

# Study of Anaemia and Iron Profile in the Subjects with Vitamin D Deficiency Comparison with Healthy Controls without Vitamin D Deficiency

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## ABSTRACT

**Introduction:** Iron and vitamin D are two essential nutrients which constitute an important worldwide health issue due to their significant roles in biochemistry. The present study was conducted to assess the prevalence and risk of anemia in a population of subjects with documented D25 deficiency compared with those with normal D25 levels.

**Material and Methods:** The present case control study was conducted among 100 subjects (50 were cases and 50 were controls) in the department of Medicine at Chatrapati Shivaji Subharti Hospital. Total serum 25(OH)D concentration was analyzed using commercially available ELISA kits. According to the manufacturer's instructions, concentrations of 25(OH)D below 25 nmol/L were classified as a deficiency, and values of 25–75 nmol/L were considered a vitamin D insufficiency. Difference between two groups was determined using chi square test and student T test for categorical data and continuous data respectively. P-value of <0.05 was considered as significant.

**Results:** Hb<13 was found in 75% of the case group (male) and 25% of the control group (male). Hb<12 was found in 64.29% of the case group (female) and 45.45% of the control group (female). Mean iron (µg/dl) among the male and female case group was less as compared to the control group with statistically significant difference as p<0.05.

**Conclusions:** The results of the present study showed that Vitamin D metabolism is dependent on iron and its deficiency might disturb Vitamin D activation.

**Keywords:** Vitamin D, Iron, Anemia, Deficiency

Recent evidence suggests that other dietary factors, such as adequate vitamin D consumption, may affect iron regulation and erythropoiesis.<sup>9-11</sup> Vitamin D repletion has been associated with dose reductions in erythrocyte-stimulating agents (ESA) and increased reticulocytosis. Vitamin D has been demonstrated in bone marrow to affect marrow function. Moreover, levels of 1,25 hydroxyvitamin D (the active form of vitamin D) are several hundred-fold higher in bone marrow compared with plasma.<sup>12</sup>

Iron is an important co-factor for many enzymes like 1 $\alpha$ -hydroxylase required for hydroxylation of 25(OH) D to 1,25(OH)<sub>2</sub> D. Despite these important observations there are very few studies which report the association of VDD with anaemia especially in this region where VDD is so rampant. Therefore, the present study was done to evaluate whether an association exists between 25-hydroxyvitamin D (D25) deficiency and iron deficiency anemia in a population. Using the database of a large integrated health plan, we sought to assess the prevalence and risk of anemia in a population of subjects with documented D25 deficiency compared with those with normal D25 levels.

## MATERIAL AND METHODS

The present case control study was conducted in the department of Medicine at Chatrapati Shivaji Subharti Hospital from February 2017 to January 2019.

The study group was consisted of 100 patients, out of which 50 were cases and 50 were controls. Patients were enrolled in the study after obtaining written informed consent from parents and approval from Institutional Ethical Committee.

## INTRODUCTION

Iron and vitamin D are two essential nutrients which constitute an important worldwide health issue due to their significant roles in biochemistry and simultaneously, the very high risk of deficiency in both of them.<sup>1,2</sup> Anemia affects 1 of every 3 individuals worldwide and is estimated to have a global disease burden surpassing that of major depression and chronic respiratory ailments<sup>3,4</sup>, the prevalence of anemia, and its associated comorbidities has been on the rise.<sup>5</sup>

Given the multifactorial etiology of anemia, it can be classified into different subtypes such as iron deficiency anemia or anemia of nutrient deficiency, and anemia of inflammation (also called anemia of chronic disease).<sup>6</sup> Approximately 50% of all anemia cases in developed countries are caused by iron deficiency. The groups particularly exposed to deficiencies of this mineral are women of reproductive age, children and adolescents.<sup>7,8</sup>

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**Inclusion criteria:**

1. Patients with documented deficiency of vitamin D including both inpatients and out patients >18years of age and less than 45 years of age.
2. Patients not having vitamin D deficiency (age and sex matched controls).

**Exclusion criteria:**

It excludes patients with following co-morbidities, some of the examples are as follows:

- 1) Known case of Ckd, Gastrointestinal bleed, Immunocompromised state, Previous history of sickle cell anemia, Thalassemia, Malignancies, Hemolytic anemia, Ulcerative colitis and Pregnant females were excluded from the study.

**Diagnosis criteria:** Anemia was defined as an Hb level <12 g/dL for both women and <13g/dl for men. Along with this iron deficiency anaemia was identified if subjects met C2 of the following criteria: serum ferritin <12 ng/ml, TIBC >450 µg/dl, % transferrin saturation <15%. In this study if serum ferritin was >12 ng/ml with elevated C-reactive protein (CRP) than MCV, RDW and % transferrin saturation levels were used to diagnose iron deficiency anaemia. All unique subjects with an Hb level drawn within 6 months of a D25 value were included in the analysis. Patients were grouped as Vitamin D Deficiency (VDD) was defined as serum 25(OH) D levels <30 nmol/L, vitamin D insufficiency (VDI) as 25(OH) D levels between 30 and 75 nmol/L and vitamin D sufficiency (VDS) as >75 nmol/L.<sup>13</sup> Investigations done were Cbc, Esr, Serum Iron, Serum Ferritin Level, Tibc (Total Iron Binding Capacity), Vitamin D Level, Urine Routine And Microscopy, Stool Routine And Microscopy, Ultrasound, Gbp/Peripheral Smear, Chest X-Ray and Ecg, S.Folic Acid/S.Vitamin B12 (Whenever Required)

**Blood Analysis:** The blood was withdrawn from the antecubital vein in the morning (between 8 and 9 a.m.) in the pre-prandial state, after overnight fasting. In order to obtain the serum for testing, blood samples were centrifuged for 10 min at a speed of 3500 rpm.

**Vitamin D and Iron Status Indices in Serum:** Total serum 25(OH)D concentration was analyzed using commercially available ELISA kits (DiASource, Louvain-La-Neuve, Belgium), according to the manufacturer's protocol. All assays were performed in duplicate. The coefficient of variation of the intra-assays for 25(OH)D concentration in this study was below 4%. The 25OH vitamin D Total ELISA kits (DiASource, Louvain-La-Neuve) have a Certificate of Proficiency issued by the vitamin D External Quality Assessment Scheme (DEQAS) Advisory Panel. According to the manufacturer's instructions, concentrations of 25(OH) D below 25 nmol/L were classified as a deficiency, and values of 25–75 nmol/L were considered a vitamin D insufficiency. The ferritin concentration was measured using the immunoturbidimetric method, enhanced with latex particles. Iron concentration and unsaturated iron binding capacity (UIBC) were determined using spectrophotometric methods

with FerroZine. All mentioned indicators were measured on a Roche Cobas Integra 400 biochemical analyzer (Roche Diagnostics, Rotkreuz, Switzerland) using original manufacturer reagent kits. Inter-assay variability for those indices did not exceed 7.8%, 1.3% and 1.7%, respectively. Total iron binding capacity (TIBC) was calculated from iron and UIBC (the sum of both indicators). Soluble transferrin receptor (sTfR) concentration was measured using immunoenzymatic commercial kits (RamcoStafford, TX, USA). Inter-assay variability for this indicator did not exceed 6.0%. All assays were performed in duplicate, in never-frozen or only once-frozen (-20° C) serum samples.

**STATISTICAL ANALYSIS**

Data were tabulated and examined using the Statistical Package for Social Sciences Version 22.0 (IBM SPSS Statistics for Mac, Armonk, NY: IBM Corp, USA). Descriptive statistical analysis had been carried out in the present study. Results on continuous measurements are presented as Mean±SD. Categorical data has been presented as frequency distribution. The statistical power calculation was based on the assumption that the data were normally distributed. P-value of <0.05 was considered as significant. Difference between two groups was determined using chi square test and student T test for categorical data and continuous data respectively.

**RESULTS**

The present study comprised of 100 patients, out of which 50 were cases and 50 were controls. In the case group, 72% and 28% of the subjects were male and female respectively. In the control group, 78% of the subjects were males and 22% of the subjects were females. The mean age of the study subjects in the case and control group was 33.72±8.71 and 35.67±9.32 years respectively as shown in table 1. Hb<13 was found in 75% of the case group (male) and 25% of the control group (male). 28.21% and 71.29% of the subjects in the case and control group were having Hb>13. Hb<12 was found in 64.29% of the case group (female) and 45.45% of the control group (female). 35.71% and 54.55% of the subjects in the case and control group were having

Variables	Case (VDD)		Control (VDS)	
	N	%	N	%
Male	36	72	39	78
Female	14	28	11	22
Total	50	100	50	100
Chi square	1.87			
p value	0.79 <sup>#</sup>			
Groups	Age			
	Mean		SD	
Case (VDD)	33.72		8.71	
Control (VDS)	35.67		9.32	
t test	0.78			
p value	0.62 <sup>#</sup>			
<sup>#</sup> : statistically insignificant, VDD: Vitamin D Deficiency, VDS: Vitamin D Sufficiency				
<b>Table-1:</b> Demographic characteristics of the study population				

Variables	Case (VDD)		Control (VDS)	
	N	%	N	%
Men				
Hb<13	27	75	11	28.21
Hb>13	9	25	28	71.79
Chi square test	16.39			
p value	<0.01*			
Women				
Hb<12	9	64.29	5	45.45
Hb>12	5	35.71	6	54.55
Chi square test	0.89			
p value	0.35			
VDD: Vitamin D Deficiency, VDS: Vitamin D Sufficiency, *: statistically significant				
<b>Table-2:</b> Hb levels among the men and women according to the study groups				

Groups	25(OH) Vit D (nmol/L)	
	Mean	SD
Men		
Case (VDD)	19.20	4.81
Control (VDS)	86.48	17.13
t test	27.31	
p value	<0.01*	
Women		
Case (VDD)	17.42	4.68
Control (VDS)	74.06	14.56
t test	25.89	
p value	<0.01*	
*: statistically significant		
<b>Table-3:</b> Mean 25(OH) vit D (nmol/L) among the men and women according to the study groups		

Groups	Ferritin (ng/ml)	
	Mean	SD
Men		
Case (VDD)	29.08	7.86
Control (VDS)	41.30	6.17
t test	9.88	
p value	<0.01*	
Women		
Case (VDD)	26.16	7.94
Control (VDS)	37.12	6.43
t test	8.12	
p value	<0.01*	
*: statistically significant		
<b>Table-4:</b> Mean ferritin (ng/ml) level among the men and women according to the study groups		

Hb>12 (table 2). Mean Hb among the male in the case group was 10.46±3.2 while in the control group (male), the same was 13.40±2.77 with statistically significant difference as p<0.05. Mean Hb among the female in the case group was 8.80±3.61 while in the control group (female), the same was 12.60±2.43 with statistically significant difference. Mean 25(OH) vit D (nmol/L) among the male in the case group was 19.20±4.81 while in the control group (male), the same was 86.48±17.13 with statistically significant

Groups	Iron (µg/dl)	
	Mean	SD
Men		
Case (VDD)	51.60	14.62
Control (VDS)	114.90	20.93
t test	28.39	
p value	<0.01*	
Women		
Case (VDD)	45.16	13.59
Control (VDS)	106.94	19.42
t test	27.88	
p value	<0.01*	
*: statistically significant		
<b>Table-5:</b> Mean iron (µg/dl) level among the men and women according to the study groups		

Groups	TIBC (µg/dl)	
	Mean	SD
Women		
Case (VDD)	470.74	48.71
Control (VDS)	361.80	56.99
t test	11.34	
p value	<0.01*	
Men		
Case (VDD)	430.62	43.58
Control (VDS)	341.38	53.71
t test	9.93	
p value	<0.01*	
*: statistically significant		
<b>Table-6:</b> Mean TIBC (µg/dl) level among the men and women according to the study groups		

Groups	Transferrin saturation (%)	
	Mean	SD
Men		
Case (VDD)	13.64	5.74
Control (VDS)	42.56	7.79
t test	18.60	
p value	<0.01*	
Women		
Case (VDD)	11.20	5.39
Control (VDS)	35.16	7.88
t test	17.96	
p value	<0.01*	
*: statistically significant		
<b>Table-7:</b> Mean transferrin saturation (%) level among the men and women according to study groups		

difference as p<0.05 (table 3). Mean ferritin (ng/ml) among the female in the case group was 26.16±7.94 while in the control group (female), the same was 37.12±6.43 with statistically significant difference as p<0.05 (table 4). Mean iron (µg/dl) among the male and female case group was less as compared to the control group with statistically significant difference as p<0.05 (table 5). Similar observations were recorded for TIBC (µg/dl) and transferrin

saturation (%) as shown in table 6 and 7 respectively.

## DISCUSSION

The association between vitamin D status and anemia is of potentially great public health importance. As is increasingly appreciated, the potential benefits of vitamin D extend well beyond skeletomuscular maintenance with growing evidence suggesting that 25OHD levels are associated with cardiovascular health, glycaemic regulation; angiotensin regulated vascular responses, immune function, and cell differentiation. Indeed, low 25OHD levels may increase the risk of heart disease, hypertension, stroke, and diabetes. Moreover, the immunomodulatory effects of vitamin D have been described in various diseases.<sup>7,16</sup>

### Vitamin D and Anemia

In the current research, Hb<13 was found in 75% of the case group (male) and 25% of the control group (male). Hb<12 was found in 64.29% of the case group (female) and 45.45% of the control group (female). Mean Hb among the male in the case group was 10.46±3.2 while in the control group (male), the same was 13.40±2.77 with statistically significant difference as p<0.05. Mean Hb among the female in the case group was 8.80±3.61 while in the control group (female), the same was 12.60±2.43 with statistically significant difference as p<0.05. Similar results were reported by John J. Sim et al<sup>17</sup> in their study. He demonstrates a greater prevalence and risk of anemia in individuals with D25 deficiency compared with those with normal D25 levels. The percentage of subjects with anemia and subjects on ESA therapy were higher in the D25 deficiency group compared with those with normal D25 levels. In addition, D25-deficient subjects also had a lower mean Hb level compared with those with normal D25 levels. A multivariate logistic regression analysis controlling for gender, age, renal insufficiency, and ESA use revealed an OR of 1.9 for anemia in subjects with D25 deficiency versus those with normal D25. Shikha sharma et al<sup>18</sup> also revealed similar results. He found a greater prevalence and risk of anaemia was observed in individuals with VDD compared to those with normal 25(OH) D levels. Another study showed no differences in levels of Hb and serum iron in vitamin D deficient (VDD+VDI) and VDS groups. However 58% of the children with iron deficiency anaemia had VDI and 39% had VDD.<sup>19</sup>

The underlying mechanisms involved in the association between VDD and anemia have evoked a huge interest from nutritionists, scientists, clinicians and the public. The exact mechanism of association of VDD with anemia is still not known. Vitamin D is reported to stimulate erythroid precursors and vitamin D receptors have been discovered in numerous non-renal target tissues including the bone marrow.<sup>11</sup> Vitamin D and its metabolites are present in many tissues, as are the receptors for the active form of vitamin D, calcitriol. Calcitriol plays a key role in the regulation of immune function by inhibiting the expression of proinflammatory cytokines by a variety of immune cells, thus providing negative feedback to prevent excessive inflammatory. In vivo and in vitro studies have demonstrated

that calcitriol reduces cytokine production leading to the reduction of inflammatory milieu and anemia.<sup>20</sup>

There is another explanation. VDD may stimulate immune cells within the bone marrow micro-environment to produce cytokines, inducing impaired erythropoiesis. Vitamin D therapy can improve altered iron homeostasis associated with anemia of chronic kidney disease in VDD patients. Furthermore, high-dose vitamin D therapy impacts systemic hepcidin levels in subjects with early stage chronic kidney disease. Vitamin D regulates the hepcidin–ferroportin axis in macrophages which may facilitate iron utilization. Based on these, further mechanism researches need to be conducted to evaluate whether these is a direct causal effect of VDD on anemia.<sup>10,21</sup>

Mean iron (µg/dl), ferritin (ng/ml) and TIBC (µg/dl) was greater in case group irrespective of gender as compared control group with statistically significant difference. These results are in accordance with the study done by Sharma S et al.<sup>18</sup> According to Sharma S et al<sup>18</sup>, subjects with VDD also had lowered levels of iron, % transferrin saturation and elevated levels of TIBC in comparison to VDI and VDS group. No difference was observed in mean levels of ferritin in any of the groups.

Grindulis et al<sup>22</sup> found a significant association between VDD and poor iron status was observed. Two-fifths of the children were anaemic, two fifths had low plasma levels of vitamin D and one-fifth had both these features. Children with VDD had significantly lower levels of Hb and serum iron. These results are consistent with our findings and suggest more than a simple overlap in between both the deficiencies.

John J. Sim et al<sup>17</sup> found that subjects with D25 deficiency had higher serum iron saturations, ferritins, and lower TIBC levels compared with those with normal D25 levels. Thus, iron deficiency based on laboratory studies could not account for the higher proportion of anemia in subjects with low D25 levels. Although iron adequacy may not be a factor of anemia, the higher levels of ferritin and lower serum albumin does suggest that malnutrition and inflammation may have been more prevalent in D25-deficient individuals which introduces a confounder for the association evaluated in this study.

There are limitations in this study, as it does not represent the general population and might be subject to selection bias. In addition, the number of subjects in the ID group was relatively small; therefore, the data are not sufficient to make an accurate comparison between the groups.

## CONCLUSION

To the best of our knowledge, this is the first study to address IDA and VDD relationship among adult population as well as among male and female. The results of the present study showed that Vitamin D metabolism is dependent on iron and its deficiency might disturb Vitamin D activation. Although there are some limitations to this study including small sample size, seasonal variation, and unanalysed hepcidin levels, we clearly demonstrate that vitamin D deficiency was much more prevalent in anemic patients regardless of

age, gender, or anemia subtype. Therefore, serum vitamin D measurement should be considered in the management of anemia. Also further studies with large sample sizes and proper designs are needed to highlight underlying cellular, molecular, and genetic mechanisms and to generate good quality evidence.

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