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Correlation between endometrial receptivity with expressions of IL-1 and VEGF in rats with polycystic ovary syndrome

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Abstract. – OBJECTIVE: To investigate the correlation between endometrial receptivity with expressions of interleukin-1 (IL-1) and vascular endothelial growth factor (VEGF) in rats with polycystic ovary syndrome (PCOS).

MATERIALS AND METHODS: A total of 24 female Sprague-Dawley (SD) 21 days old were randomly divided into control group (n=12) and PCOS group (n=12). Rats in the control group were normally raised, and PCOS model was established in rats of the PCOS group. All the rats were sacrificed when they grew to 80 days old. Immunohistochemistry was applied to detect the expressions of IL-1 and VEGF. Western blotting was performed to measure the relative expressions of IL-1 and VEGF proteins. Quantitative Polymerase Chain Reaction (qPCR) was utilized to determine the relative messenger ribonucleic acid (mRNA) expressions of IL-1 and VEGF. Data related to endometrial receptivity were detected.

RESULTS: The expression levels of IL-1 and VEGF in the PCOS group declined markedly compared with those in the control group, and the differences were statistically significant ($p<0.05$). PCOS group had notably lower protein expressions of IL-1 and VEGF than the control group, with statistically significant differences ($p<0.05$). The mRNA expressions of IL-1 and VEGF in PCOS group were significantly lower than those in the control group, displaying statistically significant differences ($p<0.05$). Compared with that in the control group, the endometrial receptivity of rats in the PCOS group was reduced evidently, and the difference was statistically significant ($p<0.05$). Both IL-1 and VEGF had positive correlations with the endometrial receptivity.

CONCLUSIONS: Both IL-1 and VEGF are positively correlated with the endometrial receptivity in the case of PCOS, which can serve as therapeutic targets for PCOS and improve endometrial receptivity in the future.

Key Words.
Polycystic ovary syndrome, Endometrial receptivity, Interleukin-1, Vascular endothelial growth factor.

Introduction

As a relatively common type of female reproductive endocrine disease, polycystic ovary syndrome (PCOS) is one of the major reasons for infertility in females. It is estimated that approximately 5-10% of women in childbearing age suffer from PCOS, accounting for 75% of the patients with anovulatory infertility. PCOS patients mainly manifest obesity, metabolic syndrome, excessive androgen, ovulatory dysfunction of the ovaries, infertility, etc.[1].

Endometrial receptivity is the receptive ability of maternal endometrium to blastocyst, which may be seriously affected in the process of PCOS[2]. An endometrium with a favorable receptivity allows the blastocyst successfully accomplishing location, adhesion, implantation and other nidation processes. The persistent low endometrial receptivity makes it difficult for blastocyst nidation in the endometrium, thereby resulting in infertility of the patients[3,4].

Therefore, effective amelioration of endometrial receptivity of PCOS patients is of great clinical significance and its relevant influencing factors require to be fully elucidated. This research aims to further clarify the correlation of endometrial receptivity with expressions of interleukin-1 (IL-1) and vascular endothelial growth factor (VEGF) in the case of PCOS.

Materials and Methods

Laboratory Animals and Grouping

A total of 24 female Sprague-Dawley (SD) 21 days old rats were bred in the Laboratory Animal Center. All the rats were divided into control group (n=12) and PCOS group (n=12) using a random number table. This study was approved by the Animal Ethics Committee of Yantai Yuhuangding Hospital Animal Center.

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Experimental Reagents and Instruments

Anti-IL-1 antibody (Abcam, Cambridge, MA, USA), anti-VEGF antibody (Abcam, Cambridge, MA, USA), immunohistochemical kit (Maixin Biotech Co., Ltd., Fuzhou, China), HE staining kit (Solarbio Science & Technology Co., Ltd., Beijing, China), AceQ quantitative Polymerase Chain Reaction (qPCR) SYBR Green Master Mix kit, HiScript II Q RT SuperMix for qPCR [+ genomic deoxyribonucleic acid (gDNA) wiper] kit, light microscope (Leica DMI 4000B/DFC425C), fluorescence qPCR instrument (ABI 7500, Applied Biosystems, Foster City, CA, USA), Image Lab image analysis system (Bio-Rad, Hercules, CA, USA) and Image-Pro image analysis system (Bio-Rad, Hercules, CA, USA).

PCOS Model Establishment

PCOS model in rats was established by reference to the methods of Lee et al[5]. The specific methods were as follows: female SD rats were weaned at the age of 21 days. They were subcutaneously injected with DEHA (6 mg per 0.1 kilograms of body weight) and 0.2 mL sesame oil once every day from the age of 24 days for consecutive 20 days. After vagina opening at the age of 70 days, two consecutive sexual cycles were examined using vaginal exfoliative cytological smear technique to observe changes in the sexual cycles. Meanwhile, the venous blood was collected from the tail at the age of 80 days to detect the serum levels of luteinizing hormone, testosterone and follicle stimulating hormone. Loss of complete changes in the sexual cycles and remarkably elevated the serum levels of the luteinizing hormone, testosterone and follicle stimulating hormone suggested the successful establishment of PCOS model in rats.

Processing Methods in Each Group

Rats in the control group were normally raised without any specific procedures, and those in the PCOS group were given normal diets and other living conditions to establish PCOS models. Rats were sacrificed at the age of 80 days to obtain specimens.

Specimen Acquisition

After successful anesthesia, the venous blood was collected, and bilateral ovaries and uteruses were removed. Rat ovary and uterus on the one side were fixed in 4% paraformaldehyde for 48 h for fixation, followed by preparation into paraffin sections. Ovary and uterus on the other side were placed into EP tubes and stored at -80°C for Western blotting assay.

Immunohistochemistry

The 5 μm-thick paraffin sections were routinely deparaffinized and dipped in water, and then citric acid buffer solution was added and heated in a microwave oven for antigen retrieval. After washing with phosphate-buffered saline (PBS), the endogenous peroxidase-blocking solution was added for 10-min incubation, followed by sealing in goat serum for 20 min after PBS wash. Next, the serum blocking buffer was shaken off, and IL-1 primary antibody (1:200) and VEGF primary antibody (1:200) were added and reacted at 4°C overnight. At the other day, sections were incubated with secondary antibodies for 10 min, reacted with a streptavidin-peroxidase solution for 10 min, and stained with diaminobenzidine (DAB) for color development. Finally, sections were counterstained with hematoxylin and mounted with neutral balsam.

Western Blotting Assay

Tissues were lysed in ice bath for 60 min for protein extraction. The protein was quantified by bicinchoninic acid (BCA) method (Pierce, Rockford, IL, USA). Then the standard curve and absorbance were measured using a microplate reader, and the protein concentration was calculated. After denaturation, the protein was separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), followed by membrane transfer and non-specific site blockage. Afterward, membranes were incubated with primary antibodies (1:1000) and secondary antibodies (1:1000). Chemiluminescent reagent was adopted for color development in the dark, and gel imaging system was utilized for analysis.

QPCR

The total ribonucleic acid (RNA) in cells or tissues was extracted using an RNA extraction kit. RNA was reversely transcribed into complementary DNA (cDNA) by the reverse transcription kit, with a reaction system of 20 μL. Reaction conditions were as follows: reaction at 51°C for 2 min, pre-denaturation at 96°C for 10 min, denaturation at 96°C for 10 s and annealing at 60°C for 30 s, with 40 cycles in total. The relative messenger RNA (mRNA) expressions of IL-1 and VEGF were calculated with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as an internal control. The detailed primer sequences were shown in Table I.

Table I. Primer sequences.

Name	Primer sequence
IL-1	Forward primer: 5' ATGGCAGAAGTACCTAAGCTC 3' Reverse primer: 5' TTAGGAAGACACAAATTGCATGGTGAACTCAGT 3'
VEGF	Forward primer: 5' TGCCCACTGAGGAGTCCAAC 3' Reverse primer: 5' TGGTTCCCGAAACGCTGAG 3'
GAPDH	Forward primer: 5' ACGGCAAGTTCAACGGCACAG 3' Reverse primer: 5' GAAGACGCCAGTAGACTCCACGAC 3'

Statistical Analysis

Statistical Product and Service Solutions 19.0 software (IBM, Armonk, NY, USA) was adopted for statistical analysis, and data were presented as mean ± standard deviation. One-way analysis of variance was performed for normally distributed data followed by Post-Hoc Test (Least Significant Difference). Correlations were analyzed using Pearson correlation analysis. $p<0.05$ suggested the difference was statistically significant.

Results

IL-1 and VEGF Expressions Detected Via Immunohistochemistry

The tissues with positive expressions of IL-1 and VEGF were stained in sepia, and the number of tissues with positive expressions of IL-1 and VEGF in the control group was larger than that in the PCOS group (Figure 1A). Quantitative data showed that PCOS group had remarkably lower positive expressions of IL-1 and VEGF than the control group, and the differences were statistically significant ($p<0.05$) (Figure 1B).

Relative Expressions of IL-1 and VEGF Proteins Detected Via Western Blotting

As shown in Figure 2A, the relative expressions of IL-1 and VEGF in the control group were higher than those in the PCOS group. Quantitative data showed lower expressions of IL-1 and VEGF in the PCOS group compared with those in the control group, displaying statistically significant differences ($p<0.05$) (Figure 2B).

MRNA Expression Detected Via qPCR

PCOS group had notably lower mRNA expressions of IL-1 and VEGF than the control group, with statistically significant differences ($p<0.05$) (Figure 3).

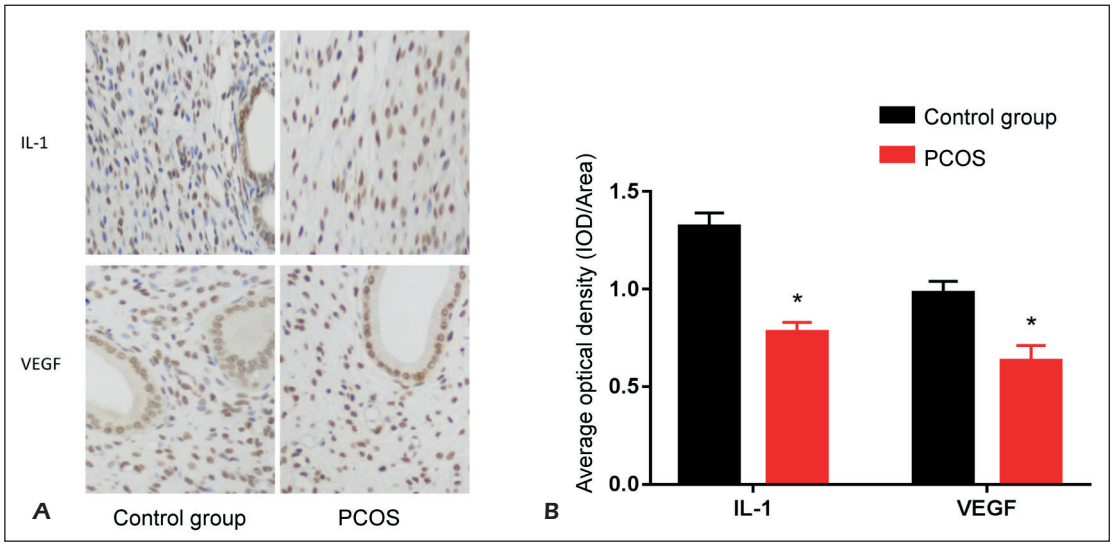

Figure 1. A, IL-1 and VEGF expressions detected via immunohistochemistry (magnification ×200). **B**, Average optical density of IL-1 and VEGF. Note: *$p<0.05$ *vs.* control group.

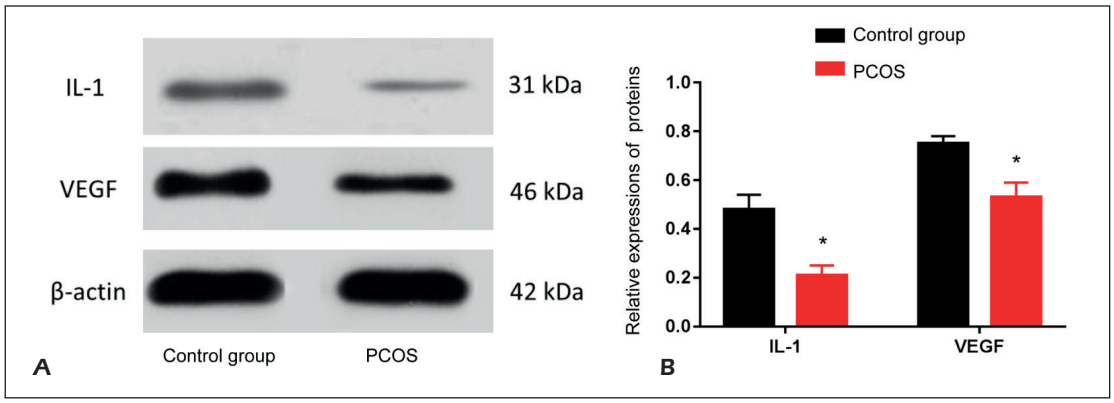

Figure 2. A, Relative protein expressions of IL-1 and VEGF detected via Western blotting. **B**, Relative protein expressions of IL-1 and VEGF. Note: *p<0.05 *vs.* control group.

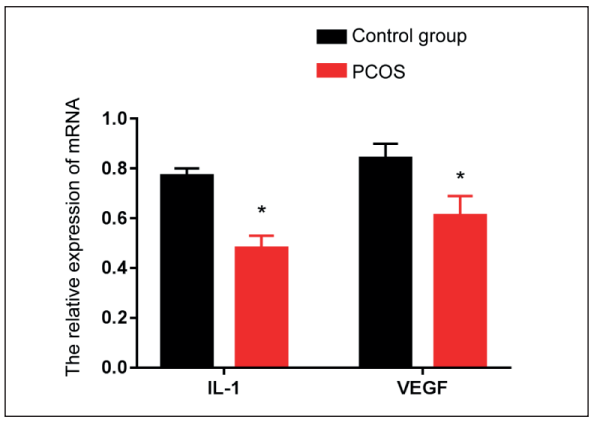

Figure 3. Relative mRNA expressions of IL-1 and VEGF. Note: *p<0.05 *vs.* control group.

Changes in Endometrial Morphology of Rats

Compared with those in the control group, the equivalent diameters, areas of endometrial gland and cavity, the area ratio of gland and stroma and mean thickness of the endometrium declined evidently in the PCOS group, and the differences were statistically significant (p<0.05) (Table II).

Correlation Analysis

IL-1 expression was positively correlated with the thickness of the endometrium (Figure 4A, r=0.899). Besides, VEGF expression also had a positive correlation with the thickness of the endometrium (Figure 4B, r=0.791).

Discussion

Current studies have argued that PCOS is a syndrome of endocrine-metabolic disorders and regulatory mechanism dysfunction caused by abnormality of endocrine regulatory function in the ovaries. The primary clinical symptoms of PCOS include hyperandrogenism, hyperinsulinemia, and insulin resistance. It is one of the main reasons of reproductive endocrine disorders and glucose metabolic disorders in women, which may induce menstrual disorders, infertility and other diseases[6,7]. Relative reports[8,9] indicated that PCOS led to a low level of endometrial receptivity, seriously influencing nidation and success rate of embryo transfer. Therefore, searching for hallmarks for endometrial receptivity is of crucial importance

Table II. Changes in endometrial morphology of rats ($\bar{\chi}$±s).

Group	Gland		Cavity		Area ratio of gland and stroma	Mean thickness of endometrium (μm)
	Equivalent diameter (μm)	Area (μm²)	Equivalent diameter (μm)	Area (μm²)		
Control group	31.88±7.66	69900±47.88	22.63±4.33	27818±27.66	0.54±0.05	78.44±21.22
PCOS group	19.89±5.92*	31893±57.38*	13.78±5.39*	11673±18.99*	0.11±0.07*	48.92±18.77*

Note: *p<0.05 *vs.* control group

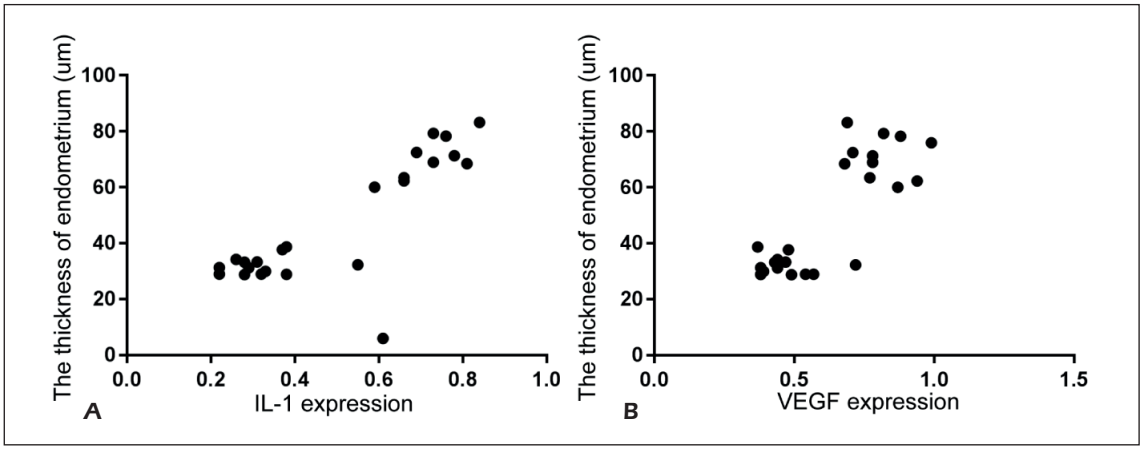

Figure 4. A, Correlation between protein expression of IL-1 and thickness of the endometrium. **B**, Correlation between protein expression of VEGF and thickness of the endometrium.

for the judgment of the endometrial receptivity state, receptivity state monitoring, amelioration of endometrial receptivity and improvement of embryo nidation[10]. It is currently believed that IL-1 is closely associated with the endometrial receptivity. Researches have revealed that IL-1 expression remarkably increases at 7-9 d after ovulation, a period that is also known as implantation window. However, the injection of IL-1 receptor antagonist during the implantation window can effectively block the process of embryo nidation[11,12]. Also, IL-1 plays a vital role in regulating human chorionic gonadotrophin, which is conducive to angiogenesis, thereby accelerating embryo growth[13,14]. Meanwhile, in-depth studies have manifested that IL-1 plays crucial roles not only during the implantation window but also in the construction of endometrial receptivity[15]. Furthermore, VEGF shows a close correlation with the endometrial receptivity and participates in placenta angiogenesis, so it is regarded as an important marker of endometrial receptivity[16,17]. Some scholars have proposed that the abundance of endometrial blood flow influences the endometrial receptivity. As a result, VEGF is presumed to be correlated with the endometrial receptivity[18]. The level of VEGF expression can directly reflect the volume of uterus blood flow. Studies[19,20] have demonstrated that VEGF exerts a specific effect on the vascular endothelial cells of the endometrium, which is capable of promoting the proliferation and migration of vascular endothelial cells and increasing vascular permeability. We believed that VEGF plays crucial roles in endometrial neovascularization.

Conclusions

We observed that both IL-1 and VEGF have a close association with the endometrial receptivity after PCOS, which can serve as therapeutic targets for PCOS and improve endometrial receptivity in the future.

Conflict of Interests

The authors declare that they have no conflict of interest.

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