Vitamin B12 deficiency, its prevalence and haematological manifestations - A study in a tertiary care hospital

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Abstract

Aim: To determine the prevalence of vitamin B12 deficiency in subjects with anaemia and elevated mean corpuscular volume.

Materials and Methods: Blood samples from 119 subjects were selected for the study based on the inclusion and exclusion criteria laid down. Blood samples were analysed on sysmex KX 21 haematology analyser. Morphological evaluation of blood cells was done on peripheral blood smear. Serum vitamin B 12 assays were done. Qualitative and quantitative variables were analysed by statistical methods.

Results: Out of 119 subjects taken for study 70 (58.8 %) were male and 49 (41.2 %) were female with mean age ± SD 40.5± 18. Vitamin B 12 deficiency was noted in 82 (69 %) of which 12 (10.1%) were in Borderline levels of deficiency, 31 (26.1%) were in deficient levels and 39 (32.8%) were in severely deficient group. Red blood cell indices were categorized as per different groups of vitamin B 12 levels. Pancytopenia was noted in 67.1% (54) of B 12 deficient individuals. Significant negative correlation was found between vitamin B 12 levels and mean corpuscular volume (r = - 0.215 .p = 0.019). A positive correlation was found between vitamin B12 and platelet count. (p= < 0.001) and also with leukocyte counts (p value < 0.001) These correlations were found to be statistically significant. Peripheral blood film examination shows macrocytosis in 76 (64%) smears and hypersegmented neutrophils in 66 (56%) smears.

Keywords: Vitamin B 12 deficiency, prevalence, Megaloblastic anaemia, Pancytopenia, Mean corpuscular volume.

Introduction

Vitamin B12 is an essential micronutrient for DNA synthesis and proliferation of haematopoietic cells of bone marrow, gastrointestinal cells, epithelial cells, cervico-vaginal cells and testicular germ cells. The daily requirement of vitamin B12 is 5–30 µg and daily absorption is 1- 5 µg.[1] Liver stores 2000 - 5000µg of B12 which lasts for 3-5 years. Nutritional cobalamin deficiency is common in India.[2] This may be attributed either due to lack of proper diet or malabsorptive states. The other causes of vitamin B12 deficiency being intrinsic factor deficiency, chronic gastritis, H. Pylori infection, blind loop syndrome, transcobalamin II deficiency, and fish tape worm infestation. Vitamin B12 deficiency may present in multiple ways from a haematological manifestation to a neurological disorder. Manifestations involving cardiac, cutaneous and skeletal systems are also noted.[3] The most common hematologic hallmark of B12 deficiency is megaloblastic anaemia.[4] Megaloblastic anaemia of B 12 deficiency is frequently observed in clinical practice but remains underestimated.[5] We observed many cases with severe anaemia and elevated MCV in our outpatient department. Most of them had pancytopenia. These individuals were subsequently found to be vitamin B12 deficient. This prompted us to carry out a study to find out the prevalence of vitamin B12 deficiency in subjects with severe anaemia having elevated MCV in our tertiary care hospital set up.

Materials and Methods

A cross sectional study was conducted in our hospital for a period of six months from January to June 2016. Blood sample from 119 subjects were selected for study based on inclusion and exclusion criteria laid down. Subjects of all age group of either sex with severe anaemia (Haemoglobin ≤7 g /dl and mean corpuscular volume ≥ 96 fl)) were included. Patients on antimetabolite therapy, anti- convulsants and proton pump inhibitor drug intake were excluded, blood samples were collected by venepuncture of anti- cubital vein. Complete blood counts with a differential count and red blood cell indices were estimated with the help of sysmex Kx21 haematology analyser. Blood smears were prepared for all cases, stained with giemsa stain and evaluated for red cell morphology – macrocytosis and hypersegmented neutrophils. Serum vitamin B 12 was measured and categorized as Group I: Normal > 240pg/ml, Group II: Borderline 170- 240 pg/ml, Group III: Deficiency < 170 pg /ml and Group IV: Severe deficiency < 100 pg/m based on symptomatic manifestations.[6] Subjects were examined clinically and a detailed history was sought.

Statistical Analysis: Descriptive statistics such as frequencies, proportions were calculated. To compare B12 deficiency and presence of pancytopenia chi-square test was done. To correlate serum Vitamin B12 levels with various parameters such as Mean corpuscular volume (MCV), Mean corpuscular

haemoglobin (MCH), Mean corpuscular haemoglobin concentration (MCHC), Leukocyte counts and platelet counts, Pearson's correlation test was done. P value of ≤ 0.05 was considered to be significant.

Results

The study population included 119 subjects. Among which 70 (58.8 %) were males and 49 (41.2 %) were females. Majority of study subjects were with the mean age of 40.5± 18.1. Vitamin B 12 deficiency was observed in 82 (69%) patients, of which 12 (10.1%) were in Borderline levels of deficiency, 31 (26.1%) were in deficient levels and 39 (32.8%) were in severely deficient levels. Normal Vitamin B12 levels were observed in 37(31.1%) individuals (Table: 1). Among the B12 deficient population, 48 (58.5 %) of them were males and 34(41.5 %) were females. Females of reproductive age group (18–35 yrs) constitute 79.4 % (27) of B 12 deficient women. Red blood cell indices are categorized as per different groups of vitamin B 12 levels (Table 2). Highest value of Mean corpuscular volume (MCV) 139 fl and Mean corpuscular haemoglobin (MCH) 46.1 pg is observed in the severely deficient group. A significant negative correlation was found between vitamin B 12 levels and mean corpuscular volume (r = - 0.215 p = 0.017). Mean corpuscular haemoglobin (MCH) and Mean corpuscular haemoglobin concentration (MCHC) were also found to be negatively correlated although statistically not significant. A positive correlation was found between vitamin B12 and platelet count. This correlation was found to be statistically significant (p= < 0.001). Vitamin B 12 and leukocyte counts were also found to have a significant positive correlations with p value < 0.001. (Table 3). Pancytopenia was observed in majority of B 12 deficient individuals constituting 67.1 % (54) and this was found to be statistically significant (p = 0.001). On peripheral blood film examination macrocytes were observed in 76 smears (64%) and hypersegmented neutrophils in 66 smears (56%) as shown in (Fig. 4, 5).

Table 1: Vitamin B12 levels measured in study population (n = 119)

Vitamin B 12 levels	n (%)	Mean	Std. Dev
Normal (> 240pg/ml)	37 (31)	634.63	530.26
Borderline deficiency (170-240 pg/ml)	12 (10.1)	190.29	22.68
Vitamin B12 deficiency (< 170pg/ml)	31 (26.1)	129.12	21.51
Severe Vit.B12 deficiency (<100pg/ml)	39 (32.8)	53.91	24.22

Table 2: Red Blood cell indices in different groups

Vitamin B 12 levels	MCV	MCH	MCHC
Normal (> 240pg/ml)	107.34± 10.6	32.28± 4.9	30.51± 3.2
Borderline deficiency (170-240 pg/ml)	108.72± 9.8	33.8 ±4.5	30.86± 2.6
Vitamin B12 deficiency (< 170pg/ml)	108.97± 12.5	32.4± 6.7	30.21 ±3.7
Severe Vit.B12 deficiency (<100pg/ml)	111.4 ±9.02	34.17 ±4.8	31.71 ±3.1

Table 3: Pearson's correlation of Vitamin B 12 (n =119)

	MCV	MCH	MCHC	White blood cells	Platelets
Correlation coefficient (r-value)	-0.218	-0.176	-0.104	0.566	0.763
p-value	0.017	0.056	0.263	<0.001	<0.001

Table 4: Comparison of haematological profiles in various studies

Parameters	Kaushik et al	Premkumar et al	Present study
Hb (g/dl)	6.39 ± 2.7	5.2 ± 1.6	4.64 ± 1.3
Total leukocyte count(cells/cmm)	4.5 ± 2.3	2.63 ± 0.88	4.2 ± 1.6
Platelet count (per cmm)	1.33 ± 1.1	82 ± 35	76 ± 34

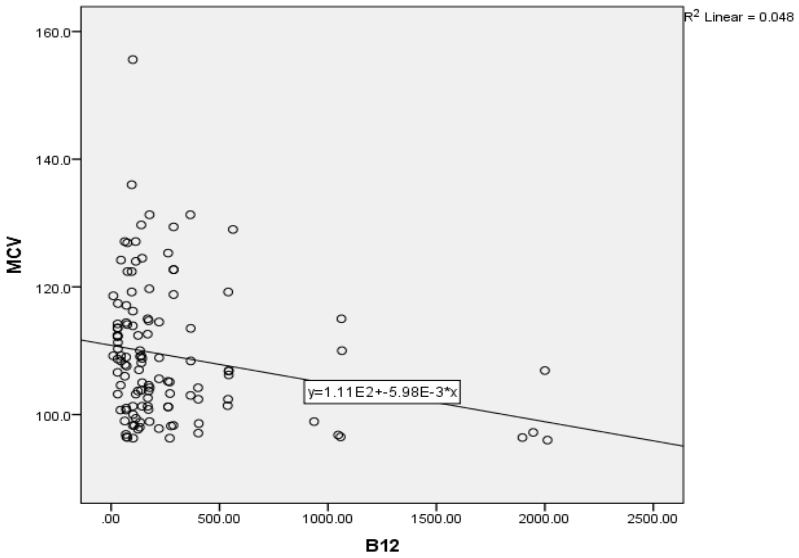

Fig. 1: Shows negative correlation between Vitamin B12 levels and MCV

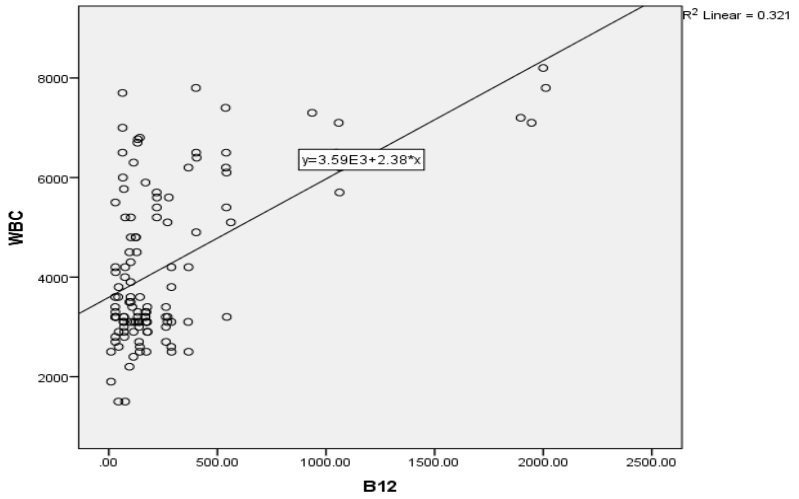

Fig. 2: Depicts positive correlation between Vitamin B12 levels and Leukocyte counts

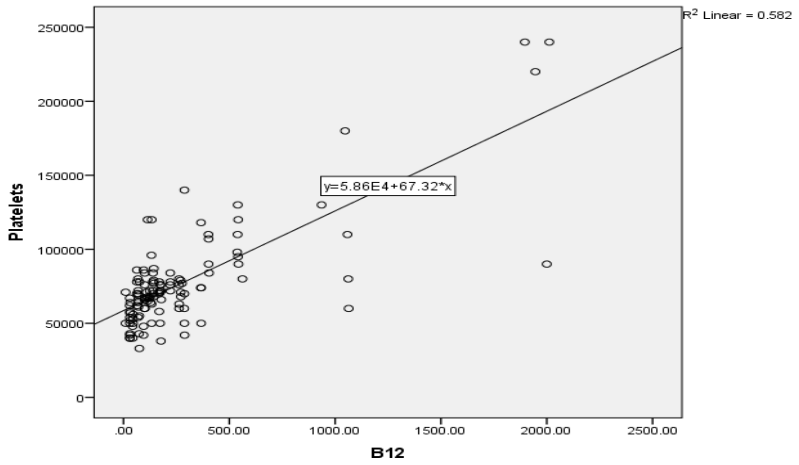

Fig. 3: Shows positive correlation between Vitamin B12 levels and platelet counts

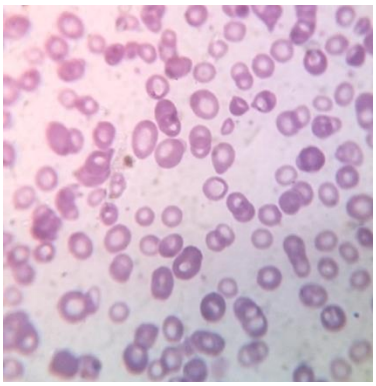

Fig. 4: Shows dimorphic picture with scattered macroovalocytes in peripheral smear

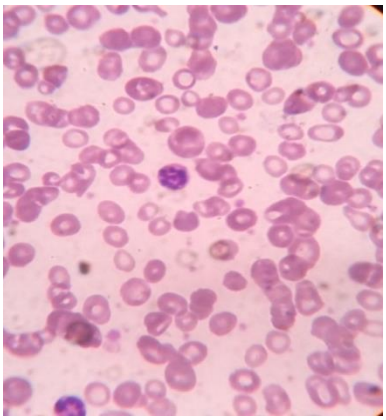

Fig. 5: Shows hypersegmented neutrophil in peripheral smear

Discussion

Vitamin B12 deficiency clinically manifests as a multisystem disorder. It commonly presents haematologically as megaloblastic anaemia and as neurological disorders. In India Folate deficiency is an important cause of megaloblastic anaemia but recent studies stress the role of vitamin B12 deficiency.[7] Reports of Khanduri et al reveal that 46.9% of non-anaemic adult subjects have either subnormal levels of B12 alone or combined Folate – B12 deficiency. This is five times more common than folate deficiency alone.[8]

The results of our study is tabulated in Table 1 showing prevalence of vitamin B 12 deficiency to be 69%. Similar findings were reported by Khanduri & Sharma (65%).[7] Ahmed et al reported vitamin B 12 deficiency in 72.6% of study population.[4] Sarode et al,[9] Kaushik Sen et al[3] and Hashim et al[10] had also observed the prevalence of B 12 deficiency to be 76% in their studies. Another hospital based study at a Tertiary care hospital at Sindh has recorded a prevalence of 57.5%.[11] An increased prevalence of 88% and 81% has been observed in the studies conducted at Delhi by Garewal et al[12] and Premkumar et al[13] respectively. On the contrary low prevalence of Vitamin B12 deficiency has been reported by Gilgit Agency of Pakistan,[14] Puneeta et al of Karnataka[15] and Rohit et al of Jaipur[16] as 31.8%, 33.9% and 36.5 % respectively. These variations in these studies could be due to the dietary practices adhered for various cultural, religious reasons and geographical differences. In India majority of the population are vegetarians. Even among nonvegetarians the consumption of nonvegetarian food is found to be low. A population based study at West Bengal[3] and Karachi[5] has reported vitamin B12 deficiency in 79 % and 85% of nonvegetarians respectively. Hence it should be emphasized that B12 deficiency occurs in nonvegetarians also. Other causes contributing to B12 deficiency are malabsorptive states such as Tropical sprue, Giardiasis, gastrointestinal infections due to H.Pylori, gastric atrophy of elderly, Autoimmune gastritis, Gastric surgeries, surgery under nitrous oxide anaesthesia and drug induced deficiency by antimetabolites, anti-convulsants, Metformins and proton pump inhibitors.

Studies of Summer et al[17] mention an incidence of B 12 deficiency to be 1-2 % in younger population and 10 – 15 % in elderly supported by other studies.[15,18] In contrast in the studies of Kaushik Sen et al[3] majority are in their 30s. In our study 70 of study subjects were younger individuals. Of which 64 (91.4%) had serum vitamin B 12 deficiency. Elderly constitutes 49 in number. Of which only 18 (36.7%.) had serum vitamin B12 deficiency. Interestingly we have observed a case of congenital megaloblastic anaemia – Imerslund Grasbeck disease in a pediatric female. This disease is an inherited failure of transport of R- Cobalamin complex by Ileum causing selective malabsorption of Cobalamin. It is usually accompanied by proteinuria. Mutations of cubilin and amnionless gene on chromosome 10 and 14 has been observed in Finnish[19] and Norwegian families.[20] Few other mutations also have been reported in Mediterranean area.[21] But this case of congenital megaloblastic anaemia is a case of rarity in our geographical distribution. A survey conducted by Pappo et al[22] in a pediatric centre have found no cases of cobalamin deficiency in their evaluation of red cell macrocytosis but one case has been reported in our study.

Regarding sex incidence, a slight male preponderance has been observed in our study. There are 70 males constituting 58.5 % of our study population. This is in contrast with other studies[3,18] wherein females predominate. Women in the child bearing group (18 – 35 yrs) constitutes 79.4 % (27) of B 12 deficient women in our study whereas 39 % (18) has been observed in the study of Puneeta et al.[15] A low level of vitamin B12 in the reproductive age group is a matter of concern as low maternal vitamin B 12 status is associated with increased risk for neural tube defects, intra uterine growth retardation and low birth weight. A serum B 12 value of 300 pg / ml should be maintained before a woman becomes pregnant. Physiological changes during pregnancy tend to decrease serum

vitamin B 12 levels. Hence a true deficiency is indicated by biochemical indices (raised serum homocysteine levels and urine methyl melonic acid). However in view of economic status, laboratory reports of serum B12 could only be interpreted in consonance with clinical findings.

Vitamin B 12 deficiency affects mainly haematologic, gastrointestinal and nervous system. Haematological manifestations could be anaemia, leukopenia, thrombocytopenia, pancytopenia, macrocytosis and hypersegmented neutrophils in peripheral smear and megaloblastosis in bone marrow. Seref et al have found haematological disorders at the rate of 96 % in an evaluation of B 12 deficient population.[18] Erythrocyte indices are used in initial evaluation of anaemic patients.[23] Traditional criteria for B 12 and folate deficiency is observation of high Mean corpuscular volume (MCV) values in erythrocyte indices. Wheeler et al have suggested that vitamin B 12 should be determined in anaemic patients with MCV more than 100 fl.[24] Even with MCV ≥ 96 fl many of the patients attending our hospital had clinical manifestations and morphological presentations in peripheral smear attributing to Vitamin B 12 deficiency. Hence we chose the inclusion criteria to be MCV ≥ 96 fl for selection of samples for our study. Erythrocyte indices were categorized according to levels of vitamin B 12 (Table 2) and highest mean value of MCV and MCH were observed in the severely deficient group (B 12 ≤ 100pg/ml) as noted in the study at Sindh.[11] A significant negative correlation of MCV with vitamin B 12 levels has been observed in our study and is depicted in Figure 1. This observation is in correlation with few other studies.[16,25,26] Confounding results have also been observed in some studies.[15,27] Rarely patients with cobalamin deficiency do not have high MCV values. This could be due to concomitant Iron deficiency, Thalassemia carrier status or anaemia of chronic disease. Hence change in MCV and MCH from existing levels to higher value but still within normal reference ranges (MCV of 90 fl replacing one of 85 fl) have to be given preference for evaluating serum Vitamin B 12 levels.[23] A hospital based study by Savage et al[28] on etiological evaluation of macrocytosis concludes the contribution of cobalamin deficiency to be 6 % for high MCV values and chemotherapy, anti-viral drugs or alcohol abuse to be 64% in contrast to our study where cobalamin deficiency stands for 69 %.

Morphological evaluation of peripheral smear revealed macrocytosis in 76 (64%) smears and a dimorphic picture in 43(36 %) smears in our study (Fig. 4). Macrocytosis has been reported to be 29.8%, 16.1% and 43 % in various other studies.[15,18,29] This variation is probably because ours is a prospective study with subjects selected on basis of elevated MCV whereas the above mentioned studies have a study population selected on basis of vitamin B 12 levels irrespective of MCV values. Co- existing Iron deficiency and inflammation also play a part in masking macrocytosis. Hypersegmented neutrophils an early sign of megaloblastosis in nutritional megaloblastic anaemia has been observed in 56% (66) of our B 12 deficient patients. (Fig. 5) Other prospective series have recorded 25.5% to 100% of patients with over 5 % hypersegmented neutrophils.[7,15]

Haematological profile for patients with B 12 deficiency in various studies are shown in Table 4 On comparison, mean value of Haemoglobin and platelet count was found to be lowest in our study than that of Kaushik et al[3] and Premkumar et al.[13] Frequency of distribution of haematological disorders along with B 12 deficiency varies in different studies. Not every patient expresses the same degree of anaemia for each level of cobalamin deficiency. Severely deficient can have surprisingly mild anaemia or even lack it. The explanation for blunted haematological response is not always apparent. Neutropenia and Thrombocytopenia has been observed in the range of 20-25 % in some studies.[3,15,18] Severely anaemic cases present with neutropenia and thrombocytopenia but is uncommon in mild anaemia. In cobalamin deficiency platelet production is only 10% of that expected from megakaryocyte mass perhaps reflecting ineffective thrombopoiesis and that too they are functionally abnormal.[1] Since we have chosen subjects with severe anaemia Hb ≤ 7 gm/ dl, increased frequency of neutropenia and almost all of them with thrombocytopenia has been evident among B 12 deficient patients in our study when compared with other studies.[3,13] A positive correlation has been observed between vitamin B12 levels and leukocyte counts (p = 0.001) (Fig. 2) and also with platelet counts (p=0.001) (Fig. 3) in our study.

Pancytopenia is diagnosed when there is reduction in all three haematopoetic cell lines. It is defined by the presence of haemoglobin < 12 gm/dl, total leukocyte count < 4000/ mm^3 and platelet count < 1, 50,000/mm^3.[30] It was found that 67% (54) of B 12 deficient individuals in our study had pancytopenia. Among them 25 (48%) belong to the category of severely B12 deficient group and most of them were found to be young males. Many Indian studies.[30-32] State that megaloblastic anaemia of B 12 deficiency remains as a significant causative factor for pancytopenia as noted in our study. On the contrary observations of a study conducted at a tertiary care centre at Kolkotta by Santra and Das[33] points it out as the least contribution towards pancytopenia. Across the world in developing countries vitamin deficiencies and infections like tuberculosis, HIV are frequent causes of pancytopenia. In contrast malignancy and marrow aplasia form the bulk of disease in developed countries.[13] Our patient population mainly of lower socio economic status and cobalamin deficiency may be attributed to be on inadequate diet. Nutritional cirrhosis leading on to hypersplenism causes increased

phagocytic trapping of haematopoetic cell lines. Concomitant tuberculosis, chronic liver disease and alcoholism all add further to marrow dysfunction causing pancytopenia. Differences in the etiology of pancytopenia reflects population characteristics, nutritional, cultural, habitual, socio economic status and geographic parameters.

The brunt of damage in deficiency of B 12 results in various clinical manifestations along with alterations in haematological parameters. Anaemia with elevated MCV that too if proceeding with multiple cell line deficiency, blood levels of B 12 should be sought for. Early screening recognizes a treatable cause and avoids irreversible late complications.

Conclusion

Majority of our study population had vitamin B 12 deficiency presenting as megaloblastic anaemia and pancytopenia. Physicians should keep in mind to assess to B 12 status in cases manifesting with such features. Current adolescent health and antenatal programmes in India include Iron and folate supplements only. It is worthwhile considering vitamin B 12 supplements in such programmes. The option of fortifying foods with vitamin B 12 may also be recommended after more population based studies are completed.

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