Profile of Vitamin B12 Deficiency and Analysis of Contributory Factors among Inpatients in a Tertiary Care Hospital in Madurai, South India

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ABSTRACT

BACKGROUND

It is well known that Vitamin B_{12} deficiency is common among vegetarians as Vitamin B_{12} is obtained predominantly from animal sources. However, recent reports show that Vitamin B_{12} deficiency is becoming more common among non-vegetarians too and surprisingly the major factor attributing to this is found to be due to dietary deficiency. We hypothesized that this could also be due to the type of non-vegetarian food consumed, cooking methods, type of utensil used, and other modifiable risk factors like smoking, alcohol and diseases causing Vitamin B_{12} deficiency. We wanted to assess the proportion of vegetarians and non-vegetarians with vit. B_{12} deficiency and analyse the contributing factors among inpatients with vitamin B_{12} deficiency in a tertiary care hospital in South India.

METHODS

This observational, prospective study was done between June and September 2019 & involved 200 Vitamin B_{12} deficiency patients in the age group of 20 - 50 years of both the genders. Patients were identified after reviewing their medical records and laboratory tests for MCV, MCH, MCHC, Hb & vitamin B_{12}. A detailed history of their food habits, practices & other relevant factors was obtained using a questionnaire. Statistical analysis was done using Mann Whitney U test.

RESULTS

There was no statistical difference ($p = 0.379$) in the vitamin B_{12} levels among vegetarians and non-vegetarians. 54.6 % of participants consumed poultry, 32.5 % consumed fruits & vegetables less than 4 times a week, 36 % & 42 % consumed fried & boiled food, 58 % used ever-silver vessels for cooking, 70.6 % used packaged milk, 23 % consumed alcohol & 21 % were smokers.

CONCLUSIONS

Vitamin B_{12} deficiency is common both among vegetarians and non-vegetarians. Among non-vegetarians, deficiency is seen more with poultry eaters, packaged milk consumers, with fried / boiled method of cooking using ever-silver vessels. Alcoholism, caffeinated beverages, smoking, presence of other diseases like diabetes, hypertension, peptic ulcer, drug intake also contributes to Vitamin B_{12} deficiency.

KEYWORDS

Vitamin B12 Deficiency, Non-Vegetarians, Vegetarians, Contributing Factors

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BACKGROUND

It is a well-established fact that vitamin B_{12} is obtained from animal sources and because of this, vitamin B_{12} deficiency is quiet common in vegetarians.[1,2] Despite this, recent findings observed an alarming increase in the incidence of vitamin B_{12} deficiency anaemia even among non-vegetarians.[3] Dietary deficiency of vitamin B_{12} was found to contribute a major role in vitamin B_{12} deficiency anaemia in non-vegetarians.

Following ingestion, vitamin B_{12} is dissociated from food proteins by hydrochloric acid and pepsin secreted by gastric parietal cells. Vitamin B_{12} then binds to salivary protein R-binder or transcobalamin I in stomach. The parietal cells produce intrinsic factor which binds with vitamin B_{12} in the duodenum after cleavage from the R-binder by the action of trypsin and other pancreatic enzymes. This binding is essential for absorption of vitamin B_{12} across the terminal ileum, mediated by the protein cubilin.[4] Any disruption in the above process results in vitamin B_{12} deficiency. A serum B_{12} above 300 pg / mL is interpreted as normal. Patients with B_{12} levels between 200 and 300 pg / mL are considered borderline, and further enzymatic testing may be helpful in diagnosis. Patients with B_{12} levels below 200 pg / mL are considered deficient.

Causes for Vitamin B_{12} deficiency in both vegetarians and non-vegetarians include defective intake, damage to parietal cells in stomach, acidic environment in duodenum and ileal diseases. Source of Vitamin B_{12} in non-vegetarians is animal foods like meat, poultry, eggs & sea foods. Apart from organic causes, in normal non vegetarians, in spite of adequate non-veg food intake, the causes for vitamin B_{12} deficiency could be due to not allowing the indoor factory farmed animals to feed on the soil, use of pesticides in farm which kill Vitamin B_{12} producing bacteria in soil, use of heavy antibiotic in animals which kills Vitamin B_{12} producing bacteria in gut of animals as well as in humans. Lifestyle changes including increased intake of fat rich junk foods, preserved foods, tea, coffee, alcohol, smoking and drugs like proton pump inhibitors, metformin could also contribute to Vitamin B_{12} deficiency.[5&6] Vitamin B_{12} (Cobalamin) is essential for DNA synthesis of red blood cells (RBC's), which helps in nuclear maturation and formation of RBC's. It is also essential for the conversion of homocysteine to methionine. Deficiency of Vitamin B_{12} results in megaloblastic anaemia due to defect in nuclear maturation, producing less & immature RBC's. In vitamin B_{12} deficiency, blood homocysteine level increases leading to atherosclerosis, increasing the risk for myocardial infarction and stroke. Methionine is essential for the methylation of myelin, DNA, RNA & neurotransmitters. Deficiency of methionine affects the myelination of the nervous system resulting in demyelination of corticospinal and dorsal column tracts. Deficiency also causes bone degradation by stimulating osteoclastic activity.

As sufficient amount of vitamin B_{12} is stored in the liver, deficiency symptoms usually take more than five years to develop. Symptoms develop early before a decrease in serum Vitamin B_{12} below the reference value was observed.[7] The onset of anaemia in case of Vitamin B_{12} deficiency is usually insidious and gradually progressive. Typically, the patient presents with smooth, beefy, red tongue often associated with diminished taste sensations. The patient may have shortness of breath, pallor, dizziness, tinnitus, fatiguability, lethargy and palpitations. Since the underlying pathologic process consists of demyelination of peripheral nerves, the spinal cord and the cerebrum, the signs and symptoms include numbness, paraesthesia, weakness, ataxia, poor finger coordination, diminished reflexes and loss of vision. Patients may come up with several mental problems like depression, memory loss, irritability, behavioural changes, panic attacks and insomnia. Untreated Vitamin B_{12} deficiency can cause pulmonary embolism, deep vein thrombosis, suppression of immune system and bone marrow failure.

The prevalence of vitamin B_{12} deficiency in Asian Indians is 70 to 80 %, especially in north Indian population is 47 %.[8] Hence the aim of this present study was to identify the proportion of non-vegetarians having B_{12} deficiency among all vitamin B_{12} deficiency patients in a tertiary care hospital in south India and to analyse the contributing factors.

Among inpatients with vitamin B_{12} deficiency in a tertiary care hospital in South India, we wanted to study the haematological profile, assess the proportion of vegetarians and non-vegetarians and analyse the contributing factors.

METHODS

This observational, cross sectional study involved 200 vitamin B_{12} deficient patients in the age group of 20 - 50 years involving both the genders, who sought admission in the General Medicine Department of a private Medical college& Hospital in South India between June 2019 - September 2019. This study was conducted after obtaining proper Institutional Ethical Committee Clearance. Patients with 1) MCV >100 fl 2) Haemoglobin < 12 gm / dL for female subjects, < 13 gm / dL for male subjects 3) WBC count < 4000 / mm^3 4) Platelet count < 150,000 / mm^3 5) Vitamin B_{12} < 200 pg / ML were included in the study.

Exclusion Criteria

1. Pregnant and lactating women. The Recommended Dietary Allowance (RDA) for vitamin B_{12} is 2.4 µg / d for adults.[9] These requirements become higher during pregnancy and lactation.
2. Elderly people who are over 50 years of age, as the Vitamin B_{12} content of healthy elderly population was found to be lower when compared with the younger age group.[10]
3. Patients with types of anaemia other than Vitamin B_{12} deficiency anaemia.
4. Patients who were on Vitamin B_{12} supplementation.
5. Patients who received blood transfusions within one month.

Data Collection Method & Tools

Medical records and laboratory tests were reviewed for identifying patients with clinical symptoms of Vitamin B_{12} deficiency anaemia and for decreased serum vitamin B_{12} levels, increase in MCV or pancytopenia. Medical records were also reviewed for pallor, glossitis, stomatitis, tingling, numbness, paraesthesia, dietary habits, personal habits, and previous history of blood transfusions, drug intake and other symptoms. Non vegetarians were defined as those consuming some meat (red meat, poultry, at least once per month) and the total of meat and fish > 1 time / week.[11] Serum vitamin B_{12} was estimated using electrochemiluminescence immune assay "ECLIA" intended for use on Elecsys and Cobase immunoassay analysers (Elecsys 2010 Modular Analytics E170 cobas e 411 Cobas E 601). After getting informed written consent from patients, values of Hb, vitamin B_{12}, complete blood count and blood indices were obtained.

Statistical Analysis

Data was analysed using SPSS v 16.0. Proportion of vegetarians and non-vegetarians was compared using Mann Whitney U test. An arbitrary cut off of 0.05 was used to interpret significance of p value.

RESULTS

Vegetarian / Non–Vegetarian	n	%
Vegetarian	92	46.0
Non-Vegetarian	108	54.0
Total	**200**	**100.0**
Gender	**n**	**%**
Male	146	73.0
Female	54	27.0
Total	**200**	**100.0**

Table 1. Proportion of Vegetarians and Non-Vegetarians & Gender Wise Distribution

Test of Normality				
	Veg / Non-Veg	Shapiro-Wilk		
		Statistic	Df	P-Value
Vitamin B12	Vegetarian	.763	92	.000
	Non-Vegetarian	.972	108	.021
Veg / Non-Veg	Vitamin B_{12} Mean Rank	Mann - Whitney U		P-Value
Vegetarian	104.40	4609.5		0.379
Non-vegetarian	97.18			

Table 2. Test of Normality & Mann-Whitney U test

History	n	%
1. Patients with Hypertension	30	15
Hypertensive patients on regular drugs	20	10
2. Patients with Diabetes Mellitus	40	20
Diabetic patients on regular drugs	30	15
3. Patients with H/O any Abdominal Surgery	35	17.5
4. Patients with H/O Peptic ulcer	15	7.5
a. Peptic ulcer patients on proton pump inhibitors	10	5
b. Peptic ulcer patients on antacids	5	2.5
5. Patients with H/O Autoimmune disease	5	2.5
6. Patients with H/O Ileal disorder	5	2.5
7. Patients with H/O Liver disorder	10	5
8. Patients with H/O Any other drug intake	20	10
9. Total number of patients consuming alcohol	46	23
a. Patients who consume brandy	17	37
b. Patients who consume beer	15	33
c. Patients who consume vodka	6	13
d. Patients who consume rum	8	17
10. Total number of smokers Cigarette / Beedi	42	21
Cigarette smokers	17	40.47
Beedi smokers	25	59.53
a. Patients who smoked 1-5 pack years of cigarette*	2	12
b. Patients who smoked 6-10 pack years of cigarette*	4	23
c. patients who smoked 11-15 pack years of cigarette*	5	29
d. Patients who smoked 16-20 pack years of cigarette*	3	18
e. Patients who smoked ≥ 21 pack years of cigarette*	3	18
f. Patients who smoked 1-5 pack years of beedi**	9	36
g. Patients who smoked 6-10 pack years of beedi**	9	36
h. Patients who smoked 11-15 pack years of beedi**	7	28

Table 3. Medical, Surgical and Personal History

* Number of cigarettes per day multiplied by duration of smoking divided by 20.
** In case of beedi smokers, the number of pack years was further divided by 4, as some articles suggest four beedis are equivalent to one cigarette. 13

Dietary Habits	n	%
1. Patients who consume milk (only milk, milk with coffee, milk with tea)	170	85
a. Patients who consume packaged milk	120	70.6
b. Patients who consume milk obtained from dairy farms	50	29.4
c. Patients who consume milk with coffee	50	29.4
d. Patients who consume milk with tea	60	35.3
e. Patients who consume only milk, without coffee or tea	60	35.3
2. Patients who consume fruits & vegetables	35	17.5
a. Patients who consume fruits & vegetables at least 2 times a day, 5 days a week		
b. Patients who consume fruits & vegetables at least once a day, 5 days a week	50	25
c. Patients who consume fruits & vegetables less than 4 times a week	65	32.5
d. Patients who consume fruits & vegetables less than 2 times a week	50	25
3. Patients who consume fish, meat, poultry	21	19.4
a. Patients who consume fish		
b. Patients who consume meat	28	26
c. Patients who consume poultry	59	54.6
4. Patients who consume fresh / preserved non-vegetarian food	99	91.7
a. Patients who consume fresh non-vegetarian food		
b. Patients who consume preserved non-vegetarian food	9	8.3
5. Patients who consume smoked / fried / boiled / grilled / non-vegetarian food	20	18.5
a. Patients who consume smoked non-vegetarian food		
b. Patients who consume fried non-vegetarian food	36	33.3
c. Patients who consume boiled non-vegetarian food	42	39
d. Patients who consume grilled non-vegetarian food	10	9.2
6. Patients who use aluminium / tin / silver / non-stick vessels for cooking	27	13.5
a. Patients who use aluminium vessels for cooking		
b. Patients who use tin vessels for cooking	40	20
c. Patients who use ever silver vessels for cooking	116	58
d. Patients who use non-stick vessels for cooking	17	8.5

Table 4. Diet History

Parameters	Mean
Haemoglobin	6 (±) 0.5 g / dL
MCV	108.4 (±) 2 fL
MCH	44 (±) 1 pg
MCHC	36 (±) 0.5 g / dL

Table 5. Haemogram Values

Shapiro wilk test and box-whisker plot revealed that vitamin B_{12} level data failed to satisfy normality assumptions. Hence, non-parametric test like Mann-Whitney U test was

used to find the difference in vitamin B_{12} level among vegetarian and non-vegetarian. On the basis of statistical significance value ($p > 0.05$), there was no difference in the vitamin B_{12} level among vegetarians and non-vegetarians.

DISCUSSION

In the present study, among the 200 vitamin B_{12} deficiency patients assessed based on their serum vitamin B_{12}, Hb content, MCV, MCH and MCHC (Table 5), 92 were vegetarians and 108 were non vegetarians (Table 1). The study results show no significant difference (p value - 0.379) in vitamin B_{12} level among vegetarian and non-vegetarian population (Table 2). This shows that vitamin B_{12} deficiency is almost equally prevalent in both vegetarian and non-vegetarian population. The results of our study differ from the results of previous studies done on vitamin B_{12} deficiency.[1,4,13] According to these studies, the prevalence of vitamin B_{12} deficiency was higher among vegetarians due to sub optimal intake of vitamin B_{12}.

Plant food is a very poor source of Vitamin $B_{12.}$ Foods rich in vitamin B_{12} include mushrooms (due to contact with B_{12} synthesizing bacteria in the soil), algae and foods fortified with vitamin $B_{12.}$ In vegetarians, deficiency is not only due to lack of non-vegetarian food, but also due to failure of regular and sufficient intake of green leafy vegetables coated with soil bacteria. Thorough washing for fear of pesticides and deep cooking reduces the level of vitamin B_{12}.[14] In our study, 32.5 % of the population consumed fruits and vegetables less than 4 times a week (Table 4).

Good sources of vitamin B_{12} include meat, fish, milk and milk products (yogurt and cheese). According to National Institute of Nutrition, Hyderabad, the amount of vitamin B_{12} for 100 g of edible portion in liver of goat, sheep is 91 mg, goat meat is 2.8 mg, egg yolk (hen) is 4.4 mg, egg whole is 1.8 mg, buffalo meat is 1.7 mg, shrimp is 9 mg, mrigal fish is 1.4 mg, cow milk is 0.14 mg, cow milk curd is 0.13 mg, skimmed milk powder is 0.3 mg.[15] Though B_{12} content of the meat is high, the bioavailability is less. The equal increase in prevalence of vitamin B_{12} deficiency in non-vegetarians could be due to the fact that in developing countries, the consumption of meat is not on a regular basis. They take meat only once a week generally as meat is expensive and the lower / middle class group cannot afford to buy. An average sized steak that is consumed by a single person in western countries will serve 6 - 8 persons after making as curry in the developing countries. Only daily meat eaters in the developing countries will have a vitamin B_{12} level similar to that of a non-vegetarian in the developed countries.[16] Among the animal foods, fish and shellfish are important contributors of vitamin B_{12}.[17] Moreover, most of the non-vegetarian population consumes more poultry when compared to meat and fish. In the present study, 54.6 % of the non-vegetarians consumed poultry and the intake of fish is only 19.4 % (Table 4). The effect of roasting and grilling on vitamin B_{12} content is minimal. However, frying causes a 32 % decrease in cobalamin content. [18] The preferred type of cooking is boiling and frying in the present study (Table 4). Only 8.3 % of the non-vegetarian participants used preserved meat. Processing of raw meat also decreases vitamin B_{12} level.[19] All these would have contributed to vitamin B_{12} deficiency in non-vegetarians.

The B_{12} status of vegetarians was mainly correlated with their intake of milk and milk products. Though vitamin B_{12} content of milk is less, the bioavailability of vitamin B_{12} is greater.[20] Half of the recommended daily intake of vitamin B_{12} is provided by 250 ml of milk. The concentrations of vitamin B_{12} in milk were affected by cow breed, season, cobalt supply, and feeding regimens. Vitamin B_{12} concentrations in milk of cows receiving a daily supplement of cobalt were higher when compared with unsupplemented cows. Heating milk at 95°C for 5 minutes, pasteurization at 75°C for 16 seconds, storing in a domestic refrigerator for nine days and day light exposure do not potentially alter vitamin B_{12} content. Whereas 30 to 40 % loss of vitamin B_{12} was observed in milk after boiling for 30 minutes or microwave heating for 5 minutes.[21] Fermentation during yogurt formation and storage of yogurt at 4° C for 14 days results in a 25 % & 26 % loss of vitamin B_{12} content. In the present study, patients who consume milk without coffee or tea is only 35.3 % and almost 70.6 % of this is packaged milk (Table 7). Coffee consumption was associated with reduced vitamin B_{12} concentration and increased homocysteine levels.[5] Coffee might increase the excretion of B-vitamins in urine. Increased consumption of coffee (29.4 %) and tea (35.3 %) by both vegetarians and non-vegetarians also could have contributed to Vitamin B_{12} deficiency.[5] Peptic ulcer especially due to *Helicobacter pylori* infection can lead to vitamin B_{12} deficiency. The infection leads to atrophic gastritis and hypochlorhydria, which results in failure of splitting of vitamin B_{12} from the food proteins and subsequent binding with R-binder.[22] Proton-pump inhibitors can also lead to vitamin B_{12} deficiency by impairing the release of vitamin. In the present study, 7.5 % of the participants had peptic ulcer (Table 3). The prevalence of vitamin B_{12} deficiency is very high among both Type I and Type II diabetes mellitus. In Type I diabetes, auto antibodies are formed against intrinsic factor and parietal cells resulting in pernicious anaemia. In Type II diabetes, vitamin B_{12} deficiency is due to the drug metformin. It causes anaemia by stimulating bacterial overgrowth in small intestine, by competitively inhibiting vitamin B_{12} absorption, by altering the intrinsic factor level and by preventing the absorption of vitamin B_{12} across ileum by binding with the cubilin receptor.[23] Out of the 200 participants in our study, almost 20 % had diabetes. Our results coincides with the results of a study conducted on the effect of metformin on vitamin B_{12} in diabetic population, where vitamin B_{12} deficiency was common among both the vegetarian (56.52 %) and non-vegetarian (35.71 %) population and the difference is not statistically significant.[24] The level of homocysteine is regulated by vitamin B_{12} and deficiency of vitamin B_{12} is associated with increased levels of homocysteine, which is an independent risk factor for high blood pressure. In our study, about 15 % of the participants were hypertensive. According to Table 3, the presence of hypertension (15 %), peptic ulcer (7.5 %), autoimmune diseases (2.5 %), abdominal surgery (17.5 %), ileal disorder (2.5 %) and drug

intake (10 %) among the study participants would have reduced the vitamin B_{12} level in both the population.

Smoking decreases serum Vitamin B_{12} concentration. In tobacco smoking patients, vitamin B_{12} levels are decreased as high amount of cyanide in tobacco smoke affects vitamin B_{12} metabolism. In smokers, there is also increased excretion of vitamin B_{12} in urine.[25] In the present study, 21 % of the male participants were smokers (Table 3). Vitamin B_{12} acts as a cofactor for the enzymes regulating homocysteine metabolism. Previous study on the effect of smoking on vitamin B_{12} in 300 male subjects showed a significant decrease in vitamin B_{12} concentration in chronic smokers as when compared with non-smokers.[26] Homocysteine was doubled. 23 % of the participants of this study were alcoholics (Table 3). Alcohol consumption reduces serum vitamin B_{12} concentration.[27] Functional B_{12} deficiency was also observed in alcoholics. They respond to vitamin B_{12} treatment inspite of normal cobalamin levels.

The type of cooking utensil used may cause changes to the contents of vitamins. Chronic use of aluminium vessel for cooking all the three meals a day for more than 10 years results in anaemia.[28] But in our study, majority of the participants used only ever silver utensils (Table 10). In the present study (Table 1), the prevalence of vitamin B_{12} deficiency was more common among male participants (73 %) when compared to female participants (27 %). The results of our study coincides with the results of two previous Indian studies which showed that men are more susceptible to vitamin B_{12} deficiency.[29 & 30] Strength of the study: The first study of its kind to measure the prevalence of vitamin B_{12} deficiency among the non-vegetarian population in south India. Limitation of the study: Because of small sample size, to extrapolate the findings to general population, large scale multicentric studies are required in the future.

CONCLUSIONS

The present study results show that the prevalence of vitamin B_{12} deficiency was nearly equal among both vegetarian and non-vegetarian population. Increasing prevalence of vitamin B_{12} among the non-vegetarian population could be predominantly due to reduced, regular consumption of milk and fish, frying of non-vegetarian food, increased consumption of beverages like coffee, smoking and alcoholism, and increased prevalence of diabetes and hypertension. It was found to be more prevalent among males than females. Identifying this deficiency in non-vegetarians could help improve their anaemia and prevent the development of long-term complications.

Data sharing statement provided by the authors is available with the full text of this article at jebmh.com.
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