Original Research Article

Vitamin D deficiency and anemia - A cross-sectional study

Sanni Kumari[1], Uday Kumar[2], Rekha Kumari[3]

[1]Junior Resident, [2]Professor And Hod, [3]Additional Professor, Department of Biochemistry, IGIMS, Patna-800014. India.
Email: sannikumari@yahoo.com

Abstract

Background: Vitamin D deficiency has a high percentage of incidence throughout the world. It is also associated with several chronic medical conditions. Vitamin D deficiency and anaemia previously has been found to be associated in various apparently healthy and diseased populations. Vitamin D has also been suggested to have an effect on erythropoiesis. Thus we aimed to investigate the link between vitamin D status and anaemia. **Methodology:** A cross-sectional analysis in the period between July 2017 and March 2018 of subjects with documented concurrent levels of 25-hydroxyvitamin-D and hemoglobin were evaluated. Vitamin D deficiency was defined as <30ng/ml and anemia was defined as hemoglobin < 11g/dl. A total of 100 subjects were included in the analysis. **Results:** Anemia was present in 48.3% of 25hydroxyvitamin D deficient subjects compared with 25% with normal 25-hydroxyvitamin D levels (p value=0.01). 25-hydroxyvitamin D-deficient subjects had a lower mean Hb (10.778±1.97 vs. 11.677±1.70; p=0.03). **Conclusion:** The observation of the present study demonstrates an association of vitamin D deficiency and a greater risk of anemia. Future studies are warranted to examine whether vitamin D directly affects erythropoiesis.
Key Word: anemia, vitamin- D**,** erythropoiesis.

***Address for Correspondence:**
Dr Sanni Kumari, Junior Resident, Department of Biochemistry, IGIMS, Patna-800014, Bihar, INDIA.
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INTRODUCTION

The role of vitamin D in health continues to be particularly associated with extra-skeletal functions, but in recent years an association between Vitamin D and anaemia has emerged, indicating the fact that there are potential roles of Vitamin D in iron homeostasis and erythropoiesis. The sequential hydroxylation of cholecalciferol in the liver and in the kidney is the essential physiology of vitamin D. This leads to the formation of 25-hydroxivitamin D (25[OH]D) and 1,25(OH)D in the liver and in the kidney respectively, the latter being the hormonally active form of vitamin[1]. To evaluate the total vitamin D level in the body serum 25(OH)D proves to be the best indicator as the metabolite is the main circulating form[2]. The higher incidence of vitamin D is associated with a number of serious diseases including heart disease, cancer and infections as this deficiency may have important implications for extra-skeletal health.[3-5] Vitamin D has also been found to have an important association with anaemia in recent years[6,7]. Anaemia is one of the major health concern globally due to its high prevalence and association with substantial morbidity and mortality.[8,9] About 2 billion people are suffering from this disease worldwide, estimated by the World Health Organisation (WHO) and approximately 50% of all anaemia cases are diagnosed as iron deficiency anaemia (IDA).[10,11]

Anaemia is attributed by a decreased concentration of erythrocytes or Hb, which leads to an impaired oxygen transport throughout the body. Furthermore, it is related with a number of chronic conditions, including kidney disease and CVD[12,13]. Based on multifactorial etiology anaemia can be classified into different subtypes such as iron deficiency anaemia or anaemia of nutrient deficiency and anaemia of inflammation[14]. The association between vitamin D status and anaemia have been observed in several different studies but only including populations

such as children, the elderly, and patients with chronic diseases or those with heart failure[6,15-17]. However, the relationship between anaemia and vitamin D status in the generally healthy adult population has not been well described. Previous studies have found that low vitamin D status had an association with anaemia risk in children, elderly population, patients with CKD and heart failure[6,16,17,18]. Vitamin D may have favourable impact on anaemia, particularly anaemia of inflammation through its down-regulatory effects on inflammatory cytokines. The mechanism underlying this relationship between vitamin D status and anaemia with inflammation involves the antimicrobial peptide hepcidin, a hormone which is involved in the regulation of Fe recycling in the body that is induced by pro-inflammatory cytokines including IL-6[19-21]. Under chronic inflammatory conditions, Fe can become sequestered within the cells of the reticuloendothelial system and become unavailable for erythropoiesis, which may ultimately lead to anaemia[22,23]. The pathway which contributes to anaemia of inflammation is through depressed erythropoiesis and reduced red blood cell (RBC) lifespan[24]. Recently, vitamin D has been reported to lower inflammatory cytokines implicated in the pathophysiology of anaemia with inflammation, and suppress expression of hepcidin mRNA[25,26]. Thus, vitamin D may reduce the risk of anaemia through its anti-inflammatory effects. Taken together, previous epidemiological studies provide strong evidence for the link between vitamin D deficiency and anaemia. However, several of these studies are limited by their cross-sectional nature. Thus the present study aimed to examine the association between vitamin D status and anaemia in a generally healthy adult population.

METHODOLOGY

It was a case-controlled, cross sectional study. This study was carried out in the Department of Biochemistry, IGIMS, Patna, between July 2017 and March 2018. We selected total 100 samples, apparently are healthy adult subjects as controls 40 and 60 patients having 25-Hydroxyvitamin D deficiency during the study period with age ranged between (30- 60) years. The data of Vitamin D3, serum iron levels, total iron binding capacity and transferring saturation in blood were measured. Anaemia was defined as an Hb level <11g/dL for both men and women. D25 deficiency was defined as a level <30 ng/ mL. Upon enrolment, participants completed questionnaires on demographic information, personal and family health history, existing health status, and medication and supplement use.

Inclusion Criteria:

Case Group:

- All patients who were presented with 25-Hydroxyvitamin D deficiency.
- Patients aged between 30 to 60 years.

Control Group:

- Apparently healthy adult population aged between 30 to 60 years.

Exclusion Criteria:

- Patients with a hospitalisation for acute or chronic disease within the previous year.
- Patients with history of substance/drug abuse or alcoholism.
- Patients having existing active malignant neoplasm.
- Patients having history of malignancy.
- Patients with uncontrolled or poorly controlled autoimmune, cardiovascular, endocrine, gastrointestinal, hematologic, infectious, inflammatory, musculoskeletal, neurologic, psychiatric or respiratory disease.

Statistical Analysis: Statistical analysis was performed using the Statistical Package for the Social Science for Windows (SPSS), version 20. Parametric and non-parametric quantitative variables were expressed as mean ± standard variation (SD) of the mean, interval and median percentiles interval respectively. The chi-square test was applied for comparing qualitative variables. The independent Student's t test was used to compare means between parametric variables. Any p value under 0.05 was considered statistically significant.

RESULTS

Total 100 samples apparently healthy were selected as study participants. Among them 60 patients were presented with 25-Hydroxyvitamin D deficiency and selected as control group. Remaining 40 patients who had a normal level of 25-Hydroxyvitamin D were selected as case group. The demographic and health status characteristics of study subjects are presented in Table 1. Study participants of case and control group were comparable in terms of age and sex. The mean age was 47.73 and 48.1 years respectively for case and control group with no significant difference (p value=0.848). 71.7% o case group and 72.5% of control group were female. Prevalence of anaemia was significantly higher (48.3%) in patients with 25-hydroxyvitamin D-deficient subjects compared with 25% with normal 25-hydroxyvitamin D levels (p<0.01). the mean 25-hydroxyvitamin D level in case and control group was 11.806±0.70 and 36.412±4.46 ng/dL respectively with statistically significant difference (p value= 0.001). The mean haemoglobin level (10.788±1.97%) is significantly lower in patients with decreased concentration of 25-

hydroxyvitamin D compared to patients (11.677±1.70%) with normal levels of 25-hydroxyvitamin D (p value=0.03). Statistically significant difference was observed among patients with normal 25-hydroxyvitamin D levels and 25-hydroxyvitamin D deficient subjects in terms of serum iron, TIBC and transferring saturation levels (p value=<0.05).

Table 1: Demographic and Health status Characteristics according to 25-Hydroxyvitamin D status

Parameters	Case Group (n=60) 25-Hydroxyvitamin D=<30ng/dL		Control Group (n=40) 25-Hydroxyvitamin D=≥30ng/dL		p value
Age (years)	**Mean**	**SD**	**Mean**	**SD**	**0.848**
	47.733	±9.06	48.100	±8.79	
Sex	**Frequency**	**%**	**Frequency**	**%**	
Male	17	28.3	11	27.5	**Chi square -0.0083**
Female	43	71.7	29	72.5	**p. value- 0.927**
Anaemia	**Frequency**	**%**	**Frequency**	**%**	
Present	29	48.3	10	25.0	**Chi square -5.492**
Absent	31	51.7	30	75.0	**P. value- 0.01**
		Biochemical Parameters			
Hb% (gm/dL)	10.778	±1.97	11.677	±1.70	**0.03**
25-(OH)D3 (ng/dL)	11.806	±0.70	36.412	±4.46	**0.001**
Serum Iron (µg/dL)	69.235	±15.71	75.912	±13.75	**0.002**
TIBC (µg/dL)	394.76	±50.69	374.320	±67.27	**0.005**
Transferrin Saturation (%)	17.699	±6.42	21.279	±6.13	**0.04**

Correlation between 25-hydroxyvitamin D levels and other biochemical parameters among case and control group is shown in Table 2. The obtained data indicated that 25-hydroxyvitamin D is associated with Hb% and serum iron level and inversely associated with TIBC. It has positive significant correlation with haemoglobin (p value=0.001), and iron level (p value=0.002) and inverse correlation with TIBC (p value=0.002) in patients with normal level of 25-hydroxyvitamin D.

Table 2: Correlation between 25-Hydroxyvitamin D and other parameters

Parameters	Hb%	Serum Iron	TIBC	Transferrin Saturation
25-Hydroxyvitamin D=<30ng/dL (case)	**.421**	**.399**	**-.213**	**.332**
p value	**0.001**	**0.002**	**0.002**	**0.009**
25-Hydroxyvitamin D=<30ng/dL (control)	**.069**	**.214**	**-.129**	**.217**
p value	**.321**	**.291**	**.428**	**.118**

DISCUSSION

The present study indicates that 25-hydroxyvitamin D deficiency or insufficiency status was associated with and increased risk of anaemia. The prevalence of anaemia was significantly higher (48.3%) in patients with 25-hydroxyvitamin D deficiency compared to subjects (25%) with normal level of 25-hydroxyvitamin D. The mean level of serum haemoglobin is significantly lower in patients with 25-hydroxyvitamin D deficiency compared to contol group. Previous studies have showed an association between vitamin D3 level and an increased risk of iron deficiency anaemia among patients without any chronic kidney disease[6,16]. The mechanism behind this association is not known clearly. This could be a potential influence of vitamin d deficiency and an increased risk of reticulocytosis and iron deficiency anaemia[7]. The findings from the present study are consistent with the hypothesized mechanism behind the vitamin D and anaemia relationship. The deficiency of vitamin D has been recommended to have an effect on erythropoiesis including cellular proliferation and differentiation and it's well documented role in the regulation of bone and mineral metabolism[27,28]. Secondly, it has been shown that vitamin D has influence on bone marrow function specially with the findings that levels of calcitriol are several hundred fold higher in bone marrow compared to plasma[29,30]. It is established that vitamin D regulates the level of systemic cytokine production and reduces the inflammatory milieu which lead to anaemia of chronic disease or inflammation[7]. For instance, vitamin D has been found to impair cytokine release and possibly exerts a direct stimulatory effect on erythroid precursors, as its receptors are also seen in different non-renal target sites, like the bone marrow[28]. So it is definite that 25(OH)D deficiency could lead to reduced local calcitriol production in the bone marrow which can limit erythropoiesis. These findings can be explained by the fact that calcitriol has a direct proliferative action on erythroid burst forming units and also up-regulates the expression of the erythropoietin receptor on erythroid progenitor cells[31-33]. So, the several non-calcemic actions

of vitamin D appear to revolve around d the improvement of anaemia. Remarkably, it is evident from the previous population-based studies that the association between vitamin D and anaemia risk may vary in terms of the cause behind anaemia as well as the racial profile of the subjects[15,34,35-37]. **Lee *et al.*** found that among Korean children, the lowest quartile of 25(OH)D was associated with increased odds of anaemia in females, but the effect was attenuated to non-significance after adjusting for iron deficiency [34]. In a cross-sectional study, conducted among 10,410 children and adolescents aged 1–21 years, **Atkinson *et al.*** observed the relationship between 25(OH)D deficiency and anaemia in a cohort of otherwise-healthy children, and also determined whether race was a modifying factor in this association[15]. The observations of their study suggested that 25(OH)D deficiency was associated with increased risk of anaemia in healthy children, but 25(OH)D-threshold levels for lower haemoglobin were lower in black children than white children. In a cross-sectional study conducted among 554 subjects aged above 17 years, **Sim *et al.*** evaluated the prevalence of anaemia in those with vitamin D deficiency compared to those who had normal levels of the vitamin[7]. They found a statistically significant difference between the prevalence of anaemia in 25(OH)D-deficient subjects and its prevalence in those with normal 25(OH)D levels. In addition, 25(OH) D-deficient subjects had lower mean haemoglobin and more prevalent use of ESA. Nevertheless, this study appears to be one of the pioneer works to investigate the relationship between vitamin D and anaemia by enrolling subjects without CKD and/or not on ESA. The observations of the previous studies confirm that changes in vitamin D metabolism are intricately related to iron deficiency, because heme-bound iron is essential in the hydroxylation process of vitamin D[38,39].

CONCLUSION

Based on our findings at the end of the study we can conclude that vitamin D deficiency is associated with the risk of anaemia in apparently healthy populations. The analysis of our study suggests that low 25(OH)D level are strongly associated with lower levels of haemoglobin. Further longitudinal studies are essential to confirm the findings of our study and to establish the mechanisms behind the observed relationship between 25(OH)D levels and anaemia.

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