Original Article:

Body iron status and association with hyperinsulinaemia and hyperandrogenism in non-obese Indian women with polycystic ovarian syndrome

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ABSTRACT

Background: Increased body iron stores, insulin and androgen levels have been reported in obese women with polycystic ovarian syndrome (PCOS). However, the status of iron and the influence of hormonal profile on iron status in non-obese women with PCOS has not been studied. The present study was thus designed to determine the iron status and hormones insulin and testosterone levels and their association with body iron status in non-obese women patients with PCOS.

Methods: The present study included 33 non-obese women diagnosed as PCOS based on National Institutes of Health consensus 1990 criteria and 31 age-matched healthy women as controls. Height, weight, body mass index, fasting plasma glucose, iron status markers i.e., serum ferritin, iron, total iron binding capacity, insulin and testosterone levels were determined.

Results: A significant increase in hormones insulin and testosterone levels ($p<0.001$) was found in patients with PCOS in comparison with controls. Similarly, a significant increase in serum ferritin levels was observed in PCOS patients compared to controls ($p<0.05$). A significant positive correlation was observed between serum insulin and testosterone levels ($p<0.05$). Similarly, a positive association was observed between serum testosterone and ferritin levels [p=0.007 odds ratio (OR) 7.0(1.715-28.568)].

Conclusions: The present study demonstrates that body iron stores, as reflected by serum ferritin concentrations, are increased even in non-obese PCOS patients. Androgen excess is associated with increased body iron stores in these patients with the possible additive effects of hyperinsulinaemia, through its perpetuating effect on increased androgens and iron-sparing effect of reduced menstrual losses.

Key Words: *Polycystic ovarian syndrome, Hyperandrogenism, Ferritin, Body iron status*

Supriya P, Bitla AR, Rajendra Prasad N, Rajagopal G, Madhusudhan Rao A, Suchitra MM, Sachan A, Srinivasa Rao PVLN. Body iron status and association with hyperinsulinaemia and hyperandrogenism in non-obese indian women with polycystic ovarian syndrome. J Clin Sci Res 2012;1:2-7.

INTRODUCTION

Polycystic ovarian syndrome (PCOS) is the most common reproductive endocrine disorder among women of reproductive age, affecting 5%-10% of population worldwide[1]. In Indian population, the incidence has been estimated to be between 4%-11% among women of reproductive age group.[2]

It is the commonest multisystem endocrinopathy having diverse aetiopathogenesis in women, causing menstrual irregularities, hirsutism and anovulatory infertility. Also, PCOS is a predominantly hyperandrogenic disorder[3] while obesity and insulin resistance are frequently associated with PCOS.[4,5] Body iron stores are increased in overweight and obese women with PCOS and other insulin resistant conditions[6]. The increase in body iron stores might contribute to the insulin resistance and β-cell dysfunction frequently found in PCOS patients.[7] This involvement appears to be bidirectional, because not only does iron accumulation favor insulin resistance but also insulin resistance may in turn facilitate iron accumulation within the body.[6,8] On the other hand, in PCOS, insulin resistance and the resultant hyperinsulinaemia exacerbate the reproductive abnormalities by increasing ovarian androgen production and decreasing serum sex hormone binding globulin[9]. Further, it may promote abnormal ovarian androgen secretion and therewith abnormal follicular development leading to dysfunctional ovarian and menstrual activity.[10] These observations suggest the impact of insulin resistance on androgen levels, which further influence body iron stores in PCOS patients.

Received: 23 December, 2011

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Although, PCOS is predominantly a hyperandrogenic disorder,[3] reports on body iron status in PCOS and its association with insulin resistance are sparse and this issue has been overlooked in either obese or non- obese women with PCOS. With respect to studies on iron status in polycystic ovarian syndrome from India, search of literature showed that there are no reports. This conclusion was drawn by searching the websites www.ncbi.nlm.nih.gov and www.medind. nic.in. The MeSH terms used in the search included, body iron status/ stores + polycystic ovarian syndrome + India, body iron status/stores + polycystic ovarian syndrome + South India, ferritin levels + polycystic ovarian syndrome + India, ferritin levels + polycystic ovarian syndrome + South India. Hence, the present study was undertaken with the aim of studying body iron status and hormonal levels and its association with iron status in non-obese women with PCOS.

MATERIAL AND METHODS

In the present study, thirty three PCOS patients attending the Endocrinology outpatient Department of Sri Venkateswara Institute of Medical Sciences, Tirupati, Andhra Pradesh, aged 20-38 years and diagnosed as PCOS based on National Institutes of Health (NIH) consensus 1990 criteria[11] were included after informed consent. The diagnostic criteria according to the NIH consensus 1990 criteria were oligomenorrhoea (≤ 9 menses/year) or amenorrhea (no menstrual periods for 3 or more months), hyperandrogenism and/or hyperandrogenaemia and after exclusion of related disorders with similar presentation like hypothyroidism [thyroid stimulating hormone (TSH) >5 mIU/mL)], hyperprolactinaemia (serum prolactin > 100 ng/mL), Cushing's syndrome (cortisol > 2 µg/dL), adrenal hyperplasia and androgen secreting tumours (testosterone levels greater than 3 times the upper reference limit associated with relevant clinical features).Women with virilization, pregnancy, those on oral contraceptives, glucocorticoids, anti- androgens, ovulation inducing agents, antidiabetic drugs or antiobesity drugs or other hormonal drugs during the previous 6 months were excluded from the study. Thirty one age matched healthy females from among the hospital staff were taken as controls. The criteria for healthy control group were absence of menstrual irregularities, hirsuitism and major medical illness. The study was approved by the Institutional Ethical Committee.

Anthropometric measurements including height, weight and body mass index (BMI) and systolic and diastolic blood pressures of all subjects were recorded. Five mL of venous blood was collected from both patients and controls, following 12 hours of overnight fasting. Four ml was transferred to a plain bottle for estimating iron, total iron binding capacity (TIBC), serum ferritin, hormones and one mL to fluoride bulb for fasting plasma glucose (FPG) estimations. The samples were allowed to stand for half-an-hour, and centrifuged at 3000 rpm for 15 minutes and the serum/plasma obtained was stored at –80 ^{o}C until analysis. The serum iron and TIBC concentrations were determined by Ferrozine method using commercially available kit (Raichem, San Marcos 92078 CA, USA) on Beckman CX-9 fully automated analyzer. Serum ferritin was estimated by indirect solid phase ELISA technique using commercial kit from UBI Magiwell (Mountain view, CA, USA). Serum insulin levels were measured by ELISA using DIAsource Ins-Easia kit from DIAsource immunoassays S.A.-Rue de I'In- dustrie,8-B-400 Nivelles-Belgium. Serum ferritin and insulin were assayed using Chemwell analyzer. Plasma testosterone was measured on specific radioimmunoas- says (RIAs) [Testo-CTK (P 3093) RIA kit , Diasorin, U.S.A.] Insulin resistance as per the homeostasis model assessment method (HOMA-IR)

was calculated using to the formula[FPG (mg/dL) × insulin (mIU/ L)]/405.[12]

Statistical Analysis: Data was expressed as mean and standard deviation. The Kolmogorov-Smirnov test was used to evaluate whether the distribution of continuous variables was normal. Differences between the two groups were analysed using unpaired student "t" test and Mann-Whitney U test as appropriate . Spearman rank correlation was used to test the correlations among the variables. Binary logistic regression was used to know the association between parameters. A p-value of < 0.05 was considered statistically significant. The statistical analysis was performed using statistical software, SPSS version 11.5 (SPSS,Inc.,Chicago IL).

RESULTS

Table 1 shows the baseline characteristics and laboratory data of PCOS patients and controls. In the present study all the PCOS patients and controls had BMI < 25 kg/m^2 indicating that they were non-obese. There were no statistically significant differences between PCOS patients and controls with respect to age, systolic blood pressure (SBP), diastolic blood pressure (DBP) and also FPG (Table 1).

Status of iron and hormonal profile

Comparisons of iron status and hormone profile between controls and PCOS patients are shown in Table 2. A marked increase in fasting serum insulin (FSI) (p=0.007), serum testosterone levels (p=0.001) and HOMA-IR (p=0.007) were observed in these PCOScases in comparison with controls. Serum ferritin levels were found to be higher in non-obese women with PCOS than controls (p=0.001) while, no significant difference was found between serum iron (p=0.386) and TIBC levels (p=0.070) in PCOS patients and the control subjects.

Associations between ferritin and hormones

A significant positive correlation was observed between FSI and serum total testosterone (r = 0.394, p=0.046) in PCOS patients. Taking the control means as the cut-off, logistic regression analysis was performed with FSI and serum total testosterone as the dependent variables and serum ferritin as the independent variable.

As shown in Table 3, serum ferritin was significantly associated with serum total testosterone [p=0.007 odds ratio (OR) (95% confidence interval 7.0 (1.715-28.568)].No association was observed with FSI levels. However, serum ferritin was significantly associated with the combination of an elevated FSI and testosterone levels [p=0.028, OR 3.9 (1.157 - 13.144)].

DISCUSSION

To the best of our knowledge, the present study documents for the first time, data regarding iron status and its relation with hormones insulin and testosterone concentrations in non-obese Indian patients with PCOS. Our study showed that, non-obese PCOS patients presented with increased body iron stores and a marked increase in hormones insulin and testosterone which are in agreement with other workers.[13-16]Marked rise in serum total testosterone in these cases may be due to excess ovarian production of androgens, which is central to the diagnosis of PCOS.[17] Yet some researchers have shown apparently normal testosterone concentrations in their PCOS cases, which may be attributed to their low to normal sex hormone binding globulin levels.[18]A variety of hypotheses have been put forward for increased body iron stores in women with PCOS. Angeles Martinez Garcia et al[19] reported that hyperinsulinaemia and insulin resistance might be responsible the increased body iron stores in PCOS. These results have

been shown both in the nonobese and in the obese subgroups, and obesity did not influence serum ferritin concentrations after controlling for both PCOS and glucose tolerance. The results of the present study also showed that serum ferritin, insulin and calculated insulin resistance levels are increased in non-obese PCOS patients though there was no significant association between them. On the other hand, a marked increase in serum testosterone levels parallel with insulin and a positive correlation between them suggests an association exists among each other in PCOS patients. This is in

Table: 1 Base-line characteristics of the PCOS cases and control subjects

Variables	Controls (n=31)	PCOS cases (n=33)	p-value
Age (Years)	24.97 ± 5.18	24.20 ± 5.2	0.48
BMI (Kg/m^2)	24.07 ± 1.83	24.30 ± 2.04	0.48
SBP (mm Hg)	113.33 ± 5.46	110.0 ± 7.74	0.06
DBP(mm Hg)	72.88 ± 6.30	72.0 ± 4.06	0.87
FPG (mg/dL)	91.73 ± 5.15	94.19 ± 7.13	0.13

PCOS = polycystic ovarian syndrome; BMI = body mass index;
SBP = systolic blood pressure; DBP = diastolic blood pressure;
FPG = fasting plasma glucose

Table 2: Body iron status and hormonal profile in the study group and control subjects

Variable	Control	PCOS cases	p-value
Serum ferritin (ng/mL)	46.66 ± 22.67	77.15 ± 61.06	0.001
Serum iron (µg/dL)	84.66 ± 27.68	93.90 ± 30.88	0.386
TIBC (µg/dL)	381.89 ± 46.01	401.67 ± 39.65	0.070
Serum testosterone (ng/dL)	0.75 ± 0.29	1.97 ± 0.47	0.001
FSI (µIU/mL)	13.77 ± 12.89	19.64 ± 10.20	0.007
HOMA-IR	3.14 ± 2.95	4.41 ± 2.36	0.007

PCOS = polycystic ovarian syndrome;
TIBC = total iron binding capacity; FSI = fasting serum insulin;
HOMA-IR= homeostasis model assessment of insulin resistance

agreement with other reports.[19] Barbieri et al[20] advocated that hyperinsulinemia plays a pathogenetic role in PCOS cases by increasing ovarian androgen production and decreasing the serum sex hormone binding globulin con- centration. Insulin may directly stimulate ovarian cytochrome P450c17a, resulting in increased 17-α hydroxylase and to a lesser extent, 17,20-lyase activity. This would lead to increased production of androstenedione, which is then converted to testosterone by the enzyme 17β reductase resulting in higher levels of free testosterone with dysfunctional ovarian and menstrual activity.[21] Therefore, it appears that insulin resistance can perpetuate androgen excess,

Table 3: Logistic regression analysis

Parameter	Regression Coefficient (B) ± SEM	Exp (B) Odds ratio	95 % CI	p-value
FSI with serum ferritin	0.866 ± 0.526	2.3	0.848-6.666	0.100
Serum total testosterone with serum ferritin	1.946 ± 0.718	7.0	1.715-28.568	0.007
Combination of elevated FSI and serum testosterone with ferritin	1.361 ± 0.620	3.9	1.157-13.144	0.028

FSI = fasting serum insulin; SEM = standard error of means; CI = confidence intervals

present in PCOS patients.[9] Both might collaborate in increasing body iron stores in these patients. The effect of androgen excess on body iron stores might result from the well known stimulatory effect of androgens on erythropoiesis, thereby increasing intestinal iron absorption[22] but may also result from the iron-sparing effect of reduced menstrual losses due to the chronic menstrual dysfunction of PCOS. Our finding of significant association between a combined increase of insulin and testosterone levels and body iron stores represented by serum ferritin levels [(p=0.028, OR 3.9(1.157-13.144)] is in support of this concept.It has recently been shown that PCOS is associated with a polymorphism in the haptoglobin α chain that reduces the antioxidant and antiinflammatory properties of this molecule.[23] It has also been shown that two haptoglobin β-chain isoforms are significantly underexpressed in PCOS plasma suggesting posttranslational modifications of haptoglobin that might contribute to haptoglobin dysfunction in PCOS[24] and thus affect iron metabolism. However, we could not include this aspect in our study. The present study demonstrates that body iron stores, as reflected by serum ferritin concentrations, are increased even in non-obese PCOS patients. Androgen excess is associated with increased body iron stores in these patients with the possible additive effects of hyperinsulinaemia, through its perpetuating effect on androgen excess. However, we could not demonstrate a direct relationship between iron stores with hyper-insulinaemia. This could probably be due to the small sample size. Another additive effect is possibly iron-sparing effect of reduced menstrual losses.

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