DOI: 10.7860/JCDR/2021/46642.14685

Original Article

Biochemistry Section

Vitamin B12 Deficiency in Eastern India: A Hospital Based Cross-sectional Study

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ABSTRACT

Introduction: The prevalence of vitamin B12 deficiency is common (around 40%) in people older than 65 years of age and in people who are strict vegetarians. The major sources of Viamin B12 are meat, fish, dairy products and fortified cereals.

Aim: To study the prevalence and common types of presentations of vitamin B12 deficiency among 20-80 years aged, non-vegetarian people attending a tertiary care hospital in a state of Eastern India.

Materials and Methods: This hospital based cross-sectional study was conducted at IPGME&R and SSKM Hospital between July 2018 to December 2019. Serum samples were collected from 478 adult patients presenting with different symptoms like pallor, fatigue, numbness and tingling sensation in limbs, memory loss, alopecia etc., which may be related to vitamin B12 deficiency. These samples were screened for serum vitamin B12 level by chemiluminescence method in ADVIA, Centaur CP (SIEMENS). Data were analysed statistically by graph pad prism 8 software. Significance of the difference between means were detected using Student's unpaired t-test and calculating the p-value (p-value <0.05 were considered as significant).

Results: Among 184 females, 50 (27.17%) were found to be Vitamin B12 deficient (VBD). Among them, 18 (36%) had mild deficiency (serum vitamin B12 level 201-220 pmol/L), 22 (44%) had moderate (serum vitamin B12 level 150-200 pmol/L) and 10 (20%) had severe deficiency (serum vitamin B12 level <150 pmol/L). A total of 35 (70%) of the VBD females were of <50 years of age. Among 294 males, 83 (28.23%) were found to be VBD. Among them, 34 (41%) had mild deficiency (serum vitamin B12 level 201-220 pmol/L), 36 (43%) had moderate (serum vitamin B12 level 150-200 pmol/L) and 13 (16%) had severe deficiency (serum vitamin B12 level <150 pmol/L). Total 43 (51.8%) of VBD male persons were of <50 years age. Among VBD female patients, 24 (48%) had neuropathy and among VBD male patients, 54 (65%) had neuropathy.

Conclusion: So, it can be concluded that if regular screening is done for serum vitamin B12 in symptomatic patients irrespective of age, a number of problems can be reduced or cured by diagnosing VBD patients and treating them with vitamin B12 either by dietary modification or medicinal supplementation.

Keywords: Non-vegetarian, Prevalence, Screening, Symptoms, Young age

INTRODUCTION

Vitamin B12 is a generic term that refers to a group of physiologically active substances chemically classified as cobalamins or corrinoids [1]. All vitamin B12 are ultimately the product of microbial synthesis. The main dietary sources are animal products like meat and meat products, fish and shell fish, dairy products and fortified cereals because plants do not produce this vitamin [1]. Normally, approximately 1 mg of vitamin B12 is stored in the liver, a quantity equivalent to the daily metabolic requirement for 2000 days. Thus, when the dietary supply of vitamin B12 is interrupted or mechanisms of absorption are impaired, vitamin B12 deficiency does not become evident for five years or longer [2]. Intrinsic Factor (IF), a glycoprotein with a molecular weight of approximately 50 kDa, produced by the gastric mucosa binds vitamin B12 and is important for its intestinal absorption. The uptake of vitamin B12 from intestine into circulation occurs via a complex mechanism [2]. Vitamin B12 from the proteins in food is liberated in the stomach with the help of secreted gastric acid and pepsin. Once liberated from food, vitamin B12 is bound in the stomach by a salivary protein known as R-protein or haptocorrins and is carried to the intestine. In the small intestine the haptocorrin is digested by pancreatic enzymes, thereby liberating the vitamin B12. The vitamin B12 binds to protein called IF, produced by gastric mucosa. When the vitamin B12-IF complex reaches the distal ileum, it binds to specific receptors on the intestinal mucosal epithelial cells and enters into them. Within the cells, vitamin B12 is dissociated from the complex and binds to the Transcobalamin II (TcII). The B12-TcII complex is transported across the cell membrane bound to a TcII receptor and then released into the plasma of the mucosal capillaries and subsequently into the blood in the portal vein [2].

Vitamin B12 deficiency is seen in patients who are vegetarians or strictly vegans [3]. Vitamin B12 deficiency is also seen in patients with pernicious anaemia because of either failure of IF secretion or secretion of biologically inactive IF. Pernicious anaemia is the end stage of an autoimmune gastritis and results in the loss of synthesis of IF leading to vitamin B12 deficiency and if untreated, complications like megaloblastic anaemia and neurological symptoms like sensory disturbances in the extremities as tingling and numbness, vibratory and position sense disturbance, motor disturbances including abnormalities of gait, cognitive changes ranging from loss of concentration to memory loss, disorientation, and frank dementia, with or without mood changes may develop [3]. Vitamin B12 deficiency is also seen in patients with malabsorption, due to deficient vitamin B12 absorption, with autoimmune disorders or in patients taking prescribed medication known to interfere with vitamin absorption or metabolism [2].

Vitamin B12 deficiency causes a range of disorders and affects all age groups. The main systems affected in vitamin B12 deficiency are the haematologic, gastrointestinal, and nervous systems [4]. Deficiency of this vitamin results in asynchrony in the maturation of the nucleus and cytoplasm resulting in ineffective erythropoiesis, intramedullary haemolysis, pancytopenia and typical morphological abnormalities in the blood and marrow cells, leading to anaemia [5,6].

Vitamin B12 deficiency also affects nervous system. The basic neuropathic change in cobalamin deficiency is the demyelination

seen in the cerebral cortex and in dorsal and lateral columns of the spinal cord [7].

It is known that the prevalence of vitamin B12 deficiency is common (around 40%) in people older than 65 years of age and in people who are strict vegetarians [2,8,9]. According to previous study, there is not much information on the extent of vitamin B12 deficiency in elderly Indians as determined by plasma vitamin B12 levels but at present there is evidence that the disease is more common than was previously believed [10,11]. According to Refsum H et al., about 3.8% of the Indian population is VBD with 75% of them having metabolic signs of cobalamin deficiency [12]. Vitamin B12 deficiency is thought to be uncommon in the Eastern parts of India including Bengal and the Eastern states as compared to the Northern and Southern parts of India [13].

Hence, this study was conducted to estimate the prevalence and to study the common types of presentations of vitamin B12 deficiency among 20-80 years aged non-vegetarian people attending a tertiary care hospital in a state of Eastern India so that, early preventive and curative measures can be taken in adult patients with minimal symptoms related to vitamin B12 deficiency.

MATERIALS AND METHODS

This hospital based cross-sectional study was conducted at IPGME&R and SSKM Hospital, Kolkata during the period between July 2018 to December 2019. All procedures followed were in accordance with the Helsinki Declaration of 1975 that was revised in 2000 [14]. Prevalence of vitamin B12 deficiency in North India is around 47% [15], taking it into consideration sample size was taken as 478.

Inclusion criteria: Study sample included non-vegetarian, adult patients aged between 20 to 80 years attending the outpatient department or inpatient department of a tertiary care hospital in a state of Eastern India, with different symptoms such as megaloblastic anaemia, neuropathy or neuropsychiatric problems, dermatological disorder, gastrointestinal problem or some other history and symptoms suggestive of possible vitamin deficiency. These samples were screened for serum vitamin B12 level.

Inclusion criteria were like the following:

1. Megaloblastic anaemia characterised by raised mean corpuscular volume (>100 fL and haemoglobin <12 gm/dL for female subjects, <13 gm/dL for male subjects) [16,17].
2. Cutaneous manifestation defined by skin hyperpigmentation at the knuckles [18-20].
3. Neuropathy [21,22].
4. Chronic diarrhoea /malabsorption [23].

Vitamin B12 levels were divided into mild (201-220 pmol/L), moderate (150-200 pmol/L) and severe deficiency (<150 pmol/L) states based on previous studies [24,25].

Exclusion criteria: Those who received blood transfusions within one month prior to presentation and those already on vitamin B12 supplementations.

Sample size: Considering confidence interval=95%, or z=1.96 and absolute precision=0.05, minimal sample size would be N=(1.96×1.96×0.47×0.53)/(0.05×0.05)=382.7 or approximate 383. We have taken 478 samples.

Method of estimation: A 3 mL of venous blood samples were collected from patients after 12 hours of fasting. Blood sample was collected by standard venipuncture technique into red topped plastic vial without anticoagulant, using aseptic precautions. Complete clot formation was ensured prior to centrifugation. Serum was separated after centrifuging at 3000 rpm for five minutes and was analysed within 24 hours. Serum vitamin B12 level was estimated by chemiluminescence method in ADVIA, Centaur CP (SIEMENS), which is a competitive immunoassay using direct chemiluminescent technology. Here, vitamin B12 from patient's sample competes with vitamin B12 labelled with acridinium ester for a limited amount of purified IF coupled to paramagnetic particle in solid phase.

The system automatically performs the following actions:

At first it washes the ancillary reagent probe with 100 µL of vitamin B12 ancillary reagent and then dispenses 100 µL of sample and 115 µL of DTT/releasing agent into a cuvette and incubates for 4.7 minutes at 37°C. It then dispenses 200 µL of solid phase and incubates for 6.3 minutes at 37°C. Then, it dispenses 200 µL of lite reagent and incubates for 3.0 minutes at 37°C and separates, aspirates, and washes the cuvettes with wash 1. It then dispenses 300 µL each of Acid Reagent (R1) and Base Reagent (R2) to initiate the chemiluminescent reaction. An inverse relationship exists between vitamin B12 in the sample and Relative Light Units (RLU) detected by the system [26-31].

STATISTICAL ANALYSIS

Statistical analysis was done by Graphpad prism 8. Mean value of different groups were calculated. Significance of the difference between means were detected using unpaired t-test and calculating the p-value (p-value <0.05 was considered as significant).

RESULTS

Sample size of the study was 478 patients, out of which 184 (38.49%) were females and 294 (61.50%) male patients with mean age 43.43±15.99 years and 46.52±16.19 years, respectively. Among 184 females, 50 (27.17%) were found to be VBD. Average vitamin B12 level in VBD females being 181 pmol/L. Among them, 18 (36%) had mild deficiency (201-220 pmol/L), 22 (44%) had moderate (150-200 pmol/L) and 10 (20%) had severe (<150 pmol/L) deficiency. Among 294 males, 83 (28.23%) were VBD. Average vitamin B12 level was 181 pmol/L in VBD males also. Among them, 34 (41%) had mild, 36 (43%) had moderate and 13 (16%) had severe deficiency [Table/Fig-1]. In this study, among 50 VBD female patients, 35 (70%) were between age group 20-49 years and 15 (30%) were ≥50 years. Among the 83 VBD male patients, 43 (51.8%) were between age group 20-49 and 40 (48.19%) were ≥50 years. Among 112 females of <50 years of age, 35 (31.25%) were VBD whereas among 72 females of 50 years or above, 15 (20.83%) were VBD. There is also significant difference (p-value=0.04) in serum vitamin B12 level between females of less than 50 years of age to those with age 50 years and above [Table/Fig-2]. Among males, 153 were <50 years of age of which 43 persons (28.10%) were VBD whereas among 141 males of 50 years or above 40 (28.36%) were VBD. No significant difference were found (p-value=0.29) in serum vitamin B12 level between males <50 years of age to those with age ≥50 years. There was also no significant difference (p-value=0.919) in serum vitamin B12 level between males and females irrespective of age, males and females of <50 years (p-value=0.122) or in males and females of ≥50 years (p-value=0.116) [Table/Fig-2].

Level of serum vitamin B12 (pmol/L)	VBD female patients n (%)	VBD male patients n (%)
<150	10 (20)	13 (16)
150-200	22 (44)	36 (43)
200-220	18 (36)	34 (41)

[Table/Fig-1]: Percentage of males and females with mild, moderate and severe vitamin B12 deficiency.

In this study, among the 50 vitamin B12 deficient female patients, more patients 24 (48%) presented with neuropathy compared to patients presented with anaemia 6 (12%) [Table/Fig-3]. Same in the case of VBD male patients was also observed. Among the 83 VBD male patients, 54 (65%) had neuropathy and 16 (19.2%) had anaemia.

DISCUSSION

In the present study, 35 (70%) among 50 VBD females and 43 (51.8%) among the 83 VBD males were between age group 20-49 years.

Population	Mean vitamin B12 level (pmol/L)	Difference between means±SEM	p-value
Females	547.1	3.719±36.62	0.9192
Males	550.8		
Female <50 years	501.6	72.37±46.67	0.1222
Male <50 years	574.0		
Female ≥50 years	617.9	-92.17±58.52	0.1168
Male ≥50 years	525.7		
Female <50 years	501.6	-116.2±57.62	0.0452
Female ≥50 years	617.9		
Male <50 years	574.0	48.30±45.83	0.2928
Male ≥50 years	525.7		

[Table/Fig-2]: Comparison of Mean vitamin B12 level between males and females of different age.
Student's t-test (unpaired) was used to calculate the p-value

Clinical features	Female n (%)	Male n (%)
Anaemia	06 (12)	16 (19.2)
Neuropathy	24 (48)	54 (65)
Gastrointestinal	05 (10)	2 (2.4)
Hepatic	2 (4)	1 (1.2)
Dermatological	05 (10)	0 (0)
Other symptoms (like generalised bodyache, knee joint pain, neck pain, low back pain, muscle weakness, vertigo, generalised tonic clonic convulsions)	8 (16)	10 (12)

[Table/Fig-3]: Percentages of different symptoms in Vitamin B12 deficient (VBD) males and females.

Significant difference (p-value=0.04) was found in serum vitamin B12 level between females of less than 50 years with age 50 years and above. Among the VBD female patients, 24 (48%) had neuropathy and 6 (12%) had anaemia whereas among male patients, it is 54 (65%) and 16 (19.2%), respectively.

In study by Allen LH, in the developed countries, 6% of those aged 60 years and above are VBD (plasma vitamin B12 level being 148 pmol/L) and in developing countries, deficiency is much more common, starting in early life and persisting across the life span and the prevalence of deficiency increasing with age [24]. In this study also, quite high percentage of VBD patients are young (70% and 51.8% in VBD females and males in 20-49 years age group, respectively). One of the common manifestations of vitamin B12 deficiency is anaemia. In this study, 6 (12%) of VBD females and 16 (19.2%) of VBD males had anaemia. Patients may have severe neurological disorders without significant haematological impairment. In a study by Issac TG et al., out of 259 patients who had vitamin B12 deficiency (<220 pmol/L),24 had moderate level 150-200 pmol/L, whereas 19 had severe (<150 pmol/L) and 17 had mild deficiency (201-220 pmol/L) of vitamin B12 [32]. Similarly, in present study among VBD females 18 (36%) had mild deficiency, 22 (44%) had moderate and 10 (20%) had severe deficiency and among VBD males 34 (41%) had mild, 36 (43%) had moderate and 13 (16%) had severe deficiency. Study by Issac TG et al., found that 23.16% of VBD patients had neuropsychiatric symptoms [32], but in present study a much higher percentage of VBD patients (48% of female VBD and 63% of male VBD) had neurological symptoms.

Vitamin B12 deficiency has dermatological manifestation also. In study by Sen K et al., the vitamin B12 levels in patients with hyperpigmentation were significantly lower than that in patients without hyperpigmentation (112±46.6 versus 167.6±36.3; p=0.04) [13]. But in contrast, in this study only 5 VBD female patients and no VBD male patients had dermatological manifestations. Studies by Kannan R and Ng MJ, and Baker SJ et al., showed the contrast findings [18-20]. Kannan R and Ng MJ, mentioned cutaneous manifestations associated with vitamin B12 deficiency are skin hyperpigmentation, vitiligo, angular stomatitis, and hair changes. A study by Baker SJ et al., studied 21 patients, 15 adults and 6 children, with hyperpigmentation all having vitamin B12 deficiency and responding to treatment with vitamin B12 either weekly or daily injections [18-20].

Limitation(s)

The percentage of vitamin B12 deficiency in elderly population may be falsely low due to their low hospital attendance. The study would have been better if we could take detailed dietary history of the patients. Further studies to see the treatment outcomes after vitamin B12 administration are also needed.

CONCLUSION(S)

From this study, we can make out that vitamin B12 deficiency is quite common in patients between 20 to 49 years age group taking non-vegetarian diet and most of them have moderate deficiency (serum vitamin B12 level between 150-200 pmol/L). A significant percentage of patients attending the tertiary care government hospital in Eastern part of India with various symptoms are VBD but there is not much difference between males and females. So, it can be concluded that if more regular screening is done of serum vitamin B12 in symptomatic patients irrespective of age, a number of problems can be reduced or cured by diagnosing VBD patients and treating them with vitamin B12 either by dietary modification or medicinal supplementation. More regular screening for serum vitamin B12 deficiency is needed in all adult persons with different symptoms to cure them from various diseases by vitamin B12 supplementation.

Acknowledgement

The authors also acknowledge the work of authors, editors and publishers of all articles, journals and books whose works have been cited or were used as reference. The authors are thankful to all the faculties and technological staffs of the Department of Biochemistry and the clinical departments of IPGME&R, Kolkata for their help, support and encouragement.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Sep 12, 2020
- Manual Googling: Mar 04, 2021
- iThenticate Software: Mar 17, 2021 (25%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. Yes

Date of Submission: **Sep 10, 2020**
Date of Peer Review: **Nov 18, 2020**
Date of Acceptance: **Mar 04, 2021**
Date of Publishing: **Apr 01, 2021**