# NONKETOTIC HYPERGLYCINEMIA IN TWO SIBLINGS WITH NEONATAL SEIZURES

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**Abstract.** Seizures are a common problem in neonates. Differential diagnoses include infection, trauma, hypoxia and congenital metabolic disorders. Among these, congenital metabolic disorder is less familiar to general pediatricians. We report two patients with nonketotic hyperglycinemia (NKH), a rare and lethal congenital metabolic disease. Transient hyperammonemia and transient hypouricemia, uncommon features found in NKH, were detected in one patient. High doses of sodium benzoate and dextromethorphan failed to modify the clinical course. Neuropathology denoted characteristic diffuse vacuolization and changes in reactive and gliotic astrocytes. The clinical course, biochemical findings, diagnostic approaches and diagnostic tests are discussed in detail. Recent modalities of treatment are reviewed. Because of its rarity and rapidly progressive course, it maybe underdiagnosed resulting in death before being recognized. Awareness of the possibility of congenital metabolic disorder in early neonatal catastrophe will increase the diagnostic rate.

## INTRODUCTION

Neonatal seizure is a common condition in general practice. Etiologies are heterogeneous, including structural malformation of the brain, electrolyte disturbances, CNS infection, hypoxic insult, and a number of hereditary metabolic diseases (Lyon *et al,* 1996). Prompt recognition is essential to successful medical intervention. It is important to establish a correct diagnosis, as therapies and prognoses are dramatically different. Diagnostic failure may lead to death, or to life-long impairment of cognition and motor development.

Nonketotic hyperglycinemia (NKH) is a rare genetic disease, inherited as an autosomal recessive trait. Tada *et al* (1969) first described a primary defect in the glycine degradation system (glycine cleavage system), which is confined to the mitochondria. As a result, glycine is accumulated in large amounts in body fluids, especially in the brain, leading to NKH (Hamosh and Johnston, 2001). Clinically, NKH is classified into three subtypes: neonatal onset, late onset, and transient form (Tada 1987; Tada *et al,* 1992).

Most patients have the neonatal phenotype, presenting in the first few days of life, with lethargy, hypotonia and myoclonic jerks, progressing to respiratory arrest and often death. For those who survive, intractable seizure and severe psychomotor retardation result.

Only a few patients have been described with transient NKH. Biochemically and clinically, these patients are indistinguishable from patients with neonatal NKH. By two to eight weeks of age, their glycine levels return to normal. Four out of five have no apparent neurologic sequelae. The transient nature is believed to be related to the immaturity of the glycine system in both the liver and the brain. Recurrence has not been reported (Tada, 1987; Tada *et al,* 1992; Homosh and Johnston, 2001). Pyridoxine-dependent seizures associated with transient NKH have been reported (Maeda *et*

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*al,* 2000). Correct diagnosis and treatment of pyridoxine-dependent seizures are essential for a dramatically different neurologic outcome.

In this report, we describe two brothers with classic NKH. One patient was treated with sodium benzoate, dextromethorphan and supportive therapies. Progressive neurological devastation was inevitable. The clinical approach, recent progress and management are reviewed.

## Case 1

PM was a 14 day-old male neonate, born at 33 weeks gestation, to a G2P1, 32 year-old woman, by cesarean section for fetal distress. Apgar scores were 7 and 8 at 1 and 5 minutes, respectively. Body weight was 2,180 g and head circumference 32 cm.

Family history revealed a non-consanguineous couple of Thai descendant. The father was aged 32 and the mother 31. The couple's first child had seizures as a neonate, and died of aspiration pneumonia at age three weeks.

Four hours after birth, grunting and subcostal retraction was observed. Generalized myoclonic seizures and hiccups occurred suddenly, necessitating intubation of an endotracheal tube and mechanical ventilation. Initial investigations included normal blood glucose 72 mg/dl, Ca 8.9 mg/dl, Na 137 mmol/l, K 4.4 mmol/l, Cl 104 mmol/l, $CO_2$ 14 mmol/l, serum AST 41 U/dl, serum ALT 11 U/dl, and normal complete blood count. Ammonia level was 359 µmol/l with a lactate level of 1.3 mmol/l. Urinalysis was normal with ketone absent. Lumbar puncture revealed clear CSF, RBC 4/mm³, WBC 4/mm³, glucose 66 mg/dl, and protein 78 mg/dl. Subsequently, blood and CSF cultures were reported negative for organisms. A combination of phenobarbital and phenytoin, and high dose (100 mg) of vitamin B6 failed to control his seizures. Symptomatic therapy included anticonvulsant drugs, intravenous fluid infusion and respiratory support.

On day 4, PM was referred to our hospital. Physical examination revealed a nondysmorphic infant with non-bulging ante-rior fontanel, 3x3 cm. Chest, abdominal and genital and extremity examinations were within normal limits. Decreased muscle tone was believed secondary to deep sedation. The repeat biochemical profile showed normal levels of electrolytes, Na 141, K 4.7, Cl 109, $CO_2$ 24, and blood glucose 137 mg/dl. Other findings were $NH_3$ levels of 23 and 30 µmol/l (normal range 9-33), lactate level 1.5 mmol/l (normal range 0.5-2.0). Initial uric acid level was low at 1.6 mg/dl, and a subsequent level was normal at 3.5 mg/dl.

EEG revealed a diffused low-voltaged background. Burst suppression pattern was not observed. Computed tomography of the brain revealed a diffuse low density of white matter in the cerebral hemisphere and cerebellum, pachygyria at both frontal lobes, and ventricles normal in appearance.

Given a classic pattern of intractable myoclonic seizure, with hiccuping and apneic episodes, normoglycemia and normal acid base balance, nonketotic hyperglycinemia (NKH) was strongly suspected. To determine a definite diagnosis of NKH, simultaneous samples of CSF and plasma were obtained (Cohen *et al,* 1989; Tanphaichitr *et al,* 2000). CSF glycine and plasma glycine levels were 125 (normal range 5 ± 2) and 1,500 µmol/l (normal range 120-560), giving a CSF-plasma glycine ratio value of 0.082, which was diagnostic for NKH.

Dextromethorphan (35 mg/kg/day) divided into four doses per day, and sodium benzoate (250 mg/kg/day) were given as an adjunct therapy to phenobarbital, phenytoin, and benzodiazepines. Despite the treatment, the myoclonic jerk continued. Hiccuping became more frequent and strong, until the 23rd day of life, when PM was mostly in an apneic condition, during a few days later.

An autopsy was performed that revealed changes in the respiratory system secondary to chronic assisted ventilation. The brain was grossly unremarkable. Histologic examination could not confirm the presence of pachygyria as seen in antemortem CT findings. However, extensive tissue rarefaction, vacuolization and

an increase in both reactive and gliotic astro-cytes were found in the white matter, espe-cially the deep white matter of all regions. In addition, microcalcifications were noted in some areas of the white matter.

## Case 2

SM was an older sibing of PM. He was described as a former full-term, normal-ap-pearing male infant. SM was born with a birth weight of 3,400 g and normal Apgar scores of 9, 10 at 1, 5 minutes. At 3 hours of life, myoclonic seizures developed. Basic investi-gations including serum Na, Ca, Mg, P, and blood glucose levels were reportedly normal. Spinal tap for CSF cell counts, protein and sugar levels were within normal ranges. Sei-zures continued and did not respond to any kind of anti-epileptic treatment. CT scan of the brain revealed delayed myelination for age with no structural abnormality. At 23 days, SM died of uncontrolled seizures and complicated pneumonia. The etiology of the seizures was not established at that time. Autopsy was not attempted.

## DISCUSSION

Two infants with myoclonic seizures at 3 and 4 hours of life were characterized. In patient 1, the diagnosis of NKH was initially made by clinical recognition of seizure pattern, comprising of myoclonic jerk, frequent and severe hiccuping and episodes of apnea . The supporting evidence was a CSF-plasma gly-cine ratio greater than 0.08, diagnostic for NKH (Tada and Kure, 1993; Hamosh and Johnston, 2001). The diagnosis in patient 2 was made retrospectively, based on clinical grounds and being a sibling of patient 1. Both neonates were extremely severe NKH cases. Although measuring the activity of the glycine cleavage complex in frozen liver tissue is rec-ommended, it was not performed in our cases due to lack of feasibility (Kure *et al,* 1998).

From the clinical point of view, other differential diagnoses of congenital metabolic disorders should be mentioned. Given the ele-vation in ammonia level in patient 1, a primary defect of the urea cycle and transient hyperammonemia associated with prematurity should be warranted. The high ammonia con-centration spontaneously subsided, suggesting a transient elevation of ammonia level. The present patient, together with those of previous reports, amount to at least five patients with NKH and transient hyperammonemia (Shiffmann *et al,* 1992; Lu *et al,* 1999). Whether or not a correlation between hyperammonemia and NKH exists, or it was an incidental finding, remains to be determined.

Due to the low uric acid level, molybde-num cofactor deficiency, another distinct dis-order causing neonatal seizure, was also con-cerned. This condition was ruled out by a subsequent normalization of uric acid level. Two high doses of vitamin B6 failed to over-come epileptic activities, making the diagnosis of pyridoxine-dependent seizures unlikely.

In NKH, glycine cleavage activity is decreased in liver and brain tissues; in ketotic hyperglycinemia, only activity in hepatic tis-sues is affected as a result of the secondary suppression of abnormal metabolite in patients with organic acidemia. Thus the absence of ketoacidosis and the normal profile of organic acid in urine is supporting evidence of NKH. Transient elevation of plasma glycine, but normal CSF glycine, can be found in patients receiv-ing anticonvulsant valproate (Belkinsopp and DuPont, 1997). A possible explanation is that fatty acid dipropylactate inhibits hepatic gly-cine metabolism (Belkinsopp and DuPont, 1977). The diagnosis of NKH cannot be established in the presence of valproate therapy (Belkinsopp and DuPont, 1977).

The onset of symptoms in NKH usually occurs between six hours to eight days, with 66% of patients being symptomatic by 48 hours (Lyon *et al,* 1996; Hamosh and Johnston, 2001). Lethargy and profound hypotonia and feeding difficulties are the first signs. Most patients have normal to increased DTR. As the course of the illness progresses, they develop myo-clonic jerks, apneic episodes and hiccups. Most infants require assisted ventilation in the first

weeks of life. Approximately 30% of cases die in the neonatal period. Those who survive usually regain spontaneous respiration by three weeks of age and can live for several months. Untreated patients develop refractory seizure, usually after three months of age (Tada and Kure, 1993).

In the first two weeks of life, the EEG is characterized by a burst-suppression pattern. However, this pattern is not diagnostic, but it is highly suggestive. On the other hand, as seen in our patients, absence of the typical EEG can not exclude NKH. Neuroimaging has usually revealed nonspecific progressive atrophy and delayed myelination (Hamosh and Johnston, 2001). Acute hydrocephalus, a megacisterna magna or posterior fossa cyst in MRI, were also described (Van Hove *et al,* 2000).

Glycine is a neurotransmitter. It has both inhibitory and exhibitory effects. Its inhibitory role in the spinal cord and brain stem is probably responsible for the apnea and hiccuping seen in this disorder. Glycine has an excitatory role in the cortex at N-methyl-D-aspartate (NMDA) receptor of glutamine, widely distributed in the brain (Ohya *et al,* 1991). This action may explain the intractable seizures and the brain damage in this condition.

There is evidence that glycine toxicity is of prenatal onset. Mothers of infants with NKH frequently report abnormal fetal movements, which are reported as persistent intrauterine hiccups. Dysgenesis of the corpus callosum frequently found in CT or autopsy (corpus callosum develops between 11 and 20 weeks of gestation) suggest early *in utero* insult and may indicate difficulty in treating this condition. In addition, diffuse vacuolization and the pathological changes of the white matter seen in patient 1 are similar to those previously described in cases of nonketotic hyperglycinemia (Shuman *et al,* 1978; Kaluza *et al,* 1999).

No effective therapy exists, but several experimental therapies directed at decreasing the glycine concentration and blocking its effect at the NMDA receptor are under investigation.

These include NMDA antagonists: ketamine, tryptophan and dextromethorphan. Sodium benzoate could lower glycine levels by being conjugated with glycine to form hippurate, which is excreted in urine (Wolff *et al,* 1986; Shiffmann *et al,* 1992; Hamosh and Johnston, 2001). Some success has been observed in decreasing seizures and improvement of consciousness, spontaneous breathing, muscle tone and feeding, but the neurological impairment is irreversible (Tada *et al,* 1992; Lyon *et al,* 1996; Hamosh and Johnston, 2001). Neuberger *et al* (2000) reported a mild atypical NKH in a 6-month-old girl who experienced seizure with continuously progressing psychomotor development after initiation of sodium benzoate and dextromethorphan treatment.

The glycine cleavage system is a group of complex enzymes composed of 4 sub-units: P- protein (periodical phosphate-dependent glycine decarboxylase), H-protein (a lipoic acid-containing protein), T-protein (a tetrahydrofolate-requiring enzyme), L-protein (lipoamide dehydrogenase) (Kikuchi, 1973). The gene for the human P protein maps to chromosome 9p13, and consists of 25 exons, encoding a protein of 1,020 amino acids. Several point mutations and frame shift mutations have been identified. The majority of Finnish patient has a common mutation G to T substitution, causing a serine to isoleucine change at codon 564 (Kure *et al,* 1992). Overall, more than 80% of patients had defects in the P-protein, and 15% in the T-protein, and a few families had defects in the H-protein (Tada and Kure, 1993; Kure *et al,* 1998). L-protein mutation has not been identified in NKH patients.

Prenatal diagnosis is possible by measuring glycine cleavage activity by chorionic villus sampling at 8-16 weeks' gestation (Hayasaka *et al,* 1990; Kure *et al,* 1992; Toone *et al,* 1992). Enzyme analysis is applicable to both P-protein and T-protein deficiencies. However, it is difficult to perform and may occasionally give a borderline result (Toone *et al,* 1994). There is an approximately 1% chance of a pregnancy with a normal CVS activity resulting in an affected child, due to a grey zone

in enzyme values (Applegarth *et al,* 2000). Amniocytes do not have glycine cleavage system activity, so they can not be used for prenatal enzyme analysis (Hayasaka *et al,* 1990). Measurements of amniotic fluid glycine and serine ratios were used in the past, but there is a significant overlap of values between affected fetuses and controls, so this method is discouraged (Mesavage *et al,* 1983). DNA diagnosis is feasible in families where the specific mutation is known (Tada and Kure, 1993; Kure *et al,* 1999a). An advantage of DNA-based diagnosis becomes more important when enzyme activity testing becomes problematic with ambiguous results (Kure *et al,* 1999b).

## ACKNOWLEDGEMENTS

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