REPRODUCTIVE ENDOCRINOLOGY

Uterine Perfusion and Hormonal Pattern in Patients with Polycystic Ovary Syndrome

SILVIA AJOSSA,^{1,2} STEFANO GUERRIERO,¹ ANNA MARIA PAOLETTI,¹ MARISA ORRÙ,¹ STEFANO FLORIS,¹ MARINA MANNIAS,¹ and GIAN BENEDETTO MELIS¹

Submitted: October 16, 2000

Accepted: April 4, 2001

Purpose: To evaluate whether polycystic ovary syndrome (PCOS) patients with different pulsatility index (PI) of uterine artery showed differences in their hormonal pattern.

Methods: Eighty-eight PCOS-affected patients and 15 controls were submitted to Doppler flow measurement of uterine artery; LH, FSH PRL, estradiol, and androgens concentration determination; and BMI evaluation during early follicular phase.

Results: The mean PI of uterine artery of PCOS patients was significantly higher than control group (2.97 ± 0.9 vs. 1.89 ± 0.2 respectively). The distribution of the PI values was significantly different in the PCOS-affected patients and in control group. The plasma levels of DHEAS and BMI were significantly higher in PCOS patients with $PI \geq 3$ than in PCOS patients with $PI < 3$.

Conclusion: High resistance in the uterine artery is present in PCOS patients, but a wide range PI values of uterine artery is present. BMI and DHEAS seem to be factors interfering with uterine perfusion.

KEY WORDS: body mass index; DHEAS; ovarian androgens; PCOS; uterine perfusion.

INTRODUCTION

Despite intensive research performed in the last few years, there is still considerable debate as to the etiology and definition of polycystic ovary syndrome (PCOS) due to the wide range of presentation of this disease (1). For many years, the diagnosis was purely clinical and relied on the presence of obesity, hirsutism, amenorrhea, and enlarged ovaries (2). The advent of radioimmunoassay revealed the hormonal characteristics of these patients: higher LH plasma levels, $LH:FSH > 2.5$, higher levels of total testosterone (total T), free testosterone (free T), and androstenedione (A) (3). Further information about PCOS has been obtained by transvaginal ultrasonography, which has defined the characteristic features of the ovaries including high number of subcapsular small follicles, augmented ovarian volume, and increased echogenicity of the ovarian stroma (4,5). This progress in making a more accurate diagnosis has led to distinguish several subgroups of PCOS caused by hypothalamic-pituitary, ovarian, or adrenal steroidogenic defects.

Several studies using transvaginal color Doppler ultrasonography have found higher impedance in the blood flow of uterine artery (3,5–8), and some authors suggested to include Color Doppler evaluation of uterine and intraovarian arteries in the traditional diagnostic examination used for the definition of this disease (5,8). On the contrary, Pinkas *et al.* (9) demonstrated that polycystic ovaries do not bear disturbance in blood flow of uterine artery. These different

¹ Department of Obstetrics and Gynecology, University of Cagliari, Cagliari, Italy.

² To whom correspondence should be addressed at Department of Obstetrics and Gynecology, University of Cagliari, Ospedale San Giovanni di Dio, Via Ospedale 46, 09124, Cagliari, Italy; e-mail: gimeli@tin.it

results may be a reflection of differences in subgroups of PCOS patients considered in the studies, thus it may be due to the different degree of involvement of hypothalamus-pituitary-ovary and/or hypothalamus-pituitary-adrenal cortex axis.

Uterine blood flow play an important role in uterine receptivity (10,11) and increased values of pulsatility index (PI) of uterine artery have been reported in infertile couples and have been related to a decreased chance to conceive in cycles of assisted reproduction (11–14). The presence of a pathologic uterine perfusion in PCOS patients may contribute to the reduced chance of conception and to the increased risk of miscarriage that characterized these patients (15).

The aim of this study was to verify whether the PCOS-affected patients with higher PI values of uterine artery were characterized by differences in their hormonal parameters.

MATERIALS AND METHODS

Prospectively, between February 1996 and June 1998 all patients with confirmed PCOS referred to our Endocrinological Center were enrolled in the study. To avoid interferences of the endocrine features of adolescence, only subjects >20 years old were enrolled (16). The diagnosis of PCOS was based on the presence of three or more of these features: LH:FSH > 2.5, high levels of total T, free T, A, hirsutism score >10, and typical ultrasonography aspect of the PCOS-ovaries (>10 cysts with 2–8 mm in diameter arranged around an echogenic stroma). All patients gave fully informed consent to participate in the study for which local ethical committee approval and Institutional Review Board approval were obtained.

All patients underwent transvaginal ultrasonography associated with Doppler flow measurement of uterine artery and serum hormone determination during the early follicular phase (Days 2–4 of the menstrual phase). The 88 enrolled patients were divided into three groups according to the PI value of uterine artery: group 1, $PI < 2$; group 2, $2 \leq PI < 3$; group 3, $PI \geq 3$.

A control group of 15 patients with normal menstrual cycle was selected according to ultrasonographic findings of normal uterus and ovaries with documented ovulation and menstrual cycle duration of 27–33 days, and all patients of this group were submitted to hormonal and color Doppler evaluation during the early follicular phase. The body mass index

(BMI = weight (kg)/height² (m²)) was evaluated in all patients.

Assessment of Uterine Blood Flow

Uterine vessels were visualized with the color Doppler technique and the ascending branch was identified lateral to the cervix at the level of the internal os. Then, a blood velocity waveforms was obtained by placing the Doppler gate on the target vessels taking care to ensure that the angle between the Doppler beam and the vessels was as close to 0° as possible. All ultrasound scans were performed using an Acuson XP/10 OB scanner (Acuson Inc., Mountain View, CA) with 7 MHz endovaginal probe. Doppler ultrasound both in the colour and spectral mode, had a frequency of 5 MHz. A high-pass filter of 125 Hz was used. The spatial peak temporal average intensity of ultrasound for B mode and Doppler examinations was <50 mW/cm², which is well within safety limits recommended by the Bioeffects Committee of the American Institute of Ultrasound in Medicine. All the blood flow evaluations were performed at the same time (8.00–10.00 A.M.) to avoid fluctuation due to circadian rhythm of uterine artery blood flow (17). The PI for uterine artery was measured. All Doppler measurements were performed by the same operator (SA) and each measurement was repeated at least three times and averaged. These mean values of PIs were used for the statistical analysis. A recording was considered satisfactory for measurement when there were at least five equally intense waveforms in a row.

The reproducibility of Doppler measurements was tested in 10 patients, where the PI was measured by the same operator three times at 10-min intervals and analyzed by the analysis of variance. The coefficient of variation was 4.9%.

We performed each evaluation in the right portion of the uterus alone since some previous studies demonstrated no difference between right and left uterine artery in spontaneous cycles (5,8,18,19).

Hormonal Analysis

On the same day of Doppler flow analysis, single blood sampling was collected from each patient and sera obtained after refrigerated centrifugation were stored at –20°C till the assay of the following hormones: LH, FSH PRL, estradiol (E₂), Total T, Free T, A, 17 α -hydroxyprogesterone (17-OHP), dehydroepiandrosterone (DHEAS), and sex-hormone binding globulin (SHBG). All assays

were performed by RIA or immunoradiometric assay (IRMA) by using the available commercial kits. To avoid interassay interference, blood samples were analyzed in the same assay.

Statistical Analysis

Student's *t* test, chi square test, and exact test for unpaired data were performed whenever appropriate and .05 was considered statistically significant. The correlation between blood flow parameters and hormonal parameters was evaluated by using regression analysis.

RESULTS

The mean PI of uterine artery of PCOS-affected patients was significantly higher than that of the control group (2.97 ± 0.9 and 1.89 ± 0.2 , respectively; $p < .05$) (Table I) and also the distribution of the PI values in the two groups was significantly different: infact the rate of $PI < 2$ was significantly lower in the PCOS-affected patients, 7% in the PCOS group and 40% in the control group ($p < .05$); the rate of PI that ranged from 2 to 3 was 59% in the PCOS group and 40% in the control group; and the rate of $PI \geq 3$ was 34% in the PCOS group and 20% in the control group.

The hormonal parameters of the two groups were reported in Table I. In PCOS-affected patients LH, Total T, Free T, and A levels were significantly higher than those of control group and also LH:FSH is significantly higher (Table I).

As for the three subgroups of PCOS-affected patients, plasma levels of LH, FSH, E_2 , P, PRL, Total T, Free T, A, 17OHP, and SHBG did not differ signifi-

Table I. Evaluated Parameters of PCOS Patients and Control Group

	PCOS group	Control group	Normal range
Age (years)	27.9 ± 4.9	28.8 ± 5.1	
BMI	20.36 ± 2.3	19.1 ± 3.8	
PI	2.97 ± 0.9	1.89 ± 0.2^a	
LH (mIU/mL)	7.27 ± 3.3	4.03 ± 1.9^a	1.5–10
FSH (mIU/mL)	4.32 ± 1.9	5.41 ± 1.1	1.3–11
LH:FSH	1.95 ± 2.0	0.87 ± 0.6	<2.5
E_2 (pg/mL)	30.8 ± 25.4	35.8 ± 10.9	30–120
P (ng/mL)	0.17 ± 1.4	0.29 ± 0.1	0.1–1.2
PRL (ng/mL)	18.76 ± 9.5	14.7 ± 2.9	2.5–18
Total T (ng/mL)	0.64 ± 0.31	0.52 ± 0.21^a	0.1–1.3
Free T (pg/mL)	2.65 ± 1.0	1.50 ± 0.71^a	0.4–3.9
DHEAS (μ g/mL)	1.65 ± 1.0	1.28 ± 0.5	1.2–3.6
17-OHP (ng/mL)	1.19 ± 0.6	0.89 ± 0.3	0.1–1
A (ng/mL)	3.52 ± 1.9	2.25 ± 0.86^a	0.4–3.3

Note. Values are Mean $\pm$ SD.

^a $p < .05$.

Table II. Evaluated Parameters of the Three Subgroups of PCOS Patients

	PI < 2	$2 \leq PI < 3$	$PI \geq 3$
% of patients	7	59	34
Age (years)	28.3 ± 3.2	27.8 ± 2.9	27.3 ± 3.8
BMI	19.59 ± 1.3	19.79 ± 1.2	21.181 ± 1.5
LH (mIU/mL)	7.68 ± 5.1	6.71 ± 5.36	9.31 ± 5.0
FSH (mIU/mL)	5.83 ± 3.33	3.81 ± 1.8	4.83 ± 1.5
LH/FSH	1.3 ± 0.2	2.04 ± 2.4	2.02 ± 1.2
E_2 (pg/mL)	24.2 ± 10.5	29.5 ± 30.0	30.6 ± 15.1
P (ng/mL)	0.15 ± 0.1	0.50 ± 0.2	0.13 ± 0.14
PRL (ng/mL)	17.05 ± 12.9	17.61 ± 9.4	15.26 ± 9.1
Total T (ng/mL)	0.72 ± 0.4	0.57 ± 0.3	0.60 ± 0.34
Free T (pg/mL)	2.49 ± 0.91	2.54 ± 1.0	2.85 ± 1.02
17OHP (ng/mL)	0.94 ± 0.4	1.20 ± 0.6	1.22 ± 0.6
A (ng/mL)	3.9 ± 2.19	3.05 ± 1.8	3.27 ± 1.9
DHEAS (μ g/mL)	1.267 ± 0.6	1.513 ± 0.9	1.968 ± 0.6

Note. Values are Mean $\pm$ SD.

cantly, but the plasma levels of DHEAS were significantly higher in the group three (Table II) and direct correlation was found between PI values of uterine artery and DHEAS plasma levels ($r = .255$, $p < .03$).

Mean BMI was similar in control and PCOS-affected patients groups (Table I), but in the PCOS-group, it was significantly higher in the group of patients with uterine $PI \geq 3$ (Table II) and direct correlation was found with PI values of uterine artery ($r = .268$, $p < .05$).

DISCUSSION

This study confirms that patients affected by PCOS have higher resistance in uterine arteries as previously described by other authors (3,5–8); however, the result of this study also demonstrates that PCOS itself does not predetermine a single uterine blood flow pattern. In fact, in the group of patients affected by this syndrome a very wide range of PI values of uterine artery is present (from 1.57 to 7.25) and PI of uterine artery ≥ 3 was found in 34% of patients. Higher BMI and DHEAS seem to be the factors affecting PI of uterine artery in PCOS patients.

The effects of BMI on uterine vascularity was previously described by Battaglia *et al.* (6), who found that obese patients with PCOS showed higher mean uterine artery PI than lean patients with PCOS. Increased body weight seems to negatively affect not only uterine perfusion but also endocrinological and clinical pattern of PCOS patients (20–22), and Franks *et al.* (22) demonstrated that the weight reduction of more than 5% is associated with an improved biochemical profile and with restoration of menstrual cyclicity.

The effect of DHEAS on uterine perfusion had not yet been explored in young nonpregnant women. DHEAS qualitatively is one of the most important adrenal steroid (23) and it is a more suitable estimate of adrenal androgen production than total urinary 17-KS (23). The adrenal cortex is responsible for more than 90% of DHEAS production and ovary only for less than 10%. About 50–60% of patients affected by PCOS are characterized by high levels of adrenal androgens (24–26), and this excess has been attributed to adrenal androgen hyperresponsiveness to ACTH or to an intrinsic adrenal dysfunction (27). Gonzales *et al.* (27) demonstrated that while the activity of cytochrome P450c 17 α may be influenced by ovarian activity and in particular by estrogen, 17,20lyase activity does not appear to be modified by ovarian factors (27). Thus, two separate adrenal abnormalities can be identified in patient affected by PCOS (27). Few studies have been performed about the effect of DHEAS on uterine perfusion. On normal full-term pregnant women a significant decrease in uterine artery PI was observed after DHEAS administration (28). These authors concluded that this rapid decrease induced by DHEAS may be not a direct effect of DHEAS but may be mediated by the rapid increase of endothelium-derived relaxing factor due to the increase of E₂, since the blood E₂ levels increased rapidly after IV injection of DHEAS and it is known that placenta uses DHEAS of both maternal and fetal origin for the production of E₂. No studies were performed to evaluate the direct effect of this hormone on uterine vascular tone in nonpregnant women.

In conclusion, our knowledge on PCOS is very far from being definitive and conclusive and a lot of information has to be added to understand the actual pathogenesis and the clinical implications of this pathology. The uterine perfusion seems to be impaired in most of the women affected by this syndrome but it is not clear, yet, which is the mechanism that determine this characteristic features.

REFERENCES

1. Mason H: Polycystic ovaries: Question of definition. *Ultrasound Obstet Gynecol* 1998;12:154–155
2. Stein IF, Leventhal ML: Amenorrhea associated with bilateral polycystic ovaries. *Am J Obstet Gynecol* 1935;29:181–191
3. Ajossa S, Paoletti AM, Guerriero S, Floris S, Mannias M, Melis GB: Effect of chronic administration of cabergoline on uterine perfusion in women with polycystic ovary syndrome. *Fertil Steril* 1999;71:314–318
4. Franks S: Polycystic ovary syndrome: A changing perspective. *Clin Endocrinol (Oxf)* 1989;31:87–120
5. Battaglia C, Artini PG, D'Ambrogio G, Genazzani AD, Genazzani AR: The role of color Doppler imaging in the diagnosis of polycystic ovary syndrome. *Am J Obstet Gynecol* 1995;172:108–113
6. Battaglia C, Artini PG, Genazzani AD, Sgherzi MR, Salvatori M, Giulini S, Volpe A: Color Doppler analysis in lean and obese women with polycystic ovary syndrome. *Ultrasound Obstet Gynecol* 1996;7:342–346
7. Battaglia C, Artini PG, Genazzani AD, Gremigni R, Salvatori M, Sgherzi MR, Giulini S, Lombardo M, Volpe A: Color Doppler analysis in oligo- and amenorrheic women with polycystic ovary syndrome. *Gynecol Endocrinol* 1997;11:105–110
8. Aalem FA, Predanic M: Transvaginal color Doppler determination of the ovarian and uterine blood flow characteristics in polycystic ovary disease. *Fertil Steril* 1996;65:510–516
9. Pinkas H, Mashiach R, Rabinerson D, Avrech OM, Rufas O, Meizze I, Ben-Rafael Z, Fish B: Doppler parameters of uterine and ovarian stromal blood flow in women with polycystic ovary syndrome and normally ovulating women undergoing controlled ovarian stimulation. *Ultrasound Obstet Gynecol* 1998;12:197–200
10. Goswamy RK, William G, Steptoe PC: Decreased uterine perfusion: A cause of infertility. *Hum Reprod* 1988;3:955–959
11. Graham RA, Seif MW, Aplin JD, Li TC, Cooke ID, Rogers AW, Dockery P: An endometrial factor in unexplained infertility. *Br Med J* 1990;200:1428–1431
12. Steer CV, Campbell S, Tan SL, Crayford T, Mills C, Mason BA, Collins WP: The use of transvaginal color flow imaging after in vitro fertilization to identify optimum uterine conditions before embryo transfer. *Fertil Steril* 1992;57:372–376
13. Tsai YC, Chang JC, Tai MJ, Kung FT, Yang LC, Chang SY: Relationship of uterine Perfusion to outcome of intrauterine Insemination. *J Ultrasound Med* 1996;15:633–636
14. Cacciatore B, Simberg N, Fusaro P, Tiitinen A: Transvaginal Doppler study of uterine artery blood flow in in vitro fertilization-embryo transfer cycles. *Fertil Steril* 1996;66:130–134
15. Chappel SC, Howles C: Reevaluation of the roles of luteinizing hormone and follicle-stimulating hormone in the ovulatory process. *Hum Reprod* 1991;6:1206–1212
16. Venturoli S, Porcu E, Fabbri R, Magrini O, Paradisi R, Pallotti G: Postmenarchal evolution of endocrine pattern and ovarian aspect in adolescent with menstrual irregularities. *Fertil Steril* 1987;48:78–85
17. Zaidi J, Jurkovic D, Campbell S, Okokon E, Tan SL: Circadian variation in uterine artery blood flow indices during the follicular phase of the menstrual cycle. *Ultrasound Obstet Gynecol* 1995;5:406–410
18. Zaidi J, Jurkovic D, Campbell S, Pittrof R, McGregor A, Tan SL: Description of circadian rhythm in uterine artery blood flow during the peri-ovulatory period. *Hum Reprod* 1995;10:1642–1646
19. Tekay A, Martkainen H, Jouppila P: Comparison of uterine blood flow characteristics between spontaneous and stimulated cycles before embryo transfer. *Hum Reprod* 1996;11:364–368
20. Acien P, Quereda F, Matallin P, Villaroya E, Lopez-Fernandez JA, Acien M, Mauri M, Alfayate R: Insulin, androgens, and obesity in women with and without polycystic ovary syndrome: A heterogeneous group of disorders. *Fertil Steril* 1999;72:32–40
21. Ciampelli M, Fulghesu AM, Cucinelli F, Pavone V, Roncisvalle E, Guido M, Caruso A, Lanzone A: Impact of insulin and body

- mass index on metabolic and endocrine variables in polycystic ovary syndrome. *Metabolism* 1999;48:167–172
22. Franks S, Kiddy D, Sharp P, Singh A, Reed M, Seppala M, Koistinen R, Hamilton-Fairley D: Obesity and polycystic ovary syndrome. *Ann N Y Acad Sci* 1991;626:201–206
 23. Lobo RA, Paul WL, Goebelsmann U: Dehydroepiandrosterone Sulfate as an indicator of adrenal androgen function. *Obstet Gynecol* 1981;57:69–73
 24. Hoffman DI, Klive K, Lobo RA: The prevalence and significance of elevated dehydroepiandrosterone sulfate levels in anovulatory women. *Fertil Steril* 1984;42:76–81
 25. Hudson RW, Lochman HA, Damby FW: 11beta-hydroxyandrostenedione: A marker of adrenal function in hirsutism. *Fertil Steril* 1990;54:1065–1071
 26. Carmina E, Koyama T, Chang L, Stanczyk FZ, Lobo RA: Does ethnicity influence the prevalence of adrenal hyperandrogenism and insulin resistance in polycystic ovary syndrome? *Am J Obstet Gynecol* 1992;167:1807–1812
 27. Gonzales F, Chang L, Horab T, Stanczyk FZ, Crickard K, Lobo RA: Adrenal dynamic responses to physiologic and pharmacologic adrenocorticotrophic hormone stimulation before and after ovarian steroid modulation in women with polycystic ovary syndrome. *Fertil Steril* 1999;71:439–444
 28. Hata T, Senoh D, Hata K, Kitao M, Masumura S: Effect of dehydroepiandrosterone sulfate on uterine artery flow velocity waveforms in term pregnancy. *Obstet Gynecol* 1995;85:118–121