



## A Study of Vitamin D Status in Insulin Resistant Type 2 Diabetics in a Tertiary Care Hospital

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### Abstract

*The aim of this study was to assess vitamin D status in insulin resistant type 2 diabetics in a tertiary care hospital. The study was conducted on thirty newly diagnosed type 2 diabetics. Age and sex matched control population was also taken into consideration. The study was conducted over a period of one year in a region where there is enough exposure to sunlight. The level of plasma 25(OH)D was measured. The level of fasting blood glucose and fasting insulin was also measured. Insulin Resistance was measured using HOMA (Homeostasis Model Assessment Method), where HOMA values greater than 2.6 are suggestive of Insulin Resistance. The mean plasma 25(OH)D level was 15.35 ± 5.1 nmol/l in diabetics, with 87% of case population being vitamin D deficient, whereas mean vitamin D is 25.7 ± 5.6 nmol/l in control population with only 17% control population diabetic. The mean Insulin Resistance is 5.8±1.9 among Diabetic Population in comparison to HOMA value of 1.4 ±0.7 among controls. There was significant difference of HOMA IR between cases and controls (p =0.006). We found a significant correlation between Vitamin D deficiency and Insulin Resistance. Our results suggest that vitamin D deficiency is prevalent among newly diagnosed Type 2 diabetics even in region where there is adequate exposure to sunlight throughout the year*

**Key Words:** 25(OH)D: 25 Hydroxy Cholecalciferol, HOMA-IR: Homeostasis Model Assessment – Insulin Resistance, T2DM: Type 2 Diabetes Mellitus, ADA: American Diabetic Association, ELISA: Enzyme Linked Immuno Absorbant Assay.

### Introduction

Diabetes is a global epidemic with highest prevalence in India<sup>[1]</sup>. According to International

Diabetes Federation, close to one-fifth of all adults with diabetes in the world live in the South-East Asia Region<sup>[1]</sup>. T2DM is a complex heterogeneous

group of metabolic disorders characterized by hyperglycemia and impaired insulin action and/or insulin secretion. The complex nature of T2DM reflects the multifaceted genetic background and the varied genetic-environmental interaction.

Vitamin D inadequacy constitutes a largely unrecognized epidemic in many populations worldwide. It has been reported in healthy children, young adults, especially African Americans, and middle-aged and elderly adults. The prevalence of vitamin D inadequacy in India is 54% to 78% as shown by the recent studies.<sup>[2,3,4]</sup> Based on basic and animal studies, vitamin D and calcium have also been suspected as modifiers of diabetes risk and important for insulin secretion and action.

A role for vitamin D in pancreatic  $\beta$ -cell function might be mediated by the binding of circulating 1,25-dihydroxyvitamin D to the  $\beta$ -cell vitamin D receptor. Alternatively, vitamin D could function through activation of 25-hydroxyvitamin D by 1- $\alpha$ -hydroxylase, which has been shown to be expressed in  $\beta$  cells.<sup>[5,6]</sup> So it becomes imperative to find innovative approaches for prevention and management of the epidemics of Diabetes and vitamin D deficiency crippling the Indian population. Hence, a correlation between these two disorders and potential area of intervention managing both the conditions together would be of utmost importance in today's era. Thus, this study is undertaken to evaluate the prevalence of vitamin D deficiency among diabetics in comparison to non diabetics.

### Materials and Methods

The patients were selected as per inclusion criteria of the study. The time period was January 2013 to June 2014. Newly diagnosed Type 2 Diabetic Individuals attending Medicine OPD and Ward were regarded as Cases and the age and sex matched Non-Diabetics were included as Controls. The study variables included Serum and whole blood of cases and controls. Cases were included by strictly maintaining the following Inclusion Criteria ie. Age :25 to 65 years; Type 2

Diabetics as per ADA Criteria; Non Diabetics as per ADA Criteria; Patients consenting to undergo various tests; The Exclusion Criteria was: Age<25 years and >65 years; Type 1 Diabetics; Patients not willing to undergo various tests; H/O Osteomalacia, Rickets, Recurrent Fracture; H/O following drug intake in recent period-Corticosteroids, Phenytoin, Anti-retroviral therapy, etc; H/O other diseases that mimics finding of Type 2 DM, and/or influences blood sugar level.

About 60 people were assessed. 30 patients of Type 2 Diabetics and 30 Non diabetic Controls. At first patients are screened on basis of History and Clinical Examination, followed by FBS, to categorize patients as Type 2 diabetic and non diabetics. Fasting Insulin and Vitamin D level are also measured in both groups.

Fasting Plasma Glucose was measured by Glucose Oxidase -Peroxidase method by Auto analyzer RANDOX DAYTONA. Serum Fasting Insulin was measured by non competitive ELISA method and Serum Vitamin D by competitive Elisa Method, and were analyzed by Erba CHEM-5 plus V2 TECAN Elisa Reader and Mathematical Calculation of Insulin resistance By HOMA Method. Suitable statistical analysis of the data obtained from the study will be performed by SPSS (Statistical Package for Social Sciences) Software

### Results

In this study, a total of 30 non diabetics and 30 diabetic patients were enrolled as per selection criteria. All the patients were subjected to detailed history taking, clinical examination, and different lab investigations including fasting sugar, fasting insulin levels and 25-hydroxy vitamin D levels and HOMA-IR was analyzed. Collected data were analyzed using suitable statistical methods and inferred in the following manner.

### Statistical Methods

Categorical variables are expressed as Number of patients and percentage of patients and compared across the groups using Chi Square test for

Independence of Attributes. Continuous variables between subjects like fasting blood sugar, fasting insulin levels, 25-hydroxy vitamin D, HOMA-IR and have been expressed as mean value  $\pm$  standard deviation and compared across the 2 groups using unpaired t test.

The statistical software SPSS version 16 has been used for the analysis and Graph Pad Prism has been used for formulation of graphs. 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.

The mean and standard deviation of the principal characteristics of the controls and the cases/diabetics like age ,sex, body mass index waist hip ratio, systolic blood pressure, diastolic blood pressure, LDL cholesterol, HDL cholesterol and serum triglyceride levels were calculated and were compared using unpaired t test. None of the characteristics were found to differ significantly among the control and the study population ( $p > 0.05$ ).

For the sake of our statistical analysis Vitamin D status would be reported as deficient if less than 20 ng/ml.<sup>[7]</sup> According to the above criteria among the control 5 out of 30 (16.7%) had vitamin D deficiency (<20 ng/ml) whereas 26 out 30 diabetics (86.7%) had vitamin D deficiency among the cases. There was a significant difference between the mean of vitamin D levels among the 2 populations with  $p = 0.007$ .

Mean levels of 30 control was  $25.78 \pm 5.6$  ng /ml whereas it was  $15.35 \pm 5.1$  ng/ml among diabetics/cases. (figure 1).

#### **Comparison of Insulin , Fasting Blood Glucose, HOMA-IR in Control and Diabetics:**

Among 30 controls the mean Insulin concentration was  $6.36 \pm 0.41$  uIU/ml. Among Cases / Diabetics the mean Insulin is  $14.9 \pm 1.1$  uIU/ml. There was significant difference between the mean of the Serum Insulin concentration of cases and controls ( $p = .0001$ ). (Figure 2)

Among 30 controls the mean fasting blood glucose is  $86.7 \pm 1.9$  mg/dl. The mean fasting blood

glucose in Diabetics is  $147 \pm 8.12$  mg/dl. There is statistically significant difference between the mean Fasting blood glucose of cases and controls ( $p = 0.0013$ ) (Figure 3)

Among 30 diabetics/cases the mean HOMA-IR is  $5.89 \pm 1.9$ . Among Control population the mean HOMA –IR is  $1.41 \pm 0.1$ . (figure 4)

Among the population the Diabetics/Cases with HOMA-IR  $> 2.6$  was taken into consideration as Insulin Resistant Diabetics<sup>[8]</sup>. Among the control population patients with HOMA-IR  $< 2.6$  was taken into consideration as Non Insulin Resistant Non Diabetics and a comparison of HOMA-IR was made among cases and controls. There was a statistically significant difference among the mean of the HOMA-IR of cases and controls ( $p = 0.006$ ) (figure 5)

#### **Correlational Analysis**

Correlation analysis between the serum 25-hydroxy vitamin D levels with HOMA-IR was performed in cases and controls. Bivariate correlation analysis between vitamin D levels and HOMA-IR in the control group revealed the following results.

A weak moderate negative correlation was found between serum vitamin D levels and insulin resistance index (HOMA-IR) with  $r = -0.358$  and 2-tailed T test shows  $p = 0.01$  among the control population.

On the other hand, Bi-variate co-relational analysis between Vitamin D and HOMA-IR in diabetics reveals that, there was moderate negative correlation between serum 25-hydroxy vitamin D levels and HOMA-IR with  $r = -0.663$  and 2 tailed test reveals  $p = 0.001$  .

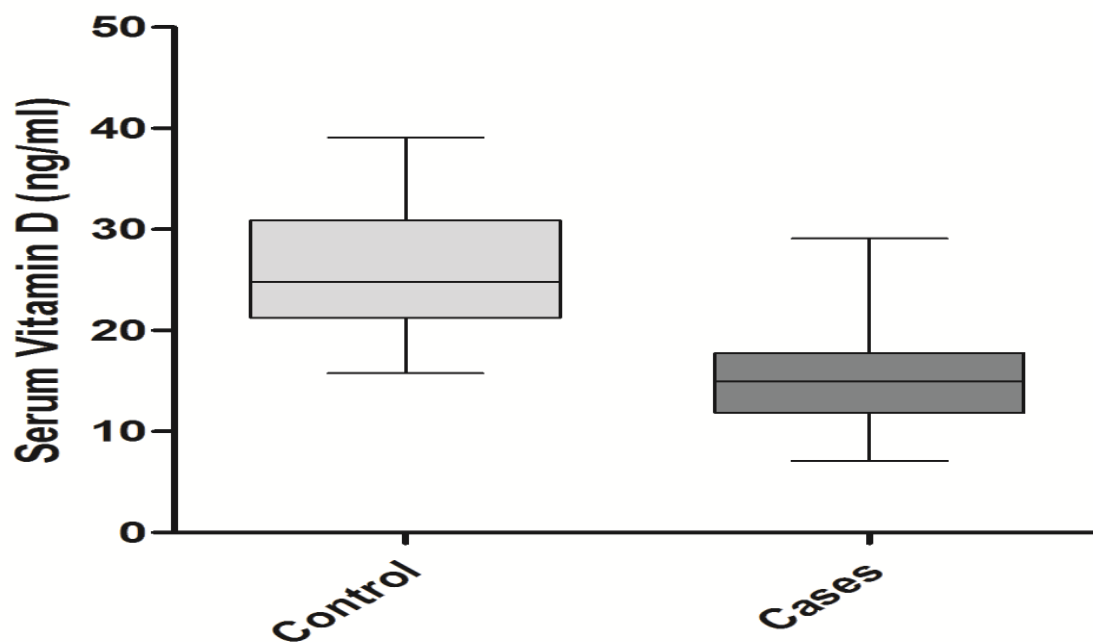


Figure 1: Graph showing Vitamin D levels in control and cases

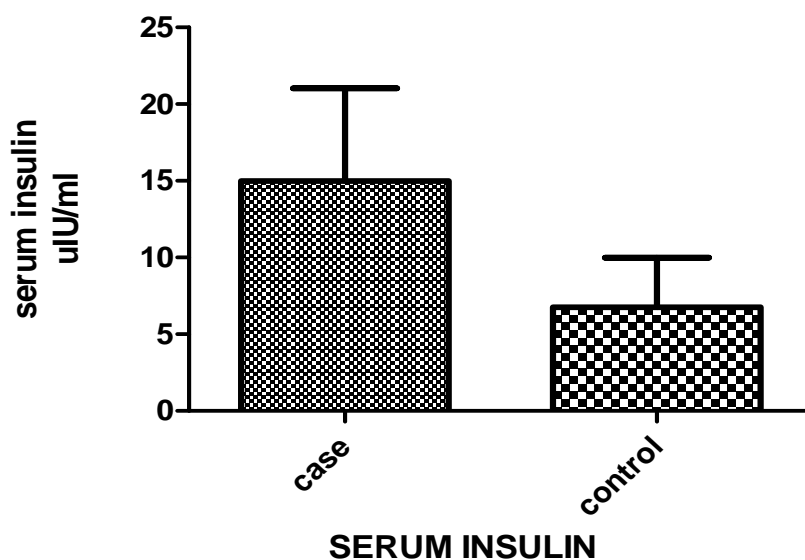


Figure 2: Graph showing serum Insulin in Cases and Controls

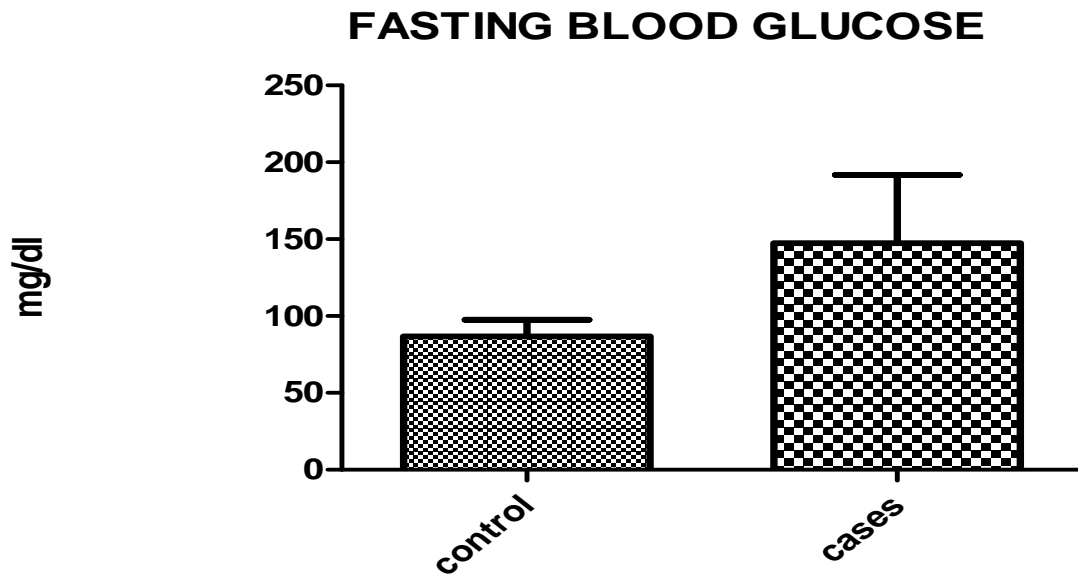


Figure 3: Graph showing comparison of Fasting Blood glucose among cases and controls (mg/dl)

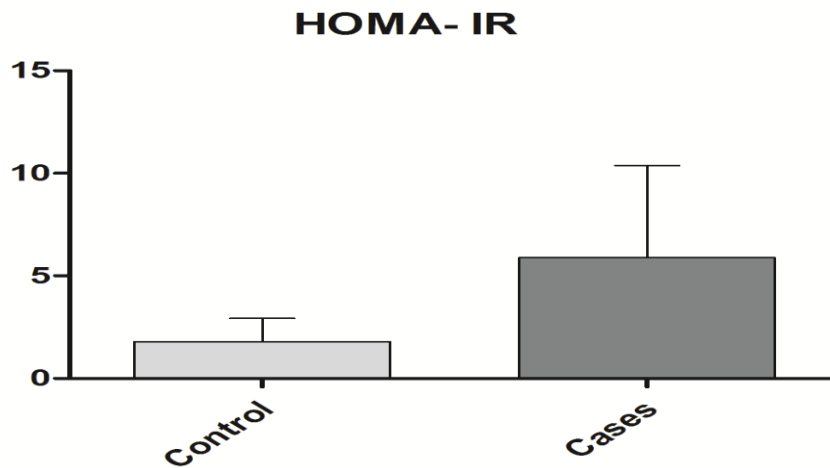


Figure 4: Graph showing HOMA-IR in control and diabetics

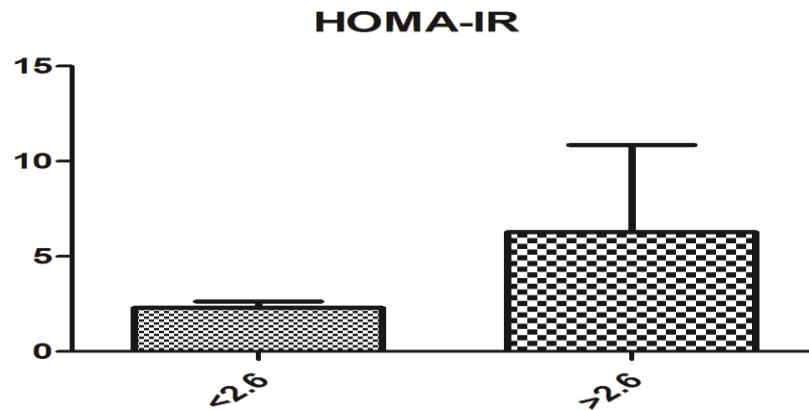


Figure 5: Graph : Comparison of HOMA-IR<2.6 and HOMA-IR>2.6 among Diabetics

## Discussion

The diabetics (study group) had a HOMA-IR of  $5.89 \pm 1.9$  compared to  $1.41 \pm 0.1$  of the control group. There is significant difference between the mean HOMA-IR of 2 population. The cut off value of HOMA-IR was taken to be 2.6, where HOMA-IR values  $> 2.6$  signifies presence of Insulin Resistance whereas HOMA-IR values  $< 2.6$  shows absence of IR. With a cut-off of 2.6, 4% of the control group was found to have insulin resistance ( $\geq 2.6$ ) compared to the cases where 90% of the diabetic population was found to have HOMA-IR  $> 2.6$ .

Hence, it is observed that higher levels of HOMA-IR, suggesting the presence of insulin resistance are associated with insufficient levels of serum vitamin D.

A study conducted by Kayaniyil and associates<sup>[9]</sup> on 712 subjects aged  $> 30$  years who were at high risk for type 2 Diabetes or Metabolic syndrome. Among these subjects Insulin Sensitivity was quantified using Matsuda Insulin Sensitivity Index for Oral Glucose tests (ISOGTT) and Insulin Resistance was measured using HOMA-IR. Beta cell dysfunction was assessed by dividing the Insulin Sensitivity Index (IGI) by HOMA-IR and by calculating Insulin Sensitivity Index-2. The study demonstrated independent association of 25(OH) D with both insulin sensitivity and Beta cell function in subjects with DM by multivariate regression analysis.

The study conducted was one of its kind as it primarily targeted the newly diagnosed population of Type 2 diabetes. The higher incidence of vitamin D deficiency is also strange in this part of India, where there is exposure to significant amount of sunlight. It could probably be due to the fact that disparate ethnicities have different optimal serum concentration of 25(OH)D., as supported by a study conducted by NHANES III data by Scragg and associates<sup>[10]</sup>. The study is also indicative of a possibility that vitamin D deficiency may be a probable cause for conversion of latent diabetes to Overt Diabetes.

The current study may have a great clinical impact. First, at risk population (obese, sedentary lifestyle) may undergo regular Vitamin D screening because Vitamin D deficiency is also implicated in other disorders like Metabolic Syndrome. Second routine screening of Vitamin D deficiency in diabetes would give us a knowledge regarding co-existing hypo-vitaminosis D. Early diagnosis would lead to earlier therapeutic recommendation. Third, supplementation of Vitamin D in DM patients may improve the therapeutic efficacy of oral hypoglycemic / insulin by correction of the insulin resistance status.

There were a few limitations of this study. First, the cross sectional design and the small sample size of the study group is not adequate to make a recommendation for the larger population of the type 2 diabetes but the definitely promising results warrant the need for larger trials. Second, the study have not accounted for confounders that may have mediated association between 25(OH) Vitamin D and Insulin sensitivity such as physical activity and calcium intake.

**Grants:** No grants

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