Megaloblastic anaemia: Prevalence and causative factors

UMA KHANDURI, ARCHNA SHARMA

ABSTRACT

Background. Megaloblastic anaemia is not uncommon in India, but data are insufficient regarding its prevalence, and causative and precipitating factors. We did a prospective study to document such data for patients of megaloblastic anaemia.

Methods. All patients presenting to our hospital over a period of 6 months with a haemoglobin <10 g/dl and/or mean corpuscular volume >95 fL and blood film findings consistent with megaloblastosis were included in the study. Demographic data, diet, drug intake, previous blood transfusion and presenting symptoms were recorded. Clinical findings were obtained from medical records of patients. Complete blood counts, blood film examination, reticulocyte count and cobalamin and folate assays were done. Results of liver function tests and bone marrow slides were available for review.

Results. Megaloblastic anaemia was diagnosed in 175 patients with anaemia. Assays were done on 120 patients (55 were lost to follow up) and results showed cobalamin deficiency in 78 patients (65%), combined cobalamin and folate deficiency in 20 patients (12%) and pure folate deficiency in 8 patients (6%). Fifteen per cent of patients had normal or high values of both vitamins, having received blood or haematinics before the diagnosis was established. The peak incidence of megaloblastic anaemia was in the age group of 10–30 years (48%), with female preponderance (71%). The predominant symptoms were fatigue, anorexia and gastritis, low grade fever, shortness of breath, palpitations and mild jaundice. Twenty-five per cent of patients were on acid-suppressing medication and 15% had previous transfusion for anaemia. Eighty-seven per cent of patients with cobalamin deficiency and 75% with folate deficiency were lactovegetarians. In the combined deficiency cohort, 71% were vegetarians and 29% were occasional non-vegetarians. Physical findings were pallor (85%), glossitis (29%), mild icterus (25%) and hyperpigmentation (18%).

Abnormal haematological findings were mean corpuscular volume 77–123 fL (9 patients had iron deficiency), red cell distribution width 16%–44%, pancytopenia in 62% of patients, reticulocyte count >2% in 42% of patients and typical megaloblastic blood films in all patients. Bone marrow smears available in 22 patients showed moderate-to-severe megaloblastosis. Thirty-two per cent of patients in whom liver function tests were done showed indirect bilirubinaemia with normal enzymes.

Conclusion. Megaloblastic anaemia was diagnosed from complete blood counts, red cell indices, blood film examination and assays of the two vitamins. Bone marrow examination was not essential for diagnosis. Cobalamin deficiency was the major cause of megaloblastosis. Aetiological factors were a diet poor in cobalamin or folate, increased requirements during the growth period and pregnancy, and the use of acid-suppressing medication. Physicians managing these patients need to be aware of the timing of blood sampling for assays, that haematinics and transfusions provide only short term benefits, and that long term follow up and diet counselling is crucial.

Natl Med J India 2007;20:172–5

St Stephen's Hospital, Tis Hazari, Delhi 110054, India
UMA KHANDURI, ARCHNA SHARMA Department of Haematology
Correspondence to UMA KHANDURI, *prakash_uma@yahoo.com*

INTRODUCTION

Megaloblastic anaemia has been recognized as a clinical entity for over a century. The first clinical description of pernicious anaemia, which is one of the known causes of megaloblastic anaemia, has been attributed to Thomas Addison in 1849.[1] Much of the early work on megaloblastic/pernicious anaemia was done on western subjects. Megaloblastic anaemia results from abnormal maturation of haematopoietic cells due to faulty DNA synthesis. Two vitamins, cobalamin (vitamin B_{12}) and folic acid are essential for DNA biosynthesis. Deficiency of either vitamin results in asynchrony in the maturation of the nucleus and cytoplasm of rapidly regenerating cells. In the haematopoietic system this asynchrony results in abnormal nuclear maturation with normal cytoplasmic maturation, apoptosis, ineffective erythropoiesis, intramedullary haemolysis, pancytopenia and typical morphological abnormalities in the blood and marrow cells.[2,3]

Megaloblastic anaemia leads to substantial morbidity if unrecognized or misdiagnosed. Its aetiology is multifactorial and may result from dietary deficiency, impaired absorption and transport or impaired utilization of these vitamins in DNA synthesis. In India with diverse ethnic populations, different dietary and social customs, the incidence of megaloblastic anaemia and its associated problems have not been adequately documented.

Severe megaloblastic anaemia is not uncommon among patients who present with symptomatic anaemia in hospitals around Delhi. We did a prospective study between April and September 2003 at St Stephen's Hospital, Delhi to document the incidence of megaloblastic anaemia in our hospital, determine which of the two vitamins was responsible, document the clinical presentation and dietary practices in affected patients, and identify any precipitating factors.

METHODS

The inclusion criteria for the study were a haemoglobin level <10 g/dl and/or a mean corpuscular volume (MCV) >95 fL along with peripheral blood film findings consistent with megaloblastosis (pancytopenia, anisopoikilocytosis, macrocytosis, tear drop cells, hypersegmented neutrophils, macropolycytes and presence of basophilic stippling, Howell–Jolly bodies or nucleated red cells with megaloblastic change).

A proforma was used to document demographic data, clinical presentation, dietary history, past history of anaemia, blood transfusions and drugs. Details of physical examination were obtained from medical records of patients. With informed consent, two blood samples were collected from each patient, 2 ml in EDTA for complete blood counts (CBC) and 5 ml clotted blood for serum. CBC were done on the day of blood sampling. Serum was separated from clotted blood and stored frozen at –25 °C until assayed in batches for cobalamin and folate levels. The laboratory tests performed were:

1. CBC using the A^{c}.T(diff) cell counter from Beckman Coulter.
2. A blood film was stained by the Leishman stain[4] and evaluated for red cell morphology, platelet count and white cell morphology by 2 haematologists. A differential count of 100 neutrophils based on the number of lobes (from 1 to >6) was done on all available blood films.
3. Reticulocyte count using 1% Brilliant Cresyl Blue for supravital staining.[5]
4. Serum folate and cobalamin levels were done on patients who were admitted to hospital and on those who attended the follow up clinic. The vitamins were assayed using competitive enzyme immunoassay on Immunoassay Analyser AIA-600 (TOSOH, Japan). For both assays, the instrument was calibrated using 5 commercial calibrators and high and low controls were run in each batch that was analysed. The normal range of cobalamin using the AIA PACK B12 was 100–700 pg/ml and folate using AIA PACK FOLATE was 3.0–22 ng/ml.
5. Liver function tests were requested by attending physicians in patients who were clinically jaundiced. These were done using the HITACHI 911 Autoanalyzer (Roche, Germany).
6. Bone marrow examination was requested by attending physicians in some patients. The slides were stained by the May Grunwald Giemsa stain.[4]

RESULTS

During the study, 26 630 blood samples were received for CBC in the laboratory. Of these 6412 samples (24%) had a haemoglobin value <10 g/dl. The number of patients who met the inclusion criteria was 175 (2.7% of patients with anaemia). Of these, 120 cases were available for review and assays as 55 patients did not come for follow up.

Based on the analysis, the patients were divided into 4 groups (Table I).

Group A: Hb <10 g/dl, MCV >95 fL, megaloblastic blood film, low cobalamin and/or folate levels.

Group B: Hb <10 g/dl, MCV >95 fL, megaloblastic blood film, normal or high cobalamin and/or folate (partially treated or transfused patients).

Group C: Hb or MCV in the normal range, megaloblastic blood film and low cobalamin and/or folate levels.

Group D: Hb <10 g/dl, MCV >95 fL, megaloblastic blood film and no assays available.

Of the 120 patients (50 men, 70 women; age range: 9 months to 80 years; Fig. 1) who had assays done for cobalamin and folate, 78 (65%) had cobalamin deficiency, 8 (6%) had folate deficiency and 14 (12%) had combined deficiency. Twenty patients (17%) had normal or high values of both vitamins, having received treatment before sampling for assays.

TABLE I. Distribution of patients in the four groups

Deficiency	Groups				Total
	A	B	C	D	
Cobalamin	46	–	32	–	78
Folate	6	–	2	–	8
Combined	11	–	3	–	14
Unknown	–	20	–	55	75
Total	63	20	37	55	175

Group A: Haemoglobin (Hb) <10 g/dl, mean corpuscular volume (MCV) >95 fL, low cobalamin and/or folate
Group B: Hb <10 g/dl, MCV >95 fL, normal or high cobalamin and/or folate
Group C: Hb or MCV normal, low cobalamin and/or folate
Group D: Hb <10 g/dl, MCV >95 fL, no assays available

Three patients were pregnant at the time of investigation and all of them had cobalamin deficiency. Two girls <1 year of age with severe anaemia had cobalamin deficiency. The nutritional status of their mothers was not known. All patients were residents of Delhi and its suburbs within a radius of 25 km from central Delhi. Eighty-seven per cent of patients with cobalamin deficiency and 75% of patients with folate deficiency were lactovegetarians. In the combined deficiency cohort, 71% were lactovegetarians. Even non-vegetarian patients ate meat only occasionally. All patients were from the middle and low income groups. A history of intake of H_2 receptor blockers or proton pump inhibitors namely ranitidine and omeprazole was obtained in 30 patients. These drugs had been prescribed or bought over-the-counter for symptomatic relief of gastritis and anorexia.

The predominant symptoms were fatigue (70%), anorexia and gastritis (60%), low grade fever (50%), cardiovascular (shortness of breath, palpitations and syncope) (30%) and yellow discoloration of eyes (20%). Paraesthesias, diarrhoea, hyperpigmentation and early graying of hair were present in <10% of patients. The duration of symptoms ranged from a few days to 3 years. Eighteen patients (15%) had received blood transfusions for anaemia, 1–3 years before the present hospital visit.

The physical signs recorded by the attending physicians included pallor (85%), glossitis (29%), mild icterus (25%) and hyperpigmentation of knuckles (18%). A detailed neurological evaluation was not recorded.

The main haematological findings are shown in Table II. The MCV ranged from 77 fL to 123 fL. Nine patients whose MCVs were <95 fL and belonged to Group C were also iron-deficient. The red cell distribution width (RDW), which is an indicator of the variation in the size of red cells, ranged from 16% to 44% (normal: up to 13.5%).

Pancytopenia was present in 74 patients (62%). Reticulocyte count was done in 74 patients and was found to be >2% in 42%.

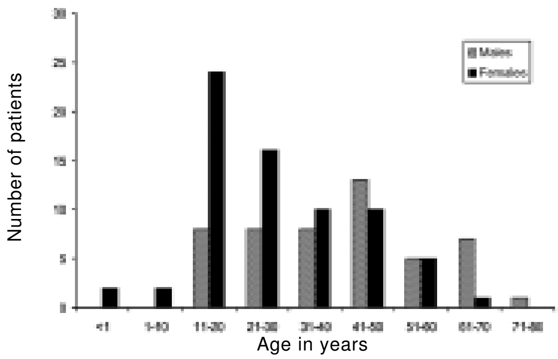

FIG 1. Age and sex distribution of patients

Blood films showed marked anisopoikilocytosis, both microcytic and macrocytic red cells, substantial number of tear drop cells, leukopenia and thrombocytopenia. Nucleated red cells with megaloblastic nuclei and red cell inclusions such as Cabot rings, basophilic stippling and multiple Howell–Jolly bodies were seen (Fig. 2).

Hypersegmentation of neutrophils (>5 lobes) was present in all blood films examined and ranged from 2% to 60% of neutrophils. Bone marrow examination was done in 22 patients. Marrow smears were moderate to markedly hypercellular with moderate-to-severe megaloblastic change in all haematopoietic precursor cells.

Liver function tests were done in 62 patients; of these 20 patients (32.2%) had raised indirect bilirubin levels with normal liver enzymes. Serum lactate dehydrogenase was requested for in only 4 patients and the levels were increased in all of them.

DISCUSSION

Based on the western literature there is a perception that folate deficiency is the main cause of megaloblastic anaemia. Only 2.7% of patients with anaemia in our hospital had megaloblastic anaemia. Cobalamin deficiency was responsible for megaloblastic anaemia in the majority of our patients (65% pure cobalamin deficiency and 12% combined deficiency) and pure folate deficiency accounted for 6%. We were unable to determine which vitamin was deficient in 17% as the patients had received haematinics and blood transfusions before sampling for the assays could be done.

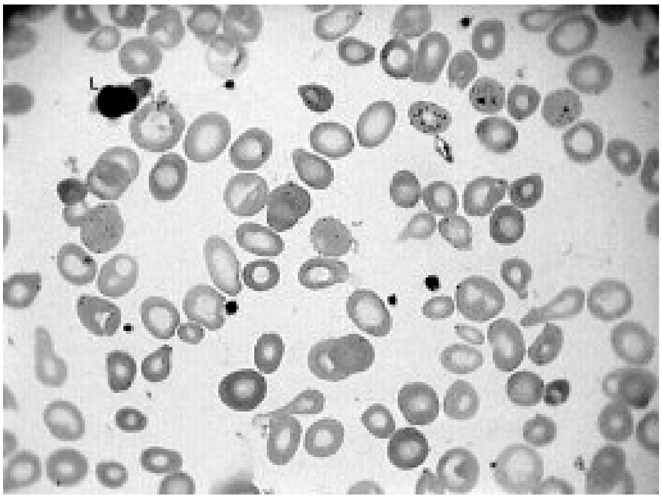

FIG 2. Peripheral blood film (×1000) showing paucity of red cells, marked anisopoikilocytosis, tear drop cells. Macrocytes were recognized by comparing the size of red blood cells with that of mature small lymphocytes (L). Platelets were reduced in number. Basophilic stippling was seen (arrow).

Megaloblastic anaemia is a chronic condition developing over a period of time and most patients are well compensated. There is no indication for urgent blood transfusion. Serum samples for assay of the 2 vitamins should be drawn before any form of therapy is given since assays alone can determine which vitamin is deficient.

The majority of our patients were lactovegetarians. The average Indian vegetarian diet is deficient in cobalamin.[7,8] An earlier pilot study reported by us had shown that 40% of normal Indian subjects with normal haemograms were cobalamin-deficient.[6] A 1973 study by WHO on the nutritional status of pregnant women in India documented iron, folate and cobalamin deficiency.[9] In obstetric practice supplementation of iron and folate is the norm. Folate supplementation alone in the presence of occult cobalamin deficiency may precipitate neurological complications.[2,10]

We attempted to identify factors that might be responsible for converting occult cobalamin deficiency into florid megaloblastic anaemia. In Caucasian and Chinese populations, megaloblastic anaemia is reported to occur in older age groups with an equal sex ratio or male preponderance.[11,12] In contrast, the peak incidence in our study was seen in the age group of 10–30 years (48% of patients) and there was a preponderance of women (71%). It is possible that the increased demand during growth spurt, puberty and child-bearing age group in a population already deficient in cobalamin precipitated the anaemia.

Gastritis, anorexia, nausea and vomiting were present in 60% of patients. The lining epithelium of the gastrointestinal tract becomes atrophic in megaloblastosis. A vicious cycle of megaloblastosis leading to atrophy of mucosa, and subsequent malabsorption of the two vitamins, worsens megaloblastic anaemia.

A history of intake of acid-suppressing medication (H_2 receptor antagonists and proton pump inhibitors) was present in 25% of our patients. These drugs had been prescribed for gastritis by their primary physicians and were often purchased from pharmacies over-the-counter without prescriptions. These drugs may play a role in malabsorption of cobalamin.[13,14] Strict regulation in prescribing and dispensing these medications should be considered. We did not document malabsorption in these patients.

For a laboratory diagnosis of megaloblastic anaemia, a CBC with red cell indices, examination of a well stained blood film and assay of the 2 vitamins are sufficient to make a definitive diagnosis. Pancytopenia was present in 62% of patients. Other authors have also observed that megaloblastic anaemia must be an important

TABLE II. Haematological data

Laboratory parameter	Deficiency			
	Cobalamin (*n*=78)	Folate (*n*=8)	Combined (*n*=14)	Unknown (*n*=20)
Haemoglobin (g/dl)				
1.5–5.0	27	2	7	10
5.1–10	41*	6	7	10
Mean (SD) MCV (fL)	106 (16)	103.9 (12.5)	106 (14.9)	110.9 (10.7)
Mean (SD) RDW (%)	23.6 (7.2)	22 (2.67)	26 (6.6)	24.7 (4.6)
White cell count <4.5×10^9/L	62.8%	62.5%	85.7%	45%
Platelet count <150×10^9/L	67.9%	62.5%	71.4%	80%
Reticulocyte count >2%	39.1%	33.3%	50%	50%

* 10 patients had haemoglobin (Hb) >10 g/dl with mean corpuscular volume (MCV) >95 fL (Hb normal range 11.5–18 g/dl and MCV normal range 82–92 fL) RDW Red cell distribution width (normal 11.5%–13.5%)
White cell count normal range 4.5–10.5x10^9/L

differential diagnosis in patients presenting with pancytopenia.[15,16] Bone marrow examination does not contribute to the diagnosis of the underlying aetiology and should be done when a diagnosis of myelodysplasia is being considered.

Liver function tests showed a mild indirect hyperbilirubinaemia with normal enzymes in 32% of the patients tested. Estimation of serum methyl malonic acid and homocysteine, which are better indicators of cobalamin and folate deficiency at the tissue level,[17–19] were not done due to cost constraints.

In conclusion, megaloblastic anaemia causes substantial morbidity in patients with anaemia. Data regarding the magnitude of the problem in different parts of India and the factors that might influence its incidence are lacking. Megaloblastic anaemia must be considered in the differential diagnosis of patients presenting with pyrexia of unknown origin, mild icterus or pancytopenia. Documentation of occult cobalamin deficiency in different ethnic and socioeconomic groups and in pregnant women needs to be done. The effect on neonates of cobalamin-deficient mothers should also be studied.

A large volume of recent literature links serum levels of homocysteine and methyl malonic acid in cobalamin and folate deficiency to occlusive cardiovascular disease and neurological manifestations.[20–23] Complete evaluation for subtle neurological signs and cardiac function needs to be done in the at-risk population to assess the deficiency of these vitamins.

Patients are being treated in the short term with haematinics and transfusions with relief of symptoms. In most instances long term follow up and diet counselling are not being done. The fortification of diet to prevent megaloblastosis needs to be taken up as a national public health issue.

ACKNOWLEDGEMENTS

We acknowledge the cooperation of the Departments of Medicine, Surgery, Paediatrics and Obstetrics and Gynaecology for referring patients for the study.

REFERENCES

1 Addison T. Anaemia—disease of the suprarenal capsules. *London Med Gazette* 1849;**43:**517–18.

2 Antony AC. Megaloblastic anemia. In: Hoffman R, Benz EJ, Shattil SJ, Furie B, Cohen HJ, Silberstein LE, *et al*. (eds). *Hematology. Basic principles and practice*. 4th ed. Edinburgh:Churchill Livingstone; 2005:519–56.

3 Carmel R. Megaloblastic anemias: Disorders of impaired DNA synthesis. In: Greer JP, Foerster J, Lukens JN, Rodgers GM, Paraskevas F, Glader B (eds). *Wintrobe's clinical hematology*. 11th ed. Philadelphia:Lippincott Williams and Wilkins; 2004:1367–95.

4 Bain BJ, Lewis SM. Preparation and staining methods for blood and bone marrow films. In: Lewis SM, Bain BJ, Bates I (eds). *Practical haematology by Dacie and Lewis*. 9th ed. Edinburgh:Churchill Livingstone; 2001:47–64.

5 Bain BJ, Bates I. Basic haematological techniques. In: Lewis SM, Bain BJ, Bates I (eds). *Dacie and Lewis Practical haematology*. 9th ed. Edinburgh:Churchill Livingstone; 2001:19–46.

6 Khanduri U, Sharma A, Joshi A. Occult cobalamin and folate deficiency in Indians. *Natl Med J India* 2005;**18:**182–3.

7 Antony AC. Vegetarianism and vitamin B-12 (cobalamin) deficiency. *Am J Clin Nutr* 2003;**78:**3–6.

8 Antony AC. Prevalence of cobalamin (vitamin B_{12}) and folate deficiency in India—*audi altera partem*. *Am J Clin Nutr* 2001;**74:**157–9.

9 Yusufji D, Mathan VI, Baker SJ. Iron, folate and vitamin B12 nutrition in pregnancy: A study of 1000 women from southern India. *Bull World Health Organ* 1973;**48:**15–22.

10 Refsum H. Folate, vitamin B12 and homocysteine in relation to birth defects and pregnancy outcome. *Br J Nutr* 2001;**85** (Suppl 2):109–13.

11 Chan JCW, Liu HSY, Kho BCS, Chu RW, Ma ESK, Ma KM, *et al*. Megaloblastic anaemia in Chinese patients: A review of 52 cases. *Hong Kong Med J* 1998;**4:**269–74.

12 Clarke R, Grimley Evans J, Schneede J, Nexo E, Bates C, Fletcher A, *et al*. Vitamin B_{12} and folate deficiency in later life. *Age Ageing* 2004;**33:**34–41.

13 Marcuard SP, Albernaz I, Khazanie PG. Omeprazole therapy causes malabsorption of cyanocobalamin (vitamin B_{12}). *Ann Intern Med* 1994;**120:**211–15.

14 Schenk BE, Kuipers EJ, Klinkenberg-Knol EC, Bloemena EC, Sandell M, Nelis GF, *et al*. Atrophic gastritis during long-term omeprazole therapy affects serum vitamin B_{12} levels. *Aliment Pharmacol Ther*1999;**13:**1343–6.

15 Sarode R, Garewal G, Marwaha N, Marwaha RK, Varma S, Ghosh K, ***et al*.** Pancytopenia in nutritional megaloblastic anaemia. A study from north-west India. *Trop Geogr Med* 1989;**41:**331–6.

16 Kumar R, Kalra SP, Kumar H, Anand AC, Madan N. Pancytopenia—a six year study. *J Assoc Physicians India* 2001;**49:**1078–81.

17 Allen RH, Stabler SP, Savage DG, Lindenbaum J. Diagnosis of cobalamin deficiency. I. Usefulness of serum methylmalonic acid and total homocysteine concentrations. *Am J Hematol* 1990;**34:**90–8.

18 Refsum H, Yajnik CS, Gadkari M, Schneede J, Vollset SE, Örning L, *et al.* Hyperhomocysteinemia and elevated methylmalonic acid indicate a high prevalence of cobalamin deficiency in Asian Indians. *Am J Clin Nutr* 2001;**74:**233–41.

19 Carmel R, Mallidi PV, Vinarskiy S, Brar S, Frouhar Z. Hyperhomocysteinemia and cobalamin deficiency in young Asian Indians in the United States. *Am J Hematol* 2002;**70:**107–14.

20 Giles WH, Croft JB, Greenlund KJ, Ford ES, Kittner SJ. Association between total homocysteine and the likelihood for a history of acute myocardial infarction by race and ethnicity: Results from the Third National Health and Nutrition Examination Survey. *Am Heart J* 2000;**139:**446–53.

21 Chambers JC, Kooner JS. Homocysteine: A novel risk factor for coronary heart disease in UK Indian. *Asians Heart* 2001;**86:**121–2.

22 Carmel R, Gott PS, Waters CH, Cairo K, Green R, Bondareff W, *et al*. The frequently low cobalamin levels in dementia usually signify treatable metabolic, neurologic and electrophysiologic abnormalties. *Eur J Haematol* 1995;**54:**245–53.

23 Karnaze DS, Carmel R. Neurologic and evoked potential abnormalities in subtle cobalamin deficiency states, including deficiency without anaemia and with normal absorption of free cobalamin. *Arch Neurol* 1990;**47:**1008–12.