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Original Article

Clinical efficacy of metformin combined with clomiphene in patients with polycystic ovary syndrome and their effect on serum sex hormones

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Abstract: Objective: To analyze the effect of metformin combined with clomiphene in patients with polycystic ovary syndrome (PCOS) and their effect on serum sex hormone levels. Methods: A total of 69 patients with PCOS treated in The People's Hospital of Yichun City from August 2016 to July 2017 were enrolled, and randomized into the clomiphene treated group (group A, n=35) and metformin plus clomiphene treated group (group B, n=34). The general clinical data of the patients were observed and recorded. The fasting serum levels of serum follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), and pituitary prolactin (PRL) were measured by ELISA. The indicators of glucose metabolism, including fasting insulin (FIN), fasting plasma glucose (FPG), homeostasis model assessment of insulin resistance (HOMA-IR), and leptin levels were also measured. At the same time, the pregnancy rate, ovulation rate, abortion rate of early pregnancy and clinical efficacy after treatments were compared between the two groups. Results: The post-treatment levels of FSH and E2 were 6.71±0.86 IU/L and 72.96±6.83 ng/L respectively in group B, and significantly higher compared to those in group A (both $P<0.05$). The LH and PRL levels in group B after treatment were 7.14±0.58 IU/L and 13.21±1.78 μg/L respectively, and were significantly lower compared to those in group A (both $P<0.05$). No significant changes were seen in the pre-treatment FIN, FPG, Leptin, and HOMA-IR between group B and group A (all $P>0.05$). After treatment, all indicators in group B were significantly lower than those in group A (all $P<0.05$; 12.31±0.86 IU/mL, 4.17±0.58 mmol/L, 3.96±0.47 ng/mL and 2.47±6.83, respectively). After treatment, the pregnancy rate and ovulation rate in group B were 55.9% and 73.5% respectively, higher than those in group A (both $P<0.05$). The abortion rate of early pregnancy in group B was 2.9% after treatment, which was not significantly different when compared to that in group A ($P>0.05$). The total number of effectively treated patients in group B was 32, making an effective rate of 94.1%, which was significantly higher than that in group A ($P<0.05$). Conclusion: Metformin combined with clomiphene have good clinical efficacy in patients with PCOS, improve the patients' endocrine function of sex hormones, and increase the ovulation rate and pregnancy rate.

Keywords: Metformin, clomiphene, polycystic ovary syndrome

Introduction

Polycystic ovary syndrome (PCOS) is a gynecological disease with abnormal fertility due to endocrine disorders. The clinical symptoms include polycystic ovaries, abnormal ovulation, hyperandrogenemia and insulin resistance. PCOS incidence is around 12%, affecting mostly women of childbearing age and seriously affecting their physical and mental health [1]. The secretions of sex hormones are abnormal in PCOS patients, with the follicular cells secreting more androgens which inhibit follicular maturation and reduce ovulation, and also induce hirsutism or acne [2].

Reddy et al. found that most PCOS patients had higher insulin levels, which interfered with glucose and lipid metabolism, and further aggravated endocrinal disorders in the patients [3]. Clomiphene is a common ovulation inducing drug which interferes with the negative feedback regulation of estrogen on hypothalamus and thus promotes ovulation [4]. Metformin can

improve insulin resistance, relieve glucose metabolism disorder in PCOS patients, and inhibit androgen secretion by follicular cells [5]. Verma et al. found that administration of clomiphene alone did not significantly alleviate insulin resistance in patients with PCOS, and also led to the thinning of endometrium in some patients which adversely affected reproductive function [6]. Clinical studies have shown that clomiphene combined with metformin can reduce endocrine imbalance and increase pregnancy rate in the treatment of patients with PCOS [7]. Since insulin resistance is an important complication of PCOS, alleviating it by metformin may improve the clinical outcome in PCOS patients.

The aim of this study was to analyze the clinical efficacy of metformin combined with clomiphene in treating PCOS.

Materials and methods

General data

This study was approved by the Ethics Committee of The People's Hospital of Yichun City, and all patients signed the informed consents. A total of 69 women with PCOS who were treated in The People's Hospital of Yichun City from August 2016 to July 2017 were enrolled. The patients were randomized into the clomiphene treated group (group A; n=35, aged 24-31 years with an average age 27.59±2.46 years) and metformin plus clomiphene treated group (group B; n=34, aged 25-32 years with an average age 26.48±3.12 years).

Inclusion criteria: (1) Women, aged 24 to 35 years; (2) hyperandrogenism; (3) less ovulation; (4) the number of follicles in the ovary was higher than 12 or the ovarian volume was significantly larger. The patients who met three of the above criteria were considered as PCOS [8].

Exclusion criteria: (1) Patients complicated with other gynecological diseases such as tubal blockage, uterine fibroids; (2) abnormal liver and kidney function; (3) patients with medication allergy.

Treatment methods

The patients in group A started clomiphene on the fifth day of the first menstrual cycle at 50 mg/d, for 3 menstrual cycles (Shanghai Hengshan Pharmaceutical Co., Ltd., China) [9]. Group B patients started metformin (US Bristol-Myers Squibb Pharmaceuticals) on the first day of menstruation thrice a day at 0.5 g/time for 3 menstrual cycles, and added clomiphene (50 mg/d) on the 5th day of the 4th menstrual cycle for 5 consecutive days, for 3 menstrual cycles [10]. There was no significant effect of the taking time of clomiphene on the efficacy evaluation of PCOS patients. The ovulation in both groups was detected regularly by ultrasound. If a patient did not menstruate for 45 consecutive days during the course of treatment, her serum human chorionic gonadotropin levels should be detected immediately; if negative, the patient was prescribed progesterone capsules (100 mg/d, for 5 consecutive days). The treatment was continued when withdrawal bleeding occurred, and immediately discontinued if the patient became pregnant during the course of treatment.

Observation indicators and detection methods

The primary observation indicators were serum levels of follicular stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL) and estradiol (E2). Commercially available ELISA kits were used to measure the hormone levels: FSH-Beijing North Institute of Biotechnology, China; LH-Nanjing JianCheng Bioengineering Institute, China; PRL-Shanghai Dahao Biotechnology Co. Ltd., China; E2-Shanghai Hengyuan Biotechnology Co. Ltd., China [4]. On the third day of a patient's menstrual cycle, 6 mL venous blood was collected intravenously and placed at 4°C for 1 h, centrifuged at 1,200 r/min for 15 min, and the serum was collected. The hormone standards were serially diluted across a gradient, and the serum samples were diluted 1:200. Sixty microliters of standard sample were added per well, and the plates were incubated at 37°C for 1 h; after washing three times with PBS, 80 μL substrate solution was added. The wells were washed again with PBS, and the reaction was stopped with the termination solution. After letting the plate sit for 0.5 h, OD of each well was measured at 450 nm with a microplate reader. Each test sample was set in triplicates, and the standard curves for each hormone were plotted to calculate their respective concentration in the samples. The related indicators of patient's glucose metabolism were measured by automatic biochemical detector (Beckman Coulter Company, USA). The

Table 1. Comparisons of general clinical data between two groups of patients

Group	Group A (n=35)	Group B (n=34)	t	P
Age (year)	27.59±2.46	26.48±3.12	2.374	0.082
BMI (kg/m^2)	24.18±1.37	24.87±1.43	3.153	0.071
Course of disease (year)	3.76±0.51	4.03±0.63	3.242	0.063
WHR	0.84±0.06	0.81±0.03	2.159	0.091
Infertile years (year)	3.12±0.17	3.23±0.14	2.439	0.076

Note: BMI, body mass index; WHR, waist-to-hip ratio.

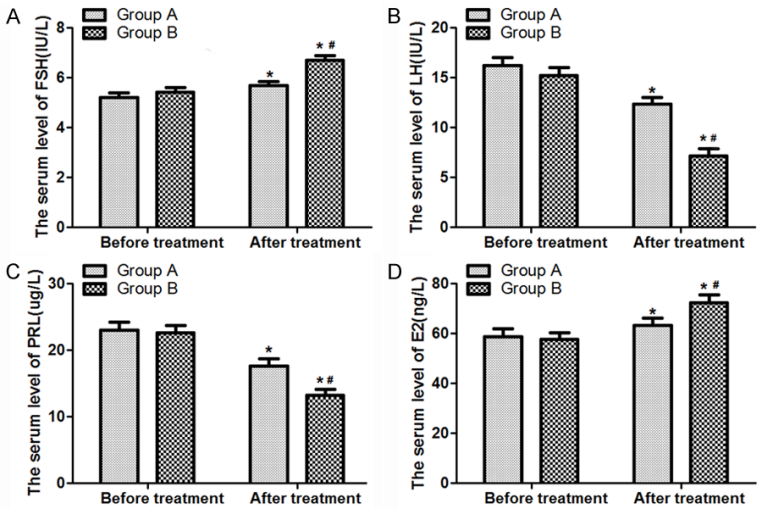

Figure 1. Serum sex hormone levels after treatment in both groups. A: Changes in serum FSH levels in both groups; B: Changes in serum LH levels in both groups; C: Changes in serum PRL levels in both groups; D: Changes in serum E2 levels in both groups. Compared with those before treatment, *P<0.05; compared with group A, #P<0.05. FSH, follicle-stimulating hormone; LH, luteinizing hormone; E2, estradiol; PRL, pituitary prolactin.

fasting plasma glucose (FPG) was detected by the hexokinase method, and the fasting serum insulin (FINS) was detected by immunoassay [5, 6]. The homeostatic model assessment-insulin resistance (HOMA-IR) was calculated as FPG * FINS/22.5, with values higher than 2.69 indicating insulin resistance [9]. Pregnancy rate=number of pregnancy cycles/total number of clinical pregnancy cycles * 100%; similarly, ovulation rate=(number of ovulation cycles/total number of ovulation cycles) * 100%, abortion rate=(number of miscarriage cycles/total number of clinical pregnancy cycles) * 100%, and total effective rate of pregnancy rate=number of case (markedly effective + effective)/total number of cases * 100%.

Secondary observation indicators included age, body mass index (BMI), course of disease, waist-to-hip ratio (WHR), and number of infertile years, and were recorded before and after treatment.

Efficacy criteria

The efficacy criteria for PCOS patients were classified as: markedly effective when the menstrual cycle was restored to normal, clinical symptoms significantly improved, and hormone secretion levels were stable and normal after treatment; effective when the menstrual cycle had recovered to a certain extent and the secretion levels of endocrine hormones had improved; invalid when the menstrual cycle was still unstable, clinical symptoms were not improved, and hormone secretion was disordered after treatment [10].

Statistical analysis

SPSS 21.0 was used for data processing, measurement data were expressed as mean ± standard deviation ($\bar{x}$ ± sd), and the data subjected to normal distribution adopted t test, which were expressed as t. The Chi square test and the Fisher exact probability method were used for quantitative data, and were represented by χ^2. P<0.05 indicates statistically significant difference.

Results

Comparisons of general data before treatment between two groups of patients

There were no significant differences between the two groups of patients in terms of age, BMI, WHR, course of disease and infertile years (all P>0.05). See **Table 1**.

Changes of serum sex hormones after treatment in both groups of patients

The post-treatment levels of FSH and E2 in group B patients were 6.71±0.86 IU/L and

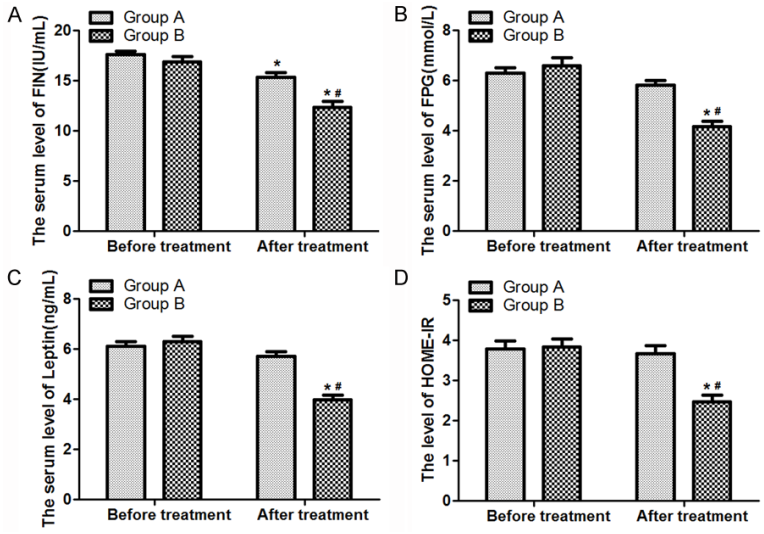

Figure 2. Glucose metabolism indicators levels after treatment in both groups. A: Changes in serum FIN levels in both groups; B: Changes in serum FPG levels in both groups; C: Changes in serum Leptin levels in both groups; D: Changes in HOMA-IR in both groups. Compared with those before treatment, *P<0.05; compared with group A, #P<0.05. FIN, fasting insulin; FPG, fasting plasma glucose; HOMA-IR, homeostasis model assessment of insulin resistance.

Table 2. Comparison of pregnancy rate, ovulation rate and abortion rate after treatment in both groups of patients (n, %)

Group	Pregnancy rate	Ovulation rate	Abortion rate
Group A (n=35)	8 (22.8)	17 (48.6)	3 (8.6)
Group B (n=34)	19 (55.9)	25 (73.5)	1 (2.9)
χ^2	5.213	4.769	2.187
P	0.023	0.029	0.062

72.96±6.83 ng/L, respectively, and were significantly higher than those in group A (both P<0.05). In contrast, LH and PRL levels in group B after treatment were 7.14±0.58 IU/L and 13.21±1.78 μg/L respectively, which were significantly lower than those in group A (both P<0.05). See **Figure 1**.

Changes of serum glucose metabolism related indicators after treatment in both groups

No significant differences were seen in the pretreatment levels of FIN, FPG, Leptin, and HOMA-IR between the two groups (all P>0.05). After treatment, all glucose metabolism indicators in group B (FIN, 12.31±0.86 IU/mL; FPG, 4.17±0.58 mmol/L; Leptin, 3.96±0.47 ng/mL; HOMA-IR, 2.47±6.83) were significantly lower compared to those in group A (all P<0.05). See **Figure 2**.

Comparison of pregnancy rate, ovulation rate and abortion rate after treatment in both groups of patients

The post-treatment pregnancy rate and ovulation rate in group B were 55.9% and 73.5%, and significantly higher than those in group A (both P<0.05). The abortion rate in group B after treatment was 2.9% which was not significantly different compared to that in group A (P>0.05). See **Table 2**.

Clinical efficacy after treatment in both groups

The total number of effective cases in group B was 32, and the total effective rate was 94.1%, which was significantly higher than that in group A (P<0.05). See **Table 3**.

Discussion

PCOS is a common gynecological disease with high morbidity and an insidious onset of symptoms, which makes early diagnosis difficult [11]. The pathogenesis of PCOS is complex, and is related to the lifestyle, genetic factors and endocrine function of the patients. The main clinical manifestations of PCOS are menstrual disorders, abnormal ovulation, along with acne and weight gain in some patients [12]. The study showed that fetus with lower birth weights could induce an increase in androgen levels, which in turn interfered with the normal physiological mechanisms of the hypothalamus-pituitary-ovary axis [13]. Clinically, PCOS is also often associated with hyperinsulinemia, which will promote LH secretion, and thus stimulate the follicular membrane of ovary to synthesize more androgen and inhibit FSH secretion [14]. A study suggested that attention should be paid to the disturbance of abnormal glucose and lipid metabolism on the secretion of sex hormones in patients with PCOS [15]. Therefore, in order to improve the clinical effi-

Table 3. Clinical efficacy after treatment in both groups

Group	Markedly effective (n, %)	Effective (n, %)	Invalid (n, %)	Total effective rate (n, %)
Group A (n=35)	20 (57.1)	9 (25.7)	6 (17.2)	29 (82.8)
Group B (n=34)	25 (73.5)	7 (20.6)	2 (5.9)	32 (94.1)
χ^2	5.617	5.264	4.857	5.428
P	0.021	0.031	0.036	0.026

cacy of PCOS treatment, it is of great value to discuss the rational medication regimen.

Clomiphene is an ovulation-promoting drug which inhibits estrogen receptor activity in hypothalamus, thereby interfering with the feedback regulation of the sex hormone on hypothalamus and accelerating estrogen secretion levels and stimulating ovulation [16]. Metformin can improve glucose metabolism in patients with PCOS and thus decrease the level of androgens. Abnormal glucose metabolism indicators, such as FIN, FPG, Leptin, and HOMA-IR, in PCOS patients are known to result in abnormal glucose and lipid metabolism. High blood glucose levels inhibit the secretion of gonadotropin-releasing hormone (GnRH), leading to increased secretion of LH [17, 18]. We found that the serum levels of FSH, LH, PR and E2 were significantly improved in patients of group B that received the clomiphene and metformin combination treatment, compared to those in patients of group A who only received clomiphene. The FSH and E2 levels were significantly higher, and those of LH and PRL were significantly lower in group B compared to those in group A, suggesting that metformin combined with clomiphene could improve the endocrinal balance in PCOS patients. In addition, FIN, FPG, Leptin, and HOMA-IR were significantly lower in group B compared to those in group A, indicating that the glucose metabolism indicators of PCOS patients were significantly improved after the combination treatment rather than clomiphene alone. Consistent with this, a study showed that clomiphene could not improve insulin levels in PCOS patients because it was often accompanied with abnormal insulin secretion, which in turn reduced its therapeutic effects [19]. Furthermore, consistent with this study, Salehi et al. found that metformin treatment could significantly inhibit insulin levels in PCOS patients, which improved the regulation of hypothalamic secretion [20]. The pregnancy rate and ovulation rate were 55.9% and 73.5% respectively after combination treatment in group B, which were higher than those in group A. The total number of effectively treated patients in group B was 32, making the total effective rate 94.1%, which was significantly higher than that in group A, indicating better clinical efficacy of the combination treatment. The disordered secretion of sex hormones in PCOS patients can change the microenvironment of follicular cells, cause follicular dysfunction, stimulate the pancreas to synthesize more insulin leading to hyperinsulinemia, and disrupt glucose metabolism. High insulin levels also promote the secretion of gonadotropins from the pituitary gland through feedback regulation, and further increase endocrinal abnormalities in PCOS patients. The combined use of metformin and clomiphene for PCOS can simultaneously regulate the patient's sex hormone secretion and glucose metabolism, and thus improve the clinical efficacy. Studies have shown that metformin can effectively improve insulin resistance, ovulation and pregnancy rate in PCOS patients, which is consistent with our findings [21, 22]. This study investigated the related mechanism of insulin resistance and sex hormone secretion disorder in PCOS patients, and further experiments were needed to improve the understanding of the pathogenesis of PCOS.

In conclusion, the combined treatment of metformin and clomiphene can effectively improve the sex hormone levels, ovulation and fertility functions in PCOS patients.

Disclosure of conflict of interest

None.

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