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Original Research Article

Study of serum calcium and vitamin D levels with hormonal profile along with biochemical profile in women with polycystic ovary syndrome

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ABSTRACT

Background: The polycystic ovary syndrome (PCOS) is one of the commonest human endocrinopathies and is increasingly recognized as a variant of the metabolic syndrome in women with the characteristic features of insulin resistance, central obesity, impaired glucose metabolism, dyslipidemia, and hypertension.

Methods: This study is mainly focused on study of parameters like gonadotropin hormonal profile, serum vitamin D and calcium levels in polycystic ovary disease (PCOD). The study comprised 45 clinically proven polycystic ovary disease patients in the age range of 19-34 years. The biochemical estimations carried out in the study were – Fasting Blood sugar, LH, FSH, prolactin, 25- OH vitamin D and calcium along with anthropometric data. The values obtained were compared with age matched equal number of healthy control female subjects from the same population.

Results: The serum concentration of calcium and vitamin D levels are decreased significantly ($P < 0.001$) when compared to controls. Insulin resistance is predominantly seen in PCOS subjects. The study outlines the importance of insulin resistance, dyslipidemia, decreased serum calcium and vitamin D levels in PCOS subjects may be a cause for the progression of polycystic ovary syndrome.

Conclusions: In the present study vitamin D deficiency is highly prevalent in PCOS women from this area compared to control women. We also relations of vitamin D status with insulin sensitivity, HDL-C, and C-reactive protein in PCOS patients, which support the increasing evidence that vitamin D deficiency is associated with multiple metabolic risk factors in PCOS women. A high prevalence of vitamin D deficiency and low calcium levels were observed in PCOS women from our population when compared to controls. Insulin resistance was predominantly seen in PCOS subjects when compared with controls, indicating the association of vitamin D levels with insulin resistance.

Keywords: 25-OH vitamin D, Gonadotropin hormones, Hypovitaminosis, Polycystic ovary syndrome, Serum calcium

INTRODUCTION

It is showed that, insulin resistance is responsible in developing polycystic ovaries in PCOS women though obesity is said to be the major cause.¹ Hence pathogenic determinants of PCOS include insulin resistance and obesity. Therefore, women with PCOS had an increased

risk for type 2 diabetes. The sequel of PCOS reach beyond reproductive health, as women affected with PCOS at long-term are at increased risk of adverse lipid profiles, type II diabetes and hypertension, as well as cardiovascular or cerebrovascular morbidity.¹ Vitamin D deficiency might be a causal factor in the pathogenesis of insulin resistance and the metabolic syndrome in

PCOS.^{2,3} In obese people vitamin D is deposited in adipose tissues, making it unavailable for the body to use. As a result, obese people are expected to have low levels of serum vitamin D. Vitamin D deficiency is associated with multiple health conditions such as diabetes, cardiovascular diseases including stroke, depression, dementia and other conditions.⁴

It is shown that the relationship between vitamin D and insulin resistance is compensated by various mechanisms; for example, low serum vitamin D levels by calcium mobilization due to elevated parathyroid hormone (PTH) secretion.^{5,6} The cellular effect of vitamin D is mediated through the intra-nuclear vitamin D receptor (VDR). VDR is expressed in variety of tissues other than the skeleton, including intestines, parathyroid glands, immune cells, pancreas, and more recently the hypothalamic pituitary axis and reproductive tract. The presence of VDR in the ovary, uterus, placenta and testis suggest a regulatory role of vitamin D in reproductive physiology vitamin D deficiency is hypothesized to contribute to a spectrum of gynecological disorders of which PCOS appears the best studied.⁵⁻⁷

A small number of observational studies identify the inverse association of serum 25-OH vitamin D and with insulin resistance, features of hyperandrogenism in women and circulating androgens in women with PCOS. Serum levels of vitamin D are reported to predict ovarian response in women undergoing ovulation induction with clomiphene citrate.^{5,6,8} This was independent of the BMI. Low levels of vitamin D were found to be associated with lower rate of follicular development and pregnancy after ovarian stimulation with 50 mg clomid.^{5,9}

Several data converge towards beneficial effect of vitamin D in metabolic disturbances in women with PCOS. Recent studies supporting the contribution of vitamin D deficiency to metabolic disturbances in women with PCOS, including insulin resistance (IR), obesity, hypertension and menstrual dysfunction have been done in western, European and Middle East countries.⁴⁻⁹ It is shown that the relationship between vitamin D and insulin resistance is compensated by various mechanisms; for example, low serum vitamin D levels by calcium mobilization due to elevated parathyroid hormone (PTH) secretion.⁶

Recent studies supporting the contribution of vitamin D deficiency to metabolic disturbances in women with PCOS, including insulin resistance (IR), obesity, hypertension and menstrual dysfunction have been done in western, European and Middle East countries.^{3,6-13} Only few studies have been done so far in India to confirm the relationship of vitamin D with insulin resistance. Therefore, our aim of this study was to evaluate the association of vitamin D deficiency along with glucose, insulin and insulin resistance in Indian women with polycystic ovary syndrome.

METHODS

The study was conducted at Karpaga Vinayaga Institute of Medical Sciences and Research Centre, Madhurnatagam, Tamil Nadu, India. The study comprises of 45 women with PCOS and equal number of age matched normal females without any present or previous history of PCOS were selected to serve as controls. PCOS patients with age range 19 to 34 years were selected for the study. All women with PCOS were come as out patients to Gynecology and Obstetrics department of Karpaga Vinayaga Institute of Medical Sciences and Research Centre, Madhurnatagam, Tamil Nadu.

Women of the control group were healthy volunteers. All the subjects were enrolled in the study after being explained the purpose of the study by concerned physician and informed consent was obtained from them. The research study was approved by institutional ethical committee. PCOS diagnosis was done on the basis of the Rotterdam criteria.¹³ According to this criterion, PCOS was defined by the presence of menstrual dysfunction i.e. oligomenorrhea (fewer than six menstrual periods in the preceding year) or amenorrhea (absence of periods for more than 6 months), and clinical hyperandrogenism (i.e. hirsutism: Ferriman-Gallwey score >6) and/or hyperandrogenemia. Furthermore, patients with any other cause of oligomenorrhea or hyperandrogenism, such as nonclassic congenital adrenal hyperplasia, androgen secreting tumours, Cushing's syndrome, or hyperprolactinaemia were excluded. Patients with congenital adrenal hyperplasia, Cushing's syndrome, hyperprolactinemia, androgen-secreting tumors and intake of any medication affecting endocrinal parameters were excluded from the study. PCOS women who were pregnant or on any contraceptive pills or oral hypoglycemic agents were also excluded from the study.

About 5 ml of blood is collected from the antecubital vein. Fasting blood samples were collected in plain, sodium fluoride and heparin tubes. Serum is separated by centrifugation. Blood samples are centrifuged at 3500 rpm for 10 min to separate serum. Serum glucose, serum total cholesterol, triglycerides, HDL, and calcium levels were analyzed using Biosystems reagent kits available for semiautomated biochemistry analyzer.

Hormones Insulin, LH, FSH, Prolactin, were estimated by enzyme linked immuno fluorescent assay (ELFA) method using minividas (Germany) fully automated hormone analyzer. For adequate quality control both normal, abnormal reference control serum solutions and calibrators were run before analyzing each test sample. Other factors affecting the results like proper functioning of instrument, glassware, cuvettes and distilled water are thoroughly checked before using.

SPSS 20.0 version is used for Statistical analysis. All the values were shown as mean and standard deviation.

RESULTS

Comparison of biochemical and hormonal parameters of women with PCOS and controls was given in Table 1. All the subjects included in the study were assessed for fasting blood sugar, fasting insulin, calcium, phosphorous and Vitamin D.

Comparisons were made between the two groups (controls and PCOS women). The biochemical findings made during the course of the study are represented in

Table 1. Mean age of the control women was 26.2±6.3 and that of PCOS patients was 26.8±6.3 (p = 0.65).

There was no significant statistical difference with respect age of the PCOS women and control women. In the present study fasting glucose showed a significant increase (p <0.0001) in PCOS women compared to controls. Calcium and 25 – OH vitamin D levels were decreased in PCOS women (p <0.01; and p <0.03 respectively) when compared to control women (Table 1).

Table 1: Comparison of biochemical and hormonal parameters in PCOS women and healthy controls.

Parameter	PCOS group (n = 45)	Control group (n = 45)	P value
Age	26.8±6.4	26.2±6.3	0.65
FBS	117±14.5	89.5±8.5	0.0001**
F. Insulin	9.4±4.5	6.9±2.3	0.001*
HOMA IR	3.8±2.0	1.9±0.8	0.0001**
T cholesterol	227±15.8	175±19.6	0.0001**
Triglycerides	188±14.9	135±28.6	0.0001**
HDL	44±12.3	57±8.9	0.0001**
Calcium	8.2±1.5	8.9±1.2	0.01*
25 – OH vitamin D	20.15±7.8	24.4±10.4	0.03*
LH	8.1±2.9	6.7±1.3	0.004**
FSH	4.5±1.8	6.2±1.9	0.0001**
LH/FSH	0.94±0.17	2.04±0.73	0.0001**
Prolactin	34.5±10.2	15.7±5.8	0.0001**

FBS – Fasting Blood Sugar; F. Insulin – Fasting insulin; HDL –High Density Lipoprotein; LH – Leutinizing Hormone; FSH – Follicle Stimulating Hormone. *, **p value is less than 0.05 which is statistically significant.

Table 2: Correlation study for biochemical and hormonal parameters among PCOS women.

	F. Glucose	F. Insulin	HOMA IR	LH	FSH	LH/FSH	Prolactin
F. Glucose	1	0.703**	0.811**	0.256**	-0.533**	0.615**	0.699**
F. Insulin		1	0.981**	0.021	-0.410**	0.409**	0.542**
HOMA IR			1	0.049	-0.469**	0.466**	0.603**
LH				1	0.244**	0.556**	0.089
FSH					1	-0.571**	-0.481**
LH/FSH						1	0.469**
Prolactin							1

It is evident from Table 1 that total cholesterol, triglycerides, HDL showed a highly significant increase (p <0.001) and decrease in HDL (p < 0.05) in cases compared to controls indicating that PCOS women had dyslipidemia. Fasting glucose, fasting insulin and insulin resistance showed a significant increase (p <0.001) in cases compared to controls. Gonadotropin hormones LH and FSH showed mild increase (p <0.0001) in PCOS women when compared to healthy controls.

Correlation study (Table 2) revealed significant positive correlation of fasting glucose and fasting insulin with insulin resistance (HOMA IR) (r = 0.811 and r = 0.981 p <0.01) and significant negative correlation with FSH (r = -

0.533 and r = -0.41, p <0.01). Fasting glucose showed positive correlation with LH/FSH ratio and prolactin as well (r = 0.615; r = 0.699; respectively and p < 0.01). LH showed positive correlation with LH/FSH ratio (r = 0.556; p<0.01). FSH showed negative correlation with LH/FSH ratio (r = -0.571; p <0.01). LH/FSH ratio showed positive correlation with prolactin (r = 0.469; p <0.01).

DISCUSSION

Polycystic ovary syndrome is a common endocrinopathy characterized by chronic anovulation, hyperandrogenism and multiple small subcapsular cystic follicles in the

ovary on ultrasonography, which affects 4 to 16% of women in reproductive age.¹⁵⁻¹⁷ It is frequently associated with insulin resistance and compensatory hyperinsulinemia. Most common abnormalities seen in PCOS are increased BMI, low HDL levels and elevated triglycerides.¹⁶ In the present study abnormal lipid profile was found in women with Polycystic Ovary Syndrome. The findings of elevated TC, TG and LDL-C were in agreement with those of Mattson et al, Wild et al and Talbott et al, Saha S et al.¹⁷⁻²⁰ PCOS may represent a major segment of the female population at a risk of cardiovascular disease which may be related to increased VLDL levels.²¹ As shown by other research workers, this increase in VLDL is basically due to insulin resistance.²¹ Insulin normally inhibits the expression of microsomal triglyceride that is responsible for apo-B and VLDL secretion. Hence, insulin resistance may be responsible for increased VLDL in PCOS individuals.²² In the present study we observe 70% of PCOS patients exhibiting an abnormal lipid profile and the mean values of cholesterol, TGL, HDL, LDL and, VLDL are increased (Table 1).

The mechanism by which Vitamin D affects insulin secretion is not known, but one of the propositions is activation of human insulin gene transcription.⁶⁻⁹ It is not surprising to see Vitamin D to affect insulin metabolism as it regulates calcium metabolism and calcium plays an essential role in insulin secretion.¹⁴⁻¹⁷ Vitamin D has many diverse actions, its role as an immunomodulatory molecule may provoke an inflammatory response in the presence of Vitamin D deficiency and inflammation is known to predispose to IR.^{19,21}

In this study, we demonstrated that women with PCOS had low serum vitamin D levels when compared to age matched controls. Previous studies also demonstrated lower levels of Vitamin D or no difference in women with PCOS when compared to age matched controls.^{17,22,8,20} In accordance with previous reports, we detected Vitamin D levels that correlated negatively with insulin levels in women with PCOS.^{14,15}

The present study includes 45 PCOS patients and 45 controls. Cut off value of HOMA IR is taken as >2.5 .^{7,13,22-26} In the present study serum insulin and HOMA IR in PCOS patients is increased when compared with controls and is highly significant ($P < 0.001$) and this is in accordance with the previous studies.^{15-19,22,27} In the present study the mean values of cholesterol, TGL, LDL and, VLDL are increased, whereas mean value of HDL was decreased.

Insulin resistance, hyper insulinemia are the factors to play an important role in the pathogenesis of PCOS. In the present study, our results showed that there is predominant insulin resistance, hypothyroidism, dyslipidemia, and increased LH/FSH ratio in women with PCOS patients when compared with control women.²³⁻²⁶ The direct effect that testosterone plays on adipocytes was investigated and induction of androgen receptor

mediated insulin resistance via testosterone was established.²⁷ The hyperandrogenism is due to increased LH and low to normal FSH levels. Due to the increase in LH and hence estrogen, FSH is negatively inhibited. Theca cell hyperplasia ensues leading to hyperandrogenemia that clinically presents as hirsutism. In our study, hirsutism is taken as one of the clinical features to diagnose hyperandrogenism. Currently, BMI has been negatively associated with the baseline levels of LH, LH pulse amplitude, and the pituitary response to GnRH.^{27,28} The negative correlation between BMI, baseline levels of LH in PCOS patients, observed in the present study, supports studies published before.^{27,28}

In our study, we observed mild increase the prolactin level (Table 1) in 70% cases, which is similar to the previous studies, Kalsum and Jalali, where 69.51% of subfertile women suffered from hyperprolactinaemia.²⁹ Nizam et al. also showed that hyperprolactinaemia is a major cause of subfertility, and treatment with drugs which lowers prolactin level showed that 24% infertile women became pregnant.³⁰ This finding is consistent with our study.

CONCLUSION

This study showed that there is predominant insulin resistance, dyslipidaemia, and increased incidence of hypothyroidism in case of PCOS women, which appears to place them at a higher risk of developing diabetes as well as cardiovascular diseases. Normal gonadotropin-ovarian axis is disturbed in PCOS women. This is reflected by the higher levels of LH, lower FSH levels and elevated level of LH: FSH ratio. The ratio of LH/FSH greater than 2.0 can be the significant in the diagnosis of PCOS women in our population. We have also observed that the mean LH levels elevated significantly, mild hyperprolactinemia and decrease in FSH, suggesting the importance of regulation of these hormones in the management and treatment of PCOS.

This study concludes that women with PCOS show high prevalence of insulin resistance and low 25-OH vitamin D levels which in turn may be responsible for the metabolic and endocrine disturbances. Hence, we suggest vitamin D supplementation might improve menstrual frequency and metabolic disturbances in women with polycystic syndrome. The present study also indicates that insulin resistance was an independent risk factor for the presence of vitamin D deficiency in women with PCOS. Further, we recommended long term follow up studies to identify the role vitamin D supplementation in patients with PCOS to confirm the beneficial role of vitamin D.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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