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ORIGINAL RESEARCH ARTICLE

STUDY OF VITAMIN B_{12} STATUS AND THE CONSEQUENTIAL CLINICO-HEMATOLOGICAL PROFILE IN HEALTHY VEGETARIAN POPULATION IN NEPAL

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ABSTRACT

Background: A strict vegetarian diet has been associated with an increased risk of vitamin B12 deficiency. Clinical manifestations of cobalamin deficiency are hematologic and neurological symptoms. The purpose of this study was to determine the prevalence of vitamin B12 deficiency in healthy vegetarian population and to study the clinical and hematological profile of vitamin B12 deficient subjects. Methods: A community based cross-sectional observational study was conducted through preplanned camps in Kathmandu Nepal. 166 vegetarian people were investigated for serum vitamin B12 level and their hematological profile was done. They were examined for neurological manifestation of vitamin B12 deficiency. Results: The mean serum vitamin B12 level of the vegetarian in this study was 271.34 ±241.74 pg/ml (mean ± SD)., 53.6 % had serum vitamin B12 deficiency. 56.5 % of male and 47.1 % of female had vitamin B12 deficiency. The risk of vitamin B12 deficiency is more in males than females with odds ratio 1.201 (CI=95%) which is not statistically significant with p-value 0.312. There was no significant difference in vitamin B12 level in different age group.The study population had mean hemoglobin of 13.12 ± 1.72 gm/dl. Anemia was present in 34.9 % of study population. 33.9 % of male and 37.3 % of female had anemia. There was no significant difference in anemia in male and female vegetarian (p-value 0.72). 40% of patient who had vitamin B12 deficiency had anemia.A significant low hemoglobin level and higher MCV were found as level of serum vitamin B12 decreased. Only one patient had macrocytic anemia.There were few symptoms but not specific to vitamin B12 deficiency. Conclusion: The result of present study show that prevalence of vitamin B12 deficiency among healthy vegetarian was 53.6% with higher prevalence in male and elder age group.Prevalence of anemia was 34.9% in this study with elderly group and females being more affected.There was significant correlation between vitamin B12 and hemoglobin level and significant negative correlation with MCV. Although, not significant, leucocytes count, lymphocyte count and platelets were low in vitamin B12 deficient population.Few clinical features were present in vitamin B12 deficient subjects but neurological features specific to vitamin B12 deficiency were not seen.

Key words: Nepal, Vegetarian population, Vitamin B_{12} deficiency

INTRODUCTION

A substantial proportion of the population of Nepal adheres to a vegetarian diet for cultural and religious reasons. Vegetarian diets are most frequently sub-classified into lactovegetarian,ovovegetarian, lactoovovegetarian (depending whether the dietincludes only dairy products, eggs, or both, respectively),and vegan diets (avoiding all animal products). A vegetarian diet is considered to promote health and longevity, attributed to the antioxidants and anti-carcinogenic substances present in them[1]. Evidence indicates that plant-based diets are associated with a reduced risk of coronary heart disease,several types of cancer, and some other chronic degenerative diseases [2].

However, it is also known that animal foods provide micronutrients that are nonexistent or available only in limited amounts in plant foods, such as vitamin B_{12}. It is a water-soluble vitamin containing the mineral cobalt, so compounds with vitamin

B_{12} activity are collectively called "cobalamins". Methylcobalamin and 5-deoxyadenosylcobalamin are the 2 metabolically active forms of vitamin B_{12} in humans, that play a fundamental role in cell division and in one-carbon metabolism[3].Dietary sources are primarily of animal origin,including meats, dairy products, and eggs.Therefore, a strict vegetarian diet has been associated with an increased risk of vitamin B_{12} deficiency.[4]

Although early noticeable symptoms of vitamin B_{12} deficiency are nonspecific, the best-known clinical manifestations of cobalamin deficiency are hematologic and neurological symptoms. The associated typical hematologic changes are easy to detect, but the dilemma is that they develop at a late stage of the condition and may even be absent.[5,6]

The true prevalence of vitamin B_{12} deficiency is difficult to estimate because reports are based on values that vary because of inclusion criteria and individual laboratory methodology.[7]It is estimated to affect 3–40% of the adult population, depending on the diagnostic criteria used.[8]Studies from different countries like India,Taiwan and other European nations have reported lower mean serum concentrations of vitamin B_{12}among vegetarians than the omnivores. Even when the mean serum concentration of vitamin B_{12} is within the normal range, a significantly higher percentage of vegetarians have serum vitamin B_{12}<200pmol/l, indicating a deficient state, than do the percentage of omnivores in the same cohorts.

The main objective of the study was to determine the prevalence of vitamin B_{12} deficiency, based on study of serum vitamin B_{12}, in healthy vegetarian population of Nepal and to study the clinico-hematological manifestations in the deficient subjects.

METHODOLOGY

This was a community based, observational study performed in one of the vegetarian communities (Marwari community) of Kathmandu, Nepal, through preplanned camps. All the vegetarians were informed regarding the camps via advertisement in the local newspaper. All the vegetarians aged >16 years with no history of use of vitamin B_{12} within past 12 months were enrolled in the study after informed consent. The subjects who had history of pernicious anemia, gastrectomy, resection of small bowel, malabsorption or recent acute blood loss (of any cause) were excluded from the study.

A total of 179 subjects participated in the study. Out of 179, 13 were excluded from the study because 5 were already receiving vitamin B_{12} supplements, 1 was known case of malabsorption and 7 did not come for clinical examination. So total subjects enrolled in this study were 166.

Blood samples were taken from all subjects using aseptic precautions into 2 separate vacutainers- one simple tube for the separation of serum, second tube containing the anticoagulant EDTA for whole blood analysis of hematological parameters.Upon arrival at the laboratory, the serum fraction was obtained by centrifugation at 4000 rpm for 5 min, aliquotted and stored at -20°C until analysis.Serum vitamin B_{12} was estimated by fully automated, Chemiluminescent Immunoassay (CLIA) analyzer. On the whole blood sample, hemoglobin, a complete blood count and peripheral smear study were done. Those participants who had serum vitamin B_{12} lower than the reference range were contacted, and detail history taking and physical examination for any neurological abnormalities was done.

Vitamin B_{12} deficiency was defined based on serum vitamin B_{12} level less than 211pg/ml. Anemia was defined as hemoglobin less than 13 g/dl in male and 12 g/dl in female. Other markers of vitamin B_{12} deficiency (homocysteine and MMA) were not measured.Descriptive statistical analysis was made of the demographic data of the patients. For quantitative variables, data were given as mean ± SD, for qualitative variables, data were given as no (%). Chi square test was used to determine the level of significance and odds ratio in grouped variables. Pearson's correlation analysis and t test was applied to determine correlation and significance in continuous variables. All statistical tests were 2-sided and p-value ≤ 0.05 was considered to be statistically significant. SPSS ver. 17 was used for the analysis.

RESULTS

The mean age of the participants was 46.81 years ±11.26 years (Range: 18 – 72 years). Out of 166 participants, 69.3 % (n=115) were male and 30.7

% (n=51) were female with male to female ratio of 2.2:1. Majority of the participants (42.2 %) were of age 31-45 years and only 12 % (n=20) were of age>60 years.

The mean serum vitamin B_{12} level was 271.34 ±241.74 pg/ml. Using the cutoff of 211 pg/ml, 53.6 % (89/166) had vitamin B_{12}deficiency. The proportion of vitamin B_{12}deficiency in male and female was not significantly different (56.5% vs. 47.1%, NS).The risk of vitamin B_{12} deficiency was more, although statistically insignificant, in males than females (OR = 1.20, P = 0.31).

Although the highest percentage of vitamin B_{12} deficiency was observed in the age category of >60 years (55 %), the distribution of vitamin B_{12} deficiency was not significantly different between the different age categories. There was no strong association of age with vitamin B_{12} level (P = 0.9).

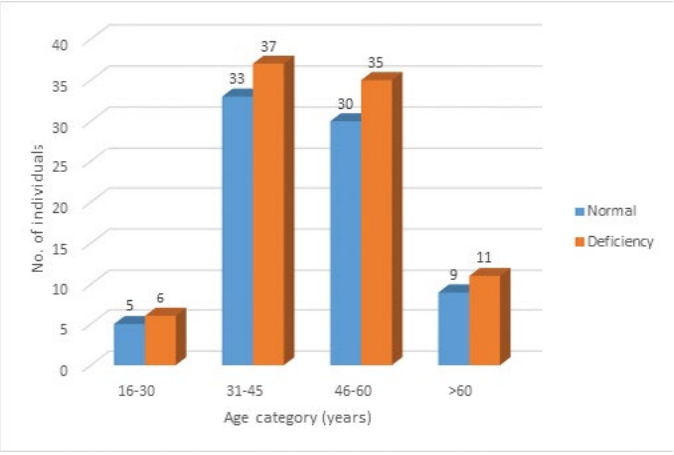

Figure 1: Distribution of vitamin B_{12} deficiency in different age categories

Vitamin B12 and hematological variables

The mean hemoglobin was 13.12 ± 1.72 g/dl (Range: 6.7 – 17.8 g/dl). Anemia was present in 34.9 % (58/166). There was no significant difference in the proportion of anemics in male and female vegetarian (33.9 % vs. 37.3 %, P= 0.72).

40% of patients who had vitamin B_{12}deficiency had anemia. There was significant positive correlation between vitamin B_{12} level and hemoglobin (Pearson's r = 0.20, P =0.01). The WBC count and platelets count was within normal range for all the subjects. The mean MCV was 88.2 ± 7.7 (Range: 75.5 – 100.0). Only 1 patient had macrocytic anemia. There was significant negative correlation between vitamin B_{12} level and MCV (Pearson's r = -0.35, P <0.001). There was no significant correlation between vitamin B_{12} levels andWBC count and platelet count.

Figure 2 shows the distribution of clinical complaints of the 89 subjects who had low vitamin B_{12} level. Paraesthesia in the lower limbs was the commonest complaint (7.9 %) followed by fatigue and tiredness. There were no subjects who complained of dyspnea, unsteady walking in darkness, altered sensation in feet while walking or features of memory impairment. Clinical examination did not reveal any significant abnormalities except pallor present in 40 % of cases.

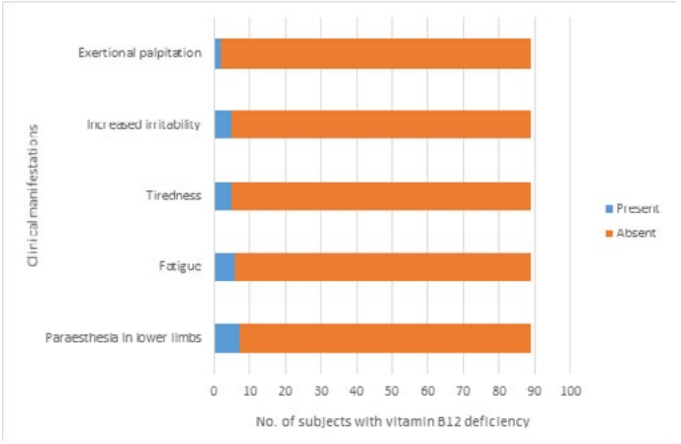

Figure 2: Distribution of clinical complaints in vitamin B_{12} deficient subjects

DISCUSSION

This study included vegetarian population (lactovegetarian) of a specific community of Kathmandu, the Marwaris, who adopt vegetarian diet since birth. The subjects were not receiving any vitamin B_{12} supplements.This study had a predominance of male vegetarians (Male: Female = 2.2 :1), most probably because the sampling technique was convenience sampling, based on the subjects that came to the health camps and there was increased participation of males in such activities.

Because vitamin B_{12} is produced in nature only by vitamin B_{12} producing microorganisms, humans must receive vitamin B_{12} solely from the diet.[9] Although there are abundant vitamin B_{12} producing bacteria colonizing the large bowel,there is no evidence to suggest that these bacteria contribute substantially

to the vitamin B_{12} needs of the individual as that organ is too distal to allow normal vitamin B_{12} absorption.[10]The recommended dietary allowance (RDA) of vitamin B_{12} for adults is 2.4 mcg. The food products, such as egg. cheese, milk, breakfast cereals (unfortified), all provide less than 1.0 mcg/ serving.[11] Hence, strict vegetarians who primarily depend on such foods are at a significant risk of developing vitamin B_{12} deficiency. Besides adequate dietary intake, other 4 factors are important for the adequate and efficient absorption of cobalamin: Acid-pepsin in the stomach (to liberate vitamin $B_{12}$from food protein and binding to R factor), Pancreatic proteases (to free cobalamin from R factors), Gastric secretion of a functional intrinsic factor (IF); a 45 kDa glycoprotein that has high affinity for cobalamin secreted by parietal cells and finally, an ileum with functioning Cobalamin-IF receptors[12.] Total body stores of cobalamin are 2 to 5 mg, approximately one-half of which is in the liver. Due to the extremely efficient enterohepatic circulation of vitamin B_{12}, the liver can store several years' worth of vitamin B_{12}. As a result, it takes years to develop vitamin B_{12} deficiency after absorption of dietary B_{12} ceases.[13]

The frequency of vitamin B_{12} deficiency in vegetarian in this study was 53.6%. This is similar to study conducted in Seventh-day Adventist ministers in Australia by Hokin BD et al in 245 vegetarians where serum level of vitamin B_{12} below the reference range of study (126-627 pg/ml) was 53%.[14]The Marwari population have a high life style and standard of living comparable to Adventist minister of Australia. Similar reports of high prevalence of vitamin B_{12} deficiency in vegetarians have been shown in different studies. In a study by Mehta et al in healthy Indian University students, serum vitamin B_{12} levels were below 200 pg/ml in 80% of the lacto vegetarians.[15]Herrmann et al reported 77% of the lactovegetarians had low serum vitamin B_{12} level.[16] Alexander D et al from New-Zealand found that 35% of the long-term vegetarians had serum vitamin B_{12} concentrations below the reference range.[17] The variation in prevalence of vitamin B_{12} deficiency in vegetarian in different part of world is mainly related to dietary habits (due to variation in the intake of milk and milk products).

The higher prevalence of vitamin B12 deficiency in males compared to females in this study (54 % vs. 46 %) has also been observed in studies elsewhere.[18]And also, most reported studies have shown a gradual rise in the incidence of vitamin B_{12} deficiency with age [19,20]. In this study the highest frequency of deficiency was observed in age group of >60 years, however there was no statistically significant difference between different age groups.

A significant low hemoglobin level and higher MCV were found as level of serum vitamin B_{12} decreased. Regardless of the mechanism, the cause of anemia in cobalamin deficiency is ineffective erythropoiesis or intramedullary hemolysis, for which enhanced apoptosis has been suggested as the possible mechanism [21].Similarly, lower leucocyte counts and platelet count were found as level of serum vitamin B_{12} decreased; but it was not significant. Although there was no significant difference in vitamin B_{12} level in different age groups, anemia was more common in elderly age group (55 %). It may be due to increased sensitivity of vitamin B_{12} deficiency in older group leading to anemia.[22]Another possible explanation is a usually undiagnosed clinical manifestation of cobalaminmalabsorption that is not so uncommon in the elderly subjects, the pernicious anemia. Although frequently causedby autoimmune destruction of gastric parietal cells, strictvegetarian diets or simply inadequate diets are also implicated for the development of this condition.[3]

Vitamin B_{12} participates as cofactor in 2 important intracellular metabolic reactions- odd chain fatty acid metabolism and folate-dependent methylation of the sulfur amino acid homocysteineto form methionine. It has been proposed that the former pathway might be important in myelin formation and in the neurologic abnormalities seen with vitaminB_{12} deficiency.[12]The later pathway is necessary for normal DNA synthesis as this reaction frees THF from its methylated form so that it can participate in purine base synthesis.[3]

Although the classic hematologic expression of vitamin B_{12} deficiency is a megaloblastic macrocytic anemia, it was remarkably rare (1/89) in this study which was consistent with other studies.[23]One of the studies has shown that up to 28 percent of affected patients may have a normal hemoglobin level, and up to 17 percent may have a normal mean corpuscular volume.[24]It is possible that the high folate intake

and co-existing iron deficiency in vegetarians mask the classic hematological manifestations of vitamin B_{12} deficiency. It is also possible that the degree of vitamin B_{12} deficiency among vegetarians is usually not severe enough to cause macrocytosis or anemia.[25]

The early symptoms of vitamin B_{12} deficiency are nonspecific and include unusual fatigue, appetite loss and numbness and tingling in the hands and feet and frequent upper respiratory infections along with others.[3]The characteristic neurologic complex associated with the deficiency is defined as myelosis funicularis, which consists of impaired perception of deep touch, pressure and vibration, persistent paraesthesia and in severe cases, appearance of pathologic reflexes.[3]A study done on 143 patients with vitamin B_{12} deficiency, 74% presented with neurologic symptoms, with the greatest percentage of isolated numbness (paraesthesia) and gait abnormalities.[26]The manifestations are attributed to a defect in myelin formation, particularly in the dorsal and lateral spinal columns[12]. In this study there were few symptoms but not specific to vitamin B_{12} deficiency also seen in other studies[22] and the typical neurological features were absent even in vegetarians with very low serum vitamin B_{12}.This is because neurologic symptoms occur late in the development of overt cobalamin deficiency.[27]One thing to note however, is that the neurological symptoms of vitamin B_{12} deficiency can occur without anemia, so early diagnosis and intervention is important to avoid irreversible damage.[28]

CONCLUSION

The population of Nepal, and mainly vegetarian consume a diet low in cobalamin. This study has shown that the prevalence of vitamin B_{12} deficiency is quite high in vegetarian population. Since this study is conducted in Marwari population who has high life style and standard of living and increase consumption of milk, the actual prevalence of vitamin B_{12} deficiency in general population may still be higher than estimated in this study. Megaloblastic anemia and neurological symptoms occur late in the development of overt cobalamin deficiency. Perhaps more important from a general health perspective are long-term effects of mild, subclinical cobalamin deficiency, which is related to cardiovascular diseases, cancer and birth defects and pregnancy complications.[29]Strict vegetarians must obtain their vitamin $B_{12}$from supplements or consumption of fortified cereal products to prevent deficiency, that contain the crystalline formulations of vitamin $B_{12,}$ which are better absorbed than naturally occurring vitamin B_{12}.[30] Further large scale studies are needed to determine the prevalence of vitamin B_{12} deficiency in Nepalese population, and, depending on the findings, national strategies in the form of food fortification or vitamin B_{12} supplementation in order to prevent long-term effects of mild, subclinical cobalamin deficiency.

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