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The effect of metformin on serum lipoprotein (a) levels and insulin resistance in South Indian women with polycystic ovary syndrome

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Abstract
Aim: The study was done to determine the effect of metformin on lipoprotein (a) levels and insulin resistance in south Indian women with polycystic ovary syndrome.
Materials and Methods: A total number of 105 PCOS cases and 95 controls were recruited for the study. Base- line Parameters such as anthropometric, reproductive hormones, insulin resistance markers, and Lipoprotein (a) levels were measured by standard methods. All 104 PCOS cases received metformin 1500 mg/day for 8 weeks. At the end of 8 week treatment, insulin resistance was measured as HOMA-IR and serum lipoprotein (a) was measured by Immunoturbidimetric method by autoanalyzer.
Results: The results showed that there was significantly decreased in serum lipoprotein (a) levels ($P<0.05$) and Insulin resistance ($P<0.01$) by Metformin treatment.
Conclusion: The present study showed that Metformin treatment may cause the prevention of cardiovascular risk in south Indian women with PCOS by lowering Lipoprotein (a) levels and Insulin resistance.

Keywords: Polycystic ovary syndrome, lipoprotein (a), insulin resistance, metformin

Introduction
Polycystic Ovary Syndrome (PCOS) is a heterogeneous multifactorial and Polygenic disorder, prevalence between 5-10% in reproductive age with unknown etiology and one of the leading causes of anovulatory infertility [1]. Women with PCOS are at increased risk for a variety of health conditions such as metabolic syndrome, cardiovascular diseases and Type II diabetes mellitus. Insulin resistance (IR) is key features in the development of each of these diseases and occurring in 50-70% of the PCOS patient [2]. Many of these woman are also obese, which further exacerbated their IR. Consistent with high prevalence IR and obesity, PCOS patient demonstrate a greater prevalence of IG T, Type II DM, dyslipidemia and chronic subclinical inflammation [3]. IR occurs in both lean and obese women with PCOS. In contract, in women without PCOS insulin resistance occurs primarily in the obese individual. This suggests that IR is an intrinsic part of the disorder [4]. Insulin resistance, hyperandrogenism and dyslipidemia are likely to be the major risk factors for cardiovascular diseases in PCOS patient [5, 6].
Actually, lipid abnormalities have been reported in up to 70% of PCOS patients and displayed different patterns depending on several factors such as PCOS phenotype, obesity, associated effects of IR and hyperandrogenism that combine with environment (diet, physical exercise) and genetic factors [7, 8, 9]. Lipoprotein (a) is a circulating lipoprotein composed of liver-derived Apo (a) covalently bound to Apo-B. Lp (a) is a modified form of LDL in which Apo-A-1 is bound to Apo- B and metabolically distinct from LDL and its levels are determined genetically. Higher plasma concentration of both Lp (a) and LDL- cholesterol was associated with increased cardiovascular risk [10]. Lp(a) is a dyslipidemic marker and independent risk factor for cardiovascular events, linked to an increased risk of myocardial infarction, stroke and coronary heart disease [11].
As per survey conducted by metropolis health care [a multinational chain of pathology laboratories) about 18% women in India are affected by PCOS [12].

For this reason on early diagnosis and proper management PCOS patient is very essential. Therefore early screening of lipoprotein (a) levels and Insulin resistance can help to prevent cardiovascular risk and associated diseases in PCOS patients. Since these patients have insulin resistance, metformin, a biguanide can be used to reduce insulin resistance. Based on the above observation, the aim of present study was undertaken to evaluate effect of metformin on lipoprotein (a) levels and IR in South Indian women with PCOS.

Materials and Methods

One hundred and four patients with PCOS aged between 20 to 35 years were recruited from outpatient department of Obstetrics and Gynecology, Jawaharlal Institute of Post Graduate Medical Education and Research (JIPMER) Puducherry, India. The control group consisted of ninety five healthy volunteer females with regular menstrual cycles aged between 20 to 35 years. The diagnosis of PCOS was made according to the ESHRE/ASRM-sponsored PCOS consensus workshop group guidelines.

The study was approved by Institute Research Council Board and followed by Human Ethical Committee. JIPMER, Puducherry, India. The written informed consent was obtained from patients and controls.

Patients with diabetes mellitus, thyroid dysfunctions, Cushing's syndrome, congenital adrenal hyperplasia, hyperprolactinemia, androgen secreting tumor, renal and liver dysfunction were excluded from the study by specific laboratory tests. Subjects with medication like ovulation induction agents, anti-androgens, antidiabetic, antiobesity, hormonal drugs and current or previous use of OC within last 6 months, smoking and alcohol intake were also excluded from the study.

All 104 PCOS patients and 95 healthy control underwent full physical examination and anthropometric measurements including weight, height and, waist and hip circumferences and were asked to complete a general questionnaire. Weight was measured with the subjects wearing right clothing without shoes, and height was measured using a stadiometer. Body Mass Index (BMI) was calculated by using the formula: weight (Kg)/height (meters). Waist circumferences (WC) were measured with the patients standing at a point mid way between lower costal margin, and iliac crest in the mid-auxiliary line. Hip circumferences were measured at the widest point over the buttocks. The presence and extent of hirsutism was quantified using the Ferrinman-Gallwey (F-G) score.

After overnight fasting, venous blood sample was obtained between 08:00 am and 08.30 am, on the 2nd day of spontaneous progesterone (medroxyprogesterone acetate 10 mg/day for 7 days) induced withdrawal bleeding for (to carry out the study during the follicular phase we had to induce the menstruation in PCOS women because they were having irregular menstrual cycles) estimation of hormones, IR marker indices and Lipoprotein (a) levels. The plasma glucose was determined by glucose oxidase-peroxidase, end point method using a commercial kit (Agape diagnostic, India) using clinical chemistry Auto analyzer (Beckman Coulter AU680, Japan).

Serum LH, FSH, insulin were determined by two-site sandwich immunoassay Chemiluminescence method by Siemens Advia Centaur CP analyzer. Serum Testosterone, Androstenedione, Progesterone, Estradiol, DHEAS were analyzed by competitive immunoassay of Chemiluminescence method by Siemens Advia Centaur CP analyzer. The SHBG was measured by competitive immunoassay of Chemiluminescence method by chemi analyzer. The Plasma Lipoprotein (a) was estimated by Immunoturbidimetric method by using commercial kit [Transasia Bio-Medical Limited, India] by autoanalyzer [Bayer Express Plus-USA]. IR was determined by Homeostasis Model Assessment for insulin resistance (HOMA-IR) = Fasting glucose (mg/dl)x fasting insulin (μIU/ml)/405.

All 104 PCOS Patients received metformin 1500 mg/day (C.I Laboratories, Calcutta-700653) along with food 8 weeks. Patients were instructed not to modify their eating habits, Lifestyles and Active level during this study. At the end of 8 weeks treatment blood sample were collected and lipoprotein (a) levels and insulin resistance were performed like pre-metformin treatment.

Statistical Analysis

All the statistical analysis was carried out using SPSS (Chicago, IL, USA) software version 16.0 for Microsoft windows. All data were presented as mean ± standard, deviation. The paired't' test was used to compare the parameters of control and cases. Independent't' test was used to compare the parameters between control and cases. Paired 't' test was used compared the parameters before and after metformin treatment. Statistical significance was considered as $P<0.05$

Results

Table 1: Anthropometrics parameters in control and PCOS Cases.

Variables	Control (n = 95)	PCOS (n = 104)
Age (years)	27±4	27.33±3.30 NS
Weight (Kg)	57±12	68±4**
Height (Cm)	1.59±0.05	1.58±3.38 NS
BMI (Kg / m^2)	22.08±1.75	27.39±1.45**
Waist circumference (Cm)	75±4	89±3*
Hip circumference (Cm)	91±5	104±4*
Waist-hip ratio	0.82±0.02	0.85±0.01*

Values on shown in mean ± standard deviation
* $p<0.05$ and ** $p<0.01$ compared to controls
NS = Not significant

Table -1 shows Anthropometric Data in control and PCOS cases. In this study all women were in the age group of 25-35 years for both cases and controls. Age distribution was almost similar for both cases and control. The average age was found to be 27 years with standard deviation of 4 years in control group and average age of women with PCOS was found to be 27 years with standard deviation of 3.3 years. t test indicates age-wise both groups were similar. The BMI was found to be more in PCOS patients and statistically significant when compared the control group ($p<0.01$). The waist hip ratio was more in PCOS cases and statistically significant when compared to the control subjects ($p<0.05$).

Table 2: Hormonal profile in control and PCOS cases

Variables	Control (n = 95)	PCOS (n = 104)
LH (μIU/ml)	5.98±1.03	13.10±7.00**
FSH (μIU/ml)	5.74±1.16	4.61±1.85*
LH / FSH	1.05±0.11	2.83±0.76*
TT (ng/dl)	36.60±8.15	63.98±16.65**
Androstenedione (ng/dl)	1.47±0.45	3.64±0.87**
Progesterone (ng/dl)	0.45±0.40	0.61±0.39*
Estradiol (pg/ml)	58.92±17.21	39.03±11.51*
SHBG (nmol/L)	62.39±8.35	42.98±11.44*
DHEAS (μg/dl)	173.12±44.72	262.64±72.33**

Values are shown in mean ± standard deviation
* $p<0.05$ and ** $p<0.01$
NS = Not significant

Table 2 shows hormonal profile between control and PCOS patients. The F-G score, LH, total testosterone, androstenedione were significantly higher in PCOS patients than controls ($p<0.01$) and similarly LH/FSH ratio and progesterone, DHEAS were also significantly higher in women with PCOS than healthy controls ($p<0.05$). The hormone FSH, estradiol and SHBG were lowered significantly in PCOS group than control group ($p<0.05$).

Table 3: Insulin resistance indices, Lipoprotein (a) levels in Control and PCOS cases

Variables	Control (n = 95)	PCOS (n = 104)
Fasting glucose (mg/dl)	83.76±6.67	106.64±12.56*
Fasting Insulin (μIU/ml)	14.27±2.92	35.61±5.31**
Glucose / Insulin	6.20±1.79	3.01±0.33**
HOMA-IR	2.92±0.53	9.49±2.36**
QUICKI	0.33±0.09	0.28±0.09*
Lipoprotein (a) (mg/dl)	17.04±4.30	24.88±7.72*

Values are shown in mean ± standard deviation
* $p<0.05$ and ** $p<0.01$ compared to controls

Table 3 depicts the insulin resistance indices and lipoprotein (a) levels in control and PCOS groups. The fasting plasma glucose and Lipoprotein (a) levels were significantly increased in PCOS cases when compared to control groups ($p<0.05$). The fasting plasma insulin and HOMA-IR were also significantly increased in PCOS patients compared to control groups ($p<0.01$). The fasting plasma glucose and insulin ratio was significantly decreased in PCOS group than control subjects ($p<0.01$). The QUICKI was significantly lowered in PCOS patients than in control subjects ($p<0.05$).

Table 4: Insulin resistance parameters, Lipoprotein (a) levels in PCOS cases (Before and after metformin treatment).

Variables	PCOS (n = 104) (Before Treatment)	PCOS (n = 104) (After Treatment)
Fasting glucose (mg/dl)	106.64±12.56	80.31±11.44*
Fasting Insulin(μIU/ml)	35.61±5.31	21.18±8.87*
Glucose / Insulin	3.01±0.33	4.48±1.91*
HOMA-IR	9.49±2.36	4.16±1.73*
QUICKI	0.28±0.09	0.31±0.02NS
Lipoprotein (a) (mg/dl)	24.88±7.72	21.17±4.44*

Values are shown in mean ± standard deviation
* $p<0.05$ compared to before treatment
NS = Not significant

Table-4 depicts the insulin resistance parameters and lipoprotein (a) levels in PCOS patients before and after treatment with metformin. The fasting glucose, fasting insulin, HOMA-IR and Lipoprotein (a) levels were significantly lowered ($p<0.05$) and fasting glucose and insulin ratio was significantly increased ($p<0.05$) in PCOS patients by metformin treatment. However QUICKI was not significantly altered.

Discussion

Polycystic ovary syndrome is an common endocrine disorder and associated with a range of reproductive, obstetric, metabolic and psychological features. Reproductive and obstetric manifestations include hyperandrogenism, menstrual dysfunction, infertility and pregnancy complications [13]. Since PCOS is associated with metabolic syndrome, they are more prevalence for the development of CVD [14]. Hyperinsulinemia is a predictor of CAD and IR has been proposed as a key factor linking to hypertension, glucose intolerance, obesity, lipid abnormalities and coronary heart disease [15].

A number of studies have been published investigating the effect of metformin on IR in women with PCOS. The first study was by Velazquez EM *et al*., (1994) in USA found that metformin treatment in 26 obese PCOS women with mean BMI of 29 Kg/m^2 for 8 weeks showed that reduced hyperinsulinemia, systolic blood pressure, and improved hormonal and reproductive abnormalities [16]. These results are in agreement with the reported findings by Diamanti-Kandarakis E *et al*., (1998). However, these findings are in contrast with two other studies which have shown no change in insulin sensitivity with Metformin treatment in PCOS patients [17, 18]. A reduction in fasting glucose and insulin levels after treatment with metformin was reported by investigators in both obese [19] and non-obese [20].

In a meta-analysis of 13 controlled studies, in different ethnic populations, it was confirmed that metformin has a significant effect in reducing fasting insulin levels, associated with a small reduction of fasting glucose [21]. It was shown that in patient with PCOS and treatment with 1500mg of metformin for 8 weeks decreases the levels of serum insulin [22, 23]. Recently, two well designed studies revealed that metformin therapy significantly reduce insulin resistant in PCOS patients [24, 25].

Not all studies have shown consistent improvement of insulin sensitivity with metformin therapy. Possible explanation for this discrepancy may be variability in the doses of metformin and the effect of metformin on BMI. Our study also indicated that the fasting glucose and fasting insulin and HOMA-IR were reduced and QUICKI and glucose / insulin ratio were increased with metformin therapy and consistent with reports like Velazquez *et al*., (1994), De leo V *et al.,* (1999) and Awartari KI *et al*., (2002).

The possible mechanism for metformin action for lowering insulin resistance by inhibiting hepatic glucose production by gluconeogenesis by direct inhibition of gluconeogenic enzymes, decrease intestinal glucose uptake and increase insulin sensitivity in peripheral tissues. [26]. Metformin has antilipolytic effects and lower circulating free fatty acids concentration, which ultimately reduce the gluconeogenesis and IR [27, 28]. Metformin likely plays its role in improving ovulation induction in women with PCOS through a variety of actions, including reducing insulin level and alter in the effect of insulin on ovarian androgen biosynthesis, theca cell proliferation and endometrial growth [29]. Metformin has

been shown to inhibit GnRH release by activation of hypothalamic AMPK pathway both in vivo and *in vitro* [30].

The Women with PCOS had higher concentration of lipoprotein (a) than age matched control groups [31, 5]. In the present study also shown that lipoprotein (a) levels significantly elevated in PCOS patients than control subjects. Our result demonstrated that higher lipoprotein (a) levels and insulin resistance in women with PCOS is associated central fat excess and high-risk for developing CVD.

In addition, recently Swetha R *et al*., (2015) shown that there was a elevated lipoprotein (a) level in PCOS patients than controls [32]. However, one study shown that there was no significant differences in lipoprotein (a) concentration between the PCOS and control groups [33]. Although insulin resistance could lead to increased level of lipoprotein (a) in PCOS patients [34], however the exact molecular mechanism of action is not completely understood.

In the present study, women with PCOS treated with metformin; there was a reduced level of lipoprotein (a). At present no adequate studies have been carried out till date the effect of metformin in PCOS patient. However there are two studies reported that a significant reduction of lipoprotein (a) levels in PCOS cases by Metformin treatment. [35, 36]. A decrease in insulin resistance by metformin treatment may reduce the lipoprotein (a) levels in PCOS patients in our study. However the mechanism of metformin's effects on lipoprotein (a) are unknown.

Conclusion

In the present study shown that IR and lipoprotein (a) levels were increased in South Indian women with PCOS than healthy controls and treatment with metformin in PCOS, there was significant decrease of IR and lipoprotein (a) levels. This is suggesting that metformin is associated with reduced cardiovascular risk in these patients. However long-term prospective studies are needed to investigate the exact role of metformin in PCOS patients in future.

Conflict of Interest

The authors confirm that this research article content has no conflict of interest.

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