Vitamin B12 deficiency in India: Mean corpuscular volume is an unreliable screening parameter

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ABSTRACT

Background. Vitamin B12 deficiency is thought to be more common than was previously believed, but there are little data from India on this. It has protean clinical manifestations, and raised mean corpuscular volume (MCV) is commonly used by physicians as an indicator for megaloblastic anaemia caused by vitamin B12 deficiency. We evaluated the clinical profiles of our patients with vitamin B12 deficiency and tried to ascertain how useful MCV and the peripheral smear were in diagnosis.

Methods. We evaluated the clinical picture, haematology indices and peripheral smear findings of 117 patients with low vitamin B12 levels. Serum folic acid, ferritin values and biopsy findings of some patients were also assessed.

Results. Patients were commonly detected to have reduced levels of serum vitamin B12 during the work-up for anaemia ($n=45$) or for neurological symptoms ($n=31$). Of the 94 cases in which smears were examined, 26 showed macrocytes and hypersegmented neutrophils were present in 24. Twenty-six patients showed a raised MCV, 50 patients had an MCV within the reference range and 28 had low MCV. Pancytopenia was present in 5 patients. Concomitant iron deficiency, as judged by serum ferritin levels, was present in 18 patients.

Conclusion. Vitamin B12 deficiency is not uncommon in India. It is often diagnosed during the work-up for a haematological disorder or for neurological symptoms. MCV is unreliable as a screening parameter for the presumed diagnosis of macrocytic anaemia, which is associated with vitamin B12 deficiency.

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INTRODUCTION

Vitamin B12 deficiency has been a well known health problem. However, now there is evidence that the disease is more common than was previously believed.[1] Vitamin B12 deficiency may present in multiple ways, from a haematological manifestation such as megaloblastic anaemia to a neurological one such as subacute combined degeneration of the spinal cord.[2,3] We had an unexpected cluster of 4 physician colleagues with vitamin B12 deficiency within a short period of time, with presentations as varied as leucopenia, unsteadiness of gait, fasciculations and numbness in fingers. This prompted us to evaluate the clinical records of our patients with reduced serum vitamin B12 levels, and correlate them with their haematological findings.

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METHODS

Approval was obtained from the hospital institutional review board (IRB) before the onset of the study. We retrieved all cases in which vitamin B12 assays had been done in our laboratory from October 2008 to February 2010. The vitamin B12 assays were done on the Beckman Coulter chemiluminescence Access 2 instrument. The vitamin B12 values were classified initially as per biological intervals used in our laboratory (normal 181–914 pg/ml, indeter-minate 146–180 pg/ml, deficient ≤145 pg/ml). Subsequently, for this study, we considered all values ≤180 pg/ml as low. Clinical and laboratory data were retrieved from the electronic health records. Haematology assays were performed on a Beckman Coulter LH 780 haematology analyser using volume conductivity and scatter technology.

RESULTS

There were 429 vitamin B12 assays performed during the period. One hundred and forty-five patients had low levels of vitamin B12. Twenty-eight were excluded from the study as we had no clinical (n=14) or haematological (n=14) data.

There were 71 men (age range 16–82 years, median 44 years) and 46 women (age range 21–85 years, median 40 years). In the latter group, there were 18 women in the age group of 18–35 years.

The indications for evaluation of vitamin B12 levels in the 117 patients were—work-up or evaluation for anaemia (n=45), neurological signs and symptoms (n=31), gastrointestinal symptoms (n=7), gynaecological conditions (n=6), non-specific complaints (n=7), fever with cytopenias (n=9), peripheral smear findings (n=7) and other haematological conditions (n=5).

The neurological symptoms included vertigo, motor tics, sensory neuropathy, ataxic hemiparesis, stroke, cervical myelopathy, subacute combined degeneration of the cord, migraine, vertigo, burning sensation in the lower limbs, dementia, giddiness and nystagmus, anxiety neurosis, transient ischaemic attack, episodic migraine and acute confusional state.

Two patients were on phenytoin, two were on methotrexate while one was on steroids and antitubercular treatment. Because some patients had already had some haematological investigations done elsewhere, complete details were not available for all of the 117 patients. As many as 46 patients had haemoglobin levels in the reference range (Table I), while mean corpuscular volume (MCV) was increased in about one-fourth of cases and decreased in about the same proportion. Leucopenia and thrombocytopenia were present in about 20% of the patients. A peripheral smear was requested for in 94 patients. Macrocytosis was seen in 28 (29.8%) patients, while a dimorphic blood picture of microcytic hypochromic cells and macrocytes was seen in 16 (17%). Twenty-six (27.7%) patients had a microcytic and hypochromic picture while 24 (25.5 %) had a normochromic, normocytic picture. Five patients (5.4%) had pancytopenia. Hypersegmented neutrophils were seen in 24 (26.1%) patients.

Two of 16 patients who had serum folic acid levels done had low values (<3 ng/ml). Six patients tested negative for anti-parietal cell and anti-intrinsic factor antibodies while three expressed anti-parietal cell antibodies but were negative for the intrinsic factor.

Eighteen of 37 patients who had serum ferritin values evaluated had reduced levels. Of the 28 patients with low MCV, ferritin was evaluated in 19 and was found to be reduced in 13. Of the

TABLE I. Haematological parameters in patients with reduced Vitamin B12 levels

Item	*n*	Range	Decreased in	Normal	Increased in
Haemoglobin (g/dl)	117	2.8–18.1	Males (<13.5), 35 Females (<12), 35	Males (13.5–18), 35 Females (12–16), 11	Males (>18), 1 Females (>16), 0
Total leucocyte count (cells/cmm)	110	1000–25 400	(<4000); 22 (20%)	(4000–11 000); 79 (71.8%)	(>4000); 9 (8.2%)
Platelet count (per cmm)	104	0.18–5.16	(<1.5 lakh); 21 (20.2%)	(1.5–4.5 lakh); 79 (76%)	(>4.5 lakh); 4 (3.9%)
Mean corpuscular volume (fl)	104	55.5–131.5	(<77); 28 (27%)	(77–97); 50 (48%)	(>97); 26 (25%)
Mean corpuscular haemoglobin (pg)	104	16–46	(<26); 31 (29.8%)	(26–34); 52 (50%)	(>34); 21 (20.2%)
Mean corpuscular haemoglobin concentration (g/dl)	104	24.2–36.2	(<33); 40 (38.5%)	(33–36); 63 (60.6%)	(>36); 1 (0.96%)
Red cell distribution width (%)	104	11.9–38	0	(11.5–14.5); 38 (36.5%)	(>14.5); 66 (63.5%)

50 patients with a normal MCV, 2 had low ferritin levels. Two patients were investigated for and found to have a beta-thalassaemia trait on electrophoresis.

Seventeen patients underwent 24 upper gastrointestinal and one lower gastrointestinal biopsies. The histopathological findings on biopsy were: unremarkable oesophageal biopsy (1), unremarkable antral biopsy (6), atrophic gastritis (1), chronic superficial gastritis (4), intestinal metaplasia (1), *Helicobacter pylori* gastritis (3), partial villous atrophy (2), subtotal villous atrophy (1), duodenal carcinoid (1), unremarkable duodenal biopsy (4) and unremarkable colonic biopsy (1).

Three patients had bone marrow trephine biopsies and aspirates. Two showed features of megaloblastic erythroid maturation while one patient had polycythaemia with normoblastic haematopoiesis.

Vitamin B12 supplements, usually injectable, were given to 88 patients. Follow-up of 48 patients showed improvement as judged by clinical or haematological evaluation. Data on the other patients were not available.

DISCUSSION

Vitamin B12 deficiency has variable clinical manifestations but is usually associated with megaloblastosis in the bone marrow, macrocytosis in the peripheral smear and a raised MCV. Pernicious anaemia is the most common form of vitamin B12 deficiency in the West. However, folate deficiency is another important cause of megaloblastic anaemia. In contrast, in India, pernicious anaemia is uncommon.[4] Though folate deficiency is an important cause of megaloblastic anaemia, recent data have suggested that vitamin B12 deficiency is also an important cause in India.[5–10]

Further, while vitamin B12 deficiency in the West is seen more commonly in the elderly and is due to malabsorption, in India it affects all age groups and is possibly related to an inadequate diet. Allen attributes low vitamin B12 levels to vegetarianism, a low intake of animal-source foods and malabsorption.[11] Malabsorption in the elderly is usually due to gastric atrophy or to *Helicobacter pylori* infection. A vegetarian diet is lacking in vitamin B12 and this can lead to megaloblastic anaemia. Although our data on the diet of patients is incomplete, many Indians are vegetarians. Even those who consider themselves as non-vegetarians usually consume meat only occasionally.[8] Classical pernicious anaemia is believed to be uncommon in India but there are a few reports of its occurrence.[4] The relatively low numbers reported in India are probably due in part to incomplete laboratory evaluation, especially with reference to anti-intrinsic factor and anti-parietal cell antibodies. It has also been suggested that there may be a link between *Helicobacter pylori* gastritis and pernicious anaemia.[12]

Carmel states that most newly diagnosed B12 deficiencies seem subclinical.[1] Given that about one-third of our patients (33.8%) who were investigated had low values, it appears that vitamin B12 deficiency is fairly common. We had 18 women (39%) in the age group of 18–35 years. It is particularly important to detect vitamin B12 deficiency in women in the child-bearing age group because low maternal vitamin B12 status is associated with a significantly increased risk for neural tube defects.[13–15] Molloy *et al*. assessed vitamin B12 status and the risk of neural tube defects in a population with high neural tube defect prevalence and no folic acid fortification. They suggested that vitamin B12 levels of >300 pg/ml should be maintained before women become pregnant.[13] It is also known that low maternal vitamin B12 levels are associated with intrauterine growth retardation and low-birth weight.[14,15] Estimating the levels of vitamin B12 during pregnancy are of little use because vitamin B12 levels are known to decrease physiologically in pregnancy; thus, low serum vitamin B12 levels in pregnancy may not indicate true deficiency.[16] During pregnancy, a finding of low serum vitamin B12 levels should ideally be followed by testing for biochemical indices of vitamin B12 deficiency (serum homocysteine and urine methylmalonic acid, both of which are raised in true vitamin B12 deficiency) before supplements are started. These are, however, expensive investigations and are not performed in most laboratories.

Not all patients with low serum vitamin B12 levels have a 'true' deficiency; some low values may be spurious for various reasons or may reflect partial deficiency of transcobalamin I.[17,18] The suspicion of vitamin B12 deficiency remains a clinical diagnosis and interpretation of laboratory reports should be done in consonance with clinical findings.[17]

In our study, a raised MCV was seen in only 28 patients while an almost equal number showed a low MCV. Though the literature is replete with data on raised MCV in megaloblastic anaemia, some recent papers have shown that this may not always be true.[9,10,19,20] This could be because of concomitant iron deficiency, thalassaemia carrier status or anaemia of chronic disease, all of which are not uncommon in India. Carmel *et al.* have shown that iron deficiency is often associated with pernicious anaemia.[21]

Hypersegmented neutrophils were observed in 24 (25.5%) of our patients, in contrast to Khanduri and Sharma's study where hypersegmented neutrophils were present in all the patients.[10] This difference was probably because their study was a prospective one with two pathologists evaluating each smear independently, while ours was a retrospective study. Also, Khanduri and Sharma selected patients with a haemoglobin level of <10 g/dl and an MCV level of >95 fl, while our study population was selected on the basis of vitamin B12 levels, irrespective of haemoglobin and MCV values. Other prospective series have recorded 43% to 100% of patients with over 5% hypersegmented neutrophils.[8,22] Many of our patients were diagnosed during the work-up for a

cytopenia or for neurological manifestations and are hence reflective of clinical practice.

Given the increasing 'discovery' of vitamin B12 deficiency, the option of fortifying foods with vitamin B12 may need to be considered. Currently, the only nutrient that is part of a mandatory fortification programme in India is iodine which is added to table salt. If at all we suggest fortification, there should be clear objectives that can be achieved by this measure. Vitamin B12 fortification is worth considering because of the link of this nutrient to megaloblastic anaemia and hyperhomocysteinaemia, which in turn increases the risk of stroke and heart disease.[1] The outcomes of this measure are likely to be reduction of subclinical vitamin B12 deficiency and reduction in vascular complications associated with hyperhomocysteinaemia. There will also be a decrease in the masking of vitamin B12-associated anaemia caused by blanket folic acid fortification. It has been suggested that fortification of foods with vitamin B12 may not be as effective as with other nutrients, because the bioavailability of B12 is limited and malabsorption has been the limiting factor in people who need it the most.[1] Yet, given that diet rather than absorption is probably the major cause in our population, this may not be such a deterrent in India.

Refsum and Smith recommend the fortification of bread or cereal on the basis of studies which have shown that in relatively healthy, middle-aged to elderly persons, low-dose vitamin B12 improves vitamin status (as determined by blood concentrations of vitamin B12) and functional markers such as total homocysteine, holotranscobolamin and methylmalonic acid. They suggest that it is possible to improve the vitamin B12 status of a large part of the population by fortification.[23] Current adolescent health and antenatal programmes in India include iron and folate supplements; few, if any, include vitamin B12. It is worthwhile considering vitamin B12 supplements in such programmes and to extend them to older women in the child-bearing age group. However, we need complete and reliable evidence of the benefits of vitamin B12 fortification before we can categorically propose it.

To conclude, vitamin B12 deficiency is not uncommon in India. An MCV value within the reference range, if used as a screening parameter, may be misleading. Physicians must keep in mind vitamin B12 deficiency in their differential diagnosis for a relatively wide spectrum of cases, particularly for cytopenias or anaemia of presumed iron deficiency type.

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REFERENCES

1 Carmel R. Efficiency and safety of fortification and supplementation with vitamin B12: Biochemical and physiological effects. *Food Nutr Bull* 2008;**29** (2 Suppl): S177–S187.
2 Hoffbrand AV. Megaloblastic anemias. In: Fauci SA, Kasper LD, Longo LD, Hauser LS, Jameson LJ, Loscalzo J (eds). *Harrison's principles of internal medicine. Volume 1*. 18th ed. Pennsylvania:McGraw-Hill; 2012:862–72.
3 Hoffbrand V, Moss PAH, Pettit JE. Megaloblastic anaemias. In: *Essential haematology*. 5th ed. Massachussetts:Wiley-Blackwell; 2006:44–57.
4 Desai HG, Antia FP. Vitamin B12 malabsorption due to intrinsic factor deficiency in Indian subjects. *Blood* 1972;**40:**747–53.
5 Sarode R, Garewal G, Marwaha N, Marwaha RK, Varma S, Ghosh K, *et al.* Pancytopenia in nutritional megaloblastic anemia: A study from north-west India. *Trop Geogr Med* 1989;**41:**331–6.
6 Gomber S, Kela K, Dhingra N. Clinico-hematatological profile of megaloblastic anemia. *Indian Pediatr* 1998;**35:**55–8.
7 Chandra J. Megaloblastic anemia: Back in focus. *Indian J Pediatr* 2010;**77:**795–9.
8 Antony AC. Vegetarianism and vitamin B-12 (cobalamin) deficiency. *Am J Clin Nutr* 2003;**78:**3–6.
9 Khanduri U, Sharma A, Joshi A. Occult cobalamin and folate deficiency in Indians. *Natl Med J India* 2005;**18:**182–3.
10 Khanduri U, Sharma A. Megaloblastic anaemia: Prevalence and causative factors. *Natl Med J India* 2007;**20:**172–5.
11 Allen L. Causes of vitamin B12 and folate deficiency. *Food Nutr Bull* 2008;**29** (2 Suppl): S20–S34.
12 Desai HG, Gupta PA. *Helicobacter pylori* link to pernicious anaemia. *J Assoc Physicians India* 2007;**55:**857–9.
13 Molloy AM, Kirke PN, Troendle JF, Burke H, Sutton M, Brody LC, *et al.* Maternal vitamin B12 status and risk of neural tube defects in a population with high neural tube defect prevalence and no folic acid fortification. *Pediatrics* 2009;**123:**917–23.
14 Muthayya S, Dwarkanath P, Mhaskar M, Mhaskar R, Thomas A, Duggan C, *et al.* The relationship of neonatal serum vitamin B12 status with birth weight. *Asia Pac J Clin Nutr* 2006;**15:**538–43.
15 Muthayya S, Kurpad AV, Duggan CP, Bosch RJ, Dwarkanath P, Mhaskar A, *et al.* Low maternal vitamin B12 status is associated with intrauterine growth retardation in urban South Indians. *Eur J Clin Nutr* 2006;**60:**791–801.
16 Pardo J, Peled Y, Bar J, Hod M, Sela BA, Rafael ZB, *et al.* Evaluation of low serum vitamin B (12) in the non-anaemic pregnant patient. *Hum Reprod* 2000;**15:**224–6.
17 Hudson B. Vitamin-B12 deficiency. *BMJ* 2010;**340:**1245–6.
18 Wickramasinghe SN. Diagnosis of megaloblastic anaemias. *Blood Rev* 2006;**20:** 299–318.
19 Sekhar J, Stabler S. Life threatening megaloblastic pancytopenia with normal mean cell volume: Case series. *Eur J Intern Med* 2007;**18:**548–50.
20 Aitelli C, Wasson L, Page R. Pernicious anemia: Presentations mimicking acute leukaemia. *South Med J* 2004;**97:**295–7.
21 Carmel R, Weiner JM, Johnson CS. Iron deficiency occurs frequently in patients with pernicious anemia. *JAMA* 1987;**257:**1081–3.
22 Chan JCW, Liu HSY, Kho BCS, Ma ESK, Ma KM, Choi PT. Megaloblastic anaemia in Chinese patients: A review of 52 cases. *Hong Kong Med J* 1998;**4:**269–74.
23 Refsum H, Smith AD. Are we ready for mandatory fortification with vitamin -B12? *Am J Clin Nutr* 2008;**88:**253–4.