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CORRELATION OF VITAMIN D WITH HOMEOSTASIS MODEL ASSESSMENT-ESTIMATED INSULIN RESISTANCE (HOMA-IR) IN POLYCYSTIC OVARY SYNDROME PATIENTS ATTENDING A TERTIARY CARE HOSPITAL

Physiology

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ABSTRACT

Introduction: Polycystic ovary syndrome (PCOS) is a common endocrine disorder in women of reproductive age. Association of vitamin D {25(OH)D3} status with metabolic and hormonal dysfunction in PCOS has been investigated by some studies but the relationship between them still remains inconclusive. Therefore, this cross-sectional study aims to analyze the relationship between vitamin D level and HOMA IR (an indicator of insulin resistance) in women with PCOS. **Aim And Objective: Aim:** To investigate the association between vitamin D levels and PCOS in our population. **Objective:** To check whether serum vitamin D level has any impact on fasting blood sugar, fasting insulin and HOMA IR in a PCOS patient attending a tertiary care hospital in Kolkata. **Materials And Methods:** Our study was a Descriptive cross sectional study. The study included 146 PCOS patients in the age group 15-45 years attending the Gynaecology OPD of a tertiary care hospital (Calcutta National Medical College and Hospital) in Kolkata. 25(OH)D3 levels were measured by ELISA. Other biochemical parameters taken were fasting blood sugar (FBS), fasting serum insulin and HOMA-IR. The association between Vitamin D level and the above mentioned parameters was studied. **Result:** In our study vitamin D levels was negatively correlated with fasting blood sugar (FBS) levels ($r=-0.879$, $p<0.001$), fasting insulin levels ($r=-0.865$, $p<0.001$) and and HOMA IR ($r=-0.897$, $p<0.001$). **Conclusion:** Our study showed that the serum 25(OH)D3 concentration was correlated with certain biochemical and endocrinal parameters like FBS, fasting insulin and HOMA IR, which are also considered as metabolic risk factors. So our study hints that vitamin D may have a decisive role in PCOS and has a potential to emerge as an associated biochemical parameter of PCOS. Multi-centre randomized controlled trials with large sample sizes are needed to explore the metabolic role of vitamin D supplementation in women with PCOS.

KEYWORDS

HOMA IR, PCOS, Vitamin D

INTRODUCTION

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women of reproductive age, characterized by:

i) ovulatory dysfunction resulting in oligo- and/or anovulation,
ii) hyperandrogenism and/or hirsutism,
iii) presence of polycystic ovarian morphology.

PCOS is also associated with chronic anovulation, irregular menses, infertility, acanthosis nigricans, insulin resistance (IR), hyper insulinemia, dyslipidemia, and central obesity, which are all risk factors for the metabolic syndrome, type 2 diabetes mellitus, and cardiovascular disease.

According to WHO, globally, prevalence estimates of PCOS are highly variable, ranging from 2.2% to as high as 26% (1). Prevalence of PCOS in India ranges from 3.7 to 22.5 per cent.

PCOS, discovered by Stein and Leventhal (2), earned its name based on the ovarian morphology. The underlying cause of PCOS is unknown. However a genetic basis that is both multifactorial and polygenic is suspected as there is a well documented aggregation of the syndrome within families. Some have suggested autosomal dominant inheritance with expression in both males and women (3). Based on the ESHRE/ ASRM Rotterdam 2003 (4) criteria PCOS can be defined as:
Two of the following three needs to be present :

- Clinical and / or biochemical hyperandrogenism
- Oligo-/ anovulation
- Polycystic ovaries

Oligo or anovulation is defined as menstrual cycles less than 21 or more than 35 days while polycystic ovarian morphology is considered diagnostically significant in the presence of ≥12 follicles with a diameter of 2–9mm or an ovarian volume of >10ml (5).

In recent years, the difference of vitamin D levels between PCOS women and healthy women, as well as the relationship between vitamin D and metabolic factors in PCOS women has remained controversial. Some studies have shown that PCOS women had lower serum 25(OH)D3 concentration than healthy women, and vitamin D deficiency was associated with increased HOMEOSTASIS MODEL ASSESSMENT-ESTIMATED INSULIN RESISTANCE (HOMA-IR), hyper-insulinemia, dyslipidemia, metabolic risk factors in patients with PCOS (6,7). It was also found to be associated with serum LH, LH:FSH ratio and testosterone (8,9).However, other scholars have found different results (10).

So far, due to the heterogeneity of the studies and their conclusion, it is not clear whether vitamin D status is associated with endocrinal/metabolic disturbances in PCOS women. Also, very less number of studies have been done on the same in Kolkata, India. Therefore, this cross-sectional study aims to analyze the relationship between vitamin D level and HOMA IR (an indicator of insulin reistance) in women with PCOS.

AIM AND OBJECTIVE:

To check whether serum vitamin D level has any impact on fasting blood sugar (FBS), fasting insulin and HOMA IR in a PCOS patient attending a tertiary care hospital in Kolkata.

MATERIALS AND METHODS

Study Setting:
Department of Gynaecology and Biochemistry of Calcutta National Medical College and Hospital (CNMC&H), Kolkata.

Study Period: May,2021 – May,2022

Study Design: Descriptive type of cross sectional study.

Sample Size: 146

Inclusion Criteria:
i) 15- 45 years female, ii) Newly diagnosed cases of PCOS, and iii) Has not undergone any treatment or intervention.

Exclusion Criteria:
i) Not willing to participate in the study. ii) Not falling in the above specified age group (15-45 years). iii) Any other systemic disease. iv) On any medications that affect their reproductive physiology (eg. OCP). v) On any medication that might affect the serum vitamin D concentration in any possible way. vi) Has other causes of hirsutism apart from PCOS like adrenal tumours, etc. vii) Has been started on some form of treatment for PCOS. viii) Chronically and terminally ill patients.

Study Variables:
- Vitamin D (independent variable)
- FBS, fasting insulin and HOMA-IR (dependent variable)

Study Tools:
1. Patient information sheet
2. Consent form
3. Pretested and predesigned case record sheet
4. Syringe and vial
5. Auto analyser for glucose and insulin level detection
6. Vitamin D kit (Diagnostic Automation, Inc. (DAI) 25-OH Vitamin D total ELISA Kit)

Sample Design And Recruitment Technique:
Purposive sampling was done over a period of one year. 146 consecutive PCOS patients (attending the Department of Gynaecology, CNMC&H) of the age group 15-45 years, who were diagnosed with PCOS as per the Rotterdam criteria and meeting other inclusion criterias was taken as subjects.

Collection Of Data:
Female patients in the age group of 15-45 years attending the above mentioned OPDs at CNMC&H were considered. Amongst them, those who had a complaint of amenorrhea and/or hirshutism was prescribed an abdominal USG. On having positive USG findings for PCOS and fulfilling the Rotterdam's criteria they were given an informed consent form. Those who gave consent were enrolled as cases. Further necessary information about the patients and their clinical condition was collected by a predesigned and pretested questionnaire. At the Biochemistry department of CNMC&H blood samples were collected on the 3rd day of their menstrual cycle for evaluating certain biochemical and endocrinal parameters. Therefore necessary data on cases for the study was collected by means of the predesigned and pretested questionnaire and the biochemical test reports.

Investigations:
Following biochemical investigations were done on serum :
- Vitamin D (25-hydroxy cholecalciferol / 25(OH)D3)
- FBS
- Fasting serum insulin

Insulin resistance (HOMA-IR) was calculated by using a specific formula.

Procedure Of Investigations:
Blood samples were collected in the morning between 8:00 am and 9.00 am after an overnight fasting on the 3rd day of menstrual cycle. 5ml of venous blood was collected from the antecubital vein in an empty vial (clotted blood/serum required for fasting insulin level) and in a vial with sodium fluoride (as anticoagulant for for estimation for fasting sugar). The samples were centrifuged for 15 min at 2500 rpm, and aliquots of serum were kept at − 20°C until assays for analysis.

For vitamin D, the Diagnostic Automation, Inc. (DAI) 25-OH Vitamin D total ELISA Kit was used which is a solid phase enzyme-linked immunosorbent assay (ELISA), based on the principle of competitive binding.

For determination of fasting glucose automated analyser (Sclavo Konelab-600i Prime Sclavo Konelab (Sclavo diagnostic International SRL, Italy) and Erba glucose reagent kit was used. Trinder's method was used for glucose estimation where glucose was oxidised to glucuronic acid and hydrogen peroxide by the enzyme glucose oxidase. The formed hydrogen peroxide (H202) was detected by a chromogenic oxygen acceptor, phenol, 4- aminophenazone(4-AP) in the presence of peroxidase (POD). The intensity of the colour formed was proportional to the glucose concentration in the sample.

For in vitro assessment of fasting insulin level ADVIA Centaur and ADVIA Centaur XP systems were used. The ADVIA Centaur insulin assay is a two site sandwich immune assay using direct chemiluminescent technology which uses constant amount of two antibodies. The first one is a lite reagent, a monoclonal mouse anti insulin antibody labelled with acridinium ester. The second antibody in the solid phase is a monoclonal mouse insulin antibody which is covalently coupled to paramagnetic particles.

HOMA-IR was then calculated to evaluate insulin resistance (IR).

HOMAIR = [fasting glucose(mg/dl) × fasting insulin(mIU/L)] / 405

Data Analysis:
The statistical software SPSS version 22 has been used for the analysis. Data was collected and entered in SPSS 22. Raw data was shown in master chart. Continuous variables are expressed as Mean, Median and Standard Deviation. Association between continuous variables are captured using Spearman's Rank Correlation Coefficient. An alpha level of 5% has been taken, i.e. if any p value is less than 0.05 it has been considered as significant.

Ethical Consideration:
Approved by the Medical Ethics Committee of Calcutta National Medical College and Hospital, Kolkata.

RESULTS
In our study, a **significant negative correlation** of serum vitamin D levels with FBS (r=-0.879, p<0.001) [Figure 1], fasting insulin levels (r= -0.865, p<0.001) [Figure 2] and HOMA IR (r=-0.897, p<0.001) [Figure 3] was observed.

Table 1: Anthropometric and biochemical parameters of the study subjects.

PARAMETERS	Age (years)	Weight (kg)	FBS (mg/dl)	Fasting insulin (mIU/L)	HOMA IR
Mean	20.36	58.48	97.26	18.16	4.38
Standard error	0.34	0.9	0.65	1.06	0.27
Standard deviation	2.99	7.97	5.70	9.35	2.35
Sample variance	8.97	63.54	32.51	87.5	5.51
Minimum	15	43	79	0.5	0.09
Maximum	26	73	108	39.66	10.09

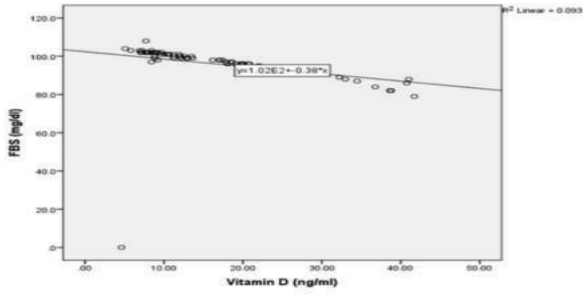

Figure 1: Scatter plot diagram of correlation between vitamin D and FBS

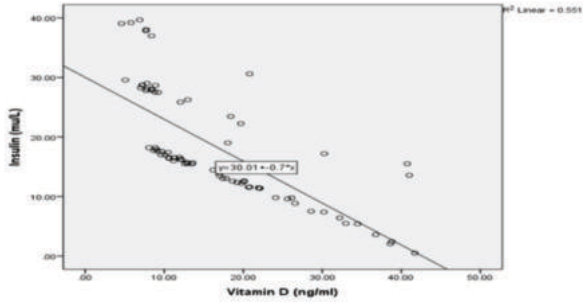

Figure 2: Scatter plot diagram of correlation between vitamin D and fasting insulin

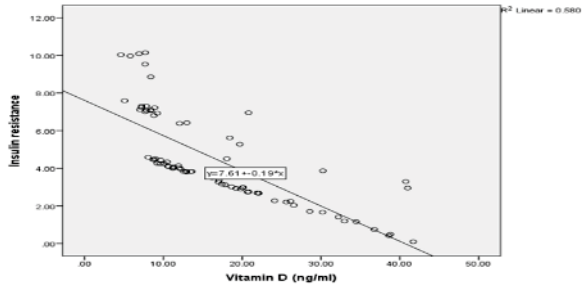

Figure 3: Scatter plot diagram of correlation between vitamin D and insulin resistance (HOMA IR)

DISCUSSION

Polycystic ovary syndrome (PCOS) is a common cause of ovarian dysfunction in women with anovulation. The main symptoms are characterized by chronic anovulation, hyperandrogenism, and/or the presence of polycystic ovary morphology from ultrasound examination. The phenotypic manifestation of this disorder is associated with various degrees of gonadotropic and metabolic abnormalities determined by the interaction of multiple genetic and environmental factors.

The potential influences of vitamin D on glucose homeostasis include the presence of specific vitamin D receptor (VDR) in pancreatic β-cells and skeletal muscle, the expression of 1-α-hydroxylase enzyme which can catalyze the conversion of 25-hydroxyvitamin D [25(OH)D3] to 1,25-dihydroxyvitamin D, and the presence of a vitamin D response element in the human insulin gene. Vitamin D deficiency can decrease the expression of insulin receptors, and cause lack of inhibition of the release of inflammatory cytokines (11). Vitamin D regulates extracellular and intracellular calcium, which is essential for insulin-mediated intracellular processes in insulin-responsive tissues such as skeletal muscle and adipose tissue. Hence vitamin D deficiency leads to IR.

Low 25(OH)D levels are found to be significantly correlated with insulin resistance in women with PCOS (12). Thus, genes involved in vitamin D metabolism have been suggested as candidate genes for the susceptibility to PCOS. A few polymorphisms in the VDR gene, such as Cdx2, Taq1, Bsm1, Apa1, and Fok1, were reported to play an influential role on insulin secretion and sensitivity in PCOS women (13).

In this present study, a strong negative correlation of vitamin D levels with serum FBS, insulin and HOMA-IR levels was observed in PCOS patients ($p<0.001$ for all).

Wehr et al., demonstrated a significant association of low 25(OH)D levels with increased levels of fasting glucose and HOMA-IR in PCOS patients (14). Wang et al., found serum 25(OH)D3 concentration to be significantly negatively correlated to fasting insulin and HOMA-IR in PCOS patients (15). Rashidi et al., found a negative correlation between vitamin D levels and insulin resistance (HOMA IR) in PCOS patients (16).

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CONCLUSION

Our study showed that the serum 25(OH)D3 concentration was correlated with certain biochemical and endocrinal parameters like FBS, fasting insulin and HOMA IR, which are also considered as metabolic risk factors. So our study hints that vitamin D may have a decisive role in PCOS and has a potential to emerge as an associated biochemical parameter of PCOS.

Multi-centre randomized controlled trials with large sample sizes are needed to explore the metabolic role of vitamin D supplementation in women with PCOS.

Conflict Of Interest

None

Source Of Funding

None

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