ABSTRACT

**Background:** Polycystic ovary syndrome (PCOS) is the most common endocrinopathy among women. Insulin resistance is now recognized as an integral component, particularly in obese. Association of vitamin D status with metabolic and hormonal dysfunction in PCOS has been investigated by some studies but the relationship between them still remains to be conclusive. The link between altered vitamin D status (if any) and androgen excess may have dependence on insulin resistance associated with PCOS.

**Aims and objectives:** The present study aims to find out interrelationship between serum Vit.D, Total Testosterone levels and Insulin resistance in PCOS cases.

**Materials and methods:** The study included 150 PCOS patients from Gynaecology and Endocrinology OPD of a tertiary care hospital of Kolkata, West Bengal, and 150 age-matched healthy volunteers. Serum 25(OH) Vit D, total testosterone and insulin levels were estimated by Enzyme Linked Immunosorbent Assay (ELISA) method. Insulin resistance was calculated from fasting serum insulin and fasting plasma glucose level with HOMA-IR formula (Homeostasis Model Assessment of Insulin Resistance).

**Results:** Our study revealed that serum vitamin D levels were significantly lower in PCOS cases in comparison to age matched control group. Significant negative correlation of vitamin D status was found with insulin resistance and androgen status in PCOS cases.

**Conclusion:** Though this is a small study, it has provided some clue into pathophysiology of PCOS. The relations and associations achieved from the results of this study sustained this explanation and 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.

**Keywords:** Vitamin D, Insulin Resistance, Total Testosterone, Polycystic Ovary Syndrome.

INTRODUCTION

Polycystic ovary syndrome is the most common endocrinopathy among women. It has been reported to have a prevalence of 6-12%. The present diagnostic criteria of this heterogeneous disorder, according to the Androgen Excess & PCOS Society (AE-PCOS society) are: hyperandrogenism (clinical &/or biochemical), ovarian dysfunction (ovulation disturbance&/or polycystic ovary morphology) and the exclusion of other causes of androgen excess or related disorders. Insulin resistance is now recognized as an integral component, particularly in obese. Approximately 50-70% of women with the condition known as PCOS were described as showing hyperinsulinemic insulin resistance (IR), which may play a major pathological role in development of syndrome.
Women with PCOS may also be at elevated risk of vitamin D deficiency (VDD). Additionally, positive associations of VDD with some well-known comorbidities of PCOS including type 2 diabetes, metabolic syndrome and cardiovascular diseases are reported. [5] These comorbidities of PCOS are placing a high financial burden on healthcare costs in the 21st century. [6,7] A diagnosis of PCOS therefore implies an increased risk of these complications developing later in life.

The link between altered vitamin D status (if any) and androgen excess may have dependence on insulin resistance associated with PCOS. Women with this ‘Hyper androgenic syndrome’ are thus an ideal group for identifying insulin resistance at an early stage and preventing its complications later in life. [8,9] In PCOS cases, insulin resistance is mainly limited to glucose metabolism, whereas other biologic actions of insulin - including those involved in steroidogenesis (e.g. in ovary) - are not impaired. Therefore, Insulin resistance increases hyperandrogenism through insulin mediated ovarian androgen hyper-production and reducing sex hormone binding globulin (SHBG) synthesis in liver. [10] At the level of the granulosa cells, insulin amplifies the response of granulosa cells to LH. Therefore these cells undergo abnormal differentiation & premature arrest of follicular growth & thus, anovulation. Vitamin D deficiency may play a key role in development of PCOS [11,12,13] although controversy remains about it, as stated by many workers.

Therefore the present study has been undertaken to find out interrelationship between serum Vit.D, Total Testosterone levels and Insulin resistance in P.C.O.S cases.

**MATERIALS AND METHODS**

**Study setting:** The study was conducted in the department of Biochemistry, Medical College, Kolkata.

**Timeline:** January 2016 to January 2017

**Informed Consent:** Written informed consent was taken from the patients as per Proforma. Demographical data, detailed history and clinical findings and laboratory investigations were recorded in the Proforma.

**Ethical Clearance:** This study was cleared by Institutional Ethics committee.

**Study group:** Study group patients are those suffering from polycystic ovary syndrome as diagnosed by criteria laid down by Androgen Excess and PCOS Society (AE-PCOS Society), attending Gynaecology and Endocrinology OPD of Medical College and Hospital, Kolkata.

**Control group:** Age matched healthy subjects not suffering from PCOS and anovulation, attending OPD Biochemistry Lab. for various investigations

**Study design:** This is a hospital based case control study.

**Inclusion criteria:** Documented cases of PCOS of reproductive age group (15-45 years) with their informed consent.

**Exclusion criteria:** Patients taking vit D and calcium containing drugs, other causes of anovulation, other endocrinopathies (e.g.: Thyroid and Parathyroid disorder, other causes of hyperandrogenism etc), renal stones, chronic kidney disease, chronic liver disease, Type 2 diabetes mellitus, patients taking oral contraceptive pill or steroid medications or patients having any malignancy were excluded from the study.

**Sample size:**
Cases- 150 PCOS patients (15-45 years)
Controls- 150 normal age matched subjects.

**Materials Required:**
Supporting laboratory instruments like the following-
XL-600 auto analyzer, Semi auto analyzer (ERBA CHEM 5 V2), ELISA reader with washer.

**Procedure:**
Patients who readily participated in the study voluntarily, were selected on the basis of inclusion and exclusion criteria, after proper consent. About 10 ml of venous blood was collected from each of study and
control group at 12 hour fasting in early morning with proper aseptic technique- 8 ml blood was taken in Plain vial with clot retractor and rest 2 ml will be taken in a Fluoride vial. The sample taken in clot activator without anticoagulant were allowed to clot and then all the tubes were centrifuged at 1500 rpm speed for 3-5 minutes for separation of serum and plasma. After separation serum was stored at -20°C in the freezer compartment and plasma was stored at 2-8°C until analysis. All the tests were done with serum obtained from clotted blood except fasting plasma glucose which was done with plasma.

1) Insulin resistance was calculated from fasting serum insulin and fasting plasma glucose level with HOMA-IR formula (Homeostasis Model Assessment of Insulin Resistance).[14]

HOMA-IR was calculated using the following formula:

\[
\text{Fasting glucose (mmol/l)} \times \text{fasting insulin (µIU/ml)} \div 22.5
\]

Women were classified as being insulin resistant or not insulin resistant in accordance with defined cutoff points for HOMA-IR ≥2.5.[15]

2) Serum 25(OH) D, total testosterone and insulin levels were estimated by Enzyme Linked Immunosorbent Assay (ELISA) method [Euroimmun kit for vitamin D, DSI (Italy) kit for Total testosterone and Accubind (Monobind Inc.USA) kit for serum insulin]

STATISTICAL ANALYSIS

Statistical analysis of the study was done by SPSS software version 20.0 and Microsoft Excel 2013 after obtaining the data at the end of the study. Values were presented as mean ± SD. P- values were indicated for the differences between groups, as analyzed using the student’s t test and p- value less than 0.05 was considered to be statistically significant. Correlation between variables was determined using linear regression analysis

RESULTS

No significant difference was found between the ages of cases and controls (p-value 0.08) by independent t-test.

Serum Vitamin D level in cases was found to be significantly lower than the serum vitamin D levels in controls (p-value<0.001) by independent t-test.

Serum insulin levels in cases were found to be significantly higher than the serum insulin levels in controls (p-value<0.001) by independent t-test.

Serum total testosterone levels in cases were found to be significantly higher than the serum total testosterone levels in controls (p-value <0.001) by independent t-test.

Significant negative correlation of serum vitamin D levels with serum insulin levels(r -0.55 when p <0.01), HOMA IR (r -0.54 when p <0.01) and serum total testosterone levels(r -0.50 when p <0.01) was observed. Significant positive correlation of serum total testosterone levels with serum insulin levels (r 0.59 when p <0.01) and HOMA-IR (r 0.58 when p value <0.01) was found.

4. COMPARISON OF SERUM VITAMIN D LEVELS BETWEEN CASES AND CONTROLS:

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>CASES (TOTAL OBSERVATIONS=190)</th>
<th>CONTROLS (TOTAL OBSERVATIONS=190)</th>
<th>p VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>SERUM VITAMIN D</td>
<td>18.75±0.35</td>
<td>26.61±0.36</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Serum Vitamin D levels in cases were found to be significantly lower than the serum vitamin D levels in controls (p-value <0.001) by independent t-test.

5. COMPARISON OF SERUM INSULIN LEVELS BETWEEN CASES AND CONTROLS

<table>
<thead>
<tr>
<th>PARAMETERS TO BE COMPARED</th>
<th>CASES(TOTAL OBSERVATIONS=190)</th>
<th>CONTROLS(TOTAL OBSERVATIONS =190)</th>
<th>t VALUE</th>
<th>p VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum insulin in µIU/ml (MEAN±S.E.)</td>
<td>38.26±0.76</td>
<td>6.93±0.37</td>
<td>13.6</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Serum insulin levels in cases were found to be significantly higher than the serum insulin levels in controls (p-value <0.001) by independent t-test.
DISCUSSION

PCOS is a syndrome associated with hyperinsulinemia and hyperandrogenism. Although vitamin D primarily plays a role in bone metabolism, it has important functions in the reproductive system. Vitamin D receptors are found in ovarian and endometrial tissues and play an important role in steroidogenesis. The present study was directed towards investigating into the relationship existing (if any) between vitamin D status observed in PCOS subjects with their levels of Androgen (Testosterone) and Insulin resistance.

The present study includes 150 PCOS patients and 150 controls. In our study no significant difference was found between the ages of cases and controls (p value 0.08) by independent t-test. Mean age of our PCOS population was 22.9 years. In the study of Hana Fakhoury et al, the mean age of the cases was 35.9 ± 5.5 years, whereas the mean age for the controls was 38.4 ± 5.0 years, which was found to be statistically significant (P = 0.01).
Pritam Kumar Saha et.al. Association of Vitamin D Status with Polycystic Ovary Syndrome in Relation to Insulin Resistance and Serum Total Testosterone

Bronstein J et al stated that 74% of cases are adolescents (13-18 yrs). PCOS may occur at a younger age in girls who develop early pubarche and thelarche. Therefore, the diagnosis and workup should be considered in young girls with risk factors suggestive of PCOS.

Studies comparing vitamin D levels between patients with PCOS and healthy women with normal ovulation have yielded conflicting results. Some studies have shown that vitamin D levels do not change in patients with PCOS. In a study by Panadis et al., there was not a difference in vitamin D levels between healthy individuals and patients in general, but obese patients with PCOS had lower vitamin D levels. Several studies have reported low levels of vitamin D levels between 11 and 31ng/ml with the majority having values <20 ng /ml (67-85%). Vitamin D deficiency is also common in the general population in many parts of the world, with 10-60% of adults having values lower than 20 ng/ml. When comparing women with PCOS to control women with similar age and BMI, Mahmoudi et al. found the women with PCOS had a significantly higher vitamin D level(29.3ng/ml in PCOS women vs 19.4 ng/ml in control women). On the other hand, Li et al. reported lower vitamin D levels, although not significant, in women with PCOS compared with women without PCOS (11 ng/ml in PCOS group vs 17 ng/ml in control group). Recently, Wehr et al. also reported lower levels in women with PCOS (n=545) compared to the control women (n=145:25.7 vs 32.0 ng/ml, respectively).

Evidence from multiple studies has shown that almost 70% of patients diagnosed with PCOS have resistance to insulin. In our study also, serum insulin levels and HOMA-IR in PCOS patients is significantly higher than those in the controls (p<0.001) and this is in accordance with the studies of Naidu J N et al. Vitamin D may enhance insulin action by enhancing insulin synthesis and release, increasing insulin receptor expression or suppression of pro-inflammatory cytokines that are believed to mediate insulin resistance. Vitamin D may also mediate insulin sensitivity by improving calcium status, increasing local production of 25(OH) Vit D. Insulin resistance increases hyperandrogenism through increase in ovarian androgen production and reduction of sex hormone binding globulin (SHBG) production. Therefore, vitamin D may play a key role in the development of PCOS. However, the underlying mechanisms require further exploration.

In order to establish causation, randomized controlled intervention trials need to be undertaken. There have been very few interventional studies evaluating the effects of vitamin D supplementation in women with PCOS.

**Limitations**

Status of SHBG and free androgen index are not included in this study. Here in this study we have considered total testosterone levels of the population as a measure of their androgen status. This limitation of the study is mainly due to limited time-interval, limited resources, etc.

In some studies, it has been suggested that obesity may have a confounding role in the relationship between 25OHD and insulin resistance in women with PCOS. There remains the future scope of investigating the role of serum vitamin D status separately in the obese and non-obese PCOS group.

Deranged extracellular calcium homeostasis in vitamin D deficiency and consequent release of parathormone and its effect in PCOS is not studied here. Differences in insulin resistance are not studied in between cases having hyperandrogenism with anovulation and ovulatory cases having hyperandrogenism with polycystic ovaries. All of these may be
considered as the future prospects of this study.

CONCLUSION

The relations and associations achieved from the results of 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. Though this is a small study, it has provided some clue into pathophysiology of PCOS. Future research with adequately powered randomized placebo-controlled double-blind studies of vitamin D supplementation in women affected by PCOS is suggested. Until then, screening women who are at risk of vitamin D deficiency and supplementation, if necessary, may be considered.

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