

# Iron Status and Lipid Profile in Irregular Cycle Women with Polycystic Ovary Syndrome

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**Abstract:** *Polycystic ovary syndrome (PCOS) is a complex and heterogeneous disorder characterized by hyperandrogenemia, hyperinsulinemia, insulin resistance, and chronic anovulation. It is the most common endocrine disorder in women of reproductive age*<sup>(1)</sup>. *Polycystic ovary syndrome is associated with insulin resistance; iron overload may lead also to insulin resistance and diabetes. Serum ferritin levels are increased in PCOS, especially when increase insulin resistance (IR), suggesting mild iron overload. Factors contributing to potential iron overload in PCOS include the iron sparing effect of chronic menstrual dysfunction and insulin resistance*<sup>(2)</sup>. *The aim of the present study is to evaluate the iron status parameters in PCOS patients as a possible cause of the associated features of PCOS*<sup>(3,4)</sup>.

**Keywords:** Iron status, lipid, polycystic ovary syndrome

## 1. Patients and Parameters

90 patients with PCOS diagnosed according to the Rotterdam revised consensus meeting in 2003 with matched control of 30 apparently healthy women were examined. Blood were aspirated from individuals in the morning, Lipid profile (TG, Total Cholesterol, LDL and HDL), Iron status (Total Iron, total iron binding capacity TIBC, serum ferritin, UIBC, Transferrin concentration, and Transferrin percentage) and other parameters hormone were determined.

## 2. Materials and Methods

After ethics committee approval, 90 women with PCOS recruited. Their age range was between 15 to 42 year. Patients with PCOS were recruited from the "Center of Fertility" in Al-Sadr Teaching Medical City in Najaf Governorate-Iraq. PCOS was defined according to the Rotterdam revised consensus meeting in 2003, it was proposed that oligomenorrhea, clinical or biochemical hyperandrogenaemia and the presence of polycystic ovaries should serve as the diagnostic criteria for PCOS (22). Control subjects were 30 healthy control women. Their age range was between 15 to 42 year with a normal menstrual cycle and with no clinical or biochemical features of hyperandrogenism<sup>(5)</sup>.

## 3. Measurements

Blood were aspirated from individuals in the morning (fasting) and collected in Gel tube for serum separation by centrifugation in order to estimate the lipid profile and Iron status parameters. Serum levels of Lipid profile (TG, Total Cholesterol, LDL and HDL) were estimated using

colorimetric method, Total Iron and total Iron Binding Capacity (TIBC) were estimated using colorimetric method by the following procedure: An excess of iron is added to the serum iron to saturate the transferrin. The unbound iron is precipitated with basic magnesium carbonate<sup>(6)</sup>. After centrifugation the iron in the supernatant was determined. The Ferritin Quantitative Test is based on a solid phase enzyme-Linked immunosorbent assay (ELISA). The assay system utilizes one rabbit anti-ferritin antibody for solid phase (microtitre wells) immobilization and a mouse monoclonal anti-ferritin antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution (6, 7).

### **Biostatistical analysis:**

- The results were presented as Mean  $\pm$  SD.
- Student's t-test was used to verify the association of biochemical parameters in patients relative to the control group.
- Significant variation was considered when the P value was less than 0.05.
- Minitab program version 15.20.000, was used to analyze the data

## 4. Results

The characteristics of the study groups are presented in table (1) which consists of the data of both patients with polycystic ovary syndrome and the control group. They include the number of women, age, waist, hip, WHR, height, BMI, weight, the number of patients with menstruation pattern (regular and irregular). It is not clear that the two groups are approximately well matched, thus results obtained could be considered creditable.

**Table 1:** Anthropometric data of enrolled patients and control groups

Parameters	PCOS patients Mean ± SD	(Range)	Control Mean ± SD	(Range)	P-Value
Age(year)	25.91±6.21	(15 - 42)	28.77±5.51	(15-42)	NS
Wt.(kg)	75.15±13.9	(50 - 110)	67.85±9.66	(50-90)	0.002
Ht.(m)	1.57±0.104	(1.40 -1.80)	1.63±0.071	(1.45-1.78)	NS
BMI(kg/m <sup>2</sup> )	30.39±6.19	(18.59 - 44.95)	25.41±4.82	(19.72 - 32.4)	0.001
Waist(cm)	101.1±14.0	(76.3 -123.19)	84.6±10.9	(68.58-106.68)	0.001
Hip (cm)	103.86±6.43	(83.82 - 114.38)	101.18±7.75	(83.82 -114.6)	NS
WHR	0.97±0.11	(0.76 - 1.17)	0.831±0.067	(0.71 - 0.97)	0.001
(regular cycle)	19	-	30	-	-
(irregularcycle)	41	-	-	-	-

Wt: weight, Ht: height, BMI: body mass index, WHR: Waist to hip ratio

The results of iron status expressed as mean ± standard deviation are presented in table (2A). There is a significant difference (p<0.05) in all iron status of PCOS patients with irregular in comparing with regular group. All parameters are increased in PCOS patients except TIBC which decrease in these patients in comparing with healthy control group.

**Table 2 (A):** The relevance of regular and irregular cycle with concentrations of biochemical parameters (Iron status) in the PCOS women group.

Parameters	PCOS with Regular Cycle (n=19) Mean ± SD	PCOS with Irregular Cycle (n=41) Mean ± SD	P-value
Total Iron (Mm)	12.67± 5.04	22.67 ±4.69	0.001
TIBC (Mm)	37.13± 8.64	47.19 ± 7.92	0.001
Ferritin	16.61±10.39	32.14 ± 20.61	0.01
TS%	56.11±14.24	63.33±16.03	0.01
UIBC	14.52 ±8.346	16.71 ±8.149	0.03
TS.c	0.093±0.021	0.092 ±0.023	NS

TIBC: total iron binding capacity, TS%: transferrin percentage, UIBC:unsaturated iron binding capacity ,TS.c: transferrin concentration.

The results of lipid profile and hormone parameters expressed as mean ± standard deviation are presented in table (2B). There is a significant difference (p<0.05) in (LDL,TG,LH, LH/FSH and Prolactin) of PCOS patients with irregular in comparing with regular group.

**Table 2 (B):** The relevance of regular and irregular cycle with concentrations of biochemical parameters (lipid profile and hormonal profile) in the PCOS women group.

parameters	PCOS with Regular Cycle (n=19) Mean ± SD	PCOS with Irregular Cycle (n=41) Mean ± SD	P-value
TC (mg/dl)	194.83 ± 24.92	195.20 ± 19.39	NS
HDL (mg/dl)	40.60 ± 4.39	39.82 ± 5.73	NS
LDL (mg/dl)	74.85±12.34	119.15 ± 23.35	0.001
TG (mg/dl)	147.7± 8.67	178.1±30.32	0.001
LH ( IU/L)	4.38 ± 1.75	11.21±2.45	0.001
FSH (IU/L)	5.41 ± 1.68	5.76± 1.26	NS
LH/FSH	1.78± 0.81	2.03±0.41	0.001
PROGSTRONE (nmol/L)	3.43 ± 0.82	3.22 ± 0.75	NS
TT (nmol/L)	2.80 ± 0.40	3.18±1.09	NS
FT (Pmol/L)	12.36 ± 2.20	13.16 ± 2.41	NS
E <sub>2</sub> (Pmol/L)	208.29 ± 71.61	209.13± 48.45	NS
Prolactin(nmol/l)	0.83±0.17	0.94 ±0.21	0.02

TC: total Cholesterol, HDL: high density lipoprotein, LDL: Low density lipoprotein, TG: triglyceride, LH: luteinizing hormone, FSH: follicular stimulating hormone, LH/FSH ratio, SHBG: sex hormone binding globulin, TT: total testosterone, FT: free testosterone, E<sub>2</sub>:estradiol

The correlation between iron status and lipid profile of PCOS patient are expressed as correlation coefficient (r-value) and (p-value) are presented in table (3A), table (3B) and table (3C) respectively. It can be noticed that the only significant correlation noticed was the negative correlation between total iron and HDL (r=-0.05, p= 0.03), TIBC and HDL (r = -0.08, p = 0.01), Ferritin and HDL (r = -0.03, p = 0.02), Ferritin and TG (r = -0.01, p= 0.04).

**Table 3 (A):** Host information data (Total Iron and TIBC) correlation with Lipid profile.

parameters	Total Iron		TIBC	
	r	p	r	p
TC(mg/dL)	0.06	0.02	0.02	0.01
HDL(mg/dL)	-0.05	0.03	-0.08	0.01
LDL(mg/dL)	0.03	0.02	0.01	0.02
TG(mg/dL)	0.13	0.01	0.05	0.01

TC: total Cholesterol, HDL: high density lipoprotein, LDL: Low density lipoprotein, TG: triglyceride

**Table 3 (B):** Host information data (UIBC and Ferritin) correlation with Lipid profile.

Parameters	UIBC		Ferritin	
	r	p	r	p
TC (mg/dL)	-0.03	NS	0.05	0.03
HDL(mg/dL)	0.06	NS	-0.03	0.02
LDL(mg/dL)	-0.04	NS	0.02	0.01
TG(mg/dL)	-0.02	NS	-0.01	0.04

TC: total Cholesterol, HDL: high density lipoprotein, LDL: Low density lipoprotein, TG: triglyceride

**Table 3 (A):** Host information data (TS.c and TS%) correlation with Lipid profile

Parameters	TS.c		TS%	
	r	p	r	p
TC (mg/dL)	-0.04	NS	0.02	NS
HDL(mg/dL)	0.05	NS	-0.03	NS
LDL(mg/dL)	-0.02	NS	0.05	NS
TG(mg/dL)	-0.02	NS	-0.01	NS

TC: total Cholesterol, HDL: high density lipoprotein, LDL: Low density lipoprotein, TG: triglyceride

## 5. Discussion

To examine the influence of Period Regularity on the concentrations of biochemical parameters in PCOS patients table (2A-2B), they were classified into two groups, i.e., those regular and irregular cycle groups. The results pointed out that (19) with regular and (41) with irregular cycle out of the 60 patients. Student's t test revealed significant increase Total Iron(p=0.001), TIBC(p=0.001), ferritin (p=0.01), TS%(p=0.01), UIBC (p=0.03), LDL (p=0.001), TG (p=0.001), LH(p=0.001), LH/FSH ratio(p=0.001), prolactin (p=0.02), levels in the patients with irregular cycle groups when compared with regular cycle groups. Other parameters(TS.c, Cholesterol, HDL, Progesterone, E2, FSH, FT and TT) failed to indicate significant variation.

Irregular cycle defined in PCOS when (cycle) there is less than 8 periods yearly or when periods longer than 6 weeks<sup>(8)</sup>. The disturbances in women period may reflect a hormonal changes that cause ovulation disturbances or fine anatomical changes in ovaries. However, the hormonal changes are present, in general, in PCOS women under study and hence the changes in hormones between PCOS<sup>(9)</sup>. Anovulation or oligo-ovulation is a very common presenting symptom of PCOS. Irregularity of menstruation, amenorrhea or oligomenorrhea, is first encountered in adolescence or present in the infertility clinic. The root cause of anovulation in PCOS is now thought to be associated with the multiple small follicles characteristic of the PCOS<sup>(9)</sup>.

Anovulation or oligo-ovulation, the irregularity of menstruation, is a common symptom presented in PCOS women. Also, is associated with significant abnormalities in early stages of follicular genesis<sup>(10)</sup>. The process during which small primordial follicle develop in to large preovulatory follicle and which culminate in ovulation<sup>(11)</sup>. The early follicle growth is accelerated, but the follicle arrest in their development when they reach 2-9 mm in diameter, a phase during which the selection of a dominant follicle would normally occur<sup>(12)</sup>, and the development of multiple small follicle resulting from hyperinsulinemia. Although a significant number of woman with excessively high serum androgen concentrations have increased adrenal gland androgen production. Androgen excess, together with LH and insulin may also be involved in the inhibition of follicle maturation towards the dominating stage<sup>(11)</sup>.

The Oligo-ovulation of PCOS frequently results in chronic oligomenorrhea or even in periods of amenorrhea. Regular menstrual losses are one of the few mechanisms by which the female body losses iron in significant quantities. The iron sparing effects of chronic oligomenorrhea might contribute to the increased iron store found in serum individual with PCOS<sup>(13)</sup>.

This result agreement with previously studied suggested a causal role of iron overload in the development of abnormalities in glucose tolerance, however this association could also be spurious, because woman with increased ferritin were those with the worse IR and the higher androgen level<sup>(14,13,15)</sup>.

Patients with polycystic ovary syndrome (PCOS) have elevated serum ferritin levels, indicating increased body iron stores<sup>(16)</sup> and this agree with present study. This iron overload, by favoring insulin resistance and pancreatic  $\beta$ -cell dysfunction, may contribute to the abnormalities in glucose metabolism frequently present in patients with PCOS<sup>(17)</sup>.

Many researchers have shown that PCOS is not only a gynecological condition affecting women of reproductive age but, also, a comprehensive syndrome with a variety of associated metabolic disorders (insulin resistance, obesity, hyperinsulinaemia and dyslipidemia) began commonly to be described as associated with PCOS, And this suggests appear in this study<sup>(18)</sup>.

This increase in tissues iron are in agreement with the result of other researches<sup>(19)</sup>. It is hypothesized that genetic factors, the absence of a regular menstrual blood loss, or even hyperinsulinemia resulting from insulin resistance, considering that insulin might stimulate intestinal iron absorption by up regulating the activity of hypoxia-inducible factor-1alpha and down regulating hepcidin expression<sup>(20)</sup>, may have contributed to the increased body iron stores and serum ferritin levels observed in PCOS patients<sup>(20)</sup>.

Elevated iron stores were positively associated with the prevalence of the metabolic syndrome and with insulin resistance<sup>(21,22)</sup>. Insulin resistance, hyperinsulinaemia, and obesity are all seen in patients with PCOS<sup>(23)</sup>.

The increased iron stores might contribute to the insulin resistance and  $\beta$ -cell dysfunction frequently found in PCOS patients, as has been proposed for insulin resistance, the metabolic syndrome, and type 2 diabetes<sup>(23)</sup>. The results of Luque-Ramírez *et al.*, suggested that insulin resistance and hyperinsulinism, and not the reduced menstrual losses secondary to from oligo- or amenorrhea, are responsible of the increased ferritin levels and body iron stores found in overweight and obese women with PCOS<sup>(19)</sup>.

Yet several of these factors might collaborate in the increased body stores observed in overweight and obese PCOS patients. As proposed for type 2 diabetes<sup>(24)</sup>, the insulin resistance intrinsic to PCOS, exacerbated by obesity and perhaps dietary influences, may facilitate iron absorption and deposition in tissues, a mechanism possibly amplified by the reduced menstrual losses of PCOS patients. Iron deposition in certain tissues increases insulin resistance, closing the vicious circle of iron overload and predisposing these women to disorders of glucose tolerance and other components of the metabolic syndrome<sup>(25)</sup>, because the periodic blood loss resulting from regular menstruation protects premenopausal women against excessive iron accumulation, oligomenorrhea and amenorrhea might contribute to the increase in ferritin observed in overweight and obese PCOS patients<sup>(25)</sup>.

However, the increase in serum ferritin levels in PCOS may be a secondary, not a pathogenic, event in PCOS. The absence of regular menstrual blood loss in PCOS patients might contribute to iron overload, as serum ferritin levels were increased in our amenorrheic patients compared with regularly menstruating women. Oxidative stress (OS) may

be increased in PCOS woman, OS increases ferritin synthesis, partly to avoid further oxidative damage, given that ferritin neutralizes the highly toxic unbound iron<sup>(24,26)</sup>.

Additionally, it's well known that in PCOS there is hyperandrogenemia which affects on erythropoiesis processes<sup>(27)</sup>. This phenomenon correlated hyperandrogenemia in PCOS with one of the important iron function<sup>(28)</sup>.

The amelioration of insulin resistance and hyperinsulinemia by metformin may explain the reduction in serum ferritin levels and iron stores found in the PCOS patients treated with this insulin sensitizer, especially when, to our best knowledge, no direct interaction of metformin with the intestinal absorption of iron has been described to date<sup>(19)</sup>. It is considered that increased iron stores contribute to insulin resistance and hyperinsulinemia by reducing hepatic insulin extraction and metabolism and by decreasing glucose uptake in muscle<sup>(29,30)</sup>. This fact revealed that the iron that precipitate in any tissue do not extracted and circulated easily. While there is accumulation of iron in certain tissues, the circulating iron is low and does not reflect the iron status in other tissues<sup>(31)</sup>.

The association of iron overload and insulin resistance may be bidirectional because the compensatory hyperinsulinaemia characteristic of insulin resistance also facilitates iron accumulation within the body<sup>(17,32)</sup>. Indeed, previously reported that body iron stores in patients with PCOS are also modulated by hyperandrogenism and menstrual dysfunction<sup>(16)</sup>.

The cause of serum lipid profile disorder may attributed to the impaired insulin action due to the insulin resistance in PCOS patients<sup>(33)</sup>. Such abnormalities are companied with the increase lipid peroxidation in this patient's the approximately 70% of woman with PCOS exhibit abnormal serum lipid level. These changes are consistent with lipid profile typically found in association with IR and MetX<sup>(34)</sup>. Abnormal lipid levels increase the risk of atherosclerosis and early cardiovascular disease (CVD) in woman with PCOS. Low HDL concentrations appear to be the predominate abnormality in PCOS patients and always be accompanied by elevated TG<sup>(34,35)</sup>.

The present findings agreement with last studies of Valkenburg *et al.*, showed when lipid changes occur in PCOS women, this may affected by obesity and hyperandrogenism<sup>(36)</sup>. In other words, obesity in PCOS women would be the most important factor for metabolic abnormalities<sup>(37)</sup>. This results are in accordance with the results of the present study.

Also this results disagreement with Bickerton *et al.*, also found no significant differences in terms of lipids between PCOS and non-PCOS women<sup>(38)</sup>. Bahceci's *et al.*, findings suggests that the level of LDL, HDL and glucose, in women with and without PCOS do not show any significant differences<sup>(39)</sup>. Similarly, Jahanfar *et al.*, in a study aimed at evaluating the genetic and environmental factors affecting lipids among twins, found no significant differences between women with and without PCOS in serum TC, HDL

and LDL and this results disagreement with the present study<sup>(40)</sup>.

## 6. Conclusion

The results of the present study concluded that:

- 1) Anthropometric parameter direct the change of Iron Status (Total Iron, TIBC, Ferritin, UIBC, TS% and TS.c) and lipid profile levels in PCOS
- 2) Iron Status level is higher in PCOS in comparing with control group.
- 3- Significant increase of the concentrations Total Iron(p=0.001), TIBC(p=0.001), ferritin (p=0.01), TS%(p=0.01), UIBC (p=0.03), LDL (p=0.001), TG (p=0.001), LH(p=0.001), LH/FSH ratio(p=0.001), prolactin (p=0.02), levels in the patients with irregular cycle groups when compared with regular cycle groups. Other parameters(TS.c, Cholesterol, HDL, Progesterone, E2, FSH, FT and TT) failed to indicate significant variation.

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