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Full Length Research Paper

Correlation analysis of inhibin B, follistatin and activin A in patients with polycystic ovary syndrome

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Polycystic ovarian syndrome is a term used to describe women who have a tendency, because of abnormal ovulation to develop multiple small cysts on their ovaries. In this study, we investigated the abnormal levels of serum inhibin B, follistatin and activin A in patients with polycystic ovary syndrome (PCOS). Serum levels of inhibin B, follistatin and activin A on cycle day 3 were measured both in PCOS group and in controls (women with regular cycle). The difference between the two groups and relationship of inhibin B, follistatin and activin A with other sex hormone, body mass index (BMI), insulin and fast glucose were analyzed. Levels of serum inhibin B in PCOS were higher (P < 0.001) (290 ± 86 pg/ml) than those of controls (172 ± 53 pg/ml) and follistatin were higher (P = 0.01) in PCOS (1317 ± 392) pg/ml than controls (804 ± 203) pg/ml. Levels of serum activin A were lower (P < 0.05) in PCOS (545 ± 44 pg/ml) than those of controls (588 ± 89 pg/ml). In PCOS women, serum inhibin B levels were positively correlated with insulin (r = 0.436, P < 0.05) and glucose (r = 0.618, P < 0.05). Serum activin A levels were negatively correlated with insulin (r = -0.442, P < 0.05). Serum follistatin levels were positively correlated with age and negatively correlated with T. Abnormal paracrine or autocrine in inhibin/follistatin/activin system may be partly responsible for the hypogenesis of pre-ovular follicles in PCOS and it is obvious that hyperinsulinemia may be affected by higher concentrations of inhibin B and lower concentrations of activin A.

Key words: Polycystic ovary syndrome, follistatin, activin, hyperinsulinemia.

INTRODUCTION

The polycystic ovary syndrome (PCOS) is a common and complex endocrine disorder affecting 5 – 10% of women of reproductive age and is characterized by hyperandrogenism and chronic anovulation with consequential menstrual irregularity and subfertility (Knochenhauer et al., 1998; Zacur, 2001). During pregnancy, women with PCOS were described to carry an increased risk for complications, that is, miscarriage, preterm labour, gestational diabetes and preeclampsia (Glueck et al., 2000; Legro, 2007). Moreover, PCOS is often associated with features of the metabolic syndrome (that is, central obesity, high blood pressure and elevated serum triglycerides, impaired glucose tolerance and subsequent increased risk of developing diabetes mellitus type 2 in later life) (Conn, Jacobs and Conway, 2000).

As many as 70% of women with PCOS exhibit insulin resistance, with the compensatory hyperinsulinemia considered to be the cause of the hyperandrogenism (Kolodziejczyk et al., 2000) . The Chinese population comprises more than one fifth of the total world population (World Health Organization (WHO report, 2004); however, few studies of the metabolic status of Chinese women with PCOS have been performed (Chang et al., 2000; Chen et al., 2006).

Polycystic ovary syndrome has varied onset and clinical presentation. Asian women display stigmata of insulin resistance at a lower body mass index (BMI) than other populations (Holte, 1998; Chang et al., 2000; Chen et al., 2006). Hence, we designed a study to determine

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Table 1. Clinical and endocrine characteristics in patients with PCOS and controls.

	PCOS	Controls	P value
Clinical			
Age (years)	26.9 ± 3.8	29.1 ± 3.5	0.003
BMI	23.7 ± 3.8	20.7 ± 2.0	0.035
Endocrine			
Oestradial (pmol/l)	82 ± 23	79 ± 25	0.411
LH (IU/l)	7.6 ± 2.2	4.2 ± 1.7	0.003
FSH (IU/l)	3.8 ± 1.6	4.9 ± 2.0	0.032
LH/FSH ratio	2.2 ± 0.9	0.89 ± 0.3	0.001
Prolactin (mIU/l)	189 ± 23	168 ± 25	0.053
Testosterone (nmol/l)	2.47 ± 0.9	1.15 ± 0.5	0.002
Fasting insulin (mIU/l)	20.2 ± 6.1	12.4 ± 3.7	0.001
Fasting glucose(mmol/l)	4.8 ± 0.08	4.5 ± 0.08	0.06
Fasting insulin/glucose	4.4 ± 0.16	2.8 ± 0.12	0.001

The data are mean ± SEM.

clinical and endocrine characteristics in patients with PCOS compared with control. We also evaluated the serum inhibin B, follistatin and activin A levels in patients with PCOS.

MATERIALS AND METHODS

Subject

Thirty-five women with PCOS age, 26.9 ± 3.8 years (mean ± S D); BMI, 23.66 ± 3.8 kg/m^2 (mean ± SD)] were recruited at the First Affiliated Hospital of Suzhou University from 2002 to 2003. PCOS was diagnosed by the combination of one clinical criteria (menstrual and/or ovulatory disturbances, or minor signs such as acne or seborrhea) with one biologic criteria (LH/FSH 2 - 3, serum LH level > 10 IU/l and/or T > 3.5 nmol/ml and/or free insulin/glucose > 3) or by the ultrasonographic criterion of PCO (LI MZ. 2001).

Controls

The control population was comprised of 26 women [age, 29.1 ± 3.5 years (mean ± SD); BMI, 20.70 ± 2.0 kg/m^2 (mean ± SD)]. Inclusion criteria: a regular menstrual cycle, normal values of E_2, FSH, LH, PRL, T and no previous use of medication or oral contraceptives during at least 3 months prior to the study.

Sampling procedure

Blood samples were taken from all controls and PCOS subjects on cycle day 3. The last menstrual period occurred either spontaneously or after withdrawal of medroxygesterone in amenorrheic or oligmenorrheic PCOS women. Serum was stored at –20°C until used.

Hormonal immunoassays

Serum concentrations of inhibin A, inhibin B, activin A and follistatin were determined using two-site enzyme-linked immunosorbent assays (ELISA). E2, FSH, LH, PRL, T were determined by two-site enzyme-linked inmmunosorbent assay (Biosource, USA). Insulin concentrations were measured by radioimmunoassay (RIA). Inter- and intra-assay coefficients of variation were all < 10%. Glucose concentrations were measured using the glucose hexokinase method.

Statistical analysis

The results were presented as the mean ± SEM of the independent experiments. ANOVA was used for the analysis of the test results (LSD test) and factorial analysis and Duncan analysis of variance at the significance levels of $p < 0.05$ and $p < 0.05$ or $p < 0.01$, respectively, were considered to indicate significance. Correlation analyses were performed using linear regression and the Pearson's correlation coefficient (*r*). These analyses were conducted using the Excel 2000 SR-1 program (Microsoft, Troy, NY) and Statistica Kernel Release 7.1 (StartSoft Inc., Tulsa, OK) for Windows.

RESULTS

Clinical and endocrine characteristics in patients with PCOS and controls

Clinical and endocrine characteristics in patients with PCOS and controls were examined (Table 1). BMI, LH, fasting insulin levels were significantly higher in PCOS group than in control group ($P < 0.05$ or $P < 0.01$), whereas serum levels of FSH was lower in PCOS group ($P < 0.05$). In addition, LH/FSH and fasting insulin/ glucose ratios were significantly higher in PCOS group than in control group (both P = 0.001). Levels of Serum inhibin B, activin A and follistatin in patients with PCOS and Control women.

Levels of Inhibin B and follistatin in the serum of PCOS were significantly higher ($P < 0.01$) than those of control (Figure 1). In addition, concentration of activin A was significantly lowers ($P < 0.05$) in PCOS (545 ± 44 pg/ml) than control (588 ± 89 pg/ml) (Figure 1).

Effect of BMI on serum hormone levels in PCOS and control

Effect of BMI on serum hormone levels in PCOS and control was shown in Table 2. Concentrations of inhibin B and follistatin were significantly ($P < 0.05$) higher in PCOS than control, whereas concentration of activin A were significantly lower in PCOS than in control ($P < 0.05$).

Correlation analysis of inhibin B, follistatin and activin A with other hormones, age, BMI, insulin and glucose

In PCOS women, serum inhibin B levels displayed positive ($P < 0.05$) correlation with insulin and glucose ($r = 0.436$ and $r = 0.618$), whereas activin A were negatively

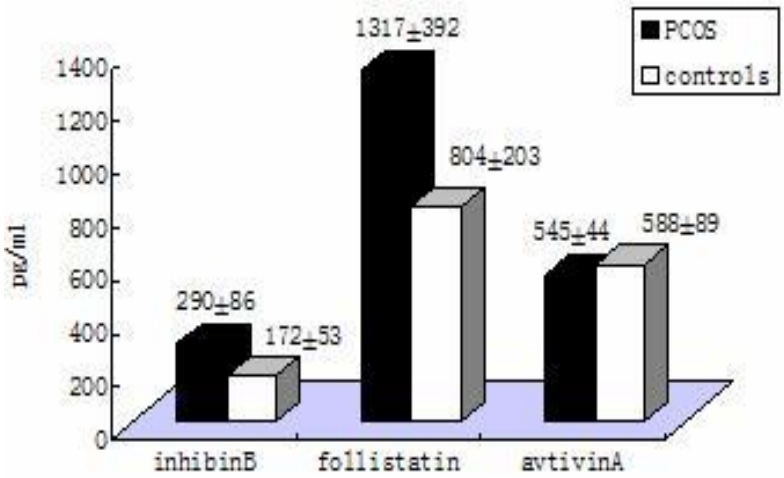

Figure 1. Serum levers of inhibinB, follitation and activinAin PCOS and controls.

Table 2. Serum levers of inhibin B, activin A, follistatin in PCOS and controls after BMI treatment.

	InhibinB	follistatin	activinA
PCOS (pg/ml)	304 ± 96	1345 ± 422	532 ± 31
Controls (pg/ml)	172 ± 53	804 ± 203	588 ± 89
P value	0.001	0.040	0.018

Table 3. Relation of inhibinB, follistatin and activinA to other hormons and BMI in PCOS and controls.

	age	BMI	insulin	glucose	E2	FSH	LH	PRL	T
PCOS (n=36)									
inhibinB	0.176	0.194	0.436*	0.618*	0.538*	-0.085	0.149	-0.358	-0.071
follistatin	0.115	-0.054	0.356	0.118	-0.314	-0.102	-0.401*	-0.079	-0.109
activinA	0.099	0.384*	-0.442*	-0.188	-0.003	-0.364*	-0.044	-0.190	0.030
Controls (n=22)									
inhibinB	-0.759**	0.147	-	-	0.420	-0.431	-0.07	0.262	0.663*
follistatin	0.473*	0.248	-	-	0.166	0.076	-0.006	-0.196	-0.572*
activinA	0.187	0.173	-	-	0.146	0.138	0.030	0.036	0.293

*$P < 0.05$, **$P < 0.01$

(P < 0.05) correlated with insulin and BMI (r = -0.442 and r = -0.364) (Table 3). In addition, inhibin B has posititive correlation with E_2 and follistatin has negative correlation with LH. In control women, serum inhibin B levels were positively correlated with T and negatively correlated with age. Serum follistatin levels were positively correlated with age and negatively correlated with T.

DISCUSSION

Available data, however, provide evidence that the development of PCOS is complex and is regulated by multiple genetic pathways and is modified by several exogenous factors. Insulin resistance plays a significant role in the pathogenesis of the PCOS and represents a link to the unfavorable cardiovascular risk profile frequently found in affected women (Önalan et al., 2005; Chen at al., 2009a and b). We found that BMI, LH, fasting insulin levels were significantly higher in PCOS group than in control group (P < 0.05 or P < 0.01), whereas serum levels of FSH was lower in PCOS group (P < 0.05) . In women with polycystic ovary disease who also have insulin resistance, glucophage (Metformin), a medi-

cation that makes cells more sensitive to insulin, has been shown to make ovulation normal. Losing weight (which can be difficult) may help to reduce the high insulin levels in the blood. For women with this condition who are overweight, weight loss can reduce insulin resistance, stimulate ovulation and improve fertility rates (Cheung and Williams, 2000).

Activin and inhibin are members of the transforming growth factor -b (TGF-b) superfamily of ligands, which also includes the bone morphogenic proteins (BMPs), müllerian inhibiting substance (MIS; antimüllerian hormone [AMH]) and growth differentiation factor 9 (GDF-9) (Duleba et al., 2000). The possible role of inhibin B, follistatin and activin A in the pathogenesis of ovary deserves investigation. We found that concentrations of inhibin B and follistatin were significantly ($P < 0.05$) higher in PCOS than control, whereas concentration of activin A were significantly lower in PCOS than in control ($P < 0.05$). Based on the present results, it can be suggested that ovarian cells have the intrinsic property of secreting higher amounts of inhibin B or follistatin than normal cells. The data had shown higher concentration of follistatin in PCOS. Body weight has been shown to affect the follistatin level in women with PCOS (Eldar- Geva, 2001); however, such an association between body weight and follistatin level was not found in another study (Norman et al., 2001; Chen et al., 2010). With regression analysis, in this study, we showed that in PCOS women, serum inhibin B levels displayed positive correlation with insulin and glucose, whereas activin A were negatively correlated with insulin and BMI. Activin A could act in an autocrine/paracrine or endocrine manner in different cells and tissues in pregnancy. Serum follistatin levels were positively correlated with age and negatively correlated with T. All tissues that secrete activin also secrete follistatin which provides a tight regulation of activin activity. When first trimester placental chorionic villous explants were cultured *in vitro*, activin A stimulated the out growth of cytotrophoblasts in to the surrounding matrix (Caniggia et al., 1997), an effect that was reversed by follistatin.

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