Doppler flow velocities of uterine and ovarian arteries & hormonal patterns in patients with Polycystic Ovary Syndrome (PCOS)

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Abstract:

Introduction: Polycystic ovary syndrome is a complex disorder characterized by elevated androgens, chronic anovulation, and polycystic picture on ultrasound it is one of the most common hormonal disorders, affecting 5%–10% of women of reproductive age. Despite many diagnostic criteria, controversies still exist in definition, evaluation and management of PCOS.

Aims and Objective: To investigate blood flow velocities in the ovarian stromal artery and uterine artery in women with Polycystic ovary syndrome (PCOS) and to correlate these velocities with clinical, biochemical and hormonal parameters.

Methods: This prospective case control study analyzed 35 consecutive patients with PCOS (cases) and 35 age-matched women without PCOS (controls).

Results: The women with PCOS had a significantly higher Weight, BMI, WHR, LH/FHS ratio, ovarian volume, and follicles in periphery of ovary and hirsutism score compared with women in the control group. The mean values of PI of ovarian stromal artery was 0.96±0.19 in cases and 2.6±0.26 in controls and the mean values of uterine artery PI is 2.8±0.41 in cases and 2±0.27 in controls (p<.05). On correlating these velocities with clinical, biochemical and hormonal parameters there was inverse correlation ovarian stromal artery PI with DHEA-S and LH. Also, there was positive significant correlation of uterine artery PI with DHEA-S.

Conclusion: Results from the study indicates that transvaginal color Doppler evaluation of uterine and intraovarian arteries can be added to the traditional endocrinologic and ultrasonographic parameters clinically used for the diagnosis of polycystic ovary syndrome.

Keywords: Doppler Flow velocities, Ovarian, Uterine, Hormonal, PCOS
INTRODUCTION

Polycystic ovary syndrome (PCOS) is one of the most common hormonal disorders, affecting 5%–10% of women of reproductive age. It is characterized by chronic anovulation, hyperandrogenism, insulin resistance, obesity, infertility and an increased risk of spontaneous abortion. Despite its prevalence and the vast amount of clinical, laboratory, and experimental data published, the etiologic mechanisms behind PCOS are not fully understood owing to the diversity of clinical and biochemical characteristics. The diagnosis of PCOS may be diagnosed on the basis of 2 of the following 3 criteria: (i) Oligo and/or anovulation, (ii) Clinical and/or biochemical signs of hyperandrogenism and (iii) Ultrasonographic PCO, after the exclusion of other pathologies having a similar clinical presentation.

According to the Rotterdam consensus definition, one of the following two criteria has to be met for the diagnosis of polycystic ovary: either 12 or more follicles measuring 2–9 mm in diameter or increased ovarian volume (>10 cm³). The presence of a single polycystic ovary (PCO) is sufficient to provide the diagnosis.

Patients with polycystic ovaries tend to have ovarian stromal velocities that are significantly higher than normal ovaries. It is also known that they are at higher risk of ovarian hyperstimulation syndrome, particularly following IVF treatment. Abnormal uterine blood flow has been observed in women with PCOS as well as in the subfertile population in general and in women with idiopathic recurrent miscarriage. Uterine blood flow in women with PCOS may be associated with the phenotypic expression of the disease and it has been suggested to be reduced in women with PCOS who have hyperandrogenism or are obese. This study was aimed to investigate blood flow velocities in the ovarian stromal artery and uterine artery in women with Polycystic ovary syndrome (PCOS) and correlate these velocities with clinical, biochemical and hormonal parameters.

MATERIAL AND METHODS

The present prospective Case control Study was conducted in the Department of Obstetrics and Gynaecology in collaboration with Department of Radiodiagnosis, Vardhman Mahavir Medical College and Safdarjung Hospital, New Delhi in 35 consecutive patients with PCOS and 35 age-matched women without PCOS who participated as controls. All the patients were recruited from the outpatient clinics of the hospital during September 2009 and December 2010. Written informed consent was obtained from all of the women before enrollment in the study. Patient's sample size was used by applying an alpha error of 5%, a power of study 80%, the fact that the prevalence of disease is 5-10% and using values in similar studies from literature, a sample size of 35 was calculated. All the enrolled patients of PCOS was made according to the European Society for Human Reproduction and Embryology and the American Society for Reproductive Medicine (ESHRE/ASRM) criteria\(^1\,^2\) PCOS was diagnosed when 2 of the following
3 features were present: oligo- and/or anovulation, clinical and/or biochemical signs of hyperandrogenism, and polycystic ovaries on ultrasound examination (the presence of 12 or more follicles of 2–9 mm in diameter and/or ovarian volume greater than 10 cm$^3$).

Controls: The control group are those having complains other than menstrual problems, not having any signs or symptoms of hyperandrogenemia. The health of the control group was determined on the basis of medical history, physical and pelvic examinations, blood chemistry, and pelvic ultrasound. None of the women in the control group had signs or symptoms according to the ESHRE/ASRM criteria.

For at least 6 months all participants before entering the study, had not taken any medication that could affect the biochemical profile or metabolic variables. The women in the control group had regular, normal menstruation (days 25–35).

METHODOLOGY
Detailed history from all participants was taken including Identification data, Reproductive history, Menstrual history, History of weight gain, History of thyroid disease, History of galactorrhea and any other significant complains.

General physical examination, Height (m), Weight (kg), Weight-Hip-Ratio: Waist-to-hip ratio, Body mass index (kg/m$^2$), Hirsutism score$^3$. Systemic examination of all systems and Pelvic examination was done.

BLOOD CHEMISTRY
Serum and plasma samples were collected from patients after an overnight fast of at least 12 hours during the early follicular phase of the menstrual cycle or on random days in amenorrhoic patients. All the parameters such as luteinising hormone, follicle-stimulating hormone, total testosterone, dehydroepiandrosterone - sulphate, Insulin Postprandial, Glucose fasting and postprandial Glucose-oxidase, peroxidase method using auto-analyser, Total cholesterol, High-density lipoprotein cholesterol, Low-density lipoprotein cholesterol, Triglycerides were measured by enzymatic colorimetric assay.

ULTRASOUND PARAMETERS
Ultrasound and Doppler analyses were performed during the follicular phase of the menstrual cycle (between the third and fifth day) using a 6.5 MHz vaginal transducer (HDI 4000) equipped with color and pulsed Doppler. All patients were evaluated at the same time of day to avoid fluctuations of the uterine artery blood flow due to the circadian rhythm. A 50-Hz filter was used to eliminate low-frequency signals originating from blood vessel wall movements. Ovarian volume was calculated using the formula for an ellipse (length $\times$ width $\times$ height $\times$ 0.523). Number of follicles of size 2-9mm in the periphery of ovary was measured. Any follicle of size >9 is not counted in calculation.

When several blood vessels were detected inside the ovarian stroma (except for those close to the surface of the ovary or near the wall of the follicle), one of the blood vessels was selected for pulsed Doppler measurements.

Uterine artery blood flow velocities were analyzed from the ascending branches of both main arteries at the level of the internal
cervical os in a longitudinal plane. At least 3 satisfactory blood flow velocity waveforms were obtained and used for statistical analysis of the average from 3 waveforms. The angle of insonation was always changed to obtain maximum color intensity. When good color signals were obtained, blood flow velocity wave forms were recorded by placing the sample volume across the vessel and entering the pulsed Doppler mode. No significant differences between the pulsatility index (PIs) of the left and right uterine and ovarian stromal arteries were observed, and therefore the average value of both was taken.

The pulsatility index (PI), defined as the difference between peak-systolic and end-diastolic flow divided by the mean maximum flow velocity was determined using calculation software.

STATISTICAL ANALYSIS

At the end of the study, the data was analysed by using Student ‘t’ test, Chi-square test, Fisher exact test and Mann Whitney tests were used wherever applicable, p value <0.05 was considered statistically significant. Analysis was done by using SPSS version 15.0.

RESULTS

The mean age of the women in the PCOS and control group was not significantly different (23.29 ± 3.33 vs 23.00 ± 3.07 years, respectively; p <0.05). Various clinical parameters such as weight (kg), Height (mt), BMI (kg/m²), WHR were taken into account and analysed between cases and controls and found to be high and statistically significant in PCOS patients (p <0.05). Height was not statistically different in cases and controls (p >0.5). In our study, 48.6% of participants were overweight in cases as compared to 2.9% in controls (p <0.05).

MODIFIED FERRIMAN-GALLWEY SCORE

Hirsutism Score was calculated with Modified Ferrimen-Gallwey Score. A score of more than 6 was taken as presence of hirsutism. Mean value of cases was 7.89±1.694 and in controls it was 2.69±1.38. Statistically, Hirsutism score was high and significant in cases in comparison to controls (p < 0.05) when comparison between cases and control was made score of more than six was present in 74.3% of cases and none in controls which was also found to be statistically significant.

MENSTRUAL HISTORY AND HISTORY OF WEIGHT GAIN

History of oligomenorrhea and amenorrhea was present in 94.33% in cases and none in controls and history of weight gain was present in 54.3% of cases which was statistically significant.

BIOCHEMICAL PARAMETERS

Mean FSH in cases was 7.371±5.30 and in controls, it was 9.24±4.57, LH was 9.53±7.11 and 7.82±5.64, LH/FSH was 1.39±0.76 and 1.03±1.06, prolactin was 11.34±6.90 and 11.62±4.87, respectively. The values of levels of FSH, LH and Prolactin were not statistically different in cases and controls. The mean values of LH/FSH ratio in cases was 1.3 and in controls is 1.03 which was statistically significant (p <0.05).

The mean values of Fasting and Postprandial Blood Sugars in cases was 93.51±8.96 and 106.40±9.83, respectively. Similarly, in controls it was 90.97±7.02 and 101.57±8.75, respectively. Comparison of postprandial
blood sugars were statistically higher in cases than in controls but less than the range for diabetic population cut-off value (fasting >126mg/dl, postprandial >200mg/dl) as per American diabetic association criteria. Mean serum insulin (PP) in cases was 5.91±6.15, testosterone was 1.41±0.71 and DHEAS was 2.32±0.91 and in controls, it was 2.82±2.36, 0.703±0.32 and 1.89±2.25 respectively. The normal range of insulin for our hospital 0-25uIU/ml but none participants from the cases and controls had serum insulin values more than 25uIU/ml.

**BIOCHEMICALHYPERANDROGENAE MIA**

The normal range of total serum testosterone in female population in our hospital is 0.1-1.2 ng/ml and DHEAS is 0.9-3.6 ug/ml. The mean value of DHEAS in cases was 2.3±.91 and 1.8±2.25 in controls (p < .05). But none of PCOS case had value >3.6ug/ml which is normal cut off for females in our hospital. The mean value of total serum testosterone in cases was 1.4±.71 and 0.70±.32 in controls which was statistically significant (p < .05). Eighteen patients had testosterone more than 1.2ng/ml i.e. biochemical hyperandrogenemia as compared to only 3 in controls which was statistically significant (P<.05). None of the variables of lipid profile were statistically different in cases and controls.

**ULTRASONOLOGICAL PARAMETERES**

The mean value of follicle number in periphery of ovary was 11.74±2.38 in cases but in controls it was 4.06±1.79. The mean value of ovarian volume was 11.81±1.42 but in controls it was 5.76±1.06 (p<.05). The number of patients in cases group having follicle number more than 12 which was one of the ultrasonographic parameter for polycystic ovary was 14 (40%) which found to be statistically significant (p<.05) but in controls none as it was one of the exclusion criteria. The number of patients in cases having ovarian volume >10 cm³ which was one of the ultrasonographic criteria for polycystic ovaries was 30 (85.7%) which was found to be statistically significant (p<.05) whereas none in control group which was one of exclusion criteria.

**DOPPLER ANALYSIS OF OVARIAN STROMAL ARTERY AND UTERINE ARTERY**

**Pulsatility Indexes of Ovarian Stromal Artery and Uterine Artery**

No significant differences between the pulsatility index (PIs) of the left and right uterine and ovarian stromal arteries were observed, and therefore the average value of both was taken (Tables 1 & 2). The mean values of PI of ovarian stromal artery was 0.96±0.19 in cases and 2.6±0.26 in controls and the mean values of uterine artery PI was 2.8±0.41 in cases and 2±0.27 in controls. PI of ovarian stromal artery was significantly low in cases as compared to controls (p<.05) and uterine artery PI was high in cases as compared to controls (p<.05).

Correlating the ovarian stromal artery pulsatility indexes with rest of parameters there was positive and significant correlation with LH, LDL in cases and negative and significant correlation with DHEAS i.e. ovarian blood flow is directly proportional to LH and LDL and inversely proportional to
DHEAS in cases. On correlating uterine artery there was positive and significant correlation with DHEAS in cases i.e. uterine artery blood flow is directly proportional to DHEAS. There was positive correlation of postprandial blood sugar with ovarian PI in controls, positive correlation of LDL with ovarian PI in cases and negative correlation with LDL in controls (Table 3).

**DISCUSSION**

Doppler ultrasound has been used for several years to study the pattern of blood flow in fetal and maternal vessels. Recently, color Doppler imaging had been used to diagnose various gynaecological disorders. Transvaginal color flow Doppler gives us an accurate tool to study the female reproductive system. The reduced distance between the probe and the pelvic structures supplies better resolution. Transvaginal color Doppler imaging facilitates the detection of small vessels in the utero-ovarian circulation and measurement of impedance to flow in this vascular tree.

In the present study, the women with PCOS had a significantly higher Weight, BMI, WHR, LH/FHS ratio, ovarian volume, and follicles in periphery of ovary and hirsutism score compared with women in the control group. The impedance in the blood flow of the uterine artery was significantly higher in women with PCOS, i.e. the uterine PI was significantly higher in PCOS group (2.8±±.41 in cases and 2±±.27 in controls) as previously reported by Battaglia et al. 1995\(^4\) (Uterine PI 3.58±±.34 in hirsute PCOS, 2.77±±.45 in non-hirsute PCOS patients); Adali et al. 2009\(^5\) (Uterine PI, 4.88±±0.96 in PCOS group and 4.11±±0.82 in controls); Aleem and Predanic 1996\(^6\) (Uterine PI 3.74±±1.01 in PCOS group and 2.43±±0.36 in controls).\(^7\)

In this study the PI of the ovarian stromal artery was significantly lower (.96±±.19 in cases and 2.6±±.26 in controls) i.e. blood flow in ovarian stromal artery was higher in patients with PCOS compared with the controls as reported by Adali et al 2009 (Ovarian PI, 1.40±±0.63 in cases and 2.90±±0.20 in controls)\(^5\) and Battaglia et al 1995.\(^4\) The low PI values indicate that ovarian stromal vessels are probably dilated and engorged and more abundant in the ovaries of women with PCOS (Loverro et al. 2001).\(^8\)

Zaidi et al 1995\(^9\) studied the intraovarian vasculature and found a higher intensity of color flow in the ovarian stroma of patients with PCOS. They postulated that the increased ovarian stromal blood flow velocity in combination with a relatively unchanged impedance to blood flow may reflect increased ovarian perfusion and thus a greater delivery of gonadotropins to the granulosa cells of the developing follicles. Study done by Mala, Ghosh and Tripathi 2009\(^7\); Resende et al. 2001\(^10\) did not find ovarian PI to be lower in PCOS patients i.e. ovarian blood flow is not increased in PCOS patients.

In the present study there was no correlation of BMI with uterine and ovarian artery blood flow as in similar studies by Aleem and Predanic 1996\(^6\) and Resende et al. 2001\(^10\). This is in contrast to as reported by Battaglia et al 1995\(^4\) who found elevated uterine PI among obese patients. They attributed this findings to the presence of hyperinsulinaemia among obese patients,
inducing a state of hyperandrogenism through enhancement of insulin-like growth factor 1 (IGF-1) receptor which, in synergism with LH, causes increased activity of cytochrome P-450 17-alpha (Insler et al. 1993). However, in non-obese patients with PCOS, a relative increase of growth hormone concentration stimulates excessive ovarian IGF-1 production which, in synergism with LH, results in hyperandrogenism by the same mechanism (Insler et al. 1993).

Elevated LH level and LH/FSH ratio, important pathophysiological features of PCOS, are responsible for hyperplasia of the ovarian thecal and stromal cells, and may also be responsible for the increased ovarian stromal vascularization by acting via catecholaminergic stimulation, neoangiogenesis, and leukocyte and cytokine activation. In the present study too, there was negative but significant correlation between of LH with ovarian PI i.e. raised LH levels is responsible for increased blood flow in ovarian stroma. But no relation was found of LH with uterine blood flow.

In the present study there was no correlation between the Ferrimen-Gallwey score, follicular number and ovarian volume and uterine or ovarian stromal artery PI. There was conflicting positive correlation of postprandial blood sugar with ovarian PI in controls, positive correlation of LDL with ovarian PI in cases and negative correlation with LDL in controls that could not be explained. As neither postprandial blood sugars nor LDL levels was higher than normal cut-off values in cases and controls these can be attributed as mere co-occidental findings.

Failure of implantation and spontaneous abortion commonly occur in women with PCOS (Glueck et al. 1999). In women with PCOS, the increased impedance of the uterine arteries, by reducing uterine perfusion, may interfere with implantation and increase the risk of spontaneous abortion. Moreover, increased androgen levels in women with PCOS may block the effects of estrogen at the endometrium. Thus, endometrial receptivity and implantation may be affected by the increased androgen levels and impaired uterine perfusion.

Women with PCOS have a high risk for future cardiovascular disease and pre-eclampsia, in which vascular dysfunction is involved.

It is well known that the risk of an excessive response to controlled ovarian hyperstimulation in infertile women with PCOS is higher than that in infertile women with normal ovaries. CONCLUSION

PCOS is complex endocrinopathy with wide ranging variations and clinical manifestations. Doppler flow velocities of ovarian and uterine arteries are not done in currently used many criteria. The present study concluded that there is increased ovarian stromal blood flow and decreased uterine perfusion in women with PCOS. These blood flow changes in uterine and ovarian artery may provide insight into pathophysiology of PCOS.
In conclusion, results from this study showed that transvaginal color Doppler evaluation of uterine and intraovarian arteries can be added to the traditional endocrinologic and ultrasonographic parameters clinically used for the diagnosis of polycystic ovary syndrome.

**TABLE 1**

**OVARIAN ARTERY PULSATILITY INDEX IN CASES/CONTROLS**

<table>
<thead>
<tr>
<th></th>
<th>Minimum value</th>
<th>Maximum value</th>
<th>Range</th>
<th>Mean</th>
<th>Standard deviation</th>
<th>Standard error of mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases</td>
<td>0.71</td>
<td>1.70</td>
<td>0.99</td>
<td>0.963</td>
<td>0.197</td>
<td>0.0333</td>
</tr>
<tr>
<td>Controls</td>
<td>1.99</td>
<td>3.15</td>
<td>1.16</td>
<td>2.69</td>
<td>0.269</td>
<td>0.0455</td>
</tr>
</tbody>
</table>

**TABLE 2**

**UTERINE ARTERY PULSATILITY INDEX IN CASES/CONTROLS**

<table>
<thead>
<tr>
<th></th>
<th>Minimum value</th>
<th>Maximum value</th>
<th>Range</th>
<th>Mean</th>
<th>Standard deviation</th>
<th>Standard error of mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases</td>
<td>2.1</td>
<td>3.6</td>
<td>1.5</td>
<td>2.877</td>
<td>.4144</td>
<td>0.701</td>
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<tr>
<td>Controls</td>
<td>1.4</td>
<td>2.8</td>
<td>1.4</td>
<td>2.024</td>
<td>.2770</td>
<td>1.468</td>
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</table>

**TABLE 3**

**CORRELATION OF OVARIAN STROMAL ARTERY AND UTERINE ARTERY PULSATILITY INDEXES WITH CLINICAL, HORMONAL, AND BIOCHEMICAL CHARACTERISTICS OF WOMEN WITH POLYCYSTIC OVARY SYNDROME AND WOMEN IN THE CONTROL GROUP.**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Ovarian stromal artery PI</th>
<th>Uterine artery PI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PCOS</td>
<td>Control</td>
</tr>
<tr>
<td>Weight</td>
<td>.019</td>
<td>.147</td>
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<tr>
<td>Height</td>
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<td>.022</td>
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<td>BMI</td>
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<td>WHR</td>
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<td>-.259</td>
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<td>Hirsutism Score</td>
<td>-.154</td>
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<td>Follicular number in periphery of ovary</td>
<td>-.027</td>
<td>-.019</td>
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<td>Ovarian volume, cm³</td>
<td>.046</td>
<td>.067</td>
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<tr>
<td>FSH follicle stimulating hormone (mIU/ml)</td>
<td>-.270</td>
<td>-.337</td>
</tr>
<tr>
<td>Characteristics</td>
<td>Ovarian stromal artery PI</td>
<td>Uterine artery PI</td>
</tr>
<tr>
<td>----------------------------------------</td>
<td>---------------------------</td>
<td>-------------------</td>
</tr>
<tr>
<td></td>
<td>PCOS</td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>LH lutenising hormone (mIU/ml)</td>
<td>-.347 (^a)</td>
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<td>LH/FSH ratio</td>
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<td>PROLACTIN (ng/ml)</td>
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<td>-.058</td>
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<td>Postprandial blood sugar (PP BS) mg/dl</td>
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<td>-.338 (^a)</td>
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<td>INSULIN (PP),uIU/ml</td>
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<td>Testosterone ng/ml</td>
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<td>-.183</td>
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<td>DHEAS ug/ml</td>
<td>-.427 (^a)</td>
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<td>Triglycerides(TG) mg/dl</td>
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<td>High density lipoproteins(HDL) mg/dl</td>
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<td>.114</td>
</tr>
<tr>
<td>Low density lipoproteins(LDL) mg/dl</td>
<td>.367 (^a)</td>
<td>-.323</td>
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<tr>
<td>Serum cholesterol mg/dl</td>
<td>-.126</td>
<td>.084</td>
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</table>

\(^a=p<.05\)

REFERENCES


