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CLINICAL STUDY

Spleen-*Yang*-deficiency patients with polycystic ovary syndrome have higher levels of visfatin

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Abstract

OBJECTIVE: To study serum visfatin levels in women with polycystic ovary syndrome (PCOS) grouped by Traditional Chinese Medicine (TCM) patterns. To study the correlations of serum visfatin levels with homeostatic model assessment insulin resistance (HOMA-IR), fasting plasma glucose (FPG), fasting insulin (FINS), body mass index (BMI), testosterone (T), total cholesterol (TC), and triglycerides (TG).

METHODS: Two hundred and twelve PCOS patients were placed into the following TCM pattern subgroups: Kidney-*Yang* deficiency (KYD) group, Spleen-*Yang* deficiency (SYD) group, stagnant Liver-*Qi* transforming into heat (SLQTH) group, and Kidney-*Yin* deficiency (KYIND) group. The correlations between serum visfatin levels and HOMA-IR, FPG, FINS, BMI, T, TC, and TG were analyzed.

RESULTS: Of all patients with PCOS, there were 82 in the KYD group (38.6%), 67 in the SYD group (31.6%), 37 in the SLQTH group (17.5%), and 26 in the KYIND group (12.3%). Visfatin levels in all PCOS subgroups were higher than those in the control group ($P<0.01$ or $P<0.05$). Among these subgroups, the visfatin levels in the SYD group were significantly higher than those in the other three TCM pattern groups ($P<0.05$). There were no statistical differences among the remaining three pattern groups. The levels of BMI, FINS, HOMA-IR, T, and TG were significantly higher in all subgroups than those in the control group ($P<0.05$). There were no significant differences in FPG and TC between all PCOS subgroups and the control group ($P>0.05$). The SYD group had higher levels of FINS and HOMA-IR compared with the KYD, SLQTH, and KYIND groups ($P<0.05$). In all subgroups, after controlling for BMI, TG, TC, and age, visfatin was positively correlated with FINS ($r=0.197$, $P=0.015$) and HOMA-IR ($r=0.173$, $P=0.033$), and was not correlated with T.

CONCLUSION: KYD and SYD patterns are most common in PCOS patients. Increased visfatin is a common pathophysiologic manifestation in PCOS patients. The SYD group had the highest levels of visfatin, and visfatin was positively correlated with FINS and HOMA-IR.

Key words: Polycystic ovary syndrome; Medicine, Chinese traditional; Pattern; Nicotinamide phosphoribosyltransferase

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a common reproductive endocrine disorder. About 50% of PCOS patients have different levels of insulin resistance (IR).[1] Traditional Chinese Medicine (TCM) has been used to treat infertility for thousands of years. Recent studies show that visfatin is associated with type 2 diabetes mellitus, IR, PCOS, and other diseases.[2-6] In this study, we studied serum visfatin levels in women with different TCM patterns and PCOS and their correlations with homeostatic model assessment insulin resistance (HOMA-IR), fasting plasma glucose (FPG), fasting insulin (FINS), body mass index (BMI), testosterone (T), total cholesterol (TC), and triglycerides (TG).

SUBJECTS AND METHODS

Patient inclusion and exclusion

The patient cohort included 212 women with PCOS in the Reproductive Center of the First Affiliated Hospital, Chongqing Medical University between June 2010 and April 2013. Diagnosis criteria for PCOS were established by the Ministry of Health of the People's Republic of China.[7] Women with PCOS were excluded if they took drugs within 6 months of the trial that could possibly affect glycolipid metabolism, such as corticosteroids, lipid-lowering drugs, anti-obesity drugs, and anti-diabetic drugs. Patients were also excluded if they had acute inflammation. Patients were grouped into four subgroups based on patterns differentiated according to TCM theory. Thirty-six infertile women served as the control group. All patients gave informed consent and the trial was approved by the Ethics Committee of The First Affiliated Hospital of Chongqing Medical University.

Criteria of TCM pattern differentiation

TCM pattern differentiation criteria were established in reference to the literature.[8,9] The main symptoms of Kidney-*Yang* deficiency (KYD) are oligomenorrhea and amenorrhea. Minor symptoms include: aversion to cold, loose stool, lassitude in the loin and legs, sexual hypoesthesia, obesity, hair growth, and gonadal dysgenesis. The tongue-pulse diagnosis for KYD is plump pale tongue with white fur, purplish dim tongue with a whitish coating, or white and thin fur, and a sunken and thin pulse. The main symptoms of Spleen-*Yang* deficiency (SYD) include oligomenorrhea, menorrhagia, or amenorrhea. Minor symptoms include: oppressed feeling in the chest and nausea, anorexia, sallow complexion, drowsiness, hypodynamia, obesity, hair growth, and gonadal dysgenesis. The tongue-pulse diagnosis of SYD is plump pale tongue with white fur, teeth-printed tongue, purplish dim tongue with whitish coating, or white and thin fur, and a thin and slippery pulse. The main symptoms of stagnant Liver-*Qi* transforming into heat (SLQTH) are irregular menstrual period, oligomenorrhea, amenorrhea, or polyhypermenorrhea. Minor symptoms include: anxiety and irritability; fullness in the chest, hypochondrium and breast; galactorrhea; bitter taste in the mouth; furuncles; and postconnubial dysgenesia. The tongue-pulse of gonadal dysgenesis include a red tongue with a thin and yellow coating, and a thin and taut pulse. The main symptoms of Kidney-*Yin* deficiency (KYIND) are delayed menstrual cycle, oligomenorrhea, amenorrhea, or advanced menstrual cycle. Minor symptoms include: feverish sensation in the palms and soles, furuncles, dry mouth, coprostasis, obesity, hair growth, postconnubial dysgenesia. The tongue-pulse diagnosis is a red tongue with a thin coating, and a thin and rapid pulse.

Measurement of circulating parameters

Patient FPG, FINS, T, TC, TG, height, and weight were measured. Fasting plasma glucose (FPG) was measured immediately by the enzymatic method using an Areoset analyzer (BS-200, Mindray, Shenzhen, China) in hospital laboratory. The Fasting serum insulin (FINS) and testosterone (T) were analyzed by the chemiluminescent immunometric assay test method using an Immulite analyzer (DXI-800, Beckman Coulter, Brea, USA) in hospital laboratory. The serum total cholesterol (TC) and triglycerides (TG) were assessed by enzymatic methods (Modular DDP, Roche, Basel, Switzerland) in hospital laboratory.

Visfatin levels were measured with an enzyme-linked immunosorbent assay kit (Bio-Swamp, Shanghai, China), following the manufacturer's instructions. The minimum detectable concentration was 1 μg/L, the intra-assay coefficient of variation was less than 9% , and the inter-assay coefficient of variation was less than 15%.

The following formulas were used to calculate BMI and HOMA-IR, respectively:

$$BMI=Weight\ (Kg)/(Height\ [m])^2$$

$$HOMA\text{-}IR=(FPG[mmol/L]\times FINS[mIU/L])/22.5.$$

Data analysis

SPSS 19.0 software (IBM, Chicago, IL, USA) was used for data analysis. Quantitative data are expressed as Mean±*SD*. The differences among groups were evaluated by one-way analysis of variance (non-parametric test were used for heterogeneity of variance). Quantitative data were compared using a *Chi*-square test. Correlations between variables were assessed by Spearman's correlation analysis and partial correlation analysis. $P<0.05$ was considered significant.

RESULTS

Classified characteristics of TCM patterns in PCOS patients

The cohort included 212 PCOS patients who had

complete information and conformed to the diagnosis criteria. The percentages of KYD, SYD, SLQTH, and KYIND were: 38.6% (*n*=82), 31.6% (*n*=67), 17.5% (*n*=37), and 12.3% (*n*=26), respectively (Table 1). There was no significant difference in percentage between the KYD group and the SYD group ($P>0.05$). However, the KYD and SYD groups had significantly higher percentages than the SLQTH and KYIND groups ($P<0.05$). In addition, no significant differences in mean age or duration of infertility were observed between the PCOS group and the control group ($P>0.05$).

Comparison of the characteristics of four TCM different pattern PCOS patients

The clinical and hormonal characteristics of different TCM patterns of PCOS and the control group are shown in Table 2. BMI, FINS, HOMA-IR, T, TG, and visfatin were significantly higher in PCOS group than those in the control group ($P<0.01$). There were no statistically significant differences in FPG and TC between the PCOS and control groups ($P>0.05$). The SYD group had significantly higher levels of FINS, visfatin, and HOMA-IR than those in the KYD, SLQTH, and KYIND groups ($P<0.05$). There were no significant differences in FINS, visfatin, and HOMA-IR among the KYD, SLQTH, and KYIND groups (KYD *vs* SLQTH, KYD *vs* KYIND, and SLQTH *vs* KYIND, $P>0.05$). The serum level of testosterone in the KYIND group was significantly higher than that in the KYD, SLQTH, and SYD groups ($P<0.05$). No significant differences in testosterone were found among the KYD, SLQTH, and SYD groups (KYD *vs* SLQTH, KYD *vs* SYD, and SLQTH *vs* SYD, $P>0.05$). Additionally, no statistically significant differences were observed in the levels of BMI, FPG, TG, and TC among the four Chinese medical syndrome groups.

Correlation analyses of clinical, biochemical, and hormonal parameters in PCOS patients

The correlation analysis of clinical, biochemical, and hormonal parameters in the PCOS group are shown in Table 3. Visfatin is positively correlated with FINS ($r=0.171$, $P=0.013$), HOMA-IR ($r=0.185$, $P=0.007$), and T ($r=0.148$, $P=0.047$). There were no correlations between visfatin and BMI, FPG, TC, or TG ($P>0.05$). No correlations were observed between TG and BMI, FINS, or HOMA-IR ($P>0.05$). TC did not show any significant correlations with FINS and HOMA-IR ($P>0.05$), and there were also no correlations between T and BMI, FPG, FINS, HOMA-IR, TG, or TC ($P>0.05$).

Table 1 Characteristics of TCM patterns in PCOS patients

Group	*n*	Age (years)	Duration of infertility (years)	*P* value (%)
KYD	82	28.3±3.9	3.4±0.5	38.6
SYD	67	29.2±5.2	3.2±0.5	31.6
SLQTH PCOS	37	27.1±3.1	3.6±0.4	17.5
KYIND	26	26.8±2.7	3.2±0.6	12.3
Controls	36	29.2±2.7	3.1±0.6	-

Notes: KYD: kidney-*Yang* deficiency group; SYD: spleen-*Yang* deficiency group; SLQTH: stagnant Liver-*Qi* transforming into heat group; KYIND: kidney-*Yin* deficiency group; PCOS: polycystic ovary syndrome; TCM: Traditional Chinese Medicine.

Table 2 Characteristics of four TCM pattern groups and control group ($\bar{x}\pm s$)

Parameter	KYD	SYD	SLQTH PCOS	KYIND	Controls
n	82	67	37	27	36
BMI (kg/m^2)	24.63±3.09[a]	25.57±3.29[a]	24.39±3.22[a]	24.64±2.95[a]	20.09±1.14
T (ng/mL)	0.51±0.17[ab]	0.54±0.24[ab]	0.52±0.21[ab]	0.67±0.22[a]	0.25±0.06
FPG (mmol/L)	5.19±0.37	5.27±0.54	5.19±0.35	5.18±0.35	5.05±0.28
FINS (mIU/L)	10.04±2.73[ac]	12.65±5.02[a]	10.02±2.18[ac]	9.98±3.24[ac]	5.86±0.67
HOMA-IR	2.31±0.65[ac]	3.00±1.36[a]	2.32±0.51[ac]	2.31±0.79[ac]	1.31±0.15
Visfatin (ng/mL)	22.89±16.66[ac]	29.10±18.74[a]	18.93±18.79[ad]	19.32±18.01[ad]	5.55±3.30
TG (mmol/L)	1.47±0.75[a]	1.64±1.12[a]	1.31±0.49[a]	1.30±0.51[a]	0.56±0.14
TC (mmol/L)	4.43±0.94	4.39±0.92	4.16±0.42	4.27±0.41	3.96±0.88

Notes: KYD: kidney-*Yang* deficiency group; SYD: spleen-*Yang* deficiency group; SLQTH: stagnant liver-*Qi* transforming into heat group; KYIND: kidney-*Yin* deficiency group; TCM: Traditional Chinese Medicine; BMI: body mass index; T: testosterone; FPG: fasting plasma glucose; FINS: fasting insulin; HOMA-IR: homeostatic model assessment insulin resistance; TG: triglycerides; TC: total cholesterol. [a]$P<0.01$, for the difference between the PCOS and control groups; [b]$P<0.05$, for the difference between the KYIND, KYD, SYD, or SLQTH groups; [c]$P<0.05$, [d]$P<0.01$ for the difference between the SYD and KYD groups, or the KYIND and SLQTH groups.

Table 3 Relationships between clinical, biochemical, and hormonal parameters

Parameter	Visfatin		T		BMI		TG		TC	
	r	*P* value	*r*	*P* value	*r*	*P* value	*r*	*P* value	*r*	*P* value
BMI	0.125	0.069	0.042	0.576	-	-	0.039	0.569	0.030	0.667
FPG	0.061	0.375	0.069	0.335	0.072	0.295	-	-	- 0.040	0.733
FINS	0.171	0.013	0.015	0.839	0.085	0.218	0.079	0.253	0.001	0.997
HOMA-IR	0.185	0.007	0.033	0.660	0.117	0.089	- 0.109	0.112	- 0.021	0.765
T	0.148	0.047	-	-	0.042	0.576	0.014	0.857	0.180	0.016
TG	- 0.047	0.492	0.014	0.857	0.039	0.569	-	-	0.305	0.000
TC	- 0.124	0.071	0.180	0.016	0.030	0.667	0.305	0.000	-	-

Notes: BMI: body mass index; FPG: fasting plasma glucose; FINS: fasting insulin; HOMA-IR: homeostatic model assessment insulin resistance; T: testosterone; TG: triglycerides; TC: total cholesterol.

Correlation analyses of clinical, biochemical, and hormonal parameters in PCOS patients after controlling for BMI, TG, TC, and age

The relationships between visfatin and clinical, biochemical, and hormonal parameters in the PCOS group after age, BMI, TG, and TC were controlled for are shown in Table 4. Partial correlation analysis shows that after controlling for BMI, TG, TC, and age, visfatin still positively correlated with FINS (r=0.197, P= 0.015) and HOMA-IR (r=0.173, P=0.033). There was no correlation between visfatin and T (P>0.05).

Table 4 Relationships between clinical, biochemical and hormonal parameters in all PCOS patients after controlling for BMI, TG, TC, and age

Statistical Parameter	FPG	FINS	HOMA-IR	T
r	- 0.021	0.197	0.173	0.142
P value	0.8	0.015	0.033	0.081

Notes: FPG: fasting plasma glucose; FINS: fasting insulin; HOMA-IR: homeostatic model assessment insulin resistance; T: testosterone. BMI: body mass index; TG: triglycerides; TC: total cholesterol.

DISCUSSION

PCOS is an endocrine disorder syndrome characterized by reproductive dysfunction and abnormal glucose metabolism. Modern TCM theory hypothesizes that the main mechanism of PCOS is the dysfunction of Kidney, Liver, and Spleen, and generational and restrictional interrelationships among the Kidney-menstruation-Ren Chong-uterus imbalance. Consistent with other reports,[10-12] our data show that, in 212 patients with PCOS, the proportions of KYD (38.6%) and SYD (31.6%) were significantly higher than those of SLQTH and KYIND (P<0.05). After analyzing the syndrome differences among 120 patients with PCOS,[10] we found that the KYD ratio was higher than other patterns (P<0.05). Xiong *et al* [11] found that SYD had the highest proportion (48%) in 105 PCOS patients, followed by kidney deficiency (32%). Therefore, we considered KYD and SYD as the dominant TCM patterns in patients with PCOS. Moreover, the pathogenesis of PCOS may be associated with Spleen-*Yang* and Kidney-*Yang* deficiency and phlegm dampness.

Visfatin was first isolated from visceral adipose tissue of human and mouse by Fukuhara *et al* [13] in 2005. Visfatin can bind to the insulin receptor and phosphorylate the tyrosine residues of the insulin receptor, insulin receptor substrate (IRS)-1, and IRS-2. This phosphorylation activates protein kinase B and the mitogen-activated protein kinase signaling pathway. Therefore, visfatin shares the same signal transduction pathways as insulin, and has similar hypoglycemic and other "insulin-like" functions. However, the correlations between visfatin and FINS and HOMA-IR are still controversial.[14-16] In the present study, we found that BMI, FINS, HOMA-IR, and visfatin in PCOS patients were higher than those in the control group (P<0.01). Increased serum visfatin in PCOS patients with abnormal glucose tolerance may be a compensatory response for insulin resistance by binding with insulin receptors to increase insulin sensitivity. Abnormal glucose tolerance and insulin resistance may be the two causes of increased serum visfatin. Correlation analysis showed that, after controlling for the influence of BMI, TG, TC, and age, visfatin was positively correlated with FINS and HOMA-IR in the PCOS group. This indicates that visfatin is positively correlated with insulin resistance in PCOS patients and might reflect the extent of insulin resistance. Therefore, obesity may be an abnormal visfatin condition, but obesity is not a determinant of visfatin levels in PCOS patients.

In PCOS patients, abnormal lipid metabolism is closely related to glucose metabolism. Similar to the observation by Tarkun *et al*,[17] we found that TG in PCOS patients was higher than that in the control group (P< 0.05). There were no differences in TC between the PCOS and control groups. Another study showed that insulin reduced the expression of adipose triglyceride lipase (ATGL) in 3T3-L1 cells, and ATGL expression in a diabetes-obesity mouse model (ob/ob, db/db) decreased by 50%.[18] This suggested that ATGL expression decreases during IR. However, the main function

of ATGL, the key enzyme of lipid metabolism, is to catalyze TG by hydrolyzing it into diglycerides and FFA. In this case, IR decreases TG hydrolysis, resulting in increased TG. Other researchers found that TG and TC in the IR group were higher than those in the non-IR group, and TG and TC were positively correlated with HOMA-IR.[19] However, the results of our study showed that TG was not correlated with FPG, FINS, or HOMA-IR. Therefore, IR may be the main reason for elevated TG *in vivo*, and may play a role in the occurrence of dyslipidemia in PCOS patients. In contrast to TG, visfatin could more effectively reflect the state of insulin resistance in PCOS women.

Hyperandrogenism is considered to be the main endocrine characteristic of PCOS patients. Previous studies found that insulin could increase the synthesis of androgen,[20,21] and visfatin was positively correlated with T in patients with PCOS.[2,14] In the present study, T levels were significantly higher in PCOS patients than those in the control group (P<0.01). T was positively correlated with visfatin, but not FINS or HOMA-IR. Consistent with others,[5,22-24] there were no correlations between T and FINS or HOMA-IR after controlling for BMI. Therefore, we believe that visfatin was not correlated with T, and had no direct links with synthesis, secretion, and metabolism of sex hormones.

In the present study, the BMI, FINS, HOMA-IR, and serum visfatin levels of PCOS patients with four different TCM syndromes were higher than those in the control group (P<0.01). The levels of visfatin, FINS, and HOMA-IR in the SYD group were higher than those in the other three groups (P<0.05). Elevated serum visfatin level is a common pathological manifestation in PCOS with different TCM syndromes, especially in SYD patients. According to TCM theory, because of spleen-*Yang* deficiency, the dysfunction of transformation and transportation, and the stasis of body fluids and food essence in the meridians and collaterals, the muscular interstitial space cannot be used by the body and there is stagnation of phlegm, which will eventually lead to obesity. The increase in adipose tissue is the most important reason for increased FINS and HOMA-IR. Our previous study retrospectively analyzed 510 patients with PCOS and found that IR in the SYD group is higher than those in the other three groups (P<0.01).[25] Based on these results, the main pathological manifestations of SYD PCOS patients are glucose and lipid metabolism disorders. The spleen is the main organ involved in metabolism, especially in glucose and lipid metabolism. Therefore, the transportation dysfunction of the spleen may be similar to the pathological mechanisms of insulin resistance in Western Medicine.

In conclusion, the patterns of KYD and SYD were most common in patients suffering from PCOS. The levels of visfatin were significantly higher in the PCOS group than those in the control group. FINS, HOMA-IR, and visfatin levels were highest in the SYD group. Visfatin was positively correlated with FINS and HOMA-IR, and it could more effectively reflect the insulin resistance situation of PCOS patients than T or TG. Therefore, visfatin may be an indicator for the management of PCOS.

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