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Study of association of Leptin, Sex hormone binding globulin (SHBG) and Glucose-insulin ratio (GIR) with 25-Hydroxycholecalciferol for insulin resistance in women with Poly Cystic Ovarian Syndrome

Dr Huma Nasrat

Akanksha Dubey Assistant Professor Department of Biochemistry Chirayu Medical College and Hospital Bhopal

ABSTRACT

Introduction- PCOS has emerged as a major endocrinological disorder in females in the reproductive age group with significant short term and long term effects such as metabolic syndrome infertility, cardiovascular disorders. Insulin resistance is one of the cardinal features of this syndrome.

Aims and objectives- To study the different biochemical and anthropometric markers of insulin resistance and to correlate with vitamin D status in PCOS patients and to explore whether vitamin D could be considered as predictor of insulin resistance in PCOS.

Materials and methods- Sixty patients of PCOS diagnosed by Rotterdam criteria were enrolled in this study.Estimation of 25- OH vitamin D was done by ELISA using 25 OH vitamin D direct ELISA kit from Immundiagnostik (Bensheim). Serum leptin level was measured by ELISA by using leptin DRG leptin sandwich ELISA kit. Sex hormone binding globulin was measured by ELISA using DRG SHBG. Insulin levels were estimated by Chemiluminescence immunoassay (CLIA) in Access 2 Beckmann Coulter.

Results- 28.34% of PCOS women showed 25(OH)D values <30 nmol/L(Vitamin D deficiency).25-OH-vit D levels were significantly different in the two groups (HOMA-IR≤ 3.9 and HOMA>3.9).We found a significant negative correlation of 25(OH)D levels with fasting insulin, HOMA-IR and glucose –insulin ratio.We found that vitamin D was the important determinant of HOMA-IR in women with PCOS.28.9 % of total variation in HOMA-IR is explained by 25-OH-vit D alone (r^2=0.289 p value 0.0001).

Conclusions- Lower levels of vitamin D are associated with higher levels of insulin resistance and thus Vitamin D deficiency might be involved in the pathogenesis of insulin resistance in PCOS.

KEYWORDS : .

INTRODUCTION

Polycystic ovary syndrome (PCOS) was first reported as Stein-Leventhal syndrome in 1935[1] and has since emerged as the most common endocrine disorder, affecting 18% of women in reproductive age group[2].PCOS may present with a constellation of clinical manifestations which include hirsutism, acne, excessive androgen production, menstrual disturbances and subsequently, anovulation and infertility[3].The potential long-term consequences of PCOS include type 2 diabetes mellitus, hypertension, and cardiovascular disease.[4]

Recent studies suggested that in addition to various insulin resistance markers like HOMA-IR, leptin, fasting insulin levels etc., Vitamin D could also modulate insulin resistance in women with PCOS apart from its role in calcium homeostasis.Insulin secretion is a calcium dependent process[5] and positive correlationof seruminsulin and insulin resistancewith serum calcium has been demonstrated[6]. Furthermore, vitamin D receptor (VDR) and calcium sensing receptor (CaSR) genes appear to be involved in maintaining calcium homeostasis[7-9].Mechanisms behind the underlying insulin resistance are the matter of intense research since the last few decades.

Thus, the objective of our study was to study the different biochemical and anthropometric markers of insulin resistance and to correlate with vitamin D status in PCOS patients and to explore whether vitamin D could be considered as predictor of insulin resistance in PCOS.

Materials and methods-

The study was conducted jointly by the department of Biochemistry in association with the department of Obstetrics and Gynaecology, Lady Hardinge Medical College, New Delhi, India after Institutional ethical clearance.

Sixty patients of PCOS diagnosed by Rotterdam criteria were enrolled in this study after informed written consent[10]. Patients with hyperprolactinemia, Cushing's syndrome, congenital adrenal hyperplasia, and androgensecretingtumours were excluded from the study.

Body mass index (BMI) of patients wascalculated from Quetelet's formula (weight in kg/height in m^2). Waist circumference was measured in a standing position midway between the lower costal margin and the iliac crest. Hip circumference was measured in a standing position at the maximum circumference over the buttocks. Under aseptic conditions, 8 mL of fasting blood sample was drawn into plain and EDTA-NaF containing vacutainers from the antecubital vein. The vacutainer containing the blood samples were kept at room temperature for 30 min and were then centrifuged for the clear separation of serum or plasma.

All routine biochemical assays including fasting plasma glucose(FPG), liver function tests (total bilirubin, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase), kidneyfunctiontests (urea,creatinine, uricacid), electrolytes (sodium, potassium,calcium,phosphate) and lipid profile(total cholesterol, triglyceride, high density lipoprotein(HDL) were performed on same day on Beckmann Coulter Synchron CX-9 fully automated analyser using standard reagents and kits.

Samples for serum insulin,25- OH vitamin D, SHBG, leptin were kept at-80°C till batch analysis.

Insulin resistance was estimated using the homeostatic model assessment-insulin resistance (HOMA-IR). HOMA-IR was calculated as the product of the fasting plasma insulin value (mU/ml) and the fasting plasma glucose value (mg/dl), divided by 405 .

Estimation of 25- OH vitamin D was done by ELISA using 25 OH vitamin D direct ELISA kit from Immundiagnostik(Bensheim). Serum leptin level was measured by ELISA by using leptin DRG leptin sandwich ELISA kit.Sex hormone binding globulin was measured by ELISA using DRG SHBG. Insulin levels were estimated by Chemiluminescence immunoassay (CLIA) in Access 2 Beckmann Coulter.

The interassay and intraassay precision for 25 –OH-vitamin D was 7 % (CV) and 7 (CV) % respectively. The specificity of the antibody for 25-OH Vitamin D was 100 %.

The interassay and intraassay precision for leptin was 5.95 % (CV) and 6.91%(CV) respectively. The specificity of the antibody for leptin was 100 %.

The interassay and intraassay precision for sex hormone binding globulin was 11.6%(CV) and8.6%(CV) respectively. The specificity of the antibody for SHBG was 100 %.

Statistical analysis

The data was analyzed by using Statistical software SPSS version 13. The values are expressed as mean±S.D with their 95 % confidence intervals. Pearson correlation, partial correlation and multiple linear regression analysis was performed to develop a regression model and to explore the predictive power of vitamin D in PCOS

RESULTS

Table I. Clinical features and demographic profile of study group (n=60)

Clinical features	Percentage (%)
Age≤19 yrs	10
Age≥19 yrs	90
Age of menarche at 11-14yrs	98
Overweight (BMI- 25-30)	23
Obesity (BMI ≥30)	30
Menstrual irregularities	96
Hyperandrogenic features	13
Infertility	50
Polycystic ovaries on ultrasound	100

Table 2. Frequency of PCOS cases (n=60) according to Vitamin D status

Vitamin D status	Vitamin D deficiency (25-OH vit D < 30 nmol/L)	Vitamin D insufficiency (25-OH vit D 30-75 nmol/L)	Vitamin D sufficiency (25-OH vit D > 75 nmol/L)
Number (%) of PCOS cases	17 (28.34 %)	26 (43.33 %)	17 (28.34 %)

Table 3.Special parameters in study group (n=60)

Parameter	Mean ±S.D
Insulin (µIU/ml)	20.44±13.84
Fasting glucose(mg/dl)	95.62±13.79
Glucose/insulin ratio	6.2 ±2.9
Leptin (ng/ml)	53.37 ±39.94
SHBG (nmol/L)	36.32±7.09
25-OH vit D (nmol/L)	52.27±25.87

Table 4. Derived parameters in study group (n=60)

Parameter	Mean ±S.D
BMI(kg/m^2)	27.1±5.8
WHR	0.805±0.07
HOMA-IR	5.42±5.59

Table 5. Comparison of 25-OH Vit D between 2 groups according to HOMA-IR values

Parameter	HOMA-IR≤3.9 (n=29) Mean±SD	HOMA-IR>3.9 (n=31) Mean±SD	*p-value* (Independent sample t test)
25-OH Vit D(nmol/L)	69.72±18.31	35.94±20.810	0.0001*

*p value <0.05 is considered statistically significant

Table 6.Correlation of 25-OH –vit- D and insulin resistance in PCOS (n=60).

Parameter	Pearson coefficient-r	p-value
Fasting plasma glucose	-0.218	0.095
Insulin	-0.619	0.000*
Glucose-insulin ratio	.440	0.000*
Leptin	-0.151	0.249
SHBG	-0.023	0.863
HOMA-IR	-0.538	0.000*
BMI	-0.130	0.321
WHR	0.135	0.303

*Correlation is significant at 0.05 level

Table 7. Multiple Linear Regression Analysis Of HOMA -IR With Insulin Resistance Markers In PCOS(n=60)

Parameter	Coefficient of determination R^2	p-value
Fasting plasma glucose	0.084	0.025*
Glucose-insulin ratio	0.046	0.101
Leptin	0.022	0.258
SHBG	0.020	0.282
25-OH vit D	**0.289**	**0.0001***
BMI	0.092	0.018*
WHR	0.014	0.372

*Correlation is significant at 0.05 level
HOMA-IR was taken as dependent variable.

Discussion

PCOS has emerged as a major endocrinological disorder in females in the reproductive age group with significant short term and long term sequelae such as metabolic syndrome infertility, cardiovascular disorders. Insulin resistance is one of the cardinal features of this syndrome. The pathogenesis underlying the insulin resistance in women with PCOS are not clearly understood. Hyperandrogenemia, leptin resistance, inflammation have been implicated. Although promising, these pathways have not totally solved the mystery behind PCOS. Hypovitaminosis D has garnered considerable interest in the recent past as a possible etiology behind insulin resistance due its pleiotropic effects on various cellular mechanisms.

According to the serum 25-OH-D levels,vitamin D status can be classified as vitamin D sufficiency(>75nmol/L),insufficiency(30-75nmol/L) and deficiency(<30nmol/L)[11]. In our study, 28.34% of PCOS women showed 25(OH) vitamin D values below this (<30 nmol/L) recommended level (Table 2).

Studies on relationship between insulin resistance and poor vitamin D status in patients with PCOShave shown conflicting results [12,13]. PCOS patients (n=60) were divided into two groups according to the HOMA-IR value (cut off 3.9)[14].25-OH vit D levels were significantly different in the two groups (HOMA-IR<3.9 and HOMA>3.9) (table 5) We found a significant negative correlation of 25(OH)D levels withfasting insulin, HOMA-IR and glucose –insulin ratio(table 6). Similar to our results Hahn et al.reported an association of low 25(OH)D levels withinsulin resistance in 120 PCOS women[15]. Apart fromthese cross-sectional findings, there is one prospective intervention study with vitamin D supplementation,which demonstrated beneficial effects ofvitamin D on insulin secretion in PCOSwomen[16]. In nonPCOS cohorts including subjectswith various BMI, vitamin D concentration wasinversely related to the prevalence of diabetes [17], plasma concentrations of glucose , insulin resistance[19]. Insulin resistance may have adverse effects in women with PCOS through its action at different sites. These include the liver through suppression of hepatic synthesis of sex hormone binding globulin[20]. Thus sex hormone binding has been proposed as a insulin resistance marker by different studies in women with PCOS.In our study we have found that sex hormone binding globulin was inversely associated with vitamin D levels in women with PCOS (table 6).Our findings are in concordance with a similar study carried out by E Wehr, S Pilz et al[21].

Our study also indicates inverse association of low 25(OH)D levels with leptin and BMI in PCOS women(table 6).These results are similar to studies conducted by E Wehr, S Pilz et al[21]. So far, it is notclear whether vitamin D insufficiency results fromobesity and/or whether obesity is a consequence ofvitamin D insufficiency. On the one hand, obesity maycontribute to low circulating vitamin D levels bytrapping vitamin D in fat tissues. There is evidence that low vitamin D levels are associated with obesity [15]and vice versa .Thus low vitamin D intake might be an independent predictor of obesity [22].Vitamin D supplementation may improve insulin resistance and

prevent other health complications, such as diabetes mellitus type 2.This has to be addressed in large intervention trials .We confirm previous findings reporting the relationship of low 25(OH)D levels with various markers of obesity in women with PCOS.

To study insulin resistance in the study groups we used linear regression analysis taking HOMA-IR as dependent variable to elucidate the most important independent predictors of insulin resistance. We found that vitamin D was the important determinant of HOMA-IR in contributing 28.9 % (r^2=0.289 *p* value 0.0001)(Table 7).These evidence suggests that vitamin D deficiency might be involved in the pathogenesis of insulin resistance in PCOS.

A number of possible mechanisms have been proposed to explain the association between vitamin D deficiency and insulin resistance. Firstly, vitamin D may exert a positive influence on insulin action by stimulating the expression of insulin receptor and thereby promoting insulin responsiveness for glucose transport .The vitamin D response element is present in the promoter of the human insulin gene and the transcription of the human insulin gene is activated by 1,25(OH)D [23]. Calcium ion fluxes have an important role in regulating secretory mechanisms in the body including insulin secretion. This implies a pivotal role of vitamin D the hormone involved in calcium metabolism in the regulation of β-cell function[24] . Finally, due to its immunomodulatoryfunctions, hypovitaminosisD might induce a higher inflammatory response, which is again associated with insulin resistance[25].

Impairment of β cell function has been seen in women with PCOS. This might be the underlined mechanism of our finding of a negative association of 25(OH)D levels and HOMA-IR, fasting insulin levels.

These theories prove beyond doubt the role of vitamin D in the etiopathogenesis of the various hallmarks of PCOS- insulin resistance, altered ovarian steroidogenesis, obesity among others. This calls for large scale intervention studies to evaluate the role of vitamin D supplementation for prevention/ management of PCOS.

Our study had a number of limitations. We evaluated only PCOS patients without a control group which might have helped in better analysis and ratification of our findings. Assessment of response to Vitamin D supplementation in the group demonstrating deficiency would have proven helpful for the substantiating our results. Financial and managerial constraints proved as limiting factors. Nonetheless, our study is a step forward in throwing light on the role of vitamin D deficiency in PCOS especially in the Indian context.

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