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Effects of exenatide combined with clomifene citrate on insulin resistance and Angiotensin II/Angiotensin-(1-7) in peripheral blood in patients with polycystic ovary syndrome.

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Abstract

Objective: To study the effects of exenatide combined with clomifene citrate on the levels of Angiotensin (Ang) II/Angiotensin-(1-7) and insulin resistance in patients with polycystic ovary syndrome (PCOS).
Methods: A total of 78 patients with PCOS who were treated in our department from January 2015 to January 2017 were included in the study. Patients were randomly divided into observation group (n=45) and control group (n=33). Patients in the observation group were treated with exenatide combined with clomifene citrate. Patients in the control group were treated with metformin combined with clomifene citrate. Serum levels of Ang II, Ang (1-7) and serum follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), testosterone (T), sex hormone binding globulin (SHBG) and dehydroepiandrosterone sulfate (DHEAS) levels were measured before and after treatment. Fasting plasma glucose (FPG) and fasting serum insulin (FINS) were measured. Homeostasis model assessment-insulin resistance (HOMA-IR) was calculated. The ovulation rate and pregnancy rate was recorded.
Results: After treatment, the levels of Ang II, Ang (1-7) and Ang II/Ang (1-7) in the observation group were lower than those before treatment (P<0.05). The ratio of Ang II, Ang (1-7) and Ang II/Ang (1-7) in the observation group was lower than that in the control group (P<0.05). The levels of LH, T and DHEAS in the two groups were lower than those before treatment (P<0.05). And the level of the observation group was lower than that of the control group, the difference was statistically significant (P<0.05). The levels of FSH, E2 and SHBG were statistically significant compared with before treatment in the two groups (P<0.05), and the level of the observation group was higher than that of the control group (P<0.05). After treatment, the levels of FPG, FINS and HOMA-IR in the observation group and the control group were significantly lower than those before treatment (P<0.05). The levels of FPG, FINS and HOMA-IR in the observation group were lower than those in the control group (P<0.05). The ovulation rate and pregnancy rate of the observation group were higher than those of the control group, the difference was statistically significant (P<0.05). There was no significant difference in the incidence of adverse reactions between the observation group and the control group (P>0.05).
Conclusion: Exenatide combined with clomifene citrate is effective in the treatment of PCOS, and can reduce the activity of renin-angiotensin system (RAS) in patients, reduce the level of insulin resistance in patients, promote ovarian ovulation. It is safe and reliable, which should be further expanded and followed-up to detect the long-term efficacy and safety in the treatment of PCOS patients.

Keywords: Polycystic ovary syndrome, Exenatide, Clomifene citrate, Angiotensin, Insulin resistance.

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Introduction

Polycystic ovary syndrome (PCOS) is a common endocrine disease, the incidence in women of childbearing age can reach about 6%. It can cause a variety of hormone secretion disorders, with a variety of clinical manifestations including obesity, hirsute, oligomenorrhea, infertility, cardiovascular disease and type 2 diabetes increased incidence [1]. PCOS patients are often accompanied by varying degrees of insulin resistance and compensatory hyperinsulinemia. Insulin has the effect of stimulating ovarian androgen secretion. The injection of insulin can increase the levels of androgen in normal women. Insulin resistance in patients with hyperinsulinemia is often accompanied by hyperandrogenism [2,3]. Therefore,

compensatory hyperinsulinemia on the one hand can cause pancreatic β depletion, on the other hand cause elevated levels of androgen in PCOS patients, which is the important reason causing various types of manifestations of PCOS. The incidence of cardiovascular disease in PCOS patients is also much higher than the normal women of childbearing age with hypertension as the representative. The study suggests that the activation of renin-angiotensin system (RAS) plays an important role in it and is closely related to insulin resistance [4]. Angiotensin II (Ang II) is the main active substance of RAS system, which can play a biological role in promoting vasoconstriction after binding to receptor. Angiotensin (1-7) (Ang (1-7)) is a terminal active substance in the RAS system. It is the metabolite of Ang II, but also its antagonist, with the role of promoting vasodilatation [5]. Exenatide is a glucagon-like peptide-1 (GLP-1) that binds directly to the islet receptor, with efficacy of promoting insulin secretion, inhibiting glucagon secretion, reducing the RAS system activity to achieve relaxation of blood vessels, and reducing the incidence of cardiovascular disease [6]. In this study, 78 patients with PCOS were treated with exenatide combined with clomifene acid citrate, and the efficacy and safety of the therapy were evaluated by observing the changes of Ang II/Ang (1-7) and insulin resistance in peripheral blood.

Materials and Methods

General data

This study was a single-center, randomized, controlled, single-blind study (for patients) that included 78 patients with PCOS who were treated in our department from January 2015 to January 2017. This study was conducted in Department of Ultrasound, Beijing Obstetrics and Gynecology Hospital, Capital Medical University, Chaoyang Dist, Beijing, P.R. China. All patients were female, aged 19 to 35 years, with mean age (25.75 ± 6.33) years, body mass index (BMI) 23.4~28.6 kg/m^2, average (25.88 ± 5.55 kg/m^2) and 65 cases of infertility. Inclusion criteria [7]: 1) With oligomenorrhea, amenorrhea or irregular uterine bleeding. 2) Has one of the following two items: hairy, acne, high androgenic manifestations or hyperandrogenism. With ovarian polycystic changes examined using ultrasound, the follicles ≥ 12 with diameters more than 2 mm, or ovarian volume >10cm^3. 3) With insulin resistance: Homeostasis model assessment-insulin resistance (HOMA-IR) index was assessed [8,9]. HOMA-IR=fasting plasma glucose (FPG) concentration (mmol/L) × fasting serum insulin (FINS) concentration (mIU/L)/22.5. The (receiver operating characteristic) ROC curve was plotted to determine the HOMA-IR threshold (IR=1.90), insulin resistance (IR) was thought to exist when it was higher than this threshold. Exclusion criteria: 1) Patients with hypothalamus, pituitary, adrenal gland, or thyroid diseases, accompanied by uncontrollable high blood pressure (diastolic blood pressure (DBP) ≥ 120 mmHg). 2) Accompanied by heart, liver, kidney diseases, or malignant tumors. 3) Patients who took hypoglycemic lipid-lowering drugs within 1 month, or took hormones or antihypertensive drugs within 3 months. Patients were divided into observation group (n=45) and control group (n=30) according to the random number table method.

Treatment programs

Patients in the observation group were treated with combination therapy: exenatide injection (American Baxter Pharmaceutical Solutions LLC, import registration number H20140822) 5 μg, Hypo, Bid. After treatment for 1 month, it was changed to 10 μg, Hypo, Bid. After treatment for 2 months, clomifene citrate capsules (Shanghai Hengshan Pharmaceutical Co., Ltd., GYZZ H31021107) 50 mg, p.o., QD was added, with 5 days for a course of treatment. The efficacy was assessed, if anovulation occurred, 50 mg clomifene citrate was added in the next course, with a total of 3 courses. The patients in the control group were treated with metformin hydrochloride tablets (Guangzhou Baiyunshan Tianxin Pharmaceutical Co., Ltd., GYZZ H44023514) 250 mg, po, Bid (if as poor blood glucose control, change to Tid or increase of 1 tablet each time, with the final daily no more than 2000 mg), once every 3 months, plus clomiphene citrate capsules (the same usage and dose with the observation group).

Detection indexes

Before and after treatment, the following indicators (for patients with oligomenorrhea, blood was collected on the 3rd to 7th morning of menstrual cycle, for patients with amenorrhea, blood was collected at any morning). Specimen treatment: 5 ml of peripheral blood samples were collected and placed in a coagulation tube. After coagulation, blood was centrifuged at a low temperature centrifuge (5427 R, Eppendorf, Germany) at 6000 rpm and at 4°C for 5 min, the supernatant was collected and stored in tube, and stored at -80°C in refrigerator for inspection. Detection criteria: 1) Ang II, Ang (1-7) levels: Human serum Ang II ELISA kit (Shanghai Qiao Yu Biotechnology Co., Ltd.) and Human Ang (1-7) ELISA kit (US Bachem) was used, Serum Ang II, Ang (1-7) levels were measured with ELISA method. 2) Serum hormone: The levels of follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2) testesterone (T), sex hormone binding globulin (SHBG) and dehydroepiandrosterone sulfate (DHEAS) levels were measured by Cobas6000 automatic electrochemiluminescence immunoassay and kit reagent (Roche, USA). 3) Glucose metabolism: Chemiluminescence was used for the detection FPG and FINS levels (Roche), and HOMA-IR value calculated. 4) Ovarian function: The ovulation rate and pregnancy rate were recorded, follicular growth and ovulation performance was confirmed with transvaginal B-ultrasound in ovulation patients.

Statistical analysis

SPSS22.0 software was used to analyze the data. For normal distribution count data, ($\bar{x}$ ± s) was used to represent. Logarithmic conversion was used for data of non-normal distribution. T-test was used for comparison between groups. The measurement data is expressed in (n (%)). Comparison

between groups using χ^2-test or Fisher exact probability method.

Results

General data

There were no significant differences in general data between the two groups (P<0.05). This study was approved by the Ethics Committee of our hospital, patients and their families were informed consent on the purpose and method of the study (Table 1).

Table 1. Comparison of general data between the two groups.

Groups	n	Age (years)	BMI (kg/m^2)	HOMA-IR	SBP (kPa)	DBP (kPa)
Observation group	45	25.92 ± 6.75	26.26 ± 5.71	5.66 ± 1.27	19.15 ± 3.44	12.36 ± 2.25
Control group	33	25.67 ± 7.33	25.74 ± 6.37	5.47 ± 1.34	18.46 ± 3.58	11.74 ± 2.31
t		9.973	0.378	0.638	0.86	1.189
P		0.921	0.706	0.526	0.392	0.238

Comparis on of serum hormone levels

The results showed that the levels of LH, T and DHEAS after treatment in the two groups were lower than those before treatment (P<0.05), and the serum levels of LH, T and DHEAS in the observation group were lower than those in the control group, The difference was statistically significant (t=5.564, P=0.000; t=2.659, P=0.010; t=2.058, P=0.043). The levels of FSH, E2 and SHBG after treatment in the two groups were significantly higher than those before treatment (P<0.05), and the levels of FSH, E2 and SHBG in the observation group were statistically higher those in control group, with significant difference (t=7.11, P=0.000; t=2.266, P=0.026; t=8.127, P=0.000) (Table 2).

Comparison of ovulation rate and pregnancy rate

The results of follow-up showed that the ovulation rate and pregnancy rate in the observation group were higher than those in the control group (P<0.05) (Table 3).

Table 2. Comparison of serum hormone levels between the two groups (x ± s).

Groups	n	Time point	FSH (IU/L)	LH (IU/L)	E2 pmol/L	T (μg/L)	SHBG (nmol/L)	DHEAS (μmol/L)
Observation group	45	Before treatment	6.27 ± 1.04	10.01 ± 1.35	125.38 ± 16.63	1.82 ± 0.23	38.71 ± 2.66	8.26 ± 20.33
		After treatment	7.06 ± 0.87	6.29 ± 1.74	140.70 ± 18.17	0.64 ± 0.12	60.30 ± 3.07	6.15 ± 19.25
Control group	33	Before treatment	6.33 ± 1.07	10.05 ± 1.11	126.79 ± 17.07	1.79 ± 0.31	39.14 ± 3.15	8.43 ± 19.86
		After treatment	6.76 ± 0.75	7.68 ± 1.67	137.52 ± 14.86	0.81 ± 0.24	55.15 ± 2.28	7.48 ± 20.47

Table 3. Comparison of ovulation rate and pregnancy rate (n (%)).

Groups	n	ovulation rate	pregnancy rate
Observation group	45	39 (86.7)	24 (53.3)
Control group	33	19 (57.6)	9 (27.3)
χ^2		8.450	5.297
P		0.004	0.021

Table 4. Comparison of glucose metabolic index ($\bar{x}$ ± s).

Groups	n	Time point	FPG (mmol/L)	FINS (mIU/L)	HOMA-IR (AU)
Observation group	45	Before treatment	7.60 ± 0.36	16.12 ± 5.82	7.02 ± 0.84
		After treatment	5.86 ± 0.38	8.67 ± 2.14	5.23 ± 0.70
Control group	33	Before treatment	7.56 ± 0.42	15.73 ± 5.27	6.93 ± 0.69
		After treatment	6.24 ± 0.35	12.16 ± 3.42	5.79 ± 0.58

Comparison of glucose metabolic index

After treatment, the levels of FPG, FINS and HOMA-IR in the observation group and the control group were significantly lower than those before treatment (P<0.05). After treatment, FPG, FINS and HOMA-IR were lower in the observation group than in the control group (t=4.510, P=0.000; t=5.532, P=0.000; t=3.747, P=0.000) (Table 4).

Comparison of Ang II and Ang (1-7) levels in the two groups

The results showed that the levels of Ang II in the observation group and the control group were significantly lower than those before treatment (t=14.104, P=0.000; t=6.299, P=0.000). After treatment, the level of Ang (1-7) in the observation group was lower than that before treatment, the difference was statistically significant (t=3.592, P=0.001), although Ang (1-7) in the control group decreased, but the difference was not statistically significant (t=1.255, P=0.214). After treatment, the

levels of Ang II and Ang (1-7) in the observation group were significantly lower than those in the control group (t=7.191, P=0.000; t=2.406, P=0.019). After treatment, the ratio of Ang II/Ang (1-7) in the observation group was lower than that before treatment (t=6.708, P=0.000). There was no significant difference in control group between t before and after treatment (t=1.509, P=0.136). After treatment, the ratio of AngII/Ang (1-7) in the observation group was lower than that in the control group, the difference was statistically significant (t=3.623, P=0.001) (Table 5).

Table 5. Comparison of Ang II and Ang (1-7) levels in the two groups (x± s).

Groups	n	Time point	AngII (ng/L)		Ang (1–7) (ng/L)		AngII/Ang (1-7)
Observation group	45	Before treatment	137.06 11.37	±	309.08 79.73	±	0.45 ± 0.03
		After treatment	101.47 12.54	±	256.67 56.49	±	0.40 ± 0.04
Control group	33	Before treatment	140.36 12.75	±	315.35 86.49	±	0.45 ± 0.07
		After treatment	121.48 11.57	±	291.08 69.71	±	0.43 ± 0.03

Adverse reactions

The adverse reactions in the observation group were mainly mild to moderate gastrointestinal discomfort. The highest incidence of nausea and fatigue, were 15.6%, followed by hot flash and breast discomfort, were 13.3%. The highest incidence of nausea and hot flashes in the control group was 15.2%, followed by breast discomfort and fatigue, were 12.1%. There was no significant difference in the incidence of adverse reactions between the two groups (P>0.05) (Table 6).

Table 6. Comparison of adverse reactions between the two groups (n (%)).

Groups	n	Nausea	Vomiting	Headache	Increased or irregular bleeding	Hot flashes	Blurred vision	Breast discomfort	Stomach ache	Fatigue	Erythra
Observation group	45	7 (15.6)	3 (6.7)	3 (6.7)	4 (8.9)	6 (13.3)	2 (4.4)	6 (13.3)	5 (11.1)	7 (15.6)	2 (4.4)
Control group	33	5 (15.2)	2 (6.1)	3 (9.1)	2 (6.1)	5 (15.2)	1 (3.0)	4 (12.1)	3 (9.1)	4 (12.1)	1 (3.0)
χ^2		0.002	0.012*	0.158*	0.214*	0.052	0.103*	0.025*	0.084*	0.185*	0.000*
P		0.961	0.355	0.301	0.306	0.820	0.429	0.265	0.284	0.239	0.429

* Fisher exact probability method

Discussion

Insulin resistance-related androgenic hyperthyroidism is the most important pathophysiological change of PCOS. 60% of PCOS patients with BMI were in the range of overweight or obesity, 70% of PCOS patients with IR or diabetes symptoms. The risk of type 2 diabetes in these patients is much higher than in the normal population [10]. At the same time, 75% of PCOS patients have increased testosterone or DHEAS [11]. The causes of insulin resistance in PCOS and the increase in androgen is not yet fully clear, but is generally believed that patients with chronic low inflammation in the body are closely related [9]. Studies have shown that there are high levels of proinflammatory factors in the body of obese patients with diabetes, and with weight loss after exercise, all levels of inflammatory factors have decreased significantly in the body [12]. PCOS is also considered a chronic inflammatory disease, the source of inflammatory factors may be adipose tissue of hyperplasia or hypertrophy. Adipose tissue has a strong, abnormal exocrine function, which can release many adipocytokines such as leptin, IL-6 and TNF-α, which can affect women's endocrine and metabolic function through a variety of inflammatory signaling pathway [12]. The abnormal increased inflammatory factors not only can directly act on the ovaries, resulting in increased androgen secretion, but also stimulate the production of androgen by the follicular cells by promoting the secretion of pituitary LH, and then trigger PCOS hirsutism, amenorrhea, infertility, and other clinical manifestations [13,14]. Although obesity or overweight may not be the true cause of PCOS for patients with normal BMI, long-term follow-up has found that the incidence of cardiovascular disease in these patients is also higher than that in the normal population, and *in vivo* inflammatory factors such as MIF, TNF-α level is also higher than normal [15]. Therefore, anti-inflammatory treatment of PCOS patients will be an effective treatment.

Exenatide has a strong anti-inflammatory effect, which can reduce the body's oxidative stress response, reduce the production of a variety of inflammatory factors including TNF-α, and then produce effects of preventing arteriosclerosis and cardiovascular disease [16]. In this study, after treatment, LH and T levels decreased in the two groups of patients, especially more significant in the observation group, suggesting that exenatide combined with clomifene citrate may reduce the pituitary LH secretion by inhibiting the body's inflammatory response in PCOS patients, thereby reducing the origin androgen production in ovarian. After treatment, the level of FSH in patients increased, further indicating that LH/FSH secretion abnormalities caused by hyperestrogenemia can be improved. The results of the final follow-up also showed that the fertility function of the observation group was better,

ovulation rate and pregnancy rate were significantly higher than the control group. Chronic inflammatory response caused abnormal increase of TNF-α, IL-6, leptin and other inflammatory factors in the body are also the main cause of IR. Studies have shown that TNF-α can cause IR by inhibiting insulin signaling with inhibiting the activation of insulin receptor substrate 1 (IRS-1) [17]. Exenatide is a glucagon-like peptide-1 agonist that inhibits pancreatic α-cell secreting glucagon while inhibiting pancreatic β-cell releasing insulin, and can also act on inhibition of appetite through blood-brain barrier, delaying gastric emptying and thus achieve the purpose of weight control [18,19]. In this study, the levels of FPG, FINS and HOMA-IR decreased, insulin resistance improved after treatment, and the improvement in the observation group was more significant, indicating that exenatide increased the insulin sensitivity in PCOS patients compared with metformin, with function of protection and recovery of pancreatic β-cell in patients.

It is generally believed that the anti-inflammatory effect of exenatide is derived from its control of arterial systolic blood pressure. Exenatide can reduce the activity of Extracellular signal-regulated protein kinases 1 and 2 (ERK1/2) and JNK signaling pathway in Ang II-mediated vascular smooth muscle cells by inhibiting the activation of RAS system, reduce the proliferation and migration of vascular smooth muscle cells, and thus improving the vasomotor ability [20]. In order to further investigate the effect of exenatide on the RAS system in PCOS patients, we examined serum Ang II and Ang (1-7) levels. The results showed that both groups of patients with Ang II and Ang (1-7) levels were reduced after treatment, indicating that two metabolic pathways of RAS system have been effectively inhibited. Compared with the control group, the ratio of Ang II/Ang (1-7) in the observation group was lower, indicating that Ang (1-7) decreased less than Ang II, and the use of exenatide could make Ang (1-7) more effective on antagonizing Ang II, improving patients' IR. In summary, exenatide combined with clomifene citrate is effective in the treatment of PCOS, and can reduce the activity of RAS in patients, reduce the level of insulin resistance in patients, promote ovulation, and be safe and reliable, and should be further expanded and follow-up to determine the program for the long-term efficacy and safety in the treatment of PCOS patients.

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