

# Study of Serum 25-Hydroxy Vitamin D Levels in Anemia – A Case Control Study

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## ABSTRACT

**Introduction:** Vitamin D is a fat-soluble vitamin. It has diverse biological effects extending beyond the maintenance of calcium and phosphorus homeostasis and ensuring the proper functioning of the body. Anemia and Vitamin D deficiency are both important health issues, however, the nature of association between them remain unresolved. The objectives of this study were (1) To estimate and compare the serum levels of Vitamin D in cases of anemia and healthy controls (2) To comprehend the association between Vitamin D levels and anemia.

**Materials and methods:** It was a case control study. Thirty (n=30) diagnosed cases of anemia (hemoglobin <11g/dl) and thirty (n=30) age and sex matched healthy controls were included in the study. 25-Hydroxy vitamin D(25-OH Vitamin D) levels were measured in venous blood by chemiluminescence immunoassay method. Other hematological parameters were also measured simultaneously. Statistical analysis was done using student 't' test. Pearson's correlation was performed to establish the relationship between study variables.

**Results:** The study showed statistically significant decrease (p<0.05) in serum 25 OH vitamin D levels in cases compared to controls. Our study showed a positive correlation between 25 OH vitamin D and hemoglobin levels.

**Conclusion:** Anemia is associated with decreased vitamin D levels. Early detection of vitamin D deficiency and supplementation may lead to improvement of anemia.

**Keywords :** Anemia, 25 OH Vitamin D, haemoglobin

## INTRODUCTION

Anemia is a major global health concern due to its high prevalence and association with substantial morbidity and mortality.<sup>1,2</sup> It is defined by a decrease in the total amount of hemoglobin or the number of red blood cells below the critical level. Despite its importance in public health and the consistent implementation of strategies to control anemia, its prevalence remains relatively unchanged.<sup>3</sup> This is attributable to several reasons: (1) the multifactorial and interactive nature of the etiologies making anemia difficult to prevent or treat, (2) the comorbidities related to anemia, such as chronic kidney disease, have an increasing frequency, and (3) the distribution of the population around the world is shifted toward the elderly, who have a high prevalence of anemia.<sup>4-6</sup> Vitamin D also known as sunshine vitamin, a fat soluble vitamin has diverse biological effects. 25 hydroxy Vitamin D (25 OH vitamin D) is a hormone precursor that is present in 2 forms. Ergocalciferol, or vitamin D<sub>2</sub>, is present in plants and some fish. Cholecalciferol, or vitamin D<sub>3</sub>, is synthesized in the skin by sunlight. In addition to absorption of calcium, recent epidemiologic studies have observed relationships between low vitamin D levels and multiple disease states,

probably caused by its anti-inflammatory and immune-modulating properties and possible effects on cytokine levels.

Vitamin D<sub>3</sub> is synthesized from 7-dehydrocholesterol in the skin. The vitamin D binding protein transports the vitamin D<sub>3</sub> to the liver where it undergoes hydroxylation to 25(OH) D (the inactive form of vitamin D) and then to the kidneys where it is hydroxylated by the enzyme 1 alpha hydroxylase to 1,25(OH)<sub>2</sub>D, its active form. This enzyme is also present in a variety of extrarenal sites, including osteoclasts, skin, colon, brain, and macrophages, which may be the cause of its broad-ranging effects.<sup>7</sup>

Clinical and physiological studies focused on its multidirectional involvement in several metabolic pathways. Vitamin D deficiency is most under diagnosed and untreated nutritional deficiency in the world. Recently the role of vitamin D in erythropoiesis has been suggested but evidences are limited in general population.<sup>8-14</sup> We hypothesized that vitamin D has an association with anemia and its supplementation along with iron may aid faster recovery.

The objectives of this study were to estimate and compare the serum levels of 25-hydroxy vitamin D(25-OH Vitamin D) in cases of anemia and healthy controls and to comprehend the association between levels of 25-OH Vitamin D and anemia.

## MATERIALS AND METHODS

It was a case control study. Thirty(n=30) diagnosed cases of anemia ( hemoglobin <11g/dl) and thirty(n=30) age and sex matched healthy controls in the age group 20-50 years, attending HSK hospital were included in the study. Duration of study was from June-August 2014. Ethical clearance was obtained from the institute's ethical clearance committee. Informed consent was taken from the subjects.

Anemia was defined by hemoglobin levels < 11g/dl - World Health Organisation criteria for defining anemia. Vitamin D deficiency was defined as serum values < 20 ng/ml<sup>15</sup> (Normal->30ng/ml, Insufficiency-20-29.9ng/ml, Deficiency<20ng/ml).

**Exclusion criteria:** Subjects with h/o blood loss –trauma / gastrointestinal loss, blood transfusion, thalassemia /sickle

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cell anemia, those having recent malaria, jaundice, malabsorption syndromes-IBD, hepatic and renal failure, those who are on recent medications glucocorticoids, antifungals, antiseizure, antiretroviral drugs, Diabetes Mellitus were excluded from the study. Elderly and postmenopausal women, pregnant women were also excluded.

**Biochemical analysis**

A sample of 5 ml venous blood was collected (3ml=plain, 2ml=EDTA –Ethylene Diamine Tetra Acetic acid). Plain blood was centrifuged and serum was separated. 25-OH Vitamin D levels were measured in serum by chemiluminescence immunoassay (CLIA) method using Snibe Maglumi 1000. If electing to test vitamin D status, serum 25 hydroxy vitamin D is the accepted biomarker.<sup>16</sup> Although 1,25-OH-D is the active circulating form of vitamin D, measuring this level is not helpful because it is quickly and tightly regulated by the kidney.

EDTA blood was used to measure red blood indices. Hemoglobin levels, Red Blood Cell count (RBC), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC) were measured using Swelab Alfa fully automated hematology analyser

**STATISTICAL ANALYSIS**

Data was expressed in terms of mean ± SD. Chi-square test was applied to estimate the difference between the two groups of population. Unpaired ‘t’-test was used to study the changes in serum 25 OH vitamin D levels between the study

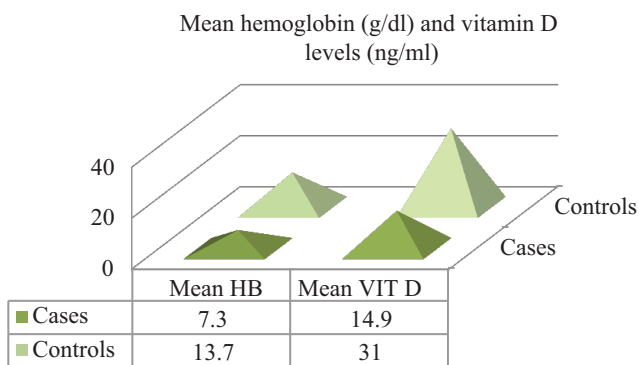
groups. Pearson correlation was performed to establish the relationship between study variables. p value <0.05 was considered statistically significant.

**RESULTS**

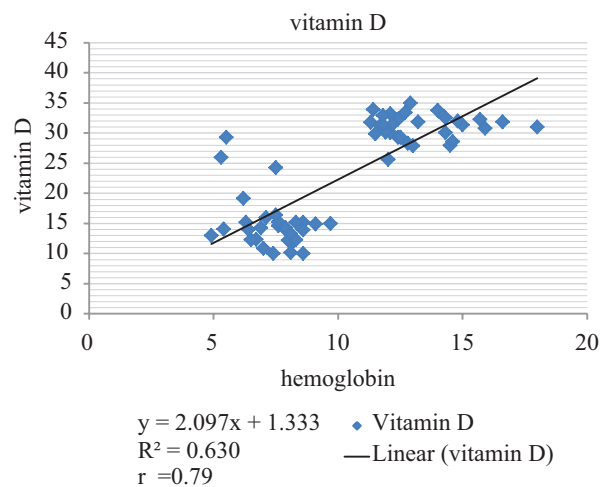
This was a comparative case control study conducted on thirty (n=30) diagnosed cases of anemia (hemoglobin <11g/dl) and 30 age and sex matched healthy controls (n=30). Serum 25-OH vitamin D was estimated, analyzed and correlated with haemoglobin and other blood indices. The results were expressed as mean ± standard deviation. The mean age (in years) of cases was 45.3±13.1 years and that of controls was 41.4±11.2 years and was not significant (p=0.20).

Table-1 shows serum hemoglobin, 25-OH vitamin D levels and other hematological parameters in both groups. The mean serum hemoglobin levels was 7.3±1.1 g/dL in cases of anemia and that in controls was 13.3 ±1.7 g/dL. The mean 25-OH vitamin D levels in cases was 14.9 ±4.4 ng/mL and that in controls was 31.0±2.08 ng/mL. Other hematological parameters such as RBC count, MCV, MCH and MCHC are also mentioned in Table 1 and figure 1.

**Correlation between 25-OH vitamin D and hemoglobin:** there was a positive correlation between hemoglobin and 25-OH Vitamin D (r=+0.794, p=0.001) and was statistically significant. (Figure 2). Similarly there was a significant positive correlation between hemoglobin and MCV. (r=+0.69, p=0.001). (Figure 3).



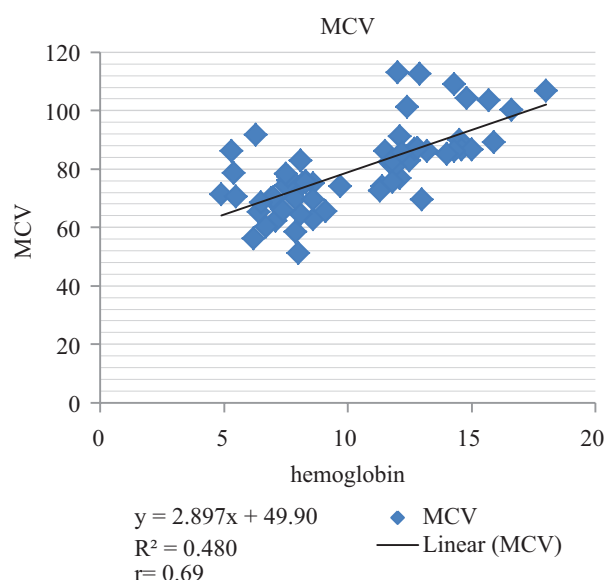
**Figure-1:** Serum hemoglobin and 25-OH vitamin D levels in both groups



**Figure-2:** Correlation between 25-OH vitamin D and hemoglobin

Characteristics	Groups	Mean ±SD	t	p	Significance
25-OH vitamin D (ng/ml)	Cases	14.9 ± 4.4	17.9	0.001	Statistically significant
	Controls	31.0 ± 2.08			
Haemoglobin(g/dl)	Cases	7.3 ± 1.1	15.6	0.001	
	Controls	13.3 ± 1.7			
RBC (millions/cu mm )	Cases	2.6 ± 0.73	9.2	0.001	
	Controls	4.1 ± 0.57			
MCV (fl)	Cases	70.5 ± 8.8	6.9	0.001	
	Controls	89.1 ± 11.7			
MCH (pg)	Cases	27.2 ± 5.4	4.2	0.001	
	Controls	32.4 ± 3.8			
MCHC (g/dl)	Cases	36.2 ± 3.0	0.46	0.001	
	Controls	36.4 ± 1.1			

**Table-1:** Serum hemoglobin, 25-OH vitamin D and other Hematological parameters in both groups



**Figure-3:** Correlation between hemoglobin and MCV

## DISCUSSION

Anemia and vitamin D deficiency are both important health issues, but their potential relationship remains less established in the general population. Our study demonstrated decreased levels of vitamin D in cases of anemia. We also found a significant positive correlation between hemoglobin and 25-OH vitamin D as well as between hemoglobin and MCV-mean corpuscular volume of the red blood cell. Vitamin D has favorable pleiotropic actions beyond its pivotal role in calcium homeostasis and bone metabolism.<sup>17</sup> Indeed, previous clinical studies have shown that the risks of various nonskeletal diseases and mortality increase as serum 25 (OH)D levels decrease.<sup>18</sup> Vitamin D supplementation has long been known to improve anemia and reduce the need for erythropoietin in dialysis patients.<sup>19,20</sup> However, the underlying mechanism has not been established to date. It is suggested that vitamin D plays a role in the suppression of the inflammatory milieu that contributes to the development of anemia. The reduction of inflammatory cytokines after vitamin D supplementation in some experimental studies supports this possibility.<sup>21,22</sup> An observational study also supports this mechanism, demonstrating that low vitamin D is associated with a high risk of anemia of chronic inflammation.<sup>23</sup> A direct effect of vitamin D on erythroid precursors is also possible. Vitamin D in hematopoietic tissues affects the proliferation of erythroid precursor cells via increased calcium permeability<sup>24</sup> or increased erythropoietin receptor expression.<sup>25</sup> Furthermore, vitamin D can affect hematopoietic tissue in a paracrine fashion because the vitamin D receptor is also expressed in bone marrow.<sup>26</sup> Anemia in itself may have predisposed patients to 25-OH vitamin D deficiency as anemic patients due to chronic fatigue may have been less likely to go outside and obtain adequate sunlight.

Vitamin D appears to be associated with anemia. One possibility is that vitamin D modulates the level of systemic cytokine production thus reducing the inflammatory milieu that leads to anemia of chronic disease. Both in vivo and in vitro studies have demonstrated that calcitriol (1,25 hydroxyvitamin D) reduces cytokine production. This may suggest a

reduced state of chronic systemic inflammation in those with normal D25 or an ineffective erythropoiesis in D25-deficient patients.<sup>9</sup> Another possible mechanism is that vitamin D directly stimulates erythroid precursors. Vitamin D receptors have been discovered in numerous non-renal target tissues including the bone marrow.<sup>27,28</sup> Normalizing tissue D25 levels may provide an adequate substrate for local tissue production of 1,25 hydroxyvitamin D in hematopoietic tissues via extra-renal tissue activity of the 1-alpha hydroxylase enzyme. Hematons (the buffy coat of bone marrow containing erythroid precursors, fibroblast, endothelial cells, lipid laden cells, and macrophages) have been demonstrated to contain significantly higher concentrations of D25 and 1,25-hydroxyvitamin D levels than bone marrow plasma.<sup>9,29</sup> High local concentrations of 1,25 hydroxyvitamin D in hematopoietic tissues may then directly activate erythroid precursor cells in a paracrine fashion. Future experimental studies are needed to establish the underlying mechanisms of the present cross-sectional study results.

## Limitations of the study

Small number of the sample. Severity of anemia was not considered (mild, moderate, severe). Anemia was not separated based on gender (females have lower hemoglobin levels). Menstrual status of female subjects was not considered.

## Conclusion

We conclude that anemia is positively correlated with vitamin D deficiency. Long term vitamin D deficiency may contribute to chronic inflammation and anemia. Vitamin D supplementation along with iron supplementation may help in faster recovery of anemia.

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