Treatment of polycystic ovarian syndrome with insulin resistance by insulin-sensitizer

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Summary

Objective: The aim of this study was to observe clinical curative effects of combination application of dimethylbiguanide and pioglitazone and single application of pioglitazone in patients with polycystic ovarian syndrome (PCOS) complicated with insulin resistance (IR). Materials and *Methods:* Forty cases of patients with PCOS complicated with IR were investigated, and 20 cases of infertile women without PCOS were taken as the control group. PCOS group was divided into group A and group B according to body mass index (BMI) to detect glucose and lipids metabolism indicators, C reactive protein (CRP), etc. There were 20 cases in group A (Pioglitazone) and 20 cases in group B (dimethylbiguanide and pioglitazone). After treatment for 12 weeks, changes of the above various indicators were compared. *Results:* After treatment, insulin resistance index and serum testosterone (T) of two groups patients with PCOS significantly reduced ($p < 0.05$). Compared to before treatment, BMI of group B significantly reduced ($p < 0.05$). For INS at two hours after treatment, group B reduced more significantly ($p < 0.05$). *Conclusion:* The combination of dimethylbiguanide and pioglitazone was more effective for the treatment of PCOS complicated with IR than simple pioglitazone; chronic inflammation occurrence was possibly one of reasons for insulin sensitivity reduction of patients with PCOS.

Key words: Polycystic ovary syndrome; Insulin resistance; Hyperinsulinemia; Insulin-sensitizer.

Introduction

Polycystic ovarian syndrome (PCOS) is one of the most common reproductive endocrine disorders for women in adolescence and childbearing age, and its main clinical features are chronic anovulation, hirsutism, obesity and infertility. Among women in childbearing age, its incidence rate is 6% to l0% [1], and it occurs in 75% of patients with anovulatory infertility [2]. Also, it is the main pathogeny for anovulatory infertility. PCOS pathogeny is still unclearly elucidated. Since Burghen *et al*. [3] firstly proposed that insulin resistance (IR) was involved in the pathophysiology process of PCOS in 1980, researches on PCOS-IR have been deepened. In recent years, more and more researches show that PCOS patients all present different extents of IR regardless of obesity or not, and the incidence rate can reach 70% [4]. IR and hyperinsulinemia (HI) play an important role in PCOS pathogenesis, and they are closely related to its long-term metabolic complications. Since this disease was initially treated by Stein and Levethal in 1935, it has lasted for 77 years to now, but a more ideal treatment method has been not found. Therefore, scholars are exploring a new treatment scheme in recent years. As a symptomatic treatment for IR, application of insulin-sensitizer in PCOS is a newer method. Especially, clinical data of thiazolidinediones (TZDs) Pioglitazone used for treating PCOS are less, and studies suggest that dimethylbiguanide and pioglitazone have a similar role in insulin sensitivity and high androgen [5]. Studies in recent years [6, 7] suggest that IR of patients with low-grade chronic inflammation and PCOS are closely associated with metabolic syndrome (MS). Insulin-sensitizer can reduce C-reactive protein (CRP) level, which further suggests that IR of PCOS is possibly an inflammatory reaction [8]. This study aimed to prospectively observe the improvement situations of endocrine, metabolic and reproductive functions of patients with PCOS complicated with IR, in case of combination application and simple application of two drugs, providing a basis for seeking the best treatment scheme of PCOS complicated with IR, and investigate the relationship of CRP with IR.

Materials and Methods

Patients

Forty cases of patients with PCOS complicated with IR and (or) HI treated in endocrine metabolism departments of Child Health Hospital of Jiangxi and the present hospital From May 2010 to June 2011 were selected. Their ages were between 18 and 34 years, and mean age was 26.04 ± 3.868 years. Among them, infertility duration of patients in childbearing age was one to eight years, and mean duration was 3.98 ± 1.97 years. In addition, 20 cases of non-PCOS infertile women were taken as the control group. For PCOS diagnostic code, the diagnostic code prepared in Rotterdam Conference of American Society for Reproductive Medicine in 2003 [9], and hyperprolactinemia, thyroid disease, Cushing's syndrome, diabetes mellitus and other endocrine diseases were excluded. Also, all patients had no chronic disease, and they did not smoke and drink and did not administer hormones for treatment in the prior three months. For

Revised manuscript accepted for publication February, 26, 2013

diagnostic code of IR, the upper 1/4 position value of Homa model insulin resistance index in normal control group (HOMA-IR) was used for judgment. If HOMA-IR ≥ 1.66 [10], fasting insulin (FINS) >15 mIU/l and (or) insulin at two hours after dining > 80mIU/l, it was diagnosed as HI. According to body mass index (BMI), 40 cases of patients with PCOS complicated with IR and (or) HI were divided into to group A (non-obesity group, BMI < 25 kg/m^2, 20 cases) and group B (obesity group, BMI ≥ 25 kg/m^2, 20 cases). For group A, Pioglitazone treatment was conducted, and combination treatment of pioglitazone and dimethylbiguanide was conducted in group B. This study was conducted in accordance with the Declaration and with approval from the Ethics Committee of the third Hospital affiliated Nan-Chang University. Written informed consent was also obtained from all participants.

Observation methods and indicators

(1) General indicators: Height (m) and bodyweight (kg) of patients were measured to calculate BMI: BMI = bodyweight (kg) / height^2 (m^2), and the work was carried out by the specially-assigned person. (2) Serologic indicators: 1) reproductive hormone: After fasting for 12 hours, phlebotomizing was conducted and examined for all patients in the 3rd day of menses or amenorrhea period (B ultrasound examination showed no dominant follicle). Chemiluminescence immunoassay was used to detect follicule-stimulating hormone (FSH), luteinizing hormone (LH), testosterone (T). 2) Glucose and lipids metabolism indicators: After fasting for eight to 12 hours, fasting elbow vein blood was drawn the next morning to detect fasting blood-glucose (FPG), fasting insulin (FINS), total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), and liver and kidney function. In addition, oral glucose tolerance test and insulin release test (blood glucose and insulin at one and two hours after administering glucose) were carried out. The full automatic biochemical analyzer was used to detect blood lipids and liver and kidney functions; Glucose oxidase method was used to detect blood glucose; radioimmunoassay was used to detect insulin. 3) CRP: detected with the full automatic biochemical analyzer. (3) B ultrasound: abdominal B ultrasound (liver, gallbladder, spleen, pancreas) examinations: observe fatty liver situations of the patients. (4) IR indicators: HOMA model was used to calculate insulin resistance index (HOMA – IR = FPG × FINS / 22.5) and insulin sensitivity index [ISI = 1 / (FINS × FPG)] for evaluating IR extent. In addition, blood glucose was re-examined in every month. If fasting blood-glucose ≥ 3.6 mmol/l, the original treatment was maintained. After treatment for 12 weeks, phlebotomizing was carried out to re-examine glucose and lipids metabolism and reproductive hormone indicators.

Drug administration method

For all patients, on the basis of constant diet control and exercise amount, group A orally administered Pioglitazone, 30 mg daily. As group A, group B additionally administered dimethylbiguanide 500mg/time after meals, three times daily. Also, the two groups of patients continuously administered drugs for 12 weeks. Before drug administration, liver and kidney functions were normal. All patients were asked to conduct contraception during drug administration and record menstrual changes and drug side-effects. At the same time, follow-up visit was carried out once every four weeks to observe menstrual changes and drug side-effects of the patients, and liver and kidney functions and blood glucose were detected regularly. If patients conceived, drug administration was immediately stopped.

Statistical analysis

SPSS19.0 software was used for statistical analysis. Firstly, normality test was carried out for measurement data. For non-normal distribution data, logarithmic transformation was carried out to convert them into normal distribution data and then analyze them. They were expressed as $\bar{x} \pm s$. For comparison between two groups, t test was used. Analysis of covariance was used for comparison between two groups after treatment. In addition, t test of paired samples was used for comparison between before and after treatment; Pearson or Partial correlation analysis was used for analysis of correlation, and HOMA-IR and CRP were respectively used as dependent variables to conducting multivariate stepwise regression analysis by combining other independent variables. For rate comparison between two groups, Fisher exact probability method was used. If $p < 0.05$, there was a significant difference.

Results

In group B, drug treatment was stopped in two cases due to pregnancy during drug administration. In the early treatment, one case in group B (namely dimethylbiguanide compatibility group) presented superior abdominal discomfort, but was tolerable. There was no apparent nausea and vomiting symptoms. Also, discomfort sense was gradually and naturally relieved. No anemia and edema occurred. During the whole treatment process, no severe side-effects such as lactic acidosis occurred. Before and after treatment, liver and kidney function of all patients had no apparent change.

Comparisons of various indicators before treatment between PCOS group and the control group

With regards to age, there was no significant difference between PCOS group and the control group, and two groups had comparability. FINS, 2hPG, lh INS, 2hINS, TG, TC, LDL-C, and IR indicator HOMA-IR of PCOS group all were significantly higher than those of the control group ($p < 0.01$), and ISI was significantly lower than that of the control group ($p < 0.01$). Also, reproductive hormone (T, LH), LH/FSH and inflammatory factor were also significantly higher than those of the control group ($p < 0.01$), and there were significant differences. In addition, BMI, FPG, and 1hPG of PCOS group were significantly higher than those of the control group ($p < 0.05$), and FSH was significantly lower than that of the control group ($p < 0.05$). The differences all had statistical significances. HDL-C of PCOS group was slightly lower than that of the control group ($p > 0.05$) and there was no significant difference (Table 1).

Comparisons of various indicators before treatment between group A and group B

Before drug administration, FINS, 2hINS and 2hPG of group B were significantly higher than those of group A ($p < 0.05$), and BMI, HOMA-IR, TG, and inflammatory factor (CRP) were significantly higher than those of group A ($p < 0.01$). The differences all had statistical significances. In addition, HDL and ISI group B were significantly lower than those of group A ($p < 0.01$), and there were significant dif-

Table 1. — *Comparison of various indicators between PCOS group and control group (x ± s).*

Group	Case	FPG (mmol/l)	1PG (mmol/l)	2PG (mmol/l)	FINS (mIU/l)	1hINS (mIU/l)	SQR2hINS	HOMA-IR	LnISI	LnTG
PCOS	40	5.01±0.51*	9.47±2.13*	7.47±1.11**	18.13±12.0**	118.24±48.9**	10.16±3.5**	4.09±2.69**	-4.31±0.69**	0.37±0.55**
Control	20	4.70±0.30	6.63±1.11	5.37±0.84	5.05±1.52	32.94±10.20	5.67±0.88	1.06±0.34	-3.43±0.18	-0.01±0.25

Group	*CHO (mmol/L)*	LDL-C (mmol/L)	HDL-C (mmol/L)	T (ng/dl)	LH (mIU/ml)	FSH (U/L)	LH/FSH	CRP (mg/dl)	BMI (Kg/m2)
PCOS	*4.73±0.8***	3.04±0.76**	1.32±0.29	81.54±29.51**	9.5±5.1**	4.71±2.03*	2.26±1.21**	2.65±2.24**	24.8±5.4*
Control	3.67±0.62	2.29±0.49	1.33±0.29	46.02±7.79	5.54±1.8	6.12±2.16	0.98±0.33	0.93±0.41	23.47±3.93

Note: vs control group,* $p < 0.05$, ** $p < 0.01$

Table 2. — *Comparisons of various indicators of group A and group B between before and after treatment (x ± s).*

Item	Non-obese group (group A)		Obese group (group B)	
	Before treatment	After treatment	Before treatment	After treatment
BMI (Kg/m^2)	20.48±2.41	20.52±2.59	29.11±3.33$$	27.48±2.76** #
FPG (mmol/L)	4.90±0.48	4.86±0.30	5.06±0.42	4.89±0.19
1hPG (mmol/L)	9.98±2.11	9.27±1.28	9.20±1.87	8.61±1.19
2hPG (mmol/L)	6.95±1.02	6.35±0.64**	8.10±0.71$	6.85±0.56**
FINS (mIU/L)	13.17±8.26	6.79±6.79**	25.20±13.86$	7.56±5.10**
1hINS (mIU/L)	109.11±44.81	59.82±25.89**	132.87±52.44	56.14±16.93**
2hINS (mIU/L)	88.85±50.68	53.70±30.33**	117.56±55.86$	40.38±22.72** #
Homa-IR	2.94±2.03	1.46±0.89**	5.64±3.02$$	1.59±1.09**
LnISI	-3.97±0.73	-3.34±0.58**	-4.72±0.52$$	-3.44±0.50**
TG (mmol/L)	1.06±0.36	1.00±0.33*	2.43±1.48$$	1.19±0.32**
TC (mmol/L)	4.27±0.73	4.21±0.51	5.03±0.40	4.68±0.57*
LDL-C (mmol/L)	2.67±0.75	2.35±0.59*	3.31±0.49	2.75±0.43**
HDL-C (mmol/L)	1.39±0.19	1.47±0.17	1.13±0.23$$	1.59±0.10**
T (ng/dl)	75.01±28.57	43.56±17.10**	77.86±32.08	36.98±12.45** #
LH (mIU/ml)	13.32±6.08	5.80±4.14**	6.80±2.97$	3.88±1.64**
FSH (U/L)	5.17±1.87	4.94±2.04	4.24±2.26	4.47±1.78
LH/FSH	2.84±1.45	1.21±0.68**	1.88±0.86$	0.92±0.33**
CRP (mg/L)	1.42±1.23		3.96±2.36$$	

Note: Comparison of group A and B before treatment, $ $p < 0.05$, $$ $p < 0.01$; vs before treatment,* $p < 0.05$, ** $p < 0.01$.
Comparasion between two groups after treatment, # $p < 0.05$.

ferences. Also, reproductive hormone (LH) and LH/FSH were significantly lower than those of group A ($p < 0.05$), and there were significant differences. For FPG, 1hPG, 1hINS, T, FSH, TC and LDL, there was no significant difference between the two groups ($p > 0.05$) (Table 2).

Comparisons of various indicators of group A and group B between before and after treatment

Compared to before treatment, BMI of group A after treatment had no apparent change, and 2hPG, insulin (FINS, 1hINS, 2hINS), IR indicator (HOMA-IR), reproductive hormone (T, LH) and LH/FSH significantly reduced and ISI significantly increased ($p < 0.01$). The differences had a statistical significance. Also, TG and LDL-C decreased ($p < 0.05$), and there were significant differences. For other indicators, there was no significant difference between before and after treatment. Compared to before treatment, BMI, 2hPG, insulin (FINS, 1hINS, 2hINS), IR indicator (HOMA-IR), blood lipids (TG, LDL-C), reproductive hormone (T, LH), and LH/FSH of group B after treatment significantly decreased and HDL-C and ISI significantly increased, and the differences all had a statistical significance ($p < 0.01$). Also, TC also decreased ($p < 0.05$), and there was a significant difference. For FSH, there was no significant difference between before and after treatment (Table 2).

Comparisons of various indicators after treatment between group A and group B

Analysis of covariance was used to control influences of BMI and pretherapy indicators. After treatment, 2hINS and T of the obesity group (group B) decreased more significantly than the non-obesity group (group A) ($p < 0.05$), and there were significant differences. After influence of pretherapy level of BMI was controlled, BMI of group B decreased more obviously. In the improvements of LH, FSH, and blood glucose and blood lipids metabolisms, there was no significant difference in the curative effect between the two groups ($p > 0.05$).

Regression analysis

Pearson correlation analysis showed that HOMA-IR was respectively and positively related to BMI, FINS and 1hINS (r was respectively 0.54, 0.99, 0.58, p <0.01), positively related to CRP (r was 0.43, p <0.05), and negatively related to LnISI (r was - 0.9, $p < 0.01$), and it had no significant correlation with other indicators. CRP was respectively and positively related to TG, TC, LDL-C and BMI (r was respectively 0.56, 0.51, 0.53 and 0.67, $p < 0.01$), positively related to FINS and Homa-IR (r was respectively 0.42 and 0.43, $p < 0.05$), negatively related to HDL-C (r was - 0.5, $p < 0.01$) and negatively related to LnISI and LH/FSH (r was respectively - 0.48 and - 0.39, $p < 0.05$), and it had no significant correlation with other indicators. Multivariate stepwise regression analysis was further carried out respectively with HOMA-IR and CRP as independent variables, and the result showed that BMI and FINS were independent related factors of HOMA-IR, and BMI was the independent related factors of CRP.

Regular menses recovery and pregnancy rate improvement after treatment

In group A, 12 cases after treatment restored regular menses (66.67%). In the follow-up period of half a year to one year, three cases were pregnant. In group B, 15 cases after treatment restored regular menses (83.33%). In the follow-up period of half a year to one year, five cases were pregnant. For the curative effect, there was no significant difference between the two group ($p > 0.05$).

Discussion

PCOS is a heterogeneous disease, and its pathogenesis is still unclear. Recent studies show that IR and HI are not only the conventional manifestations, but also its important pathophysiological basis. Also, incidence risk of type II diabetes mellitus, arteriosclerosis and cardiovascular disease increases for PCOS patients complicated with IR and HI [11, 12] and obesity will quicken the process of glucose metabolism disorder [13]. The results of this study also found that obesity extent of PCOS patients was positively associated with IR, and FPG, blood glucose and insulin levels at two hours after glucose powder administration, HOMA-IR and TG of PCOS patients in the obesity group were significantly higher than those of PCOS patients in the non-obesity group. Also, the incidence rate of glucose tolerance reduction (IGT) of PCOS patients in the obesity group was significantly higher than that of PCOS patients in the non-obesity group, indicating that the extents of IR and glucose and lipid metabolism disorder of fat PCOS patients were more serious than those of PCOS patients in the non-obesity group. It is speculated that obesity can aggregate IR.

As IR and HI play an important role in PCOS incidence, it is the key for treating PCOS to improve IR and HI. Dimethylbiguanide and pioglitazone are clinic common insulin-sensitizers, and they cannot only improve IR of PCOS patients, but also increase their ovulation rate and conception rates. For the former dimethylbiguanide, there are more reports on PCOS treatment. A majority of studies suggested that dimethylbiguanide could improve IR of PCOS, reduce androgen level and reduce blood lipids and blood glucose levels [14, 15]. The mechanism of the later improving IR is mainly to activate receptor -γ, increase insulin sensitivity, enhance conduction insulin signal system, enhance glucose transport of peripheral tissues, improve pancreatic islet beta cell function and regulate fat cells in order influence expressions and secretions of fat-derived cytokines for eliminating IR and realizing hypoglycemic effect by activating peroxisome proliferator. There are still few studies on Pioglitazone used for PCOS treatment. In this study, after PCOS patients in the non-obesity group were treated with Pioglitazone for 12 weeks, compared with pretherapy, blood glucose and insulin levels at two hours after glucose powder administration, HOMA-IR, TG, LDL, sex hormone (T, LH), and LH/FSH all significantly reduced and ISI significantly increased, suggesting that pioglitazone could reduce androgen level, restore regular menses, increase conception rate, and improve glucose and lipid abnormalities of patients with PCOS complicated with IR and reduce incidence risks of metabolic complications such as diabetes mellitus, hypertension, coronary heart disease, etc. After drug administration, BMI of the patients had no apparent change. For PCOS patients in the obesity group, after dimethylbiguanide and pioglitazone were jointly administered for 12 weeks, compared with pretherapy, their BMI, blood glucose and insulin levels at two hours after glucose powder administration, HOMA-IR, ISI, TG, TC, LDL, sex hormone (T, LH), and LH/FSH all significantly reduced, and ISI significantly increased. Studies suggest that combination of dimethylbiguanide and pioglitazone have a synergistic effect of improving IR. For the patients insensitive to dimethylbiguanide additional application of pioglitazone will improve curative effect. How is the curative effect of combination of dimethylbiguanide and pioglitazone used for PCOS treatment? There are still few studies addressing this query. Baillargeon *et al*. [16] reported that the curative effect of combination of dimethylbiguanide and rosiglitazone used for the treatment of non-obesity PCOS patients wasn't better than that of simple drug. In the present study, BMI, 2hINS and T of group B (namely combination application group) reduced more significantly than group A (pioglitazone group). Also, FINS and HOMA-IR were more apparently reduced. Although there is no significant difference, it suggests that compared with simple pioglitazone, combination application of two drugs can better reduce androgen level and improve IR of PCOS and thus correct hyperinsulinemia. In addition, LH, FSH, and blood glucose and lipid metabolisms were improved, more obviously than pioglitazone group, but there was no significant difference.

CRP is an inflammatory and acute reactive protein, and its increase is one of important indicators of predicting car-

diovascular disease and type II diabetes mellitus [17, 18]. In recent years, chronic inflammation theories on PCOS are widely concerned. The study conducted by Ruggeri *et al*. [19] and Caturegli *et al*. [20] suggested that IR and metabolic syndrome of PCOS patients were closely related to the mild chronic inflammation. In this study, CRP level of PCOS patients was significantly higher than that of the control group, suggesting that chronic inflammatory reaction was involved in the occurrence and development process of PCOS, which was in line with research result of Boulman *et al*. [21]. Person correlation analysis showed that CRP was positively associated with FINS, HOMA-IR and BMI respectively, but stepwise regression analysis indicated FINS and HOMA-IR were not independent correlation factors of CRP, while BMI was an independent correlation factor of CRP, indicating that serum CRP level and BMI had a correlation, and one reason for insulin sensitivity reduction possibly lays in the chronic inflammatory reaction.

In brief, the time length of this study was short, and sample size was small. It is necessary for observing effects of combination of dimethylbiguanide and pioglitazone on improvements of IR and metabolism and reproductive functions and further solving the infertile problem of PCOS patients to carry out larger sample-size prospective studies. At the same time, it is necessary to carry out a number of clinical studies addressing the side-effects of the two.

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