



ORIGINAL RESEARCH PAPER

Pathology

PREVALENCE OF VITAMIN B12 DEFICIENCY AND ITS CORRELATION TO THE HAEMATOLOGICAL PARAMETERS IN ANAND CITY- GUJARAT

KEY WORDS: Vitamin B12, Vegetarian, Non vegetarian, Hemoglobin and Red Blood Cell Indices.

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ABSTRACT

Vitamin B12 deficiency is a serious health problem that can result in megaloblastic anemia, inhibition of cell division, and neurological disorders. Our study aims to identify the correlation between the deficiency of vitamin B12 and the hematological parameters, also the prevalence of vitamin B12 deficiency among vegetarian and non-vegetarian people of the city of Anand in Gujarat state. We evaluated the clinical picture, hematology indices of 100 patients with reduced vitamin B12 level. In this study secondary data on age, sex, referring unit, religion and dietary history of these patients were obtained from the medical laboratory of Dr. Krunal in Anand district, Gujarat, India. We found the gender is significantly associated with hemoglobin. Vegetarian females have lower hemoglobin level than vegetarian males. Outcome of 77% of patients had normal ranges of MCV which tells us that MCV is not the only diagnostic parameter for vit.B12 deficiency. Vitamin B12 deficiency is mainly mild to moderate (100pg/ml – 210pg/ml). Severe B12 deficiency (≤ 100 pg/ml) is more involved in the younger age group (<50 years) than the older group (> 50 years). The peripheral blood film mainly shows Normochromic Normocytic RBCs during B12 deficiency in both vegetarians and non-vegetarians.

Conclusion: In our study, we concluded that the vegetarians have lower serum vitamin B12 levels compared to non-vegetarians.

INTRODUCTION

Vitamin B12 deficiency is characterized by megaloblastic anemia, fatigue, weakness, constipation, loss of appetite, and weight loss. However, in vegetarian and vegan populations, dietary insufficiency is the major cause. Furthermore, high levels of folate can mask vitamin B12 deficiency — a concern for vegetarians and vegans whose folate intake is generally high while vitamin B12 intake is low. The addition of vitamin B12 to any foods fortified with folate has been advocated to prevent masking of hematological and neurological manifestations of vitamin B12 deficiency. Subtle neurological damage (even in the absence of anemia) may be more likely in vegans because of their increased folate levels preventing early detection of vitamin B12 deficiency. Vitamin B12 deficiency can also lead to the removal of myelin from the peripheral nerves, spinal cord, cranial nerves and brain, resulting in nerve damage and nervous and psychological abnormalities.

Neurological symptoms of vitamin B12 deficiency include numbness and tingling of the hands and feet, decreased sensation, difficulties walking, loss of bowel and bladder control, memory loss, dementia, depression, general weakness and psychosis. Unless detected and treated early, these symptoms can be irreversible(Zeuschner et al., 2012).

Measurement of total vitamin B12 is simple, inexpensive and widely available, but it lacks both sensitivity and specificity. A recent report shows 46.9% of non-anemic adult subjects having subnormal levels of vitamin B12. Another study showed 60% of vegetarians and 39% of non vegetarians were B12 deficient. Erythrocyte indices have been used in the initial evaluation of anemic patients; high Mean corpuscular volume (MCV) value is a criterion for the diagnosis of folate and vitamin B12 deficiencies. There is no large study of the prevalence of B12 deficiency among patients with normocytosis or microcytosis (Björkegren & Svärdsudd, 2003).

MATERIALS AND METHODS

This cross sectional study included a total of 100 patients with reduced vitamin B12 deficiency were analyzed. The data has been collected from the Medical Laboratory of Dr. Krunal in Anand district, Gujarat, India. In this study data on vitamin B12 level, Complete Blood Cells Count, Age and Sex were collected. Informations about religious background of the patients were also gathered. Patient's data were collected. Inclusion criteria: all have vit.B12 deficiency. Exclusion criteria: other specific diseases or treatments involved.

Serum vitamin B12 levels were measured in picograms, the normal value range of serum vitamin B12 level is between 210 and 900 pg/ml according to WHO (World Health Organization) Scientific Group on Nutritional anemia(Organization, 1968, 2009).

The determination of vitamin B12 was performed on fully automated immunoassay Bekman Coulter Access-2 by Immunometric Assay method (Competitive principle) (Fernandez, Wang, Chao, & Guignon, 1990).

Complete blood cells count was done by using a fully automated - 5- part hematology analyzer (*Mindray BC – 5150*.)

Statistical Analysis:

Descriptive statistics were computed with percentages and proportion. Pearson correlation, P-value, and the mean plus or minus standard deviation ($\bar{x} \pm SD$.) by SPSS statistical computerized program under the supervision of Prof. Mansour Ali Ataa. (Professor of Biostatistics) M.Sc. from the University of Exeter – UK., and PhD from the University of Liverpool – UK.

P-value of less than 0.05 considered to be Significant.

RESULTS:

Out of the total 100 patients, there were 45(45 %) patients who

were males and 55 (55%) who were females. Our studies depicts that out of the total 100 patients there were 81(81 %) patients who followed a vegetative diet and 19 (19%) who were non vegetative, and among the vegetarian patients 35 (43%) were males and 46 (56.7%) were females and among the non-vegetarians there were 10 males (52.6%) and 9 (47.3%) were females. (See figure 1)

Out of 100 patients who were vitamin B12 deficient 4% had high MCV (>100 fL). 19% had low MCV (<76 fL) and 77% were with normal values of MCV (76 - 100 fL). Also this study had 73(73%) mild to moderate (> 100 pg/ml) vit.B12 deficiency, while 27(27%) had severe deficiency of vit.B12 (≤ 100 pg/ml).

Vegetarians and non-vegetarians among those who have severe Vit.B12 deficiency (less than 100 pg/ml) females are more dominant (16 %) than males (11%). Those who have Vit.B12 more than or equal 100 pg/ml Females are more dominant (39%) then males (34%).

There is a statistically mean difference between the two vitamin B12 deficient groups according to the sex with a (p-value of 0.000).

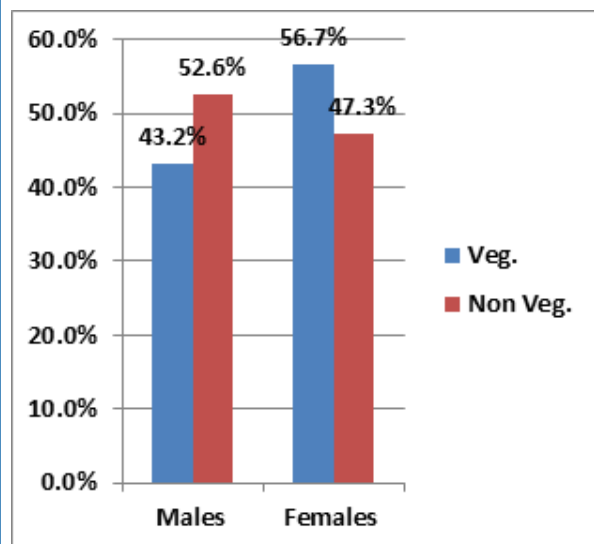


Figure (1): Percent distribution of Vitamin B12 deficiency in vegetarians and non-vegetarians according to Gender

Table (1): Distribution of vitamin B12 by the number of Patients and the mean of MCV (fl)

Vit.B12 (pg/ml)	No % of patients	Mean MCV (fl)
< 100 pg /ml	27 %	84.0
100 – 210 pg / ml	73 %	81.1

Table (1) : Out of 100 patients 73(73%) had mild to moderate (> 100 pg/ml) vit.B12 deficiency, while 27(27%) had severe deficiency of vit.B12 (≤ 100 pg/ml)

Table (2): Comparison between the mean levels of Vit.B12, Hb and MCV in Vegetarians and Non-Vegetarians

Type		B12	Hb	MCV
Vegetarians (81)	Mean	135.47	12.71	82.01
	Std. Deviation	38.68	2.10	11.20
Non Vegetarians (19)	Mean	148.89	13.41	83.36
	Std. Deviation	41.40	2.02	7.71

Table (2): the mean levels of Vit.B12, Hb and MCV were higher in Non-Vegetarians compared to Vegetarians. The mean levels were (148.89, 13.41 and 83.36) respectively in Non-Vegetarians. But in Vegetarians, the mean level were (135.47, 12.71 and 82.01) respectively.

Table (3): Relationship between Hemoglobin and MCV with

respect to the low vit.B12 level in vegetarians according to Gender.

	Vegetarians with reduced Vit.B12 level		
	Females SD.	Males SD.	P – value t-test
Hemoglobin (g/dl)	11.7 1.8	14.1 1.6	0.000
MCV (fl)	79.70 11.14	84.02 10.10	0.07

Table (3): Shows the mean of Hb g/dl for males is 14.1 and that for females is 11.7. The mean difference of Hb g/dl, is 2.46 in favor of males. In order to test whether the mean difference is significant or not, the t-test for testing the null hypothesis that, there is no significant difference in the means between vegetarian males and females, against the alternative hypothesis that the Hb g/dl mean difference is a significant difference. As can be seen from Table 6, the t-test shows that the mean difference is highly significant with (p-value == 0.000). The mean difference of MCV in both males and females is weakly significant with a (p-value = 0.07).

DISCUSSION:

The results obviously showed that vitamin B12 deficiency was higher with the vegetarian diet and the incidence was more in women 55 (55 %) than men 45 (45 %). that confirmed by the previous studies that reported The percentage of vit.B12 deficient was much higher in vegetarians than non-vegetarians(Kankonkar et al., 2004; Mangukiya, Modi, Chaurasia, Modi, & Shah, 2011; Zeuschner et al., 2012).

In the present study, the distribution of vitamin B12 deficiency shows that vit.B12 deficiency occur mainly in vegetarians 81(81%) and rarely occurs in non-vegetarians 19(19%) which agrees with the results of most studies(Kankonkar et al., 2004; Mangukiya et al., 2011).

Vegetarian Females are more exposed to vit.B12 deficiency 46(56.7%) than vegetarian Males 35(43.2%) while non-vegetarian males are exposed to vit.B12 deficiency 10(52.6%) more than non-vegetarian females 9(47.3%) which agrees with other results of previous work were done before (Farmer, 2009; SINGH, GUPTA, VENKATESAN, & ARORA).

Traditionally, vitamin B12 deficiency is suspected in subjects with macrocytic anemia, but many previous studies have shown that Microcytic anemia and macrocytic anemia are often not present in subjects with vitamin B12 deficiency. In the present study, Microcytic anemia was recorded in 19.7%, and macrocytic anemia in 3.7% of vegetarians while in non-vegetarians Microcytic anemia occurs in 10.5% and macrocytic anemia don't occur 0% (Table 3) with low total vitamin B12. Overall, Microcytic anemia or macrocytosis did not predict vitamin B12 deficiency. 84% of patients would be misdiagnosed if only MCV was used according to other studies(Loikas, 2007)

The prevalence of vit.B12 deficiency according to the vit.B12 serum level (≥ 100 pg/ml) was 39 % in females and 34 % in males, of which our results were similar to those of the other studies, the prevalence of vit.B12 deficiency in females was more than males(Lankarani et al., 2015). But in severe vitamin B12 deficiency (< 100 pg/ml) in females was 16 % and was 11 % in males; therefore, the percentage of severe deficiency is higher in females. The present study was similar to another study, where the results showed that, the prevalence of vit.B12 deficiency weren't based on age groups but rather were statistically significant in gender (P value = 0.000) (Lankarani et al., 2015)(Table 4)

Most of the populations in India are vegetarians due to cultural and religious reasons. A vegetarian diet is considered to promote health and longevity by protecting against conditions such as cardiovascular disease (CVD) and cancer; however this diet may be deficient in some nutrients. Especially a strict vegetarian diet has been associated with increased risk of vitamin B12 deficiency. (2)

Out of 100 patients with reduced vitamin B12 level, 19% had low MCV and 4% had high MCV and 77% were with normal value of

MCV, which agrees to another study(Lankarani et al., 2015).

M. R. (2012). Vitamin B12 and vegetarian diets. Medical Journal of Australia, 9, 27.

This present study shows that patients with B12 cutoff (≤ 100 pg/ml) represent 27(27%) 20(74%) of them are less than 50 years and 7(26%) of them are bigger than 50 years. while those who have a cutoff (>100 pg/ml) represent 73(73%), 52(71%) of them are less or equal to 50 years and 21(29%) are bigger than or equal 50 years. These findings agree to another study(Khodabandehloo, Vakili, Hashemian, & Zardini, 2015).

27% of patients from both categories (veg. and non veg.) had vit.B12 level less than 100pg/ml and 73% of them had vit.B12 level more than 100 pg/ml. It appears that the Mean of MCV tend to be higher in lower vit.B12 levels, which agrees with another study(Jain, Kapil, & Gupta, 2012).

A statistically significant (P value = 0.000) relationship between Hemoglobin with vegetarian males and females who have vit.B12 deficiency has been found in the present study. Females tend to have lower hemoglobin levels than males with a mean difference of 2.46. MCV has a weak mean significant difference (p value = 0.07) with vegetarian males and females who are vit.B12 deficient,(Table 1) this finding is in accordance with that of another study(Björkegren, 2003; Khanna, Lal, Kommi, & Chakraborty, 2006).

No statistical significance between Hb and MCV according to the age (Table 2) (p =value 0.2 and 0.1 respectively) has been found which tells us there is no age dependence of Hb or MCV among vit.B12 deficiency patients according to another study(Björkegren, 2003).

CONCLUSION:

In our study, we concluded that the vegetarians have lower serum vitamin B12 levels compared to non-vegetarians. Also, the mean levels of Hb and MCV in this population were higher in Non-Vegetarians compared to Vegetarians.

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REFERENCES:

1. Björkegren, K. (2003). Studies on Vitamin B12 and Folate Deficiency Markers in the Elderly: A Population-based Study. Acta Universitatis Upsaliensis.
2. Björkegren, K., & Svärdsudd, K. (2003). Reported symptoms and clinical findings in relation to serum cobalamin, folate, methylmalonic acid and total homocysteine among elderly Swedes: a population-based study. *J Intern Med*, 254(4), 343-352.
3. Farmer, B. (2009). Comparison of nutrient intakes for vegetarians, non-vegetarians, and dieters: results from the National Health and Nutrition Examination Survey 1999-2004. Masters Theses and Doctoral Dissertations, 150.
4. Fernandez, S. M., Wang, H.-P., Chao, Y.-S., & Guignon, E. F. (1990). Time-resolved fluorescence immunoassay: Google Patents.
5. Jain, R., Kapil, M., & Gupta, G. N. (2012). MCV should not be the only criteria to order vitamin B12 for anemia under evaluation. *Open Journal of Gastroenterology*, 2(04), 187.
6. Kankonkar, S., Joshi, S., Tijoriwala, S., Prabhu, R., Raikar, S., Kankonkar, R., & Dhar, H. (2004). A study of vitamin B12 deficiency in different diseases. *Bombay Hospital Journal*, 46.
7. Khanna, G., Lal, P., Kommi, K., & Chakraborty, T. (2006). A Comparison of a Vegetarian and Non-Vegetarian Diet in Indian Female Athletes in Relation to Exercise Performance. *Journal of Exercise Science and Physiotherapy*, 2, 27-34.
8. Khodabandehloo, N., Vakili, M., Hashemian, Z., & Zardini, H. Z. (2015). Determining Functional Vitamin B12 Deficiency in the Elderly. *Iranian Red Crescent Medical Journal*, 17(8).
9. Lankarani, K. B., Peymani, P., Zare, S., Tabrizi, R., Kazemi, M., & Omrani, G. (2015). Prevalence of vitamin b12 and folate deficiencies and homocysteinemia in elderly population of Shiraz, southern Iran. *Tropical Journal of Pharmaceutical Research*, 14(10), 1907-1912.
10. Loikas, S. (2007). Vitamin B12 deficiency in the aged: laboratory diagnosis, prevalence and clinical profile.
11. Mangukiyaa, S., Modi, B., Chaurasia, P., Modi, K., & Shah, R. M. (2011). Distribution of Vitamin B12 Level According to Socio-Demographic Characteristics in Patients of Dhiraj General Hospital, Pipariya, Gujarat. *National Journal of Medical Research*, 1(2), 54-56.
12. Organization, W. H. (1968). Nutritional anaemias: report of a WHO scientific group [meeting held in Geneva from 13 to 17 March 1967].
13. Organization, W. H. (2009). Global health risks: mortality and burden of disease attributable to selected major risks: World Health Organization.
14. SINGH, B., GUPTA, V. K., VENKATESAN, M., & ARORA, S. BURDEN OF VITAMIN B12 DEFICIENCY IN URBAN POPULATION IN DELHI, INDIA: A HOSPITAL BASED STUDY.
15. Zeuschner, C. L., Hokin, B. D., Marsh, K. A., Saunders, A. V., Reid, M. A., & Ramsay,