ORIGINAL ARTICLE

High Serum Ferritin Concentrations in Polycystic Ovary Syndrome Is Not Related to Insulin Resistance

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Received: 22 March 2011 - **Accepted**: 2 May 2011

ABSTRACT

OBJECTIVE: Some data are available on the association between components of the insulin resistance syndrome and serum ferritin. Insulin resistance is observed in patients with polycystic ovary syndrome (PCOS); We hypothesize that body iron stores might be increased in these women.

MATERIALS AND METHODS: Two hundred and six people (103 PCOS patients and 103 healthy women who were matched for age) entered the study. Body mass index (BMI), waist circumference and blood pressure of the participants were measured and serum androgens, gonadotropins, insulin, glucose, cholesterol, triglyceride, CRP and ferritin were evaluated.

RESULTS: Women with PCOS had a higher concentration of serum ferritin (146.5±14.6 pmol/l in PCOS vs. 129.6±21.3 pmol/l in controls, P= 0.03) and insulin as well (80.5± 62.5 Pmol/l in PCOS vs. 53.5± 29.8 Pmol/l in controls, p=0.017). No correlation was found between serum ferritin and BMI, blood pressure, waist circumference, fasting plasma glucose (FPG) and CRP. Multiple regression analysis, only showed an association between serum ferritin and severity of oligomenorrhea (r: 0.23, p=0.03).

CONCLUSION: Serum ferritin levels are increased in women with PCOS irrespective of their BMI, CRP and insulin resistance. This might be due to oligomenorrhea and less blood loss in this population.

KEY WORDS: Ferritin, PCOS, Insulin resistance, Iron store, Obesity

INTRODUCTION

Recent studies have reported a positive association between serum ferritin concentration and cardiovascular risk factors (1), insulin resistance syndrome and risk of type 2 diabetes (2-4). In our previous study in Iran, we revealed elevated serum ferritin concentration in subjects with impaired glucose tolerance (IGT), known as a pre-diabetic state (5).

Polycystic ovary syndrome (PCOS) is a common endocrine disorder among women (6) which is characterized by chronic anovulation and hyperandrogenism (7).

Insulin resistance with compensatory hyperinsulinemia is reported in obese and non-obese patients suffering from PCOS (8). Androgen overproduction has been considered as a result of hyperinsulinemia and may be followed by long-term metabolic disorders, such as impaired glucose tolerance, type 2 diabetes and cardiovascular diseases (9,10) .

It has been recognized that iron overload may play a role in insulin resistance at the cellular level (11). Considering high prevalence of insulin resistance and increased risk of type 2

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diabetes in PCOS (12, 9), we hypothesize that body iron stores might be increased in these women.

On the other hand there is not enough data about the relationship between serum ferritin concentrations in PCOS and the severity of insulin resistance and their clinical features including severity of irregular menstruation.

The objective of this case-control study was to analyze the association between high ferritin concentration and insulin resistance in PCOS. We evaluate the possible correlation of ferritin concentration with patients' metabolic variables and clinical features.

MATERIALS AND METHODS

Subjects: Two hundred and six women, aged between 15 and 40 years, including one hundred and three newly-diagnosed PCOS cases and one hundred and three healthy controls were serially enrolled in this study. All subjects were outpatients at the endocrine clinic of Vali-e-asr general hospital of Zanjan University of Medical Sciences. PCOS was diagnosed according to Rotterdam criteria (13). All subjects with such conditions as prolactinoma, congenital adrenal hyperplasia, Cushing's syndrome and virilizing ovarian or adrenal tumors were excluded. Subjects in the control group were selected from a population with the same socioeconomic condition who referred to the clinic for check-up and had normal ovulating cycles and no signs of hyperandrogenism. They were matched for age and body mass index (BMI) to the affected cases. The subjects in the control group didn't have any systemic diseases, and they didn't use any medications that might affect their reproductive physiology or iron status. All people with anemia (Hemoglobin concentration of less than 12g/dl) were excluded from the study.

All participants were notified about the goals of the study, and the participants didn't pay for the biochemical tests.

Measurements: Body weight was measured to the nearest 0.1 kg by a balanced-beam scale with subjects wearing light clothing, and height was measured by a stadiometer to the nearest 0.5 cm. BMI was calculated based on the weight/ $(\text{height})^2$ formula. Waist circumference was measured between the lowest rib and the iliac crest, at the level of umbilicus, in duplicate to the nearest millimeter using a flexible tape.

Blood pressure was measured with the subject seated using a random zero sphygmomanometer. Systolic (Korotkoff phase I) and diastolic (Korotkoff phase V) blood pressure was measured twice on the left upper arm and the mean of two measurements was used for analysis.

The score of hirsutism was determined based on Ferriman-Gallwey scoring system. Self-reported oligomenorrhea or amenorrhea was recorded. The presence of oligomenorrhea (cycles longer than 35 days) or amenorrhea (absence of menstrual bleeding for at least three usual cycle lengths) was defined as menstrual dysfunction in this study.

In all subjects, blood samples were collected between days 3 and 6 of a spontaneous menstrual cycle at 8.00- 9.00 AM, after at least 12 hours fasting. Blood sampling was done after a progesterone- induced menstruation for those women with cycles longer than 60 days. Complete blood count (CBC) was performed for all participants using a hematological analyzer (Sysmex, KX-21, Toa. Co., Japan). The basal levels of ferritin, insulin, and plasma glucose were measured, and a lipid profile was also conducted. The level of insulin resistance was determined by Homeostasis Model Assessment Index (HOMA IR), and it was calculated according to the following equation: [Insulin (μU/ml)] [FPG (mmol/L)] /22.5.

Insulin resistance was diagnosed in cases with a HOMA IR of more than 2.1(14). Insulin, luteinizing hormone (LH), follicle stimulating hormone (FSH) and testosterone levels were measured by an electrochemiluminescence immunoassay (ECLIA) using commercially available kits (Roche &DRG, German). C-reactive protein (CRP) was measured by a high-sensitivity assay, using a latex particle-enhanced immunoturbidimetric assay with analytical sensitivity of 0.175 mg/dl and

considering 5 mg/dl as the upper limit of normal. Serum ferritin was measured by electrochemiluminescence immunoassay (ECLIA) using commercially available kits (Roche Diagnostic, German) with analytical sensitivity of 0.5 ng/ml and inter-assay coefficients of variation ranged from 1.8 to 2%.

Statistical Analysis: Data are presented as means ± standard deviation. Statistical analysis was conducted using SPSS version 11.5. Proportions were compared using the chi-square test. Group means were compared using the student's t-test and Mann-Whitney test. Bivariate correlation analysis (calculation of the Pearson coefficient) was used to assess the correlation of serum ferritin levels to each parameter. Independent relationships between serum ferritin levels and those parameters to which they significantly correlated were assessed using multiple regression models. Statistical significance was set at $P \leq 0.05$.

RESULTS

Table 1 demonstrates the clinical, metabolic and hormonal characteristics of the two groups. Insulin and HOMA IR as well as ferritin concentrations increased significantly in women with PCOS compared to their matched controls. In overweight and obese subjects without PCOS, serum concentrations of cholesterol, triglyceride and insulin were higher ($p<0.01$) and HDL was lower ($p<0.01$) than those with BMI< 25kg/m^2. No significant differences were detected for the ferritin and CRP levels between obese and lean subjects in both control and PCOS groups. Table 2 shows the difference between ferritin in 2 groups of PCOS.

No difference was observed between obese subjects with PCOS and obese subjects in control group with the same BMI, for their CRP levels; but ferritin levels were higher in PCOS subjects than their BMI- matched

Table 1- Biochemical indices and clinical characteristics of women with PCOS and their matched controls (mean ± SD)

Parameter	PCOS group (n: 103)	Control group (n:103)	P value
Age(year)	24.8 ± 5.6	26.4± 6	0.4
BMI(kg/m2)	27.7 ± 5	26.2 ± 5.2	0.9
Waist (Cm)	84.8±12.8	82 ± 13	0.16
BP(mmHg)			
Systolic	114± 12	111± 11.6	0.14
Diastolic	74.5± 9.7	72.5 ±8.6	0.18
FPG(mmol/l)	4.6 ± 0.94	4.6 ± 0.5	0.4
Chol(mmol/l)	4.4 ± 0.8	4.2 ± 0.9	0.4
TG(mmol/l)	1.4± 0.8	1.2 ± 0.75	0.019
HDL Chol(mmol/l)	1.23 ± 0.3	1.22 ±0.2	0.2
Testosterone(nmol/l)	0.023 ± 0.01	0.016 ±0.01	0.07
DHEA-s(nmol/l)	11.5±5	9.7±3.7	0.02
Insulin(pmol/l)	80.5 ± 62.5	53.5 ± 29.8	0.004
HOMA IR	2.3 ± 1.9	1.5 ± 0.9	0.007
Ferritin(pmol/l)	146.5±14.6	129.6±21.3	0.03
CRP(mg/dl)	2.8±2	3.4±3	0.4

BMI: Body Mass Index; FPG:Fasting Plasma Glucose; Chol: Cholesterol; TG:Triglyceride; HDL:High Density Lipoprotein; HOMA Index :Homeostasis Model Assessment Index

Table 2-Biochemical indices of the women with PCOS and their matched controls (mean ± SD)

Parameter	Control group		PCOS group		Total	
	(BMI≥25 kg/m^2) (n:70)	(BMI<25 kg/m^2) (n:33)	(BMI≥25 kg/m^2) (n:69)	(BMI <25kg/m^2) (n: 34)	(BMI≥25 kg/m^2) (n:139)	(BMI <25 kg/m$^{2)}$ (n:67)
FPG(mg/dl)	4.7 ± 0.7	4.6± 0.48	4.5± 0.54	4.4±0.4	4.5± 0.55	4.5±0.4
Chol(mg/dl)	4.4±1.03*	3.7± 0.54	4.35±0.8	4.1±0.9	4.4 ±0.9*	3.9±0.77
TG(mg/dl)	14.4±9.4*	8.2±2.1	15.1±7.7*	9.7±5.3	14.9±8.2**	9 ±4.3
HDL Chol(mg/dl)	1.18±0.2*	1.23±0.25	1.1±0.23*	1.39±0.6	1.13±0.2*	1.3±0.5
Insulin(µU/ml)	59±29.8*	45± 23.6	87.5±76.4 *	52.8±29.8	79±62.5*	49.3±27
HOMA index	1.7± 0.9	1.3±0.7	2.5 ± 1.9*	1.5 ± 0.9	2.2 ±1.7*	1.4±0.8
Ferritin(pmol/l)	102±75	128±129	123±82.4*	157±148*	116±80	144±138

FPG, Fasting Plasma Glucose; Chol, Cholesterol; TG, Triglyceride; HDL, High Density Lipoprotein; HOMA Index, Homeostasis model assessment index

*Comparison between two groups in one category, P<0.01

** P<0.001

subjects in control group (Table2). The results confirmed after using logarithm of ferritin to normalize the distribution of this variable.

Although obese subjects with PCOS had higher insulin levels than obese participants in the control group (87.5 ±76 Vs 59 ± 29.8 pmol/l, p< 0.01), the difference between non-obese subjects with PCOS and non-obese controls was not significant (52.7±29.8 Vs 45± 23.6 pmol/l, P=0.1). In a simple linear regression analysis, ferritin was significantly correlated to insulin concentrations (r = 0.23, P = 0.01), HOMAIR (r = 0.26, P = 0.006), TG (r = 0.26, P = 0.004) and cholesterol (r = 0.23, P = 0.01) (Table 3). No correlation was found between ferritin level and CRP, BMI, waist circumference and serum androgens.

Considering the 50th percentile of ferritin concentration for healthy women (84.7 pmol/l) as the cut-off point, the risk of suffering from PCOS with high levels of ferritin was calculated to be 2.3 –fold of that for subjects with lower ferritin concentrations (OR: 2.3, CI95% 1.2-4.3, p = 0.01). The chance of having ferritin concentrations more than 50th percentile in subjects with amenorrhea or severe oligomenorrhea (less than four menstruations per year) was 2.9-fold of that for subjects with mild irregular menstruation (OR: 2.9, CI95% 1.5-5.7, p = 0.001).

Table 3- Correlations(r) between serum ferritin and other variables in all the subjects

Variable	Ferritin concentration(µg/l)	P value
BMI(Kg/m2)	0.06	0.4
BP(mmHg)	0.15	0.01
FPG(mg/dl)	0.29	0.001
TG(mg/dl)	0.08	0.01
Chol(mg/dl)	0.07	0.03

FPG: fasting plasma glucose, BMI: body mass index, BP: Blood Pressure, TG: Triglyceride, Chol: Cholesterol.

Table 4- Multiple regression of serum ferritin concentration on PCOS and other confounding variables in 176 adult women

Variables	Coefficient	CI (95%)	P-value
PCOS	0.96	0.5,1.2	0.05
BMI	0.25	0.1,0.6	0.5
HOMA-IR	21.4	-32,82	0.1

Using multiple regression analysis, we found only an association between serum ferritin and severity of menstrual dysfunction (0.23, p =0.03) (Table 4).

Insulin resistance was diagnosed in 43.6% and 23.7% of PCOS cases and subjects in the control group, respectively (p = 0.04). The odds ratio for insulin resistance was 2.4 in the subjects with PCOS (CI95%: 1.04- 5.9, P = 0.03) and 3.5(CI95%: 1.04-12, P = 0.03) in obese patients with PCOS. The risk ratio for insulin resistance was 1.4 in obese compared to non-obese subjects (CI95%:0.59-3.4, P = 0.4).

A significant correlation was found between CRP levels and BMI and waist circumference of the participants (r=0.21, p=0.02). No differences were found between PCOS patients and control subjects for their CRP levels (P = 0.462).

Based on a logistic regression analysis and considering the effect of other variables including age, BMI, waist circumference, blood glucose, insulin and CRP, the presence of oligomenorrhea was the only significant determinant of serum ferritin level.

DISCUSSION

Our study demonstrates that PCOS patients had significantly higher serum ferritin levels than healthy subjects irrespective of their insulin concentration, CRP and BMI. We found that oligomenorrhea and less blood loss in PCOS subjects might be the best explanation for their higher ferritin levels.

Although we demonstrated that PCOS subjects were more insulin resistant in our study, this difference may be BMI-dependent. We found no significant differences in HOMA IR measures of insulin resistance between the lean PCOS cases and their BMI-matched controls (p = 0.07). These data are not consistent with those from previous studies, which have shown that even in non-obese subjects, women with PCOS are significantly more insulin resistant than BMI-matched control women (12,15). The difference between our results and those from other studies may be due to the difference in the

criteria which is used for diagnosis of PCOS. Furthermore, a borderline P value was found in the present study, which may become significant if we increase the sample size.

We found that BMI is the main determinant of insulin resistance in PCOS, but changes in ferritin concentrations in these subjects are independent of BMI. These results is against previous findings suggesting ferritin as a link between obesity and insulin resistance (16,17). This difference is probably related to the method of estimation of insulin resistance in different studies. We estimated insulin resistance by calculating HOMA IR. Although this method has been suggested as a simple and accurate measure of insulin sensitivity (18), more sensitive and dynamic methods, including hyperinsulinemic isoglycemic glucose clamping and the oral glucose tolerance test (OGTT), have been recommended in most reports.

In this study, overweight and obese cases with PCOS had higher ferritin levels than BMI-matched controls. A similar result has been reported recently by two studies which suggested that increased body iron stores, expressed as increased serum ferritin concentrations, are present in women with PCOS (19,20). Héctor and colleagues (19) found higher levels of ferritin only in overweight and obese women with PCOS and not in lean subjects and concluded that these increased iron stores might contribute to the insulin resistance and β-cell dysfunction in PCOS patients, similar to the mechanism which has been proposed for insulin resistance in metabolic syndrome and type 2 diabetes (21-25).

Ferritin levels were increased in overweight and obese women with PCOS compared to overweight and obese control subjects; this was not found in lean subjects. This may be due to more severe irregular menstruations in overweight and obese subjects with PCOS compared to lean women. A significant interaction between PCOS status and the degree of obesity was found (P = 0.028).

Ferritin is considered an inflammatory marker. Therefore we measured the serum level of CRP, another inflammatory marker in these women to assess the potential confounding effect of chronic inflammation on the observed increase in ferritin levels. Our data suggested that the increased ferritin levels observed in overweight and obese PCOS patients were independent of chronic inflammation. Results recently reported by Martínez-García et al(21) is in agreement with our results for the relationship between ferritin and inflammatory factors like CRP in PCOS subjects. Héctor et al (19) also reported no association between high ferritin concentration and high levels of CRP. However higher CRP, IL-6, sICAM-1 and plasminogen activating inhibitor 1(PAI-1) as well as tumor necrosis factor-α (TNF-α) levels have been reported by Ehrmann (9). They didn't match the BMI of the cases and controls and this difference may reflect more body fat mass of PCOS subjects.

The effect of metformin on serum ferritin levels of the patients with PCOS has been reported by Luque-Ramírez et al. They concluded that serum ferritin decreased at 12 and 24 weeks of treatment in association with a marked increase in insulin sensitivity (26). They didn't find any changes in ferritin and insulin sensitivity with oral contraceptives (diane) and suggested that hyperinsulinism, and not the reduced menstrual losses is responsible for the increased body iron stores and subsequently increased serum ferritin found in overweight and obese women with PCOS.

Although we found only menstrual dysfunction to be the predictor of high serum ferritin in PCOS, Martinez Garcia et al (20) reported both menstrual dysfunction and insulin sensitivity index as the predictors of serum ferritin. The discrepancy might be explained by the difference in the designs of the studies. While our study was conducted with a case-control design and cases and controls were matched for their age and BMI, Martinez Garcia et al reported an observation in 257 pre-menopausal women who were divided into two groups and not matched for their age and BMI.

In conclusion, PCOS was found to correlate

with high ferritin levels, independent of BMI, CRP and insulin resistance. This might be due to oligomenorrhea and less blood loss in this population rather than inflammatory state and insulin resistance.

ACKNOWLEDGMENT

This study was supported by Zanjan University of Medical Sciences. We are grateful to Dr. Amirmoghadami for his assistance in laboratory measurements.

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