Serum Fetuin-A levels were increased and associated with insulin resistance in women with polycystic ovary syndrome

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Abstract

Background: Insulin resistance (IR) is a common characteristic in women with polycystic ovary syndrome (PCOS). It has been reported that circulating Fetuin-A levels were associated with IR and type 2 diabetes mellitus (T2DM). However, in PCOS women, previous reports on changes in circulating Fetuin-A concentrations were inconsistent. Methods: A cross-sectional study was conducted, including 122 PCOS women and 85 healthy women. Circulating Fetuin-A levels were determined using an ELISA kit. Results: We found that circulating Fetuin-A concentration ranged from 196.6 to 418.2 μg/L for most normal women (95 %). Women with PCOS have higher circulating Fetuin-A levels than healthy women (437.9 ± 119.2 vs. 313.8 ± 60.4 μg/L; p <0.01). Serum Fetuin-A concentrations correlated positively with WHR, LDL-C, FIns, HOMA-IR, TEST and DHEA-S. Multivariate regression analysis showed that WHR, HOMA-IR and TEST were independent influencing factors of serum Fetuin-A. Binary logistic regression reaveled that serum Fetuin-A was associated with the .occurrence of PCOS. In addition, our ROC curve analysis found that the cutoff values for Fetuin-A to predict PCOS and IR were 366.3 and 412.6 μg/L. Conclusion: Blood Fetuin-A may be a useful biomarker for screening women with PCOS and IR.

Background

Polycystic ovary syndrome (PCOS) is one of the most common endocrine and metabolic diseases in adolescent women, which has three main characteristics: anovulation (OA), hyperandrogenism (HA), clinical manifestations of HA, polycystic ovary (PCO), and most of them are accompanied by obesity and metabolic disorders. In addition to symptoms caused by hyperandrogenism and reproductive disorders, PCOS is associated with the risk of some metabolic diseases, such as obesity, dyslipidemia, chronic inflammation, metabolic syndrome (MetS), insulin resistance (IR), type 2 diabetes mellitus (T2DM), atherosclerosis and cardiovascular disease [1-4]. The pathogenesis of PCOS is complex, and its etiology remains unclear. From the definition of PCOS to its phenotype, heterogeneity is an inherent feature of PCOS, and its formation has also heterogeneity. In different phenotypes of PCOS, the relative contribution of excessive androgen and other factors, such as obesity and IR, to the development of PCOS is also different [5]. Although in the past few decades, more studies have explored the mechanism of metabolic disorders and IR in PCOS patients, the current diagnostic criteria do not include indicators reflecting metabolic disorders and IR [6-8]. Therefore, it is important to look for circulating biomarkers that reflect metabolic disorders and IR in PCOS.

Alpha-2-Heremans-Schmid glycoprotein (Fetuin-A) is a 64 kDa glycoprotein and is previously considered to be a hepatokine [9]. However, recently, some studies have found that adipose tissue can also express and secrete Fetuin-A [10-11]. Therefore, Fetuin-A is defined as both a hepatokine and an adipokine. Previous studies have shown that Fetuin-A is related to glucose and lipid metabolism and IR, including 1) inhibiting insulin receptor (InsR) phosphorylation and glucose transporter 4 (GLUT4); 2) When combined with saturated fatty acids, Fetuin-A can stimulate chronic inflammation through Toll-like receptor 4 (TLR4), leading to IR [12-14]. In human studies, it has been found that Polymorphisms in the Fetuin-A were related to T2DM [15] and circulating Fetuin-A levels are elevated or decreased, or unchanged in

obesity, T2DM, non-alcoholic fatty liver disease (NAFLD), metabolic syndrome (MetS), PCOS and cardiovascular disease (CVD), and are associated with or not associated with impaired glucose tolerance and IR [16-22]. At present, reports on the relationship between Fetuin-A and IR as well as metabolic diseases are inconsistent. Therefore, it is worth further investigation whether Fetuin-A is related to metabolic disorders and IR.

In this study, we selected newly diagnosed women with PCOS as the subjects of study, and evaluated IR and metabolic disorders in *vivo*. We compared circulating Fetuin-A levels between PCOS women and normal controls, and explored the relationship between Fetuin-A and IR as well as metabolic disorders. Our data indicate that circulating Fetuin-A may be an important biomarker for PCOS and IR.

Methods

Study population

This study was performed from December. 2018 to September. 2019. One hundred and twenty two women with PCOS (PCOS group, 19-37 years old) and eighty-five normal controls (N group, 19-32 years old) participated in the current study. The diagnosis of PCOS is based on the Rotterdam consensus criterion, which meets two of the following three criteria [23]: 1) the presence of clinical or biochemical hyperandrogenism; 2) oligovulation or anovulation; 3) ultrasound imaging of polycystic ovary. Other related diseases and disorders should be excluded. All PCOS patients were newly diagnosed without lifestyle intervention or any medication. 85 age- and BMI- matched women were recruited this study as a normal control. Normal controls had normal menstrual cycle, 21-35 days interval, normal progesterone (Prog) level in luteal phase, no hairy, acne or hair loss. Women who smoked and drank alcohol or took drugs affecting sugar/lipid/androgen metabolism within six months were excluded. Women with T2DM, thyroid disease, cardiovascular disease, liver and kidney dysfunction were excluded from the study. In the control group, blood samples were collected at the early stage of follicle (2-5 days of menstruation). The subjects were recruited from outpatient clinics, daily physical examinations or advertisements in schools or communities. Before participating in the study, all subjects signed informed consents. This study was supported by the Hospital Ethics Committee and is consistent with the institutional guidelines. The study was performed in accordance with the Helsinki Declaration.

Anthropometric and biochemical measurement

Body mass index (BMI) was calculated as weight divided by height squared. Waist circumference and hip circumference were measured by the same observer for calculation of waist-to-hip ratio (WHR). The homeostasis model assessment of insulin resistance ($HOMA\text{-}_{IR}$) was calculated using the following equations: $HOMA\text{-}_{IR}$ = fasting insulin (FIns, lU/ml) × fasting blood glucose (FBG, mmol/L)/22.5 [24]. After 12-14 hours of fasting, blood samples were obtained from all the subjects before breakfast and centrifuged at 4 ºC. Serum was stored at -80 ºC for further analysis. Blood glucose, HbA1c, insulin levels

and blood fat, including triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) were measured as in a previous population [25].

Measurements of serum Fetuin-A and sex hormonal measurements

The serum concentration of Fetuin-A was determined by enzyme-linked immunosorbent assay (ELISA), according to the manufacturer's instructions. Both intra- and inter-assay variations were 10 %, respectively. The measuring range of this kit was 9.38 - 600 ng/ml. Serum hormonal concentrations including the luteinizing hormone (LH), follicle-stimulating hormone (FSH), testosterone (TEST) were measured with electrochemiluminescence immunoassay using COBAS E immunoassay analyzers (Roche Diagnostics GmbH). Dehydroepiandrostenedione sulfate (DHEA-S) was measured using an automated analyzer (Abbott Architect; Abbott Laboratories), as in a previous reported [26].

Statistical analysis

Statistical analyses were conducted using SPSS software version 19.0 (SPSS, Chicago, IL). Results are expressed as mean ± SD or median (interquartile range) unless stated otherwise. Variables with a non-normal distribution were transformed by logarithm or square-root before analysis. Independent sample *t* test was used in comparisons between two groups. One-way ANOVA with post hoc analysis to investigate differences in body composition and other indicators between PCOS women and normal controls. Simple and multiple linear regression analysis was used to study the correlation between fasting Fetuin-A concentrations and other biomarkers. Multivariate logistic regression analysis was used to investigate the relationship between Fetuin-A and PCOS.Receiver operating characteristic (ROC) curve was drawn by SPSS 19.0 software to evaluate the sensitivity and specificity of Fetuin-A in predicting PCOS. No missing data in our experiment.Sample size was calculated using the following equations: $N = [Z\alpha/2\ \sigma/\varepsilon\mu]2$ (σ, standard; μ, mean; $Z\alpha/2 = 1.96$, $\alpha = 0.05$, $\varepsilon = 10\%$) .$p < 0.05$ was considered significant.

Results

Serum Fetuin-A concentration in PCOS and healthy women

Table 1 summarizes the demographic, anthropometric and metabolic parameters of all women in the current study. In PCOS women, BMI, WHR, blood pressure (BP), TG, TC, LDL-C, FBG, FIns and HOMA-$_{IR}$ were significantly increased compared to healthy women (Table 1). Furthermore, sex hormone levels, including LH, LH/FSH, TEST and DHEA-S, were also markedly increased in PCOS women relative to those in healthy women (Table 1). The distribution of Fetuin-A concentrations in healthy women were showed in Figure 1A. We found that circulating Fetuin-A concentration ranged from 196.6 to 418.2 μg/L for most normal women (95 %). Importantly, as shown in Figure 1B, PCOS patients have higher circulating Fetuin-A levels than healthy women. Even after adjusting for weight and age, the difference was significant (Table 1). According to BMI < 25kg/m^2 or ≥ 25kg/m^2, PCOS patients were divided into obese/overweight (ob/ow) and non-obese/overweight groups (non-ob/ow). We found that the level of Fetuin-A in obese/overweight group was significantly higher than that in non-obese/overweight group (Figure 1C). In

addition, serum Fetuin-A was divided into three tertiles (tertile 1, < 322.6 μg/L; tertile 2, 322.6-419.6 μg/L; tertile 3, >419.6 μg/L). The odds of developing PCOS were calculated by logistic regression analysis. In the tertile 2 and 3 of blood Fetuin-A, the odds ratios of developing PCOS were higher than tertile 1 (95% CI 1.28 - 5.14 for tertile 2 and 95% CI 11.8 – 113.5 for tertile 3; *vs.* tertile 1, both $p < 0.01$; Figure 1D).

Serum Fetuin-A level and its association with other parameters in the study population

We next investigated the relationship between the levels of circulating Fetuin-A and various other parameters. Serum Fetuin-A was positively correlated with WC, TG, LDL-C, FIns, HOMA-$_{IR}$, TEST and DHEA-S (Table 2). We also performed multiple stepwise regression to determine variables that had independent associations with serum Fetuin-A. The results showed that only HOMA-$_{IR}$ and TEST were independent predictors of the levels of circulating Fetuin-A (Table 2). The multiple regression equation was $Y_{Fetuin\text{-}A} = 38.9 + 10.7\ X_{HOMA\text{-}IR} + 317.4X_{WHR} + 46.7X_{TETS}$.

With the increase of serum Fetuin-A levels, the risk of PCOS increased linearly. When analyzed by means test and Cheran-Armitage trend test, the concentration of fetoglobulin A was independently associated with polycystic ovary syndrome, and Fetuin-A was independently associated with PCOS when analyzed by a Row Mean Scores test and a Cochran-Armitage trend test (Table 3). Additionally, logistic regression analysis revealed that Fetuin-A was significantly related to PCOS, even after controlling for anthropometric variables, blood lipid and so on (Table 4).

ROC curve analysis

In order to explore the prediction of PCOS and IR by blood Fetuin-A, we performed receiver operating characteristic (ROC) curve analysis. The data analyzed by ROC showed that the area under the ROC curves for PCOS (AUC_{PCOS}) was 0.81 with a specificity of 83 %, and sensitivity of 0.70 % ($p < 0.01$, Figure 2A), and AUC_{IR} was 0.82 with a specificity of 72%, and sensitivity of 81 %. The best cut-off values for Fetuin-A to detect PCOS and IR were 366.3 μg/L for PCOS and 412. 6μg/L for IR.

Discussion

In the past decade, a number of population-based studies have reported the relationship between circulating Fetuin-A concentrations and PCOS. However, the conclusions of these studies are contradictory. In previous studies, circulating Fetuin-A levels were increased, decreased or unchanged in PCOS patients compared with normal women [20, 27-31]. In the current investigate, we found that circulating Fetuin-A concentrations were markedly elevated in PCOS women compared with healthy women. Our results are consistent with those of Enli et al [28, 30-31], but contrary to those of Díaz et al [20]. The cross-sectional nature of studies and population heterogeneity in women with PCOS may be related to differences in outcomes. Furthermore, previous studies had small sample sizes, and some had no healthy controls or obese PCOS patients were not included. Therefore, in this study, we avoid these

defects. In addition, we used newly diagnosed PCOS women to avoid the effects of medication, lifestyle interventions, and duration of the disease. The reason for the rise in circulating Fetuin-A is unknown. We speculate that the metabolic disorders caused by hyperandrogenism and hyperinsulinemia may promote the synthesis and release of Fetuin-A in *vivo*. In addition, increased Fetuin-A may be derived, at least in part, by the status of low-grade inflammation, since inflammatory cytokines, such as CRP, are increased in women with PCOS.

Previous two studies found that Fetuin-A inhibits insulin receptor tyrosine kinase and Toll-like receptor 4 in liver and muscle cells to inhibit insulin signaling and stimulate inflammatory signaling pathway [13, 14]. However, population-based studies have shown that there is no correlation between fetuin-A and IR in diabetic patients, and there is no correlation between Fetuin-A and the risk of diabetes [32-33]. In the current work, we found that circulating Fetuin-A was positively correlated with $HOMA_{IR}$, FIns, WHR and TEST, suggesting that Fetuin-A was associated with hyperinsulinemia and hyperandrogenism. These data support the results of Pal *et al* and Srivas *et al*. Therefore, under the hyperinsulinemia and hyperandrogenism, elevated circulating Fetuin-A raises a question whether lowering Fetuin-A concentration is the key to improving IR. To address this question, further study is necessary.

We analyzed the ROC curve to explore the best cut-off point for predicting PCOS by circulating Fetuin-A. Our results show that cyclic Fetuin-A is a good predictor for PCOS. We therefore believe that the relationship between Fetuin-A and PCOS may be due to the high prevalence of IR in these women.

Our research also has also some limitations. Firstly, the study population is young women, so our results may not be applicable to elderly population. Secondly, our results are based on a single measurement of Fetuin-A. Without repeated measurements at different time points, this may introduce random measurement errors in determining biochemical variables. Finally, the nature of cross-sectional study makes it impossible for our results to explain the causal relationship between increased Fetuin-A levels and the occurrence of IR and PCOS.

Conclusions

In conclusion, our results suggested that serum Fetuin-A levels were increased in PCOS patients. Circulating Fetuin-A concentrations were associated with dyslipidemia, IR and ovarian hyperandrogenism in PCOS women.

Abbreviations

PCOS, Polycystic ovary syndrome; BMI, body mass index; TG, Triglyceride; HOMA-IR, homeostasis model assessment of insulin resistance;

Declarations

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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Author contributions S.L., W.H. and Y.H. researched data. H.L. reviewed and edited the manuscript. L.G. contributed to the writing of the manuscript and helpful discussion. G.Y. and X.L. are the guarantors of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Competing interests No potential conflicts of interest relevant to this article were reported.

Ethics approval and consent to participate Before participating in the study, all subjects signed informed consents. This study was supported by the Hospital Ethics Committee and is consistent with the institutional guidelines. The study was performed in accordance with the Helsinki Declaration.

Consent for publication Not applicable.

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Tables

Table 1 Main clinical features and circulating Fetuin-A levels in PCOS and control subjects

Characteristics	Controls (n=85)	PCOS (n=122)	P-value
Age (yr)	26.0 ± 3.4	25. 3 ± 3.4	0.114
BMI (kg/m^2)	21.6 ± 2.9	24.4 ± 4.4	< 0.001
WHR	0.81 ± 0.08	0.88 ± 0.10	< 0.001
SBP (mmHg)	113 ± 8	128 ± 8	< 0.001
DBP (mmHg)	75 ± 7	77 ± 7	0.045
TG (mmol/L)	0.92 (066-1.34)	1.37(1.01-2.48)	< 0.001
TC (mmol/L)	3.94 ± 0.76	4.64 ± 0.93	< 0.001
HDL-C (mmol/L)	1.23 ± 0.27	1.18 ± 0.33	0.278
LDL-C (mmol/L)	2.18 ± 0.58	2.89 ± 0.58	< 0.001
FBG (mmol/L)	4.77 ± 0.46	5.06 ± 0.91	< 0.01
FIns (mU/L)	7.40 (5.55-9.17)	18.00 (9.30-27.08)	< 0.001
$HOMA-_{IR}$	15.0 (1.15-2.04)	3.85 (1.97-5.83)	< 0.001
FSH (IU/L)	5.92 ± 2.00	5.78 ± 1.36	0.574
LH (IU/L)	6.07 ± 5.14	8.39 ± 4.94	< 0.001
LH/FSH	1.05 ± 1.09	1.50 ± 0.90	< 0.01
TEST (nmol/L)	1.01 ± 0.68	1.44 ± 0.65	< 0.001
DHEA-S (µmol/L)	4.50 ± 1.86	6.58 ± 2.93	< 0.001
Fetuin-A (µg/L)	313.8 ± 60.5	437.9 ± 119.3	< 0.001
Fetuin-A (adjusted)§	320.8 ± 11.1	433.1 ± 9.2	< 0.001

Values are given as mean ± SD or median (inter quartile range). BMI, Body mass index; WHR, Waist hip ratio; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; FBG, fasting blood glucose; FIns, fasting plasma insulin ; TG, Triglyceride; TC, Total cholesterol; HDL-C, High- density lipoprotein cholesterol; LDL-C, Low-density lipoprotein cholesterol ; HOMA-IR, homeostasis model assessment of insulin resistance; FSH, follicle-stimulating hormone; LH, luteinizing hormone; TEST, testosterone; DHEA-S, dehydroepiandrosterone sulfate. §, mean ± SE by general linear model with adjustment of age and weight.

Table 2 Correlation analysis of variables associated with circulating Fetuin-A levels in the study populations

	Simple		Multiple	
Variable	R	*P*	*b*	*P*
Age (years)	-0.155	0.088	------	------
BMI (kg/m^2)	0.164	0.072	------	------
SBP(mmHg)	-0.134	0.140	------	------
DBP(mmHg)	0.050	0.585	------	------
WHR	0.230	0.011	317.367	0.002
FPG (mmol/L)	0.029	0.755	------	------
TG (mmol/L)	0.115	0.206	------	------
TC (mmol/l)	0.090	0.323	------	------
HDL-C (mmol/L)	0.029	0.754	------	------
LDL-C (mmol/L)	0.188	0.038	------	------
FIns (pmol/L)	0.473	0.000	------	------
$HOMA-_{IR}$	0.400	0.000	10.705	0.000
FSH (IU/L)	-0.020	0.830	------	------
LH (IU/L)	-0.021	0.822	------	------
LH/FSH	-0.073	0.423	------	------
TEST (nmol/L)	0.241	0.008	46.733	0.001
DHEA-S (μmol/L)	0.187	0.039	------	------

Table 3 Row Mean Scores and Cochran-Armitage Trend Test of the impact of circulating Fetuin-A on PCOS individuals

	PCOS	
	χ^2	*p*-value
Row Mean Scores	62.7898	< 0.001
Cochran-Armitage Trend Test	7.7089	< 0.001

Table 4 Association of circulating Fetuin-A with PCOS in fully adjusted models

Model adjustments	PCOS		
	OR	95%CI	*P*
Age, SBP, DBP	1.015	1.010-1.020	0.000
Age, SBP, DBP, BMI, WHR	1.014	1.009-1.019	0.000
Age, SBP, DBP, BMI, WHR, FBG, FIns	1.011	1.005-1.017	0.000
Age, SBP, DBP, BMI, WHR, FPG, FIns, Lipid profile	1.010	1.004-1.017	0.002
Age, SBP, DBP, BMI, WHR, FPG, FIns, Lipid profile, Hormone parameters	1.008	1.001-1.016	0.036

Results of binary logistic regression analysis are presented. 95%CI, confidence interval; OR, odds ratio. Data from two subject groups were pooled to calculate logistic regression.

Figures

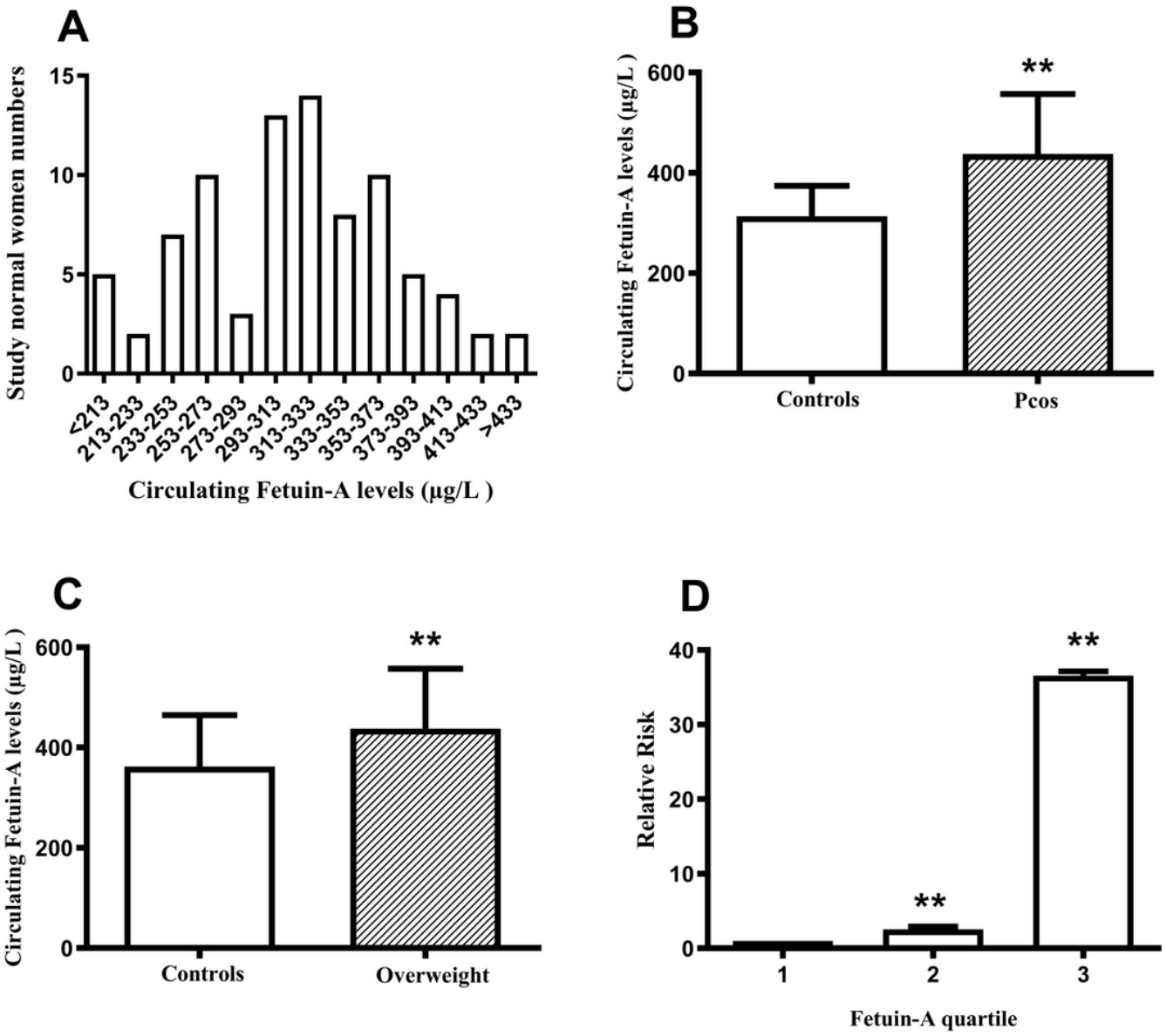

Figure 1

Serum Fetuin-A levels in study population. (A) Distribution of circulating concentration of Fetuin-A in normal women. (B) Serum Fetuin-A levels in healthy and PCOA women. (C) Circulating Fetuin-A levels in lean and over-weight subjects. (D) The odds ratio of having MetS in different tertiles of circulating Fetuin-A. Data are expressed as means ± SD. *P< 0.05, ** P<0.01 vs. Controls or tertile 1.

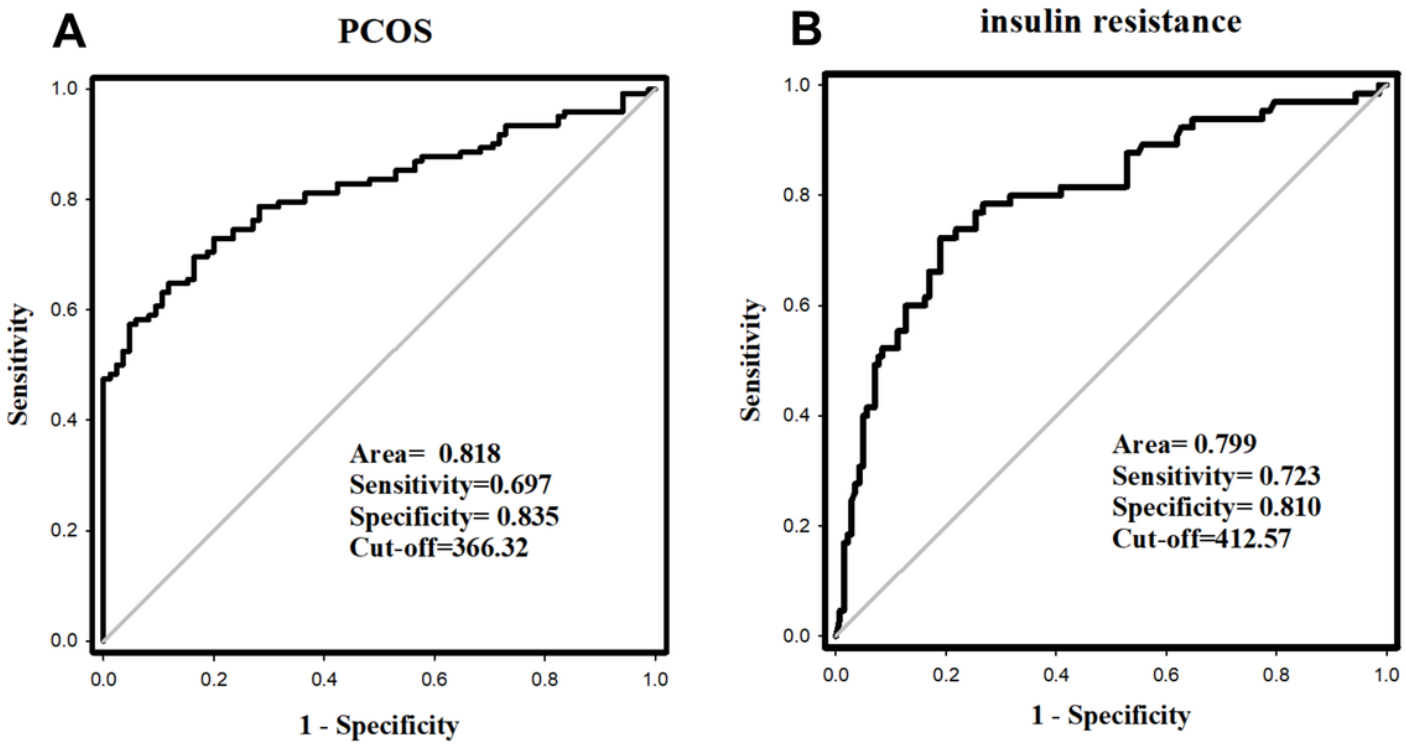

Figure 2

(A) ROC curve analysis of the prediction of PCOS. (B) ROC curve analysis of the prediction of insulin resistance.