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Effect of six-month use of oral contraceptive pills on plasminogen activator inhibitor-1 & factor VIII among women with polycystic ovary syndrome: An observational pilot study

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***Background & objectives*: Polycystic ovary syndrome (PCOS) is an endocrinopathy warranting lifelong individualized management by lifestyle and pharmacological agents mainly oral contraceptive pills (OCPs). This study was aimed to report the impact of six-month OCP use on plasminogen activator inhibitor-1 (PAI-1) and factor VIII (FVIII) in women with PCOS.**

***Methods*: PCOS women diagnosed on the basis of Rotterdam 2003 criteria, either treated with OCPs (ethinyl estradiol-0.03 mg, levonorgestrel-0.15 mg) for a period of six months (n=40) or drug-naïve (n=42), were enrolled in this study. Blood was drawn to estimate glucose, insulin levels and lipid profile. Chemiluminescence immunoassays were used to measure hormones (LH, FSH, PRL, T_4). Plasma levels of PAI-I and FVIII were measured by commercially available kits.**

***Results*: Menstrual regularity, Ferriman-Gallwey score and serum total testosterone significantly improved in the OCP group compared to drug-naïve group (P<0.01). No significant difference was observed in PAI-1 levels of the two groups; however, significant decrease in FVIII levels was observed in OCP group as compared to drug-naïve group. PAI-1 levels of OCP group correlated positively with blood glucose two hours, triglycerides and insulin two hours, while FVIII levels of OCP group correlated negatively with fasting insulin and homoeostatic model assessment-insulin resistance.**

***Interpretation & conclusions*: OCPs use has differential effect on pro-coagulant markers among women with PCOS. Well-designed, long-term, prospective, large-scale studies are prerequisite to elucidate the efficacy and safety of OCP in the treatment of PCOS.**

Key words Factor VIII - oral contraceptive pills - plasminogen activator inhibitor-1 - polycystic ovary syndrome - pro-coagulant markers

Polycystic ovary syndrome (PCOS), is a common endocrine disorder of reproductive age women globally[1] and in India also[2]. Besides the various reproductive abnormalities (chronic anovulation and hyperandrogenism), these women are prone to develop insulin resistance (IR), compensatory

hyperinsulinaemia, dyslipidaemia, hypertension, obesity[3], metabolic syndrome (MS), type 2 diabetes mellitus (T2DM) and higher risk of cardiovascular diseases (CVD)[4]. Several reports have shown dysregulation of the haemostatic system pointing towards pro-thrombotic state, including hypofibrinolysis, hypercoagulability[5,6] and endothelial and platelet dysfunction[5,7].

Plasminogen activator inhibitor-1 (PAI-1), a key regulator of fibrinolysis, is a marker of IR as plasma PAI-1 antigen levels and activity are frequently elevated in IR states, such as abdominal obesity, MS and T2DM[8]. Given the increased prevalence of IR in women with PCOS, plasma PAI-1 levels have been assessed in women with PCOS[9-11] and in some elevated levels of PAI-1 have been reported. Factor VIII (FVIII) is a vitamin K dependent coagulation factor primarily synthesized in the liver and circulates in the plasma in a non-covalent complex with von Willebrand factor. FVIII is reported as a common risk factor for venous thrombosis, coronary artery disease and stroke[12,13], with its elevated levels being linked to higher body mass index (BMI), plasma glucose, insulin, fibrinogen and triglycerides levels[14]. There are scarce data on FVIII; it has not been extensively investigated among women with PCOS.

Oral contraceptive pills (OCPs) are considered the treatment of choice for PCOS as these agents are useful in regulating menstrual cycles and ameliorating androgenic symptoms[15]. However, OCPs have been linked to derangement in lipid and glucose metabolism in addition to venous thrombosis[16,17]. This pilot study was undertaken to compare plasma levels of PAI-I and FVIII among women with PCOS receiving OCP at least for six months with drug-naïve PCOS women.

Material & Methods

This single-contact, observational pilot study was conducted from January 2014 to March 2015 in the department of Endocrinology of Sher-i-Kashmir Institute of Medical Sciences (SKIMS), Srinagar, India. The women were required to qualify Rotterdam 2003 criteria[18] for diagnosis of PCOS before enrolling into the study; therefore, of the 200 women screened during this period, only those fulfilling the inclusion criteria were selected for this study. The women were divided into two groups: OCP group (n=40); women who had received OCP (ethinyl estradiol-0.03 mg, levonorgestrel-0.15 mg) at least for 24±2 wk after the diagnosis and drug-naïve group (n=42) which included women with PCOS and were not taking any drug. The study protocol was approved by the Institutional Ethics Committee of SKIMS, Srinagar (IEC No: SIMS 131/IEC-SKIMS/2013-6479).

Clinical assessment: Consecutive women attending the clinics with complaints of oligomenorrhoea, unwanted hair growth or acne vulgaris were informed about the study and those who volunteered to be part of the study were asked to sign informed consent. Details regarding menstrual history, weight gain, unwanted hair growth, acne vulgaris, *etc.* were noted. Oligomenorrhoea was defined as number of cycles less than eight per year or interval of >35 days while amenorrhoea as cessation of menstrual cycle for six months. Anthropometry (measurement of height, weight, waist-hip ratio and blood pressure) and detailed systemic examination were done in all women. Hirsutism score was done using Ferriman-Gallwey score[19] by counting nine specified body areas. A score of >8 was taken as significant. Exclusion criteria included those with known diabetes or hypertension, Cushing's syndrome, hypothyroidism, hyperprolactinaemia and androgen-secreting tumours.

Laboratory evaluation: Blood samples for glucose and insulin were collected at 0 (4 ml), 60 (3 ml) and 120 min (2 ml) after the glucose load [oral glucose tolerance test (OGTT)] performed after 10-12 h of an overnight fasting. A fasting sample was taken for hormones [luteinizing hormone (LH), follicular-stimulating hormone (FSH), T4, thyroid-stimulating hormone (TSH), prolactin (PRL), cortisol, 17-OHP and total testosterone], lipid profile and liver and kidney function tests. The hormone estimation included 17-OHP (to rule out non-classical congenital adrenal hyperplasia), TSH and T4 (to rule out hypothyroidism), PRL (to rule out prolactinoma), cortisol (to rule out Cushing's syndrome) and total testosterone (to diagnose hyperandrogenism and to rule out androgen-secreting ovarian or adrenal tumours).

Assays: Glucose (mg/dl) was measured by glucose oxidase peroxidise method (DiaSys Diagnostic Systems, Germany). Plasma insulin was measured by electrochemiluminescence (Cobase411, Roche Diagnostics Limited, Germany), using commercially available kits. Lipid parameters such as cholesterol, triglycerides, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) were measured by colorimetric method (DiaSys Diagnostic Systems, Germany). Chemiluminescence immunoassay (ECLIA, Meditron, USA) was used to analyze LH, FSH, PRL, T4, TSH, cortisol and total testosterone using commercial

kits (BECKMAN COULTER uicel DXI 800, USA) in duplicate. ELISA was used to analyse 17-OHP using commercial kits (Diagnostics Biochem, Canada). Plasma levels of PAI were measured in duplicate using ELISA kit (SYMANSIS, New Zealand). FVIII in the plasma was estimated by coagulometric method using commercially available kit (SIEMENS, USA).

IR was estimated using homoeostatic model assessment-IR (HOMA-IR) which was calculated as product of fasting insulin value (µIU/ml) and the fasting plasma glucose value (mg/dl) divided by 405[20]. Quantitative insulin sensitivity check index (QUICKI) was used to estimate insulin sensitivity and was calculated as 1/log fasting insulin (µIU/ml)+log fasting glucose (mg/dl)[21]. Fasting glucose and insulin ratio (FGIR) was calculated as fasting plasma glucose (mg/dl)/fasting insulin (µIU/ml)[22].

Statistical analysis: Statistical analysis was done using SPSS 20 software (SPSS 20, IBM, Armonk, NY, USA). Various parameters such as anthropometry, basic biochemical, hormonal and insulin measures were compared between cases and controls using two-sampled *t* test. Pearson correlation coefficient (r) was used to analyze the association between study variables.

Table I. Comparison of various clinical and biochemical parameters of drug-naïve group and oral contraceptive pill group

Parameters	OCP group (n=40)	Drug-naïve group (n=42)
Mean age (yr)	21.69±4.23	22.01±5.04
Number of cycles/yr	11.70±2.93**	7.33±3.90
Ferriman-Gallwey score	9.12±6.47	11.29±7.33
Weight (kg)	60.30±6.7	59.92±8.28
WHR	0.92±0.08	0.94±0.09
BMI (kg/m^2)	24.30±3.56	23.81±3.11
Systolic BP (mmHg)	124.89±7.37	122.44±6.99
Diastolic BP (mmHg)	83.6±4.99	81.9±6.62
Serum total cholesterol (mg/dl)	189.87±38.43**	156.48±26.17
Serum triglycerides (mg/dl)	120±36.54	115.08±40.68
Serum HDL (mg/dl)	49.41±10.13	46.45±8.74
Serum LDL (mg/dl)	120.77±42.56**	86.21±24.99

**P<0.01 compared to drug-naïve group, values are mean±SD. WHR, waist-hip ratio; BMI, body mass index; BP, blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; OCP, oral contraceptive pill

Results & Discussion

Baseline characteristics of the study groups are summarized in Tables I and II. The characters such as mean age (years) and BMI (kg/m^2) were comparable among the two groups. However, significant difference was observed between number of cycles per year and serum total testosterone levels (P<0.01) between OCP and drug-naïve group. Serum total cholesterol, LDL cholesterol, insulin two hours (P<0.01) and QUICKI (P<0.05) were significantly higher in the OCP group compared to drug-naïve group. The difference in plasma PAI-1 levels was insignificant between the OCP-treated and drug-naïve participants. However, plasma FVIII level was significantly lower in the OCP-treated group compared to drug-naïve women (P<0.01). PAI-1 in OCP group showed significant positive correlation with blood glucose two hours (r=0.26, P=0.02), insulin two hours (r=0.28, P=0.03) and serum triglyceride level (r=0.24, P=0.01). However, FVIII levels showed significant negative correlation with fasting insulin (r=−0.34, P=0.02) and HOMA-IR (r=−0.32, P=0.030).

Women with PCOS are characterized by increased levels of pro-coagulant markers such as fibrinogen and PAI-1, which promote atherogenic processes and increase the risk of CVD in these women[23]. Several studies have reported higher PAI-1 levels in women with PCOS which correlated positively with components of IR[9,10]. Furthermore, evidence suggests that OCP use may further worsen coagulation state and risk for CVD[24,25].

There are limited studies evaluating the effect of OCPs on plasma PAI-1 levels, and the results remain controversial rather than conclusive[26-28]. In the current study, an insignificant increase in plasma PAI-1 levels was observed with six months of OCP use. However, PAI-1 levels of OCP group correlated positively with markers of MS and IR. Previous data suggest that OCP use may decrease[26,27] or have no effect[28] on PAI-1 levels. Teede *et al*[26] reported that additional decrease in PAI-1 levels occurred with the use of OCP containing cytoproterone acetate in women with PCOS. Similar to our results, Küçük *et al*[29] reported an insignificant difference in PAI-1 levels between women taking OCPs containing levonorgestrel and control women who had not received OCPs although this study was conducted among non-PCOS women.

Table II. Comparison of oral glucose tolerance test derived insulin resistance parameters, gonadotropins, total testosterone and pro-coagulant markers between drug-naïve group and oral contraceptive pill group

Parameters	OCP group (n=40)	Drug-naïve group (n=42)
Blood glucose - fasting (mg/dl)	89.57±10.28	88.85±16.91
Blood glucose - 2 h (mg/dl)	115.87±26.31	110.77±38.31
Serum insulin - fasting (µIU/ml)	18.33±24.75	13.18±10.19
Serum insulin - 2 h (µIU/ml)	65.64±54.53*	45.63±32.55
FGIR	6.74±5.84	4.88±10.30
HOMA-IR	2.89±3.04	4.05±3.99
QUICKI	0.31±0.02**	0.32±0.01
Serum LH (IU/l)	6.94±4.59	7.73±5.54
Serum FSH (IU/l)	6.23±3.32	6.16±2.00
Serum total testosterone (ng/dl)	53.57±17.03**	67.35±29.47
Plasma PAI-1 (ng/ml)	1.28±0.62	1.15±0.45
Plasma factor VIII (%)	0.57±0.33**	0.78±0.28

P<0.05, **<0.01 compared to drug-naïve group. OGTT, oral glucose tolerance test; FGIR, fasting glucose insulin ratio; QUICKI, quantitative insulin sensitivity index; LH, luteinizing hormone; FSH, follicular-stimulating hormone; PAI-1, plasminogen activator inhabitor-1; HOMA-IR, homoeostatic model assessment-IR; IR, insulin resistance

FVIII level is known to increase the pro-coagulant activity and use of OCPs further elevates FVIII levels[30]. A significant decrease in plasma FVIII levels was observed with the use of OCP containing ethinyl estradiol and levonorgestrel compared to drug-naïve PCOS patients and FVIII of OCP group correlated negatively with markers of IR in the present study. A study has reported that OCPs seem to have no effect on FVIII levels[31]. Women with OCP intake showed increase in fibrinogen and FX and a reduction in anti-thrombin III levels when compared with their control values[28]. This group also reported small but significant increases in triglycerides and triglyceride-rich lipoproteins[28].

In conclusion, results of this pilot study may not be conclusive owing to several limitations such as lack of baseline data in the OCP group, smaller number of subjects and no control group with longitudinal follow up. Despite these limitations, the data may pave way for well-designed, randomized, longitudinal studies with large cohort among women with PCOS.

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***Conflicts of Interest*:** None.

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