in OPD of Department of Obstetrics and Gynaecology, Nilratan Sircar Medical College & Hospital. Women diagnosed as PCOS with subfertility and who had given consent were the subjects of this study.

3.2. Study population

Patients having complaint of primary subfertility and diagnosed as PCOS in age group of 18 to 40 years of age, from different districts of West Bengal and adjacent states. Healthly Volunteers were also included in this study.

3.3. Criteria for inclusion

1. Diagnosed cases of PCOS, suffering from primary subfertility due to anovulation.
2. Age group 18 to 40 years.

3.4. Criteria for exclusion

Patients were excluded from this study if any of the followings was present:

1. PCOS patients not wanting pregnancy
2. Other detected factors of infertility of women like tubal factors, any known cases of endometriosis, anatomical defect in uterus, any tumour, etc.
3. Unexplained vaginal bleeding & AUB.
4. Known endocrinopathies like thyroid disorders and hyperprolactinemia.
5. Any associated known major medical comorbidities like heart disease and liver disease etc.
6. Male factor of infertility ruled out by husband's semen analysis.

3.5. Study period

12 months (April 2017 – March 2018).

3.6. Sample size

Purposive sampling was done. 55 in each group were taken initially. The number varied according to the availability of cases.

3.7. Sampling technique

Purposive sampling technique.

3.8. Sampling design

Consecutive patients were taken as study population following the inclusion and exclusion criteria, and by this way 55 patients were placed in one group, other 55 patients in another group.

3.9. Study design

A randomised double-blind clinical study was conducted after approval from Institutional Ethics Committee. Informed and written consent was taken from eligible women who agreed to participate in this study.

PCOS patients were categorized as per Rotterdam criteria. Among them patients were selected with primary subfertility due to anovulation. Other factors of primary subfertility were ruled out by certain investigations like thyroid function test, hysterosalpingogram, husband semen analysis, etc.

3.10. Study tools

1. Consent form
2. Case record form
3. Proforma of history taking

3.11. Methods

Subjects of PCOS were selected as per eligibility criteria, among them cases to be included were recruited. Women 18-40 years of age who attended the Gynaecology OPD of NRSMCH, with complaint of primary subfertility and diagnosed as cases of PCOS (by Rotterdam Criteria) were assessed for eligibility. Those who had any other factor leading to subfertility (other than ovulation factor) were excluded from the study and those who met the aforesaid inclusion criteria were enrolled into this study.

Total 100 cases were taken. They were randomised into two groups, each by computer generated randomisation. Opaque sealed envelopes were given for group allocation (A and B).

Women of Group A were given Tab.Clomiphene citrate 50mg daily for 5 days (from Day 3 to Day 7 of menstrual cycle of women, along with Tab.Metformin (500)mg 1 tablet twice daily throughout the cycle. This regimen was continued for 3 months initially, and if the patient did not conceive and the dose of tab. Clomiphene citrate was increased to 100mg daily for next 3months.

Women of Group B were given the same dose of Tab. Clomiphene citrate with initial dose of 50mg (D3 to D7 of menstrual cycle), with further increment of the dose if required in consecutive next 3 months.

Both groups of patients were followed up for their evidence of ovulation, cycle regularisation and pregnancy.

3.12. Parameters to be studied

3.12.1. General parameters

1. Chief complaint
2. History

3. Menstrual history
4. General examinations
5. Patient's weight and BMI
6. Gynaecological examinations
7. Routine blood investigations including TSH, LH, FSH, Prolactin
8. Trans vaginal sonography/ Transabdominal sonography to diagnose PCOS.

3.12.2. Parameters for ovulation

1. Basal body temperature
2. Folliculometry
3. Serum progesterone(in pre menstrual phase)

3.12.3. Parameters to rule out other causes of subfertility

1. Husband's semen analysis
2. HSG(to rule out tubal factors)
3. USG(to rule out uterine factors)
4. TSH and Prolactin

3.13. Outcomes of the study

1. Primary outcome
   (a) Incidence of ovulation
2. Secondary outcomes
   (a) Cycle regularisation
   (b) Incidence of pregnancy

3.14. Study technique

1. Approval of Institutional Ethics Committee was taken.
2. Subjects fulfilling the inclusion criteria and exempted from the exclusion criteria were included in this study.
3. The written consent was taken from eligible women who were willing to participate in the study.
4. PCOS categorization was done according to Rotterdam's Criteria
5. A proforma was made mentioning patient particulars, history, examination, findings and past records.
6. Detailed history and clinical examination was carried out.
7. Other factors of primary subfertility were ruled out.
8. Husband semen analysis was done.
9. Blood investigations were done.
10. Patients were followed up till they ovulated, conceived, failure to ovulate, failure to conceive.

3.15. Statistical analysis

1. Statistical data was expressed as number and percentage.
2. Mean and standard deviation (SD) were calculated where required.
3. Microsoft excel and GraphPad, Quickcals & MedCalc software had been used for statistical calculation.
4. Continuous variables were compared with Student's t-test, categorical variables with Chi-square test ; and p-value < 0.05 was considered as statistically significant.

4. Result and Analysis

We initially screened 200 women suffering from primary subfertility, 90 of them were excluded from the study, 52 not met the inclusion criteria, 22 declined to participate and 12 due to other reasons. 110 PCOS patients suffering from primary subfertility were randomised into two groups. Each group had 55 patients, out of which 48 patients in group A and 44 patients in group B were analysed in the study.

Table 1: Distribution of cases

Group	No. of cases	Percentage
Clomiphene citrate plus metformin	48	52.17%
Clomiphene citrate only	44	47.82%
Total	92	100

Women in Group A were given ovulation induction with tablet Clomiphene citrate 50mg one tablet once daily from day3 to day 7 of menstrual cycle, along with tablet Metformin 500mg twice daily throughout the cycle.

Women in Group B were given ovulation induction with tablet Clomiphene citrate 50mg only in same schedule as group A. (Table 1)

Table 2: Association of age of patients in two Groups

	Group		
Age (in years)	**CC+MET (N=48)**	**CC only (N=44)**	**P-Value (t-Test)**
Mean ± SD	25.23±4.01	26.57 ±4.62	**0.16**
17-24	15(31.3)%	13(29.5)%	
25-32	27(56.3)%	21(47.7)%	
33-38	6(12.5)%	10(22.7)%	

When age distribution of patients were analysed, maximum number of patients were 25-32 years, followed by 17-24years, and 33-38years respectively in descending order (Table 2).

There was no statistical significant difference between the two groups.

Table 3: Association of menstrual history in two groups

	Group		
Menstrual history	**CC+MET (N=48)**	**CC only (N=44)**	**P-Value (Chi-square-test)**
Irregular	39(81.3)%	33(75.0)%	0.64
Regular	9(18.8)%	11(25.0)%	

In this study it was observed that maximum patients were having irregular menstrual cycle. There was no statistical significant difference in terms of menstrual history in two groups. (Table 3)

Table 4: Association of BMI (normal/overweight/obese) in two groups

	Group		
BMI (normal/overweight/obese)	**CC+MET (N=48)**	**CC only (N=44)**	**P Value -Test)**
Mean ± SD	28.66±3.89	28±4.18	0.43
ormal(18.5-24.9)	6(12.5)%	10(22.7)%	
Overweight(25-29.9)	20(41.7)%	18(40.9)%	
Obese(more than 30)	22(45.8)%	16(36.4)%	

From this table it was evident that maximum patients were obese (45.8% in 1^{st} group, 36.4% in 2^{nd} group) followed by overweight (41.7% in 1^{st} group, 40.9% in 2^{nd} group) followed by normal BMI (12.5% in 1^{st} group, 22.7% in 2^{nd} group). No statistical significance was noted in the two groups. (Table 4)

Table 5: Fasting sugar and OGTT in two groups

	Group		
FBS & OGTT	**CC+MET (N=48)**	**CC only (N=44)**	**P-Value(Chi-square test)**
Deranged	17(35.4)%	27(61.4)%	0.02
Normal	31(64.6)%	17(38.6)%	

Oral Glucose Tolerance Test was performed with 75gram glucose among patients of both groups. It was observed 2^{nd} group (CC only) was having significant higher incidence of deranged OGTT (61.4%) as compared to 1^{st} group (35.4%). (P-value<0.05) (Table 5)

Mean value of ratio of LH:FSH noted in both groups were 2.79±0.65 in 1^{st} group and 2.81±0.62 in 2^{nd} group. There is no statistical significant difference noted in terms of LH:FSH ratio in two groups. (Table 6)

Serum progesterone were performed Day 21^{st} of menstrual cycle in two groups in 1^{st} cycle of ovulation induction. Mean value of progesterone noted in both groups were 4.22±4.99 in 1^{st} group and 4.05±3.84 in 2^{nd} group. There was no statistical significant difference in serum progesterone evaluation in two groups.(Table 7)

Mean value of progesterone in 3^{rd} cycle of ovulation induction in two groups were 5.98±4.48 in 1^{st} group and 6.17±4.38 in 2^{nd} group. There was no statistical significant difference in serum progesterone evaluation in two groups. (Table 8)

Mean value of progesterone in 6^{th} cycle of ovulation induction in two groups were 8.73±6.47 in 1^{st} group and 5.9±5.28 in 2^{nd} group. There was no statistical significant difference in terms of serum progesterone evaluation in two groups. (Table 9)

Ovulation induction was given to 1^{st} group of patients with Clomiphene citrate plus metformin combination, and 2^{nd} group with Clomiphene citrate only. Folliculometry was done in both group of patient during their 1^{st} cycle of treatment and evidence of ovulation was noted. It was seen that evidence of ovulation by folliculometry was higher in 1^{st} group of patients (18.8%) as compared to the 2^{nd} group (11.4%). There is no statistical significant difference noted in two groups in terms of evidence of ovulation in 1^{st} cycle of folliculometry. (Table 10)

Evidence of ovulation by folliculometry done in 3^{rd} cycle of ovulation induction, was higher in 1^{st} group (48.8%) as compared to the 2^{nd} group (41%). There was no statistical significant difference in two groups. (Table 11)

Evidence of ovulation by folliculometry in 6^{th} cycle of ovulation induction was higher in 1^{st} group of patients (50%) as compared to the 2^{nd} group (34.8%). There is no statistical significant difference in two groups.(Table 12)

When overall analysis was done in terms of occurrence of ovulation after the completion of the prescribed treatment in two groups, it was observed that the rate of ovulation was higher in 1^{st}group (83.3%) as compared to 2^{nd} group (65.9%). Ovulation induction was significantly higher in 1st group in comparision to 2^{nd} group. (P-valu0.0001). (Table 13)

Menstrual cycle was regularised, who had received treatment with combination of CC plus Metformin (89.6%), as compared to who had received CC only (38.6%). Regularisation of menstrual cycle was significantly higher in 1^{st} group. (P-value < 0.05). (Table 14)

Pregnancy rates in 1^{st} group was little higher (33.3%) as compared to 2^{nd} group (20.5%). There was no statitistical significant difference found with respect to pregnancy rates in both groups. (Table 15)

Maximum patients ovulated after the 3^{rd} cycle of treatment, and overall ovulation rate was higher in 1^{st} group as compared to 2^{nd} group. There was no statistical significance in difference in ovulation rates with respect to number of cycles of treatment given in two groups. (Table 16)

5. Discussion

The current study had been conducted in Gynaecology OPD in the Department of Obstetrics and Gynaecology in Nilratan Sircar Medical College & Hospital, Kolkata, West Bengal where 200 patients of primary sub-fertility were selected. Amongst them 110 patients so suffering from PCOS (diagnosed by Rotterdam Criteria) were opted for conducting detailed analysis. Out of them, 92 patients were randomised finally into two groups, while the rest lost to follow up and some had been discontinued the study on their own discretion.

The observation revealed that 48 (52.17%) number of patients in 1^{st} group were given ovulation induction with combination of clomiphene citrate and metformin while 44(47.82%) number of patients in 2^{nd} group were given

Table 6: LH:FSH ratio in two groups

Group	No.	Mean	Std. Dev	P-Value
CC+MET (N=48)	48	2.7917	.6510	0.84
CC only (N=44)	44	2.8182	.6203	

Table 7: Association of day 21 serum progesterone (ng/ml) in 1st cycle of OI in two Groups

Group	No.	Mean	StdDev	P-Value (Chi-square)
CC+MET (N=48)	48	4.2242	4.9891	**0.86**
CC only (N=44)	44	4.0584	3.8480	

Table 8: Association of day 21 serum progesterone (ng/ml) in 3rd cycle of OI in two Groups

Group	Obs	Mean	Std. Dev	P-Value(Chi-square)
CC+MET (N=48)	38	5.9847	4.4849	0.85
CC only (N=44)	39	6.1667	4.3832	

Table 9: Association of day 21 serum progesterone (ng/ml) in 6th cycle of OI in two Groups

Group	No.	Mean	Std Dev	P-Value(Chi-square test)
CC+MET (N=48)	19	8.7332	6.4715	0.13
CC only (N=44)	23	5.9652	5.2827	

Table 10: Evidence of ovulation in Folliculometry in 1^{st} cycle of ovulation induction in two Groups

	Group		
Folliculometry (1st cycle of OI) evidence of ovulation	**CC+MET (N=48)**	**CC only (N=44)**	**P-Value(Chi-square test)**
no	39(81.3)%	39(88.6)%	0.48
yes	9(18.8)%	5(11.4)%	

Table 11: Evidence of ovulation in Folliculometry in 3rd cycle of ovulation induction in two Groups

	Group		
Folliculometry (3rd cycle of OI) for evidence of ovulation	**CC+MET ("N=48)**	**CC only (N=44)**	**P-Value (Chi-square test)**
no	21(51.2)%	23(59.0)%	0.63
yes	20(48.8)%	16(41.0)%	

Table 12: Evidence of ovulation in Folliculometry in 6th cycle of ovulation induction in two Groups

	G roup		
Folliculometry (6th cycle of OI) evidence of ovulation	**CC+MET (N=48)**	**CC only (N=44)**	**P-Value(Chi-square)**
No	11(50.0)%	15(65.2)%	0.46
Yes	11(50.0)%	8(34.8)%	

Table 13: Ovulation in two Groups

Ovulation (Yes/No)	CC+MET (N=48)	CC only (N=44)	P-value(Chi-square test)
Yes	40 (83.3)%	29 (65.9)%	<0.0001
No	8 (16.7)%	15 (34.1)%	

Table 14: Regularisation of menstrual cycle in two Groups

	Group		
Cycle Regularisation (Yes/No)	**CC+MET (N=48)**	**CC only (N=44)**	**P-value(Chi-square test)**
No	5(10.4)%	27(61.4)%	0.03
Yes	43(89.6)%	17(38.6)%	

Table 15: Number of patients conceived after ovulation induction in two Groups

	G roup		
Conceived	**CC+MET (N=48)**	**CC only (N=44)**	**P-Value (Chi-square test)**
No	32(66.7)%	35(79.5)%	0.25
Yes	16(33.3)%	9(20.5)%	

Table 16: Association of number of cycles of treatment required in patients who ovulated after treatmen in two groups

	Group		
Cycles of treatment required	**CC+MET (N=40)**	**CC only (N=29)**	**P-Value**
Mean ±SD	3.37±1.82	3.48±1.74	0.80(t-Test)
1 cycle	9(22.5)%	5(17.2)%	0.87(Chi-square)
3 cycles	20(50.0)%	16(55.2)%	
6 cycles	11(27.5)%	8(27.6)%	0.83(chi-square)

clomiphene citrate alone.

This study manifested a significant increase in rates of ovulation with use of combination of clomiphene citrate and metformin as compared to clomiphene citrate alone.

In this present study, the age distribution of patients were maximum between 25-32 years, 56.3% in group A and 47.7% in group B, with mean of 25.23±4.01 and 26.57 ±4.62 respectively in each group. The difference in parameters like age of patients in two groups was not statistically significant (P-value=0.16).

Mean age of distribution in study done by Hardik Patel et al,[19] was 26.04±7.78, which was similar to our study. Only 8.33% were more than 31 years of age. Similar results were found in study done by Legro et al,[20] where mean age distribution was 27.9±4.

In this study maximum patients were from rural areas 64.6% and 65.9% in first and second groups respectively.

Other variables like socioeconomic status, menstrual history were comparable in both groups but not statistically significant.

The majority of patients in our study were obese with BMI 48.5% and 36.4% in first and second groups with mean value of 28.66±3.89 and 28±4.18 respectively. The observation like BMI < 25kg/m^2 was 12.5% and 22.7% in above groups in respective order.

The study conducted by Hardik Patel et al,[19] revealed that 20 patients (41.67%) had body mass index (BMI) <25 kg/m2 while 28 patients (58.33%) had BMI above 25 kg/m2. In Study done by Papa Dasari et al,[21] showed that obesity was present in 37.5% patients, which is in proximity and almost analogous to our observation. Some pieces of similar studies were carried out by Moll et al. though Dunaif et al[9] in his study had shown the incidence of obesity was higher (55.87%) and (65%) in respective order of medication while comparing to our study.

In present study, the symptoms like Acne was found to be present in (18.8%) of patients and (18.2%) of patients in Group A and B respectively. Hirsutism (by Ferriman Gallway Scale[22] was present in average 38.3% of patients in both group though they were not statistically significant.

Exactly 56.25% of patients had shown hirsutism and 45.83% of patients had acne in study done by Hardik Patel et al.[19] Study done by Papa Dasari et al,[21] showed 85% of patients had been diagnosed with hirsutism. These studies had higher incidence of hirsutism in patients of PCOS, as compared to our study.

In our study certain investigation like USG of whole abdomen, lipid profile, liver function test were also conducted though no statistical significant difference were noted amongst two groups.

Fasting Blood Sugar (mg/ml) and oral glucose tolerance test were accomplished in our study. On average the same was found to be deranged in 48.4% of patients in both the groups. The difference of FBS and OGTT in two groups respectively were statistically significant (p-value=0.02).

Serum progesterone was done in premenstrual period i.e, 21^{st} day of menstrual cycle. Values >3ng/ml indicates ovulation. In this study it was observed that mean serum progesterone was highest in 1^{st} group (8.73 ± 6.47ng/mL) at the end of 6^{th} cycle of ovulation induction. Mean progesterone in 2^{nd} group (6.17 ± 4.38 ng/mL) was highest at end of 3^{rd} cycle of ovulation induction. There was no significant difference in two groups. Israel et al.[23] found that serum progesterone was (>=3ng/ml) in premenstrual period in patients who had evidence of ovulation. Similarly, Nadji et al[24] found that plasma progesterone of >=2ng/ml was always associated with secretory endometrium and hence there was the evidence of ovulation. In our study as mean serum progesterone was > 3ng/ml, there was an evidence of ovulation. Hence, the serum progesterone deserves to be considered, an important biochemical predictor of ovulation.

In our study folliculometry was also done to ascertain the evidence of ovulation. Maximum ovulation was recorded to be occurred after 3^{rd} cycle of treatment, and 1^{st} group had experienced 48.8% and 2^{nd} group had experienced 41% of evidence of ovulation. But difference in the two groups was recorded to be not statistically significant.

The current study revealed that there was an overall incidence of ovulation which was found to be significantly

higher in 1^{st} group (83.3%) as compared to 2^{nd}(65.9%), P-value(<0.001). In study conducted by Hardik Patel et al,[19] 66.4% of patients ovulated who received clomiphene citrate and metformin as compared to 57.12% patients who received clomiphene citrate alone. In study done by Dasari et al,[21] there was significant increase in ovulation rate in group given clomiphene citrate and metformin (72%) as compared to clomiphene citrate only(29%). In studies done by Vandermolen et al (2001),[25] Kocak et al.2002),[26] Batukan et al,[27] Sturrock et al,[28] respectively, similar results were observed. There was significant increase in ovulation rate with combined medication with clomiphene citrate and metformin as compared to clomiphene citrate alone. Results of current study were almost resembling to these studies.

Whereas, study done by Ng et al (2001),[29] the ovulation rate in group given clomiphene citrate and metformin (40%) was lesser than the control group given clomiphene citrate alone (70%). This result did not match with our study. The observation made by Moll et al. (2006)[15] had concluded that adding metformin to clomiphene citrate was not associated with any incidence to establish higher ovulation rate significantly.

The current study revealed that the Menstrual cycle of patients was significantly regularised in group given clomiphene citrate(89.6%) and metformin combination as compared to clomiphene citrate alone (38.6%), (P-value=0.03). Similar result was found in study done by Bhavana V. Sontakke et al.[30] on effect of metformin on menstrual irregularities. It was found that metformin allowed resumption of normal menses in most of the patients, as 95% of patients had been recorded to be regular cycles.

Pregnancy rate was higher in 1^{st} group (33.3%) as compared to 2^{nd}(20.5%) though this difference was not statistically significant (P-value=0.25). Hardik Patel et al.[19] in his study had observed that 25.91% of patients to become conceived with combined medication of Clomiphene citrate and Metformin, as compared to 23.8% with Clomiphene citrate alone. The study conducted by Dasari et al,[21] found higher pregnancy rates (24%) in patients conceived with combination treatment of clomiphene citrate and metformin as compared to clomiphene citrate alone. Costello's review[31] showed pregnancy rate was higher (35%) in the group given combination treatment. Pregnancy rates in our study were also found to be similar with these studies.

Studies done by Vandermolen et al.[25] and Batukan et al,[27] pregnancy rates were significantly higher in group given combination therapy for ovulation induction.

Moll et al (2006)[15] in his study had showed no significant difference in pregnancy rates in two above kind of study groups.

Mean duration of ovulation was 3 months in both groups. The ovulation rates were higher (50% and 55.2%) in group A and B respectively as far as the comparison amongst 1^{st} cycle and 6^{th} cycle of ovulations were concerned in present study.

Similar observation was also recorded in study carried out by Dasari et al[21] and Batukan et al.[27] Dasari et al[21] as his study showed that mean duration of ovulation was 3.5 months in the group offered with combined medication like clomiphene citrate and metformin. Batukan et al[27] showed mean duration of ovulation was 4 months in the group given clomiphene citrate and metformin.

Salient points

1. Age of the patients were between 18-40 years in both the groups. Majority of patients were between 25-32years of age constituting 56.30% in CC plus metformin group and 47.70% in CC only group. Only 31.30% and 29.50% of the patients in respective groups were between 17-24 years and rest were between 33-38 years.
2. Most of the patients had irregular menstrual cycle, 81.3% and 75% respectively in each group.
3. BMI distribution in both the groups were comparable. Majority of them were obese having BMI >30 kg/m^2,with incidence of 45.8% and 36.4% in groupA and groupB respectively.
4. Fasting blood sugar and oral glucose tolerance test were deranged in 35.4% and 61.4% in both groups.
5. Serum progesterone was done on 21^{st} Day of menstrual cycle during 1^{st},3^{rd} and 6^{th} cycle of ovulation induction. Mean value was highest in 6^{th} cycle with value of 8.73±6.47 and 5.96±5.28 in each group respectively. Group A was having higher mean value than group B.
6. Folliculometry was done to note evidence of ovulation in 1^{st},3^{rd} and 6^{th} cycle of ovulation induction. Overall ovulation was found highest at the end of 3^{rd} cycle of ovulation induction, 48.8% and 41% respectively in each group.
7. The Primary outcome i.e, incidence of ovulation was significantly higher in group A given combination of clomiphene citrate and metformin(83.3%) with respect to group B given clomiphene citrate only (65.9%).(P-value<0.001).
8. The secondary outcomes, i.e, regularisation of menstrual cycle was found to be significantly higher in group given CC plus metformin (89.6%) as compared to CC only (38.6%). (P-value=0.03)
9. Pregnancy rates were found higher in 1^{st} group 33.3% than 2^{nd} group (20.5%). Though the result was not statistically significant. (P=0.)
10. There was no significant role of the combination regime on number of cycles required for ovulation induction. Overall maximum patients had ovulation after the end of 3^{rd} cycle 50% and 55.2% respectively in both groups. But overall, the incidence of ovulation

was higher in 1^{st} group.

6. Conclusion

Primary subfertility is one of the major complications in patients of PCOS mainly due to anovulation / oligo ovulation. In the present study one group was given Clomiphene citrate along with metformin and another group was given clomiphene citrate only for ovulation induction. It was found that combination of clomiphene citrate and metformin was more effective in induction of ovulation when compared to clomiphene citrate alone.

7. Limitations of the Study

Although this study has reached its primary aim, but there were certain limitations in the study:

1. There was distinct lack of follow up and monitoring.
2. Smaller sample size because many patients lost to follow up. Larger sample sizes are needed to add power to result obtained.
3. Patient was followed up only for 6 cycles due to lack of time
4. Pregnancy outcomes in patients who conceived couldnot be analysed due to short duration of study period.
5. Doses of clomiphene citrate varied in patients, so did their duration of therapy, thus being ineligible for meta-analysis.
6. Therefore, further RCTs using similar doses and durations of treatment are essential to conclude if the benefits can be attributed to this specific treatment regime.

8. Source of Funding

None.

9. Conflict of Interest

The authors declare no conflict of interest.

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