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A Baby with Nonketotic Hyperglycinaemia

HNT ***HUI,*** YC ***HO,*** HB ***CHAN,*** SCF ***TAM,*** NLS ***TANG,*** PLS ***IP***

Abstract Nonketotic hyperglycinaemia or nonketotic hyperglycinaemic encephalopathy (NKH) is an autosomal recessive inborn error of glycine metabolism. We report a baby with the disease treated with sodium benzoate, dextromethorphan and anticonvulsants who succumbed at 4 months of age.

Key words Neonatal seizure; Nonketotic hyperglycinaemia

Introduction

Nonketotic hyperglycinaemia or nonketotic hyperglycinaemic encephalopathy (NKH) is an autosomal recessive inborn error of glycine metabolism with poor prognosis. The inherited defect of the glycine cleavage system leads to accumulation of glycine in body fluids, such as plasma, urine and particularly, cerebrospinal fluid (CSF).[1] In the neonatal type, NKH generally presents with intractable seizures, hiccups, muscular hypotonia, and apnoea.[2] Elevation of glycine in the brain is thought to be responsible for these symptoms.

Department of Paediatrics and Adolescent Medicine, United Christian Hospital, 130 Hip Wo Street, Kwun Tong, Kowloon, Hong Kong, China

HNT **HUI** （許寧達） *MBChB, MRCP, MRCPCH*
YC **HO** （何鈺照） *MBChB, FHKCPaed, FHKAM(Paed)*
HB **CHAN** （陳衍標） *MBBS, FRCPCH, FHKCPaed*
PLS **IP** （葉麗嫦） *MBBS(Syd), FRCPCH, FHKCPaed*

Division of Clinical Biochemisty, Queen Mary Hospital, 102 Pokfulam Road, Hong Kong, China

SCF **TAM** （譚志輝） *MBBS, FRCPA, FHKCPath*

Department of Chemical Pathology, Prince of Wales Hospital, 30-32 Ngan Shing Street, Shatin, N.T., Hong Kong, China

NLS **TANG** （鄧亮生） *MBChB, MD*

Correspondence to: Dr HNT **HUI**

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We report a baby girl with a clinical course of the neonatal type of nonketotic hyperglycinaemia treated with sodium benzoate, dextromethorphan, anticonvulsants and supportive treatment.

Case Report

This female newborn was the second child of healthy non-consanguineous parents. The antenatal history was uneventful except that polyhydramnios was detected since 33 weeks of gestations. She was born at 38 weeks of gestation, with a birth weight of 3010 grams. The birth was a normal spontaneous delivery, with Apgar scores of 10 at 1 and 5 minutes.

Hypothermia and unstable temperature were noted on the first day of life. After initiation of feeding, increasing lethargy with poor feeding was observed at 24 hours. She became floppy with diminished response to painful stimuli. She was also noted to have a weak cry, repeated hiccups and paucity of movement. Pupil sizes were normal and reactive to light. Deep tendon reflexes were intact. No seizure was evident at that time. Sepsis and meningitis were suspected as the baby had unstable temperature and depressed sensorium. Sepsis screening including blood culture and lumbar puncture were performed. Broad-spectrum antibiotics (ampicillin and cefotaxime) were started.

However, her clinical condition continued to deteriorate on the next few days. She developed repeated apnoea,

desatuation (SaO_2 70-80% on 28% O_2) and carbon dioxide retention (70 mmHg) on day 3 of life. She was then managed with endotracheal intubation with mechanical ventilation. Non-contrast computed tomography of the brain (Figure 1) on day 4 of life showed decreased attenuation of white matter only. Electroencephalography on day 6 of life showed low amplitude slow waves compatible with encephalopathy.

Inborn errors of metabolism was suspected because of the disturbance in consciousness and negative sepsis screening results. No hypoglycaemia or metabolic acidosis was noted. The serum levels of ammonia, lactate and pyruvate were normal. Serum urate level was normal. Serum amino acid pattern analysed semi-quantitatively by thin layer chromatography did not reveal any abnormality. Urine for ketones, reducing substances were negative and urinary organic acids were of normal pattern. Urine for amino acid pattern was within normal limits. No definitive diagnosis could be made at that juncture and feeding was resumed via nasogastric tube.

She developed intractable seizures since day 11. The seizures were characterised by myoclonic jerks, rotational eye movements and arching of the back. She was treated with phenobarbitone but the seizures were poorly controlled. Another set of metabolic screening was repeated at that time but the results were unrevealing.

She was managed supportively. Her clinical condition improved slightly with more spontaneous eye opening. Respiratory effort improved and apnoea episodes disappeared. Withdrawal from ventilator support was achieved at 18 days of life. EEG on day 20 of life showed improved background activity but excessive sharp waves over both hemispheres compatible with interictal state. There was no response to pyridoxine administration. Vigabatrin was added to control the seizures but to no avail and the child's conscious state showed little further improvement. Repeat EEG showed poorly organised background with multiple sharp waves over both hemispheres. Repeated CT brain showed similar findings to the previous scan. Magnetic resonance imaging of brain (Figure 2) at 4 months of age showed marked thinning of the corpus callosum.

In view of the absence of documented metabolic derangement in association with the intractable seizure and encephalopathy starting in the neonatal period, and the conglomerate of emerging clinical features, NKH was highly suspected and quantitative amino acids analysis was

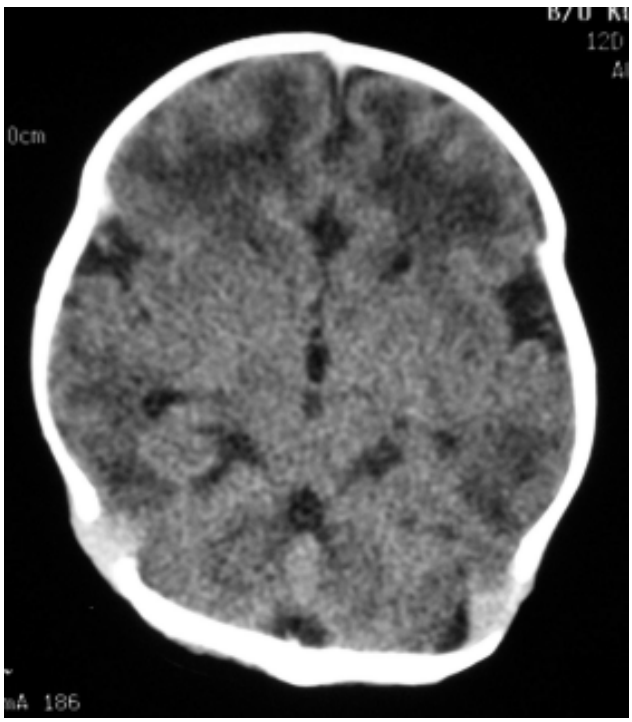

Figure 1 The white matter appeared hypodense on the pre-contrast CT brain study. The ventricular system and sulcal spaces are slightly capacious.

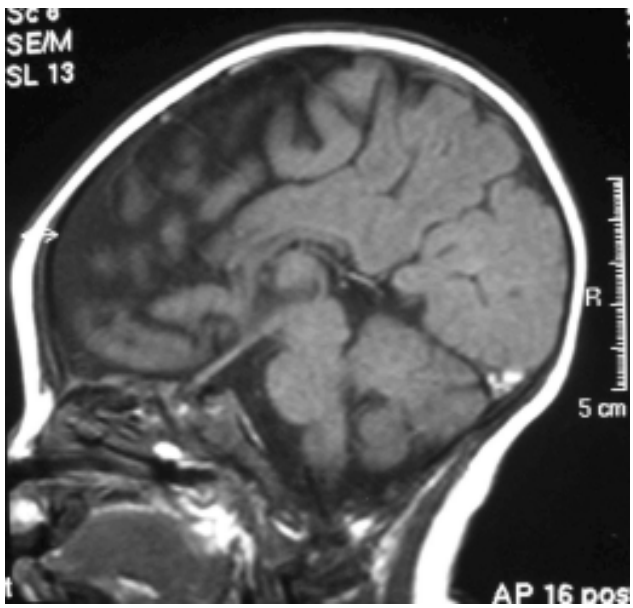

Figure 2 MRI brain showed marked thinning of the corpus callosum, particularly evident in the sagittal planes. This had involved the rostrum, body and splenium.

performed. Blood and CSF were sent for quantitative tests for glycine by reversed phase HPLC with gradient elution and spectrofluorometric detection at 6 weeks of life. A mildly elevated plasma glycine level (795 μmol/L, reference range 230-740 μmol/L) and a more markedly elevated CSF glycine level (145 μmol/L, reference range 5-10 μmol/L) were detected. The CSF-to-plasma ratio of glycine was grossly elevated to 0.18 (normal <0.04). The diagnosis of NKH was made at that juncture. The baby was then treated with sodium benzoate (500 mg/kg daily) and dextromethorphan (22.5 mg/kg daily). These medications were well tolerated by our patient.

The child's condition gradually improved in the following weeks. She had improved arousal state with more eye opening. She grimaced and cried with suctioning. Feeding improved and nasogastric tube feeding was taken off successfully. There was a decrease in both the frequency and severity of seizure. Plasma glycine level subsequently dropped to the normal range at 2 months of age and the child was then discharged home. The child had developmental progress during that period. Her neck control improved. She could respond to her mother's voice with smiling, eye opening with eye contact.

However, she was re-admitted for worsening of seizures at 3 months of age. EEG showed hypsarrhythmia. Plasma glycine level was still within the normal range and the CSF/plasma ratio dropped slightly to 0.155. She was treated with clobazam and ACTH without any improvement. Eye contact was lost and feeding was poor. Her condition deteriorated gradually and she became hypotonic and unresponsive to stimuli. She finally succumbed at 4 months of age.

Discussion

NKH is an inborn error of metabolism in which excessive amounts of glycine accumulate in body fluids leading to serious neonatal neurological disorder.

Glycine is the simplest non-essential amino acid that can be synthesised in a number of ways. It is metabolised by the glycine cleavage system, a complex enzyme system with four enzyme components.[1,3] The enzymatic defect in the cleavage system will lead to accumulation of glycine in body fluids, especially the CSF.

The major roles of glycine as a neurotransmitter lead to the neurological features of NKH. There are two neurotransmitter roles for glycine in the central nervous system, one inhibitory and one excitatory. The classic glycine receptor is inhibitory and located primarily in the spinal cord and the brain stem, producing the effects of respiratory failure, weakness and hypotonia. The second glycine receptor is excitatory and located throughout the central nervous system, including the cerebrum and cerebellum, leading to the presentation of seizures and myoclonus.

The onset of the neonatal type of nonketotic hyperglycinaemia is typically in the first few days of life, with stupor, hypotonia, seizures, multifocal myoclonus, hiccups, and finally episodes of apnoea and coma.[4] Most patients die in the neonatal period or within the first year. The few survivors have hyperreflexia, frequent hiccupping, myoclonic seizures, and severe psychomotor retardation.[5]

Because of its clinical course, the incidence of the neonatal type of NKH may be underestimated as a result of early death before the condition is being recognised. It is important that we keep in mind the possibility of inborn errors of metabolism when there is unexplained disturbance of sensorium and recurrent seizure in the early neonatal period.

The definitive diagnosis of our patient could not be made until the amino acid in CSF was analysed quantitatively for glycine level. As the screening test of amino acidopathies performed on serum and urine was only a semi-quantitative assay carried out by thin layer chromatography, abnormal pattern of amino acids would not be detected in serum or urine as the elevation in amino acid was not dramatic (plasma glycine level 795 μmol/L, reference range 230-740). It was recognised that plasma amino acids had to be increased more than double that of normal before an abnormal pattern would be detectable on thin layer chromatography. Plasma and CSF for quantitative measurement of glycine levels, which is essential for the diagnosis of NKH, should be performed at outset when the clinical suspicion of this inherited metabolic disorder is high.

Early recognition of the disease is important as early treatment may improve survival and the long-term clinical outcome.[6] Polyhydramnios may be the first symptom of NKH when CNS damage has commenced in utero and led to decreased fetal swallowing.[7] Prenatal diagnosis can be done by glycine cleavage enzyme complex assay in chorionic villus biopsies or molecular analysis in families where the mutations are known.[8]

The molecular defects in the glycine cleavage enzyme system responsible for NKH had been reported. This mitochondrial enzyme complex is composed of four

proteins encoded by four different genes, namely P-protein, H-protein, T-protein and L-protein.[8] In Caucasians, about 80% of patients had a defect in the P-protein; however, there was no molecular genetic data in Chinese NKH patients.[9] Although, molecular study could be performed in NKH, the "gold standard" for a definitive diagnosis of NKH still relies on the glycine cleavage enzyme assays performed on fresh frozen liver biopsy.[8]

The major differential diagnosis of a neonate presenting with encephalopathy but without obvious metabolic derangement in terms of acid-base disturbances include NKH, urea cycle defects, maple syrup urine disease (MSUD), pyridoxine-dependent encephalopathy, peroxisomal disorders (e.g. Zellweger syndrome), and Molybdenum cofactor deficiency. Hyperammonaemia in the absence of liver function derangement is a prominent feature in urea cycle defect that was not presented in our patient. Maple syrup urine disease is characterised by the presence of markedly elevated levels of branch-chain amino acids in plasma and ketoacids in urine that give a positive "ketostix" and a (Dinitrophenylhydrazine) DNPH test.[10] All such features were absent in our case. Pyridoxine-dependent encephalopathy is a rare disorder presenting with generalised clonic seizures shortly after birth. The seizures are particularly resistant to conventional anticonvulsants but response dramatically to the initial bolus of intravenous pyridoxine.[11] Zellweger syndrome is a rare autosomal recessive genetic disorder characterised by neonatal hypotonia and seizures, liver disease, stippling of the epiphyseal plates, renal cysts, craniofacial dysmorphisms, cataracts, impaired neurological function with psychomotor retardation.[12] Molybdenum cofactor deficiency is an inborn errors of metabolism involving cysteine catabolism. Refusal to feed, vomiting, intractable seizures and development delay develop within a few weeks after birth. The diagnosis was established by the presence of very low blood uric acid level and increased amounts of sulfite, thiosulfate, S-sulfocysteine, xanthine, and hypoxanthine in the urine.[13]

Treatment strategies of NKH include reduction of the glycine burden and the antagonism of the neurotransmitter effects of glycine (Table 1).[14]

Sodium benzoate is conjugated with glycine to form hippurate, which can be excreted in the urine. Dextromethorphan, an N-methyl-D-aspartate (NMDA) receptor antagonist, has been shown to have beneficial therapeutic effects in some patients with NKH. Hamosh et al administered sodium benzoate (at doses of 500 to 750 mg/kg/day) and dextromethorphan (at doses of 3.5 to 22.5 mg/kg/day) to 4 infants with NKH and followed them up from 3 months to 6 years. The treatment improved arousal, decreased or eliminated seizures, and allowed some developmental progress in the long term.[5,16] Treatment with dextromethorphan was associated with resolution of nystagmus and improved eye contact and interactive behavior with progression of developmental milestones without altering plasma or CSF glycine levels.[17] Treatment with strychnine and ketamine also showed some beneficial effects in anecdotal reports.[18] Other putative treatments including tryptophan and benzodiazepines could not show sustained long-term benefits.[4,5]

Our baby only showed neurological improvement for a short period of time after the initiation of treatment with sodium benzoate and dextromethorphan. As the treatment regime was only started after 6 weeks of life, by then the central nervous system probably had been severely and irreversibly damaged by the toxic effects of glycine.

Table 1 Treatment modalities of NKH[15]

Treatment	Biochemical effects	Clinical effects
Benzoate	Normalise plasma glycine level, reduce but did not normalise CSF glycine	Reduce seizure frequency
Strychnine	Competitive glycine antagonist	Improvement only in milder forms
Ketamine	Non-competitive NMDA receptor	Cessation of seizure, reappearance of swallowing and sucking, improved neurological status
Dextro-methorphan	Non-competitive NMDA receptor	Improves neurological status, cessation of seizure, EEG normalised

NMDA receptor: N-methyl-D-aspartate receptor

Conclusion

The importance of early diagnosis of nonketotic hyperglycinaemia in neonates with disturbances in consciousness cannot be overemphasised. However, the prognosis of the disease is still grave despite the putative treatments with sodium benzoate and dextromethorphan. It is important to recongnise the diagnosis early, which clinch on a high index of suspicion, as genetic counselling and prenatal diagnosis can be offered at the subsequent pregnancy.

Key Messages

1. Nonketotic hyperglycinaemia is a major differential diagnosis in neonates with disturbances in consciousness with seizures in the absence of distinct acid-base disturbances. Making the correct diagnosis is important because genetic counselling and prenatal diagnosis can be offered at the subsequent pregnancy.
2. Routine metabolic screening may not be able to detect a mild increase in amino acid if it is performed by a semi-quantitative method. Plasma and especially CSF samples are required for quantitative measurements if clinically warranted.

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