**Role of intramuscular levofolinate administration in the treatment of Hereditary folate malabsorption: report of three cases.**

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**Abstract**

Hereditary folate malabsorption is a rare autosomal recessive disorder caused by impaired active folate transport across membranes and into the central nervous system due to loss-of-function mutations in proton-coupled folate transporter (PCFT). Newborns with this condition have initially normal folate stores, but as they are unable to absorb dietary folate and use rapidly their stores because of their growth demands, symptoms appear in the early infancy. Significant neurological morbidity usually follows the initial non-specific clinical presentation and delayed initiation of treatment. High dose oral and parenteral folinic acid treatment have been previously reported in literature to improve the clinical outcome without achieving optimal cerebrospinal fluid (CSF) folate levels though. The active isomer of 5-formyltetrahydrofolate, also known as levofolinic acid, is available for administration. We report our experience in achieving normal (age dependent) CSF 5-Methyltetrahydrofolate (5-MTHF) levels following daily intramuscular administration of levofolinic acid in three patients with HFM. Follow up assessment with repeated lumbar punctures has shown a stabilization of 5-MTHF levels within normal range. Clinical features and brain MRI findings had as well either improvement or stabilization. To the best of our knowledge, we provide as well for the first time data in regards to the im levofolinate treatment dosage.

**Introduction**

Hereditary folate malabsorption (HFM; OMIM 229050) is a rare autosomal recessive disorder caused by impaired active folate transport across membranes and into the central nervous system due to loss-of-function mutations in proton-coupled folate transporter (PCFT) [1-3]. The severe systemic folate deficiency concomitant with impaired transport of folate across the blood-choroid plexus-brain barrier represents the pathophysiological basis of the disease and results in a marked deficiency of cerebrospinal fluid (CSF) folate [3-5].

Infants with HFM come to medical attention in early infancy when they present with non-specific systemic findings such as failure to thrive, feeding difficulties, diarrhea, megaloblastic anaemia and/or pancytopenia [1, 6]. One the most unique aspects of the disease is an immune deficiency which occurs in a setting of significant hypogammaglobulinemia resulting in infections with unusual organisms [6, 7]. Neurologic symptoms may present concomitantly or develop progressively without adequate treatment [1, 6]. Developmental delay, peripheral neuropathy, movement disorder, behavioral issues and seizures have been observed in the vast majority of reported patients with HFM [1, 6]. Diagnosis is suspected on the basis of the above mentioned clinical features and

haematological findings, which should prompt the clinician to measure CSF 5-methyltetrahydrofolate (5-MTHF) levels which are usually very low or undetectable. Confirmation of the diagnosis is made by detection of biallelic disease causing mutations in the SLC46A1 gene.

Early diagnosis is important for the initiation of treatment. Previous literature reports recommend high doses of oral or intramuscular (im) 5-formyltetrahydrofolate (5-formylTHF), also known as folinic acid, aiming to improve or normalize 5-MTHF CSF levels before irreversible damage occurs [1, 6]. Nevertheless, those levels are difficult to achieve even in patients who have been receiving high doses of parenteral folinic acid [8]. The active isomer of 5-formylTHF, also known as levofolinic acid, is as well available for administration. Anecdotal observations suggest that the active isomer might be more effective, especially in patients with neurological involvement and refractory seizures [1]. Herein we report 3 cases of patients with HFM who received im levofolinate treatment. Follow up assessment with repeated lumbar punctures has shown a normalization of their 5-MTHF levels and stabilization of clinical features and brain MRI findings. Based on achieving normal CSF 5-MTHF results, to the best of our knowledge, we provide for the first time data in regards to the im levofolinate treatment dosage.

**Case presentation 1**

The first patient is the only son of non-consanguineous parents of British origin, born at term following an uneventful pregnancy. This patient's clinical features were previously reported [9]. He presented to his local hospital at 2 months of age with failure to thrive, pallor, and diarrhea. He was diagnosed with Pneumocyctis jiroveci pneumonia. Initial work-up showed megaloblastic anaemia, neutropenia, undetectable serum folate with normal vitamin B12 levels. IgG levels were normal but IgA and IgM levels were low. Following initial intravenous folinic acid replacement patient was discharged home on oral folic acid supplementation of 1.5 mg/day and immunoglobulin replacement therapy.

Follow-up at 6 months of age demonstrated macrocytic anemia, neutropenia, low serum/red cell folate and no specific immunodeficiency abnormalities. The dose of oral folic acid was increased. At the age of 13 months the patient showed developmental arrest, movement disorder and signs of central motor neuron involvement. The oral folic acid supplementation was again increased. At this point patient had a normal brain MRI and a lumbar puncture was unsuccessful. Even on high doses of oral folic acid patient continued to have the same clinical features and hematologic abnormalities. Based on the above constellation of findings HFM was suspected. Undetectable CSF 5-MTHF levels were in agreement with the clinical diagnosis (Table 1). Genetic analysis confirmed the diagnosis and patient was found to be compound heterozygous, with a mutant allele from his mother (c.204-205delCC) in exon 1 resulting in a frameshift starting at position N68 with early termination of translation, and a mutant allele from his father located in exon 2 (c.1004 C>A) resulting in an A335D loss-of-function point mutation [9]. At this point patient was started on im folinic acid which led to an improvement of his clinical features; nevertheless, CSF 5-MTHF levels remained suboptimal.

Annual reviews showed delay of gross/fine motor skills, significant visual perceptual difficulty as well as slow witnessed continuous acquisition of skills with support. At the age of 5½ years the patient developed sudden mood swings, drop attacks with occasional generalized tonic-clonic seizures. EEG confirmed complex partial seizures. Patient was

started on antiepileptic treatment and im levofolinic acid was introduced. Results from a repeat lumbar puncture one year following the initiation of levofolinate have shown normal levels of 5-MTHF and brain MRI. Currently, at the age of 10 years the patient continues to have normal 5-MTHF CSF level and shows continuous developmental progress. To date, no local or systemic complications had been raised from the use of im injections.

**Table 1. Biochemical and neuroimaging findings in Patient 1**

| Age | CSF 5-MTHF | Medication | Imaging |
|---|---|---|---|
| **17months** | <1nmol/l<br>ref. 72-305 | Folinic acid, 5mg/day, im<br>(0.4mg/kg/d) | Normal intracranial appearances |
| **2years 6months** | 19nmol/l<br>(trough level)<br>ref. 72-305 | Folinic acid, 12mg/day, im<br>(0.8mg/kg/d) | Non-specific myelination delay and lack of white matter bulk |
| **3years** | 31nmol/l<br>(trough level)<br>ref. 72-305 | Folinic acid, 12mg/day, im<br>(0.71mg/kg/d) | Subcortical white matter of bilateral cerebral hemispheres better myelinated, unchanged lack of white matter bulk, no other intracranial changes |
| **5years** | 14nmol/l<br>(trough level)<br>ref. 52-178 | Folinic acid, 12mg/day, im<br>(0.6mg/kg/d) | No abnormality demonstrated |
| **6years** | 17 nmol/l<br>(trough level)<br>ref. 52-178 | Sodium Levofolinate,<br>15mg/day, im<br>(0.61mg/kg/d) | Normal intracranial and intra-spinal appearances |
| **7years** | 52 nmol/l<br>(trough level)<br>ref. 52-178 | Sodium Levofolinate,<br>20mg/day, im<br>(0.78mg/kg/d) | Normal intracranial and intra-spinal appearances. |
| **10years** | 62nmol/l<br>(trough level)<br>ref. 46-160 | Sodium Levofolinate,<br>50mg/day, im<br>(1.13mg/kg/d) | |

**Case presentation 2**

Patient 2 is the first male child of non consanguineous parents of Pakistani origin born at term after an uncomplicated pregnancy. He presented at 2 months of age with failure to thrive and irritability. On clinical examination patient presented subtle facial dysmorphism and hypospadias. Initial laboratory work-up showed severe pancytopenia requiring red blood cell and platelet transfusion. Plasma folate level was virtually zero and vitamin B12 was low. IM vitamin B12 and oral folic acid supplementation was started. Good progress was initially documented although plasma folate levels remained suboptimal and total plasma homocysteine was elevated. Vitamin B12 level normalized with im treatment within two months, however the patient was lost to follow up.

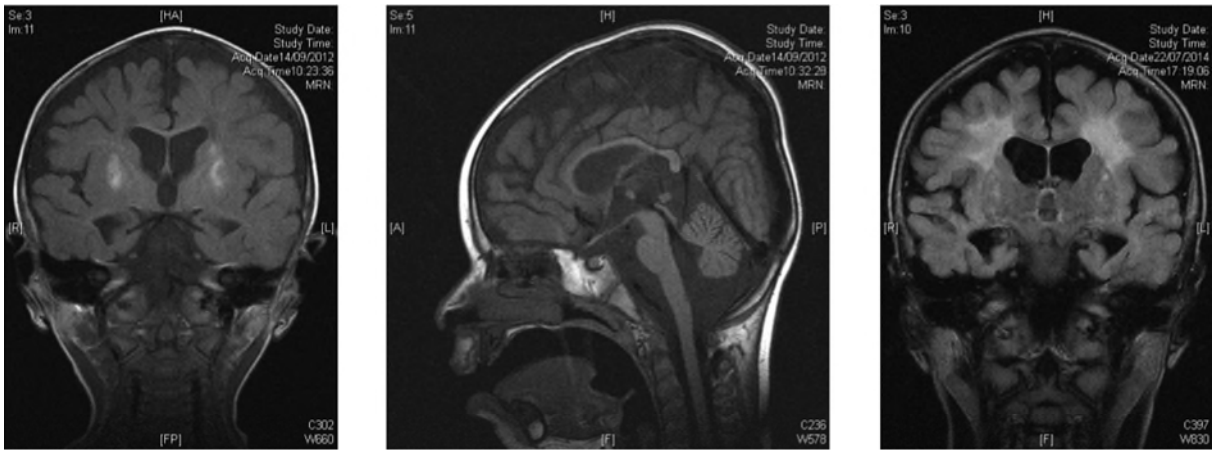
Six months later, while in Pakistan, patient underwent brain CT scan, following new onset epilepsy. The scan demonstrated basal ganglia calcification and cerebral atrophy. At

the age of 17 months the patient was admitted to PICU because of status epilepticus. Clinical examination revealed truncal hypotonia, movement disorder, and dystonia with poor visual interaction. EEG at that point showed hypsarrythymia. Because of the above constellation of findings a lumbar puncture for a full CSF work-up was done. The CSF 5-MTHF was undetectable with the rest of findings within normal range. Gene mutation analysis confirmed the diagnosis of hereditary folate malabsorption due to a homozygous c.198C>A, p.Cys66* mutation causing a premature stop codon. This mutation has been previously reported in literature in a patient who was compound heterozygous [10], but not in a homozygous state. Patient was started initially on oral folinic acid supplementation and 4 months later on im daily injections which led to clinical and biochemical improvement. (Table 2).

Although on im folinic acid therapy, patient's neurologic progress was turbulent with recurrent PICU admissions for status epilepticus, development of infantile spasms and subsequently myoclonic jerks which required combined antiepileptic drugs. At the age of 2 years the patient was started on im levofolinate treatment which led to the normalization of his 5-MTHF levels. Currently, at the age of 5 years the patient continues to have significant neurological deficit and intractable seizures, auditory and visual impairment and medication controlled movement disorder.

**Table 2. Biochemical and neuroimaging findings in Patient 2**

| **Age** | **CSF 5-MTHF** | **Medication** | **Imaging** |
|---|---|---|---|
| **17months** | <1 nmol/L<br>ref. 72-305 | Folinic acid, 10mg/day, oral | |
| **19months** | <10 nmol/L<br>(peak sample)<br>ref. 72-305 | Folinic acid, 30mg/day, oral | |
| **21months** | 11nmol/l<br>(peak sample)<br>ref. 72-305 | Folinic acid, 15mg/day, im<br>(2.5mg/kg/d) | Ponto-cerebellar hypoplasia in addition to generalised lack of cerebral volume and myelination, bilateral symmetrical mature haemorrhagic infarction of the basal ganglia, symmetrical periventricular and deep white matter change over the frontal lobes (Figure 1 ) |
| **2years** | 31nmol/l<br>(trough level)<br>ref. 52-178 | Sodium Levofolinate,<br>20mg/day, im<br>(1.3mg/kg/day) | |
| **2years 10months** | 50 nmol/l<br>(trough level)<br>ref. 52-178 | Sodium Levofolinate,<br>50mg/day, im<br>(3.3mg/kg/d) | |
| **4years 6months** | 106nmol/l<br>(trough level)<br>ref. 52-178 | Sodium Levofolinate,<br>40mg/day, im<br>(1.6mg/kg/d) | No progression of prominent white matter changes and ponto-cerebellar volume reduction |




A B C

**Figure1.** MRI brain T1 weighted image showing basal ganglia calcifications (A), ponto-cerebellar hypoplasia (B) T2 weighted image (C) showing white matter changes over the frontal lobes.

**Case presentation 3**

Patient 3 is the younger brother of Patient 2. Parents have declined antenatal testing. The pregnancy was uneventful and patient was delivered at term in good condition.
The investigations which were carried out on the second day of life showed mild thrombocytopenia, with normal serum folate red cell folate and homocysteine. CSF 5-MTHF level was nevertheless really low and levofolinate treatment was started at a dose of 5mg/kg/day as one intramuscular daily dose (Table 3). Genetic analysis confirmed the presence of the same mutation as in the case of his brother in homozygous state. The neurologic progress and the brain MRI findings are normal up to date.

**Table 3. Biochemical and neuroimaging findings in Patient 3**

| **Age** | **CSF 5-MTHF** | **Medication** | **Imaging** |
|---|---|---|---|
| **Day 2** | 12 nmol/L<br>ref. 72-305 | Sodium Levofolinate,<br>15mg/day, im<br>(5mg/kg/d) | |
| **1month** | 140nmol/L<br>(trough level)<br>ref. 72-305 | Sodium Levofolinate,<br>15mg/day, im | Brain MRI –normal |
| **6 months** | 30nmol/L<br>(trough level)<br>ref. 72-305 | Sodium Levofolinate,<br>15mg/day, im<br>(2mg/kg/d) | |

**Discussion**

HFM is a rare autosomal recessive condition affecting folate metabolism. So far 37 families have been described worldwide, out of which 30 have as well genetic confirmation

[4, 6, 11-13]. Our patients were found to have mutations already described in literature as pathogenic [9, 10]. HFM is a multisystemic disease, which emphasizes the major role of folate in different tissues and organs. Patients 'clinical features have been recently summarized [14]. Findings include poor feeding, faltering growth, megaloblastic anemia or even pancytopenia, diarrhea, immune dysfunction and neurological manifestations with progressive deterioration [1, 14, 15]. In our small cohort, Patient 1 and 2 had typical features of the disease, which in combination with the low 5-MTHF led to the diagnosis of HFM. The abnormal findings on brain MRI of Patient 2 included calcification of the basal ganglia. Intracranial calcifications have been reported in other patients with HFM, typically present in the cortex or basal ganglia [5, 16, 17].

There have been no formal studies assessing the different folate formulations, dosing and routes of administration that provide an evidence based regimen for optimal treatment. Most publications agree that parenteral administration of folinic acid is the only effective treatment for HFM. Even though anemia, immune dysfunction and gastrointestinal symptoms are reversible with the oral or parenteral administration of folinic acid and normalization of blood folate levels is easily achieved, it has been proven that CSF levels remain suboptimal [6, 7, 8, 10, 14, 15, 17]. Thus, the major challenge remains to achieve normal CSF 5-MTHF levels for treatment of neurological disease[6].

This paper presents for the first time evidence of the effective correction of CSF 5-MTHF levels using im levofolinate in three patients with HFM. Two out of three patients were treated initially with oral and/or parenteral folinic acid, the racemic stable form of folate, which led to correction of the systemic manifestations. Nevertheless, CSF folate levels remained unsatisfactory, below the age dependant values, which prompted us to use levofolinate, the pharmacologically active isomer of 5-formylTHF. Daily im administration of the medication led to the normalization of 5-MTHF levels in the CSF and those levels remain stable and within normal range so far. Clinical features and brain MRI findings had as well either improvement or stabilization. Initial dose in each patient was decided based on trough CSF 5-MTHF levels, and previous dose of folinic acid in combination with current knowledge that the biologic impact of the active isomer is twice that of the racemic compound at the same dose [1].

Finally, this paper describes the youngest patient diagnosed with HFM and treated on the second day of life. This patient had only mild thrombocytopenia without any additional systemic or neurological findings.

Our patients did not experience any local or systemic complications as a consequence of the im injections. However, lifelong daily im administration is challenging and development of alternative and effective route of administration would be welcomed by the families.

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