Varied distribution of RhD epitopes in the Indian population

S. S. KULKARNI, S. C. GUPTE, K. VASANThA, D. MOHANTY, K. GHOSH

ABSTRACT

Background. Inhabited by more than 4000 caste and tribal groups, India has an extremely heterogeneous population. For thousands of years many tribal groups have practised endogamy and are practically genetically isolated. Traditionally, polyclonal anti-D reagent has been used for RhD typing; though monoclonal antibodies are increasingly being used. As a result, blood banks find it difficult to assign the RhD status to an increasing number of people. As monoclonal anti-D typing reagents may not detect all RhD antigen epitopes, we studied the RhD antigen epitope heterogeneity in different population groups in India.

Methods. Red cells of 5315 RhD-positive individuals belonging to different castes and tribes of India were tested with 30 different epitope-specific monoclonal anti-D antibodies.

Results. No single monoclonal antibody could detect all RhD-positive red cells detected by polyclonal antisera. The highest proportion of D antigen was detected by LHM 76/55 and BRAD-8 (98%) monoclonal antibodies.

Conclusion. We need to determine the correct mix of monoclonal antibodies that will detect nearly all RhD antigens detected by polyclonal anti-D sera. Similarly, before accepting monoclonal anti-D for therapeutic use, it would be necessary to determine the appropriate ones for use in the Indian population.


INTRODUCTION

The Rh antigen varies quantitatively and qualitatively. It has been recognized that the D antigen is not a single entity but made up of antigenic determinants. Red cells of rare D-positive people who lack part of the D mosaic are called partial D variants. If exposed to the appropriate red cells these individuals can produce antibodies against the missing part of their D antigen. RhD antigen status is usually determined by testing with either polyclonal or monoclonal anti-D. The advent of human monoclonal anti-D with a unique specificity led to the concept of epitopes. Monoclonal antibodies (MAbs) provide unlimited supplies of reagents of identical specificity which are ideal for definition of partial D antigen.

Since the time MAb reagents became available in the Indian market, blood banks have been encountering more cases with doubtful RhD status. We have also observed an increasing number of cases referred to us for confirmation of the RhD group. It is possible that the reagents produced in western countries may not be suitable for India as D antigen is genetically controlled and major variations may exist in the D antigen profile of Caucasians and Indians. The incidence of different partial D variants in our population is at variance from what has been reported from western countries. Major differences between D variants in western and African populations have also been reported.

The administration of prophylactic anti-D immunoglobulin to RhD-negative women after delivery of an RhD-positive infant has been successful in reducing the incidence of Rh alloimmunization. Trials using monoclonal anti-D prophylaxis are ongoing in Western countries. Would these be suitable for anti-D immunoglobulin prophylaxis in the Indian population as the profile of D antigen epitopes can vary from population to population. The Indian population is extremely heterogeneous and is distributed among no less than 4000 castes and tribes. These castes and tribes are still largely genetically isolated because of the practice of endogamy for thousands of years. Hence, it is expected that the RhD epitopes may be distributed differentially among different caste or tribal groups. Due to these variations, we felt a need to study the D antigen epitope in an Indian population. It is vital for the safe and efficient practice of transfusion that Rh typing reagents used in India are reliable and suitable for our population.

Different epitope-specific monoclonal anti-D produced in other countries (UK, Germany, France, USA and Japan) were evaluated for reactivity with our population. Some of these MAbs are available commercially as RhD typing reagents. We studied 100 samples each from different castes and communities, as a representative sample of the population to detect the presence or absence of reactivity with MAb.

METHODS

Thirty MAbs, 27 of IgG type and 3 of IgM type were used. LHM 76/55, LHM 77/64, LHM 70/45, LHM 76/58, LHM 169/80, LHM 76/59, LHM 174/102, LHM 59/19, LHM 59/20, LHM 50/3, 7 and ESD-1 were obtained from Dr Robin Fraser, Scottish National Blood Transfusion Service; OSK-3, OSK 3–1, OSK 3–3 from Osaka Red Cross Blood Center, Japan; BRAD-1, BRAD-2, BRAD-3, BRAD-4, BRAD-5, BRAD-6, BRAD-7, BRAD-8, H 27, 2B6,
RUM-1 and AB5 from the International Blood Group Reference Laboratory (IBGRL), UK; AR and Co88 from Centre Regional de Transfusion, France; and GLR-02 and SF11D8 from Stanford University Medical Center, USA.

Blood samples of different Indian castes, communities and tribal groups were obtained largely from among the 14 million population of Mumbai city and were screened with 30 monoclonal anti-D culture supernatants. The samples collected were from various camps organized by our institution, blood banks and the antenatal outpatient department. All cells were tested with standard techniques and the antigenic cultures were organized by our institution, blood banks and the anti-D culture supernatants. The samples collected were from tribal groups were obtained largely from among the 14 million University Medical Center, USA.

**RESULTS**

Blood samples of 5315 RhD-positive subjects of different castes and communities were screened using a panel of 30 epitope-specific MAbs. A negative reaction with any monoclonal anti-D was repeated and if consistent was considered as an absence of that particular epitope. A negative reaction with 1 to 8 monoclonal anti-D was observed in 1339 samples (25.2%), of which 70 (5.22%) samples gave negative results with 5–8 MAbs. The communities which predominantly showed a negative reaction with some monoclonal anti-D were Bhundari (55%), Vatalia Prajapati (70%), Halai Lohana (38%), Hindu Mahadeo Koli (51.5%), Thakar (70%), Halai Memon (75%), Protestant Christians (40%) and Parsees (41.6%). A positive reaction with all monoclonal anti-D was obtained in 30%–92.5% of samples. In Vaishnavi, Boudha, Kshatriya and Sanchor Jain Samaj communities, about 90% of subjects showed a positive reaction with MAbs.

Thirty-six per cent and 41.7% of blood samples from Parsee and Christian communities, respectively, showed a negative reaction with MAbs. Boudha community had the lowest incidence of negative reaction with MAbs. BRAD-3, BRAD-7, H 27, SF11D8 and LHM 59/20 frequently had a negative reaction in the Parsee community while BRAD-4, BRAD-8, H 27, GLR-02, AR and LHM 77/64 frequently had a negative reaction in the Christian community. The negative reaction with some MAbs was more frequently seen in tribal groups (34.65%) compared with non-tribal subjects (23.61%) indicating the absence of these epitopes in tribal populations. Chi-square test revealed there was significant difference between these two populations (p<0.001). Among the tribals, a negative reaction with MAbs was more prominently seen in the Hindu Mahadeo Koli and Thakar communities.

The reactivity of the 30 epitope-specific MAbs panel in RhD-positive Indian population is shown in Fig. 1. The positive reaction rate of BRAD-3, BRAD-7 and H 27 was lower than that for other MAbs. None of the MAbs had a 100% positive reaction in all blood samples. BRAD-1, BRAD-6, BRAD-8, 2B6, LHM 70/45, LHM 76/55, LHM 50/3.7 and LHM 59/19.5 showed a 97%–98% positive reaction rate.

Figure 2 shows the percentage of negative reactions with MAbs among subjects with absence of reactivity to 5–8 monoclonal anti-D more frequently. The negative reactions were more often seen in the Vatalia Prajapati, Hindu Mahadeo Koli and Halai Memon communities.

**DISCUSSION**

Our study is likely to be beneficial in choosing an appropriate prophylactic monoclonal anti-D for antenatal patients in India. The screening of samples with 30 epitope-specific MAbs revealed that none of the culture supernatants reacted with all the RhD-positive red cells. This shows that no single monoclonal anti-D would give correct results in the whole population. Some MAbs such as BRAD-1, BRAD-6, BRAD-8, 2B6, LHM76/55, LHM 50/3.7, LHM 59/19.15 and LHM 70/45 showed 97%–98% reactivity with RhD-positive individuals. A blend of few of these MAbs might be able to detect all RhD-positive Indians but this will require further studies in different population groups. Of a total of

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**FIG 1.** Percentage of population positive with epitope-specific monoclonal anti-D. None of the monoclonal antibodies reacted with all RhD-positive individuals tested in the study. The negative reaction rate of BRAD-7, H 27 and BRAD-8 was less than that for other monoclonal antibodies.

**FIG 2.** Percentage negativity with 5–8 epitope-specific monoclonal anti-D in selected communities. The negative reaction with monoclonal antibodies was more frequently seen in Vatalia Prajapati and Halai Memon communities.
5315 subjects tested, 74.8% showed a positive reaction with all MAbs. A negative reaction with 1 to 8 MAbs was seen in 25.2% of subjects.

As India has enormous genetic, cultural and linguistic diversity, our population is ideal for genetic studies. We included various castes, tribes and communities among our sample. There was variation in reactivity with MAbs in various communities. The communities, which predominantly showed negative reactions with MAbs were Bhandari, Vatalia Prajapati, Halai Memon, Protestant Christians and Parsees. Analysis of subjects of different religions such as Parsees and Christians showed a negative reaction with MAbs more frequently. Both the communities are known to have a higher incidence (15–17%) of RhD-negative group.31 If MAbs of different epitope specificities used in our study are employed as reagents to test the blood samples of the above communities, more discrepant results will be obtained. As more than 90% of samples of the remaining communities showed appropriate results, there would be comparatively less problem if these MAbs are selected as reagents. The percentage of subjects showing positive reactivity with BRAD-1, BRAD-7 and H 27 anti-D was less compared with other MAbs used in our study. Only 15 of 30 MAbs showed >95% positive reaction with Rh-positive subjects in our study population. Hence, our study shows that if these MAbs are used as reagents for testing in the Indian population more discrepancies in D typing will be observed.

It is believed that tribal people who constitute about 8% of the total population are the original inhabitants of India.32 The reactivity of D antigen with MAbs was also studied in some tribal groups and compared with data from non-tribal groups. A negative reaction with MAbs was found more frequently in the tribal population than in non-tribals (p=0.001). The endogamous nature of tribal groups may explain this observation as other genetic markers also show significant difference in prevalence in the tribal groups.33 Trials have been done in UK using BRAD-3 and BRAD-5 as anti-D prophylaxis.34 These antibodies efficiently clear D-positive cells from D-negative subjects.35 Prophylactic anti-D prepared from BRAD-5 and BRAD-3 may be marketed in the near future. Hence, culture supernatants of these MAbs were included in our study. BRAD-5 reacted with 94% and BRAD-3 with 90% of the RhD-positive population studied. These results indicate that this prophylactic anti-D will fail to provide protection to a mother if her RhD-positive foetus has epitopes against which these antibodies have not been raised. It would be necessary to conduct clinical trials in the Indian population before accepting these for therapeutic use. MAbs raised against the epitopes of D antigen predominantly found in our population would be ideal for anti-D prophylaxis in India.

The incidence of weak D varies from 0.3% to 0.7% in UK and the USA and is reported as 0.016% in Chinese donors in Hong Kong.6,9,10 Muller et al.35 found significant differences in the regional distribution of the 3 most common weak D types by PCR screening in Germany. Okubo et al.36 reported the incidence of partial D to be 0.0005% in Japanese; this is lower than that in western countries. The incidence of D* was found to be 1.7% in blacks, 0.3% in whites and 0.3%–0.5% in a western Indian population.23,24 Our study shows that a single MAb against the RhD epitope will miss a large number of RhD-positive individuals in India. A mix of MAbs to different epitopes of RhD antigen will have to be worked out so that a majority of D-positive Indian population (about 99%) will be assigned the correct RhD status. Using this mix, a study on the Indian population needs to be done to assess whether a particular reagent can be used for all castes and communities in India. It would be necessary to conduct a clinical trial of prophylactic MAbs in India before accepting it for therapeutic use.

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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.


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 B₁₂) 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, macrocytocytes 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+cT (diff) cell counter from Beckman Coulter.
2. A blood film was stained by the Leishman stain 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.
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 Htach 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.

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).

<table>
<thead>
<tr>
<th>Deficiency</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>Total</th>
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<tr>
<td>Cobalamin</td>
<td>46</td>
<td>32</td>
<td>2</td>
<td>7</td>
<td>55</td>
</tr>
<tr>
<td>Folate</td>
<td>6</td>
<td>2</td>
<td>–</td>
<td>–</td>
<td>8</td>
</tr>
<tr>
<td>Combined</td>
<td>11</td>
<td>3</td>
<td>–</td>
<td>–</td>
<td>14</td>
</tr>
<tr>
<td>Unknown</td>
<td>–</td>
<td>20</td>
<td>–</td>
<td>55</td>
<td>75</td>
</tr>
<tr>
<td>Total</td>
<td>63</td>
<td>20</td>
<td>37</td>
<td>55</td>
<td>175</td>
</tr>
</tbody>
</table>

*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 H2 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%.

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Pancytopenia was present in 74 patients (62%). Reticulocyte count was done in 74 patients and was found to be >2% in 42%.
Blood films showed marked anisopikilocytosis, 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.

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. An earlier pilot study reported by us had shown that 40% of normal Indian subjects with normal haemograms were cobalamin-deficient. A 1973 study by WHO on the nutritional status of pregnant women in India documented iron, folate and cobalamin deficiency. 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.

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. 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 (H2 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. 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 complication.2,10

![Fig. 2. Peripheral blood film (×1000) showing paucity of red cells, marked anisopikilocytosis, tear drop cells. Macrococytes 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).](image)

**Table II. Haematological data**

<table>
<thead>
<tr>
<th>Laboratory parameter</th>
<th>Cobalamin (n=78)</th>
<th>Folate (n=8)</th>
<th>Combined (n=14)</th>
<th>Unknown (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Haemoglobin (g/dl)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.5–5.0</td>
<td>27</td>
<td>2</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>5.1–10</td>
<td>41*</td>
<td>6</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>Mean (SD) MCV (fL)</td>
<td>106 (16)</td>
<td>103.9 (12.5)</td>
<td>106 (14.9)</td>
<td>110.9 (10.7)</td>
</tr>
<tr>
<td>Mean (SD) RDW (%)</td>
<td>23.6 (7.2)</td>
<td>22 (2.67)</td>
<td>26 (6.6)</td>
<td>24.7 (4.6)</td>
</tr>
<tr>
<td>White cell count &lt;4.5×10⁹/L</td>
<td>62.8%</td>
<td>62.5%</td>
<td>85.7%</td>
<td>45%</td>
</tr>
<tr>
<td>Platelet count &lt;150×10⁹/L</td>
<td>67.9%</td>
<td>62.5%</td>
<td>71.4%</td>
<td>80%</td>
</tr>
<tr>
<td>Reticulocyte count &gt;2%</td>
<td>39.1%</td>
<td>33.3%</td>
<td>50%</td>
<td>50%</td>
</tr>
</tbody>
</table>

* 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).

**White cell count normal range 4.5–10.5×10⁹/L**

**Mean (SD) RDW (%) 23.6 (7.2) 22 (2.67) 26 (6.6) 24.7 (4.6)**

**Mean (SD) MCV (fL) 106 (16) 103.9 (12.5) 106 (14.9) 110.9 (10.7)**

**Haemoglobin (g/dl)**

**Platelet count <150**

**Reticulocyte count >2%**
differential diagnosis in patients presenting with pancytopenia. 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, 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 occultis cardiovascular disease and neurological manifestations. 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.

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