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Study of Effect of Metformin in PCOS Patients

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INTRODUCTION

Polycystic ovary syndrome (PCOS), also called hyperandrogenic anovulation (HA), or Stein-Leventhal syndrome, is one of the most common endocrinal disorders among females. PCOS produces symptoms in approximately 5% to 10% of women of reproductive age (approximately 12 to 45 years old)[1]. It is characterized by chronic anovulation with either oligomenorrhoea or amenorrhoea and hyperandrogenism and is the most common cause of anovulatory infertility and hirsutism (Solomon, 1999).[2] Anovulation results in irregular menstruation, amenorrhoea, and ovulation-related infertility. Hormone imbalance generally causes acne and hirsutism. Insulin resistance is associated with obesity, type 2 diabetes, and high cholesterol levels. The symptoms and severity of the syndrome vary greatly among affected women.

In 2003, a consensus workshop sponsored by ESHRE/ASRM in Rotterdam indicated PCOS to be present if any 2 out of 3 criteria are met: oligoovulation and/or anovulation, excess androgen activity ,polycystic ovaries (by gynecologic ultrasound) and other entities are excluded that would cause these. In 2006, the Androgen Excess PCOS Society suggested a tightening of the diagnostic criteria to all of excess androgen activity' Oligoovulation/anovulation and/or polycystic ovaries and exclusion of other entities that would cause excess androgen activity.

In PCOS Serum (blood) levels of androgens (male hormones), including androstenedione and testosterone may be elevated. The free testosterone level is thought to be the best measure, with ~60% of PCOS patients demonstrating supranormal levels. The Free androgen index (FAI) i.e. the ratio of testosterone to sex hormone-binding globulin (SHBG) is high and is meant to be a predictor of free testosterone, but is a poor parameter for this and is no better than testosterone alone as a marker for PCOS, possibly because FAI is correlated with the degree of obesity.

Some other blood tests are suggestive but not diagnostic. The ratio of LH (Luteinizing hormone) to FSH (Follicle-stimulating hormone) when measured in international units, is elevated in women with PCOS. Common cut-offs to designate abnormally high LH/FSH ratios are 2:1or 3:1 as tested on Day 3 of the menstrual cycle . There are often low levels of sex hormone-binding globulin, in particular among obese or overweight. Anti-Mullerian hormone (AMH) is increased in PCOS, and may become part of its diagnostic criteria.

Metformin hydrochloride a biguanide is an antihyperglycemic agent which improves glucose tolerance in NIDDM subjects, lowering both basal

and postprandial plasma glucose. Metformin HCl does not produce hypoglycemia in either diabetic or non-diabetic subjects and does not cause hyperinsulinemia. The magnitude of the decline in fasting blood glucose concentration following the institution of Metformin HCl therapy is proportional to the levels of fasting hyperglycemia. Metformin treated patients showed significant improvement in all parameters of glycemic control (fasting plasma glucose FPG, postprandial glucose PPG and HbA1c), stabilization or decrease in body weight and a tendency to improvement in the lipid profile, particularly when baseline values are abnormally elevated. Moreover by acting on the ovary and restoring normal ovarian activity Metformin positively modulates the reproduction axis namely GnRH-LH episodic release (Genazzani et al., 2004)[3]

This study was conducted with the objective to determine the effects of Metformin on clinical features, Metabolic, Endocrine profiles and Insulin sensitivity in PCOS patients

MATERIAL AND METHODS

The present was conducted in the Department of Obstetrics and Gynaecology in a medical College college over a period of 2 years after approval from ethics committee .A total of 50 obese women with PCOS were included in this study, aged 18-35 yrs, body Mass Index:>26kg/m2 ,euthyroid and not on any medications The clinical diagnosis of PCOS was made using the Rotterdam criteria when women had at least two of three criteria: Ovulatory dysfunction (oligoovulation or anovulation, Excess androgen activity (hirsutism, acne, or elevated serum androgens),Polycystic ovaries by ultrasound

Oligomenorrhoea was defined as fewer than eight menses per year or cycles longer than 35 days in length. Hirsutism defined as a Ferriman Gallwey score. Polycystic ovarian morphology diagnosis by using transvaginal ultrasound when at least one ovary had 8-12 antral follicles with a mean diameter <9 mm and/or a total ovarian volume >10 cm. Patients with thyroid disorders, Hyperprolactinemia, Cushing syndrome, Nonclassical congenital adrenal hyperplasia, Androgen-secreting tumors of the ovaries or adrenal glands ,Women with PCOS if they had taken any medication within 3 months before the study that could affect glucose or sex hormone metabolism, such as Metformin or hormonal contraception were all excluded from the study.

All the patients fulfilling selection criteria were explained about the nature of the study and a written informed consent was obtained before enrollment .After the enrollment, demographic data such as age, religion, education, socio economic status, were obtained through an interview. Detailed history including complaints, married life, obstetric, sexual, menstrual, medical and pharmacological history was documented and clinical examination was performed .Routine investigations like Hb%, TLC, DC and special investigations like Thyroid profile, Serum Prolactin, Fasting blood glucose levels,Serum Androgens and serum insulin levels and ultrasound were carried out.

The patients were then prescribed tablet Metformin for a period of 6 months. The dose of the drug was increased stepwise, from 500 mg once daily for the first week to 500 mg bid for the next week, and to 500 mg tid for a further 24 weeks. Patients were instructed not to modify their usual eating habits throughout the study. Metformin effects on menstrual abnormalities of women with PCOS were evaluated by assessing post-treatment changes in frequency of cycles. Furthermore, changes in several endocrine and metabolic features of the syndrome along with insulin sensitivity were also assessed.

Thus, before and at the end of study, the following were carried out:

1) Assessment of menstrual history, with recording of menses in the 6-month periods before the study and during treatment and if any pregnancy occurred during this period.

2) Physical examination for body weight, waist/hip ratio, hirsutism score**,** measured by a modification of the Ferriman-Gallwey method, and blood pressure, measured by a mercury sphygmomanometer with the subject in the sitting position, after a rest of at least 5 min;
3) Venous blood withdrawal after overnight fasting for serum androgens (total and free testosterone, DHEAS, Androstenidione)
4) Fasting Blood Glucose levels
5) Serum Insulin levels.

Each woman was asked to report any side-effect during the treatment.

RESULTS

Fifty females aged 18-35 years having polycystic ovarian syndrome were enrolled and studied for a period of 6 months. Tablet Metformin HCl 500 mg was given thrice daily for a period of 24 weeks (180 days) The observations were recorded on day 0 and day 180 for various parameters and they were statistically evaluated by using student (paired) t-test for comparison from day 0 to day 180.

TABLE 1 Baseline Characteristics of Patients (N=50)

Sl. No.	Characteristics	Range	Mean ± S.D.
1.	Weight (kg)	50-102	72.71± 8.90
2.	Height (m)	1.4 – 1.7	1.6 ± 0.1
3.	BMI (kg/m^2)	26 – 39.8	28.60 ± 2.64
	Blood pressure (mmHg): Systolic	90 – 150	128 ± 14.9
	Diastolic	70 – 100	83 ± 9.0
5.	Fasting Serum glucose (mg/dl)	65 – 110	92.74 ± 13.0
6.	Fasting Serum Insulin (μU/ml)	10.4 – 51	20.6 ± 11.0
7.	Serum Testosterone (ng/ml)	0.7 – 4.9	3.02 ± 0.81

Baseline characteristic features of all study cases were noted before starting them on tablet metformin.

TABLE 2 Patients Subgroups And Their Percentage (N=50)

Sl. No.	Characteristics			Day-0	Day-180
1.	Body Mass Index (BMI) kg / m2	(a)	<25	0	6(12%)
		(b)	25.0 - 29.9	43 (86%)	43 (86%)
		(c)	>30.0	7 (14%)	1 (2%)
2	Fasting serum insulin (FSI) (μU/ml)	(a)	<10	0	27 (54%)
		(b)	10-15	18 (36%)	16(32%)
		(c)	>15	32(64%)	7(14%)
3	Fasting serum glucose (FSG) (mg/dl)	(a)	<100	31 (62%)	45(90%)
		(b)	100-110	19(38%)	5(10%)

To study the effect of treatment according to the body mass index (BMI) in kg/m^2 the patients were divided into 3 groups according to the WHO criteria a) BMI <25 b) BMI between 25-29.9 and c) BMI ≥ 30. Before treatment group. Group (a) had 0 patients which increased to 6 (12%) after treatment. Group (b) had 43 (86%) patients before and after treatment. In group C initially, there were 7 (14%) patients which reduced to 1(2%) after treatment.

For studing effect on fasting serum insulin (FSI) in μU/ml. patients were divided into three groups (a) FSI < 10, (b) FSI between 10-15 and (c) FSI > 15 μU/ml. Before treatment group (a) did not have any patients but following 180 days treatment 27 (54%) patients were in this Group. Group (b) had 18 (36%) patients initially which decreased to 16 (32%) patients after treatment. Group (c) had 32(64%) patients which decreased to only 7(14%) after treatment.

For observing the fasting serum glucose (FSG) in mg / dl patients were divided into two groups (a) and (b) according to WHO criteria of FSG ≤ 110 mg / dl. Group (a) with FSG level of < 100 had initially 31 (62%) patients which increased to 45(90%) after treatment while group (b) had 19(38%) patients whose number decreased to only 5(10%) after treatment . Thus all the parameters showed improvement after treatment.

TABLE 3 Changes in Patient Variables from "Day-0" To "Day-180"

Sl. No.	Characteristics	Day-0 Mean ± S.D.	Day-180 Mean ± S.D.	P-value
1.	Weight (kg)	72.71±8.90	68.83±8.93	0.001*
2.	Body mass index (BMI) kg/m2	28.60±2.64	27.05±1.91	0.001*
3.	Blood Pressure: Systolic	128±14.9	122 ± 11.1	0.001*
	Diastolic	83 ± 9.0	82 ± 6.8	0.841
4.	Fasting serum glucose (mg/dl)	92.74 ± 13.0	86 ± 8.7	0.001*
5.	Fasting serum Insulin (μU/ml)	20.6 ± 11.0	9.8 ± 5.6	0.001*
6.	S.Testosterone	3.02 ± 0.81	2.72 ± 0.73	0.001

*P value significant.

Using student t-test (paired) for comparison from baseline (day-0) to Day-180. Thus there was statistically significant reduction in weight, BMI, BP, fasting serum glucose and insulin levels.

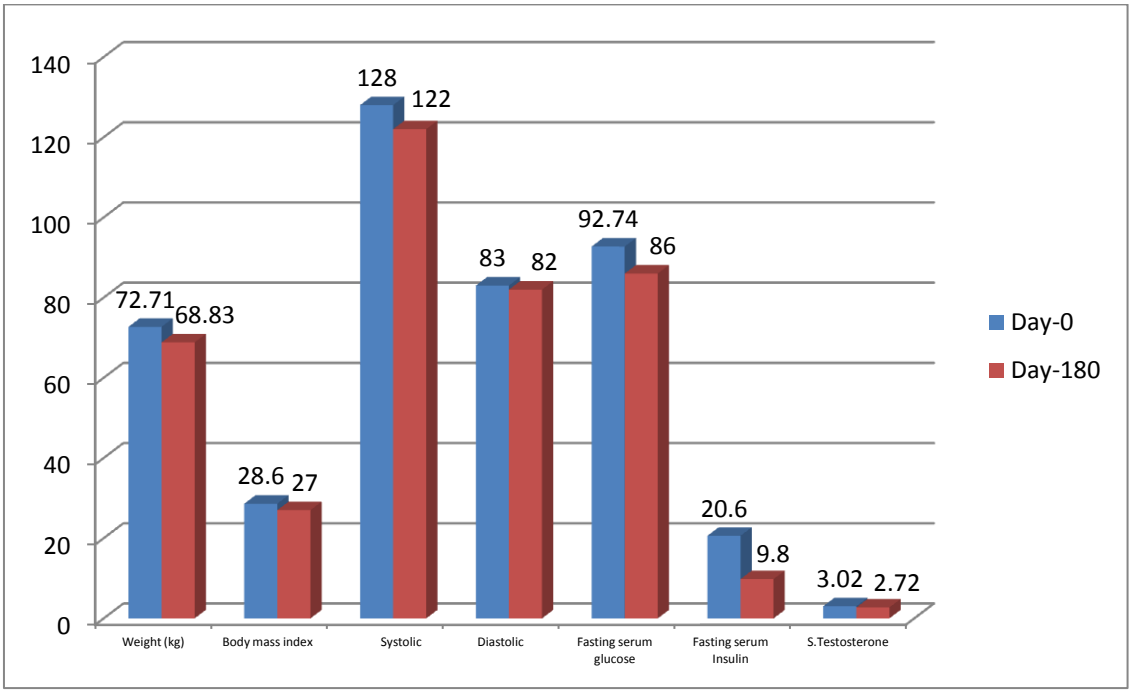
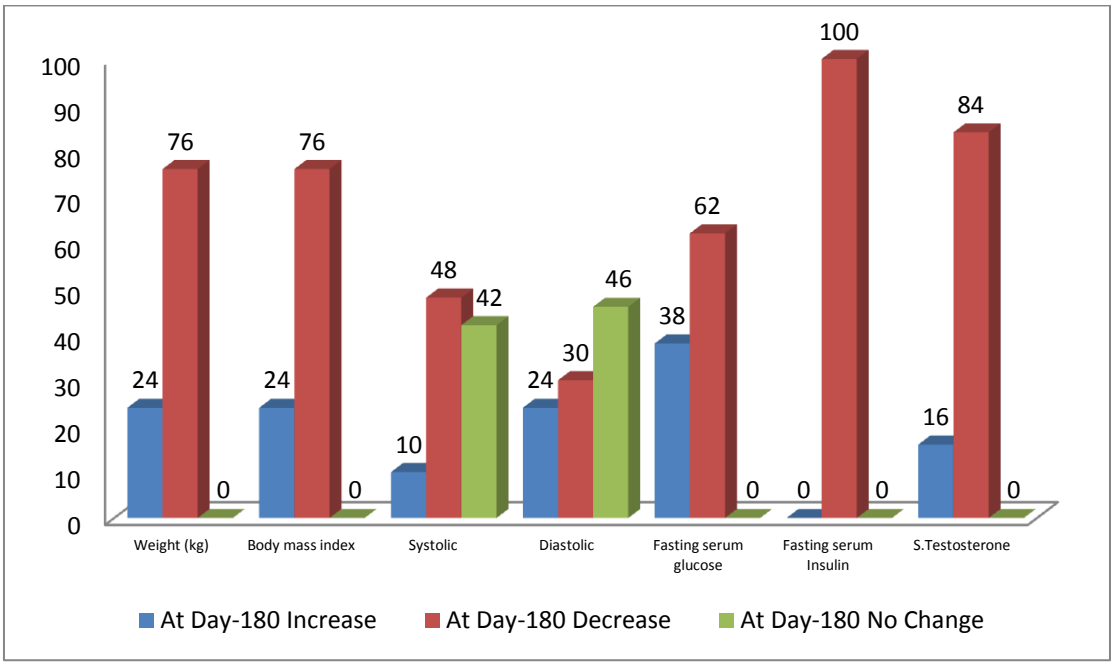


TABLE 4 Changes In Patient Variables After Treatment

Sl. No.	Characteristics	At Day-180		
		Increase	Decrease	No Change
1.	Weight (kg)	12 (24%)	38 (76%)	0
2.	Body mass index (BMI) kg/m2	12 (24%)	38 (76%)	0
3.	Blood Pressure: Systolic	5 (10%)	24 (48%)	21 (42%)
	Diastolic	12 (24%)	15 (30%)	23 (46%)
4.	Fasting serum glucose (mg/dl)	19 (38%)	31 (62%)	-
5.	Fasting serum Insulin (μU/ml)	0	50 (100%)	-
6.	S.Testosterone	8 (16%)	42 (84%)	-



Thus most of the PCOS cases had reduction in all the parameters under evaluation which was beneficial for them.

DISCUSSION

The cause of PCOS is unknown. However, insulin resistance with compensatory hyperinsulinemia is a prominent feature of the syndrome and appears to have a pathophysiologic role in the hyperandrogenism of the disorder. Both lean and obese women with PCOS show evidence of decreased insulin sensitivity but insulin resistance accompanied by compensatory hyperinsulinemia is most marked when there is interaction between obesity and the syndrome. There is ample evidence that hyperinsulinemia results in increased ovarian androgen biosynthesis in vivo and in vitro and decreased sex hormone binding globulin (SHBG) synthesis from the liver, leading to increased bioavailability of free androgens (Nestler, 1991)[4]. This excess in local ovarian androgen production augmented by hyperinsulinemia causes premature follicular atresia and anovulation (Uliger, 1996)[5].

Hyperinsulinemia may have a direct effect on the hypothalamus and/or pituitary to increase serum luteinizing hormone concentrations and therefore indirectly increase LH-dependent ovarian androgen biosynthesis possibly resulting in abnormal LH and follicle stimulating hormone release and subsequent oligomenorrhoea.. Hyperinsulinemia may also directly affect folliculogenesis and may arrest growth of antral follicles after they have reached a diameter between 5 and 8 mm.

In addition to reproductive morbidity the association of PCOS with insulin resistance and hyperinsulinemia puts patients at risk for possible long term metabolic hazards such as type II diabetes mellitus, dyslipidemia and cardiovascular disease.

Given the importance of hyperinsulinemia in the development of hyperandrogenism and disrupted folliculogenesis it seems likely that medications that act as insulin sensitizing agents may be more useful in restoration of normal endocrinologic and clinical parameters of this condition. The most extensively studied insulin sensitizing drug in the treatment of PCOS is Metformin (Seli, 2002)[6]. Metformin has been administered to women with PCOS to reduce insulin resistance and the sequelae of hyperinsulinemia including hyperandrogenism (Futterweit, 1999)[7]. We designed this study to use Metformin in our patients having PCOS, hyperinsulinemia and hyperandrogenisrn with the specific objective to investigate the effect of Metformin on the carbohydrate metabolism, along with the alterations in the physiological parameters such as body weight, body mass index, and blood pressure. Subjective evaluation regarding the cyclical disturbances, hyperandrogenic features (Acanthosis nigricans, male type baldness, acne, hirsutism) and biochemical analysis of the fasting serum glucose (FSG), fasting serum insulin (FSI) and testosterone (T) were all done to make the precise diagnosis of PCOS aided also by the ultrasound pelvis.

In our study, total patients who completed the study were 50. Out of which 35 (70%) had oligomenorrhoea and 15 (30%) had irregular cycles. None of the patients in the study had amenorrhoea. Following 6 months (180 days) therapy with Metformin HCl 500 mg thrice daily 33 (66%) patients developed regular cycles while 11 (22%) patients who previously had irregular cycles experienced improvement. Remaining 6 patients did not have any change. Velazquez et al. (1997)[8] reported the results of 22 PCOS women with chronic oligomenorrhoea or amenorrhoea, hirsutism completing 6 months of Metformin therapy 500 mg three times per day of which 21 (95.7%) of the women achieved regular menstrual cycle. Kolodziejczyk et al. (2000)[9] and Vandermolen el al. (2001)[10] also have demonstrated that Metformin administrated at a dose of 500 mg three times daily increased menstrual cyclicity, improved spontaneous ovulation and promoted fertility. Acbay (1996)[11] and Ehrmann (1997)[12] have failed to demonstrate salutary effect of metformin in PCOS. In the later study the mean body mass index of the women approached 40 Kg/m^2 and Metformin is said to be not effective in cases of morbid obesity.

In our study we found hyperandrogenic features like acanthosis nigricans in 4 (8%) patients, male type baldness in 5 (10%) patients, acne 13 (26%) patients and hirsutism 33 (66%) patients. Harborne (2003)[13] and Falsetti (2002)[14] have indicated that PCOS is a heterogenous disorder in which hyperandrogenism commonly manifests itself as hirsutism 60-83%, acne 11-43%, seborrhoea and alopecia. Conway el al. (2000) reported the incidence of male pattern baldness in approximately 8% and acanthosis nigricans in 5% of women with PCOS. Our study coincides with these results. Conway et al.[15] Marsden (2001) found that all 20 patients included in his study were hirsute and none of them had acanthosis nigricans. This is not coinciding with our study probably because as it has been mentioned by Carmina (1992)[16] that women with PCOS especially those of asian origin do not demonstrate obvious excess hair growth.

In our study, out of 50 patients, 37 (74%) patients had multiple small ovarian cysts on ultrasound pelvis. Vrbikova et al.[17] (2001) found that 17 (71%) patients out of a total of 24 patients had multifollicular ovaries while MacPaimill (2002) [18]have indicated that it is not essential that a women should have polycystic ovaries to have this syndrome. Therefore, polycystic ovaries observed on ultrasound are a sign of PCOS and not by themselves diagnostic of the disease. He depicted that polycystic ovaries are seen in 67% to

86% of the patients who have PCOS. Our finding is consistent with Vrbikova et al and MacPaimill.

In our study we found significant reduction in body weight from 72.71±8.90 to 68.88±8.53 Kg. Pasquali (2001)[19] and Glueck (1999)[20] also have found significant reduction in the body weight of the patients. The latter has shown significant reduction of weight in 28 (65.1%) patients out of a total of 43 while we have observed significant decrease in 38 (76%) patients out of a total of 50 patients.

Velazquez (1997)[8] in 22 patients found significant reduction in BMI from 26.79±4.13 to 26.22±3.99 Kg/m^2 and Glueck (1999)[20] in 43 patients observed significant reduction in BMI from 36.4 ± 7.0 to 35.1 ±6.7 Kg/m^2. These are in favour of our study as we have observed in 50 patients significant reduction in BMI from 28.60±2.64 to 27.05 ±1.91 Kg/m^2 whereas Hung (2001)[21] has observed a non significant increase in BMI from 23.8 to 24.1 Kg/m^2 in 10 PCOS patients. This differs from our results which might be due to the low mean baseline values of BMI and in the study of Hung.

Legro (2001)[22] have found that 195 women having PCOS had mean blood pressures within the normal limits. So is the condition in our study as a total of 50 patients had the blood pressures within the normal limits. Mean systolic blood pressure showed a significant reduction from 128± 14.9 to 122± 11.1 mm of Hg after 6 months of Metformin therapy. Nestler (1996)[23] on analysis of their studies have shown a significant reduction in both the systolic (from 128±2 to 127±2 mm of Hg) and diastolic (from 87±1 to 83±1 mm of Hg) blood pressures. We have also observed a decrease in diastolic blood pressure from 83±9.0 to 82±6.8 mm of Hg but it came out to be non-significant on statistical evaluation. Harborne (2003) in 52 PCOS patients found insignificant increase in both systolic and the diastolic blood pressures from 119.1 to 120/1 mm of Hg and 74.4 to 80.3 mm of Hg in contrast to our study which might be due to the longer duration of study of 12 months while our study lasted only for 6 months.

Fasting glucose level reduced significantly from 92.74±13.0 to 86±8.37 ng/dl in our study which is coinciding with the study of Morin-Papunen et al. (2000)[24]. He observed significant reduction in fasting glucose level of 8 PCOS patients from 5.2±0.1 (92.85 mg/dl) to 4.9±(U (87.5 ng/dl) mmol/L (÷mmol/L by 0.056 conversion factor to gel mg/dl) after 3 months of Metformin therapy. Whereas Marea et al. (2002)[25] in 15 PCOS women after 35-40 days of Metformin therapy and Kolodziejczyk (2000)[9] in 35 women after 12 weeks of Metformin therapy found non-significant reduction in the fasting glucose level. On the contrary Nestler (1998) [26]observed non-significant increase in the fasting glucose level from 78±3 mg/dl to 81±3 mg/dl after 35 days of treatment. Short period of this study is probably related to these results.

As regard to the fasting serum insulin level we observed a significant reduction in the mean fasting serum insulin level from20.6+11.0 to 9.80±5.6 μU/ml which is matching with the studies of Nestler (1996)[23], Kolodziejczyk (2000)[9], Morin-Papunen (2000)[24] and Marca (2002) [23].We have also observed a significant decrease in the serum testosterone levels from 3.02 ± 0.81 to 2.72 ± 0.73.which is consistent with studies done by Kazerooni et al[27] and K.H. Chou et al[28] on serum testosterone levels following Metformin therapy.

Thus hyperinsulinemia reflecting insulin resistance is a common feature in lean and obese patients with the polycystic ovary syndrome. Hyperinsulinemia contributes directly to excessive testosterone production by the ovaries and decreased synthesis of sex hormone binding globulin in liver, thereby increasing levels of total and free testosterone. Metformin therapy increases insulin sensitivity and decreases insulin levels in patients with the polycystic ovary syndrome. Improvement of hyperinsulinemia is associated with decreased levels of total and free testosterone and increased estradiol levels. Clinically

administration of Metformin improves hirsutism, normalizes menstrual cycles and induces ovulation in a substantial number of patients with the polycystic ovary syndrome (Kirpichnikov, 2002)[29].

CONCLUSION

It seems that Metformin has a direct effect on the human ovarian steroidogenesis in addition to reduction of weight and body mass index.

It is therefore concluded that Metformin treated patients showed significant improvement in all parameters (except diastolic blood pressure) indicating that Metformin is of benefit in reducing insulin resistance in PCOS cases.

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