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 of 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, previous reports on changes in circulating Fetuin-A concentrations in women with PCOS have been inconsistent.Methods: 207 subjects were screened for PCOS according to the diagnostic guideline of the Rotterdam consensus criterion. Serum Fetuin-A levels were measured using an ELISA kit. An independent t-test or Nonparametric test was used to detect differences between PCOS and control groups. The association of the serum Fetuin-A with other parameters was examined by spearman's correlation analysis. Results: Our findings show that circulating Fetuin-A concentration ranged from 196.6 to 418.2 μg/L for most women without PCOS (95 %). Women with PCOS have higher circulating Fetuin-A levels than healthy women (437.9 ± 119.3 vs. 313.8 ± 60.5 μg/L; $p < 0.01$). Serum Fetuin-A was positively correlated with BMI, WHR, TG, TC, LDL-C, HOMA-IR, LH, T, and DHEA-S. Multivariate regression analysis showed that WHR, TG, HOMA-IR, and DHEA-S were independent predictors of the levels of circulating Fetuin-A. Binary logistic regression revealed 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 for PCOS and IR.

Background

Polycystic ovary syndrome (PCOS) is one of the most common endocrine, metabolic diseases in adolescent women. It has three main characteristics: oligo or amenorrhea (OA), hyperandrogenism (HA), and/or clinical manifestations of HA, polycystic ovary (PCO), and most cases are accompanied by obesity and other metabolic disorders. In addition to symptoms caused by hyperandrogenism and reproductive disorders, increasing evidence supports the central role of insulin resistance (IR) and compensatory hyperinsulinemia in the pathogenesis of the PCOS [1] and in patients' increased risk of developing other metabolic diseases, such as obesity, dyslipidemia, chronic inflammation, metabolic syndrome (MetS), type 2 diabetes mellitus (T2DM), atherosclerosis and cardiovascular disease [2-3]. Overall, IR affects up to 70 % of women with PCOS. Despite extensive research, the mechanisms underlying IR in PCOS patients are not completely understood [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 also has 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 manifested in different ways [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 patients.

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) inhibition of insulin action through inhibition of the auto-phosphorylation of insulin receptor tyrosine kinase and glucose transporter 4 (GLUT4); 2) Combination with saturated fatty acids may cause Fetuin-A to stimulate chronic inflammation through the Toll-like receptor 4 (TLR4), leading to IR [12-14]; 3) the mRNA and protein of Fetuin-A are increased in ob/ob mice [14]. 4) impairing of adipocyte function leads to IR [15]; 5) increase of the expression of Fetuin-A is associated with endoplasmic reticulum (ER) stress leading to the development of IR [16]. In human studies, it has been found that polymorphisms in Fetuin-A were related to T2DM [17] and circulating Fetuin-A levels are elevated or decreased, or unchanged in obese patients, T2DM, non-alcoholic fatty liver disease (NAFLD), metabolic syndrome (MetS), PCOS and cardiovascular disease (CVD) are either associated with or not associated with impaired glucose tolerance and IR [18-24]. These inconsistent findings on the relationship between Fetuin-A and IR, as well as metabolic diseases, necessitate further investigation.

In this study, we selected newly diagnosed women with PCOS as the subjects of study, and evaluated the relationship between circulating Fetuin-A and IR in *vivo*.

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 [25]: 1) hyperandrogenism and/or clinical manifestations of hyperandrogenism; 2) oligo or amenorrhea; 3) ultrasound imaging of polycystic ovary. Other related diseases and disorders were excluded. All PCOS patients were newly diagnosed without lifestyle intervention or any medication. Eighty-five age-matched women have recruited in this study as normal control. Control subjects had a normal menstrual cycles (21-35 days) and had no acne or hirsutism. Their ovarian morphology was normal by ultrasonography. Normal controls had normal menstrual cycle, 21-35 days interval, normal progesterone (P4) level in luteal phase, no hairy, acne or hair loss. Exclusion criteria include T2DM, thyroid disease, cardiovascular disease, liver, and kidney dysfunction. The subjects were recruited from outpatient clinics, daily physical examinations or advertisements in schools or communities. All subjects signed informed consent before participating in the study. This study was approved by the Hospital Ethics Committee and is consistent with the institutional guidelines. The study was performed in accordance with the Helsinki Declaration.

Anthropometric 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 [26]. According to $HOMA\text{-}_{IR}$, individuals were divided into IR ($HOMA\text{-}_{IR} \geq 3.8$) and non-IR (HOMA-IR < 3.8) [27]. After 12-14 hours of fasting, blood samples were obtained from all the subjects before breakfast and centrifuged at 4 ºC. The serum was stored at -80 ºC for further analysis.

Measurements of serum Fetuin-A, sex hormone, and biochemical parameters

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 [28]. Serum hormonal concentrations, including the luteinizing hormone (LH), follicle-stimulating hormone (FSH), testosterone (T) 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 report [29].

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.

For sex hormone measurements, blood samples were collected in the early-follicular phase (day 3 to 5 of the menstrual cycle) for the control group, and after a spontaneous bleeding episode or after a period of amenorrhea longer than three months for PCOS women [30].

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 before analysis. An independent sample *t*-test or Nonparametric tests was used in comparisons between two groups. One-way ANOVA with post hoc analysis was used 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. The 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 and sex hormone levels of all women in the current study. The distribution of Fetuin-A concentrations in healthy women is shown in Figure 1A. We found that circulating Fetuin-A concentration ranged from 196.6 to 418.2 μg/L for most normal women (95 %). PCOS patients had higher circulating Fetuin-A levels than healthy women (Figure 1B, Table 1). PCOS patients were divided into obese/overweight (ob/ow) and lean groups by BMI < 25kg/m^2 or ≥ 25kg/m^2, respectively. We found that the levels of Fetuin-A in obese/overweight group were significantly higher than those in the lean group (Figure 1C). When control subjects were divided into overweight and lean groups, there was no significant difference in serum Fetuin-A (313.5 ± 60.6 *vs.* 317.4 ± 62.7μg/L), suggesting that there was no significant relationship between serum Fetuin-A level and overweight in normal women, 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).

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
T (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

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; T, testosterone; DHEA-S, dehydroepiandrosterone sulfate.

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

Next, we investigated the relationship between the levels of circulating Fetuin-A and various other parameters. Serum Fetuin-A was positively correlated with BMI, WHR, TG, TC, LDL-C, HOMA-$_{IR}$, LH, T, and DHEA-S (Table 2). Moreover, as previously reported, we also found a significant correlation between T and HOMA-$_{IR}$ (r = 0.351, p <0.01). Next we performed a multiple stepwise regression to determine variables that had independent associations with serum Fetuin-A. The results showed that only WHR, TG, HOMA-$_{IR,}$ and DHEA-S were independent predictors of the levels of circulating Fetuin-A (Table 2). The multiple regression equation was $Y_{lg10(Fetuin\text{-}A)} = 2.11 + 0.012\ X_{HOMA\text{-}IR} + 0.401X_{WHR} + 0.017X_{TG} + 0.009X_{DHEA\text{-}S}$.

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

	Simple		Multiple	
Variable	*r*	*p*	*b*	*p*
Age (years)	-0.124	0.076	------	------
BMI (kg/m^2)	0.294**	< 0.001	------	------
SBP (mmHg)	0.076	0.279	------	------
DBP (mmHg)	0.039	0.579	------	------
WHR	0.351**	< 0.001	0.401	< 0.001
TG (mmol/L)	0.293**	< 0.001	0.017	< 0.05
TC (mmol/l)	0.184**	< 0.01	------	------
HDL-C (mmol/L)	-0.106	0.128	------	------
LDL-C (mmol/L)	0.339**	< 0.001	------	------
HOMA-$_{IR}$	0.511**	< 0.001	0.012	< 0.001
FSH (IU/L)	-0.079	0.259	------	------
LH (IU/L)	0.178*	< 0.05	------	------
T (nmol/L)	0.320**	< 0.001	------	------
DHEA-S (μmol/L)	0.330**	< 0.001	0.009	< 0.01

[a]Variables with a non-normal distribution were transformed by logarithm before analysis

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 3).

Table 3 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.

ROC curve analysis

To explore the prediction of PCOS and IR by blood Fetuin-A, we performed receiver operating characteristic (ROC) curve analysis. The result showed that the area under the ROC curves for PCOS (AUC_{PCOS}) was 0.82 with a specificity of 83.5 %, and sensitivity of 69.7 % ($p < 0.01$, Figure 2A), and AUC_{IR} was 0.80 with a specificity of 81%, and sensitivity of 72.3 %. The best cut-off values for Fetuin-A to detect PCOS and IR were 366.3 μg/L for PCOS and 412. 6 μg/L for IR.

Discussion

In the past decade, several 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 [22, 31-35]. In the current investigation, we found that circulating Fetuin-A concentrations were markedly elevated in women with PCOS compared to healthy women. Our results are consistent with those of Enli et al. [33 -35], but contrary to those of Díaz et al. [22]. The cross-sectional nature of the study and population heterogeneity may be related to differences in outcomes. Furthermore, previous studies had small sample sizes, and some had no healthy controls or did not include obese patients with PCOS. In this study, we avoid these shortcomings. In addition, we used newly diagnosed PCOS women to avoid the effects of medication, lifestyle interventions, and the duration of the disease that are associated with those patients who have been under treatment. The reason for the rise in circulating Fetuin-A is unknown. We speculate that the metabolic disorders and hyperandrogenism caused by IR (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.

Two previous studies found that Fetuin-A inhibits the insulin receptor tyrosine kinase and Toll-like receptor 4 in liver and muscle cells to inhibit insulin signaling and stimulate inflammatory signaling pathways [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 [36-37]. In the current work, we find that serum Fetuin-A was positively correlated with BMI, WHR, TG, TC, LDL-C, HOMA-$_{IR}$, LH, T and DHEA-S, suggesting that Fetuin-A is associated with hyperinsulinemia and hyperandrogenism. These data support the results of Pal *et al.* and Srivas *et al.* [13]. Because circulating Fetuin-A levels were associated with both T and IR, hyperandrogenism, and IR were important characteristics of PCOS, it is of clinical significance to consider Fetuin-A a biomarker for PCOS.

Surprisingly, serum Fetuin-A concentration increased significantly in obese/overweight PCOS women but did not change in normal women with obese/overweight. This result is consistent with a previous study [38]. The reason for this is unknown. We speculated that the main factors affecting circulating Fetuin-A might be hyperinsulinemia and hyperandrogenemia, not adipose mass. In our study cohort, a small number of overweight women may have affected the results. In addition, under hyperinsulinemia and hyperandrogenism, elevated circulating Fetuin-A raises the question of whether lowering Fetuin-A concentrations are the key to improving IR and hyperandrogenism. Secondly, because serum Fetuin-A levels are related to hyperandrogenemia, it is important to observe whether circulating Fetuin-A concentration will change with the menstrual cycle due to the change of hormone levels. To address these questions, further study is necessary.

We analyzed the ROC curve to explore the best cut-off point for predicting PCOS with circulating Fetuin-A. Our results show that cyclic Fetuin-A is a good predictor for PCOS patients. 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 apply to an elderly population. Secondly, our results are based on a single measurement of Fetuin-A. Without repeated measurements at different time points, the introduction of random measurement errors in determining biochemical variables is possible.. Finally, the nature of cross-sectional studies makes it impossible for our results to explain the causal relationship between increased Fetuin-A levels and the occurrence of IR and PCOS.

Conclusion

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

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 analyzed 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 consent. 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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Figures

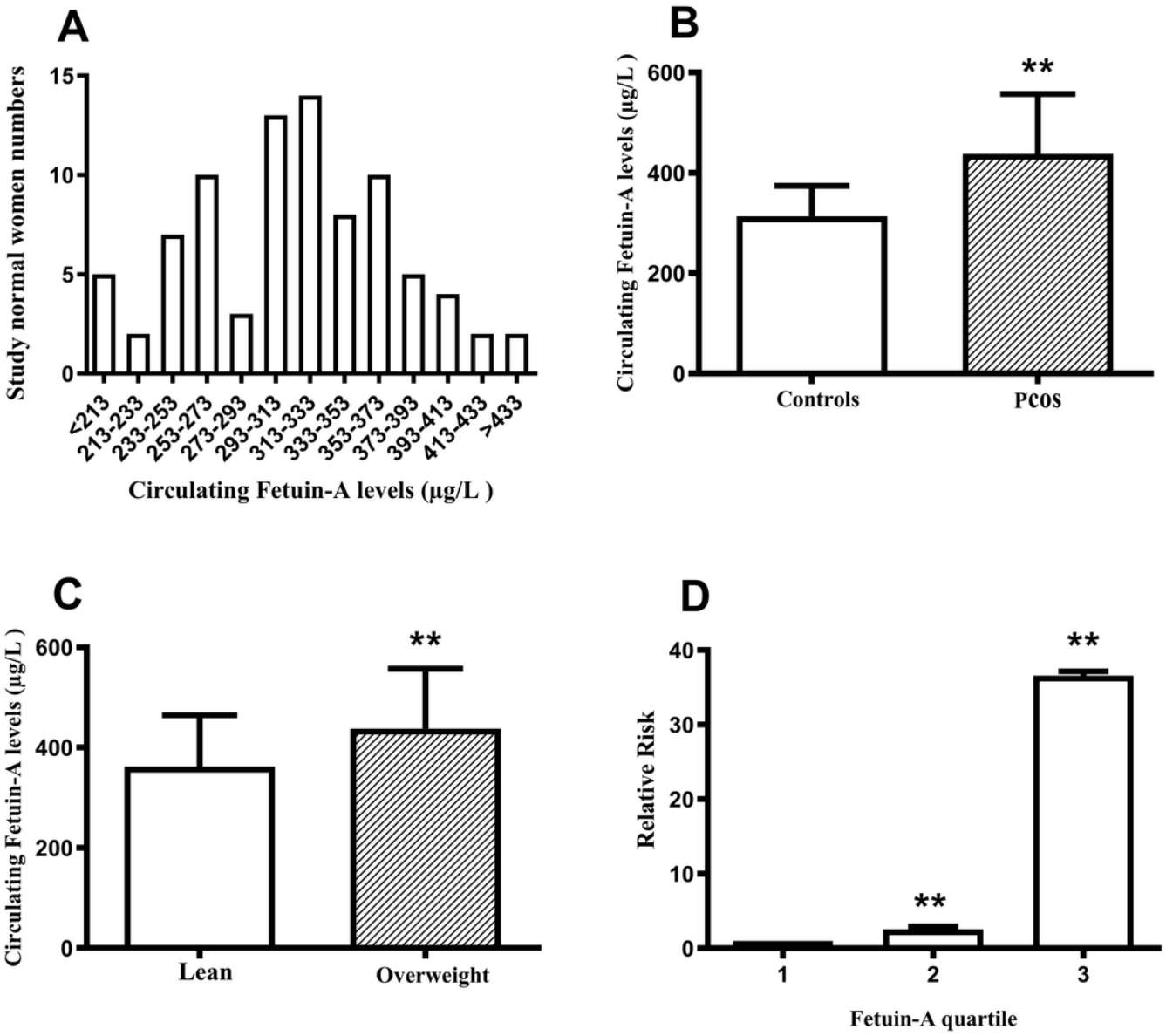

Figure 1

Serum Fetuin-A levels in the 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.

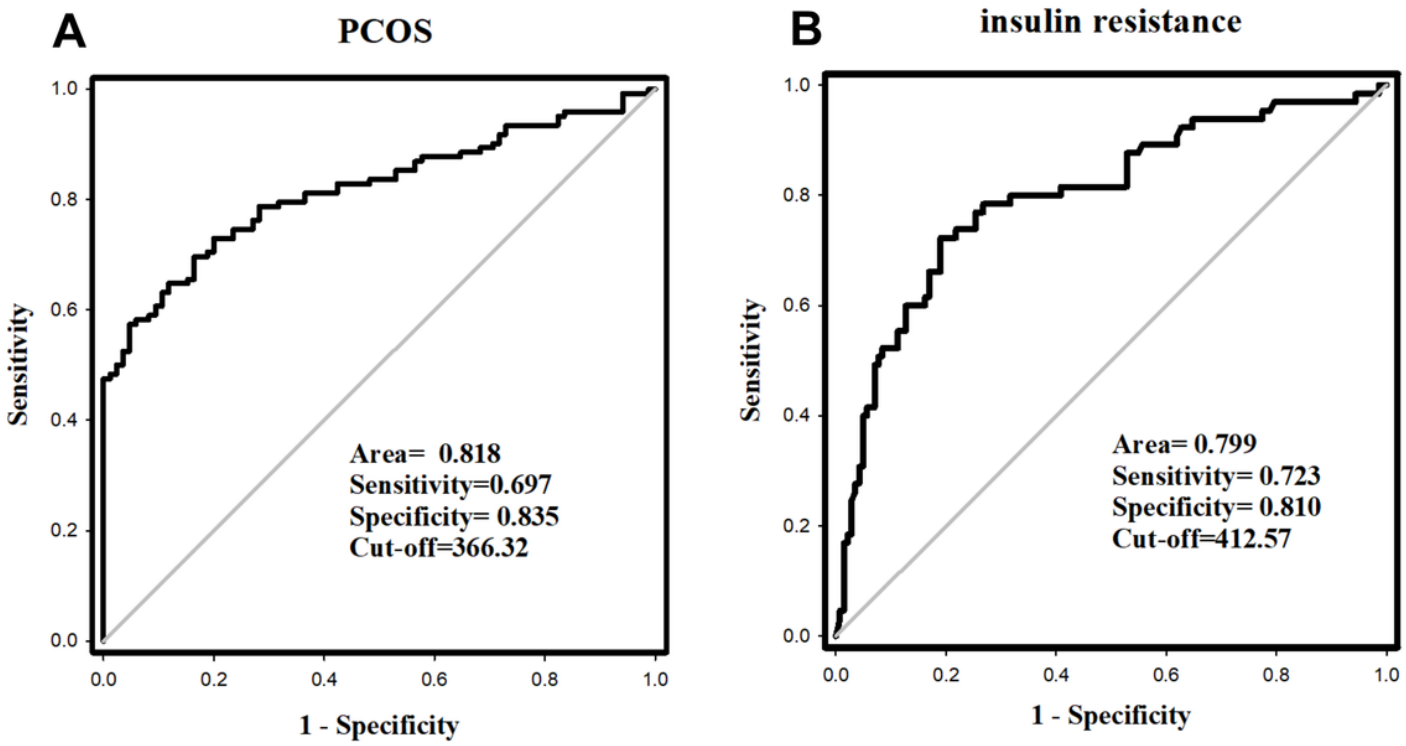

Figure 2

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