Hereditary Folate Malabsorption: A Rare Treatable Disorder with Hematological and Neurological Manifestations

Sir,

With the advent of neurogenomics, the diagnosis and treatment of rare inherited disorders have been made possible. A 20-month-old boy born to third-degree consanguineous parents presented to the pediatric neurology outpatient clinic with a history of multiple daily episodes of generalized tonic–clonic seizures, atypical absence seizures, and myoclonic jerks of one-month duration. Before the onset of seizures, he could stand with support, transfer toys, babble, and wave bye-bye. After the seizure onset, he had gradual regression of milestones involving all domains. He was treated elsewhere with antiseizure medications but had poor seizure control despite treatment with adequate doses of appropriately chosen antiseizure medications. He was born full-term by lower segment cesarean section with a birth weight of 2100 g. His two siblings succumbed in the infantile period due to severe anemia and pneumonia.

He was initially evaluated in the pediatrics outpatient clinic at 3 months of age for fever, cough, oral ulcers, and progressive pallor of one-week duration. Pallor, weight of 3800 g (<3rd centile), length of 52 cm (<3rd centile), and head circumference of 36.5 cm (10–25th centile) were documented. There was no facial dysmorphism, lymph node enlargement, or hepatosplenomegaly. There were no focal neurological deficits. The laboratory parameters at presentation are summarized in Table 1. Bone marrow aspiration was done elsewhere and a review of slides revealed normocellular marrow with marked erythroid hyperplasia, adequate megakaryocytes,

Table 1: Laboratory profile of child at presentation and follow-up

Laboratory parameters	At 3 months of age* Patient value (normative value)	At 3 months of age** Patient value (Normative value)	At 20 months of age Patient value (Normative value)
Hemoglobin	6.9 g/dL (mean 12.6 g/dL and 2 SD below mean is 11.1 g/dL)	8.4 g/dL (mean 12.6 g/dL and 2 SD below mean is 11.1 g/dL)	10.8 g/dL (mean 12.0 g/dL and 2 SD below mean is 10.5 g/dL)
Red blood cell count	NA	2.84 million/cu.mm (3.12-5.1 million/cu.mm)	4.15 million/cu.mm
Mean corpuscular volume (MCV)	NA	88.7 fL (Mean: 76 fL and 2 SD below mean: 68 fL)	80.4 fL (Mean: 78 fL and 2 SD below mean: 70 fL)
Mean corpuscular hemoglobin (MCH)	NA	29.7 pg (Range: 25-35 pg)	26.1 pg (Range: 25-29 pg)
Mean corpuscular hemoglobin concentration (MCHC)	NA	33.4 gm/dL (Range: 28-36 gm/dL)	32.5 gm/dL (mean 33.0 gm/dL and 2 SD below mean is 30 g/dL)
Total leukocyte count	4200 cells/cu.mm (5,000-19,500 cells/cu.mm)	10,200 cells/cu.mm (5,000-19,500 cells/cu.mm)	8,000 cells/cu.mm (6,000-17,500 cells/cu.mm)
Platelet count	70,000 cells/cu.mm (150,000-350,000 cells/cu.mm)	150,000 lakhs/cu.mm (150,000-350,000 cells/cu.mm)	191,000 lakhs/cu.mm (150,000-350,000 cells/cu.mm)
Reticulocyte count	NA	2.8% (Corrected)	1.7%
Serum vitamin B_{12}	>2000 pg/mL (259-1576 pg/mL)	ND	>2000 pg/mL (259-1576 pg/mL)
Serum folate	4.1 ng/mL (3.9-26.8 ng/mL)	ND	3.2 ng/mL (3.9-26.8 ng/mL)

NA- Not available, ND- Not done *Investigations done elsewhere **Investigations done at our hospital

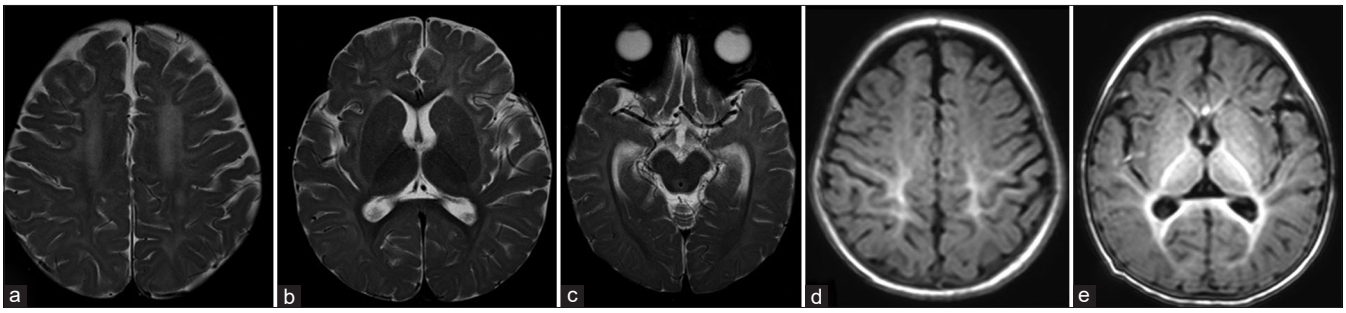

Figure 1: MRI brain at 2 years. T2 weighted images (a-c) and T1 weighted images (d and e) show anterior predominant mild T2 hyperintensity with corresponding T1 isointensity to mild hypointensity in deep and subcortical white matter suggestive of hypomyelination. The myelination of early myelinating structures including pre- and post-central white matter, posterior limb of the internal capsule, and optic radiations is normal. Note that corpus callosum is relatively spared

and a moderate increase in reticulin and he received a blood transfusion before referral.

A peripheral blood smear study showed marked anisocytosis, macrocytes and microcytes with polychromatophils, teardrop cells, and occasional fragmented red blood cells. Mild elevation of serum alanine transaminase was noted (patient: 51 U/L; normal: 5–33 U/L). He was empirically treated with vitamin B_{12}, vitamin E, and folic acid supplementation. On follow-up, he had mild developmental delay and recurrent respiratory tract infection. Hemogram, creatinine, liver function tests, homocysteine, and vitamin B12 level were normal on follow-up while folate level was 1.9 ng/mL. Early infant stimulation therapy was initiated.

Examination at 20-month of age revealed subtle dysmorphism characterized by upward slanting of eyes, depressed nasal bridge, and low set ears. His weight (8.3 kg), length (73 cm), and head circumference (40.5 cm) were below the third centile. The child had good eye contact, normal fundus, central hypotonia and extensor plantar responses. Etiological possibilities of peroxisomal disorders, lysosomal storage disorders, immunodeficiency syndromes, cobalamin or purine metabolism disorders, and mitochondrial disorders were considered based on the history, sibling deaths, and examination findings.

Laboratory evaluation at 20 months of age revealed a blood lactate 2.5 mmol/L (0.9–1.8 mmol/L), ammonia 88 µg% (27.2–115.8 µg%), and uric acid 3.5 mg/dL (1.8–4.9 mg/dL). Repeat hemogram, serum vitamin B12, and folate are summarized in Table 1. His thyroid profile and blood immunoglobulin levels were normal. Cerebrospinal fluid (CSF) glucose was 70 mg/dL (blood glucose: 114 mg/dL), protein was 28 mg/dL (6–25 mg/dL), lactate 1.5 mmol/L (1.1–2.2 mmol/L), and folate level was 0.6 ng/mL (16–21 ng/mL). Serum and CSF amino acids, urine organic

acids, serum acylcarnitine levels, and blood hexosaminidase A and B levels were normal. A repeat bone marrow aspiration and biopsy did not reveal any evidence of malignancy or storage disorder. Electroencephalogram (EEG) revealed epileptiform activity arising from the bilateral posterior head region. Brain magnetic resonance imaging (MRI) showed features of hypomyelination [Figure 1a-e] and there was no calcification on computed tomography (CT) brain. Clinical exome sequencing revealed a pathogenic homozygous nonsense variation in the exon 2 of the *SLC46A1* gene (chr17:g. 26731970G>A) that results in a stop codon and premature truncation of the protein at codon 249 (p.Arg249Ter; ENST00000440501.1) confirming the diagnosis of hereditary folate malabsorption (HFM). Parents were heterozygous carriers for the same variant. Genetic counseling was offered regarding the risk of recurrence in future pregnancies and the option of prenatal diagnostic modalities. Our patient had good seizure control after treatment with intramuscular calcium folinate injections at a dose of 1.5 mg/kg/d and optimization of doses of antiseizure medications but had slow developmental gains. A repeat CSF and serum folate levels on follow-up were 8.2 ng/mL and >20 ng/mL, respectively.

Isolated HFM (OMIM 229050), a rare autosomal recessive disorder occurs due to defective absorption and transportation of folate across the small intestine and central nervous system.[1,2] Folate is a water-soluble vitamin found in green leafy vegetables. The name was derived from the Latin word "folium" which means leaf.[2] Folic acid (vitamin B_9) is a synthetic form found in supplements and fortified food. Folate is not synthesized in humans but derived exogenously from food sources. The reduced form of folate is essential for the synthesis of amino acids, deoxyribonucleic acid (DNA), pyrimidines, and purines.[1,3] Dietary folate polyglutamates are converted to monoglutamate in the presence of hydrolase enzyme and hydrolysis occurs in the gastrointestinal lumen, brush border, or lysosomes of the enterocyte.[1] The dietary folate is absorbed across the duodenum and jejunum via active transport while folic acid is absorbed via passive diffusion.[2] The proton-coupled folate transporter (PCFT) expressed in the brush border of the small intestine governs the transport of folate across the intestine.[4] Monoglutamate enters the portal circulation and reaches the liver where it is reduced to tetrahydrofolate. Tetrahydrofolate is converted to methyl or formyl forms before entering circulation.[2] The transport of folate across various cells is governed by reduced folate carrier (RFC) or folate receptor (FR) systems with the affinity for reduced and oxidized folates, respectively.[1] The brain does not contain dihydrofolate reductase and hence reduced folates are transported across the central nervous system (CNS). The PCFT is also expressed in the choroid plexus and regulates the transport of folates into the CSF. Other possible mechanisms for the transport of folate across CNS are through FR in choroid plexus and CSF soluble folic acid-binding protein.[1] Folate deficiency may occur due to decreased nutritional intake, drug ingestion, malabsorption syndromes, inborn errors of metabolism, hemolytic anemia, and psoriasis.[1]

HFM is a rare cause of folate deficiency in children. HFM is caused by mutations in the *SLC46A1* gene that encodes PCFT.[5,6] In HFM, the gut absorption and transportation across CNS are defective. Children with HFM manifest gastrointestinal, hematological, immunodeficiency, and neurological syndromes.[1] Clinical symptoms include anemia, chronic diarrhea, failure to thrive, progressive neurologic deterioration, and oral ulcers.[1] Neurological manifestations in patients with HFM are psychomotor delay, seizures, behavioral changes, ataxia, movement disorders, and peripheral neuropathy.[7] Our proband had pancytopenia, failure to thrive, oral ulcers, recurrent infection, developmental delay, neuroregression, and seizures. The pathophysiological basis for neuropsychiatric manifestations includes decreased methylation reaction that impairs DNA synthesis and the myelination process.[1] Siblings of our patient succumbed to severe pneumonia. Recurrent infection in proband could possibly be explained by the fact that folate coenzymes are crucial for biosynthesis of purine and pyrimidine that determine DNA synthesis, thus folate deficiency results in B cell and T cell dysfunction.[1]

Children with HFM usually have low serum, erythrocyte, and CSF folate levels, and abnormally high urinary excretion of formiminoglutamic acid. Similarly, our proband had low serum and CSF folate levels. The ratio of CSF to serum folate level was less than 3:1 in our patient as reported earlier.[1] Low CSF folate level has been reported in patients with folate receptor 1 (*FOLR1*) gene mutation, 5,10-methylenetetrahydrofolate reductase deficiency, aromatic L-amino acid decarboxyalse deficiency, serine biosynthesis defects, dihydropteridine reductase deficiency, autism spectrum disorders, Rett syndrome, Aicardi-Goutieres syndrome, Kearns-Sayre syndrome, Alpers disease, mitochondrial complex I deficiency, hypomyelination with atrophy of basal ganglia, juvenile rheumatoid arthritis, and schizophrenia.[8] Low CSF folate may also occur in secondary deficiency of folate due to inadequate dietary intake, intestinal resection, malignancies, use of antifolate drugs, celiac disease, and hepatic failure.[8] Brain imaging may be normal though cerebral and basal ganglia calcification has been reported.[7,9,10] In contrast, delayed myelination was identified on neuroimaging of our patient without any calcification.

There are no standard guidelines for the management of children with HFM. Folinic acid (5-formyltetrahydrofolate) is a metabolically active, reduced folate compound while folic acid is a metabolically inactive, oxidized folate compound.[11] Single daily administration of parenteral or oral folinic acid is the preferred treatment of choice. The oral dosage of folinic acid varies from 10–28 mg/kg/day while an intramuscular dosage of folinic acid varies from 0.5–3 mg/kg/day.[5,11,12] The folinic acid is converted by

enzymatic action into active 5-methyltetrahydrofolate which enters the CSF through a reduced folate carrier.[12] With the administration of folinic acid, a rise in serum folate has been observed in patients with HFM while normalizing CSF folate levels was difficult.[12] Folic acid should be avoided in patients with HFM as it binds to the folate receptor in the choroid plexus and interferes with the transport of 5-methyltetrahydrofolate into the CSF.[5] Reversal of hematological and gastrointestinal manifestations and immune dysfunction has been documented after treatment but a reversal of neurological consequences may be difficult.[13] To conclude, the diagnosis of HFM should be considered in children with psychomotor delay and seizures with the involvement of other systems including hematological, gastrointestinal, or immunological systems.

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Conflicts of interest

There are no conflicts of interest.

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