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Effect of metformin combined with clomiphene on insulin resistance, oxidative stress response and T cell immune response in patients with PCOS

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ABSTRACT

Objective: To study the effect of metformin combined with clomiphene on insulin resistance, oxidative stress response and T cell immune response in patients with polycystic ovarian syndrome (PCOS). **Methods:** A total of 94 patients who were diagnosed with PCOS in Jingzhou Second People's Hospital between September 2014 and October 2016 were selected and randomly divided into the combined group who received the metformin combined with clomiphene therapy and the control group who received clomiphene therapy. The insulin resistance, oxidative stress response and T cell immune response were evaluated before treatment and 3 menstrual cycles after treatment. **Results:** 3 menstrual cycles after treatment, HOMA-IR level, serum F-Ins, F-CP, TOS, MDA, AOPP and IL-17 contents as well as peripheral blood ROR γ t mRNA expression of combined group were significantly lower than those before treatment while HOMA-β level, serum TAS, SOD, GSH-Px, VitC, VitE, IL-10 and TGF-β 1 contents as well as peripheral blood Foxp3 mRNA expression were significantly higher than those before treatment; HOMA-IR and HOMA-β levels, serum F-Ins, F-CP, TOS, MDA, AOPP, IL-17, TAS, SOD, GSH-Px, VitC, VitE, IL-10 and TGF-β 1 contents as well as peripheral blood Foxp3 and ROR γ t mRNA expression of control group were not different from those before treatment. **Conclusion:** Metformin combined with clomiphene can significantly improve the insulin resistance, oxidative stress response and T cell immune response in patients with PCOS.

1. Introduction

Polycystic ovary syndrome (PCOS) is a common endocrine disease in women of childbearing age, which is characterized by changes in menstruation, infertility, obesity and polytrichia. The luteal function decline and the follicular development damage are the important pathological features of PCOS[1]. Clomiphene is a drug that promotes ovulation and improves luteal function, and it can effectively correct the endocrine metabolism disorder when used for the treatment of PCOS[2]. In recent years, studies about PCOS suggest that there is obvious insulin resistance in patients with PCOS, and the activation of oxidative stress and the T cell-mediated immune response disorder are closely related to insulin resistance and impaired follicular development[3]. Metformin is an insulin sensitizer for type 2 diabetes, which can effectively improve insulin resistance[4,5]. In the following studies, we analyzed the effects of metformin combined with clomiphene on insulin resistance, oxidative stress response and T-cell immune response in PCOS patients.

2. Case information and research methods

2.1 General information of PCOS patients

A total of 94 patients who were diagnosed with PCOS in Jingzhou Second People's Hospital between September 2014 and October 2016 were selected, all patients conformed to the diagnostic criteria for PCOS, FSH 2.0 nmol/L or testosterone 2.2 nmol/L on the 2-4 d of menstruation period or without dominant follicle during amenorrhea, and B ultrasound indicated that there were more than

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12 small follicles of 2-9 mm within ovaries. The 94 enrolled patients were divided into two groups by random number table, 47 cases in each group. The combined group received metformin combined with clomiphene therapy, was 25-38 years old, and was with (14.8±1.9) small follicles; control group received clomiphene therapy, were 25-39 years old, and were with (14.5±1.7) small follicles. There was no statistically significant difference in general information between the two groups (P>0.05).

2.2 Therapy

Both groups of patients received clomiphene therapy, and the method was as follows: taking clomiphene 50-100 mg orally from the 5th day of the menstrual cycle for five consecutive days, and the treatment lasting for 3 menstrual cycles; the combined group received metformin therapy on the basis of clomiphene therapy, and the method was as follows: taking metformin tablet 500 mg orally, 3/d, for 3 menstrual cycles in a row.

2.3 Insulin resistance evaluation

Before treatment and 3 menstrual cycles after treatment, the oral glucose tolerance test was conducted respectively, the fasting venous blood as well as the venous blood 1 h and 2 h after the oral glucose was extracted to detect the contents of glucose, insulin, C-peptide, and the HOMA-IR and HOMA-β index were calculated.

2.4 Oxidative stress evaluation

Fasting venous blood before treatment and 3 menstrual cycles after treatment was taken, the radioimmunoprecipitation kits were used to determine TOS, MDA, AOPP, TAS, SOD and GSH-Px levels, and liquid chromatograph was used to detect the contents of VitC and VitE.

2.5 T cell immune response evaluation

Fasting venous blood before treatment and 3 menstrual cycles after treatment was taken, and the contents of IL-17, IL-10 and TGF-β1 were determined by enzyme-linked immunosorbent assay kit; fasting venous blood before treatment and 3 menstrual cycles after treatment was taken and cetrifuged to get mononuclear cells, and the mRNA expression of Foxp3 and ROR γt were determined by the fluorescence quantitative PCR kit.

2.6 Statistical methods

SPSS 21.0 software was used to input data, data between two groups were by t test and P<0.05 indicated statistical significance in differences.

3. Results

3.1 Insulin resistance

Before treatment and 3 menstrual cycles after treatment, analysis of insulin resistance indexes HOMA-IR, HOMA-β, F-Ins and F-CP between two groups of patients was as follows: HOMA-IR and HOMA-β levels as well as F-Ins and F-CP contents were not significantly different between two groups of patients before treatment (P>0.05); HOMA-IR level as well as F-Ins and F-CP contents of combined group 3 menstrual cycles after treatment were significantly lower than those before treatment while HOMA-β level was significantly higher than that before treatment (P<0.05); HOMA-IR and HOMA-β levels as well as F-Ins and F-CP contents of control group 3 menstrual cycles after treatment were not different from those before treatment (P>0.05).

3.2 Oxidative stress indexes

Before treatment and 3 menstrual cycles after treatment, analysis of serum oxidative stress products TOS (nmol/L), MDA (μmol/L) and AOPP (μmol/L) between two groups of patients was as follows: serum TOS, MDA and AOPP contents were not significantly different between two groups of patients before treatment (P>0.05); serum TOS, MDA and AOPP contents of combined group 3 menstrual cycles after treatment were significantly lower than those before treatment (P<0.05); serum TOS, MDA and AOPP contents of control group 3 menstrual cycles after treatment were not different from those before treatment (P>0.05).

Table 1.
Insulin resistance of two groups of patients before and after treatment.

Groups	n	Time	HOMA-IR	HOMA-β	F-Ins	F-CP
Combined group	47	Before treatment	3.91±0.47	132.16±17.72	11.52±1.77	1.48±0.20
		After treatment	2.45±0.35*#	177.52±20.35*#	6.72±0.93*#	0.92±0.12*#
Control group	47	Before treatment	3.88±0.45	133.03±15.87	11.88±1.47	1.51±0.22
		After treatment	3.83±0.42	132.73±14.51	11.60±1.48	1.47±0.18

*: comparison between before and after treatment, *P<0.05; #: comparison between combined group and control group, P<0.05.

Table 2.
Comparison of oxidative stress products between two groups of patients before and after treatment.

Groups	n	Time	TOS	MDA	AOPP
Combined group	47	Before treatment	18.02±2.26	8.29±1.05	66.82±8.93
		After treatment	13.48±1.79*#	4.87±0.57*#	40.29±5.28*#
Control group	47	Before treatment	17.97±2.06	8.41±0.98	67.15±8.29
		After treatment	17.61±1.98	8.26±0.91	66.51±8.03

*: comparison between before and after treatment, *P<0.05; #: comparison between combined group and control group, P<0.05.

Table 3.
Comparison of anti-oxidation indexes between two groups of patients before and after treatment.

Groups	*n*	Time	TAS	SOD	GSH-Px	VitC	VitE
Combined group	47	Before treatment	1.94±0.22	72.84±8.95	113.42±15.39	1.82±0.22	1.61±0.19
		After treatment	2.74±0.35*#	107.29±13.51*#	156.72±19.31*#	2.62±0.37*#	2.45±0.33*#
Control group	47	Before treatment	1.97±0.25	73.11±8.28	112.74±13.69	1.84±0.19	1.59±0.15
		After treatment	2.01±0.22	73.51±8.93	112.93±12.17	1.81±0.25	1.64±0.20

*: comparison between before and after treatment, *$P<0.05$; #: comparison between combined group and control group, $P<0.05$.

Table 4.
Comparison of T cell immunity indexes between two groups of patients before and after treatment.

Groups	*n*	Time	Foxp3	ROR γ t	IL-17	IL-10	TGF-β 1
Combined group	47	Before treatment	1.02±0.15	0.98±0.13	1.93±0.22	85.1±10.2	2.38±0.34
		After treatment	2.66±0.32*#	0.37±0.05*#	1.04±0.13*#	213.5±33.9*#	6.51±0.83*#
Control group	47	Before treatment	1.01±0.12	1.02±0.15	1.99±0.25	86.2±9.5	2.42±0.31
		After treatment	1.03±0.15	0.97±0.12	1.91±0.21	85.7±10.1	2.35±0.31

*: comparison between before and after treatment, *$P<0.05$; #: comparison between combined group and control group, $P<0.05$.

Before treatment and 3 menstrual cycles after treatment, analysis of serum anti-oxidation indexes TAS (mmol/L), SOD (U/mL), GSH-Px (U/mL), VitC (nmol/L) and VitE (nmol/L) contents between two groups of patients was as follows: serum TAS, SOD, GSH-Px, VitC and VitE contents were not significantly different between two groups of patients before treatment ($P>0.05$); serum TAS, SOD, GSH-Px, VitC and VitE contents of combined group 3 menstrual cycles after treatment were significantly higher than those before treatment ($P<0.05$); serum TAS, SOD, GSH-Px, VitC and VitE contents of control group 3 menstrual cycles after treatment were not different from those before treatment ($P>0.05$).

3.3 T cell immunity indexes

Before treatment and 3 menstrual cycles after treatment, analysis of peripheral blood Foxp3 and ROR γ t expression as well as serum IL-17 (ng/mL), IL-10 (pg/mL) and TGF-β 1 (ng/mL) contents between two groups of patients was as follows: peripheral blood Foxp3 and ROR γ t expression as well as serum IL-17, IL-10 and TGF-β 1 contents were not significantly different between two groups of patients before treatment ($P>0.05$); peripheral blood Foxp3 mRNA expression as well as serum IL-10 and TGF-β 1 contents of combined group 3 menstrual cycles after treatment were significantly higher than those before treatment while peripheral blood ROR γ t mRNA expression and serum IL-17 content were significantly lower than those before treatment ($P<0.05$); peripheral blood Foxp3 and ROR γ t expression as well as serum IL-17, IL-10 and TGF-β 1 contents of control group 3 menstrual cycles after treatment were not different from those before treatment ($P>0.05$).

4. Discussion

Insulin resistance is the pathological characteristic of PCOS discovered in recent years[6], persistent insulin resistance may cause compensatory hyperinsulinemia, and insulin can on the one hand, act directly on the follicle cells and promote the secretion of androgen[7], and on the other hand, induce androgen synthesis through the hypothalamus-pituitary-ovarian axis[8,9]. Clomiphene is a common drug for treating PCOS, which has the effects of promoting ovulation and improving luteal function, but cannot significantly improve insulin resistance. Metformin is a common clinical insulin sensitizer, which can increase insulin sensitivity and improve insulin resistance when used for type 2 diabetes. In recent years, the value of metformin for PCOS therapy has also received more and more attention[10]. In order to define the effect of metformin combined with clomiphene on the degree of insulin resistance in patients with PCOS, the insulin resistance and sensitivity-related indexes were analyzed in the stduy, and the results showed that HOMA-IR level as well as F-Ins and F-CP contents of combined group significantly decreased while HOMA-β level significantly increased after treatment; HOMA-IR and HOMA-β levels as well as F-Ins and F-CP contents of control group did not change significantly after treatment. It means that clomiphene monotherapy has no obvious improvement on the insulin resistance in patients with PCOS, and metformin combined with clomiphene therapy can effectively improve the insulin resistance and hyperinsulinemia, and reduce insulin levels in patients with PCOS.

There is a close relationship between insulin resistance and oxidative stress in PCOS patients, and persistent insulin resistance can affect glucose metabolism, and activate oxidative stress and increase oxygen free radical generation to a certain extent; oxygen free radical action on follicle cells can cause cell damage and ovarian function damage[11]. TOS is the total peroxide in the blood circulation, which can reflect the production of oxygen free radicals; MDA and AOPP are the oxidation reaction products of lipid and proteins in cells[12,13]. In the study, analysis of the changes in serum contents of oxidative stress products before and after treatment indicated that serum TOS, MDA and AOPP contents of combined group significantly decreased after treatment; serum TOS, MDA and AOPP contents of control group did not change significantly after treatment. During the continuous generation of oxygen free radicals, the enzyme antioxidants SOD and GSH-Px and non-enzyme antioxidants VitC and VitE in the body will be gradually consumed, which is characterized by the weakening of total antioxidant capacity and the declining of TAS content[14,15]. Further analysis of the changes of serum anti-oxidation indexes before and after treatment showed that serum TAS, SOD, GSH-Px, VitC and VitE contents of combined group significantly increased after treatment; serum TAS, SOD, GSH-Px, VitC and VitE contents of control group did

not significantly change after treatment. It means that clomiphene monotherapy has no obvious improvement on the oxidative stress in patients with PCOS, and metformin combined with clomiphene therapy can effectively improve the oxidative stress reaction, reduce the formation of oxidation products and enhance the antioxidant capacity in patients with PCOS.

The immune response disturbance mediated by T cells plays an important role in the occurrence and aggravation of insulin resistance. Th17 and Treg are important T cell subgroups, which maintain dynamic balance under physiological conditions[16]. Th17 is a cell mass that specifically expresses transcription factor ROR γt, and it can synthesize and secrete IL-17, induce inflammatory response and cause insulin resistance; Treg is a cell mass that specifically expresses the transcription factor Foxp3, and it can synthesize and secrete inhibitory cytokines such as IL-10 and TGF-β1, thus inhibit the differentiation and maturation of IL-17 and reduce insulin resistance[17,18]. In the study, analysis of peripheral blood Th17 and Treg transcription factor expression before and after the treatment showed that peripheral blood Foxp3 mRNA expression of combined group significantly increased while ROR γt mRNA expression significantly decreased after treatment; peripheral blood Foxp3 and ROR γt expression of control group did not change significantly after treatment. This indicates that clomiphene monotherapy has no obvious improvement on the balance of Treg/Th17 in patients with PCOS, and metformin combined with clomiphene therapy can effectively regulate the differentiation of Treg and Th17 in patients with PCOS. Further analysis of the changes in serum contents of Th17 and Treg-related cytokines showed that serum IL-10 and TGF-β1 contents of combined group significantly increased while IL-17 content significantly decreased after treatment; serum IL-17, IL-10 and TGF-β1 contents of control group did not change significantly after treatment. This further confirms that metformin combined with clomiphene can effectively regulate the balance of Treg/Th17 in patients with PCOS.

Above all, it is believed that metformin combined with clomiphene therapy for PCOS can significantly improve insulin resistance, reduce oxidative stress, reduce oxidation product generation and enhance antioxidant capacity, and it can also regulate T cell immune response and correct Th17/Treg imbalance.

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