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Exploring the significance of sex hormone-binding globulin examination in the treament of women with polycystic ovarian syndrome (PCOS)

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Summary

Objectives: To explore whether sex hormone-binding globulin (SHBG) and free androgen index (FAI) can be seen as therapeutic effect indexes of women with polycystic ovarian syndrome (PCOS). *Materials and Methods:* The body mass index (BMI), basal sexual hormones, SHBG, fasting blood glucose (FBG), and fasting insulin (FINS) were collected from 579 women with PCOS, were divided into two groups according to BMI: obese group (n = 145) and non-obese group (n =434), according to homeostasis model assessment of insulin status (HOMA-IR). Patients were then divided into four groups: A: non-obese without insulin resistance (n = 174), B: non-obese with insulin resistance (n = 260), C: obese without insulin resistance (n = 34), D: obese with insulin resistance (n = 111). A and B groups received Diane-35 alone, C and D groups received Diane-35 plus metformin for three months. Then clomiphene citrate and HMG were used to induce ovulation then compared ovulation rate and pregnancy outcome. *Results:* FAI decreased significantly and SHBG increased significantly in all groups. In A group FINS and HOMA-IR increased significantly ($p < 0.05$), but in B and D groups FINS and HOMA-IR decreased significantly ($p < 0.05$). After treatment the ovulation rate in non-obese group was higher than obese group ($p < 0.01$). Compared with non-ovulation patients, SHBG increased significantly and FAI decreased significantly in the patient with ovulation. Regarding the pregnancy outcome, FAI decreased significantly in delivery patients than spontaneous abortion patients. Furthermore, SHBG increased significantly. *Conclusion:* It was important to check SHBG and FAI during the treatment of PCOS patient. They could be used to assess whether the treatment was effective and as a guidance of clinical medication.

Key words: Sex hormone-binding globulin; Free androgen index; Insulin resistance; Polycystic ovarian syndrome.

Introduction

Polycystic ovarian syndrome (PCOS) is the most common gynecologic endocrine disorders among adolescents and women of childbearing age; its incidence rate accounted for 5%~10% of premenopausal women [1]. PCOS' basic characteristics are long-term anovulation or rare ovulation, polycystic ovaries' alteration, high androgen hormone in blood, and clinical manifestations of heterogeneity. Recent studies have found that PCOS not only affects the reproductive system, but also other systems, leading to a complex multisystem syndrome; it also results in related metabolic disorders including insulin resistance, high androgen hormone in blood, abnormal glucose metabolism, and lipid metabolism with increased cardiovascular risk [2]. Given the high incidence of PCOS and its great harm to patients, researchers are focused on how to diagnose and treat patients with PCOS, and prevent patients from long-term metabolic complications. Patients with PCOS often have anovulatory infertility; even if patients achieve pregnancy, they also encounter a higher probability of early abortion compared to normal women. Therefore, the present authors retrospectively analyzed endocrine and metabolic alterations and induction of ovulation of 579 patients with PCOS, who visited their reproductive centers. When treated, they were given oral Diane-35 and metformin hydrochloride. The authors then detected and evaluated the indexes, and assessed treatment efficacy evaluation criteria during the treatment of PCOS; this information can provide a basis for the clinical medication.

Materials and Methods

Study design and populations

The authors collected 579 patients with PCOS in Reproductive Center of Zhongshan City Bo'ai Hospital from October 2006 to October 2012, aged from 20 to 39 years; the average age was 27.93 years, the standard deviation of age was 3.70 years. They diagnosed PCOS according to Rotterdam's criteria formulated in 2003 [3] they ruled out those patients as having late-onset congenital adrenal hyperplasia, or Cushing's syndrome, or hyperprolactinemia, or thyroid disease and hormone secretion tumor.

The authors divided 579 PCOS patients into two groups, namely obese group and non-obese group, according to the principle when patients' body mass index (BMI) was greater than or equal to 25 kg/m^2, they considered a patient as obese, otherwise they considered a patient as non-obese. Thus there were 145 patients in obese group and 434 patients in non-obese group among

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the total 579 patients. They also assessed degree of insulin resistance (IR) to target organ using homeostasis model assessment of insulin status (HOMA-IR) [4], HOMA-IR is equal to fasting insulin (FINS) value multiplied fasting blood glucose (FBG) value, then divided 22.5. HOMA-IR ≥ 1.95 is diagnosed as insulin resistance in the present center. According to this, they further divided 579 patients with PCOS into altogether four groups, namely group A, group B, group C, and group D. The non-obese without insulin resistance of group A consisted of 171 patients. The non-obese with insulin resistance of group B consisted of 263 patients. The obese without insulin resistance of group C consisted of 34 patients. The obese with insulin resistance of group D consisted of 111 patients.

Ultrasound diagnosis of PCOS

All subjects were in the follicular phase, when patients had amenorrhea; if the authors did not see the sizes of follicles that were greater than ten mm and corpus luteum in two ovaries using B ultrasound on pelvic cavity, they considered these patients to be in the follicular phase, then their blood was drawn. There were special messenger recording the sizes of uterine and ovarian, sizes and numbers of antral follicle in each side of ovary by transvaginal ultrasonography color Doppler ultrasonic diagnostic apparatus, which was used for the ultrasound diagnosis of PCOS.

Determination of reproductive endocrine hormones

The authors collected ten ml fasting elbow vein blood of each subject from the third to fifth day of the menstrual cycle, however in the amenorrhea patients, the date was not restricted. Then centrifugally collected serum, was preserved at -20°C, and then used to test LH, FSH, estradiol (E2), T, prolactin (PRL), sex hormone-binding globulin (SHBG), fasting glucose (FG), fasting insulin (FINS), and the authors calculated FAI and HOMA-IR. Also these hormones were determinated by chemiluminescence method with automatic electrochemistry luminescence immunity analyzer and reagents; the difference between the same batch number of reagent's datas was less than 2.8%, and the difference between the different ones was 4.3%.

Definitions of diseases

The diagnosis criteria for PCOS referred to one revised by European Society of Human Reproduction and Embryology, and Rotterdam working group of American Society for Reproductive Medicine (ESHRE /ASRM) [3], when a patient has two of the following three articles, she can be diagnosed as having PCOS, namely: 1) anovulation or rare ovulation; 2) clinical manifestations of high androgen hormone in blood (such as hirsutism, acne) and (or) biochemical basis; 3) manifestations of polycystic ovary under B ultrasonic, in which one side or both sides of the ovary has 12 or more small follicles whose diameter is 2~9 mm, and (or) ovarian volume was greater than ten ml. And there was exclusion of hyperprolactinemia and other endocrine diseases such as high androgen hormone in blood, namely Cushing's syndrome, congenital adrenal hyperplasia (CAH), ovarian or adrenal tumor, and so on.

Therapy methods

The authors divided the 579 patients with PCOS into two groups, namely insulin resistance group and no insulin resistance group. Patients in insulin resistance group began to take orally Diane-35, which included two mg cyproterone acetate and 0.035 mg ethinyl estradiol for each tablet, at the fifth day of menstruation, one tablet daily, and continued to take orally Diane-35 at the fifth day of next menstruation. At the same time patients in insulin resistance group took orally metformin hydrochloride enteric-coated tablets, twice a day, each time 500 mg, and continuously. After three months of taking the tablets, indexes were detected and observed. Patients in no insulin resistance group began to take orally Diane-35 at the fifth day of menstruation, one tablet daily, and continued to take orally Diane-35 at the fifth day of next menstruation, one tablet daily, continuously. After three months of taking the tablets, indexes were detected and observed. At four months after the treatment, the authors began to induce patients ovulation with CC for five days, once daily, once 100 mg, at the fifth day of menstruation. When it was seventh day of menstrual cycle, they injected 75 U HMG in the patients and adjusted the dosage of HMG according to follicular development degree. They then began to monitor the sizes of follicle one time the other day, using B ultrasound from ninth to 11th day. When follicles' diameter was greater than equal to 15 mm, they changed to daily continuous observation until ovulation. When follicles' diameter was up to 18 mm, they intramuscularly injected 10, 000U human chorionic gonadotropin (HCG) in the patients, and after injection the patients engaged in sexual activity twice in 24-48 hours. The authors then rechecked ovulation after 36 hours from the HCG injection with B ultrasound. B ultrasound showed signs of dominant follicle ovulation: collapse, reduced volume, contour clear follicle echo area disappeared, detecting a small amount of fluid in the pelvic cavity. After 72nd hour from the HCG injection, if there was no dominant follicle ovulation, then they were diagnosed as having luteinized unruptured follicle syndrome (LUFS). After two weeks of the ovulation, the authors detected HCG in patients' urine with urine pregnancy test; if negative, the patients received the above ovulation method for two months, and if positive, their blood HCG levels were assessed. The authors examinated and determined the number of gestational sacs and fetal heart sounds with B ultrasound after five weeks of ovulation, and recorded patients' delivery.

Statistical analysis

A SPSS 13.0 statistic analysis software was used to analyze the data. The values of LH, FSH, E2, T, and PRL represented normal distribution, shown in ±s and the differences of the indexes' values between groups were used by the independent t samples T test for comparison. The values of FAI, FINS, and HOMA-IR and other indicators showed non-normal distribution and numerical values were represented with median and inter-quartile rang (Md ± IQR); the differences of the indexes' values between groups were used by the non-parametric Wilcoxon test and logistic regression analysis for statistically assessment. The differences of sensitivity and specificity between groups were assessed by chi-square test for comparison. The AUC-ROC was used by Medcalc statistical software for analysis. The differences of ovulation and pregnancy delivery between groups were used by t-test to compare. When p was less than 0.05, the difference between groups was significant and had statistical value.

Results

Analysis of biochemical changes before and after treatment in 579 PCOS patients between groups

After treatment, the values of FAI in four groups were lower than those before therapy and there were significant differences. Compared with those before treatment, SHBG levels in four groups were elevated and there were significant differences. After treatment, LH levels and LH/FSH ratios in group A, or group B, or group C were lower than those before therapy and there were significant differences. However, compared with those before treatment, HOMA-IR and FINS in group A were elevated, while HOMA-IR

Table 1. — *Comparison of various biochemical indexes between before and after treatment of different methods in non-obese group ($\bar{x} \pm s$).*

Groups	N (cases)	LH (IU/l)		LH/FSH		T (nmol/l)	
		Before cure	After cure	Before cure	After cure	Before cure	After cure
A	171	11.79 ± 6.22	7.13 ± 3.36#	1.81 ± 0.93	1.12 ± 0.58*	1.90 ± 0.72	1.60 ± 0.64#
B	263	10.12 ± 5.62	6.74 ± 2.79#	1.65 ± 0.91	1.11 ± 0.50#	2.01 ± 0.77	1.60 ± 0.61#

Groups	N (cases)	SHBG (nmol/l)		FAI		FBG (mmol/l)	
		Before cure	After cure	Before cure	After cure	Before cure	After cure
A	171	52.82 ± 26.22	116.93 ± 58.83#	4.55 ± 2.89	1.77 ± 1.25#	4.88 ± 0.50	4.86 ± 0.55
B	263	46.15 ± 26.33	109.42 ± 54.83#	5.53 ± 3.30	2.02 ± 1.85#	5.29 ± 0.62	5.17 ± 0.79

Groups	N (cases)	FINS (mU/l)		HOMA-IR	
		Before cure	After cure	Before cure	After cure
A	171	6.14 ± 1.70	6.70 ± 2.71#	1.33 ± 0.38	1.44 ± 0.61#
B	263	14.08 ± 5.05	9.73 ± 4.035#	3.30 ± 1.24	2.23 ± 1.01#

*Compared between before and after treatment of two groups, #$p < 0.01$, *$p < 0.05$.

Table 2. — *Comparison of various biochemical indexes between before and after treatment of different methods in obese group ($\bar{x} \pm s$).*

Groups	N (cases)	LH (IU/l)		LH/FSH		T (nmol/l)	
		Before cure	After cure	Before cure	After cure	Before cure	After cure
C	34	8.47 ± 3.96	6.07 ± 2.34#	1.41 ± 0.67	0.93 ± 0.35#	1.90 ± 0.81	1.74 ± 0.55
D	111	7.68 ± 4.50	6.93 ± 3.82	1.24 ± 0.70	1.12 ± 0.53	2.00 ± 0.90	1.58 ± 0.66#

Groups	N (cases)	SHBG (nmol/l)		FAI		FBG (mmol/l)	
		Before cure	After cure	Before cure	After cure	Before cure	After cure
C	34	57.16 ± 40.28	117.60 ± 62.20#	4.55 ± 2.88	2.02 ± 1.70#	4.90±0.57	4.88 ± 0.51
D	111	35.61 ± 23.88	107.63 ± 52.17#	7.15 ± 4.55	2.08 ± 1.77#	5.36±0.80	5.03 ± 0.56#

Groups	N (cases)	FINS(mU/l)		HOMA-IR	
		Before cure	After cure	Before cure	After cure
C	34	7.31 ± 1.89	7.79 ± 2.17	1.56 ± 0.31	1.72 ± 0.58
D	111	19.40 ± 9.34	11.32 ± 5.05#	4.68 ± 2.70	2.58 ± 1.30#

*Compared between before and after treatment of two groups, #$p < 0.01$, *$p < 0.05$.

and FINS in group B and group C were decreased, and there were all significant differences (as shown in Tables 1 and 2).

Analysis of ovulation status after treatment of 579 patients with PCOS

As shown in Table 3, after treatment there were 373 patients with ovulation and 61 patients with no anovulation in non-obese group; there were 101 patients with ovulation and 44 patients with no anovulation in obese group. Ovulation rate in non-obese group was 85.94%, while ovulation rate in obese group it was 69.66%; the difference of ovulation rates between two groups was significant ($p < 0.01$). After treatment of ovulation, SHBG levels of patients with ovulation in both groups were higher than those of patents with anovulation in both groups, while FAI and FINS of patients with ovulation in both groups were lower than those of patients with anovulation in both groups, and there were all significant differences. LH level and LH/FSH ratio of patients with ovulation in non-obese group were lower than those of patients with anovulation, while HOMA-IR of patients with ovulation in both groups were higher than those of patients with anovulation, and there were all significant differences.

Comparison of indicators after treatment of patients in obese group and in non-obese group between delivery of live birth patients and abortion patients

After treatment, there were 147 patients that had delivery and 42 patients that had miscarriage in non-obese

Table 3. — *Comparison of various biochemical indexes between before and after treatment of patients in non-obese group and in obese group ($\bar{x} \pm s$).*

Groups	N (cases)	LH (IU/l)		LH/FSH		FBG (mmol/l)	
		Ovulation	Anovulation	Ovulation	Anovulation	Ovulation	Anovulation
Non obesity	434	6.74 ± 2.82	7.92 ± 3.94#	1.07 ± 0.48	1.35 ± 0.64#	5.04 ± 0.72	5.13 ± 0.74
Obesity	145	6.43 ± 2.74	7.42 ± 4.88	1.03 ± 0.41	1.19 ± 0.65	4.96 ± 0.53	5.13 ± 0.58

Groups	N (cases)	T (nmol/l)		SHBG (nmol/l)	
		Ovulation	Anovulation	Ovulation	Anovulation
Non obesity	434	1.58 ± 0.61	1.70 ± 0.77	120.99±57.70	76.91 ± 51.14#
Obesity	145	1.57 ± 0.62	1.71 ± 0.67	126.12±48.64	72.91 ± 49.72#

Groups	N (cases)	FAI		FINS (mU/l)		HOMA-IR	
		Ovulation	Anovulation	Ovulation	Anovulation	Ovulation	Anovulation
Non obesity	434	1.68 ± 1.21	3.42 ± 2.80#	8.39 ± 3.81	9.42 ± 4.10*	1.89 ± 0.96	2.12 ± 0.91
Obesity	145	1.48 ± 0.90	3.41 ± 2.38#	9.76 ± 3.96	12.16 ± 5.98*	2.18 ± 1.10	2.84 ± 1.54#

*Compared between ovulation and anovulation patients of two groups, #$p < 0.01$, *$p < 0.05$.

Table 4. — *Comparison of various indexes after treatment of patients in non-obese group and in obese group between delivery of live births patients and abortion patients ($\bar{x} \pm s$).*

Groups	N (cases)	LH (IU/l)		LH/FSH		T (nmol/l)	
		Delivery	Miscarriage	Delivery	Miscarriage	Delivery	Miscarriage
Non obese	189	6.88 ± 2.97	7.43 ± 3.04	1.11 ± 0.52	1.16 ± 0.45	1.50 ± 0.59	1.76 ± 0.59*
Obese	62	7.42 ± 3.40	6.05 ± 1.91	1.10 ± 0.45	1.07 ± 0.34	1.49 ± 0.57	1.92 ± 0.51*

Groups	N (cases)	SHBG (nmol/l)		FAI		FBG (mmol/l)	
		Delivery	Miscarriage	Delivery	Miscarriage	Delivery	Miscarriage
Non obese	189	128.33±61.40	105.85 ± 48.55*	1.54 ± 1.14	2.02 ± 1.21#	4.96 ± 0.71	5.16 ± 1.16
Obese	62	123.46±35.22	97.80 ± 50.25*	1.30 ± 0.59	2.62 ± 1.90#	4.98 ± 0.61	5.01 ± 0.52

Groups	N (cases)	FINS (mU/l)		HOMA-IR	
		Delivery	Miscarriage	Delivery	Miscarriage
Non obese	189	7.67 ± 3.29	9.40 ± 3.71#	1.71 ± 0.91	2.15 ± 0.94*
Obese	62	8.89 ± 2.73	12.3 ± 4.96#	2.0 ± 0.79	2.76 ± 1.29*

*Compared between miscarriage and delivery patients of two groups, #$p < 0.01$, *$p < 0.05$.

group; there were 40 patients that had delivery and 22 patients that had miscarriage in obese group. After the differences were compared between biochemical indicators when patients in obese group or in non-obese group had delivery of live births or in pregnancies whose gestational age was less than 12 weeks, and in ones with spontaneous abortions after treatment. It was shown that T, FAI, FINS, HOMA-IR, and other indicators when patients in obese group or in non-obese group had delivery of live births or pregnancy were all lower than those that had spontaneous abortions after treatment and there were significant differences. While SHBG level of patients that had delivery was higher than those that had spontaneous abortions, and there were significant differences (Table 4).

Discussion

Patients with PCOS complicated with infertility is mainly caused by ovulation obstacle, and their infertility is related to insulin resistance, high androgen hormone levels in blood, and high level of LH. Women with oli-gomenorrhea or amenorrhea have about a 90% chance of being diagnosed with PCOS [1]. Therefore it is extremely important to adopt earlier treatment before ovulation induction.

Diane-35 is commonly used drug for treating patients with PCOS, which contains cyproterone acetate, has strong progesterone activity, so it can reduce ovarian androgen secretion through the inhibition of LH secretion and bind to androgen receptors of target cells, thereby blocking androgen action of peripheral target organs. It can also inhibit 5 alpha reductase activity in skin. It has been reported that

cyproterone acetate increased ability of insulin growth factor-1 (IGF-1) binding protein, decreased the level of free insulin growth factor, thereby reducing synergy of IGF-1 in androgen synthesis process [5]. Diane-35 contains estrogen that can increase SHBG levels, and reduce free androgen levels. However, the present study found that Diane-35 lowered the level of androgen, at the same time, it did not improve insulin sensitivity, can even cause abnormal glucose tolerance in non-obese patients with PCOS; this result was similar to ones reported in the literature [6-8]. Considering that cyproterone acetate in Diane-35 has glucocorticoid activity and promotes gluconeogenesis, and is against insulin action, therefore non-obese patients with PCOS should be considered to use oral contraceptives having no glucocorticoid activity, while PCOS patients with insulin resistance should use insulin sensitizer metformin, thereby lowering insulin levels, reducing insulin resistance, improving ovarian function, and glucose metabolism function of PCOS patients, treating high androgen hormone levels in blood, and restoring menstruation and ovulation function.

The present research showed that SHBG levels in patients with ovulation after ovulation induction was much more elevated than in patients that were anovulatory, while the levels of FAI and FINS in patients with ovulation after ovulation induction were lower than in patients that were anovulatory, and there were significant differences between two groups. Abroad there have been similar reports [9]. This study suggests that SHBG and FAI levels can all be considered as effective treatment indexes to evaluate whether treatment measures both in either obese or non-obese patients with PCOS are effective or not.

This research showed that the levels of T and FAI and FINS and HOMA-IR in abortion group were higher than that in delivery group after treatment, while the SHBG level was lower than that in delivery group. The reason why patients with PCOS had abortion may be related to high androgen hormone levels in blood and insulin resistance. SHBG level decreasing in patients with PCOS elevates the level of free androgens in serum, which further promotes expression of the androgen receptor (AR) in the local endometrial. AR has similar effect to progesterone receptor (PR), also reduces the expression of integrin α V β3 in PCOS patients, which may be associated with implant failure and high abortion rates of patients with PCOS. Homeobox gene HOXA10 is one of the molecular markers on endometrial receptivity, and is regulated by steroid hormone. Some research has found that testosterone of ovarian origin in vitro down-regulated HOXA10 expression in endometrial cells of Ishikawa, and found that there was HOXA10 mRNA expression decrease in endometrial biopsy specimens of PCOS patients, which might also be one cause of endometrial receptivity found in patients with PCOS [10, 11]. Recent studies suggest that insulin resistance elevating HOMA-IR may leaded to recurrent spontaneous abortion [12]. Obesity is one of the risk factors of spontaneous abortion [13], and obese patients with PCOS have varying degrees of insulin resistance [14].

Insulin resistance resulted in PCOS patients' endometrial hyperplasia abnormal and function defect. Early pregnancy immune inhibitory glycoprotein glycodelin may inhibit the immune response of endometrial to embryos the insulin-like growth factor-binding protein-1 (IGFBP-1) is advantageous to the embryo in the adhesion process of maternal-fetal interface [15], but hyperinsulinemia has a negative impact on the pre-implantation environment by reducing the expression of glycodelin and IGFBP-1 [11]. Jennifer *et al.* [16] observed that IGFBP-1 and glycodelin in PCOS patients' serum during their early pregnancy were significantly lower than that in the control group. Thus speculated that the two reduced proteins may be related to occurrence of spontaneous abortion of PCOS patients. Hyperinsulinemia also can upregulate the level of plasminogen activator inhibitor-1 (PAI-1), so that induced thrombosis affects the placental blood supply, making the trophoblast dysplasia lead to miscarriage [17]. Yilmaz *et al.* [18] found that PCOS patients have hyperhomocysteinemia which was positively associated with insulin resistance; hyperinsulinemia leads to hyperhomocysteinemia. Hyperhomocysteinemia may increase vascular endothelial oxidative stress response, activation of platelets, promote thrombosis, stimulate vascular smooth muscle cell proliferation, promote endothelial apoptosis, interfere with maternal-fetal interface endometrial blood flow, and vascular integrity, rendering the endometrial environment conducive to embryo growth, or more likely lead to early abortion [19-21]. In addition, there is subtle relationship between hyperinsulinemia and high androgen hormone levels in blood; hyperinsulinemia inhibits hepatic SHBG synthesis, causing free androgen increased in the body. The reports suggested that through research on intima of women with PCOS, SHBG expression decreased in endometrial stromal of PCOS patients with insulin resistance may lead to abnormal steroid environment, and also to change of regulation mechanism, thus leading to abortion. Insulin resistance and hyperinsulinemia might become a central link of abortion in overweight patients with PCOS [22-24]. Combining the present research with practical clinical significance, the authors believe that SHBG and FAI can be used as an effective therapeutic evaluation index during ovulation treatment and artificial pregnancy.

In conclusion, this study suggests that whether there is insulin resistance or not, SHBG and FAI can be used as an effective treatment evaluation index, both in either obese or non-obese patients with PCOS, which can cue information of ovulation treatment and pregnancy outcome. Monitoring the levels of SHBG and FAI can guide clinical medication during the treatment of PCOS patients and can thus reduce the abortion rate, which has an important significance in guiding aristogenesis and good fostering.

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