

## Assessment of vitamin B<sub>12</sub> level in Female patients

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### Abstract

**Background:** Vitamin B<sub>12</sub> is a biologically active corrinoid, a group of cobalt containing compounds with macrocyclic pyrrol rings. Vitamin B<sub>12</sub> deficiency is characterized by megaloblastic anemia, fatigue, weakness, constipation, loss of appetite, and weight loss. It is also associated with osteoporosis, infertility and postpartum depression in female. Neurological changes, such as numbness and tingling in the hands and feet, can also occur.

**Materials and Methods:** Records of the patients who tested for vitamin B<sub>12</sub> during April 2011 to September 2011 were analyzed. Secondary data on vitamin B<sub>12</sub> level, age, referring unit, religion and dietary history of these patients were obtained from hospital records.

**Results:** Elderly female and vegetarians are at substantial risk to develop B<sub>12</sub> deficiency. The magnitude of the prevalence of B<sub>12</sub> deficiency estimated in our population strengthens the argument that B<sub>12</sub> deficiency is more prevalent in Indian female.

**Keywords:** Vitamin B<sub>12</sub>, Lacto vegetarians, Fluorometric enzyme immunoassay.

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## 1. Introduction

Our general health relies on many different factors. From a healthy diet and lifestyle, to our genetic make-up and environmental factors, all are at play. Vitamin B<sub>12</sub> is considered unique in that it is the only one of the thirteen essential vitamins that contains trace mineral called *cobalt*. Hence, it is sometimes referred to by its scientific name, *cobalamin* [1].

Vitamin B<sub>12</sub> is essential for synthesis of s-adenosyl methionine and is involved in the metabolism of proteins, phospholipids and neurotransmitters [2]. There are two pathways for absorption of vitamin B<sub>12</sub>, intrinsic factor associated and passive diffusion. The first pathway is an active process, which requires an intact stomach, intrinsic factor, pancreatic enzymes, and normally functioning terminal ileum.

Vitamin B<sub>12</sub>, bound to protein in food, is released by the activity of hydrochloric acid and gastric protease in the stomach. Atrophic gastritis, a condition affecting older adults, decreases secretion of hydrochloric acid in the

stomach, resulting in decreased absorption of vitamin B<sub>12</sub> [3]. Decreased hydrochloric acid levels might also increase the growth of normal intestinal bacteria that use vitamin B<sub>12</sub>, further reducing the amount of vitamin B<sub>12</sub> available to the body [4]. Vitamin B<sub>12</sub> deficiency is characterized by megaloblastic anemia, fatigue, weakness, constipation, loss of appetite, and weight loss. Neurological changes, such as numbness and tingling in the hands and feet, can also occur [5]. The neurological symptoms of vitamin B<sub>12</sub> deficiency can occur without anemia, so early diagnosis and intervention is important to avoid irreversible damage [6].

While there are many different causes of female infertility, B<sub>12</sub> deficiency is one factor that is often overlooked. In some case reports, doctors have reported that an ovulation (a failure to release an egg during the monthly cycle) occurs in some women with B<sub>12</sub> deficiency due to pernicious anemia [7]. Studies suggest that vitamin B<sub>12</sub> may benefit bone health, while low levels of B<sub>12</sub> can lead to the development of osteoporosis [8]. A deficiency in B<sub>12</sub> can attack the nerves in our body stripping them of their

myelin coating and disrupting communication between cells in the brain and other parts of the nervous system. Because B<sub>12</sub> depletion affects nerve cells in our brains that control how we feel, think and behave, it is not surprising that a B<sub>12</sub> deficiency has been linked to postpartum depression [9].

Vitamin B<sub>12</sub>, apart from causing neuropsychiatric symptoms, leads to hyperhomocysteinemia and methylmalonic acidemia which can have serious health implications. Low serum vitamin B<sub>12</sub> levels have low sensitivity and specificity in terms of tissue deficiency [6]. Homocysteine and methylmalonic acid estimations are adjunct and aid in diagnosis of B<sub>12</sub> deficiency but still serum vitamin B<sub>12</sub> measurement is the extensively applied standard method by practical purposes.

It is known that more vegetarians or elderly people suffer from vitamin B<sub>12</sub> deficiency compared to omnivores or younger adults. Because natural sources of vitamin B<sub>12</sub> in human diets have known to be restricted to animal-origin food, it has been believed that those people with low animal food diets are more susceptible to cobalamin deficiency [10].

## 2. Materials and methods

The study was conducted in the Department of Biochemistry of a SBKS MI & RC, Pipariya Gujarat. The prospective descriptive study was carried out over a period of 6 month from April 2011 to September 2011. All the records of the patients where vitamin B<sub>12</sub> was assayed were screened.

Approval was taken from research ethics committee of S.B.K.S Medical College, Waghodia Vadodara, before starting the study.

### 2.1 Inclusion criteria:

- I) Age >18 year
- II) Only Female
- III) Indoor patients of Dhiraj General Hospital.

### 2.2 Exclusion Criteria:

- I) Patient with acute pancreatitis, hyperlipidemia, gastrointestinal bleeding.
- II) Co-morbid major psychiatric diagnosis.
- III) Patients with Diabetes mellitus, taking lipid lowering drugs
- IV) Patients with history of renal disorder.

Data on vitamin B<sub>12</sub> level, age, referring unit, religion and dietary history of the patients were collected. Only those who consumed meat or fish were considered as non vegetarians. Lacto vegetarians as well as lacto-ovovegetarians were grouped under vegetarians.

After consent was given venous blood sample were collected, after 12 hours overnight fasting. Each test tube containing a blood sample was immersed in crushed ice before analysis. The samples were centrifuged for 10 min at a speed of 3000 R.P.M. (R -8 C Laboratory Centrifuge), to separate the serum, into a 12 ×75 mm test tube.

As per the protocol of our laboratory, blood sample for vitamin B<sub>12</sub> was drawn after an overnight fasting. Serum vitamin B<sub>12</sub> levels (normal range 211- 946 pg/ml) were estimated by AIA 360 using flourometric enzyme immunoassay using commercially available ST AIA Pack B<sub>12</sub> kits. The biochemical vitamin B<sub>12</sub> deficiency was defined at a concentration below <211 pg/ml [11,12]

Descriptive statistics were obtained for all variables. Chi square test was applied to find the association between B<sub>12</sub> values of different age, sex and diet groups. Calculations were done using by Microsoft excel and SPSS software, version 12.0 for Windows. A p value <0.05 was considered statistically significant.

## 3. Results

A total of 100 female patients screened for serum vitamin B<sub>12</sub> levels were enrolled. Mean levels of B<sub>12</sub> in 100 subjects were observed to be 536.8. If 211 pg/ml was taken as cut off for deficiency state, total 38 out of 100 subjects (38%) turned out to be B<sub>12</sub> deficient.

**Table 1: Age distribution in Female in vegetarian and Non-vegetarian**

Sr. No	Particulars	Vitamin B <sub>12</sub> levels (pg/ml)		Total N=100	Statistical value and level of significance
		<211	>211		
1	Female	38 38%	62 62%	100	
2	>50 years	20 50%	20 50%	40	Chi value= 4.07 P= 0.05 Significant
	<50 years	18 30%	42 70%	60	
3	Vegetarian	29 46.8%	33 53.2%	62	Chi value= 5.33 P= 0.02 Significant
	Nonvegetarian	9 23.7%	29 76.3%	38	

Average age of subjects in our study was 42 years (range 25 to 65 years). Forty subjects out of a total of 100 screened were more than 50 years of age (40%). B<sub>12</sub> was less than 211pg/ml in 20/40 (50%) as compare to 18/60

(30%) in <50 years of age group. Age >50 years appeared to increase the risk for vitamin B<sub>12</sub> deficiency (chi square value=4.07, table value=3.84, p= 0.04) (Table 1).

A total of 100, out of which 62 (62%) were vegetarians and 38 (38%) were non vegetarians. Among the vegetarian group, 29 out of 62 (46.8%) had vitamin B<sub>12</sub> level less than 211 pg/ml where as only 9 of 38 non vegetarians (23.7%) had B<sub>12</sub> deficiency (Table 1). Vegetarian dietary habit was found to be a substantial risk factor for B<sub>12</sub> deficiency (Chi square value=5.33, table value=3.84, p= 0.02) in our population.

#### 4. Discussion

Deficiency of vitamin B<sub>12</sub> is very common because of inadequate dietary intake and/or malabsorption. The deficiency state has a very wide presentation and can cause or exacerbate neuropsychiatric and other vague symptoms. In early stage vitamin B<sub>12</sub> deficiency might present with subtle and slight cognitive impairments. Hence early recognition becomes crucial for preventing irreversible damage.

Present study of West Indian population (n=100) depicted that 38% of the total subjects (38 out of 100) had vitamin B<sub>12</sub> deficiency (levels <211pg/ml). Although varying data have come into picture regarding prevalence, our finding is in consistence with a study where 47% of the Asian Indians had B<sub>12</sub> deficiency confirming the high prevalence of this magnitude in Indians [12]. Though this study by Gupta *et al* was carried out in south Indians residing in Canada [12], prevalence was quite similar to our study indicating that there are other factors beyond vegetarian diet that may possibly be responsible for this deficiency.

In the present study a total of 40 subjects were found to be more than 50 years of age and out of that 20 (50%) were found to be B<sub>12</sub> deficient (B<sub>12</sub> <211 pg/ml) and 31 were observed to have serum vitamin B<sub>12</sub> levels less than 350 pg/ml (70%). The prevalence rate observed in elderly population was higher than the total population. However it was much higher than that observed in Finnish elderly population [13]. This can be explained by occurrence of higher prevalence of vitamin B<sub>12</sub> in Indians probably due to the dietary habits. In our study, the estimated risk of deficiency was higher among elderly people. According to our data 52.6% of the subjects diagnosed to be B<sub>12</sub> deficient were >60 years. However the age seemed to offer a substantial risk for developing B<sub>12</sub> deficiency in West Indians. This could be attributed to dietary limitations due to vegetarian dietary habits and lower socio economic status.

By dietary data history it could be assessed that vegetarian dietetic practice offered considerable risk for developing B<sub>12</sub> deficiency, rate being 46.8% at 211 pg/ml cut off level. This is in consistence with several studies [14, 15].

In the present study vitamin B<sub>12</sub> measurement was used as the first-line test and the definition of vitamin B<sub>12</sub> deficiency was based on low level of serum vitamin B<sub>12</sub>

although measurements of metabolites such as methylmalonic acid and homocysteine have been shown to be more sensitive in the diagnosis of vitamin B<sub>12</sub> deficiency [16].

There are few limitations in our study. First, since it is a hospital based study, the population visiting the tertiary hospital cannot be considered representative of the West Indians. A number of large population based studies are required to validate our study findings in a broader perspective. Secondly since we have analyzed only the vitamin B<sub>12</sub> levels of our population, the subjects having sub clinical deficiency (elevated homocysteine and methylmalonic acid with normal B<sub>12</sub> levels) might have been missed out. Despite these limitations, prevalence observed in the present study was found to be highly significant and consistent with several other studies addressing the problem of vitamin B<sub>12</sub> deficiency. However large population based studies may provide a better outlook about the magnitude of this problem.

#### 5. Conclusion

Role of vitamins in maintenance of female health has received tremendous attention in recent times. Vitamin B<sub>12</sub> deficiency is not just a laboratory finding but a clinically relevant issue which needs to be explored. Elderly female and vegetarians are at substantial risk to develop B<sub>12</sub> deficiency. The magnitude of the prevalence of B<sub>12</sub> deficiency estimated in our population strengthens the argument that B<sub>12</sub> deficiency is more prevalent in Indian female. Hence there is a need to include screening of vitamin B<sub>12</sub> in routine clinical set up. Despite the plausible biochemical mechanism, further studies, based on laboratory findings along with clinical features will aid in understanding this irrefutable important subject.

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