

Research Article

Serum Vascular Endothelial Growth Factor in Polycystic Ovary Syndrome and its Relation to Ovarian Drilling

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ABSTRACT

Polycystic ovary syndrome (PCOS) is common endocrine disorders that cause anovulation and consequent subfertility. The Objective of this study is to determine the serum level of the angiogenetic factor vascular endothelial growth factor (VEGF) and the relationship between VEGF and other hormones before and after laparoscopic ovarian drilling (LOD) in Egyptian women with polycystic ovary syndrome (PCOS). A prospective controlled clinical study was conducted on 40 clomiphene-resistant women with PCOS diagnosed by ultrasound examination and a history of oligomenorrhea, hirsutism and obesity were studied, and they were scheduled for LOD and a control group of 22 fertile regularly menstruating women. Serum level of VEGF and other hormones were measured and compared between the two groups, and before and after LOD in the PCOS group. Serum VEGF concentrations in women with normal ovaries was significantly lower than in women with PCOS before as well as after ovarian drilling. No difference was found in the serum VEGF levels in women with PCOS before and after ovarian drilling. Serum LH, LH/FSH ratio, estradiol and testosterone were significantly lower in women with normal ovaries in comparison to women with PCOS before as well as after ovarian drilling, while LH, LH/FSH and testosterone significantly decrease in PCOS patients after ovarian drilling. There were significant positive correlations between VEGF and LH and LH/FSH in PCOS before ovarian drilling. VEGF levels in women with PCOS are higher than in normal women, and ovarian drilling does not affect these levels. Higher serum levels of VEGF in PCOS women may be related to the increased vascularity, which plays a pivotal role in ovarian pathology.

Keywords: Polycystic ovary syndrome, vascular endothelial growth factor, Laparoscopic ovarian drilling.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a common endocrine disorder that affects 5–10% of women of reproductive age [1]. The polycystic ovary syndrome (PCOS) is characterized by infertility, oligomenorrhea and hyperandrogenism. Biochemical abnormalities include hyperandrogenism, acyclic estrogen production, increased levels of luteinizing hormone (LH), decreased levels of sex hormone-binding globulin (SHBG) and hyperinsulinemia. The heterogeneity of the clinical and laboratory features strongly speaks for the multifactorial etiology of the syndrome [2].

The revised definition of PCOS requires the presence of 2 of the following 3 diagnostic criteria: oligo-ovulation and/or anovulation, clinical and/or biochemical features of hyperandrogenism, and the presence of polycystic ovary morphology, in addition to its reproductive features, PCOS is associated with an increased risk of obesity, insulin resistance, and type 2 diabetes mellitus [3]. To make a diagnosis of polycystic ovaries from transvaginal scanning, 12 or more follicles measuring 2–9mm in diameter or increased ovarian volume (>10 cm³) must be

present. It is well established that increased ovarian mass, supported by new blood vessel proliferation in stroma and theca, is a key feature of PCOS. Recent studies suggest a role for angiogenetic factors in this phenomenon [4].

Clomiphene citrate (CC) is considered the first-line medical treatment for infertile women with normogonadotrophic anovulation. The next option for women with CC-resistant polycystic ovarian disease (CCR-PCOD) is usually either gonadotrophin therapy or laparoscopic ovarian drilling (LOD). Both modalities have their own inherent merits and demerits but success rates in terms of pregnancies and live births are comparable [5,6] Laparoscopic ovarian drilling is a one day surgery that has been established as an effective second line method of ovulation induction in clomiphene citrate (CC) resistant PCOS patients with high ovulation (80%) and pregnancy rate (60–80%) [7].

Vascular endothelial growth factor (VEGF), also known as vascular permeability factor, is a potent angiogenic factor which is a mitogen for vascular endothelium. VEGF may be involved in the physiological regulation of ovarian

Table 1: Serum concentration of hormones and VEGF (mean \pm SD) in patients with PCOS (before and after LOD) in comparison with concentration in healthy fertile women.

Variable	Group I Normal Women (n=22)	Group II Women with PCOS (n=40)	
		Pre LOD	Post LOD
Age year	30.5 \pm 4.5	29.2 \pm 4.3	
BMI kg/m ²	23.6 \pm 2.1	27.5 \pm 4.5	
FSH mIU/ml	6.96 \pm 1.11	6.34 \pm 2.83	6.33 \pm 2.44
LH mIU/ml	4.14 \pm 0.66	12.57 \pm 4.28 *	9.35 \pm 3.12*§
LH / FSH	0.60 \pm 0.07	2.37 \pm 1.03*	1.61 \pm 0.58*§
Testosterone ng/ml	1.16 \pm 0.44	2.79 \pm 1.6*	1.98 \pm 1.13*§
Estradiol pg/ml	110.4 \pm 31.7	203.28 \pm 73.89*	195.82 \pm 71.8*
VEGF ng/ml	5.78 \pm 1.58	29.9 \pm 7.27*	27.84 \pm 7.03*

* $P < 0.001$ significant in comparison to controls (group I)

§ $P < 0.001$ significant in comparison between pre LOD and post LOD

angiogenesis. VEGF is expressed and secreted in the human ovary in a manner that suggests a role for this growth factor in both cyclic angiogenesis and regulation of vascular permeability, both of which are critical for ovarian folliculogenesis and normal reproductive function [8]. Increased expression of VEGF has been described in the hyperthecotic stroma of polycystic ovaries [9,10], which have been shown to be associated with increased ovarian stromal blood flow [11]. High vascularization may lead to abnormal growth of the theca interna (which is the site for androgen steroidogenesis) with subsequent hyperandrogenemia [12]. Angiogenesis within the reproductive system may be coordinated by gonadotropins and/or locally produced steroids and proteins. Vascular endothelial growth factor (VEGF) may have a role in cyclic angiogenesis and regulation of vascular permeability, which are critical for ovarian folliculogenesis. Abnormality in ovarian angiogenesis may contribute to anovulation and infertility and may be involved in the increased predilection to ovarian hyperstimulation in PCOS [13].

The aim of the present study was to determine the serum level of VEGF and find the relationship between VEGF and other hormones before and after LOD in Egyptian women with PCOS.

Patients and Methods: The present study was performed at Ain Shams University Maternity Hospital, Egypt. The studied groups of sixty-two subjects their age ranged from 22 -33 years, divided into two groups: **group I**; forty clomiphene citrate resistant PCOS infertile patients (CCR-PCOS) who were scheduled for LOD by the treating physician; and **group II**; twenty-two healthy fertile women who were attending an outpatient clinic for contraception. Their body mass index (BMI) was calculated as weight in kilograms divided by the square of the height in meters. Informed consent was obtained from all study participants, and the study was approved by the local ethics committee of Ain Shams University Hospital. Diagnosis of PCOS was based on clinical manifestations (oligomenorrhea, hirsutism, and obesity) and standard ultrasound criteria. Ovaries were classified as normal or polycystic according to ultrasonographic criteria established by Dewailly et al. [14]. By using a transvaginal probe, the total number of follicles of 2–9 mm in diameter was counted in both ovaries. The threshold was set at 12,

thereby defining polycystic ovaries by the presence of 12 or more follicles (mean of both ovaries). If the mean follicle number was less than 12, the ovaries were categorized as normal, while CCR was defined by the presence of persistent anovulation in spite of taking 150 mg CC/day for 5 days during 6 consecutive cycles [15]. Women over 35 years old, having other infertility factor(s), and/ or with tubal obstruction, or evident pelvic endometriosis discovered during laparoscopy were excluded from the study. None of the healthy controls had symptoms of hyperandrogenism, a history of menstrual dysfunction, infertility, or signs of PCOS by ultrasound examination.

LOD was carried out in the early follicular phase of the menstrual cycle. Each ovary was cauterized at 4 points, for 4 seconds at each point, by using 40W of power with a high-frequency monopolar microneedle, regardless of the size of the ovary. The whole length of the needle (10 mm) was inserted into the ovary to ensure stromal damage. On the morning of day 3 of the same cycle in which the operation was to be done, a blood sample was taken from each patient before LOD. A second sample was taken on day 3 of the first postoperative cycle after LOD.

Blood samples were obtained also by venipuncture on cycle day 3 from the control group. After collection, the blood samples were allowed to clot at room temperature for 30 min, followed by centrifugation at 1200 rpm for 10 min. Serum was stored in aliquots at -80°C until assayed. -Serum follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were measured by using the luminescence-based immunoassay Immulite 1 (Diagnostic Product Corporation, Los Angeles, CA, USA). The procedures and interpretation of the assays were performed in accordance with the manufacturer's recommendations. The assay sensitivity was 0.1 mU/mL. The intra- and inter-assay coefficients of variance were 3% and 8%, respectively. Testosterone was measured by using an ELISA kit (Orion Diagnostica, Espoo, Finland) with a sensitivity of 0.05–0.02 ng/mL. Estradiol was measured by using a solid-phase radioimmunoassay I125 kit (Diagnostic Products Corporation, Los Angeles, CA, USA) with a sensitivity of 6 pg/mL.

-Assay of VEGF was done by using a Quantikine VEGF ELISA kit (R&D Systems, Minneapolis, MN, USA) with a sensitivity of 0.195 ng/mL.

Table 2: Correlation of VEGF serum concentration with hormones in patients with PCOS before and after LOD

Comparison	Pre-LOD		Post-LOD	
	r	P	r	P
FSH mIU/ml	-0.089	NS	0.212	NS
LH mIU/ml	0.561	< 0.001	0.135	NS
LH / FSH	0.566	< 0.001	0.15	NS
Testosterone ng/ml	0.136	NS	0.07	NS
Estradiol pg/ml	0.271	NS	0.000	NS

NS= Non significant ($P < 0.05$)

Statistical analysis: Statistical analysis was performed using IBM SPSS Statistics for Windows version 20 (IBM Corp, Armonk, NY). Data were analyzed using Student's *t*-test, analysis of variance (ANOVA) was used to address differences between PCOS and controls. Pearson correlation coefficients were used to test the correlation between variables. $P < .05$ was considered significant.

RESULTS

Clinical and biochemical characteristics and data of our study subjects are shown in Table 1. The mean age of women with PCOS was 29.2 ± 4.3 years, whereas that of the women with normal ovaries was 30.5 ± 4.5 years. The mean body mass index of women with PCOS (27.5 ± 4.5) was higher than that of the control women (23.6 ± 2.1).

Serum VEGF concentrations in women with normal ovaries (5.78 ± 1.58 ng/mL) were significantly lower (P value < 0.001) than in women with PCOS before (29.9 ± 7.27) as well as after ovarian drilling (27.84 ± 7.03). No difference was found in the serum VEGF levels in women with PCOS before and after ovarian drilling (29.9 ± 7.27 and 27.84 ± 7.03 ng/ml respectively). Serum LH, LH/FSH ratio, estradiol and testosterone were significantly lower (P value 0.001) in women with normal ovaries in comparison to women with PCOS before as well as after ovarian drilling, while LH, LH/FSH and testosterone significantly decrease ($P < 0.001$) in PCOS patients after ovarian drilling.

Pearson correlation analysis was performed to evaluate for correlations between VEGF and other hormonal study: There were significant positive correlations between VEGF and LH and LH/FSH in PCOS before ovarian drilling. There were non-significant correlation between VEGF and the other suited hormones (testosterone, FSH, and estradiol) (table2).

DISCUSSION

The polycystic ovary syndrome (PCOS) is a common and complex endocrinopathy, which is characterized by chronic anovulation, oligomenorrhoea, and hyperandrogenism [16]. Vascular endothelial growth factor (VEGF) is a dimeric heparin-binding glycoprotein, which plays a pivotal role in ovarian physiology and pathology [17]. Literatures have focused on VEGF as one of the most likely candidates for accentuated angiogenesis in PCOS. A direct correlation between serum VEGF levels and Doppler indices of ovarian blood flow has been reported in PCOS women, suggesting a role for the growth factor in the stromal hypervascularity observed in this syndrome. The elevated serum VEGF concentrations in

women with PCOS may explain in part the dense hypercellular and highly vascularized stroma of PCOS as demonstrated by Doppler blood flow. The increased vascularity may result from overexpression of ovarian VEGF in women with PCOS. This hypothesis is supported by demonstration of a strong immunohistochemical staining of VEGF in the ovarian stroma of three patients with PCOS [18]. Some cases of anovulation are treated by clomiphene citrate, other are resistant to it. Laparoscopic treatment of clomiphene citrate-resistant anovulatory women is effective in achieving a return of ovulation and pregnancy [19].

In our study we found that serum VEGF concentrations in women with PCOS were significantly higher than in women with normal ovaries with no significant change in its level in pre-LOD and post-LOD. This was consistent with Agrawal et al. [20] and Tulandi et al. [21] who found that there was no difference in serum VEGF levels before and after ovarian drilling. This finding suggests that ovarian drilling does not affect VEGF production.

Agrawal et al. [22] found that greater amount of VEGF was released from cultured granulosa lutein cells of women with polycystic ovaries than from cells of women with normal ovaries. The increase occurred in all culture conditions, including the unstimulated state. Elevated serum VEGF concentrations in women with polycystic ovaries or PCOS were consistent with immunohistochemical demonstration of extensive VEGF staining in the hyperthecotic stroma of the polycystic ovaries. Since serum VEGF concentrations correlate with ovarian stromal blood flow, these changes in blood may be significant in relation to the characteristically exuberant response to ovarian stimulation with gonadotropins of the polycystic ovaries or PCOS. In addition, VEGF levels were increased in women with polycystic ovaries and those with PCOS, suggesting that this elevation is a constitutive feature of polycystic ovaries rather than a result of stimulation by LH.

Amin et al. [23] stated that great VEGF production observed in PCOS patients [6,23] may be involved in their increased risk of ovarian hyperstimulation syndrome (OHSS); the pathogenesis of OHSS seems to be related to an excess of cytokine and growth factors, especially VEGF during gonadotropin treatments.

In our study we found that LH, LH/FSH and testosterone significantly decrease in PCOS patients after ovarian drilling, this was in agreement with Felemban et al. [24] who found that laparoscopic treatment is increasingly recommended as an early treatment option for women with clomiphene-resistant PCOS, the reduction in serum LH

levels after LOD is reported to be the main mechanism by which reproductive outcome is improved. Abuelghar et al. [25] demonstrated that the use of androstenedione, LH and FSH as simple reliable predictors of spontaneous ovulation after LOD as they can predict the success of LOD, defined as spontaneous ovulation within the first 8 postoperative weeks.

CONCLUSION

Our study provides evidence that VEGF levels in women with PCOS are higher than in normal women, suggesting that this elevation is a constitutive feature of polycystic ovaries rather than a result of stimulation by LH, and the laparoscopic ovarian drilling does not affect these levels. Higher serum levels of VEGF in PCOS women may be related to the increased vascularity which plays a pivotal role in ovarian pathology. Also the study found that LH, LH/FSH and testosterone significantly decrease in PCOS patients after ovarian drilling, So that laparoscopic treatment is increasingly recommended as an early treatment option for women with PCOS.

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