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Original Research Article

Vitamin D Levels in Vegetarian Patients Diagnosed Anemia with Vitamin B_{12} Deficiency

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Abstract

Background: Vitamin B12 deficiency has been a well known health problem. Recently, there are certain studies proving it more common as previously understood. Many have sorted for vegetarian food, with about 10% of the population deciding not to consume animal foods. This depends on ethical, ecological and religious belief, but also on health reasons. When the reasons are ethical, individuals ignore on nutritional value. The link between vitamin D and anemia has emerged in recent years, indicating potential roles for vitamin D in iron homeostasis and erythropoiesis. Very few studies exist on vitamin D levels in anemia. Studies are including vitamin D and vitamin B12 are significantly few. Hence, the present this study aims to find the trend of vitamin D levels in vegetarians having vitamin B12 deficiency. ***Methods:*** All clinical and lab data of cases from Jan 2017 to May 2018 from the lab in which Vitamin B12 assay has been done was obtained from lab records. The vitamin B12 assay was done on immunoassay analyzer Roche e411 with chemilumeiscence method. Vitamin B12 levels <180ng/dl was considered deficient. The same was again grouped based on Vitamin D levels as deficient and insufficient. ***Results*:** The percentage of subjects in deficient group with low vitamin B12 levels had decreased haemoglobin <11 was 35% when compared with sufficient of 9.4%, P value = 0.02 was significantly correlating. Similarly the MCV(>97) and MCHC(<33) had higher percentage in insufficient Vit D3<30 group with 47.3% and 56.3% respectively. Both parameters also have significant P value being < 0.05. ***Conclusion*:** The study found that apart from vitamin B12 deficiency, there are higher chances of these patients being associated with vitamin D3 deficiency.
Keywords: Vitamin B12 deficiency, haemoglobin, anemia.
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INTRODUCTION

Anemia is known to affect more than 1.6 billion people worldwide and about 80 % is contributed by South Asia and Africa alone [1-3]. The prevalence of anemia in India is particularly high, where 50 % of reproductive age women, 59 % of pregnant women, 25 % of men, 40 % of adolescent girls, and 70 % of children under five years are anemic [4, 5]. Iron deficiency is the most common among all. As studies done in India found that iron deficiency is a major cause [6]. Vitamin B12 and folate deficiency have also been identified by some studies [7, 8]. Vitamin B12 deficiency has been a well known health problem. Recently, there are certain studies proving it more common as previously understood [9]. Vitamin B12 deficiency expresses itself, from haematological manifestation such as megaloblastic anaemia to a neurological one such as subacute combined degeneration of the spinal cord [10, 11]. Vitamin B12, also called cobalamin (Cbl), is present only in animal foods. This deficiency is common among vegetarians and is the result of a very low intake [12]. Recently, many have sorted for vegetarian food, with about 10% of the population deciding not to consume animal foods [13]. This depends on ethical, ecological and religious belief, but also on health reasons. When the reasons are ethical, individuals ignore on nutritional value [14]. Studies have show that the reduction or exclusion of animal foods may reduce the risk of Coronary Heart Disease (CHD) and Type 2 Diabetes (T2D) through modifiable factors such as body mass, serum glucose, blood pressure and serum lipid profile. These disorders contribute to a high mortality rate in Western countries [15-19]. However, the risk of possible nutritional deficiencies in a non-balanced vegetarian diet, the absence of important nutrients that can neutralise these advantages [20]. The link between vitamin D and anemia has emerged in recent years, indicating potential roles for vitamin D in iron homeostasis and erythropoiesis. This association has been described in several observational studies in various healthy and diseased populations [21-27], the mechanism may be due to the action of vitamin D on inflammatory cytokines and the antimicrobial peptide, hepcidin (the hormone responsible for regulating systemic iron concentrations) [28, 29]. Percentage of vegetarians in north Karnataka is high due to religious factors. Many studies on anemia due to vitamin b12 deficiency have been done as quoted earlier. Very few studies exist on vitamin D levels in anemia. Studies including vitamin D3 and vitamin B12 are significantly few. Hence, the present this study aims to find the trend of vitamin D3 levels in vegetarians having vitamin B12 deficiency.

Methods and study design

This study included only vegetarians. The anemia was confirmed with clinical data, serum Vitamin B12 levels and Haematology profiles. We retrieved all clinical and lab data of cases from Jan 2017 to May 2018 from the lab in which Vitamin B12 assay has been done. The vitamin B12 assay was done on immunoassay analyzer Roche e411 with chemilumeiscence method. The number of cases included accounted to 125. These cases had also record of vitamin D3 assay done on same instrument and method. Haematological profile was done on Sysmex 3-part Differential Automated Haematology Analyzer. Institutional ethical approval was obtained and retrieved data was analysed on SPSS software. The vitamin B12 values <180pg/mL was considered as deficient. Based on vitamin D3 levels 125 included cases were classified into 3 groups as Deficient (<20ng/dL), Insufficient (21-29ng/dL) and Sufficient (>30ng/dL) (table 1). For ease to compare, it was again classified as Vitamin D3 <30ng/dl (Vit D3 <30) and Vitamin D3 >30ng/dL (Vit D3 >/= 30) (Table-2) [30]. Haematology profile included haemoglobin, total leukocyte count, platelet count (TLC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC).

The chi-square and Fisher's exact tests were used to compare the categorical variables, Student's t test was used to assess for continuous variables. Significance was evaluated using a two-sided p value of less than 0.05.

Results

Baseline characteristics and demographics along with hematologic profile are described in Table-1.

A total of 125 cases were studied and analysed. All cases, having Vitamin B12 deficiency were only included (Vit B12 <180ng/dl).

Table-1: Baseline characteristics and demographics along with hematologic profile

Vit B12 <180ng/dl	Deficient (<20)	Insufficient(21-29)	Sufficient(>30)
Number of individuals	31	53	41
Mean D25 level	9+/- 5	26+/-3	46+/-8
Mean Age (years)	65.3+/-18	62.1+/-13	64.2+/-8
Female percentage	41.3%	31.4%	27.3%
Haemoglobin (g/dl)(<11)	33.7%	36.9%	9.4%
Total leucocyte count (cells/cmm)(<4000)	8.7%	11.5%	9.8%
Platelet count (per cmm)(<1.5 lakh)	1.3%	1.2%	0.3%
Mean corpuscular volume (fl)(>97)	33.3%	61.4%	17.3%
Mean corpuscular haemoglobin (pg)(<26)	63.3%	41.4%	47.3%
Mean corpuscular haemoglobin concentration (g/dl)(<33)	51.3%	61.4%	17.3%

31 cases had severe deficiency (vit D3 < 20) with mean age at 65.3. The percentage of vitamin deficiency was more in broader classification (Table-1). Whereas, female percentage was higher in Vit D3 <30 was 36.2% (Table-2). This shows that finding tendency of vitamin D in females more common in presence of vitamin B12 deficiency. Mean age average in all cases was 63.9+/-16 with 80yrs being oldest case. 84 subjects were Vit D3<30 deficient and 41 had sufficient Vit D3 (>30). Among subjects with Vit D3 deficiency (D3 levels <30 ng/ml), 47.7% had an MCV >97 fl with p=<0.10 vs. 17.3 % when compared with sufficient group. Haemoglobin levels <11g/dl was 35.3% in deficient when compared sufficient of 9.4% (p =0.02) which is significant. Mean vitamin D3 17.5ng/dL in deficient and 46ng/dL in sufficient. The percent of low Mean corpuscular haemoglobin concentration (MCHC) (< 33 g/dL) was 35% which is when compared with sufficient group (17%)

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Table-2: Modified grouping of Table 1 based on John J. Sim *et al.*, [32] comparing Vitamin B12 with vitamin D3 levels

Vit B12 <180ng/dl	Deficient (Vit D3<30ng/dL)	Sufficient (Vit D3>/=30ng/dL)	P value
Number of individuals(n=125)	84 (67.2%)	41(32.8%)	
Mean D25 level	17.5+/-4	46+/-8	
Mean Age (years)	63.7+/-15	64.2+/-8	0.04
Female percentage	36.2%	27.3%	0.16
Haemoglobin (g/dl)(<11)	35.3%	9.4%	0.02
Total leucocyte count (cells/cmm)(<4000)	10.1%	9.8%	0.052
Platelet count (per cmm)(<1.5 lakh)	1.2%	0.3%	0.30
Mean corpuscular volume (fl)(>97)	47.3%	17.3%	0.10
Mean corpuscular haemoglobin (pg)(<26)	52.3%	47.3%	0.03
Mean corpuscular haemoglobin concentration (g/dl)(<33)	56.3%	17.3%	0.06

P value < 0.05 is significant

There are a greater number of individuals with vitamin D3 deficiency in vitamin B12 deficiency anemia, since the percentage of individuals falling under vitamin D3 deficiency is 67% when compared with sufficient 41%. The percentage of subjects in insufficient group with low vitamin B12 levels having decreased haemoglobin <11 was 35% when compared with sufficient of 9.4%, P value = 0.02 was significantly correlating (Table-2). Similarly the MCV (>97) and MCHC (<33) had higher percentage in insufficient Vit D3<30 group with 47.3% and 56.3% respectively. Both parameters also have significant P value being < 0.05 (Table-2).

Discussion

The study made us find a relative association between vitamin D3 deficiency and anemia with Vitamin B12 deficiency. Establishing the relationship is difficult since there are lot of other factors like anemia might have created less mobility which leads to less exposure to sun resulting in Vitamin D3 deficiency. Majority of the patient were elderly group and had restricted movement, might also have contributed to less exposure to sun. Patients also have low vitamin B12 with normal haemoglobin levels were also found to have vitamin D3 deficiency. Vitamin D appears to be associated with anemia; though the mechanism is unknown. 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 Vitamin D3 affects cytokine production [31]. John J Sim *et al.*, [32] observed that patients with normal Vitamin D3 levels had lower ferritin levels than D25-deficient patients. Which may suggest a reduced state of chronic systemic inflammation in those with normal D3 or an ineffective erythropoiesis in D3-deficient patients. Our study found that vitamin B12 deficient levels had low vitamin D3 levels in elderly and female population, the percentage of low Hb, with high MCV and decreased MCHC shows that finding of anemia with Vitamin B12 deficiency has more probability of having vitamin D3 deficiency also. Baseline characteristics show higher percentage of vitamin D deficiency in aged and female population.

The choice of not eating foods of animal origin from the diet due to religious factors is common in north Karnataka region. Also factors relating to health and local availability of animal foods due to local restrictions also decrease consumption. Though the vegetarian diet is good, but not consuming food of animal origin can lead to anemia with vitamin B12 deficiency with added vitamin D deficiency [33]. Environmental and health reasons, also pose doubts over whether a number of these restrictions could be detrimental or useful [34]. The vegetarian diet can be sustainable at all.

Stages of life and in all physiological conditions, including infancy, pregnancy, lactation, senescence and sports [35]. Compared to non-vegetarians, vegetarians have reduced body mass index (BMI), Serum cholesterol, serum glucose and blood pressure with a lower mortality rate due to ischemic heart disease (IHD) [36, 37]. However, underestimating the correct supplementation of cobalamin (Cbl) can nullify these benefits [38].

Conclusion

Vitamin B12 deficiency has variable features but is usually associated with megaloblastosis in the bone marrow, macrocytosis in the peripheral smear and a raised MCV. Pernicious anaemia is being most common form of vitamin B12 deficiency in the Western countries. It also seen in folate deficiency. In contrast, in India, pernicious anaemia is uncommon. Recent data have suggested that vitamin B12 deficiency is also an important cause in India [34-38]. North Karnataka population being more vegetarians due to religious factors, finding vitamin B12 is common. The study found that apart from vitamin B12 deficiency, there are higher chances of these patients associated with vitamin

D3 deficiency. The study need further evaluation on large scale proving vitamin D3 deficiency commonly associated with vitamin B12 deficiency in these regions.

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