ORIGINAL ARTICLE

A Subgroup Analysis of Effect of Metformin in Indian Women with PCOS

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Abstract

To analyze the variations in effects of metformin on ovarian function in different subgroups of Indian women with PCOS. Sixty PCOS patients were divided into treatment (metformin 500mg TDS for 2 months) and placebo groups. Parameters were assessed at baseline and after 2 months. Patients in the metformin group were further divided into subgroups according to the Body Mass Index (BMI), Serum Insulin and Serum Testosterone levels. Statistical analysis was done by Wilcoxan's Rank Sum test and paired t test. In subgroup analysis, obese patients (BMI>30 kg/m2) had more severe abnormalities. Hyperinsulinemic patients (S.Insulin > 25 µIU/ml) had far better response to metformin than normoinsulinemic patients. Hyperandrogenic patients (S. Testosterone > 2pg/ml) responded poorly as compared to normoandrogenic patients. Patients with high insulin levels and low testosterone levels are better responders to metformin. Obesity also has implications on PCOS. Further studies are required to assess the effect of these parameters on treatment of PCOS.

Key Words

PCOS, Metformin, Indian women, Hyperinsulinemia, Hyperandrogenism

Introduction

Polycystic ovarian syndrome (PCOS) is a heterogenous endocrinological disorder affecting 5-10% women of reproductive age (1) PCOS is characterized by hyperandrogenism, menstrual irregularities, chronic anovulation and infertility (2). It is now evident that insulin resistance is a cardinal feature of PCOS and serves as a link between hyperinsulinemia and hyperandrogenism (2). Given the role of hyperinsulinemia in development of hyperandrogenism and disrupted folliculogenesis, it seems likely that insulin-sensitizing agents may be useful in restoration of normal endocrinological levels and clinical features of PCOS (3). It is seen that metformin benefits PCOS women by decreasing S. Insulin, S. Androgens and increasing ovulation rate and regularizing the menstrual cyclicity (4, 5). In this study, the variations in effect of metformin were assessed on the basis of variations in BMI, Serum Insulin levels and Serum Testosterone levels.

Material and Methods

Study was carried out on 60 women between 15-35 years with polycystic ovarian syndrome at the Department of Pharmacology and Department of Obstetrics and Gynecology, Lady Hardinge Medical College, and Smt. Suchitra Kriplani Hospital, New Delhi. No patient had any other endocrinopathy. They were nonalcoholic, nonsmoker and had not been on any medication for at least 3 months before the study. No patient had cardiac, renal or hepatic disease. Institute ethical committee, Lady Hardinge Medical College, New Delhi, approved the protocol of the study. Written consent of all the patients was taken before enrollment.

At the time of enrollment, all routine investigations were done. Anthropometric measurements (Weight, Height, BMI, Waist/Hip circumference) and F-G hirsuitism scoring were noted. After overnight fasting, Oral glucose tolerance test (OGTT) was done and samples were drawn for serum insulin, serum leptin, serum progesterone and serum testosterone. Patients were divided equally into 2 groups, one test group and the other control group. The test group received 500mg Metformin tablets thrice a day (Xmet 500 mg, Glenmark Pharmaceuticals Ltd.) for 2 months. The control group received placebo. The treatment was started in the menstrual phase or the follicular phase (as seen in USG). The patients were asked

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to maintain a menstrual calender and to note any side effects throughout the study period. Patients were followed up every month for follicle monitoring by USG from 10th day of the last menstrual period at 2 day interval for 5-6 times. Any pregnancy during the treatment period was recorded. At the end of 2 months, all the routine investigations, USG, OGTT, Anthropometric measurements, Hirsuitism scoring was recorded. Venous blood sample was taken for estimation of progesterone, testosterone, insulin and leptin. The subgroups were made in the metformin group and analysis was done for evaluation of variations in effect of metformin on the basis of BMI, S. Testosterone and S. Insulin between the subgroups and within each subgroup before and after 2 months of treatment. The hormonal assays were carried out by ELISA technique.

Statistical Analysis

't' test and Chi square test were employed for statistical analysis of the parameters between and within the two study groups at the baseline and after 2 months of treatment. Wilcoxan rank sum (Mann-Whitney) test and Paired t test were employed for subgroup analysis. Statistical analysis was done by SPSS version 10.0 statistical software. The value of $p<0.05$ was taken as significant.

Results

Obese and Non-Obese Groups (*Table1*)

BMI of 30 kg/m2 was taken as the cutoff point and 2 subgroups were made in the Metformin group. (obese > 30kg/m2 and non-obese < 30kg/m2). It was observed that obese patients had higher baseline insulin levels than non-obese patients. Obese patients also had higher baseline testosterone levels; higher fasting glucose and higher 2hrs glucose. They also had higher progesterone levels and higher leptin levels. The t test could not be applied in obese subgroup as the no of observations was very small. t test was applied only in nonobese subgroup and ($p<0.001$) was achieved in the nonobese subgroup at baseline and after 2 months. In obese subgroup, all 4(100%) patients achieved ovulation and regular cycles, while in non obese subgroup, 24 (92.3%) patients had ovulation and 25 (96.1%) patients had regular cycles. An unexpected finding was that the serum progesterone was higher in obese subgroup than non-obese subgroup, which couldn't be explained. Our results thus established that obese patients with PCOS have more severe abnormalities than non obese patients and there is a statistically significant response in non obese group to the metformin.

Hyper & Normoinsulinemic Groups (*Table 2*)

The patients were classified into 2 groups on the basis of S. insulin levels. Patients with S insulin more than 25 IU/ml were considered to be hyperinsulinemic and patients with S. insulin levels less than 25 IU/ml were considered as normoinsulinemic. It was observed that patients in both the subgroups had no significant difference in the clinical, biochemical and hormonal parameters at baseline. However after treatment, it was noted that hyperinsulinemic patients had a significant decrease ($p<0.01$) in testosterone as compared to normoinsulinemic group. Also ovulation was present in all 13 (100%) hyperinsulinemic patients while only 15(88.2%) patients had ovulation and 16(94%) patients had regular cycles in normoinsulinemic patients.

High & Normal Testosterone Groups (*Table 3*)

The patients were classified into 2 groups on the basis of S. testosterone levels. Patients with S. testosterone levels more than 2 pg/ml were taken as hyperandrogenic and patients with S. testosterone levels less than 2 pg/ml were taken as normoandrogenic group. It was observed that the group with normal testosterone had significant ($p<0.001$) reduction in insulin levels after treatment, whereas hyperandrogenic group had an increase in insulin levels. Similarly, the OGTT fasting levels reduced significantly ($p<0.001$) in normoandrogenic group, while in hyperandrogenic group, the OGTT fasting levels increased. Another significant finding was that leptin levels were higher in hyperandrogenic group as compared to normoandrogenic group at the baseline. Also, the progesterone levels increased significantly ($p<0.001$) in normoandrogenic group after 2 months of treatment, while the progesterone levels in hyperandrogenic group just reached the cut off point for ovulation after the treatment. In terms of ovulation and menstrual cyclicity, however, all 5 (100%) patients in hyperandrogenic group had achieved ovulation, while in normoandrogenic group only 23 (92%) patients achieved ovulation and 24 (96%) patients had regular cycles.

Discussion

Our study was done with the aim to assess the variations in effect of metformin with variations in BMI, Serum Insulin levels and Serum Testosterone levels. In the obese subgroups, it was observed that clinical and hormonal irregularities were more severe in obese patients (>30kg/m2) as compared to non-obese patients. In both the groups, there was a reduction in severity of clinical and endocrinal abnormalities (Table 3). As the number of patients in obese subgroup was very low, we could not assess the statistical significance. Maciel et al has reported that non obese women with PCOS responded better than obese women with metformin (6). This suggests that obesity has implications on the features of PCOS.

Both the insulin subgroups showed similar responses to metformin therapy except that there was a significant

Table 1. Subgroup Analysis in Metformin Group Done on the Basis of BMI levels

Parameters	BMI =30 kg/m² (n=4)		BMI <30 kg/m² (n=26)	
	Baseline	After 2 months	Baseline	After 2 months
	Median (Min-Max)	Median (Min-Max)	Median (Min-Max)	Median (Min-Max)
Weight (kg)	96 (80-106)	87 (74-100)	60 (33-78) † †	57 (35-75)*** † †
WHR	0.71 (0.68-0.78)	0.67 (0.66-0.69)	0.71 (0.64-0.78)	0.7 (0.58-0.77)***
FG Score	21 (5-25)	17.5 (5-23)	10.5 (4-25)	10 (4-20)***
OGTT F (mg/dl)	113.5 (109-130)	98.5 (85-125)	99 (69-130) †	89.5 (70-110)***
OGTT PP (mg/dl)	163.5 (150-190)	138 (130-173)	140 (109-169) †	139 (100-149)**
Insulin (µIU/ml)	30.75 (10.5-31.5)	21 (9.5-25)	20 (8.5-43.5)	13.3 (6.25-46)***
Leptin (ng/ml)	18.25 (7-22)	12 (4-14)	9 (0.5-20) †	4 (1-15)*** †
Progesterone (ng/ml)	2 (0.6-2.5)	4.5 (4-6)	0.5 (0.1-4)	4.8 (1-20)***
Testosterone (pg/ml)	1.05 (0.3-5)	0.41 (0.1-2.5)	0.91 (0.2-3)	0.1 (0.1-3)**
Ovulation Present n(%)	0	4(100)	0	24(92.3)
M. Cycles regular n(%)	0	4(100)	0	25(96.1)

,† - P< 0.05, **, † †- P< 0.01, *,††† - P< 0.001 †- Comparison of values in the metformin group at baseline and after 2 months *-Comparison of values within the same group at baseline and after 2 months*

Table 2. Subgroup Analysis in Metformin Group Done on the Basis of S. Insulin

Parameters	S. Insulin = 25µIU/ml (n=13)		S. Insulin < 25µIU/ml (n=17)	
	Baseline	After 2 months	Baseline	After 2 months
	Median (Min-Max)	Median (Min-Max)	Median (Min-Max)	Median (Min-Max)
Weight (kg)	60 (45-100)	58 (45-106)**	61 (33-87)	60 (35-75)**
BMI (kg/m²)	22.2 (17.3-39.2)	21.4 (17.3-36.2)**	25.6 (13.2-34.8)	24.3 (13.2-34.8)**
WHR	0.71 (0.64-0.78)	0.67 (0.58-0.77)**	0.73 (0.65-0.78)	0.71 (0.62-0.76)**
FG Score	10 (4-25)	12 (5-25)**	12 (5-25)	11 (5-20)**
OGTT F (mg/dl)	110 (80-130)	95 (80-125)**	98 (69-130)	90 (70-110)**
OGTT PP (mg/dl)	145 (109-190)	139 (100-173)**	139 (109-165)	135 (100-149)**
Leptin (ng/ml)	10 (0.5-18.5)	4 (1-14)**	10 (1.5-22)	5 (2-15)**
Progesterone (ng/ml)	0.5 (0.1-2.5)	4.5 (3-20)**	0.5 (0.15-4)	4.6 (1-12)***
Testosterone (pg/ml)	0.93 (0.2-5)	0.1 (0.1-2.5)**	0.9 (0.3-2.53)	0.2 (0.1-3)
Ovulation n(%)	0	13(100)	0	15(88.2)
M. Cycles Regular n(%)	0	13(100)	0	16(94)

,† - P< 0.05, **, † †- P< 0.01, *,††† - P< 0.001 †: Comparison of values in the metformin group at baseline and after 2 months * : Comparison of values within the same group at baseline and after 2 months.*

decrease in S. Testosterone levels in hyperinsulinemic patients leading to 100% ovulation. This pointed that patients with higher plasma insulin levels responded better to metformin therapy. Similar results were obtained in a study done by Moghetti et al, who found that responders to metformin therapy had significantly higher fasting plasma insulin levels (7). In testosterone subgroups, normoandrogenic patients had better response in decreasing the insulin and glucose levels and increasing the S. progesterone levels than hyperandrogenic patients. As the number of patients in hyperandrogenic subgroup was less, we could not assess the statistical significance.

Table 3. Subgroup Analysis in Metformin Group Done on the Basis of S.Testosterone Levels

	S.Testosterone = 2pg/ml (n=5)		S.Testosterone < 2pg/ml (n=25)	
	Baseline	After 2 months	Baseline	After 2 months
	Median (Min-Max)	Median (Min-Max)	Median (Min-Max)	Median (Min-Max)
Weight (kg)	76 (33-105)	74 (35-100)	60 (45-106)	58 (45-98)***
BMI (kg/m^2)	27.8 (13.2-36.2)	26.7 (14-34.4)	24.4 (17.3-39)	24 (16.5-36.2)***
WHR	0.69 (0.65-0.73)	0.67 (0.66-0.71)	0.71 (0.64-0.78)	0.7 (0.58-0.77)***
FG Score	13 (5-22)	12 (5-20)	11 (4-25)	10 (4-23)***
OGTT F (mg/dl)	85 (69-130)	89 (70-125)	101 (80-130)	95 (80-110)***
OGTT PP (mg/dl)	133 (126-190)	129 (112-173)	143 (109-169)	139 (100-149)**
Insulin (µIU/ml)	20 (19.2-31.5)	20.5 (6.25-21.5)	21.5 (8.5-43.5)	13.5 (8-46)***
Leptin (ng/ml)	13 (8-18.5)	7 (2.5-14)	7.5 (0.5-22)	4 (1-15)***
Progesterone (ng/ml)	0.69 (0.15-3)	3 (2.5-4.5)	0.5 (0.1-4)	5 (1-20) ***†
Ovulation Present n(%)	0	5(100)	0	23(92)
M. Cycles regular n(%)	0	5(100)	0	24(96)

,† - P< 0.05, **, † †- P< 0.01, *,††† - P< 0.001 †: Comparison of values in the metformin group at baseline and after 2 months * : Comparison of values within the same group at baseline and after 2 months.*

This result is similar to a study done by Pirwany *et al* (8), who reported an increase in ovulation rate and decrease in S. testosterone and S. Insulin concentration in normoandrogenic patients.

Conclusion

We concluded in the present study that hyperinsulinemia and normoandrogenimia are favorable conditions, as far as response to metformin therapy is considered. Obesity has also got implications on PCOS. However, further studies are required to throw more light on the effect of these parameters on effect of metformin in PCOS.

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