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"Therapeutic Vitamin B12 trial reversing neuroregression– Identifying the right time"

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Contribution of each author:

In patient care (establishing clinical diagnosis, planning investigations, management and follow-up) and writing the manuscript. To specify the extent of involvement.

- Dr. Hema Kandruwas involved in collecting the information for the initial draft of the manuscript, assessing and follow up of the child.
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Abstract

Background: Vitamin B12 plays a crucial role in the development of the fetal brain. Vitamin B12 deficiency manifests in infancy with varied neurodevelopmental manifestations ranging from nonspecific symptoms to neuroregression. In children with neuroregression, absence of either low serum vitamin B12 levels or macrocytosis, does not rule out a deficiency and may lead to missed diagnosis of a treatable condition with consequent severe neurodevelopmental morbidity. In this boy, the clinical findings along with low maternal vitamin b12 levels in the mother, therapeutic response and radiological recovery on follow up support the diagnosis of vitamin B12 deficiency.

Case Presentation: A 13-month-old boy who was predominantly on breastfeeds, presented with neuroregression and seizures. Initial assessment revealed pallor, skin hyperpigmentation, microcephaly, hypotonia and significant psychomotor delay. Laboratory investigations showed macrocytic anaemia, with elevated urinary methylmalonic acid levels(MMA). Serum vitamin B12 and folic acid levels were however normal in the child. MRI revealed global cerebral atrophy. On maternal screening vitamin B12 levels was significantly low.

Management and Outcome: Both the child and mother were treated withvitamin B12 and lost to follow up due to the pandemic. A review at 3 years 8 months showed significant improvement in the gross, fine motor and cognition, but persistent delay in the language domain. Repeat MRI showed reversal of atrophy and myelination appropriate for age.

Conclusion: Being a single case report, the findings of B12 treatment related reversal of neuroregression and cerebral atrophy will require confirmation with further experimental or analytical studies.

Keywords :

megaloblastic anaemia, neuroregression, vitamin B12 deficiency

Introduction:

Vitamin B12 deficiency in infants though rare can manifest with both haematological and neurological symptoms. In infants, aetiology, clinical features and radio imaging varies in comparison to adults and their presentation is mostly non-specific which can mask the diagnosis. (1) There is a high prevalence of B12 deficiency in Indians, especially among pregnant and lactating mothers ranging between 52% - 74%. (2) Adults may remain asymptomatic for many years despite vitamin B12 deficient diet due to adequate endogenous stores, whereas infants who have very limited hepatic reserves,develop symptoms within months of birth. (3)Affected infants are on exclusive breastfeeding by motherson a strict vegetarian diet, often a poor resource for vitamin B12. The involvement of the central nervous system has been poorly understood, however, demyelination, delay in myelination, impaired methylation or lactate accumulation in peripheral nerves, spinal cord and cerebrum have all been the proposed theories. (4) The affected infants who have normal developmental milestones in the initial 4-6 months of life, later present with non-specific symptoms such as irritability, progressive lethargy, feeding or weaning difficulties, refusal to accept solid foods, pallor, failure to thrive, hypotonia with hyperpigmentation of skin and lustreless scalp hair.(5)If not recognised and treated at this stage, neurodevelopmental slowing and neuro-regression sets in and symptoms are often unmasked as acute neurological deterioration associated with intercurrent infection.(5) Haematological evaluation reveals macrocytic anaemia and megaloblastic bone marrow. Metabolic screening suggests elevated serum homocysteine levels and urinary methylmalonic acid excretion which is the hallmark of the condition with serum low levels of vitaminB12. Mothers are often asymptomatic with low serum vitaminB12 levels when screened. MRI in these infants reveal white matter loss and delayed myelination. (6)secondary to severe vitamin B12 deficiency.\nMETHODS: Twenty-one infants aged 4-24 months with B12 deficiencies who were admitted to our clinic between May 2013 and May 2018 were included in the study. MRI, bone marrow aspiration and the Denver-II Developmental Screening Test were performed in all infants.\nRESULTS: The mean age of the infants was 12.3 months, and the mean B12 level was 70.15 ± 32.15 ng/L. Hypotonia and neurodevelopmental retardation, and anaemia were present in all patients. Their bone marrow examinations were compatible with megaloblastic anaemia. Twelve patients had microcephaly, seven had tremor and one patient died of severe sepsis. Almost all patients were fed with breast milk and their mothers were also malnourished. Nine (42.9% Affected infants respond dramatically to vitaminB12 therapy with rapid improvement with regain of milestones, however, long-term neuro deficits may result if the diagnosis and timely treatment is delayed. (7) We report a 13-month-old toddler with neuroregression and treatment of vitamin B12 deficiency who on follow up showed significant clinical and radiological improvement, however, language deficits persisted.

Case Presentation:

A 13 months old boy, brought with developmental regression and seizures 1 month before presentation. The child was second born tonon-consanguineous parents at term via caesarean section with a birth weight of 2.75kg. He was exclusively breastfed until 8 months of age followed by complementary dietwhich was nutritionally inadequate. Following normal development in early infancy, neuroregression was evident by loss of ability to come to sit or roll over by one year. He continued to respond verbally with the mother with differential sounds and no

loss of visual tracking. Examination revealed an underweight child with skin hyperpigmentation. Neurological examination revealed microcephaly, hypotonia, with brisk reflexes. Developmental assessment was conducted by different trained professionals initially and on follow up using Comm DEALL developmental checklist (CDDC) as mentioned (see Table 1). Ophthalmological and hearing assessment was within normal limits. Baseline investigations, (see Table 2) donesuggestive of macrocytic anaemia and mother had low serum vitamin B12 levels. Screening for metabolic disorders revealedmild hyperlactatemia, hyperammonaemia, normal serum homocysteine levels, and tandem mass spectroscopy was normal. Urineorganic acid levels showed elevated methylmalonic acid levels. Electroencephalogram (EEG) was abnormal with frontal epileptiform discharges, cranial MRI showed diffuse prominent subarachnoid spaces, fissures and cisterns, thinning of the corpus callosum, global cerebral atrophy, leukoencephalopathy with delayed myelination/ hypomyelination. (See Figure 1)

Management and Outcome:

Child was started on parenteral daily vitamin B12 250mcg given intramuscularly for 14 days as per recommendation, followed by slow taper with weekly doses for 4 weeks and fortnightly doses for a month and maintenance dose once a month with monitoringalong with additional folic acid supplements. Mother was given 500mcgvitaminB12 oral supplementationdaily for 2 months.On follow up at six weeks of initiation of therapy, significant improvement was observed with clearing of skin hyperpigmentation, improved feeding, activity and emergence of regressed milestones with improved tone.

Child was lost to follow-up due to covid pandemic. A follow up review at 3 years and 8 months of age child was symptomatically better but mother had discontinued all the medications including vitamin B12 after 2 months of initiation of therapy. In view of clinical seizures, child was started on sodium valproate by a paediatrician elsewhere. A detailed assessment revealed head circumference appropriate for age and normal development for age in gross and fine motor domain, however, delay persisted in a language domain (see Table 1). Investigations revealed a normocytic hypochromic blood picture. (see Table 2) Repeat serum B12 levels were normal. Tandem mass spectroscopy was normal and urine showed persisting elevated methylmalonic acid levels.Follow-up EEG was suggestive of epileptic encephalopathy, a repeat cranial MRI showed a reversal of cerebral cortical atrophy as compared to the previous study with normal spectroscopy. (See Figure 1) Child was discharged on home-based stimulation, speech therapy and continuation of anti-seizure medication.

The child's caregivers were advised long-term anti-epileptic medications but did not comply after a seizure free period. The child suffered a second episode of neuroregression at 5 years of age following recurrent seizures with status epilepticus and severe hypoxic encephalopathy. Neuroimaging repeated during this episode was consistent with severe hypoxic encephalopathy changes and a repeat vitamin B12 and metabolic evaluation including methylmalonic acid levels was within normal limits. Genetic evaluation was completed with whole exome sequencing and was inconclusive. Child was later on supported with intensive developmental therapy and has been regaining milestones assessed on follow up. Need for long term anti-epileptic medications with good compliance has been counselled.

Discussion:

Vitamin B12 deficiency in infants was described in the early 1960s from India by Jadhav et al, prominent findings were megaloblastic anaemia, neuroregression and skin hyperpigmentation among the few case studies reported worldwide. (8)Vitamin B12 a water-soluble complex organic compound, is essential for the production and

maintenance of RBCs and myelination of nerve cells, and plays important role in fetal brain development. Vitamin B12 deficiency can affect infants and children resulting in cognitive and intellectual problems depending upon the area of the brain affected. (9) Various theories have been proposed to explain the underlying mechanism of neurological dysfunction secondary to vitamin B12 deficiency, namely altered S-adenosylmethionine: S-adenosylhomocysteine (SAM: SAH) ratio, cytokines imbalance with increased TNF-a and decreased EGF (Epidermal growth factor) leading to loss of neural integrity that compromises brain development in infants. (10) While most adults remain asymptomatic for a longer duration, breastfeeding infants have limited hepatic reserves become symptomatic within few months after birth and peak by 4-10 months of age. Bicytopenia with anemia and thrombocytopenia is usually observed in vitamin B12 deficiency. However, treatment or supplementation of high dose vitamin B12 or an intercurrent infection can also cause thrombocytosis as observed in our child.(11) Laboratory investigations with the establishment of low serum B12 levels and elevated levels of methylmalonic acid shall be cornerstone of diagnostics, but normal levels of serum B12 or MMA do not exclude symptomatic B12 deficiency similar to our child.(12)Seizuresare reported with B12 deficiency although the exact mechanism are unknown and probably related to increased excitability of the damaged myelinated neuron to glutamate. (13)we aimed to evaluate patients with seizures who were found to have vitamin B12 deficiency and whose seizures resolved with vitamin B12 treatment., Methods: A total of 26 infants were included in this retrospective study. The patients were evaluated in terms of clinical findings, laboratory tests including homocysteine, electrophysiological studies, neuroimaging studies, and other neurological examination findings., Results: Of 26 patients, 14 (53.8%Korenkeet al reported an infant manifested as acute encephalopathy, epilepsy, microcephaly and megaloblastic anaemia at 4 months of age secondary to subclinical pernicious anaemia in mother and showed response to vitamin B12 therapy similarly.(14) Ursula et al, reported a 14-month-old,with severe vitamin B12 deficiency related symptoms who showed complete disappearance of structural abnormalities following therapy, however continued to have cognitive and language delay at the end of 2 years of age. (3) Nadia et al, reported 2 infants with vitamin B12 deficiency who showed complete recovery following therapy. (4) Cezary et al in their case study observed a 7-month-old child with megaloblastic anaemia and neurological regression, who had remarkable response in general condition and blood picture, however, continued to have long-term psychomotor disability due to a delay in diagnosis. A similar trajectory was seen in our index child, as the non-specific symptoms improved within weeks of initiation of treatment but the psychodevelopmental evaluation showed deficits in language domain. Thus, it is evident that the long-term prognosis and outcome of vitamin B-12 deficiency in children depend on the severity and duration of the deficient state. (7) There is limited data on long-term follow-up of developmental outcomes following therapy in these children. Pearson et al followed a 32-month-old child, found to have an intellectual delay at the end of 6 years of age. (7) The neuroimaging features in vitamin B12 deficiency can vary from diffuse or focal cortical atrophy, thinning of the corpus callosum, structural abnormalities and delayed myelination. (1) Asipayam et al, studied 21 hypotonic infants aged between 4- 24 months with B12 deficiency, who were predominantly breastfed, and had similar findings comparable to our index case, and all responded to therapy but lacks follow up of long-term sequelae. Among 21 children, 12 had microcephaly, and all had retardation in personal social, language, fine and gross motor abilities. MRI findings in

his study also revealed cerebral atrophy in all infants, corpus callosum thinning, frontoparietal, frontotemporal atrophy and delayed myelination in 2 infants each. (6) Neuroimaging in our child showed remarkable recovery which also correlated with clinical improvement, language deficits persist requiring therapy.

Limitation:Neurodevelopmental follow up and speech stimulation could not be done as the child was lost to follow up during the covid pandemic. Since this is a single case report with follow up, similar studies to compare the radiological recovery is limited.

Conclusion:

Early recognition of infants with vitamin B12 deficiency is critical because treatment can partially or completely reverse the neurological and radiological impairment, thus preventing the progression of irreversible neurological deficits, and this needs to be substantiated with further experimental or analytical studies.

Lessons learnt:

Vitamin B12 deficiency when recognised early and treated can prevent irreversible neurological damage.

Long term follow-up of these children is essential to identify persistent deficits requiring continued intervention.

Paediatricians need to have high suspicion and screen all breastfed infants with developmental concerns for vitamin B12 deficiency.

Figures

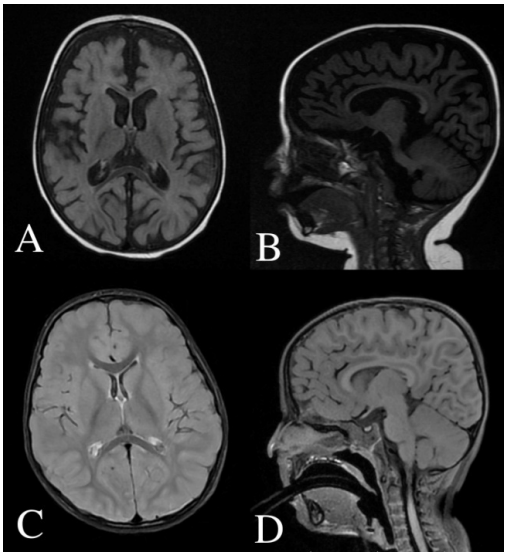

Figure-1

A- T2 Flair(axial)- Global cerebral atrophy with diffuse sulcal space widening in bilateral cerebral hemispheres with prominent subarachnoid spaces and fissures and basal citerns.

B- T1 Flair(sagittal)- Thinning of corpus callosum and myelinated.

C- T2 Flair (axial) Showing previously noted prominent cortical sulcal spaces, basal cisternal spaces appearsto be normal with reversal of cerebral cortical atrophy.

D- T1 Flair(sagittal)- Corpus callosum appears normal in size

Tables:

Table-1– Developmental profile

DOMAIN	At initial presentation* Chronological age: 13 months 6 days		On follow up* Chronological age: 3 years 8months (44m)	
	Milestones	Developmental quotient (DQ)	Milestones	Developmental quotient (DQ)
Gross Motor	Head Holding-3m Rolling over-6m Sitting with support-7m Crawling-11m Regression at 1 year Lost the ability to crawl, sitting independently and rolling over	DQ-47	Walking and running Climbs stairs and jumps Plays ball	DQ-97:
Fine Motor	Reaches out for objects-6m Transfer objects-7m Plays with toys-8m Stopped reaching objects	DQ-46	Spontaneous scribble Opensdoors, draws Plays with clay Picks thread	DQ-77
Language	Cooing - 3m Babbling - 8-9 months Stopped babbling	DQ-26	Follows simple commands Communication through pointing Jargon speech	DQ-32
Cognition	Recognizes mother Plays with rattle Stopped exploring	DQ-42	Recognizes self in photograph. Knows where things usually belong. Chooses picture books.	DQ-84

*Assessment performed using COMMDEALL developmental checklist

Table-2 - Laboratory profile

Laboratory Parameters	At initial presentation	On follow up
Hemoglobin (10.5-14g/dL)	9.2	11.4
White blood cell count, (6-14x10^3 mm^3)	11510	12110
Neutrophils (54-62%)	24%	50%
Lymphocytes (25-33%)	70%	44%
Platelet count (150-400x10^3 mm^3)	656	415
Red blood cell count (4.5-6.5x10^6 mm^3)	2.76	5.08
Hematocrit (32-42%)	30	35.9
ESR (0-10mm/hr)	9	
Mean corpuscular volume (72-88fL)	108	70
Serum Vitamin B12	313 (187-883pg/ml)	40.8 (25.1-165pmol/L)
Serum Folic acid, (3.1-20.5ng/mL)	17.6	
Plasma Ammonia (11-35 µmol/L)	136	39
Serum Homocysteine (5.46-16.2µmol/L)	9.54	
Plasma Lactate (0.5-1.6mmol/L)	5.6	1.2
Tandem Mass Spectrometry (TMS)	Normal	Normal
Urinary Methylmalonic acid levels (<3.6mmol/mol of creatinine)	8.46	24.86
Peripheral blood smear	RBC- Normocytic normochromic with oval macrocytes, target cells, tear drop cells, polychromatophils WBC- Normal in number, few hypersegmented neutrophils Platelets- Increased	RBC- Normocytic hypochromic with few polychromatophils WBC- Normal Platelets- Adequate

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