Megaloblastic Anemia: Back in Focus

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ABSTRACT

Megaloblastic anemia (MA), in most instances in developing countries, results from deficiency of vitamin B12 or folic acid. Over the last two to three decades, incidence of MA seems to be increasing. Of the two micronutrients, folic acid deficiency contributed to MA in a large majority of cases. Now deficiency of B12 is far more common. In addition to anemia, occurrence of neutropenia and/or thrombocytopenia is increasingly being reported. Among cases presenting with pancytopenia, MA stands out as an important (commonest cause in some series) cause. This article focuses on these and certain other aspects of MA. Possible causes of increasing incidence of MA are discussed. Observations on other clinical features like neurocognitive dysfunction, associated hyperhomocysteinemia and occurrence of tremors and thrombocytosis during treatment are highlighted.

Key words: Megaloblastic anemia; Pancytopenia; B12 deficiency; Folic acid deficiency

Megaloblastic anemia (MA) is a distinct type of anemia characterized by macrocytic RBCs and typical morphological changes in RBC precursors. The RBC precursors are larger than the cells of same stage and exhibit disparity in nuclear-cytoplasmic maturation. Basic underlying pathogenetic mechanism in MA is deficiency of folic acid (FA) and/or vitamin B12 at the cellular level with resultant impairment of DNA synthesis. In developing countries, most cases of MA result from nutritional deficiency of these micronutrients.

Other than MA, deficiency of these hematopoietic micronutrients in children has been incriminated to cause neuro-developmental dysfunction, abnormal movements and failure to thrive. Neural tube defects result from deficiency of folate in mothers during pregnancy. Because of these ill effects, folate has since long been included in the supplementation programmes to control anemia.

At community level most cases of nutritional anemia result from deficiency of iron and can be prevented or treated with iron administration. From public health viewpoint, deficiency of folate and B12 has been regarded to contribute little to nutritional anemia. Over the last two decades, role of these micronutrients and their deficiency has been brought to the forefront by various workers. Presentation of patients with MA with pancytopenia, vasculo-toxic effects of hyperhomocysteinemia resulting from folate and/or B12 deficiency and possible role of their deficiency in causing bone loss leading to osteopenia, osteoporosis and pathological fractures has brought B12 and folate deficiency and MA back in focus.1,2

HISTORICAL BACKGROUND: INCREASING PREVALENCE OF MA

Nutritional MA in adults is known for long. Jadhav et al have been credited to report MA in six South Indian infants for the first time in world literature in 1962.3,4 However, stray reports of MA in Indian children can be found in Indian literature even before that.

MA resulting from deficiency of folate and B12 appears to be increasing over the last two decades, although evidence for this may not be forthcoming easily as varying criteria have been used in the literature to point towards anemia resulting from deficiency of FA or vit B12. Some studies have considered macrocytosis of red cells as indicative of this type of anemia, while others have defined MA by presence of megaloblastic changes in the bone marrow. Still others have used subnormal micronutrient levels to define this type of anemia. Table 1 shows the proportion of cases contributed by macrocytic anemia or MA/FA- vit. B12 deficiency as observed in various series on nutritional anemia in children or during survey of micronutrient deficiency. While proportion of these cases seems to be increasing over the yrs, of particular interest is the observation by Gera et al which shows an almost four fold rise in proportion of macrocytic anemia cases over less than a decade at one center-2 % in 1991 and 7.8% in 1999 (Table 1). A recent report shows...
46.9% of non-anemic adult subjects having subnormal levels of B12 or folate- B12 deficiency being five times more common than that of folate.12

Further, the timing of the reports reveals a renewed interest in the subject over last two decades. Observations on adults and children from other developing countries including Pakistan, Zimbabwe, Mexico and Guatemala during this period shows that the trend in India may be reflecting a more generalized phenomenon.13-16 The increase in prevalence of MA is also supported by observation that MA accounts for maximum/a large number of cases of pancytopenia in many Indian series (see details below.17-20

RELATIVE PREVALENCE OF B12/FOLATE DEFICIENCY: B12 DEFICIENCY BECOMING MORE COMMON

Deficiency of B12 or folate can not be differentiated easily from one another as the clinical and hematological features resulting from the deficiency of both micronutrients are similar. Estimation of serum levels are required to be certain of the deficient micronutrient. Serum B12 and folate assay used to be cumbersome dependent upon biological methods, hence they were not performed widely. Currently, most widely used methods is radioimmunoassay which because of its high cost is not routinely available in developing countries. HPLC based assay have also become available. The problem is further compounded by the wide range over which the levels are obtained in normal individuals and normal values obtained in patients with frank megaloblastic morphology and severe anemia. Combined deficiency is also seen in many cases.17, 18, 21-23

Table 2 shows the proportion of cases according to micronutrient deficiency (the studies have not been differentiated whether they are on cases with megaloblastic anemia or population based studies as under comparison are the proportions of deficiency of folate and B12). The Indian series from 1965 shows that isolated B12 or combined deficiency was present in nearly 7% and 5 % instances while folate deficiency accounted for nearly 55%.24 However, Sarode et al from Chandigarh, reported B12 deficiency in nearly 85% cases with megaloblastic anemia (adults included).25 The later studies from other parts of the country have also highlighted that B12 deficiency is far more common than folate deficiency. A study from our hospital on cases with nutritional anemia shows B12 deficiency in 19 % cases and folate deficiency in 12 %. In addition nearly 35 % cases had levels of B12 which could be classified as low.10

Higher prevalence of B12 deficiency among population groups and cases with MA from India also reflects a more widespread trend. A study from Mexico showed B12 deficiency in 19% - 41% of various population subgroups while no cases with folate deficiency were observed.16 Another study on Guatemalan lactating mothers, revealed B12 deficiency in 46% compared to 9% prevalence of folate deficiency.15 Series on MA patients from Pakistan and Zimbabwe revealed B12 deficiency in

### Table 1. Prevalence of MA, Macrocytic Anemia or B12/Folate Deficiency

<table>
<thead>
<tr>
<th>No and Series</th>
<th>Year</th>
<th>Proportion of cases (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Ghai et al</td>
<td>1956</td>
<td>19.1</td>
</tr>
<tr>
<td>2. Dalal et al</td>
<td>1969</td>
<td>18.0</td>
</tr>
<tr>
<td>3. Ghai et al</td>
<td>1977</td>
<td>02.0</td>
</tr>
<tr>
<td>4. Sharma et al</td>
<td>1985</td>
<td>24.0</td>
</tr>
<tr>
<td>5. Gomber et al</td>
<td>1998</td>
<td>42.1</td>
</tr>
<tr>
<td>6. Chaudhry et al</td>
<td>2001</td>
<td>27.1</td>
</tr>
<tr>
<td>7. Gera et al</td>
<td>1991</td>
<td>02.0</td>
</tr>
<tr>
<td>8. Gera et al</td>
<td>1999</td>
<td>07.8</td>
</tr>
</tbody>
</table>

### Table 2. Relative Prevalence of Vitamin B12 and Folate Deficiency

<table>
<thead>
<tr>
<th>Indian Studies</th>
<th>Year/ Country</th>
<th>Population/Study group</th>
<th>Folate Def. (%)</th>
<th>B12 def. (%)</th>
<th>Combined deficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bhende et al24</td>
<td>1965</td>
<td>Nutr. MA</td>
<td>54.9</td>
<td>7.0</td>
<td>5.3</td>
</tr>
<tr>
<td>Mittal et al23</td>
<td>1969</td>
<td>MA, child</td>
<td>57.1</td>
<td>22.4</td>
<td>10.2</td>
</tr>
<tr>
<td>Sarode et al17</td>
<td>1989</td>
<td>MA, all ages</td>
<td>6.8</td>
<td>76.4</td>
<td>8.8</td>
</tr>
<tr>
<td>Gomber et al24</td>
<td>1998</td>
<td>MA, children</td>
<td>10.0</td>
<td>50.0</td>
<td>20</td>
</tr>
<tr>
<td>Gomber et al 19</td>
<td>1998</td>
<td>Preschool</td>
<td>2.2</td>
<td>36.6</td>
<td>2.2</td>
</tr>
<tr>
<td>Chaudhry MW10</td>
<td>2001</td>
<td>Anemic child</td>
<td>12.0</td>
<td>19.0</td>
<td>14.0</td>
</tr>
<tr>
<td>Chandra et al17</td>
<td>2002</td>
<td>MA, children</td>
<td>20.0</td>
<td>32.0</td>
<td>30.0</td>
</tr>
<tr>
<td>Khundari et al12</td>
<td>2005</td>
<td>Gen. Population</td>
<td>6.8</td>
<td>33.0</td>
<td>8.3</td>
</tr>
<tr>
<td>Other countries</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mukibi et al14</td>
<td>1992, Zimbabwe</td>
<td>MA, all ages</td>
<td>17.0</td>
<td>51.0</td>
<td>32.0</td>
</tr>
<tr>
<td>Maddood-ul-Mannan et al13</td>
<td>1995, Pakistan</td>
<td>MA, all ages</td>
<td>8.0</td>
<td>56.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Allen et al22</td>
<td>1995, Mexico</td>
<td>Gen. population</td>
<td>20</td>
<td>-</td>
<td>19-41</td>
</tr>
<tr>
<td>Casterline et al13</td>
<td>1997, Guatemala</td>
<td>Lactating mothers</td>
<td>9.0*</td>
<td>46.7*</td>
<td>-</td>
</tr>
<tr>
<td>Garcia-Casal et al22</td>
<td>2005, Venezuela</td>
<td>Gen. Population/Pregnancy</td>
<td>30.036.3</td>
<td>11.461.3</td>
<td>-</td>
</tr>
</tbody>
</table>

*Includes deficient and low levels of folate and cobalamin
over 50% cases while folate deficiency was seen in only 8% and 17% cases, respectively. These studies including adults may have a direct relevance to pediatric population as the most common cause of B12 deficiency in young children is the maternal deficiency leading to decreased stores at birth and its consequences. Stabler and Allen, in a review have highlighted B12 deficiency as a worldwide problem with breast-fed infants of B12 deficient mothers being at highest risk. They also suggested that possibility of intermittent B12 supplementation for combatting nutritional anemia should be explored.

Reasons for increasing B12 deficiency are also not very clear. Indians of low socio-economic strata who eat virtually no food of animal origin are regarded as most vulnerable to have low B12 levels. Baker and others in studies from South India observed that in these areas, B12 levels in the blood were lower than observed in west but surprisingly MA resulting from these low levels were uncommon. It was hypothesized that bulk of B12 is derived from bacterial contamination of food and water. Since free intrinsic factor is available in small intestine, endogenously produced B12 may be absorbed and may contribute to individual’s daily B12 requirement. Brit et al on the other hand, observed higher prevalence of MA among vegetarian Indian settlers in UK. They postulated that this may be a “complication of migration to a more sanitized environment” resulting in diminished bacterial contamination of small intestine leading to reduced endogenously available B12. How much within the country population migration from rural areas to urban areas has contributed to increased prevalence of B12 deficiency has not been explored.

Whatever may be the cause, B12 deficiency now appears to have displaced folate deficiency to second place as far as causing nutritional anemia is concerned.

**ETIOLOGY OF MA/B12-FOLATE DEFICIENCY**

In India and other developing countries, most cases of MA are caused by nutritional deficiency of folate, B12 or both. Pernicious anemia due to intrinsic factor deficiency, malabsorption resulting in folate deficiency (celiac disease) and certain inborn errors of metabolism e.g., methylmalonic aciduria (B12 deficiency) and methylene tetrahydrofolate reductase deficiency (folate) account for minority of cases.

Nutritional deficiency is far more common in vegetarian than in non-vegetarian families. B12 deficiency seen in infants and young children has been particularly related to maternal deficiency which results in poor body stores at the time of birth. These underprivileged infants who are exclusively/predominantly breastfed for prolonged period (in some instances as much as 3-5 years) tend to develop B12 deficiency as the breast milk content of B12 in these mothers is far below normal (poverty-vegetarianism-exclusive prolonged breast feeding axis). Cobalamin content of breast milk is lower in vegetarian mothers and is positively correlated with their serum cobalamin levels. We have documented a good co-relation between serum levels of the mothers and their suckling infants and young children. From the developed countries, cases of MA are being reported among infants born to vegetarian mothers.

Increasing B12 and folate deficiency may not be entirely related to poverty and vegetarianism. Biochemical evidence of recent B12 malabsorption has been documented in some populations. Giardia infection, particularly acquired in Asian countries has been demonstrated to cause folate malabsorption. H. pylori infection has been incriminated to cause B12 malabsorption among adults.

**CLINICAL PROFILE: ANEMIA AND BEYOND**

Cases of MA are commonly from poor vegetarian families. Most infants and young children with MA tend to be moderately or severely malnourished as they are not receiving adequate complementary feeding. Anemia, anorexia, irritability, easy fatigability are clinical features common to other causes of anemia.

Clinical features peculiar to MA include hyperpigmentation of knuckles and terminal phalanges (observed in Asian communities), enlargement of liver and spleen (seen in up to 25% cases). Petechial and other hemorrhagic manifestations have been reported in up to 25% cases. Presence of bleeding with severe anemia makes them clinically indistinguishable from aplastic anemia. Cases with MA may mimic acute leukemia due to presence of hepatosplenomegaly.

Tremors have been described to occur as a distinct entity in children of north and central India – The infantile tremors syndrome. Similar cases with tremors have recently been described from other countries. These cases mostly have macrocytic anemia and developmental regression in addition to tremors. Cases of MA not presenting with tremors also exhibit developmental retardation/regression in association with severe anemia. Recently, a group of infants have been described having microcephaly and developmental retardation even before anemia became clinically evident. Occurrence of abnormal movements in association with hypotonia, psychomotor retardation, apathy and failure to thrive is being reported in western literature as well. These cases have been shown to have diffuse frontotemporal cortical atrophy on MRI of brain. Impairment of cognitive function and persistence of neurological consequences even after treatment are major areas of
Laboratory investigations reveal macrocytic anemia. In addition, neutropenia is seen in 17-49% cases in various series. Similarly, platelet counts are decreased – observed in as many as 44-80% cases. It is thus not surprising that the cases with MA manifest with pancytopenia and mimic aplastic anemia. From India, in various series of patients presenting with pancytopenia, MA has been found to be more common cause compared to aplastic anemia and acute leukemia. Sarode et al were among the first to report this observation in patients of all age groups from a referral hospital. In another series from a referral hospital, MA accounted for 22.28% cases of pancytopenia seen over 6 yrs. This was a common cause, second only to aplastic anemia. Khunger et al have observed MA accounting for over 72% cases (all age groups) with pancytopenia. In the present series of 109 patients of pediatric age group presenting with pancytopenia, MA was the single most common cause (28.4%). Aplastic anemia and acute leukemia accounted for 20% and 21% cases, respectively. It is currently recommended that MA should be carefully excluded in cases presenting with pancytopenia.

Serum or red cell levels of folate and B12 are required to identify the deficient micronutrient. Serum levels of homocysteine are increased in deficiency of folate and B12 but levels of methylmalonic acid (MMA) are increased in B12 deficiency only (even increased urinary levels of MMA are considered diagnostic of B12 deficiency). Very high specificity of methyl-malonicaciduria in B12 deficiency has recently been confirmed. The authors also observed high specificity of FIGLU test in folate deficiency. The authors advocated for using these relatively inexpensive tests for diagnosis of B12 and folate deficiency. This assumes significance in light of the fact that levels of B12 and folate are quite variable, difficult to measure and influenced by even subclinical hepatic dysfunction.

TREATMENT

Like any other deficiency state, replacement therapy is required. Anemia and other cytopenias respond to administration of very small doses of drugs. However, since folate is available as a 5 mg tablet and its over dose is not associated with adverse effects, 5 mg daily dose has been used.

For B12 deficiency, parenteral administration of B12 is usually recommended. Intramuscular 1mg dose has been traditionally used but with this high dose cases have developed tremors and other extrapyramidal symptoms. We observed development of tremors in 6 out of 51 cases within few hrs of administration of 1 mg dose. However, these tremors and other neurological signs have been self-limiting but their appearance may influence the compliance. Sudden increase in the levels of neurotransmitters has been offered as the plausible explanation for this neurological phenomenon. The duration of treatment is not as standardized as in case of iron deficiency anemia. Six to eight wk treatment is usually described as sufficient. Decrease in MCV, reticulocytosis, and improvement in platelet and neutrophil counts are observed within few days. In the follow up, some cases developed thrombocytosis which resulted in stroke in one patient. Hence careful monitoring of blood counts is required during treatment.

CONCLUSIONS

It is very obvious that there is “resurgence” of articles on folate-B12 deficiency/MA over the last two decades. This clearly reflects renewed interest in the subject as more and more cases are being seen in clinical practice. The phenomenon appears to be widespread. Many developing countries like India have anemia control/prophylaxis programs and in addition there is overall improvement in general standard of living. In light of these facts, the causes for increased incidence of folate-B12 deficiency and /or MA needs to be elucidated. As pointed out earlier, over the last few yrs, B12 deficiency has taken over as more common micronutrient deficiency as compared to folate. Even this shift needs to be explained. Role of malabsorption and within country population migration towards urban areas contributing to diminished endogenous B12 synthesis needs to be elucidated.

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5. Ghai OP. Aetiology of anemias in infancy and childhood (in New Delhi) Indian J Child Health 1956; 95-100.
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