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Vitamin B12 Deficiency and its Correlations with Clinical, Haematological and Electrophysiological Parameters: Study from a Tertiary Care Hospital of Odisha

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Abstract

Background: Vitamin B12 is also called 'Cobalamine' has vital role for hematopoietic, neurological and cardio vascular function. The objective is to Planned to study the correlation between the levels of vitamin 12 deficiency and its clinical, haematological; and electrophysiological parameters. **Subjects and Methods**: Present study was a cross sectional observational study which included 50 adult cases of both gender. Case selection was based on clinical feature and supported by laboratory evidences. **Results:** Total 50 patients were included in the study, male to female ratio was 2.12:1, mean age of presentation was 38.9 years (range 21 - 68 years). The majority of cases 18 (36%) were between 21 - 30 years followed by 11 (22%) of cases between 41 - 50 years. Most were pure vegetarians 33(66%). Most common symptoms were generated weakness, easy fatiguability in all cases (100%) followed by dyspnoea in 40 (80%) cases. Most common non-neurological signs of presentation were pallor all cases (100%), followed by hyperpigmentation of skin and knuckles, glossitis and stomatitis in 26 (52%) and 24 (48%) cases respectively. Severe vitamin B12 deficiency was found in 12 (24%) cases, moderate deficiency in 30 (60%) and mild deficiency in 8(16%) cases. Anaemia, leukopenia, thrombocytopenia, pancytopenia and bicytopenia were found in 50 (100%), 25 (50%), 34 (68%), 22 (44%) and 15 (30%) cases respectively. Neurological features were found in 16 (32%) cases. **Conclusion:** Vitamin B12 deficiency is associated with varied multi-systemic manifestations. It is not only a laboratory finding but also an issue of clinical significance to lower morbidity.Its prevalence can not be underestimated in our regoin and should receive approprite attention as regards to treatment and prevention.

Keywords: Vitamin B12 deficiency, Megaloblastic anemia, Neuro-cognitive function, cardiovascular function.

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Introduction

Vitamin B12 (cobalamin) is a water-soluble vitamin that is crucial for normal neurologic function, red blood cell production, and DNA synthesis. Hematological presentation of cobalamin deficiency ranges from the incidental finding of increase of mean corpuscular volume and neutrophil hypersegmentation to symptoms due to severe anemia, such as irritability, dyspnea on exertion, fatigue, numbness or tingling feet and hands, muscle weakness, gastro intestinal symptoms and serious neurological consequences.

Neuropsychiatric symptoms may precede hematologic signs and are represented by myelopathy, neuropathy, dementia and, less often, optic nerve atrophy. The spinal cord manifestation, subacute combined degeneration (SCD), is characterized by symmetric dysesthesia, disturbance of position sense and spastic paraparesis or quadriparesis. As the human body is capable of storing vitamin B12 for years, deficiency is not very commonly found. Most often low serum vitamin 12 level or megaloblastic bone marrow or both are used as the tools to diagnose vitamin B12 deficiency.[1,2] There has been much controversy about the burden of vitamin B12 deficiency in the general population and normal range as well.[3,4] Themain causes of vitamin B12 deficiency include lack of intrmsic factor and other intestinal factors (e.g malabsorption), rare genetic disorders, drug intake or indaquate intake of B12.

Data regarding the magnitude of the problem in different parts of India and the factors that might influence its incidence are lacking. There is limited data regarding the clinical presentation of vitamin B12 deficiency megdoblastic anaemia and its neurological consequences in Indian population. Hence the present study was undertaken to study to determine the

level of serum vitamin B12 in all cases of anemia with suspected B12 deficiency and to correlate with clinical, haematological and elctrophysiological parameters.

Subjects and Methods

A hospital based cross-sectional, observational study was carried out at M.K.C.G Medical college and hospital during the period of April 2016 to October 2017 in the department of general medicine. Prior permission was taken from Institutional Ethics Committee. Consecutively admitted 50 adult cases of both sex, who fulfilled the inclusion criteria were enrolled. Inclusion Criteria**:** All patients of anemia with or without neurological manifestations with vitamin B12 deficiency : Haemoglobin level <13 g/dl for male,<12 for non-pregnant female and / MCV >97 fl, peripheral blood smear and Bone marrow findings with features of megaloblastic anemia (pancytopenia, anisopoikilocytosis, macrocytosis, tear drop cells, hypersegmented neutrophils, macropolycytes and presence of basophilic stippling, Howell–Jolly bodies or nucleated red cells with megaloblastic change),Serum vitamin B12 levels < 200 pg/ml.Patients with iron deficiency anemia, chronic blood loss i.e. hemorrhoids, gastric ulcer,patients who had received blood transfusion or haematinics/empirical vitamin B12 treatment oral or injectable in past <6months,acute blood loss and pregnant women were excluded from the study.

After informed consent from each patient, a proforma was used to document demographic, clinical presentation, dietary history, past history of anaemia, blood transfusion drug intake and alcoholisim. A detailed general and systemic examination was done of each patient. Standardized neurological examination was performed in all cases with suspected neurological disease. Patients were screened for dementia using Mini Mental State Examination (MMSE). The diagnosis of dementia was established on the basis of Diagnostic and Statistical Manual (DSM IV) criteria. All of the blood sample was collected in the morning, after 8-12 hr of overnight fasting. Two blood samples were collected from each patient, 2 ml in EDTA for complete blood counts (CBC) and 5 ml clotted blood for serum and biochemical examinatio. EDTA anticoagulated blood was processed through automated hematology analyzer – Beckman coulter AcTdiff2 and hematological parameters were obtained. Peripheral blood smear was stained by Leishman stain for all the cases and examined in detail and patients were distributed according to their smear findings into having macrocytosis, macro-ovalocytosis with/without hyper segmented neutrophils. The biochemical examination included blood glucose, lipid profile, liver function test and renal function test. 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. [5]

All patients were divided into mild, moderate and severe deficiency category according to their vitamin B12 levels. (Mild: 150-199 pg/ml, moderate: 100-149 pg/ml, severe: <100 pg/ml). Bone marrow sample obtained by routine bone marrow aspiration procedures by Salah's needle. Staining of bone marrow aspirate was done with Leishman stain. Nerve conduction studies were done in 8 symptomatic patients. Magnetic resonance imaging (MRI) of the brain and/or spinal cord was done in 4 cases

Statistical analysis

The measurements obtained by the above-mentioned studies were used to calculate mean values for each patient and all cases as a whole. Continuous variables were expressed as mean/SD and categorical variables were expressed as percentage Student's test was used for both independent variables. The paired sample t-test was performed to compare the difference of means between groups. P<0.05 was considered significant. Data were analyzed with SPSS softwareversion 13 (SPSS, Chicago, IL).

Results

After examining 193 patients and excluding as per the criteria given finally 50 patients were selected for study out of which 34(68%) were male and 16 (32%)were females. Male to female ratio was 2.12:1. The mean age of presentation of megaloblastic anemia was 38.9 years (range of 21 -68 yrs). Most of cases 18 (36%) of megaloblastic anaemia were among the 21-30 year age group followed by 11(22%) cases of 41-50 yrs. Maximum patients i.e 33 (66%) were pure vegetarian and 17 (34 %) patients were taking non vegetarian diet. 0ut of all 14 (28%)cases were chronic alcoholics, all were male and nonvegeterians.20% were smokers.

The major symptoms at presentation were dyspnea 40 (80%), anorexia and gastritis 25 (50%), palpitation and syncope 15 (30%), tingling and numbness 16 (32%), weakness of lower limbs 10(20%), and heart failure in 8(16%) of patients. Major non-neurological signs at presentations were pallor all cases (100%), hyperpigmentations of skin and knuckles 26(52%), glossitis and stomatitis 24(48%),edema 14(28%),Icterus 12 (24%),splenomegaly11(22%), hepatomegaly 10(20%), and congestive heart failure in 8(16%)of cases.

The percentages of patients and levels of vitamin B12 deficiency are shown in [Table 1]. The mean serum vitamin B12 was 126+17.2pg/ml (range of 79.6 pg/ml to 179pg/ml). The mean serum Vitamin B12 level was 114.29+21.21pg/ml in pancytopenia cases,126.1+25.45pg/ml in bicytopenia and 144.77+39.60 pg/ml in anemia (monocytopenia) cases. Hence the study showed that the serum vitamin B12 level decreased

with degree of cytopenias which was statistticaly significant (p=0.0129). [Table 2]

Hematological parameters are shown in [Table 3]

Peripheral blood smear findings are depicted in [Table 4]

Comparision of peripheral smear findings with severity of vitamin B12 deficiency are shown in [Table 5].

Bone marrow examination revealed, it was hypercellular with erythroid hyperplasia with reversal of M:E ratio in 44(88%) patients and normocellular in 6 (12%) patients.In all the 50 patients bone marrow showed megaloblastic maturation consisting of large megaloblastic erythroblasts with open sieve like chromatin, Nuclear fragmentation, paranuclear halo, Howel jolly bodies. There was preponderance of early and intermediate megaloblasts as compared to late megaloblasts. In patients with dimorphic anaemia both megaloblasts and micronormoblasts were seen. In myeloid cells giant metamyelocytes and band forms seen. Megakaryocytes show hypersegmentation and fragmentation with decreased thrombocytopoiesis. [Table 6] depicts correlation of diet with hematological parameters and vitamin assays.

The different neurological features are described in [Table 7]

Nerve conduction study done in 8nos of patient are described in [Table 8]

Magnetic resonance imaging was performed in 4/16 patients. There were two cases with the classical degeneration on spinal MRI with white matter hyperintense lesions in the posterior tracts on T2 weighted images. Diffuse cerebral atrophy was seen in two cases with cognitive impairment.

Discussion

In this study of 50 cases of megaloblastic anemia due to Vitamin B12 deficiency were taken for study. There was bimodal age distribution of cases with the majority of cases (18) 36% of megaloblastic anaemia were among the 21-30 year age group followed by 22%(11) case of 41-50 yrs.age group. Thus megaloblastic anaemia is more common during reproductive age group due to increased demand of Vitamins. 34 (68%) cases of megaloblastic anaemia were male while 16 (32%) cases were female. Approximately male to female ratio was 2.12:1. Thus our study is in agreement with the study by Unnikrishnan V et al (2008) and Shidappa G et al (2014) and Barik S et al (2015) but not agreed to the study of khanduri U et al (2007) may be due to geographical variations of food habits of the patients.[6–9]

Among 50 patients with Vitamin B12 deficiency 33(66%) were vegetarians and remaining 17 (34%) were having non-vegetarian diet. In study by BarikS et al (2015)(82%) cases of megaloblastic anemia were vegetarian.[8] In contrast to our finding Unnikrishnan et al reported a higher prevalence in non-vegetarians than vegans.[6]

The major symptoms and signs of our cohort are in accordance with the study of Barik S et al (2015),[8] khanduri U et al (2007),[9] and Gupta R et al (2009).[10] The neurological manifestations presented in [Table 7] differs from the series reportedby Bhole CA et al (2014),[11] who reported neurological features in 54.4% cases, out of which paresthesia in 60,3%,neuropathy in 60.3%,myeloneuropathy in 19%,neuropsychiatry manifestation in18.18%,and optic atrophy in 4.5% cases which may be due to variable sample size and geography**.**

In the present study we found that 25 (50%) patients had leucopenia (TLC <4000/cmm) and 34 (68%) patients had thrombocytopenia. (Platelet count <1.5 lacs/cmm. Our study is in agreement with the study of khanduri U et al (2007),[9] Chandra et al (2015),[12] Haq S et al (2012) and siddiqui B et al (2015).[13,14]

This study found haemoglobin ranging from 1.8gm% to 6.3 gm% and average 4.1gm%, average MCV 112.95 ranging from 99.1-134.5fl), total leucocyte count average was 4157 /cmm (ranging from 1170-10600), average platelet count of 1.11 lacs/cmm (ranging 0.12 -5.9 lacs/cmm). RDW (CV) which is an indicator of anisopoikilocytosis was increased in all cases with range of 15.9%-35 %(normal upto 13.5%). The mean reticulocyte count was 0.056% which indicate hypoproliferativeanemia. Thus our study is in agreement with the study Unnikrishnan V et al (2008) and by Veda P. (2013).[6,15]

Peripheral blood smear examination revealed macrocytosis in all 50 cases, macroovalocytes were seen in 43(86%) cases. These were large oval /egg shaped red cells. Dimorphic RBCs on P.S. consisting of predominantly macro-ovalocytes as well as microcytic hypochromic RBCs were found in 17(34%) cases. Hyper segmented neutrophils consisting of >5 lobes in more than 5% of neutrophills were seen in 34(68%) cases. Some degree of anisopoikilocytosis and poikilocytosis was found in all cases. Presence of tear drop cells, Howell jolly bodies, Cabot rings and basophilic stippling with some nucleated red cells were found in 20% cases. Thus our study is in agreement with the study of Unnikrishnan V et al (2008), khanduri U et al (2007), Gupta R et al (2009), Haq S et al(2012), Veda P. (2013).[6,9,10,13,15]

However there are many limitations of the study.Sample size was small so result cannot be generalized to general population as data was collected from a single centre.Further large multicentric studies are needed to confirm these findings in other parts of country.

Table 1: Levels of Vitamin B12 deficiency

Severity (pg/ml)	Male	Female	Total (%)
Mild199-150	5	3	8 (16)
Moderate149-100	20	10	30 (60)
Severe<100	9	3	12 (24)
Total	34	16	50 (100)

Table 2: Comparision of serum vitamin B12 level with hematological presentation

Presentation	N=50	Vitamin b12 level (pg/ml) Mean+ S.D
Anemia	13	144.77+39.60
Bicytopenia	15	126.1 + 25.45
Pancytopenia	22	114.2 + 21.21

Table 3: 3: Haematological parameters in cases of megaloblastic anaemia

Sr. no	Presentation	No. of cases	Percentage
1	Anemia	50	100
2	Leucopenia	25	50
3	Thrombocytopenia	34	68
4	Pancytopenia	22	44
5	Bicytopenia	15	30

Table 4: Peripheral Blood Smear findings

Haematological parameters	Male	Female	Total
Pancytopenia	16	6	22
Raised MCV	34	16	50
PBS. Macrocytes	34 30	16 13	50 43
PBS. Macroovalocytosis			
Hypersegmented neutrophils	24	10	34
Dimorphic anemia	10	7	17

Table 5: Comparision of peripheral smear findings with severity of vitamin B12 deficiency

Severity of vit. B12 deficiency	No. of patients (n=50)	Macrocytosis	Macro ovalocytes	Hypersegmented Neutrophils
Mild	8	8	5	4
Moderate	30	30	26	20
Severe	12	12	12	10

Table 6: Correlation of diet with hematological parameters and vitamin B12 assays

Hematological Parameters	Vegeterian (n=33) Mean+ S.D	Non Vegeterian Mean+ S.D (n= 17)	P. value
Haemoglobin(gm%)	4.27+0.49	3.744+2.76	0.2853
MCV	112.63+0.92	113.55+1.91	0.0253
Vitamin b12 level	126.04+0.42	125.21+22.63	0.7515

Table 7: Distribution of Neurological Features (Total (16/50)

Clinical parameter	No	Percentage (%)
Pavesthesia	12	75
Peripheral Neuropathy	6	37.5
Myelopathy	1	6.2
Mycloneuropathy	1	6.2
Neuropsy chiotric manifestation	8	50
Cognitive impairment	6	37.5

Table 8: Summary of nerve conduction study in patients with neurological manifestations

Results of NCV	No. of patients (n=8)
Demyelinating sensori-motor neuropathy	5
Axonal sensori- motor neuropathy	-
Mixed (axonal and demyelinating) neuropathy	2
Normal	1

Conclusion

Vitamin B12 deficiency now appears to be more common than previously thought. The various studies done earlier and the present study highlight the rise in incidences of vitamin B12 deficiency which needs to be elucidated. In setting of limited laboratory facilities the investigation should be chosen giving importance to clinical presentation. An elevated MCV supported by peripheral smear findings of macrocytosis, macroovalocytosis and hypersegmented neutrophils and if these findings are associated with clinical features of B12 deficiency then patients can be empirically treated by vitamin B12. Also low vitamin B12 levels along with peripheral smear findings of megaloblastic anaemia, rules out the need for invasive procedure of bone marrow examination for which usually patients refuse to give consent. Hence, the investigation parameters should be chosen in accordance to clinical presentation.The government should acknowledge the burden of vitamin B12 deficiency and implement it in the anaemia control program..Data regarding the magnitude of the problem and the factors that might influence its incidence are lacking. Vitamin B12 deficiency causing megaloblastic anaemia is associated with other systemic manifestation and the morbidity is more. The Physician must keep in mind vitamin B12 deficiency in their differential diagnosis of anaemia.

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