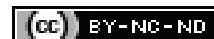


Role of Vitamin D and Haemoglobin Levels in Prediabetic Patients- A Retrospective Study

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ABSTRACT

Introduction: The 25-hydroxy (OH)₂D₃ (Vitamin D, Cholecalciferol) and Haemoglobin (Hb) level have a significant role in the regulation of immune response. A decrease in Vitamin D and haemoglobin concentrations is a common risk factor for diabetes. The link between Vitamin D and insulin resistance has been studied more thoroughly in patients with diabetes mellitus, although data from people with prediabetes is scarce.

Aim: To study the association of Vitamin D and Hb levels in prediabetes patients.

Materials and Methods: This retrospective study was conducted in the Sree Balaji Medical College and Hospital, Chennai from July 2020 to December 2020. Total 100 subjects with prediabetes conditions were included and their Vitamin D and Hb levels were measured. The patients who gave written informed consent for prediabetes screening according to ADA-2020 recommendations for prediabetes were included and their Vitamin D and Hb level

done as part of the routine check-up were collected. Data were statistically analysed using Analysis of Variance (ANOVA) and Chi-square test.

Results: Data of total 100 patients was analysed, including 68 female patients and 32 male patients. Out of 100 patients, 84 were aged between 25-50 years and 16 were aged between 51-65 years. The difference between male and female were statistically significant with p-value <0.01 (for Hb) and p value 0.05 (Vit-D), respectively. A significant difference in mean Hb level when compared among Vitamin D deficient (0.0-19.99 IU/mL) level, insufficient (20-29.99 IU/mL) and sufficient (30-99.99 IU/mL) subjects (p=0.008) was observed.

Conclusion: Low levels of Vitamin D is significantly associated with low Hb level. The patients with deficient Vitamin D level showed a significantly low mean Hb level compared to insufficient and sufficient Vitamin D level.

Keywords: Cholecalciferol, Diabetes, Immune response, Insulin resistance

INTRODUCTION

Prediabetes is a multifactorial metabolic condition that affects more than just blood glucose levels. It increases the risk of type 2 diabetes by three to ten times. Detecting and treating this condition is critical for preventing or delaying the onset of type 2 diabetes [1]. Diabetes type 2 risk increases 50% among people with prediabetes [2]. A seco-steroid prohormone, 25(OH) D₃ (Vitamin D) is a fat-soluble steroid prohormone produced by the skin. It promotes calcium absorption in the gut to keep serum calcium and phosphate concentrations sufficient for proper bone mineralisation [3]. The iron-containing oxygen-transport metalloprotein haemoglobin (Hb) is an important erythrocyte protein that is affected by many factors, including pH deviations, temperature increases, and chemical modifications that can cause functional changes in Hb [4]. The active form of Vitamin D binds to the Vitamin D receptors in beta cells in the pancreas to affect insulin secretion [5]. Vitamin D supplementation has been proposed as an effective intervention to decrease diabetes risk in people with low Vitamin D levels [6].

A decrease in haemoglobin concentrations is a common risk factor for prediabetes to diabetes. Vitamin D levels appear to be inversely connected with the likelihood of prediabetes and Haemoglobin A1c (HbA1c) levels in people with prediabetes, particularly in obese people, according to some observational evidence [7,8]. From a clinical viewpoint, it is interesting to evaluate the association of Hb concentrations and Vitamin D in prediabetes patients between 25-65 age groups as this age group is more prone to Hb and Vitamin D deficiency.

Study by Sim JJ et al., showed that Vitamin D has an important association with anaemia, the low Hb level is also noteworthy [9]. The Hb analysis helps to tell the glucose bound to Hb, and such combination can be estimated by doing HbA1c test, which is a

diabetes predictor mark test, and Vitamin D, the proinflammatory hormone, can maintain bone marrow function and can reduce metabolic disorders like diabetes. Therefore, the association of these parameters in suspected diabetes patients can help to improve their physical health status, also can reduce/delay the risk of early occurrence of metabolic diseases [9].

The link between Vitamin D, haemoglobin and insulin resistance has been studied more thoroughly in patients with diabetes mellitus [7-9], although data from people with prediabetes is scarce. Nevertheless, the relationship between haemoglobin and Vitamin D level in the prediabetes adult population has not been well described. Therefore, the study's goal was to estimate Vitamin D level and Haemoglobin level in patients with prediabetes.

MATERIALS AND METHODS

This retrospective study was carried out in Sree Balaji Medical College and Hospital, Chennai, Tamil Nadu, India from July 2020 to December 2020. The data was collected from the patients visiting the Outpatient Department (OPD) and was analysed during the same time. Research was carried out after approval by the Institutional Human Ethical Committee [Ref.No. 002/SBMC/IHEC/2017/985]. Written informed consent was taken from all the study participant.

Inclusion criteria: Retrospective data were collected from total of 100 patients aged between >25 years and <65 years, who gave written informed consent for prediabetes screening according to American Diabetes Association (ADA) recommendations for prediabetes [10] and got Vitamin D and Haemoglobin level done as part of the routine check-up were included.

Exclusion criteria: Data from those patients suffering from any other medical problems like malignancy, autoimmune disease, Pregnant or lactating women were excluded from the study.

A total of 100 patients, who presented in the department, were enrolled in the study by purposive sampling.

Study Procedure

A standardised proforma was given to obtain details pertaining to family history and medical history. An amount of 5 mL of peripheral blood was obtained in a sterile vacutainer by a trained phlebotomist. The collected blood was allowed to clot and centrifuged at 2000 rpm for 15 minutes in a refrigerated centrifuge. The supernatant containing serum was collected, aliquoted into a 0.5 µL sterile polypropylene tubes, and stored in -20°C for further use. Vitamin D was measured by Chemiluminescence immunoassay (CLIA) method in Beckman Coulter DXi 600 and Hb measured by spectrophotometric principle in Yumizen H 500 fully automated haematology analyser, respectively and the data of Vitamin D, Hb was collected in the clinical chemistry laboratory.

Vitamin D deficiency was defined as Vitamin D deficient (<20 IU/mL) level, insufficient (20-<30 IU/mL) and sufficient (30-100 IU/mL) levels [11] and normal range of haemoglobin in men was 13-17 mg/dL and in women was 12-15 mg/dL [12].

STATISTICAL ANALYSIS

Statistical analysis was performed using the Statistical Package for the Social Science (SPSS), version 20.0. The association and statistical significance was analysed by Chi-square and two-way Analysis of Variance (ANOVA) test for Vitamin D and Hb level in the study subjects.

RESULTS

The study was conducted to compare the Vitamin D levels in subjects with Hb levels in prediabetes patients between 25-65 years age groups. Out of total 100 patients, 68 patients were females and 32 were males and the percentage wise distribution according to the gender and 25-50 age, 51-65 age group categories is mentioned in [Table/Fig-1].

Characteristics		Age groups (years)	
		25-50 (n)	51-65 (n)
Total (N=100)		84	16
Sex	Male (n=32)	21	11
	Female (n=68)	63	5

[Table/Fig-1]: Demographic comparison between 25-50 age and 51-65 age groups.

[Table/Fig-2] shows association between the gender, Vitamin D, and Hb concentration according to the biological reference intervals of both parameters. Out of 32 male patients, maximum 10 patients were having Hb concentration between 13-13.99 mg/dL, followed by the 8 patients in 14-14.99 mg/dL group. In females, maximum patients 28 were having Hb between 12-12.99 mg/dL. The difference between male and female were statistically significant with p-value <0.01 for Hb and p-value 0.05 for Vitamin D, respectively [Table/Fig-2].

[Table/Fig-3] represents the comparison between mean Vitamin D level and Hb levels according to biological reference interval of Hb in patients with all age groups and it shows a statistically significant by two-way ANOVA test (p=0.046).

Variables	Hb concentrations (mg/dL)						Vitamin D concentrations (IU/mL)		
Sex	0-11.99	12-12.99	13-13.99	14-14.99	15-15.99	16-16.99	0-19.99	20-29.99	30-99.99
Male(N=32)	1	2	10	8	7	4	3	18	11
Female (N=68)	16	28	15	4	4	1	21	32	15
Asymptotic Significance 2-sided)	p<0.01						p=0.05		

[Table/Fig-2]: Association between the sex vs Vitamin D and Hb concentration in all the age groups. Bold p-values are significant; Chi-square test used to calculate the level of significance

Hb level (mg/dL)	N	Mean value of Vitamin D (IU/mL)	Standard deviation (IU/mL)	
0 -11.99	17	20.55	7.69	
12-12.99	30	26.52	11.98	
13-13.99	25	28.31	9.53	
14-14.99	12	30.16	5.86	
15-15.99	11	22.55	4.63	
16-16.99	5	24.46	3.91	
ANOVA	Sum of squares	Difference	F	Significance
Between groups	999.602	5	2.354	0.046*
Within groups	7944.405	94		

[Table/Fig-3]: Comparison between Vitamin D and Hb level based on Hb reference Interval.

*Significant at the level of 0.05

[Table/Fig-4] shows a significant difference (p-value 0.008) in mean Hb level when compared among 25(OH) D deficient (0.0-19.99 IU/mL) level, insufficient (20-29.99 IU/mL) and sufficient (30-99.99 IU/mL) subjects (p=0.008).

Vitamin D level (IU/mL)	N	Mean Hb level (mg/dL)	Standard deviation (mg/dL)	
0-19.99	24	12.18	1.95	
20-29.99	50	13.45	1.80	
30-99.99	26	13.96	0.990	
Total	100	25.85	9.50	
ANOVA	Sum of squares	Difference	F	Significance
Between groups	28.280	2	5.063	0.008
Within groups	270.921	97		

[Table/Fig-4]: Comparison between Hb and Vitamin D level based on Vitamin D reference Interval.

Bold p-value is significant

DISCUSSION

The present study indicates that 25-hydroxy Vitamin D deficiency status was associated with low haemoglobin level in all the age groups and which leads to the increased risk of anaemia as well as the hypovitaminosis condition. The mechanism between the association of these two parameters in prediabetes was unknown, however the study gives a positive sign that if patients are lying in prediabetes condition then their Vitamin D level and Hb levels can be monitored randomly because these parameters play a vital role in humans in all age groups. India currently has the highest number of diabetics in the world, and this number is likely to continue to climb in the coming years. As a result, investigations on prediabetes to diabetes-related complications are essential for assessing the disease's burden. So, the present study aimed to focus prediabetes patients with the examination of biomarkers such as Vitamin D and Hb levels variations in their life cycle.

One of the studies concluded that Vitamin D deficiency has an effect on erythropoiesis including cellular proliferation and differentiation and its well-documented role in regulating bone and mineral metabolism [13,14]. Sim JJ et al., evaluated the prevalence of anaemia in people with Vitamin D deficiency in comparison to those with normal levels of Vitamin D and the study concluded that statistically significant difference was shown between the prevalence of anaemia and

Vitamin D deficient people, having low haemoglobin level and higher usage of erythrocyte-stimulating agents when compared to persons with normal level of Vitamin D [9]. The observations of the Azizi-Soleiman F et al., confirmed that, there is a positive relationship between iron and Vitamin D because the haeme-bound iron is required for the hydroxylation process of Vitamin D, so variations in Vitamin D metabolism are closely linked to iron deficiency [15]. One of the Vitamin D and bone marrow related studies stated that Vitamin D has influence on bone marrow function particularly with the findings that levels of calcitriol are several a hundred times higher in bone marrow compared to plasma Vitamin D level [16].

In recent years, it has also been discovered that Vitamin D has a strong link to anaemia, the low Hb level. The prevalence of Vitamin D deficiency in subgroups of elderly persons with anaemia by Perlstein TS et al., summarised that in elderly people, moderate Vitamin D deficiency is linked to anaemia in general and anaemia in inflammation in particular [17]. Around 2 billion individuals worldwide suffer from this disease, with Iron Deficiency Anaemia (IDA) accounting for roughly half of all cases IDA [18,19]. Vitamin D has been shown to reduce inflammatory cytokines and suppress hepcidin mRNA expression, both of which are implicated in the pathogenesis of anaemia with inflammation. As a result of its anti-inflammatory properties, Vitamin D may help prevent anaemia [20,21]. According to Manickam B et al., study report, Vitamin D deficiency can decrease muscle strength and physical activity, which can lead to hyperglycemia and obesity. In the same way, Insulin resistance, which is a pathogenic mechanism of type 2 Diabetes Mellitus, is exacerbated by Vitamin D insufficiency [22].

Limitation(s)

One of the main limitations that has been faced in the current study was the small sample size and bias in answers shared by the study subjects also poor awareness about prediabetes condition and the management.

CONCLUSION(S)

Low level of Vitamin D is significantly associated with low Hb level. The patients with deficient Vitamin D level showed a significantly low mean Hb level compared to insufficient and sufficient Vitamin D level. The people with prediabetes should evaluate their Vitamin D and Haemoglobin levels on a regular basis since these parameters are important in humans of all ages. The findings of this study will aid in the detection and treatment of this kind of prediabetes, which is crucial in avoiding or delaying the onset of type 2 diabetes. In addition, providing general awareness by the government health department about prediabetes and the importance of taking supplementation of Vitamin D and Haemoglobin after prior doctor

consultation helps to improve the patient's knowledge as well the reduction in the diabetes patients number globally.

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