Research Article

Effect of sustained released metformin therapy on phenotypic and biochemical markers of insulin resistance in polycystic ovary syndrome in South Indian women

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ABSTRACT

Background: Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in young women. Insulin resistance (IR) may play a substantial part in the pathogenesis of PCOS, which leads to type 2 diabetes mellitus (T2DM), cardiovascular disorders and ovarian cancer. Metformin is an insulin sensitizing agent, however its role in PCOS is still controversial.

Methods: Sixty women newly diagnosed with PCOS and healthy age matched controls between 18 to 45 years were enrolled after obtaining informed consent. Women in the PCOS group were started on metformin-SR 1gram orally, which was then increased to 1.5 grams after two weeks and continued for 6 months. Fasting blood sugar (FBS), fasting insulin (FI), SHBG, TT, free androgen index (FAI), homeostatic model assessment of Insulin resistance (HOMA-IR), homeostatic model assessment of β- cell function (HOMA-B), homeostatic model assessment of Insulin sensitivity (HOMA-S) and quantitative insulin sensitivity check index (QUICKI) were measured in the control group as well as PCOS group before and after metformin therapy.

Results: After six months of metformin-SR therapy, PCOS group showed significant reduction in FI, HOMA-IR, HOMA- β, HOMA-S QUICKI, TT and FAI and significant increase in SHBG levels.

Conclusions: Six months of metformin-SR therapy favorably altered markers of IR, TT, SHBG, anovulation and hyperandrogenism in normoglycemic women with PCOS.

Keywords: PCOS, Metformin, Insulin, HOMA, SHBG, Total testosterone

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a common endocrine disorder of women in the reproductive age with the prevalence of 5-10% all over the world characterized by chronic anovulation and hyperandrogenism. Even though the aetiology of the syndrome is not fully understood.[1]

Women with PCOS are more prone to T2DM, dyslipidaemia, premature atherosclerosis, and endometrial carcinoma. Insulin resistance (IR) is one of the common factors contributing to the pathogenesis of these abnormalities. The major cause of IR is excess weight and physical inactivity.[2] Approximately 50-70% of women with PCOS have IR independent of obesity.[3] In India the prevalence of IR in PCOS women is about 76.9%.[4] HOMA-IR is a surrogate marker; acanthosis

nigricans (AN) is a phenotypic marker for measuring IR.[5,6]

SHBG is a testosterone binding glycoprotein synthesized in liver. SHBG is reduced in IR and is a sensitive marker for the measurement of IR. Women with PCOS have lower SHBG due to IR irrespective of testosterone levels.[7] The rise in serum levels of testosterone in PCOS may be due to excess ovarian production of androgens.[8]

Targeting IR by using insulin sensitizing agent such as metformin, used to treat T2DM. In general metformin inhibits hepatic gluconeogenesis, enhances glucose uptake and utilization in peripheral tissues such as skeletal muscle and adipocytes.[9,10] Metformin may also improve ovulation and reduce circulating androgen levels in PCOS women.[11]

We tried to observe the difference between phenotypic, biochemical and hormonal data in newly diagnosed women with PCOS before and after Met-SR therapy and its impact on IR.

METHODS

Sixty women newly diagnosed with PCOS, healthy age matched controls between 18-45 years were recruited after obtaining written informed consent from the outpatient clinics of Departments of Endocrinology and Reproductive Medicine at Sri Ramachandra University. The diagnosis of PCOS was based on the Rotterdam /American Society for Reproductive Medicine (ASRM) consensus criteria.[12] Patients with known T2DM, impaired fasting glucose (IFG), impaired glucose tolerance (IGT), thyroid disorders, autoimmune diseases, adrenal, kidney and liver diseases, cardiovascular disease, those planning pregnancy or pregnant, taking oral contraceptive pills (OCP) or metformin, steroidal and non-steroidal anti-inflammatory medications were excluded. This study protocol was approved by the institutional ethics committee of Sri Ramachandra University.

Study subjects were enrolled after baseline measurements' including anthropometry and laboratory parameters from their previous reports, questionnaires regarding the family history of CVD, T2DM, and PCOS was given to each subject. Blood samples from all the subjects were collected in the early follicular phase (between days 3-7) after a 12 hours overnight fasting in EDTA coated and plain vacutainer. Samples were allowed to clot adequately before centrifugation at 2000 RPM (revolutions per minute) for 10 min and the plasma were separated immediately to estimate the glucose and hormonal levels. The remaining sample was frozen at -80[0]c for further investigations.[8] Subjects recruited in the PCOS group were treated with 1gm of Met-SR tablets orally daily for one week which was then titrated to 1.5gm per day and continued for six months. All parameters were repeated in PCOS subjects after Met-SR

therapy. Finally values were compared between the groups.

The study groups were interviewed for a detailed menstrual history including age of menarche, menstrual regularity, duration and number of cycles per year. Oligomenorrhea was defined as inter-menstrual interval of >35 days or a total of < 8 menses per year, AN was defined as hyper pigmented skin over the dorsal surface of the neck and intertriginous area.

Anthropometric measurements

Anthropometric parameters such as weight, height, waist circumference, hip circumference, waist hip ratio (WHR) and body mass index (BMI) were measured by standard methods.

Hormonal assays

Plasma glucose was estimated by standard glucose oxidase and peroxidase method (GOD-POD method), and hormones were measured in duplicate by ELISA method by using Bio-RADi Mark Micro plate Reader (S/N 13848), Rayto Micro plate washer RT- 2600 C and GeNei Micro plate Shaker SLM-MPS-250. Insulin was estimated by Cal biotech (catalog No IS130D), Total testosterone (cat. No CAN-TE-250) and SHBG (cat. No CAN-SHBG-4010) were estimated by Diagnostics Biochem Canada ELISA kits.

Insulin resistance assessment

Insulin resistance was estimated by HOMA-IR method using the formula [HOMAIR = fasting glucose (mg/dl) X fasting insulin (µIU/ml)/405). β-cell function was estimated using HOMA-B by calculating 3.08 X insulin/glucose-3.5. The quantitative insulin sensitivity check index (QUICKI) was used to estimate insulin sensitivity (IS) by calculating 1/[log fasting insulin (µIU/ml) + log fasting glucose (mg/dl)] and HOMA-S was calculated by 146/Insulin x glucose. The free androgen index (FAI) was calculated by total testosterone/SHBG X 100.[8,13]

Statistical analysis

All results were expressed as mean±SD. Statistical analysis was performed using Graph Pad Prism-5. Differences between the control and PCOS groups were analysed by independent t-test and difference with in the PCOS group before and after therapy were measured by paired t-test. P-value of <0.05 was considered statistically significant.

RESULTS

In the base line women with PCOS showed significant higher physical characters such as weight, BMI, WHR. and significant higher insulin indices such as FBS, FI, IR,

and hyper androgenic characters TT, FAI levels when compared to control group (P<0.05), lower levels of SHBG and insulin sensitivity markers such as HOMA-S, QUICKI index were observed in PCOS group (<0.05).

There is no significant difference age between the two groups. IR of PCOS group showed significant correlation with BMI and non-significant correlation with SHBG and TT (Table 1 and 2).

Table 1: Anthropometric and Metabolic and Hormonal levels in control and PCOS before and after therapy groups.

Parameter	Control (n=60)	PCOS (n=60)		P1	P2
		Before Therapy	After therapy		
Age (years)	26.08±3.78	24.75±3.64	-----------------	0.05	-----
Weight (kg)	55.45±7.02	61.95 ± 11.98	59.05±10.00	<0.05*	<0.05*
BMI (Kg/m^2)	23.77±3.28	25.76±4.93	24.67±4.12	<0.05*	<0.05*
WHR	0.78±0.04	0.81±0.05	0.79±0.05	<0.05*	<0.05*
FBS (mg/dl)	87.63±10.11	94.73±6.80	86.43±6.93	<0.05*	<0.05*
FI (μIU/ml)	10.65±2.48	14.28±1.47	11.62±1.55	<0.05*	<0.05*
HOMA-IR	1.36±0.32	1.85±0.99	1.48±0.20	<0.05*	<0.05*
HOMA-β	128.05±31.13	132.68±19.24	137.87±23.35	0.32 ns	<0.05*
HOMA-S	77.92±19.08	54.50±5.93	68.74±9.55	<0.05*	<0.05*
QUICKI	0.33±0.01	0.31±0.01	0.33±0.01	<0.05*	<0.05*
TT (nmol/l)	1.77±0.51	2.65±0.74	2.44±0.73	<0.05*	<0.05*
SHBG (nmol/l)	69.79±5.39	51.84±12.25	59.17±11.94	<0.05*	<0.05*
FAI	2.56±0.11	5.19±1.15	4.15±0.96	<0.05*	<0.05*
Oligomenorrhoea	0	41	21	<0.05*	<0.05*
Acanthosis nigricans	0	26	12	<0.05*	<0.05*

Data were expressed in Mean±SD by using t-test; clinical indices were done by Chi-squire test. PCOS BT = PCOS before therapy; PCOS AT= PCOS after therapy; P1 = P-value between control and PCOS BT groups; P2 = P-value between PCOS BT and PCOS AT; * = statistically significant (P <0.05), ns = statistically non-significant (P >0.05).
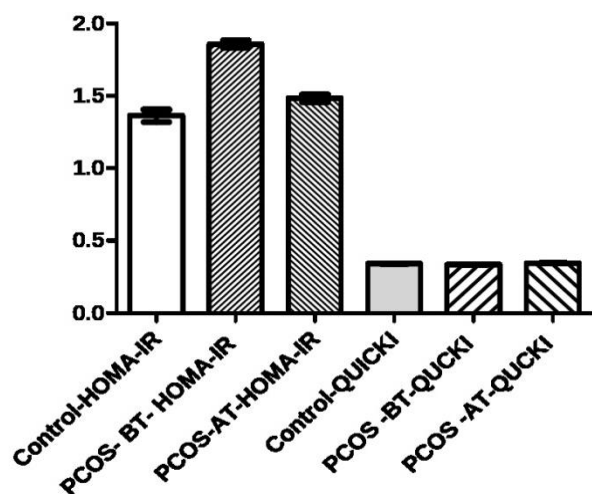
Data were expressed in Mean±SD, PCOS BT = PCOS before therapy; PCOS AT= PCOS after therapy HOMA-IR = Homeostatic Model Assessment of Insulin Resistance; QUICKI= Quantitative Insulin Sensitivity Index

**Figure 1a: Insulin resistance parameters of the control and PCOS groups.**

After six months of Met-SR therapy the PCOS group showed normalization of menstrual cycle and decrease in

AN and showed significant reduction in weight, BMI, WHR, FBS, FI, HOMA-IR, TT and FAI was a significant increase in β-cell function, Insulin sensitivity index and SHBG levels. There was correlation of BMI, SHBG and TT with HOMA-IR (Table 1, 2 & Figure 1a, 1b, 1c).

Table 2: Correlation between BMI and hormonal levels with insulin resistance.
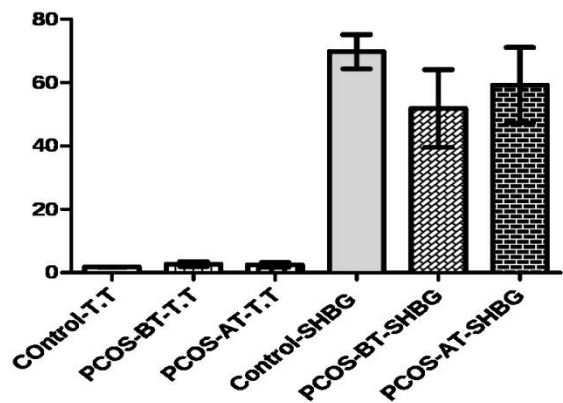
Parameters	PCOS group (n= 60)			
	Before therapy		After therapy	
	r-value	p-value	r-value	p-value
HOMA-IR vs BMI	0.29	<0.05*	0.28	<0.05*
HOMA-IR vs SHBG	0.13	0.30 ns	0.26	<0.05*
HOMA- IR vs TT	0.17	0.17 ns	0.24	<0.05*

All the values were expressed by Pearson's correlation. * = statistically significant (P <0.05), ns = statistically non-significant (P >0.05).
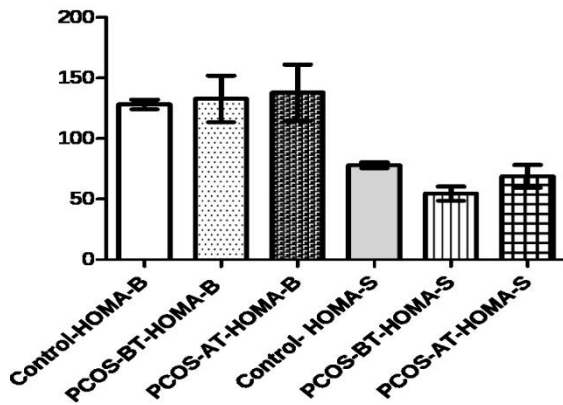
Data were expressed in Mean±SD, PCOS BT = PCOS before therapy; PCOS AT= PCOS after therapy HOMA-B = Homeostatic Model Assessment of β-cell function; HOMA-S = Homeostatic Model Assessment of Insulin Sensitivity

Figure 1b: Insulin Sensitivity and β-cell parameters of the control and PCOS groups.

Data were expressed in Mean±SD, PCOS BT = PCOS before therapy; PCOS AT= PCOS after therapy TT= Total testosterone; SHBG= Sex hormone binding globulin.

Figure 1c: Hyper androgenic parameters of the control and PCOS groups.

DISCUSSION

To our knowledge, this is the first study to report all parameters of insulin dynamics in the south Asian population with PCOS and the effect of Met-SR on these parameters. PCOS women were more obese (BMI of 25.75±4.93) when compared to controls (23.77±3.28). Met-SR therapy was significantly decreased BMI in PCOS women (from 25.75±4.93 to 24.67±4.12), which was similar to others.[5]

Met-SR therapy also reduced TT levels significantly in agreement with the previous studies.[14,15] It also improved menstrual abnormalities in women with PCOS, which was similar to other studies.[16,17] Metformin may reduce the severity of AN, In the present study we confirmed that six months of Met-SR therapy had enough to treat AN in PCOS women.

We have also demonstrated that metformin-SR therapy the PCOS group showed significant decrease in HOMA-IR, which is similar to the previous studies.[18] We also observed the improvement in β- cell function. This may be due to reduction in IR. Met-SR therapy also showed reduction testosterone, increase SHBG levels, ovulatory frequency and menstrual irregularities, which is similar to other studies.[19,20]

In the present study we have confirmed that, women with PCOS showed correlation between IR and BMI before and after therapy, and also observed a positive correlation between SHBG and TT with HOMA-IR after six months of Met-SR therapy.

CONCLUSIONS

Six months of Met-SR therapy seems to be effective in decreasing BMI, FBS, FI IR, SHBG and FAI. All function together these may lead to improvement in the menstrual irregularities and hyperandrogenism in normoglycemic PCOS women.

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