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BURDEN OF VITAMIN B12 DEFICIENCY IN URBAN POPULATION IN DELHI, INDIA: A HOSPITAL BASED STUDY

Corresponding Author

SARIKA ARORA

Department of Biochemistry, GB Pant Hospital, New Delhi, INDIA

Co Authors

BHAWNA SINGH, VINOD K GUPTA AND MALLIKA VENKATESAN

Department of Biochemistry, GB Pant Hospital, New Delhi, INDIA

ABSTRACT

Vitamin B12 deficiency is common in elderly and vegetarians, but whether screening should be carried out only for these high risk groups or for the entire population, is controversial. Vitamin B12 assays performed on 422 patients in Biochemistry laboratory of tertiary care hospital over a period of 1 year were reviewed. Individuals were considered to be vitamin B-12 deficient if vitamin B-12 was < 200 pg/ml and borderline deficiency if levels were <350 pg/ml. Women and vegetarians were found to have higher risk of vitamin B12 deficiency (OR: 0.62, 95% CI: 0.41 to 0.93; OR: 4.68, 95% CI: 2.39 to 9.15 respectively) whereas in contrast to earlier studies, no effect of age was noticed in our population (OR: 1.23, 95% CI: 0.64 to 2.38). However more investigations are required to elucidate etiological factors associated with substantial heterogeneity in vitamin B-12 levels.

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KEYWORDS

Vitamin B12, Cobalamin; Deficiency; Gender; Age; Diet

INTRODUCTION

Vitamin B12 is essential for synthesis of s-adenosyl methionine and is involved in the metabolism of proteins, phospholipids and neuro transmitters. Its deficiency leads to several neurological manifestations and affects all age groups [1]. Vitamin B12 deficiency may present with fatigue, weakness, numbness, decreased memory, irritability, confusion and depression, although initial symptoms might often be vague [2-4]. Even though the human body can store vitamin B12 to last for up to five years, its deficiency is not very uncommon. The diagnosis is frequently made on the basis of a low serum vitamin B12 level or megaloblastic bone marrow or both[5,6]. There has been much debate over establishing a universally accepted normal range for vitamin B12 and till date prevalence of B12 deficiency in the general population has not been well established[7,8].

Vitamin B12, apart from causing neuropsychiatric symptoms, leads to hyperhomocysteinemia and methylmalonic acidemia which can have serious health implications. Low serum vitamin B12 levels have low sensitivity and specificity in terms of tissue deficiency [9]. Homocysteine and methylmalonic acid estimations are adjunct and aid in diagnosis of B12 deficiency but still serum vitamin B12 measurement is the extensively applied standard method by practical purposes.

The racial, religious, ethnic and socioeconomic heterogeneity of the people in India greatly influences their dietary habits. We aimed to estimate the prevalence of vitamin B12 deficiency and its common risk factors in North Indian population attending the tertiary care hospital in Delhi.

MATERIALS AND METHODS

The study was conducted in the Department of Biochemistry of a tertiary care teaching hospital in North India. The retrospective and prospective descriptive study was carried out over a period of one year from October 2008 to September 2009. All the records of the patients where vitamin B12 was assayed were screened. Data on vitamin B12 level, age and sex was collected. Information about referring unit and religious background and dietary history of the patients was also gathered. Only those who consumed meat or fish were considered as non vegetarians. Lacto vegetarians as well as lacto-ovovegetarians were grouped under vegetarians.

As per the protocol of our laboratory, blood sample for vitamin B12 was drawn after an overnight fasting. Serum vitamin B12 levels (normal range 211- 946 pg/ml) were estimated by Roche Elecsys modular Cobas e 411 using electrochemiluminescence immuno assay (Manheim Germany) using commercially available kits from Roche. The biochemical vitamin B12 deficiency was defined at a concentration below <200 pg/ml [10, 11] whereas at levels 350 pg/ml it was taken as borderline deficiency [12].

Descriptive statistics were obtained for all variables. Student's t test was applied to find the comparison between B12 values. The Odds ratios (OR) were adjusted for sex, age

(years) and diet and OR and confidence intervals were calculated to examine the relationship between above mentioned factors for the presence or absence of B12 deficiency. Calculations were done using SPSS software, version 12.0 for Windows.

RESULTS

A total of 422 patients screened for serum vitamin B12 levels were enrolled. Out of the total 422 patients, there were 214 men (50.7%) and 208 women (49.3%).

Table 1 depicts serum B12 levels and percentage of men and women studied. Mean±SEM levels of B12 in 422 subjects were observed to be 285.1±17.71 (range: 23 – 1887 pg/ml). Men had mean value of 323.5±27.4 (range: 30 – 1789 pg/ml) and women had 245.6±21.8 (range: 23 – 1887 pg/ml).

If 200 pg/ml was taken as cut off for deficiency state, total 182 out of 422 subjects (43%) turned out to be B12 deficient. In this group, 82 (19.4%) were men with mean levels of 127.8±7.22 pg/ml and 100 (23.6%) were women with mean of 118.2±7.1 pg/ml and no significant difference was observed between the two vitamin B12 deficient groups (p=0.346).

It was observed that 328/422 (77.7%) subjects had B12 levels less than 350 pg/ml. Using this cut off value, 152 males (36%) and 174 females (41.2%) were included in the category of vitamin B12 deficient. There was no significant difference in the levels between males and females (191.7±9.4 vs 184.6±9.6 respectively, p = 0.598).

Table 1
Vitamin B12 levels in total subjects according to sex

	<200 pg/ml		<350 pg/ml		Total	
	n (%)	mean±SEM	n (%)	mean±SEM	n (%)	mean±SEM
Male	82 (19.4%)	127.8±7.2	152 (36%)	191.7±9.4	214 (50.7%)	323.5±27.4
Female	100 (23.6%)	118.2±7.1	174 (41.2%)	184.6±9.6	208 (49.3%)	245.6±21.8
Total	182 (43%)	122.5±5.06	328 (77.7%)	187.9±6.7	422 (100%)	285.1±17.71

% - percentage out of total study subjects (422).

Gender appeared to contribute towards the B12 deficiency according to our study, females being more prone to develop B12 deficiency (Odds ratio 0.62, 95% confidence interval 0.41 to 0.93 at 200pg/ml, p< 0.05 and odds ratio 0.48, 95% confidence interval 0.29 to 0.78, p<0.05 at 350 pg/ml).

Average age of subjects in our study was 41 years (range 16 to 74 years). Forty six subjects out of a total of 422 screened were more than 60 years of age (10.9%). B12 was less than 200pg/ml in 22/46 (47.8%) and 12.1% of the total study population. Age >60 years did not appear to increase the risk for vitamin B12 deficiency (Odds ratio 1.23, 95% confidence interval 0.64 to 2.38, p =0.6). In the present study, the B12 deficienct groups (< 200pg/ml as well as <350 pg/ml), majority of the subjects

belonged to age groups 21-40 and 41-60 years (table 3). About 35 to 45% population belonged to this age group as compared to 15 to 18% of >60 years.

Table 2
Serum vitamin B12 distribution according to age and diet

	<200 pg/ml n (%)	Total subjects n (%)
>60 years	22/182 (12.1%)	46/422 (10.9%)
<60 years	160/182 (87.9%)	376/422 (89.1%)
Total	182	422
Vegetarians	59/107 (55%)	107/184 (58.2%)
Non vegetarians	16/77 (20.8%)	77/184 (41.8%)

% percentage out of denominator

Dietary data could be retrieved in 43.6% (184/422) of patients only, out of which 107 (58.2%) were vegetarians and 77 (41.8%) were non vegetarians. Among the vegetarian group, 59 out of 107 (55%) had vitamin B12 level less than 200 pg/ml where as only 16 of 77 non vegetarians (20.8%) had B12 deficiency (table2). Vegetarian dietary habit was found to be a substantial risk factor for B12 deficiency (Odds ratio 4.68, 95% confidence interval 2.39 to 9.15, $p<0.05$) in our population.

Table 3
Age distribution in low serum vitamin B12 groups

	<200 pg/ml			
	<20 years	21-40 years	41-60 years	>60 years
N=182	6	64	79	33
%	3.3%	35.16%	43.4%	18.13%
	<350 pg/ml			
N=328	10	115	154	49
%	3.04%	35.06%	46.95%	14.93%

DISCUSSION

Deficiency of vitamin B12 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. It has been observed that vitamin B12 deficiency is far more prevalent than expected and majority of the cases remain undiagnosed. In early stage vitamin B12 deficiency might present with subtle and slight cognitive impairments. Hence early recognition becomes crucial for preventing irreversible damage.

Dietary vitamin B12 deficiency is a severe problem in the Indian subcontinent [13] as seen in this study. The mean vitamin B12 level was observed to be 285.1 pg/ml which itself was on a lower side of the normal range. Present study of urban north Indian population (n=422) depicted that 43% of the total subjects (182 out of 422) had vitamin B12 deficiency (levels <200pg/ml). It was observed that when cut off value was raised to 350 pg/ml, 77.7% population was found to be vitamin B12 deficient. Although varying data have come into picture regarding prevalence, our finding is in consistence with a study where 47% of the Asian Indians had B12 deficiency confirming the high prevalence of this magnitude in Indians [14]. Though this study by Gupta et al was carried out in south Indians residing in Canada [14], prevalence was quite similar to our study indicating that there are other factors beyond vegetarian diet that may possibly be responsible for this deficiency. An earlier study has also demonstrated that vitamin B12 levels in Indians residing in United States are lower when compared to non-Indians in United States [15].

Over years various groups have proposed higher values as cut off for the deficiency state. In Japan, the lowest acceptable level for vitamin B12 in blood has been raised from 200 pg/ml to 550 pg/ml [16]. There is evidence that B12 deficiency might clinically present at a much higher B12 concentration i.e. in low normal range (500-600 pg/ml). Lindenbaum et al observed that many individuals presented with deficiency symptoms at serum vitamin B12 levels as high as 350 pg/mL [12] where as another group, Tiggelen et al recommended levels to be 600 pg/ml [17].

B12 deficiency was observed in 19.4% men and 23.7% women in the present study suggesting that risk of developing B12 deficiency is affected by gender. This is in contrast to a study conducted in Finnish elderly population where male gender was observed to increase the probability for vitamin B12 deficiency [18]. In another study conducted on South Asian patients the risk appeared to be similar for men and women [14].

Undiagnosed vitamin B12 deficiency becomes more prevalent and clinically evident with advancing age. In the present study a total of 46 subjects were found to be more that 60 years of age and out of that 22 (47.8%) were found to be B12 deficient (B12 <200 pg/ml) and 31 were observed to have serum vitamin B12 levels less than 350 pg/ml (67.9%). The prevalence rate observed in elderly population was not different from that of the total population. However it was much higher than that observed in Finnish elderly population (N= 1048) where low serum total vitamin B12 (<200 pg/ml) was observed in 12% and total vitamin B12 (200-340 pg/ml) in 38.1 % of the subjects [18]. This can be explained by occurrence of higher prevalence of vitamin B12 in Indians probably due to the dietary habits. However the bias due to inclusion of less number of elderly subjects in the present study cannot be ruled out. Several other studies done in western population have reported a lower prevalence in the general population ranging from 3% to 5% [19, 20] and from 5% to 35% [18, 21, 22] among people older than 65 years as compared to prevalence rates seen in the present study.

However, in the present study only 10.9% of the total subjects were >60 years.

Although no specific risk group can be identified in terms of age, undiagnosed vitamin B12 deficiency has been found to be common in the aged. However, the study population screened by us was younger than populations in some previous studies. Although only 10.9% of the total subjects were >60 years, 47.8% of them were B12 deficient (B12 <200pg/ml). The estimated risk of deficiency was higher among elderly people. According to our data 12.1% of the subjects diagnosed to be B12 deficient were >60 years. This was however in consistence with a population based study where the prevalence of vitamin B12 deficiency was 12% in subjects >60 years of age [18]. However the age seemed to offer no substantial risk for developing B12 deficiency in north Indians. Furthermore, in present study 35% of the B12 deficient population belonged to the age 21-40 years whereas 43 % (< 200pg/ml) to 46 % (< 350pg/ml) were in 41 -60 years group. This prevalence is quiet high in the reproductive age group. This could be attributed to dietary limitations due to vegetarian dietary habits and lower socio economic status.

Vegetarian diet has always been suspected to contribute towards the development of B12 deficiency. Although dietary data history recovered was partial in the present survey, but it could be assessed that vegetarian dietetic practice offered considerable risk for developing B12 deficiency, rate being 55% and 58% when cut off levels were 200 pg/ml and 350 pg/ml respectively. This is in consistence with several studies [23, 24].

In the present study vitamin B12 measurement was used as the first-line test and the definition of vitamin B12 deficiency was based on low level of serum vitamin B12 although measurements of metabolites such as methylmalonic acid and homocysteine have been shown to be more sensitive in the diagnosis of vitamin B12 deficiency [7, 25, 26].

There are few limitations in our study. First, since we have analyzed only the vitamin B12 levels of our population, the subjects having sub clinical deficiency (elevated homocysteine and methylmalonic acid with normal B12 levels) might have been missed out. Secondly, since it is a hospital based study, the population visiting the tertiary hospital cannot be considered representative of the north Indians. Thirdly, dietary information could not be retrieved in all cases which might contribute to observed bias of higher prevalence of B12 deficiency in vegetarian subjects. Also subjects considered as non vegetarians might be occasional meat consumers. A number of large population based studies are required to validate our study findings in a broader perspective. 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 B12 deficiency. However large population based studies may provide a better outlook about the magnitude of this problem.

CONCLUSION

Role of vitamins in maintenance of health has received tremendous attention in recent times. Vitamin B12 deficiency is not just a laboratory finding but a clinically relevant issue which needs to be explored. Female gender and vegetarians are at substantial risk to develop B12 deficiency. The magnitude of the prevalence of B12 deficiency estimated in our population strengthens the argument that B12 deficiency is more prevalent in Indians. Hence there is a need to include screening of vitamin B12 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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