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Ovarian Stromal Hypertrophy in Polycystic Ovary Syndrome

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OBJECTIVE: To investigate the relationship between the ovarian stromal area and clinical hormonal characteristics in women with polycystic ovary syndrome (PCOS).
STUDY DESIGN: Twenty-eight women with PCOS (group 1) and 26 healthy women (group 2) participated in this study. For measuring the ovarian stromal area, transvaginal ultrasonography was performed on all women during the early follicular phase of the menstrual cycle. Venous blood was sampled from the women to determine serum follicle stimulating hormone, luteinizing hormone (LH), estradiol, androstenedione, free testosterone (FT), total testosterone (TT), 17α-hydroxyprogesterone, dehydroepiandrosterone sulfate, and fasting insulin and glucose levels. Two-tailed t *and Pearson correlation tests were used for statistical analysis.*
*RESULTS: Women with PCOS were heavier, and their serum FT, TT and LH levels were significantly higher than in the normals (*P<.001, P<.012 *and* P<.001, *respectively). The ovarian stromal area measured by transvaginal ultrasonography was also significantly larger than in the normals (*P<.001*). Only basal serum insulin levels seemed to correlate positively with the ovarian stromal area in women with PCOS (*r=.43 P=.09*).*
CONCLUSION: Although transvaginal ultrasonography has played an important role in the evaluation of women with PCOS, we could not demonstrate a relationship between the ovarian stromal area and hormonal characteristics of PCOS. Therefore, transvaginal ultrasonography and hormonal parameters must be used as complementary diagnostic methods in women with PCOS. (J Reprod Med 1998;43:893–897)

We did not observe any association between stromal area and hormonal parameters of PCOS except a mild but nonsignificant association with serum insulin levels.

Keywords: polycystic ovary syndrome, ovarian diseases, hypertrophy.

Introduction

Polycystic ovary syndrome (PCOS), as first described by Stein and Leventhal, was defined as the clinical presentation of amenorrhea, hirsutism, obesity and infertility with the finding of enlarged ovaries at the time of exploratory laparotomy: they were polycystic, with thickened stroma on histologic examination.[1] Since 1935, there has been much confusion with respect to the etiology and pathophysiology of PCOS. However, based on the literature, in a significant subset of patients this disorder

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may be caused by a conjunction of two independent genetic defects: one that produces elevated luteinizing hormone (LH) secretion and another that produces insulin resistance. Thus, PCOS develops as a result of the synergistic action of increased LH levels and hyperinsulinemia on the ovary.[2] These two defects cause increased secretion of androgens and their precursors from the ovarian stroma, especially from atretic follicles and thecal cells.[3]

Stromal area should not be used alone but as a complementary finding in the diagnosis of PCOS.

Although it has been reported that transvaginal ultrasonography has a specificity of 91% for the diagnosis of PCOS, no consensus exists regarding the precise diagnostic criteria.[4–9] Because it has been reported that polycystic ovaries are seen in 22% of normal, regularly menstruating, non-PCOS patients and even in some patients with hypogonadotropic hypogonadism,[10,11] clinicians suspected the significance of polycystic ovaries for the diagnosis of PCOS. However, Takahashi et al compared histologic findings on specimens from 20 patients with PCOS undergoing ovarian wedge resection by laparotomy with ultrasonographic images and showed that the histopathologic and ultrasonographic images were consistent at a rate of 90%.[12] Another paper reported that ultrasonographic findings correlated with endocrinologic dysfunction in patients with PCOS.[13]

The purpose of this study was to evaluate (1) the relationship between serum androgen levels and ovarian stromal area detected by transvaginal ultrasonography in patients with PCOS, and (2) the relationship between LH and insulin, which are synergistically responsible for hyperandrogenemia in patients with PCOS, and ovarian stromal area.

Materials and Methods

Subjects

Twenty-eight women with PCOS (group 1) and 26 healthy women (group 2) participated in this study. Each subject underwent a screening history and physical examination. Healthy women were free of any hyperandrogenemic complaints, signs or symptoms and had been menstruating regularly. The diagnosis of PCOS was made on the basis of clinical (infertility, obesity, hirsutism and oligoamenorrhea), endocrinologic (high total testosterone [TT], androstenedione [A], LH) and ultrasonographic findings,[8] and other disorders that are known to cause hyperandrogenism—such as prolactinoma, thyroid dysfunction, Cushing syndrome, androgen-secreting tumors and late-onset congenital adrenal hyperplasia (LOCAH)—were also excluded. The adrenocorticotropic hormone (ACTH) stimulation test was performed on all patients with PCOS to exclude LOCAH. Serum 17α-hydroxyprogesterone (17-OHP) levels were determined before and after an intravenous injection of 0.25 mg synthetic ACTH.[14] No patient had received any medications for at least three months prior to enrollment. Their informed consent was obtained.

Protocol

After a carbohydrate diet of 250 g/d for three days, fasting blood samples were drawn between 8:00 and 10:00 AM in early-follicular-phase spontaneous bleeding or withdrawal bleeding induced with medroxyprogesterone acetate. Fasting serum glucose was determined. Samples were centrifuged at 2,000 rpm for 10 minutes and serum stored at -20°C for hormone level measurements (follicle stimulating hormone [FSH], LH, estradiol [E_2], A, TT, free testosterone [FT], 17α-hydroxyprogesterone [17-OHP], dehydroepiandrosterone sulfate [DHEAS], insulin).

Serum glucose levels were measured by the glucose-oxidase method using a Beckman analyzer. Serum FSH, LH, E_2, A, TT, FT, 17-OHP, DHEAS and insulin levels were measured by radioimmunoassay (Coat-a-Count, Diagnostic Products Corporation, Los Angeles, California). The double antibody technique was used for FSH and LH measurements, and the single antibody technique was used for other hormones.

Coefficients of variation of the serum LH kit were 2.3%, 3.6% and 7.0% for low, medium and high values, respectively. These coefficients were 3.1%, 3.9% and 6.5% for FSH; 9.2%, 10.4% and 12.9% for TT; 4.3%, 4.0% and 3.2% for FT; 4.0%, 5.0% and 5.6% for 17-OHP; 3.9%, 4.1% and 5.3% for DHEAS; 7.0%, 5.8% and 5.6% for E_2; 5.0%, 3.3% and 8.3% for A; and 10.0%, 7.1% and 4.9% for insulin, respectively.

Ovaries were scanned by one radiologist (C.A.) transvaginally by the use of real-time sonography with a 5-MHz probe through a sector of 200° (Kretz, Zipf, Austria). Polycystic-appearing ovaries were

defined sonographically as the presence of multiple (>10), small (2–8 mm in diameter) follicles in the periphery (in one plane) and increased stromal echogenicity as described by Adams et al.[8]

Stromal areas were measured in the early follicular phase, as described by Dewailley et al.[13] A longitudinal cut in the middle of the ovary was selected, and an outline of the ovary was shaped. After the selection and exclusion of the gray scale corresponding to the microcysts, the hyperechogenic stromal area was encircled. Ultrasonography gave the area in square millimeters on the screen with the software. The stromal area of a person was defined as the mean of both stromal areas.

Statistical Analysis

All values are given as mean±SD. A paired *t* test was used for comparisons between groups. Relationships between stromal area and hormonal parameters were analyzed with Pearson's correlation test.

Results

Table I summarizes body mass index (BMI) values, serum hormone values and stromal areas in the two groups. Women with PCOS had significantly higher BMI values than the control group (28.69±7.54 versus 23.96±4.34, *P*=.008). The hormones that were significantly higher in the PCOS group were FT (*P*<.001), TT (*P*=.012) and LH (*P*<.001). Women with PCOS had a significantly larger stromal area than the control group (*P*<.001).

Correlations between stromal area and serum hormone levels were analyzed; the results are shown in Table II. Significant correlations were found between stromal area and serum LH, TT, 17-OHP in the control group (*r*=.39 *P*=.049; *r*=.48, *P*=.013; and *r*=.43, *P*=.038, respectively). These hormones were observed to increase with increasing stromal area. However, no correlation was found between stromal area and serum hormone levels in the PCOS group. Although a correlation between stromal area and serum insulin level was not significant, they seemed to be related (*r*=.43 *P*=.09).

Table I *Comparison of Hormonal Parameters Between Women with Polycystic Ovaries and the Control Groups*

Parameter	PCO	Control	*P*
BMI (kg/m²)	28.69±7.54	23.96±4.34	.008*
FSH (IU/L)	6.4±2.3	7.5±2.7	.19
LH (IU/L)	11.2±6.8	3.4±1.9	<.001*
E_2 (pg/mL)	63.1±57.2	63.9±85.9	.97
FT (pmol/mL)	3.1±1.3	1.5±0.7	.001*
TT (nmol/L)	1.1±1	0.4±0.2	.012
A (nmol/L)	1.6±0.6	1.3±0.4	.6
DHEAS (µ/dL)	305.7±171.7	229.9±107.2	.11
17-OHP (mg/dL)	1.5±1	1.1±0.9	.12
Glucose (mg/dL)	93±12.5	89.2±7	.31
Insulin (µU/mL)	13.1±10.2	10.3±9.9	.4
Stromal area (mm²)	2.5±1.2	0.8±0.3	<.001*

*P<.05.
PCO=polycystic ovaries.

When evaluating a patient with PCOS, all the clinical, biochemical and ultrasonographic findings should be interpreted separately.

Discussion

The origin of hyperandrogenemia in PCOS is accepted as the ovarian stroma. There is both *in vitro* and *in vivo* evidence that elevated LH and hyperinsulinemia act synergistically to enhance androgen secretion from the ovarian stroma.[2] It has also been shown that the synergistic effect of these two hormones causes hyperandrogenemia through stromal and thecal hyperplasia and that this in turn causes follicular atresia.[3] However, elevated LH and insulin resistance are not present in all women with PCOS. Also, the significance of ultrasonographic appearance in the diagnosis of PCOS is debatable. The presence of multiple (>10), small (2–8 mm in diameter) follicles in the periphery (in one plane), increased stromal area, increased ovarian surface area, increased ovarian volume, diffusely distributed multiple follicles and, in some cases, larger follicles are the ultrasonographic criteria reported in the literature.[4–11]

Because ultrasonography is widely accepted and used by gynecologists, associations between ultrasonographic properties and clinical, hormonal and histopathologic properties of PCOS are still being investigated to determine the best predictive appearance for the diagnosis of PCOS.[7,9,12,13]

Because LH and insulin are known to affect ovarian stroma, an association between these hormones and ovarian stroma seems logical. Although we did not observe a significant association in our study, stromal area and serum insulin level seemed to be related. Although some authors could not show any association either,[9,13] Pache et al reported an associ-

Table II *Relationships Between Ovarian Stromal Area and BMI, Glucose and Other Hormonal Parameters*

	PCO		Control	
Parameter	***r***	***P***	***r***	***P***
BMI	.29	.14	.11	.58
FSH	.005	.97	.05	.79
LH	.02	.91	.39	.049*
E_2	.09	.68	.08	.7
FT	.25	.39	.01	.95
TT	.05	.83	.48	.013*
A	.48	.32	.17	.46
DHEAS	.16	.53	.02	.93
17-OHP	.32	.18	.43	.038*
Glucose	.24	.41	.28	.2
Insulin	.43	.09	.04	.85

*p<.05.
PCO=polycystic ovaries.

ation between LH and stromal area.[7] There may be two reasons why we could not show an association between LH and stromal area: (1) serum LH is usually but not always elevated in PCOS,[15] and (2) mutations in the LH gene may cause synthesis of a variant of LH with higher bioactivity.[16,17]

It is known that hyperinsulinemia due to insulin resistance is more common in women with PCOS.[15] It has been reported that insulin causes hyperandrogenemia through IGF-1 receptors on the theca and granulosa cells of the stroma.[18] However, we could observe only a minimal association between insulin and stromal area; it was not significant. We think that this association would be significant if a larger group were studied and it would be consistent with observations by Pache et al.[7]

We did not observe any association between stromal area and TT or A in women with PCOS, whereas we found a correlation between stromal area and TT in the control group. The source of increased A and TT is the ovarian stroma.[2,3] One of the important ultrasonographic properties of PCOS is increased stromal area, as observed in our cases.[8,10] Although an association between stromal area and TT or A seems logical, Dewailly et al could only show such an association with A but not with TT.[13] However, Tabbakh et al could not show any association between ultrasonographic findings and serum androgen levels.[9] We think that the lack of correlation between stromal area and androgens is due to the fact that the source of hyperandrogenemia in PCOS is not only the ovaries but also the adrenals and peripheral tissues and also that these organs' contributions to hyperandrogenemia might show heterogeneity between patients.

In conclusion, although we observed a significant difference with respect to stromal area between patients with PCOS and those in the control group, probably due to the heterogeneity of the syndrome, we did not observe any association between stromal area and hormonal parameters of PCOS except a mild but nonsignificant association with serum insulin levels. Stromal area should not be used alone but as a complementary finding in the diagnosis of PCOS. We think that when evaluating a patient with PCOS, all the clinical, biochemical and ultrasonographic findings should be interpreted separately.

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