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Low-grade chronic inflammation in the peripheral blood and ovaries of women with polycystic ovarian syndrome

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ABSTRACT

The purpose of this study was to investigate chronic inflammation in the peripheral blood and ovaries of patients with polycystic ovary syndrome (PCOS). 86 PCOS patients and 50 controls were randomly enrolled in the study. Serum follicle stimulating hormone (FSH), luteinizing hormone (LH), testosterone (T), blood routine test, lipid metabolism index, inflammation cytokines were detected. Ovary samples from PCOS group and control group were collected for macrophage and lymphocyte immunohistochemistry staining. Patients with PCOS showed significantly higher serum CRP, lymphocytes, monocytes, eosinophilic granulocytes, as well as higher triglycerides (TG), TNF-α and IL-6. PCOS ovary had greater number of macrophages and lymphocytes immersed throughout. In conclusion, PCOS patients exhibited hypertriglyceridemia and chronic inflammation, with elevated peripheral lymphocytes, monocytes, and eosinophilic granulocytes. In addition, their ovaries showed persistent chronic inflammation with a larger number of inflammatory cells immersed.

1. Introduction

Polycystic ovarian syndrome (PCOS) affects around 5–10% of premenopausal women [1]. Women with PCOS often suffer from hyperandrogenism and anovulation. A common metabolic component is hyperinsulinemic insulin resistance. Women with PCOS exhibit a 30–40% decrease in insulin sensitivity, comparable to that seen in subjects with type 2 diabetes mellitus, and up to 40% of premenopausal women with PCOS have either impaired glucose tolerance or type 2 diabetes [2]. Insulin resistance which has been associated with an increased incidence of cardiovascular disease and atherosclerosis is now considered to be an inflammatory disorder. Chronic inflammation factors have been correlated with type 2 diabetes mellitus [3]. The only paper examining ovarian inflammation in PCOS patients showed that T-lymphocytes are markedly reduced by 60% in spite of small sample size [4].

In the PCOS ovary, infiltrating and inherent white cells secrete various inflammatory factors to promote follicle growth. Lymphocytes and macrophages were significantly increased in the sheep ovary following laser drilling of the ovarian capsule. This distribution of leukocyte subtypes was examined to determine a possible pattern in patients with PCOS [5]. Migration of leukocytes into the laser-drilled site was observed as early as 6 h after laser drilling and the total number of leukocytes in the site was found to increase up to the 12th day after surgery. In the earlier period, polymorphonuclear leukocytes were the dominant leukocyte subtype, while macrophages and lymphocytes were the major cellular components on the 12th day and later. These results show that the tissue changes in the ovary following laser drilling are consistent with a local inflammatory reaction, suggesting that part of the effectiveness of the laser drilling in patients with PCOS may be attributed to the secretory products of these leukocytes.

Consequently, the low-grade chronic inflammation in the peripheral blood and ovaries of women with PCOS were studied with the aim of contributing to an anti-inflammation therapy for women with this affliction. This study focused on haematological indexes, lipid metabolism and inflammation factors in the peripheral, also inflammation cells immersed in PCOS ovary were investigated. The inter-relationship between inflammation, lipid metabolic disturbance and steroid hormones was evaluated.

2. Materials and methods

The study protocols and consent forms were approved by the Research Ethics Committees of The First Affiliated Hospital of Sun Yat-sen University. Written, informed consent was obtained from each patient before study participation. PCOS was defined according to the Rotterdan criteria: (i) menstrual dysfunction; (ii) hyperan-

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drogenism; (iii) polycystic ovaries and exclusion of other related disorders, such as hyperprolactinaemia, non-classic adrenal hyperplasia or thyroid disease. Patients in control group had regular menstrual cycle and normal ovarian morphology detected by ultrasounds, and undergoing assisted reproductive technology due to male factors like asthenospermia and oligozoospermia.

2.1. Blood samples

86 cases of PCOS patients and 50 control patients were enrolled in the study, with age ranged from 20 to 38 years. Plasma samples were obtained in early follicular period and tested for FSH, LH, T, blood routine test, triglyceride (TG), low density lipoprotein (LDL), high density lipoprotein (HDL), cholesterol, apolipoprotein-A (Apo-A), apolipoprotein-B (Apo-B), fasting blood glucose (FBG), fasting blood insulin (FBI), CRP, TNF-α and IL-6. Steroid hormone (Abbott, USA) were detected by chemiluminescent immunoassay. FBG (Human, Germany) was detected by oxidase colorimetry. TG, LDL and HDL (Japan's First Chemical, Japan) were detected by the enzymic method. Apo-A and Apo-B (Japan's First Chemical, Japan), and CRP (Orion Diagnostica, Finland) were detected by immunity turbidimetry. TNF-α and IL-6 (BioSource, USA) were detected by ELISA.

2.2. Ovary tissue

53 PCOS ovarian samples and 48 control ovary masses from the pathology department were enrolled based on ovarian histological morphology. The patients (21–45 years old) had undergone ovarian wedge resection for treatment of PCOS or a laparoscopic ovary biopsy to diagnose primary infertility during the follicular phase of the menstrual cycle. None of the patients had been prescribed hormones or medications known to influence reproductive function, or had any evidence of infection or inflammation. All the ovary samples were embedded in paraffin for histological studies.

2.3. Immunohistochemistry staining

Paraffin sections (4 μm) were deparaffinized in xylene and dehydrated in descending concentrations of ethanol, antigen retrieval were carried out at 110 °C for 3 min, followed by phosphate-buffered saline rinse. After pretreatment, the sections were blocked with 5% goat serum for 20 min at room temperature and then incubated in a humidified chamber at 37 °C for 1 h with primary antibodies diluted (1:100) in PBS. The primary antibodies were mouse anti-human CD3 antibody (Dako, USA) or mouse anti-human CD14 antibody (Invitrogen, USA). After three times rinse in PBS, biotinylated goat anti-mouse immunoglobulins (ChemMate™ EnVision™ Detection Kit, Dako, USA) were applied to the sections for 30 min at 37 °C. The final reaction product was visualised as a brown stain (3,3′-diaminobenzidine, Dako, USA) and haematoxylin was used as a nuclear counterstain. Negative control sections incubated without primary antibody were uniformly negative. Eight randomly chosen ovarian stroma (magnification 400×) within each slide were counted for positive staining cells by two independent observers

2.4. Statistical analysis

Parametric data were reported as the mean ± SD and the significance of differences was determined by *t*-test; Nonparametric data were reported as medium (range) and the significance of differences was determined by Mann Whitney test. $P < 0.05$ was considered significant.

3. Results

3.1. Chronic inflammation and hyperlipidemia in PCOS patients

Haematological indexes clearly revealed higher chronic inflammation in the PCOS group, as lymphocytes, monocytes and eosinophilic granulocytes were significantly elevated ($P < 0.001$, $P = 0.049$ and $P = 0.002$, separately). Accompany with disturbed haematological indexes, significantly increased plasma inflammatory factors were also found in the PCOS group, such as CRP, TNF-α and IL-6 ($P = 0.001$, $P = 0.043$ and $P < 0.001$ separately). Meanwhile, PCOS patients also showed hypertriglyceridemia which is coincident with simultaneous higher BMI ($P = 0.019$) (Table 1).

3.2. Macrophages and lymphocytes immerse in follicular phase ovaries

Semi-quantitative evaluation for immersed chronic inflammation cell was carried out by two independent observers. Eight randomly chosen ovarian stroma field (by magnification 400×) in each slide were calculated for positive staining cells. PCOS ovaries showed significantly larger number of macrophages immersed [2.292 (0.500–5.600) per field vs. 0.929 (0.0–4.333) per field]. The number of lymphocytes was also inclined to be increased although not statistic significant. The results were summarized in Table 2 and Fig. 1.

Table 1
Comparison of chronic inflammation and dyslipidemia (by *t*-test).

	PCOS group	Control group	*P* Value
Patient characteristics			
Cases	86	50	
Age (y)	29.526 ± 2.818	30.479 ± 3.957	0.124
BMI (kg/m^2)	22.988 ± 6.566	20.816 ± 2.353	0.037
FSH (IU/L)	5.235 ± 1.585	5.821 ± 1.394	0.036
LH (IU/L)	11.188 ± 6.428	3.830 ± 1.853	<0.001
T (ng/mL)	0.805 ± 0.325	0.456 ± 0.183	<0.001
Chronic inflammation			
Leukocyte (10^9/L)	6.879 ± 2.251	6.385 ± 1.480	0.182
Neutrophilic granulocyte (10^9/L)	3.806 ± 1.856	3.908 ± 1.368	0.743
Lymphocyte (10^9/L)	2.524 ± 0.641	1.987 ± 0.578	<0.001
Monocyte (10^9/L)	0.435 ± 0.156	0.382 ± 0.116	0.049
Eosinophilic granulocyte (10^9/L)	0.152 ± 0.144	0.083 ± 0.068	0.002
IL-6 (ng/L)	1.234 ± 1.614	0.513 ± 0.402	<0.001
TNF-α(ng/L)	2.312 ± 1.762	1.751 ± 1.725	0.043
CRP (mg/L)	2.536 ± 2.971	0.959 ± 1.146	0.001
Dyslipidemia			
TG (mmol/L)	1.176 ± 0.735	0.883 ± 0.504	0.019
HDL (mmol/L)	1.444 ± 0.141	1.504 ± 0.310	0.530
LDL (mmol/L)	2.989 ± 0.951	2.888 ± 0.680	0.530
Cholesterol (mmol/L)	4.765 ± 0.956	4.555 ± 0.799	0.222
Apo-A (g/L)	1.567 ± 0.463	1.675 ± 0.357	0.180
Apo-B (g/L)	0.783 ± 0.218	0.759 ± 0.219	0.566
Apo-B/APO-B	2.163 ± 0.887	2.402 ± 0.817	0.140

Table 2
Comparison of chronic inflammation cells immersion in ovarian stroma.

	PCOS group	Control group	*P* Value
Case	35	32	
Age (y)	29.923 ± 3.452	30.26 ± 3.292	0.173
BMI (kg/m^2)	23.413 ± 3.759	22.868 ± 2.484	0.730
Macrophages (/HP)	2.292 (0.500–5.600)	0.929 (0.0–4.333)	0.0013
Lymphocytes (/HP)	1.000 (0.111–3.750)	1.000 (0–2.750)	0.134

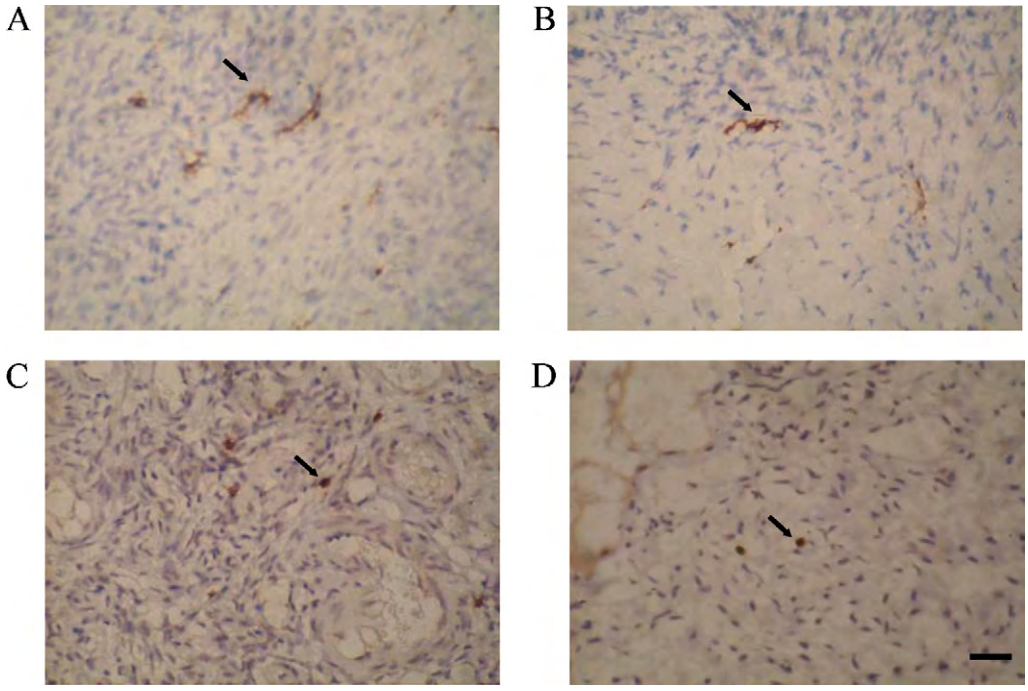

Fig. 1. (A) Macrophage in a PCOS ovary. (B) Macrophage in an ovary of the control group. (C) Lymphocyte in a PCOS ovary. (D) Lymphocyte in an ovary of the control group. The magnification for these figures is 400×, scale bar = 50 μm.

4. Discussion

In the present study, PCOS patients showed higher BMI with hypertriglyceridemia, typical elevated luteinizing hormone and testosterone. Significantly higher serum lymphocytes, monocytes, eosinophilic granulocytes, as well as higher CRP, TNF-α and IL-6 revealed peripheral inflammation condition. In addition, their ovaries showed persistent chronic inflammation with a larger number of inflammatory cells immerse.

Inflammatory cytokines like lymphokine, TNF-α and IL-6 are secreted by lymphocytes and macrophages. These cytokines would in turn activate macrophages and lymphocytes to promote further cytokines secretion and thus get into vicious circle. As TNF-α and IL-6 could also induce insulin resistance, stimulated the production of androgen [6] and cause hypothalamic-pituitary-ovarian axis secretion disorder [7], the increased number of lymphocytes in the present PCOS group could be an initiating factor of chronic inflammation and disturbed hormone spectrum.

Macrophages and lymphocytes are the main white cells immersed in ovary tissue. Their numbers varied within the ovary and along the menstrual cycle. Developing follicles possess just a few, while these cells assembled in progressive atretic follicles' granular and theca layers to the utmost [8]. Activated lymphocytes and macrophages secrete several cytokines that might induce apoptosis, such as IL-1, IL-6, IL-10, IL-12, TNF-α, GM-CSF and IGF-I [9]. Lymphocytes facilitate cytotoxicity, mediate follicle cell apoptosis, and cause follicle atresis. Macrophages phagocytose apoptotic follicular cells. So in present research, elevated macrophages and lymphocytes in the PCOS ovaries could induce cell apoptosis by various cytokines acting on granular and theca cells, consequently dominant follicles cannot be generated.

Inflammation found in peripheral and ovarian in situ might be an initial pathophysiology change with PCOS patients. It further induced insulin resistance and HPO axis dysfunction; also might induce ovarian anovulation by apoptosis.

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