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Original Article

Association of IL-29 with the lipid metabolic features of polycystic ovary syndrome

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Abstract: Polycystic ovary syndrome (PCOS), the most prevalent metabolic endocrine disease in women of childbearing age, is also defined by chronic inflammation. Interleukin-29 (IL-29), an essential mediator of the inflammation process, is thought to play a critical role in the pathogenesis of PCOS. The aim of our study was to explore the relationship between IL-29 and PCOS, as well as the lipid metabolic features. Serum samples from 32 patients diagnosed with PCOS and 45 age- and BMI-matched healthy women were collected, and the level of IL-29 was detected by using enzyme linked immune sorbent assay (ELISA). Here, levels of hormones and lipid metabolism showed significant differences between PCOS and healthy women. Concentrations of IL-29, Cholesterol (Chol), Triglycerides (TG) and low density lipoprotein cholesterol (LDL-C) were higher, while high density lipoprotein cholesterol (HDL-C) was lower in serum of PCOS patients compared to controls. As a single biomarker, the area under the curve (AUC) of IL-29 was 0.727 with sensitivity of 0.872, meaning that the diagnostic value of IL-29 for PCOS was better than testosterone (0.818) and luteinizing hormone (LH)/follicle stimulating hormone (FSH) ratio (0.655) alone. In contrast, the combination of IL-29 and testosterone showed higher AUC (0.870) and sensitivity (0.886) than IL-29 or testosterone alone for PCOS detection. Furthermore, the spearman's correlation analysis demonstrated that IL-29 was positively correlated with Chol, TG and LDL-C, but negatively correlated with HDL-C. In conclusion, we suggested for the first time that IL-29 may be used as a novel biomarker for PCOS detection and lipid metabolism disorders. These data may provide novel insight into the pathogenesis of metabolic syndrome related to PCOS and the assessment of the risk of cardiovascular diseases for PCOS patients.

Keywords: PCOS, IL-29, lipid metabolism

Introduction

Polycystic ovary syndrome (PCOS), characterized by anovulation, hyperandrogenism and polycystic ovaries, is one of the most common fertility-related disorders in women of childbearing age [1]. Its prevalence ranges from 5% to 10% worldwide [2]. Patients with PCOS often have associated metabolic disorders, such as diabetes, insulin resistance, and physiological and reproductive consequences, like inflammation, obesity [3], infertility or miscarriage [4]. It is a complicated and heterogeneous syndrome, and women with PCOS are at an increased risk for type 2 diabetes and cardiovascular diseases [5, 6].

Currently, evidences suggest that PCOS could be identified as a pro-inflammatory state or chronic low-grade inflammation [7] that leads to the metabolic dysfunctions associated with the syndrome. Elevated inflammation mediators, such as chemokines [8], cytokines [9] and white blood cells [10], were found in the serum of PCOS patients. It was found that serum levels of tumor necrosis factor α (TNF-α) [11], interleukin-18 (IL-18) [12], and hypersensitive C-reactive protein (CRP) [13] were increased in patients with PCOS. Cytokines, a main feature of inflammation, may exert a critical role in the pathogenesis of PCOS. Niu reported that cytokine profiles in the follicular fluid of PCOS patients were correlated with lipid metabolism [14]. Foroozanfard F found that inflammatory factors were associated with PCOS, which showed that the serum level of interleukin-17 (IL-17) was directly correlated with increased blood pressure in PCOS patients [15]. Peng

Table 1. The clinical and biochemical data in PCOS patients and healthy women

Variables	PCOS patients (n=32)	Healthy controls (n=45)	*P* value
Ages (years)	27.30±3.89	28.62±3.93	0.159
BMI (kg/m^2)	24.91±4.62	25.44±3.41	0.353
Testosterone (ng/mL)	0.75±0.17	0.44±0.13	0.000***
Progesterone (ng/mL)	0.55±0.38	0.80±0.36	0.007**
Estradiol (pg/mL)	49.54±19.33	51.08±29.03	0.794
LH (mIU/mL)	9.41±7.29	6.56±3.69	0.043*
FSH (mIU/mL)	5.43±1.79	5.89±1.91	0.296
Total cholesterol (mmol/L)	5.24±1.07	4.21±0.52	0.000***
Triglyceride (mmol/L)	1.85±1.21	0.70±0.25	0.000***
LDL-C (mmol/L)	2.98±0.75	2.12±0.44	0.000***
HDL-C (mmol/L)	1.24±0.29	1.39±0.22	0.028*

Note: Data were shown as mean ± S.E.M. *P<0.05, **P<0.01, ***P<0.001.

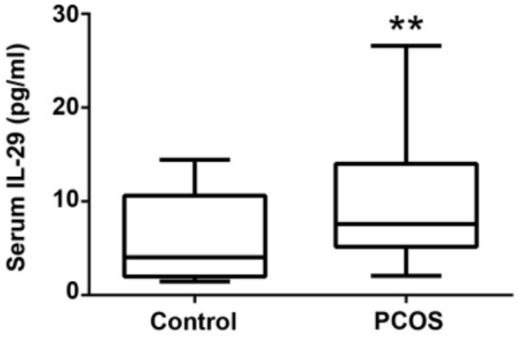

Figure 1. Increased expression of IL-29 in serum from patients with PCOS and healthy controls. ELISA assay for the level of IL-29 in serum of PCOS patients and healthy women. **P<0.01.

indicated that a high serum level of interleukin-6 (IL-6) may be a useful biomarker for treatment monitoring, but it was not an intrinsic characteristic of PCOS [16]. Interleukin-29 (IL-29) is a key proinflammatory cytokine for chronic inflammation. However, the role of IL-29 in the development and metabolism of PCOS has not been reported. Therefore, understanding the function of IL-29 in PCOS and its correlation with lipid metabolism may give us a novel mechanism of the occurrence of PCOS and its associated metabolic dysfunctions.

In this study, we aimed to explore the relationship between IL-29 and lipid metabolism by investigating the diagnostic utility of IL-29 for PCOS and its predictive value for cardiovascular diseases. First, we used ELISA to measure the concentration of IL-29 in serum, and we found that it was elevated significantly in patients with PCOS. Based on this, the serum level of IL-29 may be useful as a potential novel biomarker for PCOS detection. The combination of IL-29 and testosterone presented the highest diagnostic value for PCOS diagnosis. Moreover, IL-29 was positively correlated with indexes for lipid metabolism such as cholesterol (Chol), triglyceride (TG) and low-density lipoprotein cholesterol (LDL-C). On the other hand, it was negatively correlated with high-density lipoprotein cholesterol (HDL-C), which may act as a marker to predict the risk of cardiovascular disease for PCOS patients.

Material and methods

Ethics statement

The study was approved by the institutional review board of the First Affiliated Hospital of Dalian Medical University, and all participants provided informed consent. Methods were performed in accordance with the approved guidelines.

Serum sample

The serum samples from 32 patients diagnosed with PCOS and 45 healthy women matched by age and BMI were collected from the First Affiliated Hospital of Dalian Medical University. The diagnostic criteria was followed the Rotterdam standard of 2003: amenorrhea or oligomenorrhea, biochemical or clinical evidence of hyperandrogenism, and polycystic ovaries detected by ultrasound. Healthy women were those who did not exhibit gynecological disorders like Cushing's syndrome, congenital adrenal hyperplasia, androgen-related tumors and endocrine dysfunctions, and who had a normal and regular menstrual cycle. No one received any medication before. The serum was stored at -80°C for further analysis. The

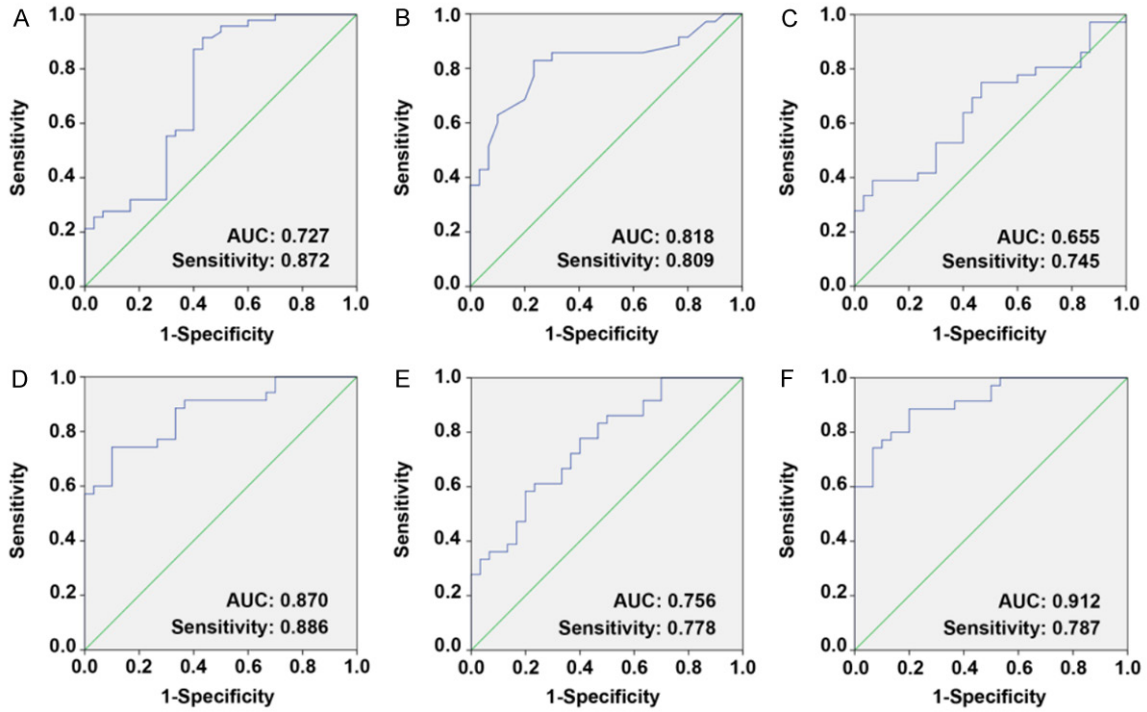

Figure 2. ROC curve analysis of IL-29, testosterone, LH/FSH ratio in PCOS prediction. A. ROC curve analysis of serum IL-29 as a single biomarker for predicting PCOS. B. ROC curve analysis of serum testosterone as a single biomarker for PCOS prediction. C. ROC curve analysis of LH/FSH ratio as a single biomarker for predicting PCOS. D. ROC curve analysis of the combination of serum IL-29 and testosterone for PCOS prediction. E. ROC curve analysis of the combination of serum IL-29 and LH/FSH ratio for predicting PCOS. F. ROC curve analysis of the combination of IL-29, testosterone and LH/FSH ratio for PCOS prediction.

levels of hormones (testosterone, progesterone, estradiol, luteinizing hormone (LH) and follicle stimulating hormone (FSH)) and indexes for lipid metabolism (Chol, TG, LDL-C and HDL-C) in serum were detected.

ELISA

Serum level of IL-29 in patients with PCOS and healthy women were measured with a human ELISA kit (Elabscience, Wuhan, China). The detection was carried out according to the manufacturer's instructions. Each sample was detected in triplicate.

Statistical analysis

Student's *t*-test was applied to analyze the difference in IL-29 level between the control group and PCOS patients. Receiver operating characteristic (ROC) curve analysis was used to determine the diagnostic value of IL-29 for PCOS detection. Spearman's correlation analysis was employed to analyze the correlation between IL-29 and indexes of lipid metabolism in patients with PCOS. Data analysis was conducted with SPSS 22.0 (SPSS, Chicago, USA), and data were presented as the mean ± S.E.M. $P<0.05$ was considered as statistically significant.

Results

The level of IL-29 in serum of PCOS patients

The clinical and biochemical data for PCOS patients and healthy women were shown in **Table 1**. The levels of testosterone and LH were higher in PCOS patients compared to healthy controls, while progesterone was lower. Estradiol and FSH showed no significant difference between these two groups. For lipid metabolism, indexes of Chol, TG and LDL-C were greatly increased in PCOS patients, and HDL-C was reduced compared to the controls. The serum level of IL-29 was significantly lower in patients with PCOS than healthy women (**Figure 1**). The results suggested that the serum level

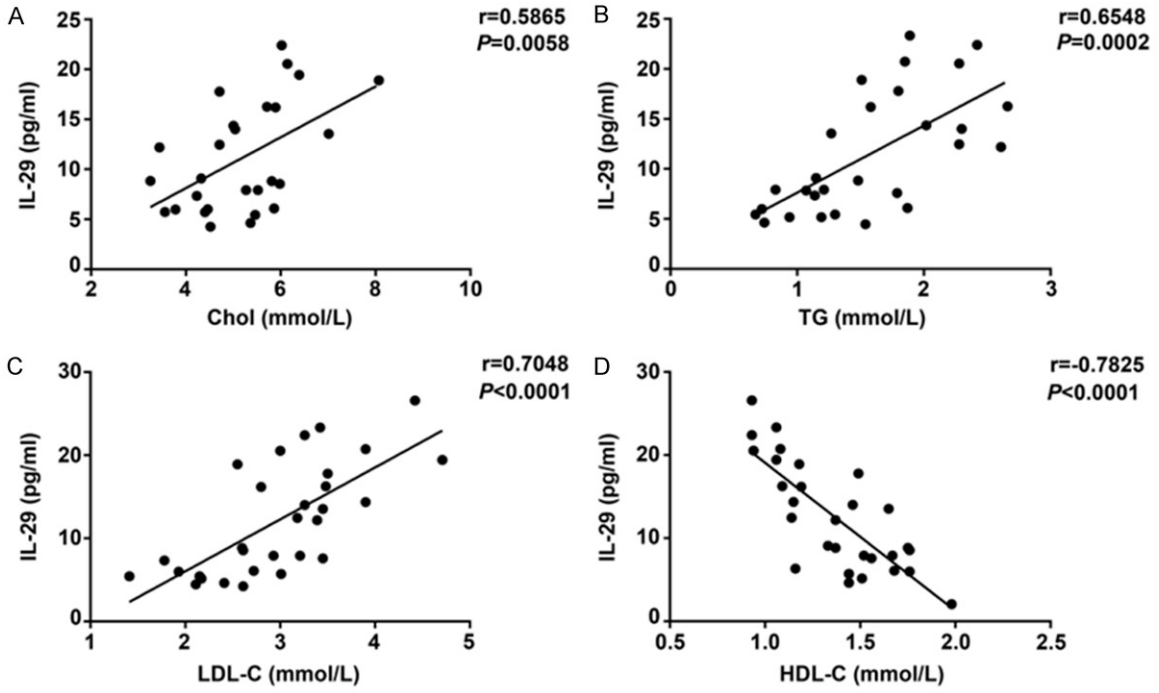

Figure 3. Correlation analysis between IL-29 and parameters for lipid metabolism. A. Correlation analysis of IL-29 and Chol (r=0.5865, *P*=0.0058). B. Correlation analysis of IL-29 and TG (r=0.6548, *P*=0.0002). C. Correlation analysis of IL-29 and LDL-C (r=0.7048, *P*<0.0001). D. Correlation analysis of IL-29 and HDL-C (r=-0.7825, *P*<0.0001).

of IL-29 was significantly increased and may be a contributor of PCOS.

The diagnostic value of IL-29 for PCOS

To determine the diagnostic value of IL-29 in PCOS detection, a ROC curve was constructed, and the area under the curve (AUC) was calculated. First, we evaluated the diagnostic performance of IL-29, testosterone and LH/FSH ratio as single biomarker in detecting PCOS. The AUCs of IL-29 (**Figure 2A**), testosterone (**Figure 2B**) and LH/FSH ratio (**Figure 2C**) were 0.727 (95% CI: 0.602-0.851), 0.818 (95% CI: 0.712-0.924) and 0.655 (95% CI: 0.523-0.787), respectively. The diagnostic sensitivity of IL-29 was 0.872, which was better than testosterone (0.829) and LH/FSH ratio (0.745). The AUCs of different multibiomarker combinations ranged from 0.756 to 0.912. The combination of IL-29 and testosterone (**Figure 2D**) increased the diagnostic value from 0.727 to 0.870 and the sensitivity from 0.809 to 0.886, and this was better than the combination of IL-29 and LH/FSH ratio (AUC: 0.756; sensitivity: 0.778) (**Figure 2E**). However, the addition of LH/FSH ratio to the combination panel of IL-29 and testosterone didn't improve the sensitivity in diagnosing patients with PCOS (AUC: 0.912; sensitivity: 0.787) (**Figure 2F**). Taken together, IL-29 may serve as a potentially novel biomarker for PCOS detection, and the combination of IL-29 and testosterone demonstrated the highest diagnostic utility for discriminating PCOS patients from healthy women.

The relationship between IL-29 and Chol, TG, LDL-C, and HDL-C

To investigate the relationship between IL-29 and lipid metabolism in PCOS patients, the spearman's correlation analysis was performed. The results showed that increased IL-29 was positively correlated with increased Chol (**Figure 3A**), TG (**Figure 3B**) and LDL-C (**Figure 3C**), with correlation coefficients of r=0.5865, *P*=0.0058 for Chol; r=0.6548, *P*=0.0002 for TG; and r=0.7048, *P*<0.0001 for LDL-C, and negatively correlated with HDL-C (r=0.78245, *P*<0.0001) (**Figure 3D**). These data suggested that IL-29 was closely related to lipid metabolism in patients with PCOS.

Discussion

Polycystic ovary syndrome, which results from genetic and environmental interactions, is a complex metabolic syndrome includes obesity, insulin resistance and dyslipidemia [17]. Among these, dyslipidemia is one of the most common metabolic disorders in PCOS patients [18]. The patients with dyslipidemia showed increased triglyceride, VLDL and LDL-C, and decreased HDL-C. In this study, we provided an extensive profile of lipid metabolism in serum of PCOS patients. The results showed that levels of Chol, TG and LDL-C were elevated, while HDL-C was decreased in patients with PCOS compared to healthy controls (**Table 1**). The lipid disorders may contribute to the occurrence of PCOS.

Increased levels of inflammatory markers are one of the main features of chronic inflammation [19]. Since it is an inflammatory disorder, increased levels of inflammatory markers in serum were observed in PCOS patients [20]. White blood cells (WBC), is one marker of inflammation and associated with cardiovascular risk factors and lipid profile. Orio F Jr enrolled 150 PCOS patients and 150 healthy women paired by age and BMI for WBC count, and found that PCOS patients had an increased WBC count [21]. Interleukin-18 (IL-18), closely correlated with metabolic syndrome, has been a critical predictor for cardiovascular mortality. Escobar-Morreale HF reported that IL-18 level was increased in PCOS patients and correlated with obesity and testosterone level [22]. Younis A found elevated IL-6, MCP-1 levels, and lower TNF-α level in patients with PCOS which were influenced by ovarian stimulation [23]. These highlighted that the inflammatory markers may reflect the inflammatory status of PCOS. Here, we selected another cytokine, IL-29, to investigate the association between its expression profile and the pathogenesis of PCOS (**Figure 1**). The ELISA result demonstrated that serum level of IL-29 was increased in PCOS patients, and it may act as one of the pathogenic factor for PCOS.

Currently, the diagnosis of PCOS mainly depends on ultrasound inspection and testosterone measurement in women with suspicious clinical manifestations [24]. As the heterogeneity of clinical symptoms and hormone levels, it is necessary to discover new biomarkers with high accuracy that are capable of discriminating PCOS patients from healthy women. Altered cytokine expression has been found to be associated with PCOS, and it could be used to classify PCOS [25]. Therefore, we evaluated the diagnostic accuracy of IL-29, testosterone, and LH/FSH ratio for PCOS detection (**Figure 2**). The AUC for IL-29 (0.727) was significantly higher than LH/FSH ratio (0.655) but lower than testosterone (0.818). It improved the diagnostic utility compared to that of LH/FSH ratio, but it was not sufficient to be clinically useful as a single biomarker. Thus, a multibiomarker panel might be a better strategy for PCOS detection. The AUC of the combination of IL-29 and testosterone elevated from 0.727 to 0.912, and the sensitivity increased from 0.809 to 0.886. These observations suggested that IL-29 may be useful in improving the diagnostic performance of testosterone in PCOS detection. Furthermore, IL-29 was positively correlated with Chol, TG and LDL-C, and negatively correlated with HDL-C in PCOS (**Figure 3**). Therefore, IL-29 and its contribution to the clinical syndrome of PCOS patients reflected the lipid metabolism states and the risk of developing cardiovascular diseases of PCOS patients.

In summary, our data suggested that increased serum level of IL-29 was correlated with lipid metabolism. This provided new insight into the pathogenesis of PCOS. The combination of IL-29 and testosterone increased the accuracy of PCOS detection. In addition, IL-29 was closely correlated with HDL-C, which could serve as a novel predictor for the risk of cardiovascular diseases in PCOS patients. Therefore, understanding the role of IL-29 in metabolic disorders would allow us to understand the etiology of PCOS better.

Acknowledgements

Mulin Liu designed and performed major experiments, analyzed the experiment data; Sicong Dong helped to do the experiments and analyze the data. Shijun Li initiated, organized and designed the study, analyzed the data and wrote the manuscript. All authors commented on the manuscript.

Disclosure of conflict of interest

None.

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