11 *Reappraisal of Megaloblastic Anemia in Tropics*

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Abstract: Megaloblastic anemia is characterized by macrocytosis (MCV >100fL) and presence of megaloblasts in the bone marrow. The main causes are: B_{12} and folic acid deficiencies, drugs and myelodysplasia (MDS). But majority are due to either B_{12} and/or folic acid deficiency. The source of folic acid is fruits and vegetables, the daily requirement is 50 μg, absorption is from the proximal jejunum, the body store 5-20 mg and its function is to transfer I carbon moieties like methyl and formyl groups for the synthesis of purines, dTMP and methionine. The source of cobalamin is meat and dairy products, the daily requirement is 2.5 μg, absorption is from the terminal ileum after combining with IF, the body store is 4 mg and is required for the synthesis of methionine from homocysteine. The main causes of cobalamin and folic acid deficiencies in India are nutritional deficiency, diseases and surgery of the stomach and small intestine, drugs and MDS. Pernicious anemia is uncommon in India. Recent studies show that absorption of B_{12} in the elderly people is defective due to defective release of cobalamin from the food. Acute megaloblastic state can occur within a few days in critically ill patients and due to nitrous oxide. Cobalamin deficiency may occur without hematological abnormalities.

Clinical manifestations include hematological, gastrointestinal and neurological features. Diagnosis of megaloblastic anemia depends on demonstration of macrocytosis (MCV>100fL), hypersegmentation of neutrophils, megaloblasts in the bone marrow and decreased level of the vitamins in the serum. The normal level of cobalamin in the serum is 300-900 pg/mL (<200 indicate significant deficiency) and serum folic acid level is 6-20 ng/mL (< 4 significant). Schilling test is very useful to detect IF abnormalities but not relevant for India. Serum homocysteine and methyl malonic acid levels are increased in cobalamin deficiency but only homocysteine levels are increased in folic acid deficiency. These tests measure tissue levels of the vitamins.

Since the major cause of megaloblastic anemia in India is nutritional, time should be taken to prescribe a good diet containing these vitamins. Cobalamin cannot be synthesized in the body. Oral cobalamin and folic acid is sufficient in addition to the diet supplements. If abnormalities of IF are detected, intramuscular cobalamin is indicated and may have to be taken life long. Pregnant women should be given folic acid throughout the period of gestation. Food fortification of folic acid is being planned. Patients diagnosed to have pernicious anemia should be followed up because they are more likely to develop gastric carcinoma. Neurological defects in cobalamin deficiency may not revert back completely even after optimal treatment if there is neuronal death. Elderly people should be given prophylactic B_{12}. Physicians, NGOs and the national government should take up health education and public awareness.

DEFINITION

Megaloblastic anemia is a group of anemias characterized by macrocytic anemia and the presence of megaloblasts in the bone marrow. These abnormalities are mainly due to a disorder in maturation of the early red cells, which is in turn due to an impaired DNA synthesis of the nucleus.

Macrocytic anemias have the MCV (mean corpuscular volume) more than 100 fL and macrocytes in the peripheral blood film. The pronormoblasts in the bone marrow (earliest recognizable cells in the erythroid series) develop into either normoblasts (normal) or megaloblasts (abnormal). Their characteristic loose chromatin network of the nucleus that is more dispersed recognizes megaloblasts (Figs 11.1 to 11.3).

CLASSIFICATION AND ETIOLOGY

Main causes of megaloblastic anemias (nuclear maturation defects with low reticulocyte production index- RPI- and MCV > 100 fL).

1. B_{12} deficiency
2. Folic acid deficiency
3. Drugs
4. Myelodysplasia (refractory anemia).

Most of the megaloblastic anemias are due to deficiencies of vitamin$_{12}$ and/or folic acid. The two vitamins are closely interrelated metabolically and their disorders are sometimes difficult to differentiate.

Physiology of the Two Vitamins

- Folic acid (pteroyl monoglutamic acid)
- Body stores: 5-20 mg (half in the liver).
- Absorption: Proximal jejunum.
- Daily requirement: 50 μg.
- Source: Fruits and vegetables.
- Function: Function of folic acid is to transfer (donate and accept) 1-carbon moieties like methyl and formyl groups to various organic compounds for the synthesis of purines, deoxythymidylate monophosphate (dTMP) and methionine.

Cobalamin

Body stores: 4 mg (2 mg in liver)

Daily requirement: 2.5 μg

Dietary source: Animal products—meat and dairy products. It is not synthesized in the body.

Absorption: Cobalamin in the food combines with R-factor (found in saliva, gastric juice) and forms a stable complex. In the duodenum R-factor cobalamin complex is digested. The released cobalamin combines with the intrinsic factor (IF), which is secreted from the parietal cells of the stomach. The IF cobalamin complex gets absorbed from the distal ileum. In the ileal mucosal cell IF, is destroyed and cobalamin gets transferred to transcobalamin (TC II). This is secreted into the circulation and is rapidly taken up by liver, bone marrow and other cells.

Cobalamin exists in two metabolically active forms—methyl cobalamin and adenosyl cobalamin. The therapeutic preparation available contains inactive cyanocobalamin (vit $B_{12)}$, which must be converted into the active forms.

Function: Methyl cobalamin is required for methionine synthesis from homocysteine. When this reaction is impaired due to cobalamin deficiency folate metabolism is deranged.

Cobalamin attached to methionine synthase accepts the methyl group from methyl THF as methyl cobalamin and transfers to homocysteine. This is how the metabolism of these two

vitamins is interrelated (Fig. 11.4). There is considerable evidence to support that if there is a deficiency of cobalamin there is trapping of methyl THF (folate trap) that has no alternate metabolic role or route. After some period this folate leaks from the cells and tissue folate deficiency develops resulting in megaloblastic hematopoiesis. So in cobalamin deficiency, tissue folate stores are substantially reduced despite normal or supra normal serum folate levels. Sometimes large doses of folic acid can produce partial hematological remission in patients with cobalamin deficiency.

Adenosyl cobalamin is required for conversion of methyl malonyl CoA to succinyl CoA. Deficiency of this active form leads to accumulation of large amounts of methyl melonyl CoA leading to synthesis and incorporation of non-physiological fatty acids into neuronal lipids. This may contribute to the neurological complications. Further methionine is necessary for the formation of choline and choline containing phospholipids. Nervous system damage is partially due to decreased methionine production.

Common Causes of Cobalamin Deficiency in India (Table 11.1)

Table 11.1: Common causes of cobalamin deficiency in India

1. Inadequate intake (nutritional)
2. Malabsorption
   - A. Defective release of cobalamin from food.
     - Gastric achlorhydria
     - Partial gastrectomy
     - Drugs blocking acid secretion
   - B. Inadequate production of IF
     - Total gastrectomy
   - C. Disorders of terminal ileum
     - Tropical and nontropical sprue
     - Intestinal resection
     - Granulomatous diseases
   - D. Competition for cobalamin
     - Bacteria (Blind loop syndrome)
   - E. Drugs
     - Colchicines, neomycin

Rarer Causes of Cobalamin Deficiency in India (Table 11.2)

Table 11.2: Rarer causes of cobalamin deficiency in India

- Pernicious anemia
- Congenital absence or functional abnormality of IF
- Regional enteritis
- Neoplasm
- Selective cobalamin malabsorption
- Fish tapeworm infection
- Nitrous oxide
- Transcobalamin II deficiency
- Congenital enzyme defects

Common Causes of Folic Acid Deficiency in India (Table 11.3)

Table 11.3: Causes of folic acid deficiency in India

1. Inadequate intake (nutritional)
2. Increased requirements
   - A. Infancy and children
   - B. Pregnancy

C. Malignancy
D. Chronic hemolytic anemia
E. Chronic exfoliative dermatitis
F. Hemodialysis

3. Malabsorption
   A. Tropical and nontropical sprue
   B. Drugs. Phenytoin and barbiturates
4. Impaired metabolism
   A. Inhibitors of dihydrofolate reductase
      - Methotrexate, Pyrimethamine, Triamterine
      - Pentamidine, Trimethoprim
   B. Alcohol

Uncommon causes

Rare enzyme defects.

Causes of Megaloblastic Anemia Other than Cobalamin and Folate Deficiency (Table 11.4)

Table 11.4: Causes of megaloblastic anemia other than cobalamin and folate deficiency

1. Drugs impairing DNA metabolism
   A. Purine antagonist-6 MP, azathioprine
   B. Pyramidine antagonist-5 FU, cytosine arabinoside
   C. Others—Procarbazine, hydroxy urea, acyclovir, zidovudine
2. Metabolic disorders (rare)
   - Hereditary orotic aciduria
   - Lesch-Nyhan syndrome
3. Megaloblastic anemia of unknown etiology
   A. Refractory megaloblastic anemia in MDS
   B. Erythroleukemia
   C. Congenital dyserythropoietic anemia

The main causes of megaloblastic anemia vary in various geographical settings. In the western world one of the commonest cause of cobalamin deficiency is pernicious anemia which is due to an absence of IF which in turn is due to either an atrophy of the gastric mucosa or autoimmune destruction of parietal cells of the stomach. But pernicious anemia is very uncommon in India. A three-month analysis (July to September 2006) of complete blood count, at KIMS Hospital, revealed 7742 cases of anemia (Hb $\leq$ 10 gm%), of which 6399 (82.65%) cases were normocytic anemia, 1332 (17.20%) were microcytic anemia and only 11 (0.14%) cases were found to be of macrocytic anemia, probably reflecting the decreasing prevalence of microcytic and macrocytic anemia. This observation has to be extrapolated to settings other than corporate tertiary care centers (like ours), to know the significance of these findings. *The prevalence of normocytic anemia, which is predominant in western countries, is quickly catching up in Indian scenario too. In India, though definite statistics are not available, the commonest cause of cobalamin and folic acid deficiency seems to be nutritional deficiency.* The deficiency of folic acid is enhanced in children and pregnant women. Physicians are now more aware of conditions like MDS in which refractory megaloblastic anemia may be the initial presentation.

Recent studies show that the incidence of cobalamin deficiency due to defective release of the vitamin from food is increasingly seen in older persons above the age of 65 and 70 years of age. Cobalamin in the food is tightly bound to enzymes in the meat. This is split and the vitamin is released by the hydrochloric acid in the stomach. In the aged persons there is achlorhydria and the cobalamin is not properly released from the food. Multivitamin tablets contain crystalline B_{12}, which can be absorbed by such gastric mucosa with achlorhydria.

Other causes in India would be drugs, gastric and small intestine surgery and tropical sprue causing malabsorption of cobalamin and folic acid. *Occasionally, a full-blown megaloblastic state (acute megaloblastic disease) can develop over the course of just a few days. This can occur in patients*

requiring intensive care receiving multiple transfusions, dialysis or TPN or following nitrous oxide anesthesia. Nitrous oxide as an anesthetic agent can destroy endogenous cobalamin stores.

It is important to remember that sometimes deficiency of cobalamin can occur without hematological abnormalities especially in the elderly. The chance of this type of non-hematological presentation is increased with folic acid food fortification (which is now enforced in countries like the US) because folic acid can mask the hematological effects of cobalamin deficiency.

Clinical features of megaloblastic anemia

1. Hematological
2. Gastrointestinal
3. Neurological

The hematological features are due to the anemia and depend on the severity of the anemia present. These include palpitations, vertigo, tinnitus, dyspnea, light headedness, angina and symptoms of CCF, pallor, icterus, tachycardia, cardiomegaly and systolic flow murmurs. Rarely there may be purpura if thrombocytopenia is present. The mild jaundice, which is present, is due to increase in indirect bilirubin, which in turn is due to the severe ineffective erythropoiesis.

The gastrointestinal manifestations are due to the effect of the vitamin deficiency (cobalamin or folic acid) on rapidly proliferating gastrointestinal epithelium. There is anorexia, moderate weight loss, soreness of the tongue, which will be smooth and beefy red. There may be diarrhea due to megaloblastosis of the small intestine and these result in malabsorption.

The neurological manifestations are due to demyelination followed by axonal degeneration and neuronal death involving peripheral nerves, corticospinal tracts and posterior column of the spinal cord and the cerebrum. Symptoms and signs include paresthesia in the extremities, weakness, ataxia, absent reflexes especially ankle jerks, positive Romberg and Babinski signs, and loss of joint and position sense. The neurological features are not usually seen with folic acid deficiency.

Diffuse hyperpigmentation, especially of the palm and sole, are found in Indian patients.

DIAGNOSIS OF MEGALOBLASTIC ANEMIA

Presence of macrocytosis (MCV >100fL) in presence of anemia suggests megaloblastic anemia. Other causes of macrocytosis include: Liver diseases, hemolytic anemia, alcoholism, hypothyroidism and aplastic anemia. In these conditions macrocytosis is not marked (MCV between 100 and 110fL). If MCV is more than 110fL megaloblastic anemia is more likely. When there is co-existing iron deficiency or thalassemia the macrocytosis is less marked. The reticulocyte production index (RPI) is low. In severe cases of megaloblastic anemia there may be reduction in the leukocyte count and the platelet count. The blood smear shows marked anisopoikilocytosis with macro-ovulocytes (large oval and fully hemoglobinized red blood cells). The neutrophils show hypersegmentation of the nucleus (Fig. 11.5). Even a single neutrophil with a nucleus more than six lobes should raise an immediate suspicion of megaloblastic anemia.

The bone marrow is hypercellular with decreased ME ratio (normal ratio 4:1) (Fig. 11.3). The iron staining of the bone marrow is usually increased. But in India many of these cases may show no stainable iron in the marrow due to coexisting iron deficiency. The characteristic cell among the RBC precursors is the megaloblast, which differs from the normal normoblasts in its nuclear features. The nuclear chromatin of the megaloblast is more dispersed (less condensed) and condenses in a peculiar fenestrated pattern. Abnormal mitosis may be seen.

There is marked ineffective erythropoiesis and more than 90% of the RBC precursors are destroyed in the bone marrow before their release (normally about 10-15% are destroyed) into the blood stream. As a result there is an increase in the unconjugated serum bilirubin and lactic dehydrogenase. Once the megaloblastic anemia is established the next step is to do a serum B_{12} and folic acid level to find out the specific vitamin deficiency. Normal range of cobalamin in the serum is 300-900 pg/mL and values < 200 indicate clinically significant deficiency. Measurement

of cobalamin bound to TC II is ideal but such assays are not routinely available. The normal serum folic acid level is 6-20 ng/mL and values <4 ng are considered to be diagnostic of folate deficiency. Measurement of RBC folate level is better because it is not subject to short-term fluctuation in folate intake and is a better index of folate stores than serum folate level.

If cobalamin deficiency is detected the next step is to find out the cause of the deficiency by Schilling test. This test is not usually done in India since cobalamin deficiency in India is mostly nutritional. Radioactive cobalamin is given by mouth to the patient followed by intramuscular injection of cobalamin to saturate the body stores. The proportion of the orally administered radioactive cobalamin excreted in the urine in the next 24 hours provides an accurate measure of the absorption of the vitamin. If this is less, then cobalamin is not properly absorbed. The next step of the test is to repeat the test after giving the patient labeled cobalamin bound to IF. If the urinary excretion approaches normal then the abnormality is a deficiency of the IF and the underlying condition is pernicious anemia or some other type of IF deficiency.

If the urinary excretion is still less (indicating decreased cobalamin absorption) the absorptive defect may be due to bacterial overgrowth, which can be corrected by administering antibiotics. The Schilling test will yield equally reliable information even after treating the patient with parenteral cobalamin. The defective release of the vitamin from the food—a recent observation—can be inferred by repeating the Schilling test with radioactive cobalamin scrambled with an egg. The usual test will be normal while mixing food will make it abnormal showing that food decreases absorption.

Serum homocysteine and methyl malonic acid levels are elevated in cobalamin deficiency while only homocysteine level is increased in folic acid deficiency. These tests measure the tissue levels of the vitamins and thus may demonstrate early deficiency when the serum levels may be still normal. This is very important in detecting subtle cobalamin deficiency in elderly people who develop neuropsychiatric abnormalities. These elderly people may have no hematological abnormalities and the serum level of cobalamin may be normal.

MANAGEMENT OF MEGALOBLASTIC ANEMIA

In India, the commonest cause for megaloblastic anemia is nutritional deficiency of the two vitamins cobalamin and folic acid. It is usual to have a co-existing iron deficiency also. So it is very important to correct all these deficiencies simultaneously. It is desirable to deworm the patient at the onset of treatment with a broad spectrum anti-helminthic like albendazole 400 mg single dose to be taken at bedtime.

A diet containing all the three hematopoietic factors should be prescribed to the patient. Meat, dairy products (contain cobalamin), vegetables and fruits (contain folic acid) should be advised. Green leafy vegetables, dates, black grapes, meat, liver, onion and jaggery contain iron. Oral supplements of vit B_{12} (2 mg) and folic acid (1-5 mg) will be enough in nutritional deficiencies in addition to the prescribed diet. The supplementations should be continued for 3-6 months. In a severe case parenteral B_{12} may be necessary.

Underlying diseases responsible for the megaloblastic anemia like bacterial over growth, and diseases of the ileum should be treated.

If the cobalamin deficiency is due to malabsorption or IF deficiency the vitamin has to be given parenterally. The therapeutic preparation is cyanocobalamin and the dose is 1000 μg given intramuscularly once a week for 8 weeks. This is followed by 1000 μg every month for varying period of time depending upon the cause. If the disease is pernicious anemia or resection of the ileum or the stomach the treatment has to be taken life long. The response to treatment is dramatic with a brisk reticulocytosis on the 4th day, peaking on the 7th day, with steady improvement in the hemoglobin over the next several weeks. The patient will experience an increase in strength and an improved sense of well-being. If there is not enough reticulocyte

response, co-existing iron-folate deficiency, infection or hypothyroidism should be suspected. Hypokalemia, salt retention and thrombocytosis may be seen in the early course of treatment. Occasionally a patient with marked anemia may require one or two units of packed red cell transfusions. But this has to be given very carefully avoiding fluid overload and cardiac failure. The neurological deficits may not be corrected completely even by optimal therapy if it has proceeded to the stage of neuronal death. There is also a potential for the development of gastric carcinoma in pernicious anemia on follow-up. Folic acid in large doses may correct the megaloblastic anemia of cobalamin deficiency but may worsen the neurological abnormalities. Therefore, if there is a deficiency of both cobalamin and folic acid, cobalamin deficiency should be corrected first. Some experts now believe that small amount of vit B_{12} (0.1 mg of oral crystalline B_{12}) should be given prophylactically daily for elderly people over the age of 65 years. This is in view of the recent observation of defective cobalamin absorption in older people.

Folic acid deficiency is also treated by replacement therapy. The oral dose is between 1 and 5 mg depending on the severity. Parenteral folate is rarely required. The hematological and clinical responses are similar. The duration of treatment depends on the cause of deficiency. Conditions like chronic malnutrition as found in India and chronic hemolytic anemia folic acid replacement should be given indefinitely. Pregnant women should be given prophylactic folic acid throughout pregnancy and lactation. From 1998 folic acid fortification of food grains is enforced in countries like US. Similar steps are being planned and discussed in India.

Megaloblastic anemia produced by various drugs can be treated by reducing the dose or stopping the offending drugs. Effects of folic acid antagonists like methotrexate can be counteracted by folinic acid in a dose of 100-200 mg/day. This circumvents the block in folate metabolism by providing a form of folate which can be converted to 5, 10 methylene THF. Pyridoxine up to 300 mg may be tried in megaloblastic form of sideroblastic anemia. Erythroleukemia should be treated as any other form of AML.

SUMMARY, CONCLUSIONS AND FUTURE

In summary, we have come a long way in our understanding about the various aspects of megaloblastic anemia. From a serious killing and disabling disease of the past it has mellowed down to an easily manageable disease in the present day thanks to these newer understandings. The Indian scenario is entirely different from the western world especially regarding the causes of megaloblastic anemia. In India the main cause is nutritional deficiency. Cobalamin deficiency may be more among the vegetarians in India. Through health education they should be encouraged to take more dairy products. Folic acid deficiency is more pronounced in children and pregnant mothers in India since regular pre-natal follow-up has not become a routine in many parts. B_{12} and folic acid estimation in the serum is not available in many parts and are quite costly. The average cost of estimation of serum vitamin B_{12} and folic acid costs Rs.300 to 750 each, while cost of estimating IF antibody and parietal cell antibody level is Rs.1250 each. The cost of these investigations should be reasonable and widely available in the future. A well-prescribed diet containing cobalamin and folic acid is the mainstay of treating megaloblastic anemia in India. Folic acid fortification of the food is a good idea and should be executed without delay. Pregnant mothers and elderly people should be given prophylactic folic acid and cobalamin. Physicians, NGOs and the national government should take up health education and public awareness of megaloblastic anemia and its effective prevention and management by a well-prescribed diet.

FURTHER READING

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