Effect of Metformin on Hormonal and Biochemical Profile in PCOS Before and After Therapy

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Abstract Insulin resistance and the resultant hyperinsulinemia exacerbate the reproductive abnormalities of Polycystic Ovarian Syndrome by increasing ovarian androgen productions and decreasing serum sex hormone binding globulin. The present study was conducted to estimate serum insulin and testosterone level in 44 PCOS cases and 32 control patients. Simultaneously the role of metformin (an insulin sensitizing agent) in modulating insulin resistance and serum androgen level was also analyzed. A significant rise in serum insulin and testosterone ($P < 0.001$) was observed in cases in comparison to control. Fasting Plasma Glucose to insulin ratio, a marker of insulin resistance revealed a significant fall in PCOS group. Follow up of cases with metformin for 3 months revealed a significant fall in serum insulin ($P < 0.05$) with improvement in insulin resistance along with a nonsignificant fall in testosterone level. Serum insulin registered a significant positive correlation ($P < 0.05$) with serum testosterone revealing its etiological association. Thus administration of drugs ameliorating insulin levels is expected to provide new therapeutic modality for PCOS.

Keywords Polycystic ovarian syndrome · Hyperandrogenism · Metformin

Introduction

Polycystic Ovarian Syndrome is the most common reproductive endocrine disorder among women of reproductive age, affecting 5–10% of population worldwide [1]. It is the commonest multisystem endocrinopathy having diverse etiopathogenesis in women, causing menstrual irregularities, hirsutism and anovulatory infertility. In Indian population the incidence has been estimated to be between 4 and 11% among women of reproductive age group [2]. PCOS is associated with insulin resistance, obesity, dyslipidemia and infertility [3]. Recently some theories depict genetic and intraovarian origin associated with environmental factors such as diet and altered life style [4, 5]. Several data support the hypothesis that insulin resistance and associated hyperinsulinemia play a pathogenic role in PCOS [6]. Hyperinsulinemia may promote abnormal ovarian androgen secretion and therewith abnormal follicular development leading to dysfunctional ovarian and menstrual activity [7]. These observations suggested the role of insulin sensitizing agents like metformin in improving these manifestations [8]. Some researchers demonstrated improvement in reproductive abnormalities in their group of patients [9], while others failed to observe any clinical or biochemical changes after metformin [10].

This discrepancy in the above observations created an interest for evaluating the role of insulin resistance for various biochemical & pathological manifestations of PCOS as well as the role of metformin as an insulin sensitizing agent for altering these manifestations in these cases.

Materials and Methods

The study was conducted in the Department of Biochemistry, S.C.B. Medical College, Cuttack from February 2006 to July

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2007. 44 PCOS patients within age group 15–35 years attending the OPD & indoor of O&G Department of S.C.B Medical College, Cuttack & 32 age matched healthy female controls from hospital staffs were included in this study group.

The diagnosis of PCOS was made from the history of chronic oligomenorrhoea (cycle length > 35 days, or less than 9 cycles per year), amenorrhoea (cycle length > 12wks), infertility with hirsutism or acne, and with an ultrasonographic findings of polycystic ovaries [11].

Women with prior history of glucose intolerance (including gestational diabetes) or NIDDM, hyperprolactinemia, thyroid dysfunction, late onset congenital adrenal hyperplasia, cushing's syndrome or patients taking medications to alter the hormonal or biochemical profiles were excluded from the study. All patients included in the study were not pregnant.

Blood was collected from both controls and cases and was estimated for routine biochemical parameters (Fasting plasma glucose, 2 h PGPG & lipid profile) using automated analyzer Flexor-XL. Specific tests for serum Insulin and serum Testosterone were also performed using ELISA [12, 13].

Out of these 44 PCOS patients, 22 women were administered with metformin (an insulin sensitizing agent) at a dose of 500 mg tds for a period of 3 months. After completion of treatment, the biochemical parameters, serum insulin and serum testosterone values were analyzed again and compared with their pre-intervention values respectively. The results obtained were analyzed by student's t test, Wilcoxon signed ranks test and Mann–Whitney U test, Pearson correlation coefficient using SPSS-15. This study protocol has been approved by the institutional ethical committee, S.C.B Medical College Cuttack.

Result

In the present study about 43.2% of PCOS cases were in the age group of 21–25 years. 38.6% of these PCOS

patients had BMI ≥ 25 kg/m² showing obesity, whereas only 25% of controls had obesity with BMI ≥ 25 kg/m².

Table 1 revealed no significant difference in FPG & 2 h PGPG in PCOS patients as compared to control. That may be due to relatively young age group patients in this study, because β -cell dysfunction worsens with age [14]. Marked dyslipidemia was obvious in these patients in comparison to control, which may be attributed to insulin resistance [15].

The hormone profile reveals a marked rise in fasting serum insulin in these PCOS cases in comparison to control ($P < 0.001$), which was in agreement with other workers showing the role of hyperinsulinemia in the pathogenesis of PCOS [3, 16]. Marked rise in serum total testosterone in these cases ($P < 0.001$) may be due to excess ovarian production of androgens, which is central to the diagnosis of PCOS [17]. Yet some researchers have shown apparently normal testosterone concentrations in their PCOS cases, which may be attributed to their low to normal sex hormone binding globulin levels [18].

Mean value of fasting plasma glucose to insulin ratio (G/I), a good measure of insulin sensitivity was observed to be 4.34 in PCOS cases. Taking this fasting G/I ratio of ≤ 4.5 as abnormal [19], 50% of cases and 9.4% of controls were determined as insulin resistant, having this ratio to be ≤ 4.5 .

After administration of metformin to 22 PCOS cases at a dose of 500 mg TDS for a period of 3 months, only 10% of PCOS patients revealed improvement in glucose tolerance, whereas no significant alteration was noted in mean FPG & PGPG values. Serum HDL registered a significant rise ($P < 0.05$) in these cases after metformin therapy. All other lipid parameters documented no marked difference after therapy. Similar observations have been registered by other workers [20], who were in agreement that metformin reduces insulin resistance which in turn modifies dyslipidemia in PCOS cases (Table 2).

Overall change in BMI was statistically insignificant after therapy. However percentage of patients presented with obesity having BMI ≥ 25 kg/m² was reduced from 36.4 to 27.3%. Obesity was worsened with increase in BMI

Table 1 Biochemical and hormonal parameters in study group

Sl. No.	Parameter (Mean $\pm$ SD)	Control ($n = 32$)	PCOS cases ($n = 44$)
1	FPG (mg/dl)	75.62 $\pm$ 9.79	78.80 $\pm$ 8.77
2	PGPG (mg/dl)	82.73 $\pm$ 10.23	84.52 $\pm$ 11.92
3	Total Cholesterol (mg/dl)	140.21 $\pm$ 15.10	165.13 $\pm$ 28.34**
4	TG (mg/dl)	115.09 $\pm$ 24.46	144.0 $\pm$ 53.4*
5	LDL (mg/dl)	67.78 $\pm$ 15.20	92.63 $\pm$ 23.77**
6	HDL (mg/dl)	49.8 $\pm$ 4.84	43.77 $\pm$ 5.75**
7	VLDL (mg/dl)	22.96 $\pm$ 4.91	28.65 $\pm$ 10.84*
8	Serum insulin (μ IU/ml)	8.03 $\pm$ 3.16	14.73 $\pm$ 7.09**
9	Serum Testosterone (total) (ng/ml)	0.5 $\pm$ 0.22	1.24 $\pm$ 0.70**

** $P < 0.001$

* $P < 0.05$

Table 2 Metabolic and hormonal changes before and after therapy in 22 pcos cases ($n = 22$)

Sl. No.	Parameters (Mean $\pm$ SD)	Before therapy	After therapy
1	FPG (mg/dl)	74.82 $\pm$ 8.49	76.7 $\pm$ 9.25
2	PPPG (mg/dl)	85.59 $\pm$ 12.47	88.04 $\pm$ 11.66
3	Total Cholesterol (mg/dl)	165.82 $\pm$ 29.82	161.27 $\pm$ 29.55
4	TG (mg/dl)	154.64 $\pm$ 44.73	151.68 $\pm$ 42.51
5	LDL (mg/dl)	91.82 $\pm$ 26.22	87.50 $\pm$ 25.34*
6	HDL (mg/dl)	42.9 $\pm$ 5.38	47.64 $\pm$ 3.98**
7	VLDL (mg/dl)	30.91 $\pm$ 8.97	30.18 $\pm$ 8.41
8	Serum Insulin (μ IU/ml)	18.67 $\pm$ 6.02	16.00 $\pm$ 5.12**
9	Serum total testosterone (ng/ml)	1.46 $\pm$ 0.71	1.45 $\pm$ 0.70

** $P < 0.001$ * $P < 0.05$

only in one patient, which might have been due to decreased physical activity during treatment period.

After 3 months of metformin therapy, serum hormone status documented a marked fall in fasting serum insulin ($P < 0.001$), similar to the results of other researchers [20–22] and was in concurrence with the opinion that being an insulin sensitizer, metformin exerts its effect by promoting peripheral glucose utilization. Others have documented no significant change in serum insulin level after therapy [10]. Study of Barbieri et al. [23] demonstrated the direct stimulatory effect of insulin on ovarian androgen production in PCOS women. Insulin infusion studies have shown a clear association existing between serum insulin and testosterone levels in cases of PCOS, suggestive of a cause and effect relationship. The present study also revealed a decline in serum testosterone level, yet it was not significant.

Our study also revealed insulin resistance measured as fasting glucose to insulin ratio to be reduced from 77.27% of cases to 40.1% after metformin therapy, showing an improvement in insulin sensitivity. The median value of this ratio raised from 4.19 before therapy to 4.68 after 3 months of metformin therapy crossing the limit value of 4.5 which was statistically significant ($P < 0.05$) and in agreement with others [22, 24] (Table 3).

Significant positive association ($r = 0.44$, $P < 0.05$) between fasting serum insulin and serum total testosterone before metformin therapy in PCOS cases in the present study (Fig. 1), shows the etiological role of hyperinsulinemia in stimulating ovarian androgen production [25]. However following metformin therapy the association was though positive, yet it was not significant.

Table 3 FPG/insulin ratio before and after therapy ($n = 22$)

FPG/Insulin ratio	Before Treatment		After Treatment	
	No.	%	No.	%
≤ 4.5	17	77.27	9	40.1
> 4.5	5	22.73	13	59.9
Total	22	100	22	100

Fig. 1 Correlation of serum insulin with serum testosterone before therapy ($r = 0.44$, $P < 0.05$)

Discussion

PCOS has been a subject of research and debate over past six decades. Insulin resistance accompanied by compensatory hyperinsulinemia is a common feature of PCOS and both obese and non-obese women with the syndrome are more insulin resistant and hyperinsulinemic than age and weight matched normal women [19]. Insulin resistance in muscles and adipose tissues increases plasma FFA and insulin concentration, that stimulate synthesis and secretion of VLDL in the liver resulting in hypertriglyceridemia, which in turn enhances post-prandial accumulation of lipoproteins (LDL, VLDL) in plasma with lowering of HDL cholesterol [20, 21]. Hyperinsulinemia plays a pathogenetic role in PCOS cases by increasing ovarian androgen production and decreasing the serum sex hormone binding globulin concentration [23]. Insulin may directly stimulate ovarian cytochrome P450c17 α , resulting in increased 17- α hydroxylase and to a lesser extent, 17,

20-lyase activity. This would lead to increased production of androstenedione, which is then converted to testosterone by the enzyme 17 β reductase resulting in higher levels of free testosterone with dysfunctional ovarian and menstrual activity. Insulin may also stimulate ovarian androgen production by enhancing serum luteinizing hormone pulses and activity that enhances ovarian cytochrome P450c17 α activity in PCOS women as evidenced by identification of insulin receptors in human pituitary tissue [26].

Administration of metformin an insulin sensitizing agent has beneficial role in lowering serum testosterone level by exerting its action over serum insulin and increasing insulin sensitivity of tissues in PCOS [20, 22]. There is mild clinical improvement in terms of regularity of menstrual cycle. It may have the added benefit of improving at least some features of syndrome X; such as dyslipidemia, hypertension and obesity [21]. However the definitive clinical improvement as characterized by reduction of hirsutism, acne, and increased fertility can be assessed if the follow up study will be continued still longer.

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