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Phoenixin-14 concentrations are increased in association with luteinizing hormone and nesfatin-1 concentrations in women with polycystic ovary syndrome

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ABSTRACT

Background: Polycystic ovary syndrome (PCOS) is commonly characterized by obesity, insulin resistance (IR), hyperandrogenemia and hirsutism. Following the reported relationship between phoenixin-14 and gonadotropin production in rat hypothalamic-pituitary-gonadal axis, the present study was designed to investigate the circulating concentrations of phoenixin-14 and their associations with the concentrations of sex hormones including luteinizing hormone (LH), follicular stimulating hormone (FSH), estradiol (E_2), progesterone (P_4) and total testosterone (TT) in PCOS patients.

Methods: A total of 41 women with diagnosed PCOS using Rotterdam criteria and 37 healthy individuals were enrolled in the study.

Results: Serum phoenixin-14 concentration in PCOS patients (n = 41) was 0.515 ± 0.044 ng/ml, significantly higher than that in healthy controls (0.289 ± 0.046 ng/ml, n = 37). PCOS patients had higher serum LH, dehydroepiandrosterone and fasting blood glucose concentrations, and higher index of homeostasis model of assessment-IR than those in healthy women. Correlation analysis showed significantly positive correlations of phoenixin-14 with LH, FSH, TT, P_4, BMI and nesfatin-1 concentrations, and significantly negative correlations with E_2 and serum insulin (FSI) concentrations, respectively.

Conclusions: Compared to control women, PCOS patients had significantly increased serum phoenixin-14, LH and androgen concentrations. The positive correlations of phoenixin-14 concentrations with LH and TT concentrations suggest a possible role of phoenixin-14 in the development of PCOS.

1. Introduction

Polycystic ovary syndrome (PCOS) is a multi-factorial endocrine disorder with a complex pathogenesis such as increased luteinizing hormone (LH) concentrations, Insulin resistance (IR) and a compensatory hyperinsulinemia. PCOS women were frequently overweight or obese with increased risk of type-2 diabetes, impaired glucose tolerance (IGT) and cardiovascular disease [1,2]. Hyperandrogenism and chronic anovulation were also associated with the syndrome, found in approximately 7% women of reproductive age [3,4].

In normal circumstances, final maturation as well as ovulation occurs upon LH stimulation. In PCOS, neuroendocrine abnormality may include rapid GnRH pulse frequency, which favors LH frequency and pulse amplitude over FSH production. This abnormality contribute to increased circulating LH/FSH ratio and is commonly observed in lean, but not obese, women with PCOS [5,6]. In girls with hyperandrogenism the increased LH pulses and accelerated daytime pulse secretion of LH were observed during early puberty, which indicated that disruption of the normal pulsatile GnRH secretion might underlie PCOS development in some patients [7]. In the ovaries, the increased LH/FSH ratio and the resistance to FSH are associated with enhanced androgens hypersecretion in ovarian follicle theca cells, which impairs follicular development

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and suppresses the inhibition of GnRH pulse frequency by progesterone, further stimulate the development of the PCOS phenotype [7]. Hypersecretion of LH is also detrimental to normal follicular growth, which might cause premature luteinization of granulosa cells and further related to changes (hypertrophy, lipid accumulation) in the follicle that generally occur after ovulation [8]. Under the increased stimulatory effect of LH, hypersecretion of theca cell-derived androgens promote the initiation of primordial follicle growth and number of small growing antral follicles, and, further impairs follicular maturation [9]. LH stimulatory effect on theca cells is further accelerated by the gonadotropic action of insulin on theca cells, either directly (through the insulin receptor) or indirectly through the IGF-1 receptor [10]. However, the effects of many regulatory proteins on the pathogenesis of PCOS are still unclear.

Phoenixin-14 and phoenixin-20 are novel endogenous neuropeptides which have recently been isolated and identified [11,12]. Phoenixin-14 is a 14 residue peptide and was found in multiple species including human, rat, mouse, porcine and canine [11,12]; whereas phoenixin-20 is a 20 residue peptide, an N-terminal extended phoenixin-14, and differs in one amino acid between the coding region of human, canine or porcine sequences [12]. In vitro study applied to anterior pituitary cells demonstrated that phoenixin might up-regulate pituitary gonadotropins including FSH and LH secretion by modulating the expression of the gonadotropin-releasing hormone (GnRH) receptor in the pituitary and potentiated the up-regulation of that receptor by GnRH itself [12]. In vivo knockdown of phoenixin expression in hypothalamus using small interfering RNA delayed the onset of estrus in female rats along with a reduction in pituitary GnRH receptor expression, suggesting the role of phoenixin in normal ovarian cyclicity [12].

Since phoenixin-14 was identified as an important component in initiating GnRH secretion at puberty [13] and the women with PCOS demonstrate higher LH concentrations compared with ovulatory women without the syndrome, it can be therefore hypothesized that phoenixin-14 might play an important role in the regulation of LH secretion by modulating the expression of the GnRH receptor in hypothalamus. To date no data have been reported on screening phoenixin-14 in women with and without PCOS.

2. Patients and methods

The study was approved by the ethics committee board of Zhejiang Women's Hospital, School of Medicine, Zhejiang University, Hangzhou, China. We recruited 78 voluntary women (37 controls and 41 PCOS patients), aged 24–35 years from the outpatient endocrine clinic of Zhejiang Women's Hospital in this study. All women voluntarily participated in the study and provided written informed consent. The diagnosis of PCOS was based according to the criteria of the Rotterdam ESHRE/ASRM-sponsored PCOS consensus workshop group (2003), which require two of the following 3 manifestations: 1) oligo- or anovulation, 2) clinical and/or biochemical signs of hyperandrogenism ($>$ 2.08 nmol/l), and 3) polycystic ovaries on ultrasound examination (the presence of $\geq$ 12 follicles measuring 2–9 mm in diameter and/or ovarian volume $>$ 10 cm^3) [14]. Patients suffering from thyroid dysfunctions, Cushing's syndrome, enzyme deficiency (21-hydroxylase in particular), androgen-secreting tumor, decreased ovarian reserve (primary ovarian insufficiency), or type 1 or type 2 diabetes were excluded. None of the subjects was taking any medication for at least 3 months before the study. All the patients had clinical and/or biochemical hyperandrogenism and chronic anovulation, and mostly the patients had polycystic ovaries on ultrasound.

The control group consisted of healthy women who had regular menstrual cycles (26–30 days) without evidence of above mentioned endocrine disturbances based on the similar initial laboratory workup as the PCOS patients and no history of any drug intake for at least 3 months. Additional exclusion criteria for both the groups included current smokers and those who consumed alcohol.

Blood samples were collected at 9:00 am after an overnight fast between the 3rd and 5th days of a spontaneous bleeding episode of the PCOS group and of a menstrual cycle of the controls. In all the women, basal serum concentrations of follicle-stimulating hormone (FSH), luteinizing hormone (LH), progesterone (P_4), prolactin (PRL), total testosterone (TT), free testosterone (Free TT), fasting blood glucose (FBG), fasting serum insulin (FSI), androstenedione (AS) and dehydroepiandrosterone sulfate (DHEA-S) were measured. Serum total cholesterol (TCHOL), high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol and triglycerides (TG) concentrations were also measured. Of the hormonal parameters, LH, FSH, E_2, TT, Free TT, AS, DHEA-S, FBG, FSI, PRL and P_4 were measured in an Immulite 2006 auto analyzer (IEMA; Siemens Healthcare Corp.) and lipids in an Olympus AU2700 clinical chemistry analyzer (Optical Co., Ltd.), using the kits recommended by the manufacturers. HOMA-IR index was calculated with the standard formula: HOMA-IR = fasting concentration of insulin (mIU/ml) $\times$ fasting concentration of glucose (mmol/l) / 22.5 [15].

Phoenixin-14, nesfatin-1 and kisspeptin concentrations were also measured in the blood samples in the same experimental series using commercial ELISA kits (Shanghai Westang Bio-Tech CO. LTD), respectively.

SPSS ver 15.0 was used for statistical analyses. Continuous variables were expressed as mean $\pm$ SD. The *t*-test was carried out for comparison between control and PCOS patients. The correlation between the parameters was analyzed with the Pearson method. Differences were considered significant at $p < 0.05$.

3. Results

3.1. Demographical characteristics and biochemical values of control women and PCOS patients

Comparisons of demographical characteristics and biochemical values of PCOS patients and healthy controls are shown in Table 1. The PCOS patients and controls showed no differences in terms of mean age, BMI, HDL, LDL, TG and TCHOL. However, the PCOS patients had significantly higher FBG than the control women (Table 1).

3.2. Baseline hormone concentrations in control women and PCOS patients

Compared with control group, the PCOS patients had significantly higher serum concentrations of AS, DHEA-S, TT, Free TT, LH, PRL, FSI as well as HOMA-IR, whereas no significant difference was found in P_4, FSH and E_2 concentrations between the 2 groups (Table 2).

Table 1
Demographical characteristics and biochemical values of controls and PCOS patients.

Parameter	Control (n = 37)	PCOS patients (n = 41)
Age (year)	29.8 ± 0.8	30.2 ± 0.8
BMI (kg/cm^2)	21.07 ± 0.48	22.60 ± 0.78
FBG (mmol/L)	5.07 ± 0.30	6.33 ± 0.51*
HDL (mmol/L)	0.98 ± 0.03	0.90 ± 0.05
LDL (mmol/L)	2.40 ± 0.11	2.09 ± 0.13
TG (mmol/L)	1.42 ± 0.18	1.70 ± 0.25
TCHOL (mmol/L)	4.01 ± 0.13	3.88 ± 0.20

Abbreviations: BMI body mass index, FBG fasting blood glucose, FSI fasting serum insulin, HOMA-IR homeostasis model of assessment-insulin resistance, HDL high density lipoprotein, LDL low density lipoprotein, TG triglyceride, TCHOL total cholesterol. Data are presented as mean ± standard deviation.

* $p < 0.05$ compared with control.