ORIGINAL RESEARCH

A Hospital Based Prospective Study to Estimate the Prevalence of Vitamin B12 Deficiency in Vegetarian Outpatients Between 18-60 Years of Age Presenting at a Tertiary Care Centre

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ABSTRACT
Background: Vegetarianism has been well known and commonly found in India since ancient times. Animal products provide the only dietary source of vitamin B_{12}. Vegetarianism is a well-known risk factor for vitamin B_{12} deficiency. The aim of this study to determine the prevalence of vitamin B_{12} deficiency among vegetarian outpatients, between the age groups of 18 and 60 years, visiting the internal medicine outpatient department of a tertiary level teaching hospital.
Materials& Methods: A hospital based prospective study in department of medicine at government S.K. Medical College, Sikar, Rajasthan, India during one year period. A prevalence study for any factor is best done in the community – in a lot of situations that would reduce the bias of a hospital study. This being a hospital study has its own limitations. However as far as possible we wanted to choose a population in our hospital survey that would most closely reflect the community or the general population at largeVegetarian patients were defined as patients that had been consuming a diet devoid of any form of meat, at least for three completed years prior to the date of recruitment. At the point of contact with the subject in the outpatient department he or she also underwent certain biochemical and haematological tests that included serum vitamin B12 and folate levels, and basic haemograms.
Results: In our study population, 61.25% had levels below 200, 22.5% had levels between200and300and16.25%hadlevelsabove300 pmol/L. The mean vitamin B12 levels in the three groupsrespectively were 144.8, 269.2 and 233.4. There was no statistically significant differencebetweenthe groupsbyANOVA. At various laboratory parameters among the three varieties of dietconsumers – however the numbers were too small for any statistical analysis betweenthem. Mean corpuscular values of less than or equal to 100fl were

categorised asnormocytic category and anything higher than that was considered as qualifying formacrocytosis. A folate level cut-off at 9.5 was also taken to look for prediction ofvitaminB12 deficiency.

Conclusion: The vegetarian diet can be sustainable at all stages of life and in all physiological conditions, including infancy, pregnancy, lactation, senescence and sports. However, underestimating the correct supplementation of cobalamin (Cbl) can nullify these benefits. It is also necessary that the diet be balanced and nutritionally adequate to reduce the risks of other deficiencies which could indirectly affect the absorption of Cbl.

Keywords: Vegetarian Diet, Vitamin B_{12}, Cobalamin, MCV, Anemia.

INTRODUCTION

Vitamin B12 is an essential nutrient that is used by the body to act as cofactors in certain key reactions within the cells. Cobalamin along with folate is needed for synthesis of DNA required in cells undergoing rapid turnover, such as haematopoetic and enteric lining cells. The physiological consequences of a deficiency in any of the above nutrients are increased homocysteine, reduced methionine and impaired formation of tetrahydrofolate. These changes ultimately lead to the characteristic neurological and haematological manifestations, not to mention certain events like vascular thromboses that may be seen even in the absence of overt vitamin B12 deficiency.[1]

Vegetarianism has been well known and commonly found in India since ancient times. It has probably in many ways got entangled with the socio-religious psyche of the people. A survey published in The Hindu a few years ago claimed that 31% of Indians are pure vegetarians and another 9% are vegetarians who eat egg – in other words, 40% of the country does not consume meat or fish. The tendency towards vegetarianism apparently seems to be more in land-locked states like Rajasthan, Haryana, Punjab, Uttar Pradesh etc and less in the coastal states like Kerala, Andhra Pradesh, Tamil Nadu, West Bengal etc. People have also claimed that India houses more vegetarians than all vegetarians put together all over the world. In view of the above, India becomes a nation likely to harbor vitamin B12 deficiency in profusion among the masses.[2]

A worldwide prevalence for a condition such as this is probably difficult to come by – however discrete reports in various articles have quoted prevalence in various regions of the world. Review of such data reveals that the numbers differ in various age groups.[1]

Animal products provide the only dietary source of vitamin B_{12}. Vegetarianism is a well-known risk factor for vitamin B_{12} deficiency. Other important etiological considerations are pernicious anaemia and malabsorption. The treatment is simply supplementing the vitamin in the body with extremely satisfying results at minimum costs and adverse effects. The clues to vitamin B_{12} deficiency in the outpatient department though occasionally glaringly simple might in many situations be more subtle and easily missed - looking at the prevalence of anaemia in vitamin B12 deficient people. These further drives home the point that among the varied spectrum of manifestations of vitamin B_{12} deficiency there may be no evidence of anaemia, with other features being present, even with very low levels of vitamin B_{12}. This group would include a lot of fairly asymptomatic individuals with low vitamin B_{12} levels – the exact clinical correlates of such a situation remain to be clearly elucidated.[3]

The aim of this study to determine the prevalence of vitamin B_{12} deficiency among vegetarian outpatients, between the age groups of 18 and 60 years, visiting the internal medicine out patient department of a tertiary level teaching hospital.

MATERIALS& METHODS

A hospital based prospective study in department of medicine at government S.K. Medical College, Sikar, Rajasthan, India during one year period. A prevalence study for any factor is best done in the community – in a lot ofsituations that would reduce the bias of a hospital study. This being a hospital study hasits own limitations. However as far as possible we wanted to choose a population in ourhospital survey that would most closely reflect the community or the general populationat large. Hence for our study, we excluded people who were significantly ill or peoplewith multiple interacting problems. We also excluded the elderly as in that populationthere would be the elements of age, poor absorption and poor nutrition confounding theresults. Vegetarian patients were defined as patients that had been consuming a diet devoid ofany form of meat, at least for three completed years prior to the date of recruitment.

METHODOLOGY

A history and physical examination were done for all patients (N=80) at the time of thefirst contact and enrollment. Detailed collection of data was performed for all the subjectsin the following areas:

1. Epidemiological and geographic profile
2. Demographic profile and the reason for attending the outpatient clinic
3. Dietary profiles, that were sub divided into three groups
4. Clinical profile including specific symptoms and signs

Data regarding the epidemiologic profile, the dietary profile, the clinical profile and the level of awareness were recorded in a preformed proforma. The proforma was filled in by the investigator at the time of the first interview and contact.

The neuropsychiatric profile of the subjects was assessed by two investigator administered questionnaires:

[1] Hospital Anxiety and Depression Score – This has been validated before in various situations.[4-7]

[2] General Health Questionnaire – 12; this has also been validated before.[6]

The above two questionnaires could have been used as self administered forms, but for the sake of standardization, they were administered by the investigator who was constant for the entire population of study.

At the point of contact with the subject in the outpatient department he or she also underwent certain biochemical and haematological tests that included serum vitamin B12 and folate levels, and basic haemograms.

BIOCHEMICAL ESTIMATION OF VITAMIN B12

Vitamin B12 in the serum samples was measured by an Elecsys 2010 Vitamin B12 assay (MODULAR ANALYTICS 2010). The Elecsys vitamin B12 assay employs a competitive test principle using intrinsic factor specific for vitamin B12. Vitamin B12 in the sample

competes with the added vitamin B12 labelled with biotin for the binding sites on the ruthenium-labelled intrinsic factor complex.

STATISTICAL ANALYSIS

Data entry was done using the Statistical Package for the Social Sciences (SPSS) software package (version 22.0). The chi-square test was used for comparison of categorical variables. Odds ratios (OR) and confidence intervals (CI) were calculated and a 'p' value less than 0.05 was considered statistically significant.

RESULTS

The characterization of vitamin B12 deficiency was made according to well known standards(75-77). The following three categories were looked at:

- >300pg/mL(>221pmol/L)—normal result; cobalamin deficiency is unlikely (i.e., probability of 1to 5 percent)
- 200 to 300 pg/mL (148 to 241 pmol/L) — borderline result; cobalamin deficiency possible
- <200 pg/mL (<148 pmol/L) — low; consistent with cobalamin deficiency (specificity of 95 to 100percent)

The mean vitamin B12 level in the population was 265.82 pg/mL and the vast majority of the subjects had vitamin B12 levels between 125and 250 pg/mL.

In our study population, 61.25% had levels below 200, 22.5% had levels between 200 and 300 and 16.25% had levels above 300pmol/L.

The mean values of folate in the low, borderline and normal vitamin B12 groups were 8.96, 9.87 and 13.27 ng/mL. The mean folate level was significantly different between the groups by one way ANOVA, with a $p<0.001**$. Thus, the mean folate concentration was significantly higher in the normal B12 group compared to the lowB12 group. Hence as is clear from Figure 1, there was a clear correlation between the folate levels and the B12 levels that was statistically significant. Patients with lower B12 levels had lower folate levels.

Figure 1: Correlation between folate and B12 values in the entire study population (p=0.001)
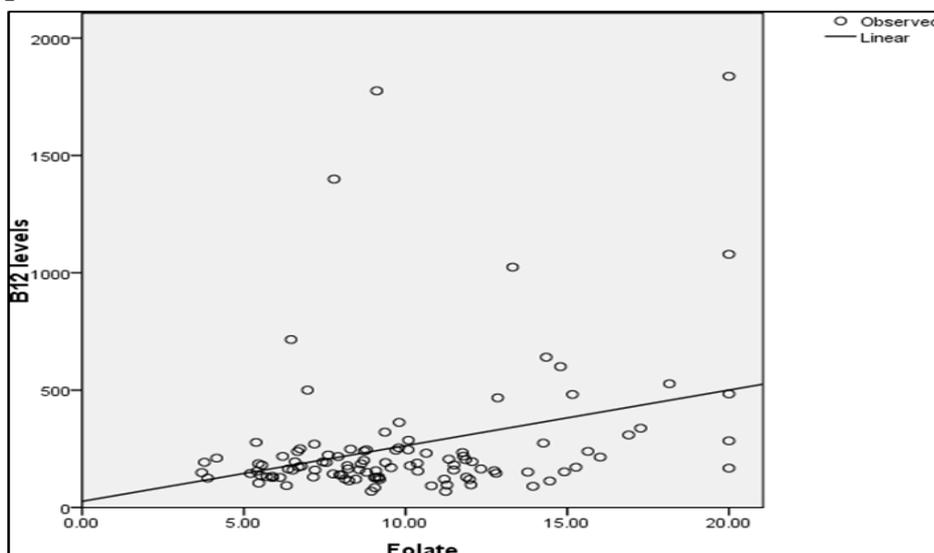
We had classified the subject population into three categories: pure vegetarians (vegans), vegetarians who consumed milk products and vegetarians who consumed milk products and eggs (eggeterians). The mean vitamin B12 levels in the three groups respectively were 144.8, 269.2 and 233.4. There was no statistically significant difference between the groups by ANOVA. At various laboratory parameters among the three varieties of diet consumers – however the numbers were too small for any statistical analysis between them(table: 2).

Table 1: Vitamin B12 categories among the genders

Vitamin B12 category	Males – (N=38)	Females – (N=42)	Total
Low	26 (68.42%)	23 (54.76%)	49 (61.25%)
Borderline	5 (13.15%)	13 (30.95%)	18 (22.5%)
Normal	7 (18.42%)	6 (14.28%)	13 (16.25%)

Table2: Laboratory parameters amongst the three dietary groups

Parameter	Pure vegetarian (N=4)	Vegetarian with milk products (N=72)	Vegetarian with milk products and eggs (N=4)
Average B12 value	144.8	269.2	233.4
Low B12	3(75%)	43 (59.72%)	2 (50%)
Average MCV	92.36	89.57	91.10
Macrocytosis	2(50%)	8 (11.11%)	1(25%)
Average Hb	12.92	13.08	12.99
Anaemia (Hb<12)	1(25%)	21 (29.16)	2(50%)

We used odds ratios and Chi square tests for studying categorical variables (table3) as are shown below. For this analysis we assumed people who had vitaminB12 levels below 200 to be in the "low" vitamin B12 category and all the others to be in the "normal" B12 category.

Table 3: Odds ratios of clinical parameters

Parameter		Numbers (Percentages)		Odds ratio	Confidence intervals	P
		Low B12 group	Borderline and normal			
Gender	**Male**	26 (68.42%)	12 (31.57%)	1.62	0.760-3.643	>0.05
	Female	23 (54.76%)	19 (45.23%)			
Hyper pigmentation	**Present**	4 (8.16%)	3 (9.67%)	0.99	0.254-3.578	>0.05
	Absent	45 (91.8%)	28 (90.32%)			
Glossitis	**Present**	22 (64.7%)	12 (35.29%)	1.332	0.623-2.987	>0.05
	Absent	27 (58.69%)	19 (41.30%)			
Paraesthesias	**Present**	20 (57.14%)	15 (42.85%)	0.822	0.376-1.778	>0.05
	Absent	29 (64.44%)	16 (35.55%)			
MCV	**>100**	9 (90%)	1 (10%)	10.06	1.275-80.06	<0.05*
	<100	40 (57.14)	30 (42.85)			

Folate	<9.5 ng/mL	31 (70.45%)	13 (29.54%)	2.86	1.234-6.163	<0.05*
	>9.5 ng/mL	18 (50%)	18 (50%)			

The "borderline' category was not used, both for statistical problems and small numbers as well as for the fact that the concept of "borderline" vitamin B12 levels is neither very uniform not very well characterized in other studies. Mean corpuscular values of less than or equal to 100fl were categorised as normocytic category and anything higher than that was considered as qualifying formacrocytosis. A folate level cut-off at 9.5 was also taken to look for prediction ofvitaminB12 deficiency.

During the clinical interview we had done an assessment of the prevalence ofanxiety and the depression within the study population with the help of two widely usedscoring systems – namely the General Health Questionnaire – 12, and the HospitalAnxietyandDepressionScore. The GHQ-12 theoretically can have scores between 0 and 36, with scores upto 15being taken as normal, 16-20 suggesting evidence of distress and 21 and above signifying severedistress. The GHQ-12 scores & the HADS-A scores were correlated with the vitamin B12values within the entire study population – there was no such significant correlation were shown in figure 2&3.

Figure 2: Correlation between GHQ-12 scores and B12 levels in the entire population
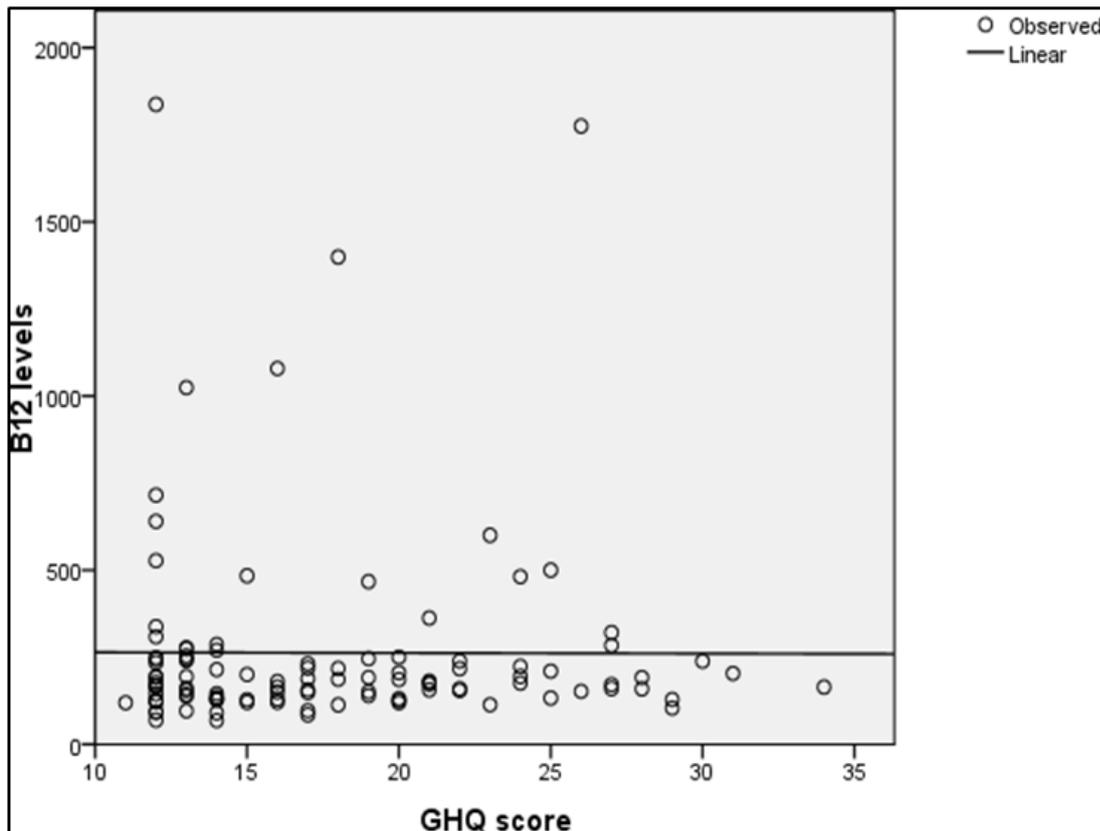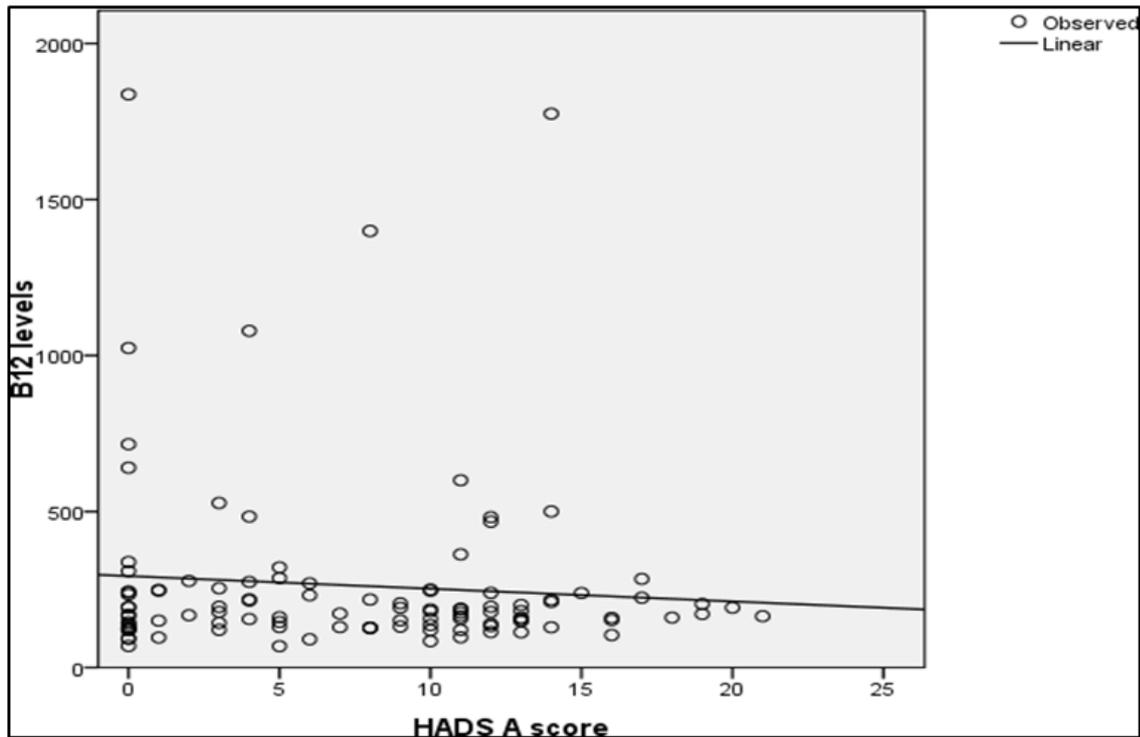
Figure 3: Correlation between HADS-A scoresandB12levels

DISCUSSION

Vitamin B12 is an essential nutrient that is used by the body to act as cofactors in certain key reactions within the cells. Cobalamin along with folate is needed for synthesis of DNA required in cells undergoing rapid turnover, such as haematopoetic and enteric lining cells. The physiological consequences of a deficiency in any of the above nutrients are increased homocysteine, reduced methionine and impaired formation of tetrahydrofolate. These changes ultimately lead to the characteristic neurological and haematological manifestations, not to mention certain events like vascular thromboses that may be seen even in the absence of overt vitamin B12 deficiency. Animal products provide the only dietary source of vitamin B12. Vegetarianism is a well-known risk factor for vitamin B12 deficiency. Other important etiological considerations are pernicious anaemia and malabsorption. The treatment is simply supplementing the vitamin in the body with extremely satisfying results at minimum costs and adverse effects.[8]

In our study showed men and women were almost equal in number and the overall age distribution appeared uniform. All subjects were Hindus. We had excluded the elderly age group and the paediatric age group. Owing to this being a hospital-based study with all its inherent biases the question of generalizability to the community at large is debatable.

The mean vitamin B12 level in the population was 265.82 pg/mL and the vast majority of the subjects had vitamin B12 levels between 125and 250 pg/mL. In our study population, 61.25% had levels below 200, 22.5% had levels between200and300and16.25%hadlevelsabove300. This is comparable to the community study by Yajnik et al[9], in which 67% of the population had a low B12 level.

Hence from this data it is clear that absolutely low levels of vitamin B12 among vegetarians, are rampant in the country, and the percentages rise even higher if we look at borderline B12 levels. We know from previous studies that even with low normal B12levels (i.e. in the borderline levels) there are biochemical changes that initiate within the body that can potentially be damaging. Hence almost 84% of the population studied here are at a risk for possible end organ damage. This is a large number, and it remains to be seen if the same trend is being duplicated in other parts of the country as well.

Folate deficiency may not always coexist with B12 deficiency, their physiology and metabolism being distinct. Serum folate levels typically take very short whiles to normalise – for example a single meal maybe enough to normalize serum folate even if tissue stores are low. Hence it has been argued before that red cell folate is a better indicator of tissue folate stores. Furthermore, folate is obtained from greens and not solely obtained from animal products. Folate deficiency is typically seen in subjects who are chronic alcoholics and those who are on medications that might antagonise folate metabolism – none of those scenarios where prevalent in our study population. Hence it was not surprising that we did not pickup any folate deficiency.[10]

Glossitis, paraesthesias and pallor where the most common clinical features amongst the subjects with low vitamin B12 levels. Hyper pigmentation and jaundice were seen to be less frequent in the B12 deficient group. No clinical feature could actually predict low B12 levels in the population. Diabetes and hypertension were not very frequent among the groups. Nobody had any other major comorbidity. Psychiatric symptoms have been analyzed separately in a later section.

An extremely low level of awareness of the vegetarian population under study, regarding this condition of vitamin B12 deficiency, was clearly apparent from the analysis of the questionnaires. Given the fact that the prevalence of the condition is high, and vegetarianism is common in India[2], it becomes all the more important to assess the awareness of this condition in the community, for public health benefits. There may be important clinical and therapeutic implications of this in the field of prevention of this condition. This maybe an important issue to look into, in future studies.

One important aspect of this study was to look at the neuropsychiatric symptomatology in our study population. Though neuropsychiatric manifestations have been described in the B12 deficient, there is not much data as to the patterns and prevalences of these symptoms. With both our screening tools (i.e. the General Health Questionnaire – 12, and the Hospital Anxiety and Depression Score), we found a high level of anxiety and depression in our study population. Unfortunately, there were no major statistical differences between the low, borderline and normal B12 categories in terms of characterization and degree of neuropsychiatric symptoms – none of them were found to be able to significantly predict B12 deficiency. Correlation studies between the anxiety and depression scores and the B12 levels in the population were not found to be statistically significant.[11]

CONCLUSION

The choice of limiting or removing foods of animal origin from the diet is increasing in popularity due to ethical, environmental and health reasons, posing doubts over whether a number of these restrictions could be detrimental or useful. The vegetarian diet can be

sustainable at all stages of life and in all physiological conditions, including infancy, pregnancy, lactation, senescence and sports. However, underestimating the correct supplementation of cobalamin (Cbl) can nullify these benefits. It is also necessary that the diet be balanced and nutritionally adequate to reduce the risks of other deficiencies which could indirectly affect the absorption of Cbl. Studies on the use of plant foods to increase the Cbl intake are promising, but still require more data.

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