

A STUDY OF CORRELATION BETWEEN VITAMIN B12 DEFICIENCY AND ITS CLINICAL, HAEMATOLOGICAL AND ELECTROPHYSIOLOGICAL PARAMETERS

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ABSTRACT:

Objectives: Vitamin B12 is a micronutrient essential for appropriate neuro-cognitive, hemopoetic and cardiovascular function. This study was planned to find correlation between vitamin B12 deficiency and its clinical, haematological and electrophysiological parameters.

Material and Methods: This study was a cross sectional study which included 125 patients who had attended Maharana Bhupal Govt. Hospital, Udaipur from 1st November 2013 to 31st August 2014. The study included all cases of anaemia with or without neurological manifestation with suspected B12 deficiency, anaemia with history of chronic alcoholism, smoking, tobacco chewing, beetle nut chewers, chronic diarrhoea and Past history of gastric surgery.

Results: Total 125 patients were included in study, among them 53 were male and 72 were females. Majority 69 (55.2%) patients were more than 60 years of age. Eighty six (68.8%) patients had severe Vitamin B12 deficiency, among them 42(63.6%) were females. Neurological complains like parenthesis, ataxia, decreased vision, psychiatric and cognitive impairment were observed in 68 (54.4%) patients among them parenthesis were present in three fifth (60.29%) patients. Reduced vision was present in only 3 (5.8%) patients. Nineteen percent patients had myeloneuropathy and Optic atrophy was noted in all 3 patients who were presented with reduced vision.

Conclusion: Deficiency disorders of vitamins associate with plethora of clinical manifestations. Old age person, female gender and vegetarians are at considerable risk to develop B12 deficiency.

Key Words: Vitamin B12, hemopoetic, neuro-cognitive function, cardiovascular function, megaloblastic anaemia.

INTRODUCTION

Vitamin B12 is considered as an essential micronutrient for optimal hematopoietic, cardiovascular and neurocognitive function. In type 1 and type 2 diabetes mellitus cases, biochemical and clinical vitamin B12 deficiency

has been demonstrated & highly prevalent. It presents with various clinical manifestations ranging from megaloblastic anaemia, pancytopenia, impaired memory, delirium, dementia, peripheral neuropathy, and sub acute

combined degeneration of the spinal cord.

Deficiency of vitamin B12 is not very common as the human body can store vitamin B12 last for up to five years. The commonly used investigations are – Low serum vitamin B12 level or megaloblastic bone marrow or both which are the basis of diagnosis of deficiency of vitamin B12 (1, 2). There has been much dispute over the normal range of vitamin B12 and also about the burden of B12 deficiency in the general population. (3, 4) Hyperhomocysteinemia and methylmalonic acidemia are the other serious consequences of Vitamin B12 deficiency. Low serum vitamin B12 levels have low sensitivity and specificity in terms of tissue deficiency (5). Homocysteine and methylmalonic acid assessments are additional help in diagnosis of B12 deficiency but still serum vitamin B12 estimation is the commonly applied and standard method for clinical purposes.

Several studies have found high incidence of vitamin B12 and folate deficiency in elderly in western population. (6, 7) However, no such data are available in elderly Indian population. Vitamin B12 and folate deficiency may be more prevalent in Indian people. Probable reason for this is, *Helicobacter pylori* infection along with vegetarian diet, increasing ageing population, and different socio-economic strata. There are two studies so far in India showing prevalence of vitamin B12 and folate deficiency. One study conducted by Yagnik et al, investigated the prevalence of low vitamin B12 concentration and hyperhomocysteinemia in rural and urban Indian men living in and around Pune, Maharashtra. (8) The analysis showed vitamin B12 level of < 150 pmol / ml in 67 per cent of men and hyperhomocysteinemia (<15 μ mol/l) in 58 per

cent subjects. They also reported 4.4 and 3.0 times higher risk of low vitamin B12 concentration and hyperhomocysteinemia respectively in vegetarians. (8)

Shobha et al presented that dietary intake would be low in Indian population because majority of population were vegetarian (57%) so daily vitamin B12 intake was 3.5 times higher than the FDA recommendation. Also, average daily dietary folate intake was almost equal to the FDA recommended daily intake of folate. However, no correlation was detected between plasma vitamin B12 and cognitive status in elderly population. (9)

Cognitive impairment is multifactorial in origin sharing modifiable and non modifiable risk factors. As there are no curative treatments for it, the only possibility is that treatment may delay or slow down disease progression. It is highly suitable to identify such modifiable risk factors like vitamin B12, hyperhomocysteinemia, and folate deficiency etc. These nutrition related risk factors not only have been associated with lower cognitive function in old age, but incipient dementia may also change dietary habits, i.e. Malnutrition can be a consequence rather than a cause of cognitive impairment. In the absence of curative treatment for dementia, vitamin B12 and folate may be relevant to the clinical course of dementia and should be considered for therapeutic intervention. Longitudinal studies should also be undertaken to explore the association between nutrition status and cognitive impairment, and whether it is possible to inhibit or delay the onset of dementia by dietary modifications. Hence, at present, vitamin B supplementation should be taken for the management of documented deficiency states of Vitamin B.

Dementia, an old age disease, is growing at a shocking rate and, increasing the personal and financial costs with an adverse impact on health care expenditure, productivity of families so it becoming a public health problem. Some study proves that ageing is a risk factor for dementia; (10) and the prevalence increases with advancing age. (11) In Europe, prevalence of dementia among people older than 65 yr is about 10 per cent. (12) Currently no curative treatment is available for dementia, a number of attempts have been made to find out the non genetic risk factors that may be modified to limit it. (11) Increased homocysteine levels in conjunction with low levels of folate, vitamin B12, which act together to control homocysteine, have been found to associate with decreased cognitive performance. The prevalence of high total serum homocysteine is associated with age and elderly population is affected more with hyperhomocysteinemia, (12) which negatively correlates with cognitive status. Deficiency of the B vitamins (folate, vitamin B6 and vitamin B12) may play a vital role in progression of cognitive impairment in the elderly. So, higher plasma homocysteine concentration is a sensitive marker for vitamin B12 and folate deficiency (11), because methylation cycle generates methionine from homocysteine which is totally absent from any dietary source. Both folate and vitamin B12 are required in the methylation of homocysteine to methionine and in the re-methylation and synthesis of S-adenosylmethionine, a major methyl donor in the central nervous system. Hence, research till date suggests that hyperhomocysteinemia due to disturbed monocarbon metabolism may contribute to cognitive impairment and Alzheimer's disease (12).

We have prospectively assessed patients with B12 deficiency associated neurological syndrome and found out their clinical and electrophysiological findings.

MATERIALS & METHODS:

This was a hospital based cross sectional study which included 125 patients who had attended indoor and outdoor of Maharana Bhupal Govt. Hospital, Udaipur from 1st November 2013 to 31st August 2014. The study included all cases of anemia with or without neurological manifestation with suspected B12 deficiency, anemia with history of chronic alcoholism, smoking, tobacco chewing, beetle nut chewing, chronic diarrhoea and past history of gastric surgery. Participants were excluded those with isolated iron deficiency anaemia, chronic blood loss i.e. haemorrhoids, gastric ulcer, acute blood loss and Patient / attendants who did not give consent.

Blood sample for vitamin B₁₂ had drawn after an overnight fasting. Serum vitamin B12 levels (normal range 211- 946 pg/ml) were estimated by Roche Elecsys modular Cobase 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 (13). The venepuncture had done in the cubital fossa and about 3 ml of blood was drawn using perfectly dry and sterile syringes and the blood had been transferred to dried blank vacutainers. The samples had been analysed the same day. Peripheral blood film had prepared for microscopic examination using Arenath count, hypersegmented neutrophills to specify type of anaemia.

Clinical neurological examination for dementia would be done with MMSE .A Nerve conduction study has been done by using electrophysiology to see evidence of neuropathy.

RESULTS:

Total 125 patients were included in study, among them 53 were male and 72 were females & majority 69 (55.2%) patients were more than 60 years of age, among them 42 (60.86%) were female. Almost (94.45) patients were Vegetarian, alcoholic (59.2%) and smoker (48.8%).

Table 1: Grading of Vitamin B12 deficiency states:

| Severity | Males | Females | Total (%) |
|---------------------------|-------|---------|------------|
| Mild (200-300) | 9 | 7 | 16 (12.8%) |
| Moderate (100-200) | 20 | 23 | 43 (34.4%) |
| Severe (<100) | 24 | 42 | 66 (52.8%) |
| Total | 53 | 72 | 125 (100%) |

Out of 125 patients include in study 86 (68.8%) patients had severe Vitamin B12 deficiency, among them 42(63.6%) were females, however mild form of deficiency was noted in few patients 16 (12.8%).

Neurological complains like parenthesis, ataxia, decreased vision, psychiatric and cognitive impairment were observed in 68 (54.4%) patients among them parenthesis were present in three fifth (60.29%) patients. Reduced vision was present in only 3 (5.8%) patients.

Nineteen percent patients had myelo-neuropathy and optic atrophy was noted in all 3 patients who were presented with reduced vision while

neuropsychiatric manifestations like depression, emotional liability, and reduced attention span and personality changes observed in 12(18.18%) patients. (Table 2)

Table 2: Distribution of Neurological manifestations during Physical examination:

| Clinical parameter | Males | Females | Total (%) |
|--|-------|---------|-------------|
| Myelopathy | 3 | 3 | 6 (9.5%) |
| Neuropathy | 15 | 23 | 38 (60.31%) |
| Myeloneuropathy | 5 | 7 | 12 (19.04%) |
| Neuropsychiatric manifestations | 5 | 7 | 12 (18.18%) |
| Normal examination despite paresthesias | 0 | 5 | 5 (12.10%) |
| Optic atrophy | 2 | 1 | 3 (4.5%) |

Majority of patients (78.9%) had constitutional symptoms like weakness, fatigue, anorexia, weight loss while gastrointestinal symptoms were present in 53.33% patients.

Among the non-neurological signs majority of patient's i.e. 112(89.47%) patients had general symptoms like pallor or hyper pigmentation of palm. Gastrointestinal signs like beefy red tongue, loss of papilla over tongue were present in 42 (61.4%) patients. Cardiovascular signs like tachycardia were present in 42 (36.84%) patients.

Figure 1: Distribution of Neurological symptoms of Vitamin B12 deficiency:

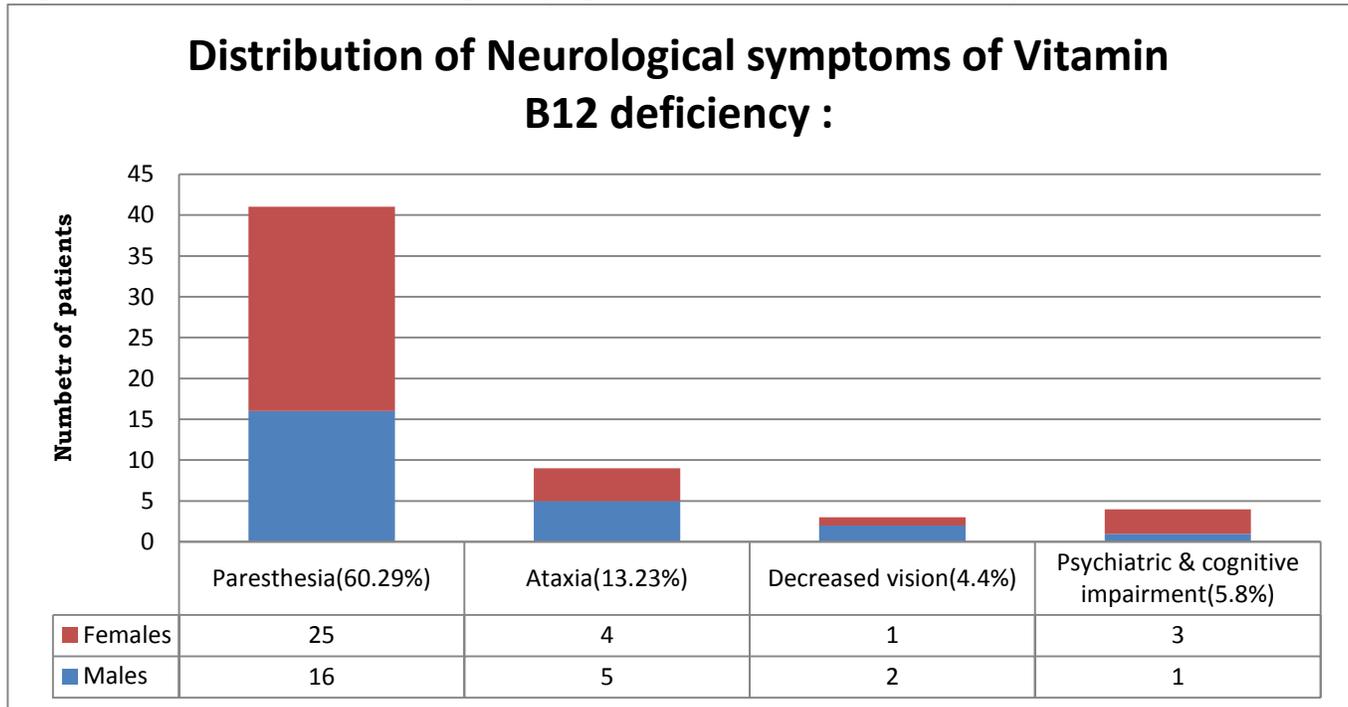
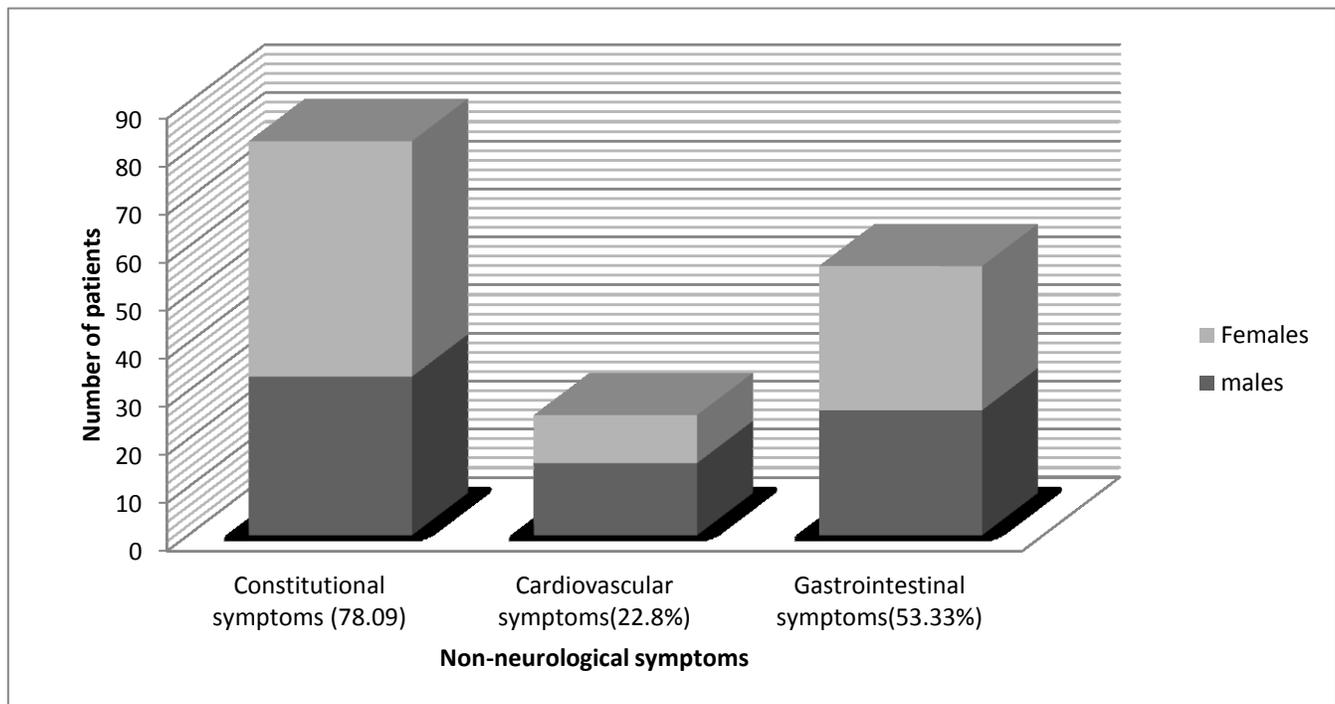


Figure 2: Distribution of Non-neurological symptoms of Vitamin B12 deficiency:



The major 112 (89.6%) haematological manifestation except anaemia was raised MCV but pancytopenia was observed in 48 (38.4%) of patients and 44 (35.2%) patients had associated iron deficiency anaemia. (Table 3)

Other investigations: Nerve conduction study was performed in 43 patients, among them pure sensory axonal neuropathy was found in 28, pure motor axonal pattern in 8, mixed axonal pattern in 4 and demyelinating pattern was observed in 3 patients. MRI Brain and Spinal cord was performed in 21 Patients, which was abnormal in

DISCUSSION:

The incidence of B12 and folate deficiency in India is unknown but recent evidence suggests that it is commoner than we thought. Some years ago Dastur et al (9) reported 5 cases with neurological manifestation of B12 deficiency, over several years in Bombay. Jeejeebhoy et al (10) found 8 cases and showed that they were due mostly to malabsorption syndrome. Wadia et al (11) randomly studied every 10th admission to the medical general wards. Among 450 cases, B12 deficiency (level < 300 pg/ml) was detected in 0.88%. 3.8% of cases had levels near the lower limit of normal. In our study total 500 patients with anaemia were selected among them 125 patients had Vitamin B12 deficiency.

The deficiency was more frequent in females than males but in Finnish elderly population study the deficiency was more common in males. Another study was conducted in south Asian patients the risk appeared to be similar in men and women. (8) The incidence and severity of B12 deficiency increase with age, specially more common in elderly females, and 69(52.2%) patients from our study had age more than 60

14 patients showing either cerebral and cerebellar atrophy or spinal cord lesions.

Remaining seven patients had normal MRI imaging. Bone marrow aspiration study was performed in 40 patients, hypercellular marrow was found in 27 patients, hypocellular in 7 and normocellular in five patients. Upper GI endoscopy with biopsy was performed in total 43 patients, among them atrophic gastritis or peptic ulcer disease was noted in 32 patients and study was normal in 11 patients.

Table 3: Distribution of Haematological other findings in Patients with anaemia due to Vitamin B12 deficiency:

| Clinical parameter | Males | Females | Total (%) |
|--|-------|---------|----------------|
| Pancytopenia | 23 | 25 | 48 (38.4%) |
| Raised MCV>100fl | 49 | 63 | 112 (89.6%) |
| PBF (Macrocytes & hypersegmented neutrophils) | 46 | 64 | 110 (88%) |
| Diamorphic anemia | 20 | 19 | 39 (31.2%) |
| Normal or reduced MCV | 2 | 11 | 13 (10.4%) |
| Associated IDA | 21 | 23 | 44 (35.2%) |

years, similar observations were found in Finnish elderly population study (9). Yao et al, (12) while studying older cases, investigated that 33% of such were truly deficient. In our study 52.8% patients had severe deficiency with serum

levels less than 100 pg/ml and mild deficiency was observed in only 12.8% of patients.

Vitamin B12 deficiency in diet is a frequent problem in the India (14) as seen in this study. Deficiency was more frequent in pure vegetarians than in non vegetarians (15, 16, 17). In our study 94.8% patients were pure vegetarian. All the cases described by Dastur et al and Jeejeebhoy et al were vegetarians.(10, 11) Low B12 and folate serum levels are not final evidence of deficiency, it is now accepted that levels of B12 between 200-300 pg/ml. It may be stated that even in the developed countries it is found that B12 deficiency is fairly common. Some studies reveal an incidence of 1-2% in the younger population and 10-15% in the elderly over 65 years of age. (18) As a result, there is much discussion in USA on cobalamine fortification of breakfast cereals. We found deficiency common in alcoholic patients in Udaipur region, 59.2% of patients were alcoholic, and 48.8% were smokers or tobacco chewer but this was not noted by Dastur et al.(10) .

Limitation: Sample size was small so result cannot be generalized to general population as data was collected from a single hospital of the city.

CONCLUSION:

Deficiency disorders of vitamins associate with plethora of clinical manifestations. Old age person, female gender and vegetarians are at considerable risk to develop B12 deficiency.

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 considerable 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 this study suggests the screening of vitamin B12 should be included in routine clinical set up.

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REFERENCES:

1. Misra UK, Kalita J, Das A. Vitamin B12 deficiency neurological syndromes: A clinical, MRI and electrodiagnostic study. *Electromyogr Clin Neurophysiol*, 43:57-64, (2003).
2. Pandey S, Kalita J, Misra UK. A sequential study of visual evoked potential in patients with vitamin B12 deficiency neurological syndrome. *Clin Neurophysiol*, 115:914-918, (2004).
3. Snow C. Laboratory diagnosis of vitamin B12 and folate deficiency. *Arch Intern Med*, 159:1289-1298, (1999).
4. Carmel R. Current concepts in cobalamin deficiency. *Ann Rev Med*, 51:357-375, (2000).
5. Stabler SP, Lindenbaum J, Allen RH.

- Vitamin B₁₂ deficiency in the elderly: current dilemmas. *Am J Clin Nutr*, 66:741–749, (1997).
6. Harrison's principles of internal medicine 18th edition.
 7. Gupta AK, Damji A, Uppaluri A. Vitamin B₁₂ deficiency Prevalence among South Asians at a Toronto clinic. *Can Fam Physician*, 50:743-747, (2004).
 8. Loikas S, Koskinen P, Irjala K, Löppönen M, Isoaho R, Kivelä SL et al. Vitamin B₁₂ deficiency in the aged: a population-based study. *Age Ageing* 2007; 36(2):177-183.
 9. Dastur DK, Quaddros EV, Wadia NH et al: Effect of vegetarianism and smoking on vitamin B₁₂, thiocyanate, and folate levels in the blood of normal subjects. *BMJ* 1972; 3: 260-264.
 10. Jeejeebhoy KN, Wadia NH, Desai HG et al : Role of vitamin B₁₂ deficiency in tropical nutritional neuromyelopathy. *J Neurol Neurosurg Psychiatry* 1967; 30 : 7.
 11. Wadia RS, Kharche M, Udar M et al: Vitamin B₁₂ and folate deficiency in a hospital population. *Ann Indian Acad Neurol* 1996; 124: 469-476.
 12. Yao Y, Yao SL, Yao SS et al: Prevalence of vitamin B₁₂ deficiency among geriatric out patients. *J Fam Pract* 1992; 35 : 524-528
 13. Parnetti L, Bottiglieri T, Lowenthal D. Role of homocysteine in age-related vascular and non vascular diseases. *Ageing* 1997; 9:241–57.
 14. Stabler SP, Allen RH, Savage DG, Lindenbaum J. Clinical spectrum and diagnosis of cobalamin deficiency. *Blood* 1990; 76:871–81.
 15. Heaton EB, Savage DG, Brust JC, Garrett TJ, Lindenbaum J. Neurologic aspects of cobalamin deficiency. *Medicine (Baltimore)*. 1991; 70(4):229-45.
 16. Oh R, Brown DL. Vitamin B₁₂ deficiency. *Am Fam Physician* 2003; 67(5): 979–86.
 17. Mezzano D, Kosiel K, Martinez C, Cuevas A, Panes O, Aranda E et al. Cardiovascular risk factors in vegetarians: normalization of hyperhomocysteinemia with vitamin B(12) and reduction of platelet aggregation with n-3 fatty acids. *Thromb Res* 2000; 100:153-60.
 18. Sumner AE, Chin MM, Abraham JL et al: Elevated methylmalonic acid and total homocysteine levels show high prevalence of vitamin B₁₂ deficiency after gastric surgery. *Ann Intern Med* 1996; 124: 469-476.