Original Article

Relationship between proinflammatory cytokines and clomiphene resistance in patients with polycystic ovary syndrome

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Background: Polycystic ovary syndrome (PCOS) is a common endocrine disorder in women of childbearing age. We aimed to investigate the correlations between proinflammatory cytokine levels and clomiphene resistance in patients with PCOS.

Methods: Between January 2019 and January 2020, a total of 72 PCOS patients who attended our department were administered clomiphene 50–100 mg/day on days 5–9 of the menstrual cycle to induce ovulation. Patients were divided into a clomiphene-sensitive group (n=42) and a clomiphene-resistant group (n=30). The proinflammatory cytokines interleukin-23 (IL-23), monocyte chemotactic protein-1 (MCP-1), tumor necrosis factor α (TNF-α), angiopoietin-2, and adiponectin were measured by an enzyme-linked immunosorbent assay (ELISA). The receiver-operating characteristic curve was employed to determine the capability of various parameters to distinguish clomiphene resistance. The univariate logistic regression analysis was used to analyze the associations between pro-inflammatory cytokine levels and clomiphene resistance in PCOS.

Results: Serum IL-23 levels showed no statistically significant difference between the clomiphene-sensitive and the clomiphene-resistant groups, whereas MCP-1, TNF-α, angiopoietin-2, and adiponectin levels were significantly different between the two groups (all $P<0.05$). Univariate logistic regression analysis indicated that elevated serum TNF-α [odds ratio (OR) =1.88, $P<0.001$], decreased angiopoietin-2 (OR =0.61, $P=0.007$), and adiponectin (OR =0.39, $P=0.012$) levels were significantly associated with clomiphene resistance. The sensitivity of TNF-α and angiopoietin-2 to distinguish clomiphene resistance in patients with PCOS was 100%. Moreover, the diagnostic efficiency of TNF-α and adiponectin in identifying clomiphene resistance was significantly higher than that of MCP-1 and angiopoietin-2 (all $P<0.05$).

Conclusions: Abnormal TNF-α, angiopoietin-2, and adiponectin levels may be associated with clomiphene resistance in PCOS patients. The serum proinflammatory cytokines TNF-α and adiponectin are helpful discriminators of clomiphene resistance in PCOS patients.

Keywords: Polycystic ovary syndrome (PCOS); clomiphene; proinflammatory cytokine

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Introduction

Polycystic ovary syndrome (PCOS) is a common reproductive system disease in women of childbearing age. It is characterized by oligo-ovulation, excessive androgen, polycystic ovaries, and insulin resistance. PCOS is also the leading cause of anovulatory infertility. Clomiphene is currently the first-line drug of choice for PCOS treatment, inducing the reactive release of gonadotropin-releasing hormones by antagonizing the effect of estrogen to promote ovulation. Although the drug has few adverse reactions, about 15–40% of PCOS patients do not respond to clomiphene treatment. In particular, PCOS patients with obesity, hyperandrogenemia, and insulin resistance are more prone to clomiphene resistance (1).

Interleukin (IL)-23 and monocyte chemotactic protein-1 (MCP-1) can recruit inflammatory cells such as granulocytes, lymphocytes and macrophages to the site of inflammation. Tumor necrosis factor α (TNF-α) is an inflammatory factor secreted by macrophages, which has multiple functions such as preventing epidemic pathogens, killing tumors, and immunomodulating. Angiopoietin-2 can regulate ovarian tissue angiogenesis, and adiponectin can play a physiological role by regulating blood sugar, lipid metabolism and insulin sensitivity. An increasing number of studies have shown that the chronic inflammatory response is an important pathogenesis of PCOS. There are many cytokines that are critical to maintaining the dynamic balance of the hypothalamic-pituitary glandular axis, the normal menstrual cycle, and ovary function. Disordered proinflammatory factor levels in patients with PCOS may be the potential pathogenesis of PCOS (2). Although there have been reports about PCOS and inflammatory response and inflammatory factors, the existing literature mostly only explored a single inflammatory factor, and previous studies mostly involved European and American populations. Therefore, this study intends to explore the correlation between changes in proinflammatory factors and clomiphene resistance in patients with PCOS. We present the following article in accordance with the STARD reporting checklist (available at https://dx.doi.org/10.21037/apm-21-3031).

Methods

Research subjects

We used a retrospective analysis to select 72 patients with PCOS treated with clomiphene in the First Hospital of Lanzhou University between January 2019 and January 2020. The inclusion criteria were as follows: (I) A clear diagnosis of PCOS according to the diagnostic criteria formulated by the endocrinology branch of the Society of Obstetrics and Gynecology of the Chinese Medical Association in 2011; (II) patients aged 20–40 years; (III) patients without diabetes mellitus, evidenced by a fasting plasma glucose level <7 mmol/L and an oral glucose tolerance test (OGTT) 2 hours postprandial blood glucose level <11.1 mmol/L; (IV) exclusion of infertility caused by other factors, such as abnormal sperm, oviduct effusion, etc.; (V) the patient had not taken hormone drugs in the 1 month before blood sampling. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of The First Hospital of Lanzhou University (No. LDYYLL2019-24) and informed consent was taken from all the patients.

Clomiphene administration protocol

The patient was given 50 mg clomiphene from the 5th to the 9th day of the menstrual cycle. Transvaginal B-ultrasound was used to detect ovulation from the 11th day to the 14th day of the menstrual cycle, and the progesterone level was recorded on the 21st day. If the dominant follicle was <1 cm and the blood progesterone level was <5 ng/mL, 100 mg of clomiphene was given at the same time in the next menstrual cycle. If the patient failed to ovulate after three cycles of clomiphene treatment, they were considered clomiphene resistant. Otherwise, they were considered clomiphene sensitive.

Collection of routine clinical and laboratory examination results

The patients' routine clinical and laboratory examination data were collected through the electronic database system, such as age, body mass index, Ferriman-Gallwey score, etc. The day before clomiphene treatment, fasting peripheral venous blood was taken to detect blood glucose, insulin, sex hormone, and high-sensitivity C-reactive protein levels. The steady-state model determined the patient's insulin resistance (IR) according to the formula: IR = fasting blood glucose (mmol/L) × fasting insulin (mIU/mL)/22.5.

Detection of proinflammatory cytokines

The day before clomiphene treatment, IL-23, MCP-1,

TNF-α, angiopoietin-2, and adiponectin were detected by an enzyme-linked immunosorbent assay (ELISA) kit.

Statistical analysis

Normally distributed measurement data were expressed as the mean ± standard deviation and were compared using independent sample *t*-tests. The measurement data that were not normally distributed were expressed as the median (quartile) and were compared using the nonparametric Mann-Whitney U test. Categorical data were expressed as rate or percentage and compared using the chi-square test or Fisher exact probability method. Receiver operating characteristic (ROC) curves, the optimal cut-off value, area under the curve (AUC), and sensitivity and specificity of each index were used to distinguish clomiphene resistance in PCOS patients. Univariate logistic regression was used to determine the relationship between each index and clomiphene resistance in patients with PCOS. A two-tailed P value <0.05 indicated a statistically significant difference.

Results

General clinical and laboratory data of patients

Compared with the clomiphene-sensitive group, the patients in the clomiphene-resistant group had higher Ferriman-Gallwey scores, more sinus follicle counts, and higher luteinizing hormone, cholesterol, and low-density lipoprotein levels (all P<0.05, *Table 1*).

Comparison of serum proinflammatory factors between the clomiphene-sensitive and clomiphene-resistant groups

As shown in *Table 2*, there was no significant difference in serum IL-23 levels between the PCOS clomiphene-resistant and clomiphene-sensitive groups. The levels of MCP-1, TNF-α, angiopoietin-2, and adiponectin showed a significant difference between the two groups (all P<0.05).

Relationship between proinflammatory factors and clomiphene resistance in patients with PCOS

Univariate logistic regression analysis showed that increased serum TNF-α and angiopoietin-2, as well as decreased adiponectin, were significantly correlated with clomiphene resistance in patients with PCOS (all P<0.05, *Table 3*).

Efficacy of proinflammatory factors in differentiating clomiphene resistance in patients with PCOS

As shown in *Table 4* and *Figure 1*, the sensitivity of TNF-α and angiopoietin-2 to distinguish clomiphene resistance in patients with PCOS was 100% [95% confidence interval (CI): 88.4–100.0%]. In addition, the AUC of TNF-α and angiopoietin-2 was significantly higher than that of MCP-1 and angiopoietin-2 in PCOS patients with or without clomiphene resistance (all P<0.05).

Discussion

The pathogenesis of PCOS is complex. Some believe that a chronic inflammatory response is the core mechanism driving the occurrence and development of PCOS (3). For example, many studies have found that inflammatory indexes, such as the C-reactive protein and leukocyte count, are significantly elevated in PCOS patients (4). Although clomiphene, an estrogen antagonist, is the classic drug for PCOS-related infertility treatment, some patients have no response to it. Given that pro-inflammatory cytokines play an important role in the pathogenesis of PCOS, we aimed to verify the hypothesis that levels of serum proinflammatory factors could be related to clomiphene resistance in patients with PCOS in this study.

The comparison of clinical and laboratory results between the clomiphene sensitive and control groups indicated significant differences in Ferriman-Gallwey scores, sinus follicle counts, luteinizing hormone, and cholesterol levels. The results are consistent with the research conclusions of Sachdeva *et al.* (5). Their team also found that the general sinus follicle count and luteinizing hormone were significantly higher in PCOS patients with clomiphene resistance than those sensitive to clomiphene (6).

IL-23 and MCP-1 are classic pro-inflammatory factors, which are significantly elevated in the body's chronic inflammatory response. IL-23 is mainly secreted by macrophages, dendritic cells, and endothelial cells and can promote the activation of Th1 cells and enhance the activity of CD8 + T cells and natural killer cells. Previous studies have shown that IL-23 is significantly increased in patients with PCOS (7). In this study, there was no significant difference in IL-23 between clomiphene-resistant and clomiphene-sensitive PCOS patients, suggesting that IL-23 is irrelevant to clomiphene resistance. The primary biological function of MCP-1 is to recruit monocytes,

Table 1 Comparison of general clinical and laboratory data between clomiphene-resistant and clomiphene-sensitive groups

Indexes	Clomiphene-resistant group (n=30)	Clomiphene-sensitive group (n=42)	*t*	P
Age (years)	28.67±4.07	29.07±4.66	–0.38	0.70
Body mass index (kg/m^2)	26.38±3.13	23.99±4.40	2.69	0.009
Waist/hip ratio	0.88±0.07	0.86±0.06	1.33	0.19
Ferriman-Gallwey score	15.51±2.69	12.31±2.49	5.21	<0.001
Sinus follicle count (n)	13.83±2.84	11.45±2.83	3.51	0.001
Testosterone (nmol/L)	2.30±1.03	2.20±0.84	0.48	0.63
Follicle stimulating hormone (mIU/mL)	5.63±1.76	5.29±2.60	0.66	0.52
Luteinizing hormone (mIU/mL)	16.10±5.03	11.47±4.58	4.06	<0.001
HOMA-IR	2.45±1.28	1.97±1.31	1.54	0.13
Triglyceride (mmol/L)	1.57±0.67	1.44±0.33	1.07	0.29
Cholesterol (mmol/L)	5.01±0.83	3.69±0.85	6.54	<0.001
Low density lipoprotein (mmol/L)	3.10±0.72	2.71±0.47	2.61	0.01
High density lipoprotein (mmol/L)	1.14±0.18	1.32±0.21	–3.86	<0.001
OGTT result				
0 h glucose	5.15±0.35	5.17±0.27	–0.28	0.78
1 h glucose	9.00±3.13	8.94±3.07	0.07	0.94
2 h glucose	7.85±2.18	6.98±3.06	1.41	0.16
0 h serum insulin (uIU/mL)	9.48±3.27	9.66±3.97	–0.21	0.84
1 h serum insulin (uIU/mL)	80.81±21.22	90.22±22.36	–1.80	0.08
2 h serum insulin (uIU/mL)	62.08±11.10	67.63±18.22	–1.60	0.11

OGTT, Glucose tolerance test; HOMA-IR, the homeostasis model assessment of insulin resistance.

Table 2 Comparison of serum proinflammatory factors between PCOS clomiphene-resistant and clomiphene-sensitive groups

Indexes	Clomiphene-resistant group (n=30)	Clomiphene-sensitive group (n=42)	*t*	P
IL-23 (ng/L)	42.32±10.05	49.56±21.19	–1.93	0.06
MCP-1 (ng/mL)	125.16±62.55	102.22±47.34	1.69	0.10
TNF-α (ng/L)	53.29±4.06	45.30±5.30	6.94	<0.001
Angiopoietin-2 (ng/L)	3387.15±1041.72	4747.85±1615.87	–4.05	<0.001
Adiponectin (ng/mL)	12,36±1.99	16.65±2.59	–7.61	<0.001

IL-23, interleukin-23; MCP-1, monocyte chemotactic protein-1; TNF-α, tumor necrosis factor α.

neutrophils, and lymphocytes to the effector site to produce an inflammatory response. A recent meta-analysis of 11 studies including 529 PCOS patients showed that the level of MCP-1 was significantly increased in PCOS patients, with no significant difference between obese and non-obese PCOS patients (8).

TNF-α is a cytokine produced in the acute phase of the body's inflammatory response. Serum TNF-α is significantly elevated in patients with PCOS and is related to hyperandrogenemia and insulin resistance (9). A meta-analysis of 1,046 patients with PCOS and 914 controls (10) also confirmed that TNF-α was elevated in patients with

Table 3 Univariate analysis of serum proinflammatory factor level and clomiphene resistance in patients with PCOS

Indexes	OR (95% CI)	P
MCP-1 (ng/mL)	1.04 (0.94–1.43)	0.10
TNF-α (ng/L)	1.88 (1.45–3.02)	<0.001
Angiopoietin-2 (ng/L)	0.61 (0.27–0.89)	0.007
Adiponectin (ng/mL)	0.39 (0.21–0.59)	0.012

CI, confidence interval; OR, odds ratio; MCP-1, monocyte chemotactic protein-1; TNF-α, tumor necrosis factor α.

Table 4 ROC curve results of serum MCP-1, TNF-α, angiopoietin-2, and adiponectin in distinguishing clomiphene resistance in patients with PCOS

Indexes	Cut-off	AUC (95% CI)	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)
MCP-1	129.7 ng/mL	0.65 (0.53–0.76)	53.3 (34.3–71.7)	78.6 (63.2–89.7)
TNF-α	46.4 ng/L	0.94 (0.86–0.98)	100 (88.4–100.0)	76.2 (60.5–87.9)
Angiopoietin-2	4,869.4 ng/L	0.75 (0.64–0.85)	100 (88.4–100.0)	47.6 (32.0–63.6)
Adiponectin	13.9 ng/mL	0.92 (0.83–0.97)	83.3 (65.3–94.4)	90.5 (77.4–97.3)

AUC, area under curve; CI, confidence interval; Cut-off, optimum cut-off value; MCP-1, monocyte chemotactic protein-1; TNF-α, tumor necrosis factor α.

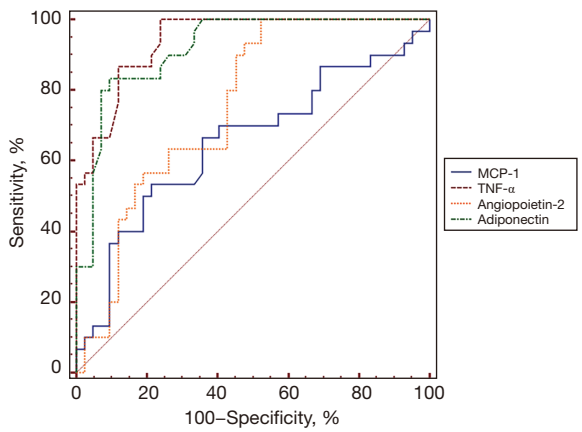

Figure 1 ROC curve results of serum MCP-1, TNF-α, angiopoietin-2, and adiponectin in distinguishing clomiphene resistance in patients with PCOS. ROC, receiver operating characteristic curve; MCP-1, monocyte chemotactic protein-1; TNF-α, tumor necrosis factor α; PCOS, Polycystic ovary syndrome.

PCOS. Seyam *et al.* showed that serum TNF-α in patients with clomiphene resistance is a potential indicator of the inflammatory state of PCOS patients (11). Similarly, the results of this study also showed that TNF-α is related to clomiphene resistance in PCOS and is the most effective discriminator of clomiphene resistance in PCOS patients.

Proinflammatory factor angiopoietin-2 plays a role in regulating the angiogenesis of tissues and organs (12). Under normal physiological conditions, the level of angiopoietin-2 increases gradually with the increase of follicles. When follicles mature, the level of angiopoietin-2 gradually decreases, suggesting that it has a vital role in follicle development (13). Previous animal models and human studies have shown that angiopoietin-2 was significantly reduced in PCOS, resulting in a significant increase in ovarian vascular density (14,15). In this study, the level of angiopoietin-2 in clomiphene-resistant PCOS patients was significantly lower than that of clomiphene-sensitive PCOS patients. Rajendiran *et al.* (16) also showed that the level of serum angiopoietin-2 in patients with PCOS was an independent risk factor for clomiphene resistance.

Adiponectin is a cytokine secreted by adipocytes and has a physiological role in regulating blood glucose, lipid metabolism, and insulin sensitivity. After controlling for confounding factors, Toulis *et al.* found that the serum adiponectin level in patients with PCOS was significantly lower than that of normal controls (17). Wang and colleagues found that serum adiponectin levels could predict the sensitivity of PCOS patients to clomiphene (18), which is consistent with the results of this study.

In conclusion, this study explored the correlation between serum proinflammatory factor levels and clomiphene sensitivity in patients with PCOS. The results indicated that the imbalance of serum TNF-α, angiopoietin-2, and adiponectin expression might be significantly related to clomiphene resistance in patients with PCOS. The serum proinflammatory factors TNF-α and adiponectin are helpful in identifying clomiphene resistance in patients with PCOS. In addition, targeted regulation of these cytokines may contribute to the personalized treatment of PCOS.

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Footnote

Reporting Checklist: The authors have completed the STARD reporting checklist. Available at https://dx.doi.org/10.21037/apm-21-3031

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://dx.doi.org/10.21037/apm-21-3031). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of The First Hospital of Lanzhou University (No. LDYYLL2019-24), and all patients gave written informed consent.

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