ORIGINAL ARTICLE

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Vitamin B12 and Non-vegetarianism: Myth versus Reality

ABSTRACT

Background: Vitamin B12 deficiency has traditionally been linked to a vegetarian diet in medical texts, for decades. This alone, is probably only the tip of the iceberg. Hence, there is a dire need to know the other possible causes of this deficiency in our country. The number of such studies so far is rather scant. **Objectives:** Our study aims to measure the prevalence of Vitamin B12 deficiency in healthy volunteers and to compare the levels between vegetarians and non-vegetarians, for statistical significance. **Materials and Methods:** We carried out a comparative, single-center study to analyze the levels of B12 in vegetarians and non-vegetarians. This was done at a Local Medical College in Mumbai, India, from March 2018. Data were collected over a period of 1 year. A sample size of 102 healthy volunteers was screened. We included students from the college between the ages of 18–22 years. They were divided into two groups, namely, vegetarian and non-vegetarian based on their dietary patterns. **Results:** Fifty subjects were on a vegetarian diet and 52 were on a non-vegetarian diet. Seventy-five individuals (73.5%) were found to be B12 deficient. About 78% of vegetarians and 59.3% of non-vegetarians were deficient. However, statistical analysis showed no significant difference between the mean and median serum B12 levels between vegetarian (148.46) and non-vegetarian (172.02) groups ($P > 0.05$). **Conclusion:** Vitamin B12 deficiency is rampant amongst both vegetarian and non-vegetarian Indians. Clinicians should have a high index of suspicion in not only vegetarian but also non-vegetarian populations.

Key words: B12 deficiency, Anemia, Vegetarian, Non-vegetarian

BACKGROUND

Historically, it has been taught that B12 deficiency is seen mainly in vegetarians. However, with increasing health checks, a large percentage of the population, both vegetarian and non-vegetarian is being diagnosed with Vitamin B12 deficiency. Studies show that subclinical B12 deficiency can affect between 2.5% and 26% of the general population.[1]

In India, Vitamin B12 deficiency is even more common. A review by Malik and Trilok-Kumar included articles between the years 2000 and 2019 for the prevalence of B12 deficiency in healthy adult and elderly Indian population. The deficiency prevalence reached 78.5% and 61.7% among adults and elderly, respectively.[2]

A simple intervention of correcting B12 deficiency can prevent a host of major diseases. Macrocytic anemia, peripheral neuropathy, chronic fatigue, dementia, and subacute combined degeneration of the cord are among its major manifestations.

Our study focuses on nutritional deficiency of Vitamin B12 between vegetarian and non-vegetarian subjects. We have also endeavored to give a better understanding of the multifactorial causes of this rampant condition. We have incorporated some of these factors in our discussion.

Objective

1. To measure the prevalence of Vitamin B12 deficiency in healthy volunteers of a medical college
2. To compare the levels of the serum B12 levels between vegetarians and non-vegetarians for statistical significance.

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MATERIALS AND METHODS

The study design is a comparative single-center study to analyze the levels of B12 in vegetarians and non-vegetarians. This study was conducted at a Local Medical College in Mumbai, India, from March 2018. Data were collected over a period of 1 year. A registered dietician recorded the dietary details of each subject.

For this study, a sample size of 102 healthy volunteers was screened. The study included students from the college

between the ages of 18–22 years. Informed consent was taken. A registered dietician enquired about the detailed dietary habits of the subjects. Individuals were divided into two groups vegetarian and non-vegetarian based on their dietary patterns. People who consumed any form of meat or fish at least once per week were considered non-vegetarian. Vegetarians traditionally include lacto-vegetarians, ovo-vegetarians, and vegans. However, all vegetarians in our study were lacto-vegetarian and none were ovo-vegetarian or vegan.

In our study, 50 subjects were on a vegetarian diet and 52 were on a non-vegetarian diet. On an average, non-vegetarians consumed 50–100 g of meat/fish once to thrice per week. Intake of fortified foods or mushroom was not significant in both groups.

For the evaluation of the serum Vitamin B12 levels, blood samples were collected and measured. Serum B12 levels <200 pg/mL were taken as deficient. Although Holotranscobalamin is a more sensitive indicator, being the active form of B12, it could not be measured due to financial constraints.

Study population

For inclusion in the comparative study, the subjects had to be (a) healthy asymptomatic individuals and (b) above 18 years of age.

Under the exclusion criteria were subjects who had (a) consumed B12 supplements, proton pump inhibitors, H2 receptor antagonists, metformin, antacids, antibiotics, probiotics, and anticonvulsants within the past 6 months, (b) had any other comorbid conditions, and (c) pregnant individuals and children.

Statistical analysis

Data were analyzed using the Statistical Package for the Social Sciences, Version 15.0 package. Data were given as mean, standard deviation, and N for continuous data and number and percentage for categorical data.

Comparison of mean of two groups was carried out by Student's unpaired *t*-test for numerical normal data and by Mann–Whitney U-test for abnormal data. Chi-square test was applied to compare percentages of two groups. All statistical tests were two tailed. Alpha (α) level of significance was taken as $P < 0.05$.

RESULTS

Out of the 102 healthy volunteers screened, 75 individuals (73.5%) were found to be B12 deficient. About 78% of vegetarians and 59.3% of non-vegetarians were deficient [Table 1 and Graph 1].

However, statistical analysis showed no significant difference between the mean and median serum B12 levels between vegetarian (148.46) and non-vegetarian (172.02) groups ($P > 0.05$), as shown in Tables 2 and 3 and Graphs 2 and 3].

Table 1: Distribution of B12 levels in the non-vegetarian and vegetarian groups

B12 value range (pg/mL)	Non-vegetarian (*n*=52) (%)	Vegetarian (*n*=50) (%)	Total (*n*=102) (%)
0–50	1 (1.9)	2 (4.0)	3 (2.9)
51–100	7 (13.5)	18 (36.0)	25 (24.5)
101–150	20 (38.5)	10 (20.0)	30 (29.4)
151–200	8 (15.4)	9 (18.0)	17 (16.7)
201–250	7 (13.5)	4 (8.0)	11 (10.8)
251–300	4 (7.7)	3 (6.0)	7 (6.9)
301–350	4 (7.7)	1 (2.0)	5 (4.9)
>350	1 (1.9)	3 (6.0)	4 (3.9)
Total	52 (100.0)	50 (100.0)	102 (100.0)

Table 2: Comparison of mean B12 between 2 groups by parametric *t*-test

Data: Mean±Standard deviation		
Non-vegetarian (*n*=52)	Vegetarian (*n*=50)	*t*-value, significance and *P*-value
172.02±88.96	148.46±91.61	*t*=1.3, NS, *P*=0.12

Table 3: Comparison of Median B12 between 2 groups by non-parametric Mann–Whitney U-test

Data: Median (range)		
Non-vegetarian (*n*=52)	Vegetarian (*n*=50)	Z-value, significance and *P*-value
143 (49,524)	127 (30,394)	Z=1.7, NS, *P*=0.09

DISCUSSION

Vitamins are essential micronutrients required for the metabolism and growth of the body. Vitamin deficiencies are a fairly common problem globally. Vitamin B12 deficiency has been recognized as a serious health concern since several years. However, in clinical practice, we have noticed a significant increase in the diagnosis of Vitamin B12 deficiency in the recent years, in both clinical and subclinical cases.

This was supported by the results of our study showing a significant 73.5% of asymptomatic healthy subjects, who were found to be Vitamin B12 deficient, on routine screening.

Vitamin B12 cannot be synthesized by the human body. Its main source is animal meat especially organ meats such as liver and kidney or animal products such as milk, cheese, and eggs. It is absent from plant derived food sources.[3] Harrison's Principles of Internal Medicine in its 21st edition mentions boldly and unambiguously "Foods of non-animal origin are free from cobalamin unless contaminated by bacteria."[4]

Historically, it has been taught that B12 deficiency is seen mainly in the vegetarian population, as the amount of B12 in vegetarian food is low, and may not be enough to meet bodily requirement. This is supported by several studies.[3,5,6]

As doctors, we have been conditioned to screen and suspect Vitamin B12 deficiency, only in the vegetarian population. With increasing routine health checks, a large percentage of the population is being diagnosed with Vitamin B12 deficiency.

About 78% of vegetarians and 59.3% of non-vegetarians screened in our study were found to be B12 deficient. Our study also showed no significant difference between the mean serum B12 levels between vegetarian (148.46) and non-vegetarian (172.02) groups ($P > 0.05$).

Some of the dietary reasons of similar B12 values seen in the vegetarian and non-vegetarian groups included:

1. Most of the non-vegetarians consumed non-vegetarian food only 1–3 times a week
2. The amount of non-vegetarian food consumed was small
3. Most of the vegetarians were lactovegetarians.

It is possible that the consumption of non-vegetarian food in India is limited, compared to our Western counterparts and includes more of chicken than the red or organ meats consumed by the Western population. Furthermore, the portions consumed by the Indian population are considerably lesser. Table 4 shows the B12 content in various animal proteins, showing a much higher proportion of B12 in organ meats.

Data also suggest that cooking methods used such as boiling, frying, and microwaving certain meats can significantly reduce the amount of B12 in it.[9] These methods are commonly used in Indian cooking of meat.

Besides nutritional diet-related deficiency, high prevalence of gut abnormalities may also contribute to B12 deficiency. The digestion and absorption of B12 (cobalamin) in the gut is a complex process. Food bound B12 is released by the action of gastric acid and pepsin in the stomach and taken up by transcobalamin I and then transported to the duodenum.[10]

In the duodenum, the pancreatic enzymes breakdown transcobalamin I and liberate B12 which joins intrinsic factor secreted by the parietal cells of the stomach.[11] The Intrinsic factor-cobalamin complex is then transported to the ileum. Intrinsic factor is necessary for uptake of Vitamin B12 in the terminal ileum. Once B12 is absorbed through the ileal brush border that it dissociates from intrinsic factor, and enters the circulation where it binds transcobalamin II. Transcobalamin II is responsible for delivery of cobalamin to peripheral tissues and the liver.[12,13]

Table 4: B12 content in foods[7,8]

Food	Micrograms per serving
Cheese, cheddar, 100 g	0.8
Milk, 2% milk fat, 1 cup	0.5
Egg, whole, boiled, 1 large	1.1
Chicken, 100 g	0.3
Lamb, 100 g	2.6
Chicken liver, 100 g	44.4
Lamb liver, 100 g	90.1
Lamb kidney, 100 g	52.4

High prevalence of malabsorptive syndromes, especially those affecting the ileum such as tuberculosis and inflammatory bowel diseases, may affect the absorption of B12. Small intestinal bacterial overgrowth is being diagnosed with increasing frequency. It may cause B12 deficiency due to utilization of B12 by abnormal microbiome.[14]

Autoimmune diseases, such as Pernicious anemia, impair B12 absorption by reducing production of intrinsic factor. This is fortunately uncommon in India. Intestinal diseases affecting the absorption of B12 may be contributing to the increasing numbers of B12 deficiency in both vegetarian and non-vegetarian populations.[15-17]

The role of the gut microbiome in vitamin B12 synthesis is worth discussing. About 20% of gut bacteria including *Pseudomonas denitrificans*, *Bacillus megaterium*, and *Propionibacterium freudenreichi*, *Bacteroides fragilis*, *Prevotella copri*, *Clostridium difficile*, *Faecalibacterium prausnitzii*, *Ruminococcus lactaris*, *Bifidobacterium animalis*, *Bifidobacterium infantis*, *Bifidobacterium longum*, and *Fusobacterium varium* are capable of producing B12. However, these bacteria are mostly colonic commensals and as B12 is absorbed in the ileum as discussed above, B12 produced in the colon may not be available for absorption.[18]

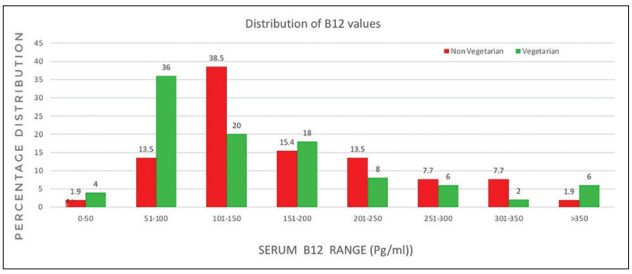
Salivary bacteria are continuously swallowed, can spread and colonize other areas, and influence the gut microbiome. A significant association between the oral and gut microbiota has been observed.[19,20]

Some of the above discussed B12 producing bacteria such as *B. megaterium*, *Prevotella,* and *Bifidiobacterium* species are also found in the oral cavity in saliva and may be contributing to the endogenous production of B12 in the human gut which may be available for absorption.[21-23]

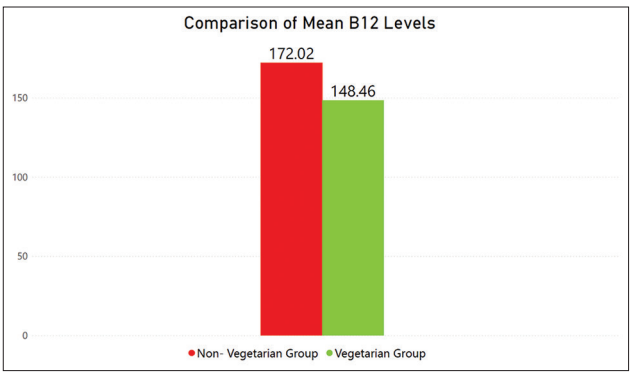
Rampant antibiotic use and misuse in the recent years has led to significant changes in gut microbial diversity that could have impacted certain B12 producing species of the gut microbiome, like *Bifidiobacterium*, contributing to increasing B12 deficiency. However, more studies are needed to prove this association.[24,25]

Another reason, we thought, could be playing a role in the growing trend of B12 deficiency in both vegetarians and non-vegetarians, is the use of reverse osmosis (RO) filters. People have changed their water drinking habits. Majority of people in cities use filters at home. This could be an important cause. These filters remove important vitamins like B12 from the water, and possibly even microorganisms responsible for endogenous B12 production. A study by Gupta *et al*. has shown this association.[26] However, in our study, we did not get data on the use of RO filters and that is a limitation of our study.

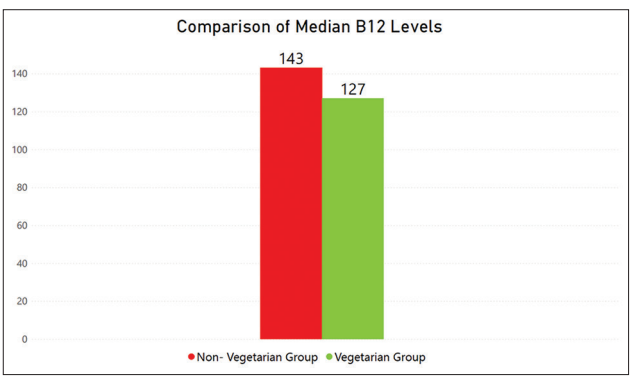
Hence, it is clear that there could be several other confounding factors besides diet which may contribute to Vitamin B12 deficiency and just the fact that a patient consumes a non-vegetarian diet is not adequate to ensure normal B12 levels.



Graph 1: Distribution of B12 levels in the non-vegetarian and vegetarian groups.



Graph 2: Comparison of Mean B12 between 2 groups by parametric t test



Graph 3: Comparison of Median B12 between 2 groups by non-parametric Mann Whitney U test. Data: Median (range)

Another study done by Ingole *et al*. did show significant B12 deficiency in non-vegetarians. However, some of their patients were consuming medications such as metformin and proton-pump inhibitors.[27]

There is a clear paucity of data showing significant B12 deficiency in people consuming non-vegetarian diets and the reasons behind the same. This warrants further studies. This would encourage physicians to suspect, screen, and correct even non-vegetarians for vitamin B12 deficiency contrary to historic teachings.

CONCLUSION

Vitamin B12 deficiency is rampant amongst both vegetarian and non-vegetarian Indians. Clinicians should have a high index of suspicion in not only the vegetarian but also the non-vegetarian population.

Further detailed studies, along the points indicated in the discussion above, provide fertile areas for basic and observational studies in future. The extreme paucity of such papers mandates further research.

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