Research Article

COMPARISON OF MONO AND COMBINED EFFECT OF METFORMIN ON OVULATORY RATE IN INFERTILE POLYCYSTIC OVARIAN SYNDROME WOMEN

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ABSTRACT

Polycystic ovary syndrome affects 7 – 8 % of women and may be the most common cause of infertility. For more than year's metformin and clomiphene citrate has been the used as a treatment for ovulatory disorders. In recent days aromatase inhibitors have been suggested as an alternative treatment to clomiphene citrate. The aim of this study was to compare the mono and combined effects of metformin and metformin - letrozole on enhanced ovulation and pregnancy rate in infertile patients with polycystic ovary syndrome (PCOS). The patients were visited infertility clinic and examined by gynecologists. Healthy normal cycled women conclude as a control (group I), untreated PCOS patients selected for (group II), All patients in both groups (III and IV) were received 1500 mg metformin per day (500 mg three times a day) for 6–8 weeks. At the end of this period, the patients in the Group IV were received 2.5 mg letrozole for 5 days from day 3 of their menstrual cycle. Primary outcome measures: biochemical and hormonal changes. Secondary outcome measures: Ovulation induction and Pregnancy rate. In this study, the metformin – letrozole group patients LH, FSH and Testosterone levels were significantly reduced after the treatment period. Some biochemical parameters also come stepping back to the normal level. Ovulation occurred in 32 patients (64%) of the metformin – letrozole group and in 16 patients (32%) of the metformin group, which showed a statistically significant difference ($P<0.05$). A non-significant increase in pregnancy rate was observed in the metformin – letrozole group. Combined therapy of metformin gives beneficial effect on Ovulation rate than the mono therapy of metformin.

Keywords: Letrozole, Ovulation induction, Polycystic ovary Syndrome.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder, affecting 5–10% of women in the reproductive age.[1-3] The characteristic clinical features of PCOS are menstrual irregularities, which include amenorrhea, oligomenorrhea or other signs of irregular uterine bleeding, and signs of excess androgen such as hirsutism, acne and obesity.[4] Acne is a common sign of hyperandrogenism.

Hirsutism is another significant clinical marker of androgen excess and varies with ethnicity. Hirsutism is defined as the presence of excessive terminal hairs in androgen-dependent areas of a woman's body. It has to be differentiated from hypertrichosis, which is usually familiar in nature and associated with endocrine dysfunction such as thyroid malfunction, or drugs such as phenytoin, and manifested by excessive and uniform growth of hair, which is not restricted to androgen-dependent areas only.[5]

Previously it was thought that PCOS only affects women of child-bearing age because the diagnosis was only made in women who underwent investigations due to complaints of infertility. It is now becoming clear that PCOS or its early stages affect women at any age, even during adolescence.[6] At this time, it remains undiagnosed because oral contraceptives are prescribed to regulate menstrual cycle until these women visit the clinic for the treatment of infertility.[7]

A large number of studies have proved the association of PCOS with increased risk of cardiovascular disease[8-10]. The presence of insulin resistance is said to be responsible for PCOS, which occurs as a consequence of disturbed carbohydrate and lipid metabolism and hence provides the link between increased risk of cardiovascular events and PCOS. Similarly, hypertension can also be considered as one of the manifestations of insulin resistance, which in turn may also be related with high body mass index (BMI).[11]

Metformin increases the number of insulin receptors, but doesn't affect insulin concentration. Thus, it doesn't lead to hypoglycemia in normoglycemic cases. Metformin is used in PCOS cases in order to decrease plasma LH, hyperinsulinemia, and ovarian androgen levels[5, 27 12-14]. Metformin increases the ovulatory rate.

Clomiphene citrate (CC) has been the most widely used and standard drug for the treatment of infertility since its introduction into clinical practice in the 1960 s. It is known that clomiphene results in an ovulation rate of 60–85%, but a conception rate of only about 20%.[15] About 20–25% of women are resistant to CC and do not ovulate.[16] CC has a long half life of 5–7 days and this may have a negative effect on the cervical mucus and endometrium.[17]

Aromatase inhibitors have been suggested as an alternative treatment to clomiphene as the discrepancy between ovulation and pregnancy rates with clomiphene citrate has been attributed to its anti-estrogenic action and estrogen receptor depletion. The aromatase inhibitors do not possess the adverse anti-estrogenic effects of clomiphene but, by suppressing estrogen production, mimic the central reduction of negative feedback through which clomiphene works. Letrozole, the most prevalently used anti aromatase for this indication, has been shown to be effective, in early trials, in inducing ovulation and pregnancy in women with anovulatory PCOS and inadequate clomiphene response[16] and improving ovarian response to FSH in poor responders[25]. Evidence from larger trials is still awaited, but some encouragement may be taken from the solidity of the working hypothesis and the success of the preliminary results.

Therefore, the current study was planned to compare the combined effects of metformin and metformin – letrozole on ovulation and better pregnancy rate in south Indian women's with polycystic ovary syndrome.

PATIENTS AND METHODS

All patients between the age of 27 and 37 years who attended an infertility clinic with a suspicion of PCOS (specifically, complaining of infertility, menstrual dysfunction or dermatological problems), were included in the study. The study sample was collected from various infertility clinics in Tamilnadu, India. The study was approved by the Scientific Ethics Committee, Coimbatore, Tamilnadu. An informed written consent was obtained from each patient. The inclusion criteria were, infertility for at least one year, having patent tubes on hysterosalpingogram, and normal semen analysis of the patients' husbands. None of the women had received any hormonal or infertility therapy for at least 6 months before enrollment to the study. Both drugs were administered during the 3rd-7th days of a menstrual cycle.

Serum inhibin B was measured by enzyme-linked immunosorbent assay and automated Chemiluminescence immunoassay systems were used for the determination of LH, FSH, TSH, and testosterone, insulin (IMMULITE 2000, DPC Biermann, Bad Nauheim, and Germany). Levels of serum glucose, total-C, HDL-C and TG were determined by the calorimetric method using a Cobas Mira Plus autoanalyser (Roche Diagnostics, Mannheim, Germany). LDL-C and VLDL-C levels were calculated by the Friedwald formula. These levels were obtained on day 3 of spontaneous or withdrawal cycle.

Transvaginal ultrasound examination were performed prior to starting the treatment on day 3 of the cycle, day 10-12 of the cycle, and more often as needed with a 5-MHZ vaginal transducer attached to a Aloko Scanner (Model SSD-500, Aloka Co, LTd, Tokyo, Japan). Follicular diameter was determined by calculating the mean of two perpendicular diameters measured at the largest plane of the follicle. Intramuscular hCG (Profasi®, Serono factory, Swiss or Pregnyl®, Organon factory Nederland) at the dose of 10,000 IU administered to trigger ovulation when at least one mature follicle (≥18mm) was developed followed by timed intercourse.

Serum β-hCG was measured 5 days after a missed period. The pregnancy rate was calculated on the basis of a positive result of a serum β-hCG level >10mIU/ml. Ultrasound was performed 2 to 4 weeks after a positive pregnancy test to confirm clinical pregnancy by fetal cardiac activity and number of gestational sacs. The follow-up continued from clinical pregnancy confirmation by ultrasonography until delivery or pregnancy loss. Ovulatory rate, pregnancy rate, miscarriage rate, and rate of multiple pregnancies in both treated groups were evaluated.

Statistical Analysis

The data are reported as the mean +/- SD or the median, depending on their distribution. The differences in quantitative variables between groups were assessed by means of the unpaired t test. One way Analysis of variance [ANOVA] was performed followed by multiple comparisons using the scheffe test. Comparison of a variable between two groups was assessed by Mann-Whitney Test. A p value of <0.05 using a two-tailed test was taken as being of significance for all statistical tests. All data were analyzed with a statistical software package (SPSS, version 13.0 for windows).

RESULTS

The population consisted of 200 subjects (Female population) divided into four groups was selected. Patients visited with infertility problem in various hospitals in various cities, Tamil nadu, India with suspected PCOS patients was selected as source of population based on the inclusion and exclusion criteria. The control subjects were selected based on inclusion and exclusion criteria. They were not receiving any drugs at the time of the study. General health characteristics such as age, body weight, BMI, hirsutism, menstrual status were investigated by a self-administered questionnaire.

The demographic characteristics like body weight, BMI, hirsutism, menstrual cycle status were significantly increased in after treatment (data's not shown).

Table 1 shows the significantly ($p<0.001$) increased in the level of blood glucose, total cholesterol, triglycerides, were observed in both treated PCOS subjects as compared to control and untreated PCOS subjects, but the level of glucose, cholesterol and triglycerides concentration were significantly ($p<0.001$) decreased in PCOS treated group IV compared to treated group III and group II subjects.

The serum cholesterol and triglycerides levels were significantly ($P<0.001$) increased in control (I) and Untreated group (II). These levels were significantly ($P<0.001$) decreased in treatment (III and IV) subjects. The

serum level HDL, LDL and VLDL levels were not significantly differ in before treatment and after treatment groups.

Table 2 shows the average mean levels TSH, Insulin, LH, FSH, Testosterone and inhibin B were observed in both treated PCOS subjects as compared to control and untreated PCOS subjects, but the level of Insulin, LH, FSH and Testosterone were significantly (p<0.001) decreased in PCOS treated group IV compared to treated group III and untreated PCOS subjects (II). It shows metformin – letrozole treatment better result compare with metformin treatment group (table 2). The level of TSH in both PCOS group (treated and untreated) compare with control there is no statistical significant. The Inhibin B levels were showed no significant difference in group I with group III. But, the levels were significantly (p< 0.001) increased after the treatment (III and IV).

Table 1: Comparison of Biochemical changes in control, baseline and treated polycystic ovarian syndrome patients.

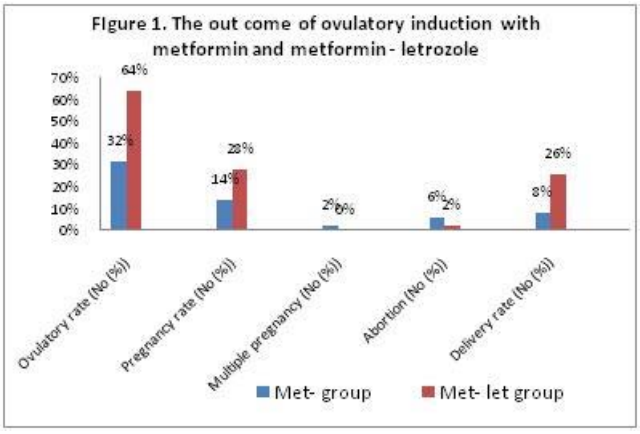
Parameters	Group I control	Group II Un treated	Group III Treated - met	Group IV Treated– met-let
Glucose mg/ dl	82.5 ± 7.7	129 ± 32.7$^{\$\$\$}$	92.6 ± 6.8$^{\#\#\#***}$	86.8 ± 5.7^{NS‡‡‡,†††}
Cholesterol mg/ dl	172 ± 18.1	258 ± 55$^{\$\$\$}$	174 ± 26^{NS***}	175 ± 30.5^{NS‡‡‡,NS}
Triglyceride mg/ dl	85 ± 13.8	139 ± 44.4$^{\$\$\$}$	89.5±13.1^{NS***}	84.3 ± 11^{NS‡‡‡,†}
D-HDL (mg/dl)	46.9 ± 2.1	48.4 ± 6.2^{NS}	47.5 ± 4.9^{NS}	45.7 ± 4.1*‡†
D-LDL (mg/dl)	66.2 ± 30.6	87.1 ± 41.2$^{\$}$	80.2 ± 36.9^{NS}	73.8 ± 28.4^{NS‡}
VLDL (mg/dl)	28.3 ± 11.1	32.9 ± 14.9^{NS}	26.6 ± 8.2^{NS}	25.6 ± 8.2^{NS‡,†}

Values are given as mean ± SD from fifty subjects in each group
Group IV compare with Group I significant at the present -*P <0.5, **P<0.01, ***P<0.001, NS –Non significant
Group III compare with Group I significant at the present -$^{\#}$P <0.5, $^{\#\#}$P<0.01, $^{\#\#\#}$P<0.001, NS –Non significant
Group II compare with Group I significant at the present -$^{\$}$P <0.5, $^{\$\$}$P<0.01, $^{\$\$\$}$P<0.001, NS –Non significant
Group III compare with Group II significant at the present -*P <0.5, **P<0.01, ***P<0.001, NS –Non significant
Group IV compare with Group II significant at the present -‡P <0.5, ‡‡P<0.01, ‡‡‡P<0.001, NS –Non significant
Group IV compare with Group III significant at the present -†P <0.5, ††P<0.01, †††P<0.001, NS –Non significant

Table 2: Comparison of endocrinological changes in control, baseline and treated polycystic ovarian syndrome patients.

Parameters	Group I control	Group II Un treated	Group III Treated - met	Group IV Treated – met-let
Insulin µu / ml	7.4 ± 1.6	16.4 ± 5$$$	9.0 ± 1.7###***	8.2 ±1.3**‡‡‡, †
LH U/ L	5.4 ±2.1	25.6± 6.8$$$	15.2 ± 3.8###***	11.2 ± 2.0***‡‡‡, †††
FSH U/ L	7.2 ± 1.4	12.2± 2.0$$$	10.9 ± 1.9###**	9.9 ±1.7***‡‡‡, †
Testosterone nmol / L	0.9 ± 0.48	4.3 ± 2.0$$$	3.1 ± 1.4###**	1.2 ± 0.56**‡‡‡, †
Inhibin B pg/ml	140.8+/-22.19	69.5+/-33.96,$^{\$\$\$}$	134.1+/-25.85^{NS***}	120.4+/40.09**‡‡‡ †

Values are given as mean ± SD from fifty subjects in each group
Group IV compare with Group I significant at the present -*P <0.5, **P<0.01, ***P<0.001, NS –Non significant
Group III compare with Group I significant at the present -$^{\#}$P <0.5, $^{\#\#}$P<0.01, $^{\#\#\#}$P<0.001, NS –Non significant
Group II compare with Group I significant at the present -$^{\$}$P <0.5, $^{\$\$}$P<0.01, $^{\$\$\$}$P<0.001, NS –Non significant
Group III compare with Group II significant at the present -*P <0.5, **P<0.01, ***P<0.001, NS –Non significant
Group IV compare with Group II significant at the present -‡P <0.5, ‡‡P<0.01, ‡‡‡P<0.001, NS –Non significant
Group IV compare with Group III significant at the present -†P <0.5, ††P<0.01, †††P<0.001, NS –Non significant



The secondary outcome of the ovulatory induction of the present study was presented in figure 1. From this study, ovulatory induction with metformin(III) and metformin-letrozole (IV) patients, the ovulation rates and delivery rates were significantly (p<0.05) differ. Another outcome of Pregnancy rate, multiple pregnancy rates and, miscarriage rates were statistically non –significant with treated groups (III & IV). According to this study, the results showed group IV gives the Enhanced ovulatory outcome.

DISCUSSION

PCOS is characterized by chronic anovulation, hyperandrogenism, and polycystic ovaries[18-20] Although considerable progress has been made toward a better understanding of the pathogenesis of this syndrome, the exact causes still is unknown.[18,19] Obesity has been implicated in the pathophysiology of PCOS due to its association with hypertension, hyperglycemia, and dyslipidemia.

Our study showed significant difference in cholesterol and triglyceride level of metformin – letrozole group patients.

High triglycerides and low HDL-C levels are closely linked to insulin resistance and are independent predictors of myocardial infarction and cardiovascular Disease.[24] It has been suggested that a triglycerides/ HDL-C ratio may be used as a simple metabolic marker to identify overweight individuals who are insulin-resistant.[32]

The HOMA-IR index is a validated estimate of insulin resistance.[33] Some Published studies have shown HOMA-IR indices in normal subjects to be 1.7. Subjects with impaired fasting glucose (IFG) have a value of 2.6, and impaired glucose tolerances (IGT) have values of 2.4**.**[34] In our study**,** we have been demonstrating the improvement in both insulin levels after the treatment period. Metformin decreases fasting glucose level by decreasing hepatic glucose output. Its use in PCOS patients, corrects the response to oral glucose tolerance, thus decreasing insulin level.[35-36]

In this study, observed improvements with regard to the clinical effects of androgens including hirsutism and acne. These findings agree with most, but not all, previous reports**.**[37] These effects may be related to a decreased LH and improvement of hyperinsulinemia, resulting in lower ovarian androgen production.[38] Aromatase enzyme has direct effect on the ovaries and increase follicular sensitivity to FSH. The level of ovarian aromatase is low in these patients. Multiple small ovarian follicles are due to high androgen level. In addition, androgens increase FSH receptors, and therefore increase FSH sensitivity.

In this study, the metformin – letrozole group patients LH, FSH and Testosterone levels were significant reduction after the treatment period. In a previous study of 10 subjects, Fruzzetti *et al.*[37] observed a statistically non-significant trend toward lower LH level. Since hyperinsulinemia and hyperandrogenism may alter the secretion of gonadotrophins in favor of an increase in LH, these drugs were lower LH secretion by reducing insulin and/or androgen levels.

In this present finding were showed significant increase in Inhibin B levels after the treatment. Some study confer, Inhibin signals via a type II activin receptor and type III TGF receptor (betaglycan) complex[25], and both have been identified on granulosa and thecal cells[29] of growing antral follicles. In rodent models, administration of inhibin to adult ovaries accelerated antral follicle development, whereas administration of activin increased follicular atresia[29]. Taken together, these studies suggest that defective inhibin biosynthesis may be functionally related to follicular arrest in PCOS.

For the first time Mitwally and Casper reported the use of letrozole in 12 patients with PCOS; ovulation occurred in 9 of the patients (75%), and pregnancy was occurred in three patients (25%).[16] In the present study, ovulatory rate was 64% in metformin - letrozole group and 32% in metformin group that was a statistically significant difference ($P<0.05$). Bayar's study showed the same ovulatory rate in both groups.[20] Cytochrome P450-C17a, which is a key enzyme in androgen synthesis, has an increased activity in PCOS patients due to increased levels of insulin. Metformin decreases the activity of this enzyme, thus increasing the response to ovulation induction Kolstad *et al.*[38] studied the relationship between menstrual cycle pattern and fertility. Thus the observed improvement in menstrual frequency can be viewed as an indication of improvement of ovulation rate and potential fecundity[22,23]. Aromatase enzyme converts androstenedione to estrone and then to E2 in peripheral tissues. Therefore aromatase inhibitors prevent estrogen production in these tissues. According to noted mechanism, some of selective aromatase inhibitors such as letrozole are used to induce ovulation especially in infertile women with PCOS[39, 40].

Pregnancy rate was not significantly different in the present study between the two groups (28% in metformin - letrozole group and 14% in metformin group (figure 1), however, we found a non significant increase in pregnancy rate similar to Tulandi's study[23]. In another study Haya Al-Fezan, *et al.,*[17] studied total 238 cycles of superovulation and IUI in women with idiopathic infertility. Patients were randomized to receive 7.5 mg of letrozole or 100 mg of clomiphene daily. There was no significant difference between the total number of developing follicles in the letrozole (5.7±3.7) and in the clomiphene groups (4.8±2.3). No difference was found in the endometrial thickness between the two groups (7.1±0.2 mm in the letrozole group, 8.2±5.9 mm in the clomiphene group). Addition of aromatase inhibitors to gonadotropins in controlled ovarian stimulation decrease gonadotropin requirements without negative effect on pregnancy rate.[25, 26]

In the present study, pregnancy loss was not significantly different in the both groups (nil in metformin-letrozole and *2*% in metformin (figure 1). This finding is similar with Mitwally's study that showed similar miscarriage rate in letrozole group compared with all other groups receiving current ovarian stimulation protocols.[27] Another report showed higher miscarriage rate with clomiphene.[17] Letrozole use has been associated with a significantly lower rate of multiple pregnancies compared with clomiphene in some studies.[27] In the present study, there was no multiple gestation in metformin-letrozole group and only one multiple gestation in metformin groups. The approximate half-life of letrozole is 45 hours (range 30-60 hours), which is shorter than the half-life of clomiphene (5-7 days)[28, 29] Letrozole should be cleared from the body completely by the time of embryo implantation. Thus, the exposure with the drug predates the critical fetal developmental period[30].

Based on the half-life of letrozole and anastrozole, administration in the early follicular phase should result in clearance of the agents before implantation takes place. Casper turned his attention to anastrozole, an aromatase inhibitor with pharmacokinetic properties similar to letrozole and slightly different chemical structure that has not been demonstrated to be associated with any

teratogenic, mutagenic, or clastogenic activity *in vivo* or *in vitro.*[31]

In conclusion, the present study found that ovulatory rate is higher with metformin - letrozole compared with metformin. A non-significant increase in pregnancy rate was observed in metformin-letrozole group. Miscarriage rate and multiple pregnancy rates were comparable in both groups. Aromatase inhibitors are effective for ovulation induction or augmentation of ovulation and administration of them in early follicular phase could be safe for ovulation induction. Potential advantages of Combined therapy of letrozole include reduced multiple pregnancies, absence of anti estrogenic adverse effects, and the subsequent need for less intensive monitoring. From this study were proposing that, combined therapy of metformin – letrozole was an excellent option for the anovulatory PCOS patients to improve their ovulatory function.

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