Int J Biol Med Res. 2011; 2(2): 490-496

Contents lists available at BioMedSciDirect Publications

International Journal of Biological & Medical Research

Journal homepage: www.biomedscidirect.com

Original Article

Comparison of Biochemical and hormonal changes in Metformin – clomiphine citrate and Metformin – Letrozole in PCOS south Indian women

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ARTICLE INFO

Keywords:
Polycystic ovary Syndrome
Clomiphene citrate
Letrozole
Ovulation induction
Insulin sensitizing agent

ABSTRACT

To determine whether metformin- clomifene citrate and metformin – letrozole combined drug treatment has beneficial effects on biochemical and hormonal changes in infertile polycystic ovary syndrome (PCOS) Women in an infertility clinic. All patients between the age of 27 and 37 years who attended a infertility clinic with a suspicion of PCOS. PCOS patients in both groups were received 1500 mg metformin (Glucophage, Merck, West Drayton, UK) a day (500 mg three times a day) for 6–8 weeks. If pregnancy occurred, the patient was excluded from the study. However, in case of failure of pregnancy after the end of this period, the patients in the group III were given 100 mg clomiphene citrate and group IV patients received 2.5 mg letrozole (Femara, Novartis, Quebec, Canada) from 3-7 days of their menstrual cycle. The demographic characteristics like body weight, BMI, hirsutism, menstural status was significantly ($p<0.001$) decreased, Biochemical parameters such as glucose, cholesterol have the significant difference (Fig II) But, lipid profile have not significantly differ after the treatment period. Endocrinological parameters of thyroid hormones were not significantly differ. But, Insulin , LH, FSH and testosterone hormones have a significant difference after the treatment period. In the present study we suggest, Comparatively Metformin – letrozole have a better result in demographic, Biochemical and endocrinoligical parameter. Especially PCOS patient androgen metabolisms were highly alter. Its exceedingly helpful to induce the ovulation and pregnancy level of infertile PCOS patients.

1. Introduction

Polycystic ovary syndrome (PCOS) is a common condition characterized by hirsutism, anovulation and menstrual irregularity. There is a broad clinical spectrum with compromised fertility an important issue for patients. Given that insulin resistance is thought to play a key part in the syndrome [1, 2], it is theoretically appealing to treat PCOS by ameliorating insulin resistance. Metformin acts by decreasing gluconeogenesis as well as increasing peripheral utilization of glucose in the presence of endogenous insulin [3].Metformin has not been associated with any congenital abnormalities when given to diabetic women who have subsequently become pregnant [4,5].

Current fertility treatment clomiphene citrate has been used as the first line treatment for ovulatory disorders.[6-8]. Clomiphene is easy to use and results in ovulation in most patients (60%- 90%), but the pregnancy rates are disappointing (10%-40%). This has been attributed to its peripheral antiestrogenic effects on the endometrium, cervical mucus, and other undetermined fertility factors.[9-11] Insulin resistance and hyperinsulinemia are common features in women with PCOS. Those patients who are resistant to clomiphene require alternative treatments. Insulin-sensitizing agents (e.g. metformin) alone or in combination with clomiphene can restore ovulation.

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PCOS patients treated with hMG or pure FSH may induce several ovulatory follicles, which may lead to multiple pregnancies and ovarian hyperstimulation syndrome.[12,13] Therefore, a simple oral treatment that could be used without risk of ovarian hyperstimulation and with minimal need to monitoring would be the preferred treatment.[12]

Aromatase inhibitors originally were developed for the treatment of breast cancer. Aromatase is a cytochrome P-450 hemoprotein that catalyzed the rate- limiting step in estrogen synthesis. That is the 3-hydroxylation step in the conversion of androstenedione and testosterone to estrone and estradiol, respectively. [6] Anastrozole and letrozole are selective aromatase inhibitors. They are reversible and highly potent that can decrease estrogen levels by 97% - 99% by using the doses of 1 to 5 mg per day.7 Whereas clomiphene stimulates endogenous FSH secretion by inhibition of central estrogen negative feedback via estrogen receptor antagonism, the effect of aromatase inhibitors is due to inhibition of peripheral estrogen production.[10]

Therefore, administration of an aromatase inhibitor in the early phase of the menstrual cycle results in the release of the hypothalamic/ pituitary axis from estrogenic negative feedback, increasing gonadotropin secretion and resulting in stimulation of ovarian follicle development.[6,10,12,14,15,16]. In the present study, we compared the combined effects of metformin - clomiphene and metformin - letrozole in PCOS patients and find out the biochemical & hormonal changes and pregnancy rate.

2. Materials and Methods

.1. Sample collection

The study was approved by the Scientific Ethics Committee, Tamilnadu. An informed written consent was obtained from each patient. We were chosen among 200 PCOS patients attending the infertility clinic of various infertility Hospital, in Tamilnadu. The major criteria for diagnosis of PCOS were oligo- and/or anovulation, clinical or biochemical, signs of hyper and ogenism and polycystic ovaries which is in accord with the revised 2003 Rotterdam criteria of PCOS. 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. Exclusion criteria included patients with a history of liver and kidney failure, cardiovascular disease.

The patients were visited and examined by gynaecologists. Healthy normal cycled women conclude as a control (group I), untreated PCOS patients selected for (group II), metformin–clomiphene citrate treated patients as (group III) metformin–letrozole treated patients as (groupIV).All patients of both groups received 1500 mg metformin (Glucophage, Merck, West Drayton, UK) a day (500 mg three times a day) for 6–8 weeks. If pregnancy occurred, the patient was excluded from the study. However, in case of failure of pregnancy after the end of this period, the patients in the metformin–clomiphene group (Group III) were given 100 mg clomiphene citrate for 5 days starting from day 3 of their menstrual cycle, and those in the metformin–letrozole group (Group IV) received 2.5 mg letrozole (Femara, Novartis, Quebec, Canada) for 5 days from day 3 of their menstrual cycle.

The condition of the ovaries was determined by transvaginal sonography every other day from day 12 of the cycle by a sonographist. A total of 10,000 IU of HCG was administered to those in whom at least one ovarian follicle was

2.1.Biophysico & Biochemical Parameters

All blood samples were obtained in the morning between 0800 and 0900 h after an overnight fast and resting in bed. In particular, at baseline blood samples were obtained during the early proliferative phase (second through third day) of the P-induced withdrawal uterine bleeding (for the cases) or the spontaneous uterine bleeding (for the controls), whereas throughout the study, they were obtained randomly in anovulatory PCOS patients and during the early proliferative phase of the spontaneous uterine bleeding in both controls and ovulatory PCOS patients.

Blood samples (5 ml) were collected into tubes containing EDTA after a 12-h fast and a 30-min resting period in the supine position and immediately centrifuged at 4 C for 20 min at 1600 x g, and plasma samples were stored at –20 C.

In all patients, weight and height were measured to calculate BMI using the formula: weight (kg)/height (m) [2]. The presence of acne and hirsutism was also assessed. The presence of comedowns (blackheads) on the face, neck, upper chest, upper back or upper arm was classed as acne. A modified Ferriman and Gallwey score was used to assess the growth of terminal hairs on upper lip, sideburn area , chin, lower jaw and neck, upper arm, chest, upper abdomen and lower abdomen [6]. Hirsutism was considered to be present where women scored >7.

2.3. Biochemical analyses

Automated chemi luminescence immunoassay systems were used for the determination of LH, FSH, TSH, testosterone, insulin (IMMULITE 2000, DPC Biermann, Bad Nauheim, Germany). Glucose (GOD-PAP method), Cholesterol (CHOD -PAP method), Triglyceride (GPO - PAP method). Levels of HDL-C 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.

2.4. 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 comparisions using the scheffe test.Comparison of a variable between two groups were 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 analysed with a statistical software package .(SPSS, version 13.0 for windows).

3. Results

The population consisted of 200 subjects (Female population) divided into four groups was selected. Treated polycystic ovarian syndrome patients with metformin – clomiphene citrate drugs (PCOS-MC; n=50) and treated polycystic ovarian syndrome patients with metformin - letrozole drugs (PCOS- ML); n=50) PCOS were compared with untreated PCOS patients (UTPCOS; n=50) and control subjects were collected from normal healthy female patients (C; n=50). 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, menstural status, ovulatory outcomes, biochemical and hormonal parameters were investigated by a self-administered questionnaire. The average mean levels of body weight, BMI, hirsutism, menstural status in control, untreated PCOS patients and treated PCOS patients in metformin - and metformin - –clomiphene citrate - letrozole subjects are presented in Table 1.

Table 1 . Demographic changes in normal women, pcos women, pcos women treated with met- cc and met- let.

Parameters	GroupI	Group II	Group III	Group IV
Body weight	54 ± 4.8	71 ± 10.9$^{\$\$\$}$	57 ± 5$^{\#***}$	56 ± 4.8*,^{‡‡‡,NS}
BMI	22 ± 2.6	28.4 ± 1.5$^{\$\$\$}$	26 ± 1.7$^{\#\#\#***}$	23.6 ± 1.3*‡‡‡,†††
Hirsutism	1.98 ±0.55	6.7 ± 1.1$^{\$\$\$}$	5.9 ± 0.72$^{\#\#\#***}$	4.1 ± 1.0**‡‡‡,†††
Menstural cycle %	100	30$^{\$\$\$}$	50$^{\#\#\#,NS}$	67^{**,‡‡‡,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, $^{\dagger\dagger\dagger P}$<0.001, NS –Non significant

The demographic characteristics like body weight, BMI, hirsutism, menstural status was significantly (p<0.001) decreased in the both treated PCOS group, untreated PCOS patients when compared with control subjects. However, the level of untreated PCOS patient body weight and hirsutism were higher in control groups and treated PCOS patient groups (Figure I).

Figure 1. Demographic changes in normal women, pcos women, pcos women treated with metformin - cc and metformin - let.

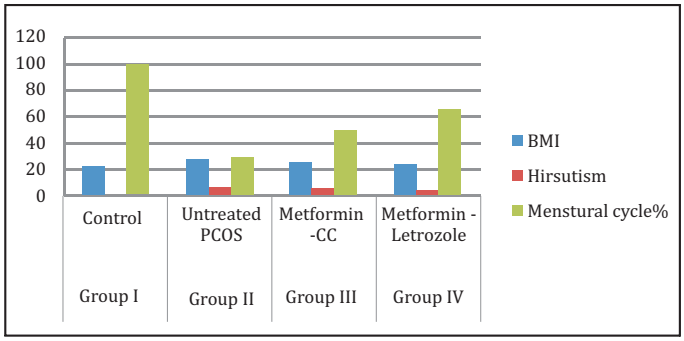


Table II shows the significantly increased in the level of blood glucose, total cholesterol, triglycerides, LDL, HDL and VLDL-C were observed in both treated PCOS subjects as compared to control, but the level of glucose, cholesterol and triglycerides concentration were significantly (p<0.001) decreased(Fig IIa) in PCOS treated metformin- letrozole group compared to treated PCOS metformin - clomiphene citrate group. The level of HDL, cholesterol in treated PCOS groups compare with control there is no statistical significant(Fig IIIa).

Figure II a. Biochemical change in normal women, pcos women, pcos women treated with metformin - cc and metformin - letrozole.

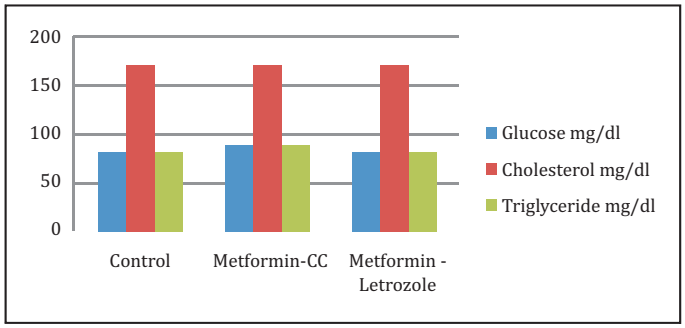


Figure III a. Comparison of lipoprotein changes in normal women, pcos women treated with metformin - cc and metformin - letrozole.

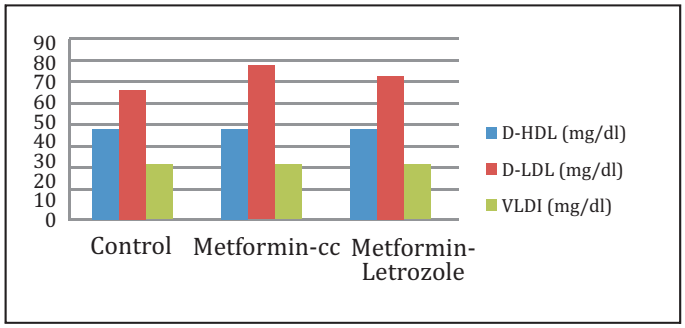
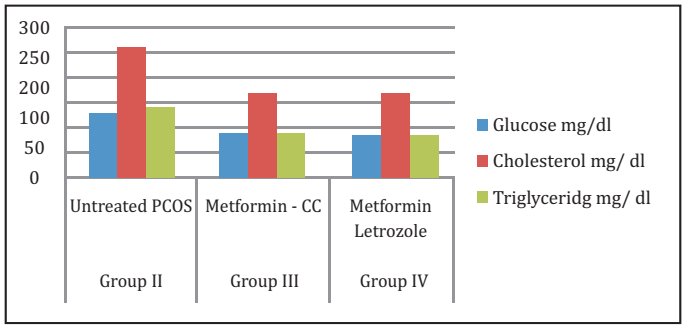


Table III shows Comparison of Biochemical and hormonal changes in untreated pcos women and , pcos women treated with metformin - clomiphene citrate and metformin- letrozole group. the significantly increased in the level of blood glucose, total cholesterol, triglycerides, LDL, HDL and VLDL-C were observed in both treated PCOS subjects as compared to untreated PCOS subjects, but the level of glucose, cholesterol and triglycerides (Fig IIb)

Figure II b. Comparison of Bio-chemical hormonal change in Untreated pcos women, pcos women treated with metformin - cc and metformin - letrozole.



concentration were significantly (p<0.001) decreased in PCOS treated metformin- letrozole group compared to treated PCOS metformin - clomiphene citrate group and untreated PCOS subjects. The level of HDL cholesterol in both treated PCOS groups compare with untreated PCOS groups were non significantly different(Fig IIIb).

Figure III b. Comparison of lipoprotein changes in Untreated pcos women, pcos women treated with metformin-cc and metformin - letrozole.

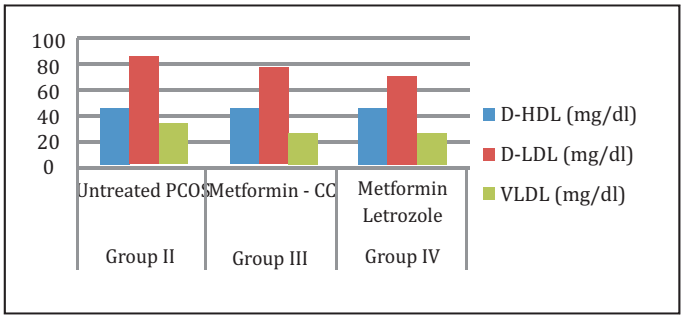


Table II . Comparison of Biochemical and hormonal changes in normal women, pcos women treated with metformin- cc and metformin- letrozole.

Parameters	Group I	Group II	Group III
Glucose mg/ dl	82.5 ± 7.7	89.6 ± 6.8###***	84.8 ± 5.7^{NS‡‡‡,†††}
Cholesterol mg/ dl	172 ± 18.1	169 ± 26^{NS}***	173 ± 30.5^{NS‡‡‡,NS}
Triglyceride mg/ dl	85 ± 13.8	89.5 ± 13.1^{NS}***	84.3 ± 11^{NS‡‡‡,†}
D-LDL (mg/dl)	46 ± 9.5	45.4 ± 6.2^{NS}	45.9 ± 6.8^{NS}
D-LDL (mg/dl)	66.2 ± 30.6	78.2 ± 36.9^{NS}	71.8 ± 28.4^{NS‡}
VLDL (mg/dl)	66.2 ± 30.6	26.6 ± 8.2^{NS}	25.6 ± 8.2^{NS‡,†}
Total T3	28.3 ± 11.1	102.6 ± 15.3^{NS}	112 ± 34.1^{NS}
Total T4	116 ± 30.4	9.4 ± 1.36^{NS}	9.87 ± 1.52^{NS}
TSH	9.96 ± 1.24	3.5 ± 4.16 NS*	2.6 ± 0.84^{NS,†}
Insulin µu / ml	2.61 ± 1.1	9.0 ± 1.7$^{###***}$	8.2 ±1.3**‡‡‡,†
LH U/ L	7.4 ± 1.6	15.2 ± 3.8$^{###***}$	11.2 ± 2.0***‡‡‡,†††
FSH U/ L	5.4 ±2.1	10.9 ± 1.9$^{###**}$	9.9 ±1.7***‡‡‡,†
Testosterone nmol / L	7.2 ± 1.4	3.1 ± 1.4$^{###**}$	1.2 ± 0.56**‡‡‡,†

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 IV compare with Group III significant at the present -†P <0.5, ††P<0.01, †††P<0.001, NS –Non significant

The hormonal levels of T3, T4, TSH, Insulin, LH, FSH and Testosterone were observed in both treated PCOS subjects as compared to control subjects, but the level of Insulin, LH, FSH and Testosterone were significantly decreased in PCOS treated metformin - letrozole group compared to treated PCOS metformin - clomiphene citrate group (p<0.001).

Table III . Comparison of Biochemical and hormonal changes in Untreated pcos women, pcos women treated with metformin - cc and metformin- letrozole.

Parameters	Group II	Group III	Group IV
Glucose mg/ dl	129 ± 32.7$^{\$\$\$}$	89.6 ± 6.8###***	84.8 ± 5.7^{NS‡‡‡,†††}
Cholesterol mg/ dl	258 ± 55$^{\$\$\$}$	169 ± 26^{NS}***	173 ± 30.5 ^{NS‡‡‡,NS}
Triglyceride mg/ dl	139 ± 44.4$^{\$\$\$}$	89.5 ± 13.1^{NS}***	84.3 ± 11^{NS‡‡‡,†}
D-HDL (mg/dl)	46 ± 12.2 ^{NS}	45.4 ± 6.2^{NS}	45.9 ± 6.8 ^{NS}
D-LDL (mg/dl)	87.1 ± 41.2$^{\$}$	78.2 ± 36.9^{NS}	71.8 ± 28.4 ^{NS‡}
VLDL (mg/dl)	32.9 ± 14.9 ^{NS}	26.6 ± 8.2^{NS}	25.6 ± 8.2 ^{NS‡,†}
Total T3	128.6 ± 70.7 ^{NS}	102.6 ± 15.3^{NS}	112 ± 34.1 ^{NS}
Total T4	10.2 ± 2.6 ^{NS}	9.4 ± 1.36 ^{NS}	9.87 ± 1.52 ^{NS}
TSH	3.37 ± 4.44 ^{NS}	3.5 ± 4.16 NS*	2.6 ± 0.84 ^{NS,†}
Insulin µu / ml	16.4 ± 5$^{\$\$\$}$	9.0 ± 1.7$^{###***}$	8.2 ±1.3**‡‡‡,†
LH U/ L	25.6± 6.8$^{\$\$\$}$	15.2 ± 3.8$^{###***}$	11.2 ± 2.0***‡‡‡,†††
FSH U/ L	12.2± 2.0$^{\$\$\$}$	10.9 ± 1.9$^{###**}$	9.9 ±1.7***‡‡‡,†
Testosterone nmol / L	4.3 ± 2.0$^{\$\$\$}$	3.1 ± 1.4$^{###**}$	1.2 ± 0.56**‡‡‡,†

Values are given as mean ± SD from fifty subjects in each group

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 level of T3, T4, TSH in both treated PCOS groups were compare with control there is no statistical significant(Fig IV a). The endocrinological parameters such as T3, T4, TSH, Insulin, LH, FSH and Testosterones average mean levels were observed in both treated PCOS subjects as compared to and untreated PCOS subjects, but the level of Insulin, LH, FSH and Testosterone were significantly decreased in PCOS treated metformin - letrozole group compared to treated PCOS metformin - clomiphene citrate group and untreated PCOS subjects (p<0.001).

Figure IV A. Comparison of hormonal change in normal women, pcos women treated with metformin - cc and metformin - letrozole.

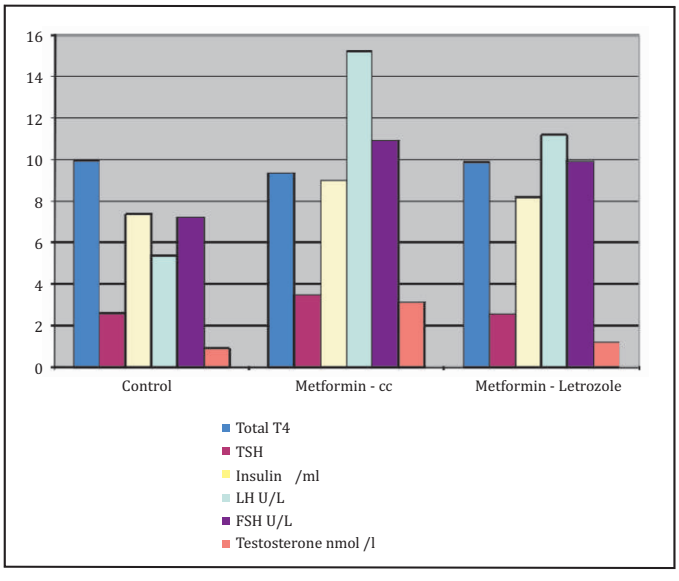
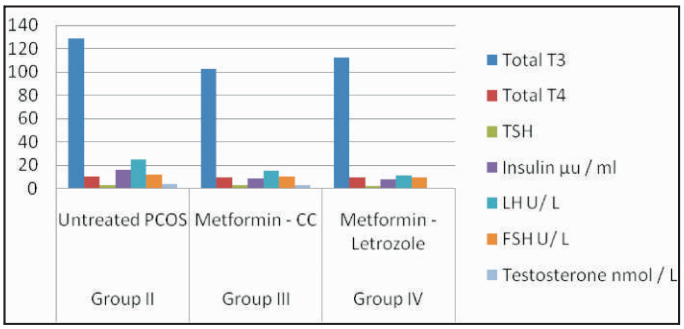


Figure IV b. Comparison of hormonal change in untreated pcos women, treated pcos women with Metformin - cc and metformin - Lterozole.



It shows (Fig IV b) metformin – letrozole treatment better result compare with metformin - clomiphene citrate treatment group. The level of T3, T4, TSH in both treated PCOS group compare with untreated PCOS group was non significantly different (Table III).

4.Discussion

Polycystic ovarian syndrome accompanied by hyperandrogenemia and hyperinsulinemia is seen in 5–10% of the population [17]. It is probably the most common endocrinological disorder amongst women of child bearing age. US studies revealed that PCOS is observed in 35% of amenorrhea cases, 85% of oligomenorrhea cases, 95% of hirsutism cases, and 75% of congenital adrenal hyperplasia cases. The most common characteristics of PCOS are chronic anovulation, hyperandrogenism, and infertility [18, 19].

Although obesity is a major predisposing factor for hirsutism in PCOS through the androgen excess mechanism, we did find the

significant difference in Ferimann and Gallwey score for hirsutism and BMI in the treated groups from the untreated PCOS group and control group. But, Silfen et al. [20] did not find a significant difference for Ferimann and Gallwey score between obese and non obese patients. 51.6% of patients had the clinical phenotype of hirsutism and 38.7% had acne. Acne was found in all the patients with hirsutism that means acne was always associated with hirsutism, but hirsutism was present exclusively in eight patients as a single clinical feature for hyperandrogenism.

At the end of the study period, the menstrual cycles over the time-course of the study increased significantly with a median of improvement of the menstrual cycle in both treated groups(III & IV) (Table I). However, there was significant difference between the groups (Table I). Patients who menstruated <4 weeks from starting treatment were not considered to have ovulated in response to the study. A number of studies have used menstrual frequency as an assessment of reproductive function women with PCOS and an improvement in menstrual regularity is considered to be a good surrogate for ovarian function and ovulatory frequency in women with PCOS [21-23]. Kolstad et al.[24] 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.

Hyperinsulinemia plays a pivot role in development of hyperandrogenemia in PCOS patients. Insulin directly stimulates androgen production from theca-cells [27, 28]. Sex hormone binding globulin level in the liver is decreased, and the level of free testosterone is increased [29]. At the same time, hyperinsulinemia increases IGF-1 by inhibiting insulin-like growth factor (IGG-1) binding protein, produced by the liver, and thus, androgen production from theca-cells is stimulated [30]. Metformin helps to 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. [25, 26].In our study suggest that after the treatment both glucose and insulin levels are deceased.

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 [31,32].

Crave et al. (1995) [36] have demonstrated that metformin administration has no additional benefit over the effect of diet on the lipid profile. Fleming et al., [22] have demonstrated an increase in HDL-C and a decrease in LDL-C within 14 weeks of metformin treatment. In our study metformin – clomiphene citrate group patients cholesterol levels were highly different from the meformin – letrozole group. While there was a slight changes were observed in other lipid profile parameters. 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 [37].

In this study, the metformin – letrozole group patients LH, FSH and Testosterone levels were significant reduction after the treatment period. It has been demonstrated that suppression of androgens with gonadotropin-releasing hormone agonists in women with PCOS does not alter lipoprotein levels [38]. But, in some study Metformin has not been shown to influence androgen concentrations in PCOS women[17].

Metformin may lower the androgen level in follicular fluid, affecting local levels of IGFs, and may produce improved ovarian stimulation. The lesser total number of follicles, but the greater proportion mature follicles was found on the day of HCG [33 - 35].

5. Conclusion

In conclusion, the present study found that combatively metformin – letrozole treated patients have the better result in demographic parameter and the glucose ,lipid profiles. In PCOS patients Androgen metabolism was highly alter, after the treatment period its suppress to the normal levels. Improvement of the menstrual frequency and ovulatory induction leads to better pregnancy rate. Potential advantages of Aromatase inhibitor of letrozole include reduced the adverse effect such as multiple pregnancies, absence of anti estrogenic adverse effects, and the subsequent need for less intensive monitoring. Therefore, it seems reasonable to use a treatment that is equally effective in induction of ovulation in infertile PCOS patients.

6. References

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