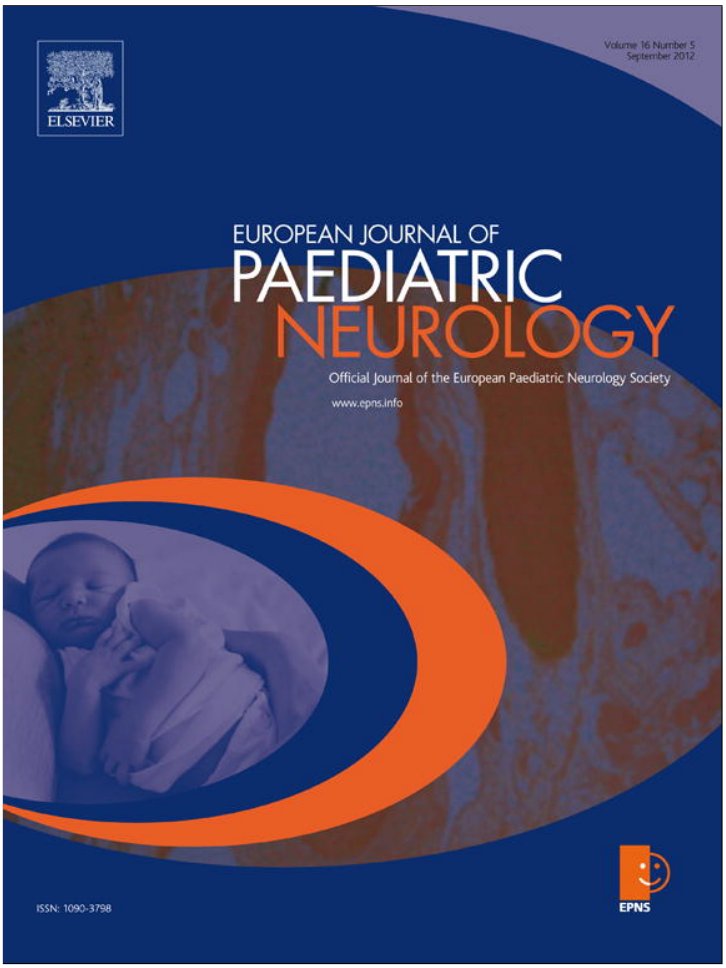

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Original article

Ketogenic diet in early myoclonic encephalopathy due to non ketotic hyperglycinemia

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ABSTRACT

Non ketotic hyperglycinemia is a rare inborn error of glycine metabolism due to deficient activity of glycine cleavage system, a multienzymatic complex consisting of four protein subunits: the P-protein, the H-protein, the T-protein and the L-protein. The neonatal form of non ketotic hyperglycinemia presents in the first days of life with encephalopathy, seizures, multifocal myoclonus and characteristic "hiccups". Rapid progression may lead to intractable seizures, coma and respiratory failure requiring mechanical ventilation. Clinical trial with scavenges drugs decreasing glycine levels such as sodium benzoate, and with drugs reducing NMDA receptors excitatory properties, such as ketamine and dextromethorphan, have been tried but the outcome is usually poor; antiepileptic therapy, moreover, is unable to control epileptic seizures. Ketogenic diet has been successfully tried for refractory epilepsy in pediatric patients. We report three cases affected by neonatal non ketotic hyperglycinemia and early myoclonic encephalopathy treated with ketogenic diet. In our patients ketogenic diet, in association with standard pharmacological therapy, determined dramatic reduction of seizures and improved quality of life.

1. Introduction

Non Ketotic Hyperglycinemia (NKH) is a rare inborn error of glycine metabolism due to a deficiency of the glycine cleavage system, a mithocondrial enzyme complex made up of four proteins encoded on four different chromosomes. In particular they are the P-protein pyridoxal phosphate containing, glycine decarboxylase, (GLDC), H-protein, that is a hydrogen carrier protein (lipoic acid containing, codified by GCSH), T-protein (tetrahydrofolate-requiring, aminomethyltranferase, AMT), and L-protein (lipoamide dehydrogenase).[1] Most patients (>80%) exhibit defects at level of GLDC, whereas up to 15% show defect of AMT gene.[2,3] Rare cases are caused by H-protein deficiency[4]; it does seem that L-protein deficiency must be a very rare cause of NKH, but it should always be considered when an NKH patient also has increased branched-chain amino acids

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and pyruvate.[1] The neonatal form of NKH is characterized by the appearance, in the very first days of life, of lethargy, myoclonic jerks and characteristic "hiccups". Rapid progression may lead to intractable seizures, coma and respiratory failure requiring mechanical ventilation. The outcome is usually poor, some patients dying during the newborn period; survivors usually exhibit severe mental retardation and intractable seizures.[5] Deficient glycine cleavage system activity leads to accumulation in central nervous system of large amounts of glycine, that allosterically activate N-methyl-D-aspartate (NMDA) receptors of hippocampus, cerebral cortex, olfactory bulb and cerebellum, with an excitotoxic effect, causing intractable seizures. Conversely, in brainstem and spinal cord the accumulation of large amounts of glycine produces an inhibitory effect, causing apnea, hiccups and diffuse hypotonia. The neurologic damage associated with NKH is mostly attributed to NMDA receptor overstimulation.[5] NKH is diagnosed on the basis of elevated levels of glycine in blood, urine, and cerebrospinal fluid (CSF), after the exclusion of organic aciduria. In classical neonatal NKH, a glycine index (CSF/plasma glycine ratio) of more than 0.08 is considered diagnostic.[1] There is some clinical report on the use of scavenges drugs decreasing glycine levels such as high-dose sodium benzoate, or with drugs reducing NMDA receptors excitatory properties, such as ketamine and dextromethorphan, but the outcome didn't show any significant improvement, and even when treatment is started early it remains unsatisfactory, with the disease following its natural course and seizures presenting severe drug-resistance.[5–8]There is a single case report signaling a benefit in seizures and clinical pattern with ketamine and dextromethorphan, but up to date no treatments have been proven to prevent neurologic sequelae.[5] Furthermore, antiepileptic therapy is usually unable to control epileptic seizures.

Ketogenic diet (KD) has been successfully tried for refractory seizures in pediatric patients suffering from different epileptic syndromes; it mimics the biochemical response to starvation when ketone bodies rather than glucose, become the main fuel for the brain energy demand.[9] It is considered the therapy of choice for few specific pathologies, and it should be taken into consideration in any case of childhood epilepsy with severe drug resistance, even if its exact mechanism of action on epileptic seizures is still poorly understood.[10]

The aim of this paper is to describe the clinical and neurological outcome of three children affected by early myoclonic encephalopathy due to NKH, and whose seizures demonstrated dramatic responses to KD.

2. Patients and methods

From the inpatient of the metabolic unit of "Bambino Gesù" Children's Hospital in Rome we selected three consecutive children diagnosed with neonatal NKH on the basis of elevated cerebrospinal fluid (CSF) glycine/serum glycine ratio (>0.08). Parents' informed consent was requested before initiating the KD. According to the recent Italian Consensus on KD,[10] all the chidren underwent pediatric, neurologic and nutritional consults before the introduction of this regimen; furthermore they all have been screened for disorders of fatty acid transport and oxidation. They all underwent polygraphic video-EEG recordings, both during wakefulness and sleep, and magnetic resonance brain study. Concentration of glycine in CSF and plasma have been assessed before and two months after initiation of KD. In the baseline period, one month before KD's introduction, antiepileptic drugs (AEDs) and standard therapy remained unchanged. None of our patients has been treated with Sodium Valproate. Children were generally started on a 4:1 ketogenic diet as ketocal® formula alone or supporting about the 80% of the daily caloric amount. Children poorly complying with ketocal® milk were shifted to a classic 4:1 ketogenic diet. The children were fasted in the hospital for 24 h until 4 + ketonuria was obtained. Urine ketones and glucose blood levels were checked and daily recorded by parents at home using ketostix and dextrostix strips. Clinical examinations, nutritional assessment, laboratory exams, awake and sleep video-EEG recordings were performed every month, or earlier if necessary. Seizure frequency was documented by parental seizures' diaries. The efficacy of the diet was assessed after 1, 3, 6 and 9 months. Two patients underwent molecular analysis for GLDC, AMT and GCSH genes.

2.1. *Patients case reports*

Case n° 1 is a female born at term by healthy Italian non-consaguineous parents. At birth she was hypotonic and lethargic. At 2 days of age she presented myoclonic seizures and epileptic tonic spasms. Video-EEG recording was performed in neonatal intensive care unit in the same day, demonstrating a suppression-burst pattern. Metabolic investigations showed increased plasma glycine levels (1572 μmol/l), with a liquoral glycine value of 286 μmol/l and a CSF/plasma ratio of 0.18, leading to the diagnosis of NKH. Epileptic seizures appeared to be resistant to i.v. administration of midazolam, phenobarbitone and phenytoin (see Fig. 1). Brain MRI showed corpus callosum hypoplasia and brain spectroscopy documented an increase in glycine peak at 3.56 ppm. At five days of age she started standard therapy with sodium benzoate (500 mg/kg/day) and dextromethorphan (3.5 mg/kg/day), with mild decrease of glycine level after 15 days, and a mild increase of CSF/plasma glycine ratio. Despite these changes in glycine levels and the administration of several AEDs (levetiracetam, carbamazepine, clobazam), EEG continued to show a suppression-burst pattern and the child continued to present multidaily seizures, above all myoclonic jerks and tonic epileptic spasms. Later, molecular analysis confirmed the clinical diagnosis of NKH, showing a homozygous mutation on GLDC gene (c.2175 > G (p.Tyr725X). When she was 4 months of age, we decided to add KD to standard and antiepileptic therapy, obtaining a dramatic seizures frequency reduction already in the first week, even allowing the child to be discharged from hospital one week later. At 10 months of follow-up a reduction in seizure frequency >50% was maintained, and glycine levels showed a further decrease (see Fig. 2). The child also appeared more alert. Her neurological conditions, however, remained deteriorated, with the presence of a spastic tetraparesis and a complete inability to walk and speak. KD appeared to be well tolerated with good dietary compliance and in absence of relevant adverse events.

Case n° 2 is a male, born at term by healthy Italian non-consaguineous parents. At birth he was hypotonic, areactive, apneic and unable to swallow. In his second day of life he

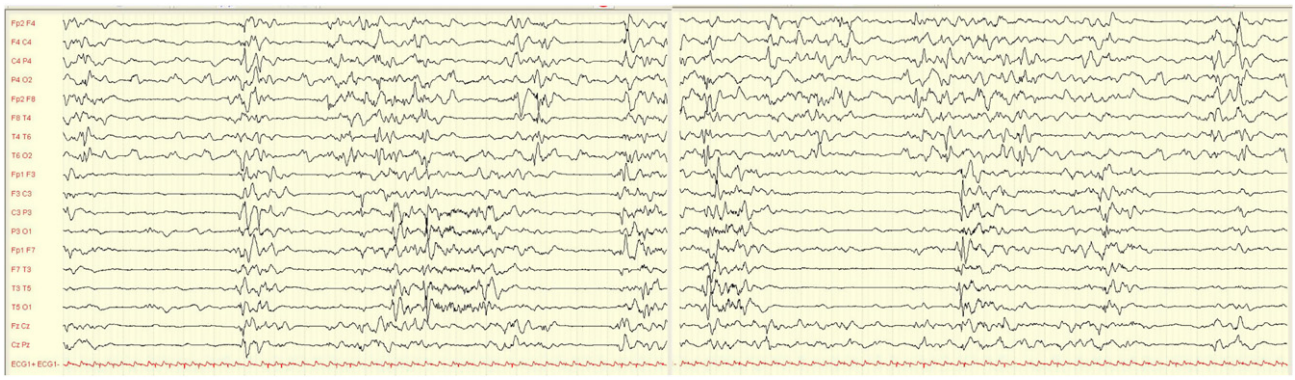

Fig. 1 – Sleep EEG of patient 1 before intiating KD, showing asynchronous suppression-burst pattern.

was admitted to the neonatal intensive care unit because of myoclonic jerks and infantile spasms. EEG showed a suppression burst pattern, and brain MRI revealed a corpus callosum agenesia, and brain spectroscopy documented an increase in glycine peak at 3.6 ppm. Metabolic investigation revealed a CSF/plasma glycine ratio of 0.27, thus allowing the diagnosis of NKH. Anticonvulsant drugs such as phenobarbital, midazolam, and phenytoin didn't show any efficacy in seizures control. At 15 days of life standard therapy was introduced (sodium benzoate 500 mg/kg/day and dextrometorphan 3.5 mg/kg/day), in addition to phenobarbital and levetiracetam, without any apparent clinical or neurological benefit, even if a slow change in glycine levels and CSF/plasma ratio was observed. Despite the increase of dextromethorphan up to 10 mg/kg/day there was only a slight and temporary efficacy on seizures frequency. The child's disease seemed to be progressive, with severe hypotonia, drug-resistant myoclonic seizures and epileptic spasms presenting many times per day, with a lack in the acquisition of the developmental milestones. At 3 months of age we decided to start KD, and 10 days after seizure frequency appeared to be reduced of >50%. At our last follow-up he was 12 months old, seizures reduction was confirmed, but brief daily focal motor seizures and tonic epileptic spasms persisted. A severe psychomotor delay was present, with no autonomous walking and significant dystonia. KD appeared to be well tolerated with no significant adverse events.

Case n° 3 is a male born at term by healthy Italian non-consanguineous parents. Since birth he showed severe hypotonia, apnea, lethargy, myoclonic jerks and tonic spasms. EEG documented a suppression burst pattern. Seizures proved to be resistant to all the commonest AEDs, also including topiramate, clonazepam, levetiracetam, phenobarbitone and midazolam, and when he was 4 months of age he was referred to our hospital presenting a status epilepticus with migrating

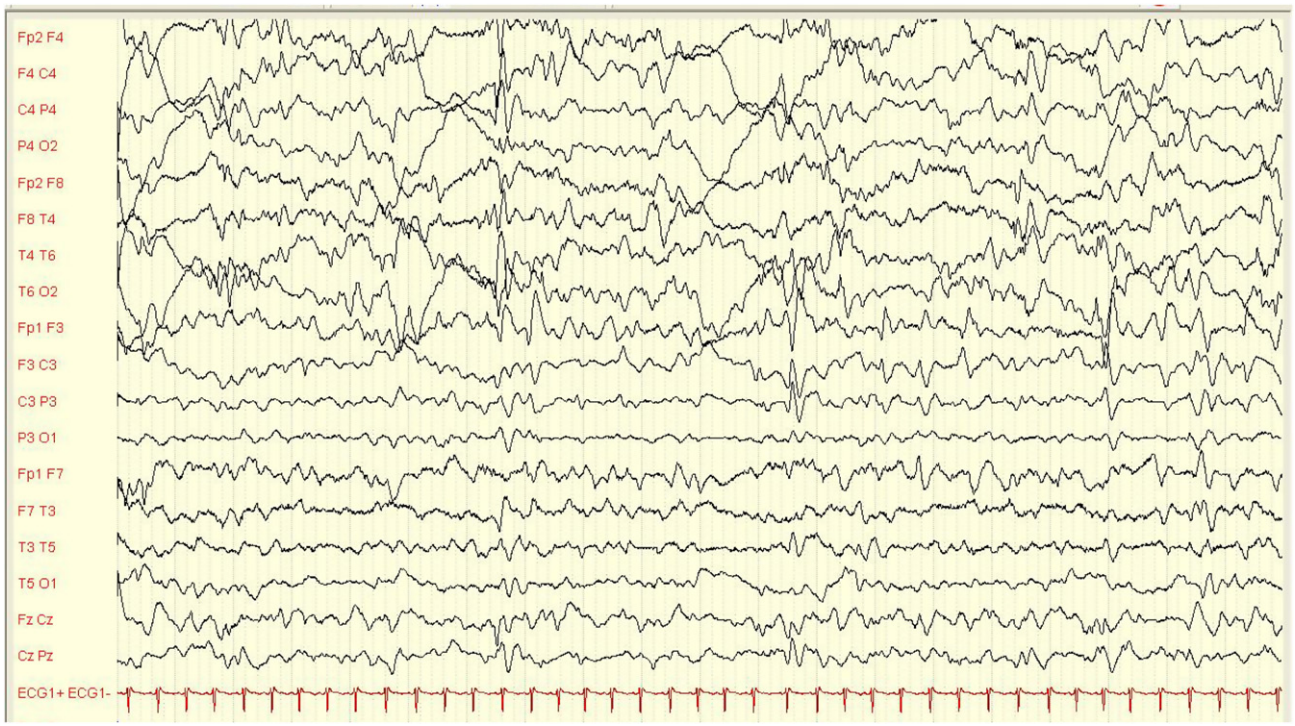

Fig. 2 – Sleep EEG during KD showing abnormal diffuse background activity with multifocal spikes and slow waves.

partial seizures, myoclonic jerks and epileptic spasms. EEG showed an asynchronous suppression burst pattern. CSF/plasma glycine ratio was 0.37, thus allowing the diagnosis of NKH. A later molecular analysis showed homozygous mutatiot in exon 8 of ATM gene (p.Arg320His). His brain MRI showed corpus callosum hypoplasia, and brain spectroscopy revealed an increase in the glycine peak at 3.56 ppm. Sodium benzoate (500 mg/kg/day) was added to phenobarbitone and clobazam without efficacy; one week later we introduced in combination dextrometorphan (3.5 mg/kg/day), thus performing the standard therapy, observing only a mild seizures reduction; EEG remained unchanged. At 6 months of age, we started KD in association with standard therapy and AEDs. One-month later seizure frequency was reduced of more than 50%, and this improvement persisted through the 9 months of follow-up, also glycine levels seemed to lower after KD was started. During his last visit he underwent a new EEG recording, which showed a continuous pattern of delta and theta activity with multifocal spikes. Dystonia appeared to be severe, the child never learned to walk nor to speak. He didn't experience any significant adverse event due to KD, and the compliance to the dietary regimen was always good.

Glycine levels in plasma and CSF at baseline, during standard therapy and two months after the beginning of KD are summarized in Table 1.

3. Discussion

Early myoclonic encephalopathy is characterized by erratic and massive myoclonus, focal motor seizures and tonic spasms presenting in the neonatal period. It can be associated with some metabolic disorders, including NKH. In our patients seizures were represented by focal or fragmentary erratic myoclonus, focal motor seizures, massive myoclonus, and repetitive tonic spasms, with a very high frequency, tending to assume a subcontinuous trend.

The suppression-burst pattern revealed by EEG recordings was frequently associated with the occurrence of seizures.

Treatments for neonatal NKH remain unsatisfactory, even when initiated early[11,12]; prospective treatment with oral sodium benzoate and the N-methyl-D-aspartate receptors antagonist ketamine and dextromethorphan can favorably modify the early neonatal course of severe NKH, but they do not prevent poor long-term outcomes, suggesting a role for glycine inducing prenatal injury and ongoing postnatal damage.[7,13]

KD is a well-known non pharmacologic treatment for childhood epilepsy not amenable to drugs[14,15]; it is considered the therapy of choice for some epilepsies secondary to metabolic diseases, such as glucose transporter protein 1 (GLUT-1) deficiency syndrome, and pyruvate dehydrogenase deficiency.[16–19] However, it should be strongly considered in children who failed two or three AEDs, regardless of age.[10] Single reports mention the clinical benefits of the diet in metabolic diseases such as phosphofructokinase deficiency, glycogenosis type V, and mitochondrial respiratory chain complex disorders.[20–22]

The mechanism of KD action against epileptic seizures and epileptic syndrome, however, still remains unknown, despite decades of researches.[23] The primary goal of KD therapy is achieving ketosis, with ketone bodies modulating the neurotoxic cascade triggered by NMDA receptor activation.[24,25] Furthermore, β-hydroxybutyrate, one ketone body, may increase concentration of GABA, the major cerebral inhibitory neurotransmitter.[5] Moreover, KD decreases reactive oxygen species production by brain mitochondria, thus preventing oxidative injury, and it has been observed an increased density of mitochondria in the hippocampus with subsequent enhancement in brain energy reserve.[26,27] An indirect confirmation of mitochondrial dysfunction in NKH comes from a recent *in vitro* experimental model of glycine toxicity on the young rat brain, showing that glycine severely impairs brain bioenergetics reducing mitochondrial respiratory chain activity.[28]In a recent study performed on animal models affected by succinic semialdehyde dehydrogenase deficiency it has been demonstrated that KD can be a successful treatment, leading to the hypothesis that KD could increase mitochonrdria's number and restore hippocampal ATP.[29] This positive effect on mitochondria number and function may play a role in KD efficacy on NKH, but there are no sufficient data to definitely support this hypothesis.

All these data suggest that ketone bodies, and in particular β-hydroxybutyrate could exert a beneficial effect in pharmacoresistant epilepsies through an indirect neuroprotective effect rather than a direct anticonvulsivant mechanism, as it is also suggested by a recent animal model of chronic ketosis.[30] From this point of view, KD could be efficacious for the treatment of seizures associated with metabolic abnormalities, such as in NKH. Therefore, in our three patients, the association of KD with the standard therapy, determined a dramatic effect on seizures

Table 1 – Clinical and biochemical characteristics of the three patients before and after the introduction of ketogenic diet.

Pt and gender	Age at test	Treatment	CSF glycine (μmol/l)	Plasma glycine (μmol/l)	CSF/plasma ratio
1, F	2 d	No therapy	286	1572	0.18
	45 d	ST + PB + CLB	257	907	0.28
	6 m	ST + KD + PB + CLB	92	238	0.38
2, M	6 d	No therapy	251	919	0.27
	38 d	ST + PB + LEV + CLB	220	753	0.29
	5 m	KD + vs	115	298	0.38
3, M	4 m	PB + MDZ + LEV	102	974	0.10
	4.5 m	ST + PB + CBZ	96	867	0.11
	7 m	KD + ST + PB + CLB	38	141	0.27

d → days; m → months; SD → Standard Therapy; PB → phenobarbitale; CLB → clobazam; KD → Ketogenic Diet; LEV → Levetiracetam; MDZ → Midazolam.

reduction, both in frequency and severity, and also ameliorated alertness.

In our patients we observed during ketogenic diet a decrease of plasma and CSF glycine levels, but we don't still know the exact mechanism underlying these changes. All types of seizures such as myoclonic jerks, tonic spasms and partial seizures decreased a few days after KD was started. Seizures reduction was maintained during the entire follow-up. All three children, however, continued to present a very severe neurological phenotype, characterized by spastic teraparesis and severe psychomotor delay. This may be due to the brain damage becoming irreversible in the very first few days from birth.[11] KD was well tolerated with no significant adverse events.

Our preliminary data suggest that KD in combination with standard therapy could be considered as a valuable therapeutic option aiming to determine a reduction in seizure frequency and severity, with an improvement in patients' and families' quality of life. Larger studies are needed to better analyze the possible antiepileptic role of KD in early myoclonic encephalopathy associated with neonatal NKH.

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