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NONKETOTIC HYPERGLYCINEMIA – CASE REPORT

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REZUMAT

HIPERGLICINEMIA NONCETOTICĂ – RAPORTARE DE CAZ CLINIC

Introducere: Hiperglicinemia noncetotică (HNC) sau "encefalopatia glicinică" este o eroare înnăscută a metabolismului glicinei, cauzată de un defect în sistemul de clivare a glicinei (SCG), conducând spre acumularea acestui aminoacid în lichidele și țesuturile organismului. SCG este un complex mitocondrial multienzimatic ce constă din patru subunități proteice: proteina P, proteina H, proteina T și proteina L, codificate de patru gene diferite. Incidența la naștere a HNK la nivel mondial a fost estimată la 1:76,000.

Caz clinic: Raportăm o pacientă (2 luni) care se prezenta cu encefalopatie progresivă, convulsii intractabile și progresie rapidă spre comă. Rezultatele analizei petelor uscate de sânge prin cromatografie lichidă cuplată cu tandem mass spectroscopie au fost sugestive pentru hiperglicinemie. Drept urmare, au fost apreciați aminoacizii în fluidele biologice umane (lichid cefalorahidian (LCR), plasmă, urină) prin cromatografie de schimb ionic, identificându-se niveluri ridicate ale glicinei în fluidele analizate. Raportul concentrației glicinei în LCR și plasmă, criteriu biochimic pentru HNK a fost 0,147 (normal <0,02), valoare caracteristică pentru forma neonatală. Monitorizarea biochimică a evidențiat o descreștere semnificativă a glicinei în plasmă și LCR după folosirea unei diete hipoglicinice. Suplimentarea terapiei cu benzoat de sodiu a demonstrat o descreștere continuă a nivelului glicinei în plasmă, dar o creștere în LCR și respectiv a raportului concentrației glicinei în LCR și plasmă, indicând o evoluție slabă și un prognostic negativ. Totodată, rezultatele obținute denotă eficiența benzoatului de sodiu prin identificarea cantităților ridicate de acid hipuric în urină.

Concluzii: Pentru confirmarea diagnosticului sunt necesare investigații enzimatice și genetice ale sistemului de clivare a glicinei. Un diagnostic timpuriu permite nu doar o abordare terapeutică individuală, dar și consilierea genetică adecvată cu posibilitatea diagnosticului prenatal. Prognosticul este mai informativ când este evaluat în funcție de nivelul glicinei în LCR și de raportul concentrației glicinei în LCR și în plasmă. Copiii care supraviețuiesc au deseori întârzieri de dezvoltare și convulsii refractare.

Cuvinte-cheie: Hiperglicinemie noncetotică, glicină, sistem de clivare a glicinei, encefalopatie, convulsii, comă.

РЕЗЮМЕ

НЕКЕТОТИЧЕСКАЯ ГИПЕРГЛИЦИНЕМИЯ - КЛИНИЧЕСКИЙ СЛУЧАЙ

Введение: Некетотическая гиперглицинемия (NKH) или «глициновая энцефалопатия» является врожденным нарушением обмена глицина, вызванный дефектом в системе расщепления глицина (glycine cleavage system - GCS), который метаболизирует аминокислоту глицин, что приводит к его накоплению в тканях и жидкостях организма. GCS представляет собой митохондриальный мультиферментный комплекс, состоящий из четырех белковых субъединиц: P-белок, H-белок, T-белок и L-белок, кодируемый четырьмя различными генами. Встречаемость заболеваемости во всем мире была оценена в 1:76.000.

Клинический случай: Представляется пациент (2 мес.) с энцефалопатией, судорогами и быстрым прогрессированием до комы. Результаты исследования сухих пятнен крови методом тандемной масс-спектрометрии дали основание предположить гиперглицинемию. Следовательно, был проведен аминокислотный анализ в биологических жидкостях (спинномозговая жидкость (СМЖ), плазма, моча) с помощью ионообменной хроматографии, которая выявила повышенный уровень глицина в плазме, моче и СМЖ. Повышенное соотношение глицина в СМЖ к плазме - 0,147 (в норме <0,02) было очевидным и характерным для неонатальной формы NKH. Мониторинг аминокислотного состава показал снижение глицина в плазме и СМЖ после диеты с низким содержанием глицина. После дополнения бензоата натрия к терапии было отмечено продолжительное снижение глицина в плазме, но повышение уровеня глицина в СМЖ и соотношения глицина в СМЖ к плазме, что указывает на плохой исход. В то же время, выявление больших концентраций гиппуровой кислоты в моче указывает на эффективность бензоата на снижение глицина в плазме.

Заключение: Подтверждающий диагноз требует ферментативного и генетического исследования системы расщепления глицина. Ранний диагноз позволяет не только подбор терапевтического ведения, но и провести надлежащее генетическое консультирование с возможностью пренатальной диагностики.

Ключевые слова: некетотическая гиперглицинемия, глицин, фермент расщепления глицина, энцефалопатия, судороги, кома.

Introduction

Glycine (Gly) is one of the non-essential amino acids, essential for a healthy digestive system, for the development and quality of human skeletal muscles, tissues, and structural integrity, as well as for the synthesis of nucleic acids. Glycine also plays a role as an inhibitory neurotransmitter in your central nervous system, particularly in the spinal cord, in the brainstem, and the retina. It is used for the biosynthesis of glutathione, creatine and many non-protein compounds, such as porphyrins and purines [1].

Levels of glycine are primarily regulated by enzymatic degradation. The primary disorder of the glycine is a deficiency of the main catabolic enzyme, the glycine cleavage system (GCS). Glycine is part of many biochemical pathways, but deficiency of the GCS removes the main catabolic breakdown of glycine resulting in increased levels of glycine. The disorder is known as nonketotic hyperglycinemia (NKH, OMIM: 605899), or glycine encephalopathy, inherited as an autosomal recessive trait. All forms of the disorder are characterized by cerebral dysfunction [2]. The GCS breaks glycine down into carbon dioxide and ammonia, and a methyl group is transferred to tetrahydrofolate creating methylene-tetrahydrofolate. The GCS is a mitochondrial enzyme complex consists of the products of four genes - three proteins and one carrier protein [3]: (1) a pyridoxal phosphate-containing protein or glycine decarboxylase (P protein); (2) aminomethyltransferase (T protein); (3) dihydrolipoamide dehydrogenase (L protein); and (4) the hydrogen carrier protein or a lipoic acid-containing protein (H protein). More than 80% of NKH patients have a defect in P-protein, up to 15% have a T-protein defect [4] [5], and H-protein defficiency is rare [6].

In NKH, the enzyme responsible is usually expressed only in the brain and liver, and the diagnosis is made based on the accumulation of glycine in the cerebrospinal fluid. Most patients present in the early days of life with life-threatening illness [7]. The accumulation of glycine in the brain and neuronal tissue is responsible for many of the clinical signs and symptoms of NKH. Glycine is both an excitatory and inhibitory neurotransmitter and has an excitatory effect when binding to the N-methyl-D-aspartate (NMDA) receptor. The excitatory actions cause seizures, while the inhibitory actions cause hypotonia and lethargy. Classical NKH occurs most frequently in the neonatal period (less than one week of age) and less often in the infantile stage (over one week of age) [8].

The incidence of NKH is underestimated, as many patients die undiagnosed. Using publicly available population genotypes, the birth frequency of NKH worldwide was estimated at 1:60,000-76,000 [9] [10].

Biomarkers for diagnosis: The diagnosis of NKH relies on amino acid analysis, which should reveal an elevation of glycine in plasma, urine, and CSF. However, due to diurnal variation and differences in disease severity, plasma glycine may not be significantly elevated in all cases. With the exception of some mild late-onset cases of NKH, glycine is always elevated in CSF. Therefore, an elevated glycine CSF:plasma ratio, usually greater than 0.08 in classical NKH, and greater than 0.04 in atypical NKH can be used for diagnosis [22, 23]. However, there have been rare cases of atypical NKH without this elevation [6]. Usually urine organic acid and plasma acylcarnitine profiles are normal [11] [12] [22].

Case report

We report on a patient (girl, 2 mo old) who was born at term, per vias naturalis, with a normal birth weight

(3800 g) and Apgar scores 7/8 points. This is the second child in a non-consanguineous couple, without suggestive family history. The first child is a healthy 11 years old girl. Within the first 24 hours after birth, the patient became apathetic, drowsy and she was transferred in the intensive care unit connected to artificial ventilation for three weeks. From the third day of life, the child started to develop intractable seizures. Consequently, she entered into a coma for a month. During first month of life she was hospitalized in an unspecialized in inborn errors of metabolism medical unit. The EEG showed excessive discontinuity of cerebral activity in the form of burst-suppression pattern and separated in the frontal region with an emphasis on the left isolated acute hypervoluted sharp waves of epileptiform character. CT scan showed cerebral mixed hydrocephalus. She was identified with positive CMV and she received antiviral treatment.

At 2 months of life, she has been transferred to third level hospital where has been seen by a specialist in inborn errors of metabolism and dry blood spots have been collected to perform liquid chromatography-tandem mass spectrometry (LC-MS/MS). At that time the patient was obnubilated. The ammonia levels were in normal ranges (34-68 μM/L), but the patient felt into a profound comatose state. Blood spot sample analyzed using LC-MS/MS in CytoGenomic Medical Laboratory revealed elevated glycine values of 1085.9 μM/L (normal: <650.0 μM/L) and normal levels for other analyzed parameters. Amino acids analysis in biological fluids (CFS, plasma, urine) has been performed by ion-exchange liquid chromatography (IELC) at the Institute of Physiology and Sanocreatology, Chisinau, Moldova. Glycine levels were high in all analyzed fluids, exceeding 5 fold above the normal ranges in plasma (normal: 125-450 μM/L), 10 fold – in CSF (normal: <20 μM/L) and 2 fold above the normal ranges in urine. The biochemical hallmark of NKH - elevated glycine CSF:plasma ratio of 0.147 (normal<0.02) [21] was evident. These results are consistent with the neonatal form of NKH. The NMR spectroscopy of urine performed at "Petru Poni" Institute of Macromolecular Chemistry, Iasi, Romania, confirmed the high levels of glycine and revealed normal levels of other organic acids.

Consequently, the treatment has been individualized according to the biochemical diagnosis. So, a low glycine diet has been initiated with a special formula and after a month the amino acids have been quantified in plasma, CSF and urine. The glycine levels decreased significantly in plasma and CSF (by 2.5 fold), but the glycine CSF:plasma ratio remained elevated (0.197), which was appreciated as a poor outcome and was in accordance with clinical evolution. At the same time, the glycine level was appreciated in dry blood spots by LC-MS/MS, being in the normal ranges (461.56 μM/L). The result of NMR spectroscopy showed a decrease of glycine in urine. Being on the diet the clinical manifestations improved, the patient became conscious, but the seizures continued. After a month of low glycine diet, the sodium benzoate 200 mg/kg/day administration has been supplemented for a month with a biochemical retesting. As a result, glycine levels have been decreased in plasma, but it was observed an increasing of the glycine levels in CSF and urine, withal the CSF:plasma glycine ratio (1.77) has been increased as well. The result of NMR spectroscopy showed high amounts of glycine and hippuric acid in the urine. The hippuric acid was identified in urine after the sodium benzoate supplementation. As a consequence of this therapeutic approach, the respiration became spontaneous, the clinical manifestations of the child improved a little in dynamics, in general remain as a severe form of NKH and the seizures are continuous.

Discussion

Nonketotic hyperglycinemia (NKH), also known as glycine encephalopathy, is an autosomal recessive inborn error of glycine metabolism due to a deficiency of the glycine cleavage system (GCS) [4]. Glycine acts both as an excitatory and an inhibitory neurotransmitter. Large amounts of glycine accumulating in the brain have an excitatory effect at the NMDA receptor channel complex located in the hippocampus, cerebral cortex, olfactory bulb, and cerebellum. Overstimulation of these receptors may cause intractable seizures and brain damage [4]. For the diagnosis of NKH, glycine concentration should be analyzed simultaneously in plasma and CSF samples, allowing determination of CSF:plasma glycine ratio. A NKH diagnosis can also be made based on molecular and enzymatic studies. For molecular studies, all three genes GLDC, AMT, and GCSH, coding for the P-, T-, and H-proteins respectively, should be analyzed, either by sequencing or by targeted mutational analysis [13][11]. Mutations in GLDC account for 75%–80% of patients, while mutations in AMT account for 15%–20%, and mutations in GCSH explain less than 1% of NKH [14]. It is important to take into account the substantial genetic heterogeneity of NKH. Genetic mutations have a strong impact on outcome in NKH [15], and large intragenic genetic heterogeneity complicates evaluating the impact of treatment intervention in NKH [16]. Patients with 2 mutations without any residual activity always have severe NKH, make no developmental progress, and have a severe seizure disorder, regardless of treatment even when initiated from birth [17]. The ultimate confirmation of an NKH diagnosis can be made by measuring GCS activity in liver tissue obtained by either biopsy or autopsy [14]. In cases of suspected NKH during pregnancy, enzyme analysis or molecular testing may be conducted on a tissue for chorionic villus sampling [18]. Early diagnosis might be important for genetic counseling and for atypical later forms, where early intervention could be beneficial or with the possibility of prenatal diagnosis.

Treatment for NKH is aimed: (1) to decrease the tissue glycine levels, (2) to treat seizures, and (3) to ameliorate excitotoxicity at the NMDA receptor. No treatment

prevents or repairs neurological damage [4] [11]. The available interventions have attained these aims with reasonable success for the first, with moderate success for the second, and with limited success for the third [19]. The sodium benzoate treatment is used to decrease the accumulation of glycine with or without a glycine restricted diet. Oral sodium benzoate was not very effective since glycine is not metabolized in the brain and conjugation with it can take place only in liver mitochondria, so it helps to decrease plasma glycine levels but has no effect on glycine levels in CSF [20].

Prognosis of Nonketotic Hyperglycinemia: A majority of patients die in the neonatal period, presumably many without a diagnosis. In the first days of life, there is a rapid progression to deep coma and respiratory arrest. In those who survive the early crisis, there is little evidence of psychomotor development. Intractable seizures are as a rule. Neonates are hypotonic or flaccid. Eventually, they become spastic, hyperreflexic, and often opisthotonic. Most are microcephalic [8].

Conclusion

The diagnosis of NKH was made based on clinical manifestations of child with neonatal progressive encephalopathy, deep coma and intractable seizures and complex metabolic work-up. The neonatal screening from DBS using LC-MS/MS was only indicative to follow the complex metabolic work-up through the amino acids evaluation in body fluids as plasma, CSF and urine. The glycine CSF:plasma ratio was the most important value for diagnosis, monitoring and prognosis of disease. The low glycine diet was appreciated as the most effective therapy to decrease plasma and CSF glycine levels, while the sodium benzoate supplementation helped to decrease plasma, but not CSF glycine level. In the presented case, the glycine CSF:plasma ratio remained increased determining severe form and poor evolution.

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