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Megaloblastic anemia in children: Case series from a single institution and literature review

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ABSTRACT

Folic acid and cobalamin are B-group vitamins that play an essential role in many cellular processes. Deficiency in one or both of these vitamins causes megaloblastic anemia, a very rare anemia in children, which is characterized by the presence of megaloblasts. Vitamin B_{12} deficiency can be caused by a low intake, decreased absorption, or impaired use.

We present five cases of megaloblastic anemia that illustrate the onset of anemia at different ages and in different pathologies.

We concluded that megaloblastic anemia is a rare anemia diagnosed in children, with multiple causes, occurring in the context of a vegetarian diet, or associated with an autoimmune disease, protein-calorie malnutrition of the mother during pregnancy and megaloblastic anemia of the mother associated with lack of substitution during pregnancy and during breastfeeding.

Keywords: megaloblastic anemia, megaloblasts, vitamin B_{12}, folic acid

Abbreviations
Hgb – Hemoglobin
MCHC – mean corpuscular hemoglobin concentration
MCV – mean corpuscular volume

INTRODUCTION

Megaloblastic anemia is a macrocytic anemia caused by deficiency of cobalamin, folate, or both (1). The average age of occurrence is 60 years; it is rare in children, except in underdeveloped countries, where due to the socio-economic situation and the lack of vitamin B_{12} in food, it is more common. It is also commonly associated with autoimmune diseases (2). Vitamin B_{12} is a water-soluble vitamin and plays a major role in metabolic reactions. People are totally dependent on vitamin B_{12} from the diet. Food sources of vitamin B_{12} are almost exclusively of animal sources (3).

Vitamin B_{12} deficiency can occur in breastfed infants by vegetarian mothers or with megaloblastic anemia; it can also be caused by intestinal malabsorption in Crohn's disease, celiac disease, chronic pancreatitis, Diphyllobothrium latum infestation or after resection of the terminal ileum (1,4). Malabsorption of vitamin B_{12} caused by intrinsic factor deficiency is very rare in children. Folic acid deficiency is also caused by poor nutrition, malabsorption or increased needs.

Megaloblastic anemia is an anemia that is characterized by the presence of precursor cells, megaloblast in the bone marrow and macrocytic red cells in the peripheral blood. These megaloblasts arise because of impaired DNA synthesis followed by ineffective erythropoiesis. Other changes include low hemoglobin and erythrocyte count, elevated mean corpuscular volume (MCV > 100 fL), in-

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creased mean corpuscular hemoglobin concentration (MCHC), thrombocytopenia, low reticulocyte count. A low serum vitamin B12 (VN = 191-663 pg/ml) or folic acid (VN = 4.6-18.7 ng/ml) supports the positive diagnosis (5).

Depending on the type of deficiency, vitamin B_{12} substitution treatment consists initially in one intramuscular loading dose each week for 1 month, then a maintenance dose or taking folic acid orally to correct anemia and megaloblastosis (6).

We report four cases of megaloblastic anemia, diagnosed in a Tertiary Hospital of Pediatrics, Emergency Hospital Tg. Mures, Romania between 2015-2018.

The informed consent was obtained from the patients' mothers (legal guardians) for publication of these cases.

CASE SERIES

Case 1

15 year old female patient, with predominantly vegetarian diet, was hospitalized for fatigability at small/moderate effort and paleness, a hematological malignancy being initially suspected. Clinical examination is showed in Table 1 and her laboratory findings confirmed severe vitamin B_{12} deficiency, as seen in Table 2. Peripheral smear and bone marrow are described in Table 3, characteristics of bone marrow are shown in Figure 1. Red cell transfusion was initiated, subsequently vitamin B_{12} intramuscular for 5 days, evolution being favorable. The case was interpreted as a megaloblastic anemia due to nutrition mistakes.

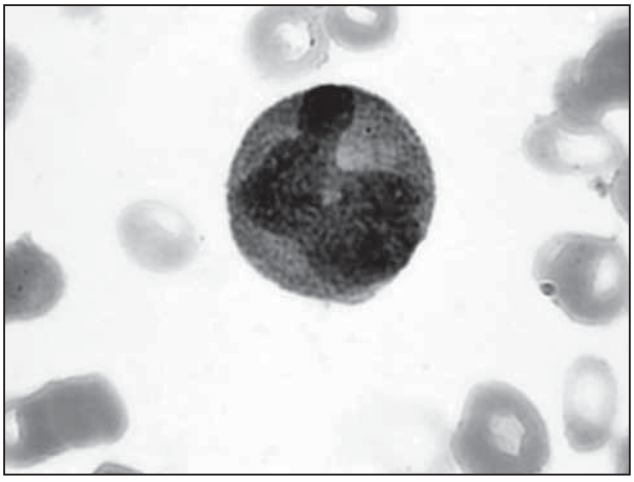

***FIGURE 1**. Bone marrow aspiration showing hypercellularity with giant metamyelocytes*

Case 2

Infant male, age four months, was hospitalized for accentuated pallor of skin and mucosa. Family history showed that during the pregnancy the mother was diagnosed with mixed iron deficiency anemia and vitamin B_{12} and folic acid deficiency, having a strict vegetarian diet. The infant's history reveals a physiological term birth, born naturally, birth weight 3,000 g, Apgar score 9/1min, exclusively breastfed until present, vaccinated, at 4 months diagnosed with hypotonic syndrome. Clinical examination is showed in Table 1 and laboratory studies are shown in Table 2 and Table 3, confirming severe anemia due to vitamin B_{12} deficiency. The aspect of bone marrow is shown in Figure 2. Hemoglobin electrophoresis was performed: Hgb A 89.8% Hgb A_2 4.6%, Hgb F 5.6%. Red blood cells and platelet transfusion was initiated, vitamin B_{12} intramuscularly. The case was interpreted as a megaloblastic anemia and beta – thalassemia minor.

***TABLE 1.** Clinical findings of case reports*

	Case 1	Case 2	Case 3	Case 4	Case 5
Age	15 years	4 months	6 months	11 years	8 months
Sex	F	M	F	F	F
General state	fa gability, poor appe te	poor appe te	poor appe te, PI <0.6 (dystrophy grade III)	good	Poor appe te, PI= 0.76 (dystrophy grade II)
Skin tone	pallor, sallow tone	pallor, mild jaudince	marked pallor, facial edema, decreased turgor	pallor	pallor
Mucosal abnormali es	paleness, depapillated smooth, red tongue, mild gingival hypertrophy	pallor	pallor, conjunc val edema	candidiasis, commisural rhagades	pallor
Muscle tone	normal	hypotony	generalized muscle weakness	normal	mild hypotony
Osteoar cular abnormali es	normal	normal	rickety chest, AF =2/1cm, depressed	normal	normal
Splenomegaly	No	Yes	No	No	No
Stool	normal	normal	Chronic diarrhea	normal	stool with mucus
PI = ponderal index, AF = anterior fontanele					

TABLE 2. *Laboratory indings of case reports*

	Case 1	Case 2	Case 3	Case 4	Case 5
Hgb (g/dl)	5.6	4.9	6.8	11.4	6.9
MCV (fL)	97.7	73.7	97.5	107.0	98.1
MCHC (g/dl)	35.0	37.6	37.2	33.8	31.4
Leucocites (/mm^3)	4,900	7,000	3,900	6,200	5,260
Platelets (/mm^3)	132,000	56,000	88,000	68,000	131,000
Re culocites (‰)	12	8	6	30	10
Vitamin B_{12} (pg/ml)	33	<50	-	56	91
Homocistein (µmol/l)	51.91	-	-	-	-
Folate (ng/ml)	-	16.4	-	-	30.02
Sideremia (µmol/l)	19.90	29.69	13.06	19.92	15.85

Hgb = Hemoglobin, MCV = mean corpuscular volume, MCHC = mean corpuscular hemoglobin concentra on
Vitamin B_{12} normal values 191-663 pg/ml, Homocistein normal values < 10 µmol/l,
Folate normal values 4.6-18.7 ng/ml

TABLE 3. *Characteristics of peripheral smear and bone marrow of case reports*

Peripheral smear	Case 1	Case 2	Case 3	Case 4	Case 5
Macro-ovalocytes	+	+	+	+	+
Anisocytosis	+	+	+	-	+
Poikilocytosis	-	+	+	-	-
Erythrocytes with Cabot rings	+	+	-	-	-
Howell-Jolly bodies	+	+	-	-	+
Macrothrombocytes	+	+	-	+	-
Hypersegmented neutrophils	+	-	-	+	-
Bone marrow					
Erythroid hyperplasia	+	+	-	+	+
Giant metamielocytes	+	+	+	+	+
Megaloblastosis	+	+	+	+	+
Howell-Jolly bodies	+	+	-	+	+
Hypersegmented neutrophils	+	-	+	+	-

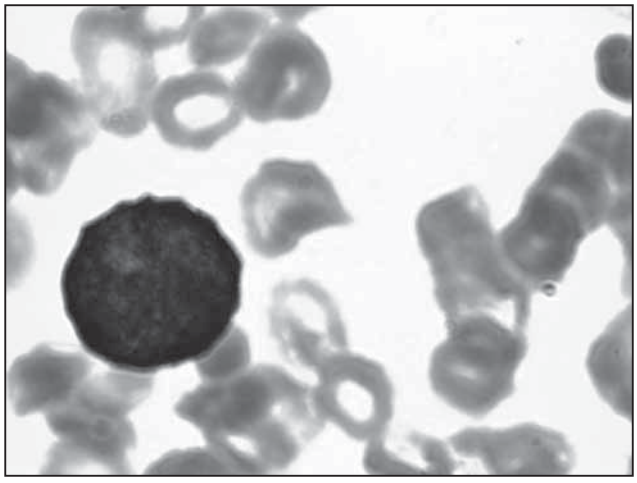

FIGURE 2*. Bone marrow aspiration showing erythroblastic series with moderate hyperplasia with megaloblasts*

Case 3

Female infant, age 6 months, hospitalized for capricious appetite, downward weight curve, semi consistent stools, marked paleness of skin and mucosa. From personal history we retain physiological term birth, Apgar score 10/1 min, birth weight 3,200 g, breastfed exclusively for a month, later with goat milk, vaccinated, non-diversified, admitted to the Pediatric Clinic at age 2 months with bronchiolitis.

Clinical examination is showed in Table 1 and her laboratory abnormalities are shown in Table 2. Peripheral smear and bone marrow are described in Table 3, confirming megaloblastic anemia. Bone marrow aspect is shown in Figure 3. Folic acid was administered orally and vitamin B_{12} mcg intramuscularly, although folate and vitamin B_{12} dosing was not conducted for objective reasons, due to low compliance and because the mother was discharged upon request. Mother was also counseled about the diet modification. In this case, megaloblastic anemia is interpreted in the context of protein-calorie malnutrition, multiple food mistakes and poor socio-economic conditions.

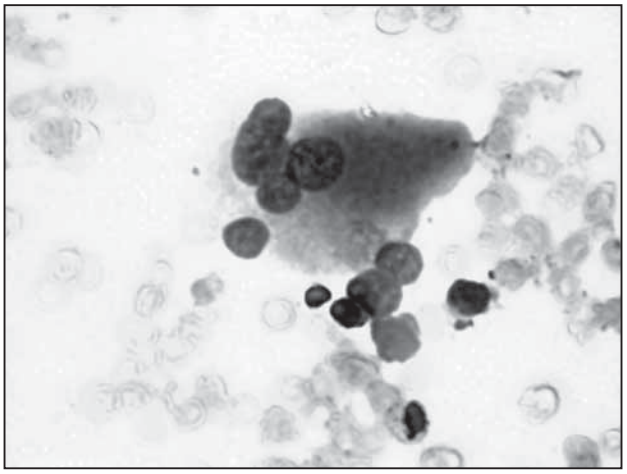

FIGURE 3. *Bone marrow aspiration showing megakaryocytes with nucleus explosion*

Case 4

11 years old female, diagnosed at age 6 weeks with mucocutaneous candidiasis, and after the age of 10 with autoimmune type I poliendocrinopaty, during multiple endocrinology assessments, patient presents thrombocytopenia, which is why she was hospitalized for hematological evaluation. Personal pathological history: mucocutaneous candidiasis, hypoparathyroidism, primary adrenal insufficiency. Clinical examination abnormalities are showed in Table 1 and laboratory studies are shown in Table 2 and Table 3, confirming megaloblastic anemia. Peripheral smear aspect is shown in Figure 4. Substitution treatment was initiated with vitamin B_{12} intramuscularly. In this case, megaloblastic anemia was interpreted as part of the autoimmune poliendocrinopaty complex.

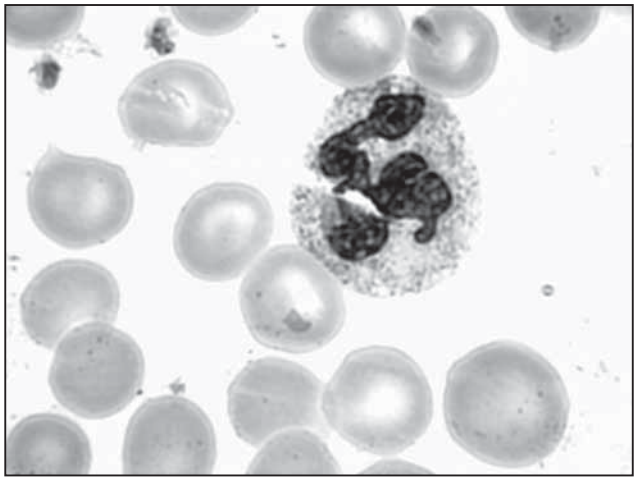

FIGURE 4*. Peripheral smear showing macrocytosis and hypersegmented neutrophils*

Case 5

8-month-old female infant with mother known with megaloblastic anemia was admitted for poor appetite and slow weighing curve, with no apparent pathological history. Clinical examination abnormalities are presented in Table I, and laboratory investigations are presented in Table 2 and Table 3, confirming megaloblastic anemia. The appearance of the peripheral smear is shown in Figure 5. Substitution treatment was initiated with red blood cells, followed by vitamin B_{12} intramuscular. This case was interpreted as a megaloblastic anemia in an infant whose mother was diagnosed with megaloblastic anemia and had prompt response to vitamin B_{12} substitution.

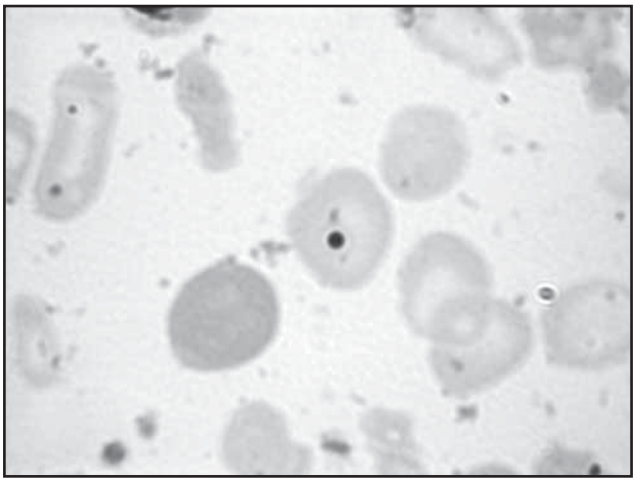

FIGURE 5. *Peripheral blood smear showing erythrocytes with Howell-Jolly bodies*

DISCUSSIONS

Megaloblastic anemia frequency varies according to geographical regions, being much higher in the Netherlands, Scandinavian countries, USA, Canada versus Southern and Eastern Europe where it is very low; however, it is not excluded that this anemia is widespread compared to the data, and that probably not all the cases are diagnosed (6). Due to folate deficiency, megaloblastic anemia is frequently attested in underdeveloped countries with a low quality of life and insufficient nutrition (7).

Infants with megaloblastic anemia may have skin paleness, moderate jaundice, can be more irritable, with capricious appetite, while older children may complain of numbness and weakness; these symptoms have been described in the five cases presented, which are similar to many reported studies (8-10).

Megaloblastic anemia is an important cause of cytopenias. The initial workup are a blood count and a peripheral smear (11); revealing: a low value of hemoglobin and red blood cell count, increased

MCV (> 100 fL), normal mean corpuscular hemoglobin concentration (MCHC), thrombocytopenia, decreased number of reticulocytes, peripheral smear with macro-ovalocytes, anisocytosis, poikilocytosis, red blood cells with Cabot rings, Howell-Jolly bodies, large platelets, hypersegmented neutrophils (1,2,4).

Sometimes signs of hemolysis are detected with increased value of lactic acid dehydrogenase and indirect bilirubin, that can be attributed to intramedullary destruction of red blood cells (12). Bone marrow smear reveals erythroid hyperplasia, giant metamielocytes, megaloblastosis, Howell-Jolly bodies, hypersegmented neutrophils (6). Low serum levels of vitamin B_{12} and folic acid sustain the diagnosis.

The five cases presented illustrate the onset of megaloblastic anemia at different ages and in different pathologies. Thus, the first two patients have the onset of the disease at 14 years and 4 months respectively, with pale skin and fatigue. Laboratory evaluation in both cases reveal a severe anemia and thrombocytopenia, blood smear with macrocytosis, and the bone marrow aspect pleading for megaloblastic anemia, vitamin B_{12} deficiency confirmed by dosing. In the first case, megaloblastic anemia was due to predominantly vegetarian food. Ravikumar et al. reported that in a group of 40 adolescents with megaloblastic anemia, 82.5% had vegetarian or a predominantly vegetarian diet pattern (9). Suarez T et al. have reported iron, folic acid and vitamin B_{12} deficiencies in 34.6%, 90.9% and 18.1% respectively of the anemic adolescents aged 12-19 years (13). Food fortification with folic acid, vitamin B_{12} and other micronutrients is a low cost and could be an effective measure to control anemia in high-risk populations. The 4 month old baby was fed only naturally, the cause of vitamin B_{12} deficiency being the strictly vegetarian diet of the mother during pregnancy and later, accompanied by lack of vitamin supplementation. Megaloblastic anemia due to vitamin B_{12} deficiency in exclusively breastfed infants born to vegan mothers has been reported by other authors and it is an important cause of neuromotor retardation (14-18). Performing hemoglobin electrophoresis, this patient also had an associated minor beta-thalassemia, which masked the macrocytosis and thus explained the lower value of MCV (73.7 fL). The fifth patient also falls into the category of megaloblastic anemia caused by the lack of vitamin supplementation, but in the context of a maternal megaloblastic anemia.

The next two patients were diagnosed by chance with megaloblastic anemia, in the context of associated pathology. Thus, the 3rd patient is diagnosed with malabsorption syndrome, IIIrd degree dystrophy, food mistakes, and peripheral smear with aspect of megaloblastic anemia, raised the suspicion, confirmed by bone marrow aspirate. In an Indian study, conducted on a sample of 29 infants with megaloblastic anemia, protein-calorie malnutrition has been documented in 14 cases. Vitamin B_{12} and folic acid dosage was done in only 10 cases, showing either a combined deficit of vitamin B_{12} and folate deficiency or only isolated vitamin B_{12} or folic acid deficiency. In the remaining patients the diagnosis of megaloblastic anemia was stated on peripheral smear and bone marrow appearance (19).

Case 4 represents an 11 year old patient diagnosed with autoimmune type I poliendocrinopaty, with associated and unexplained thrombocytopenia, which leads us to carry out investigations such as blood and marrow examination and eventually the dosage of vitamin B_{12}, which confirms the diagnosis. The literature reminds us that autoimmune diseases can be a trigger of megaloblastic anemia. There were reported cases of megaloblastic anemia associated with autoimmune hemolytic anemia, rheumatoid arthritis or autoimmune thyroiditis, Sjögren's syndrome and systemic lupus erythematosus (20-22).

Megaloblastic anemia is generally suspected in children having anemia with raised MCV and should be strongly suspected in all children having bicytopenia or pancytopenia (23). This case series described the varied presentations of the megaloblastic anemia in children and in different pathologies.

CONCLUSIONS

Megaloblastic anemia is a rarely diagnosed anemia in children, probably under diagnosed, with multiple causes, occurring in the context of a vegetarian diet, or associated with an autoimmune disease, poor socio-economic conditions, protein-calorie malnutrition, vegetarian nutrition of the mother during pregnancy associated with lack of substitution during pregnancy and during breastfeeding and last but not least maternal megaloblastic anemia.

Supplementation with vitamin B_{12} should be offered to all mothers during pregnancy and postnatal during breastfeeding beside iron and folic acid substitution, especially if the mother is vegetarian or comes from low socio-economic conditions.

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