

HYPERINSULINISM-HYPERAMMONEMIA SYNDROME IN AN INFANT WITH SEIZURES

Strajnar A¹, Tasek MZ², Podkrajsek KT^{3,4}, Battelino T^{2,5}, Groselj U^{2,*}

***Corresponding Author:** Assistant Professor Urh Groselj, M.D., Ph.D., Department of Pediatric Endocrinology, Diabetes and Metabolic Diseases, University Children's Hospital, University Medical Centre Ljubljana, Bohoriceva 20, 1000 Ljubljana, Slovenia. Tel: +386-1-5229270. Fax: +386-1-2320190. E-mail: urh.groselj@kclj.si

ABSTRACT

Hyperinsulinism-hyperammonemia syndrome (HI/HA) is the second most common form of persistent hyperinsulinemic hypoglycemia of infancy (PHHI). The main clinical characteristics of HI/HA syndrome are repeated episodes of symptomatic hypoglycemia, but not usually severe. Consequently, children with HI/HA syndrome are frequently not recognized in the first months of life. An 8-month-old boy was admitted to a hospital due to hypoglycemia seizures. He also had asymptomatic hyperammonemia with no signs of lethargy or headaches. Genetic testing revealed autosomal dominant syndrome, a mutation in the *GLUD1* gene (p.Arg274Cys). The boy started treatment with diazoxide. Subsequent growth and neurological development were normal. Hypoglycemic symptoms in HI/HA syndrome may vary from being non specific to severe. As hypoglycemia could lead to brain injury and impairment of neurological development, timely diagnosis and management are essential. If transient hypoglycemia is ruled out, metabolic disorders must be taken into account.

Keywords: Diazoxide; *GLUD1* gene; Glutamate dehydrogenase; Hyperinsulinism-hyperammonemia (HI/HA) syndrome; persistent hyperinsulinemic hypoglycemia of infancy.

INTRODUCTION

The 8-month-old male patient was admitted to our hospital because of seizures. He was an only child of non consanguineous parents and the conception was not assisted. The pregnancy was normal. The father was followed at our department over 20 years ago during his childhood due to recurrent hypoglycemia episodes, often with collapses, which usually happened after eating protein rich food; however, no hypoglycemia episodes or any other health problems persisted into the father's adulthood. The boy was born at term with normal birth parameters. Postnatally, the patient was healthy until this admission to the hospital. He was vaccinated according to the national program. His psychomotor development was apparently normal.

On the day of admission, the mother found the child's body jerking during his afternoon rest period. She tried to wake him but he was less alert. Then she saw him turn his eyes backwards. The body jerks lasted for 5 min. He also had difficulty breathing. The boy had his last meal half an hour before mother found him jerking. He had previously had two similar short episodes with twitches, always after afternoon rest, but they had ceased by themselves so parents had not sought any medical attention at those times.

At the outpatient clinic, a doctor reported the boy did not respond to pain but had the sucking reflex. The oxygen saturation was 85.0%, breathing rate was 20/min., heart rate 166/min., blood sugar 2.8 mmol/L (50.0 mg/dL).

At admission to the hospital, the oxygen saturation increased to 99.0% and the blood sugar level to 3.7 mmol/L (66.0 mg/dL) without therapeutic intervention. The child was still less responsive and his gaze was not fixed but after time he became alert and responded to all stimuli adequately. Jerking ceased. Somatic status was normal with no organomegaly. Nutritional status was also normal with

¹ University Children's Hospital, University Medical Centre Ljubljana, Ljubljana, Slovenia

² Department of Pediatric Endocrinology, Diabetes and Metabolic Diseases, University Children's Hospital, University Medical Centre Ljubljana, Ljubljana, Slovenia

³ Unit of Special Laboratory Diagnostics, University Children's Hospital, University Medical Centre Ljubljana, Ljubljana, Slovenia

⁴ Institute of Biochemistry, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

⁵ Department of Pediatrics, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

body weight 7.92 kg (20 percentile), body height 71 cm (43 percentile) and head circumference 44 cm (25 percentile).

Initial laboratory tests with lactate, pyruvate, cortisol, blood gases analysis, plasma amino acids, acylcarnitines, free fatty acids, urea and creatinine, ions, liver function tests, hemogram, C-reactive protein (CRP), were all in normal value ranges, with the exception of blood sugar and ammonia, which was 110.0 $\mu\text{mol/L}$ (reference: ammonia values 9.0-33.0 $\mu\text{mol/L}$).

Hospital Course. During the initial hospitalization, the boy was in a good mood and had a good appetite. He had normal stool. He had no seizures. Brain ultrasound, electroencephalogram (EEG), brain magnetic resonance imaging (MRI) and electrocardiogram (ECG) were normal. The laboratory tests revealed occasional hypoglycemia (the lowest value 2.2 mmol/L; 40.0 mg/dL) and persistent hyperammonemia (in a range from 95.0 to 139.0 $\mu\text{mol/L}$). Insulin level was 2.31 mE/L (reference: insulin values 2.0-29.1 mE/L). During the first hospitalization, we also performed a fasting test and protein loading test.

During the fasting test, hypoglycemia 2.3 mmol/L (42.0 mg/dL) was revealed after 11 hours of fasting. Blood ammonia was 100.0 $\mu\text{mol/L}$ at that time, insulin level 2.0 mE/L and ketones 1.6 mmol/L. All other laboratory examinations were normal with the exception of adequate ketosis after fasting.

We performed the protein-loading test [1] to exclude a possible urea cycle disorder. Two hours after the loading test with 35 g/m² proteins, blood ammonia changed from 100.0 $\mu\text{mol/L}$ to 142.0 $\mu\text{mol/L}$; other laboratory examinations were normal.

During the second hospitalization after three weeks, we performed a therapeutic experiment with diazoxide 3 mg/kg, which prevented postprandial hypoglycemia but ammonia levels remained high (in a range from 114.0 to 148.0 $\mu\text{mol/L}$). Thus, the child was diagnosed with hyperinsulinism-hyperammonemia (HI/HA) syndrome and started treatment with 10.0 mg/kg diazoxide per day and protein restriction up to 1.2 g/kg per day to avoid the high protein meals. Later, no postprandial hypoglycemias were recorded. The patient's growth and neurological development at the age of 2 years were within the normal ranges.

Final Diagnosis. The clinical diagnosis of HI/HA syndrome was anticipated and genetic confirmation was provided with a next generation sequencing (NGS) panel test related to neonatal hypoglycemia. Nucleotide variant NM_005271.3: c.820C>T was detected in the *GLUD1* gene, resulting in an arginine to cysteine amino acid substitution at the position 274 (NP_005262.1: p.Arg274Cys). The variant has previously been reported as p.Arg221Cys

(as numbered by mature protein amino acids) in patients with HI/HA syndrome [2,3]. Therefore, genetic analysis results confirmed the autosomal dominant syndrome to be HI/HA. Interestingly, the mutation was not detected in his parents.

DISCUSSION

Recurrent hypoglycemia in early infancy is most frequently caused by congenital hyperinsulinism (CHI) [4]. Transient forms of CHI are mainly a consequence of other disorders such as gestational diabetes, perinatal asphyxia or intrauterine growth retardation [5]; persistent forms are known as persistent hyperinsulinemic hypoglycemia of infancy (PHHI) (Table 1) [6]. The incidence of PHHI is estimated to be around 1/35,000-40,000 [7]. Persistent hyperinsulinemic hypoglycemia of infancy is characterized by unsuppressed insulin secretion, in spite of a low level of blood glucose, most frequently presenting in newborns with mild or severe hypoglycemia [8]. Clinical symptoms of hypoglycemia could be non specific (*e.g.*, lethargy, irritability, poor feeding) or in some cases severe (apnea, seizures or coma) [9]. As hypoglycemia could lead to brain injury and impairment of neurological development, timely diagnosis and management are essential to prevent the sequelae [8].

Hyperinsulinism-hyperammonemia syndrome is the second most frequent cause of PHHI, after the pancreatic β -cell K_{ATP} channel defects [10]. The main clinical characteristics of HI/HA syndrome are repeated episodes of symptomatic hypoglycemia [10,11]. Hypoglycemia is usually less severe as compared to that observed in the defects of the K_{ATP} channel and frequently not diagnosed in the first months of life [10]. Our patient first displayed convulsions at 8 months. This could also be a result of the higher protein intake with the solid food introduction at this age; the normal results of brain ultrasound, EEG, brain MRI and ECG ruled out most other possible causes.

Hyperinsulinism-hyperammonemia syndrome is a consequence of a mutated *GLUD1* gene for the mitochondrial enzyme glutamate dehydrogenase (GDH). Glutamate dehydrogenase is substantially expressed in liver, pancreatic β -cells, kidney and in brain. It catalyzes the oxidative deamination of glutamate to α -ketoglutarate and ammonia [10,12]. The GDH is allosterically activated by leucine and adenosine diphosphate (ADP) and is inhibited by guanosine triphosphate (GTP) [13]. In the pancreatic β -cells, α -ketoglutarate is metabolized in the tricarboxylic acid (Krebs) cycle, increasing the cellular ATP concentration that closes the ATP-sensitive potassium channels. The resulting cell membrane depolarization causes Ca^{2+} influx

Table 1. Disorders associated with persistent hyperinsulinemic hypoglycemia of infancy.

| | General Characteristics | | Laboratory and Clinical Characteristics |
|--|--|---|--|
| | Gene | Mode of Inheritance | |
| PHHI due to K_{ATP} channel mutation | Kir6.2: <i>KCNJ11</i> gene; SUR: <i>ABCC8</i> gene; imprinted region | Diffuse form: AR or AD; focal form: heterozygous paternal mutation, clonal loss of maternal 11p15.1 | ↑ Glucose requirement (up to 30.0 mg/kg/ min.); during hypoglycemia: ↓ ketone bodies, ↓ FFA (serum), insulin incompletely suppressed, normal blood gases and lactate, n/↑ ammonium, IGFBP-1 (<120.0 mg/mL), normal glucagon response |
| Glucokinase activating mutations | <i>GCK</i> gene | AD | Heterozygous: familial mild non progressive hyperglycemia, gestational diabetes; homozygous: neonatal diabetes |
| HI/HA syndrome | <i>GLUD1</i> gene | AD | Hyperammonemia (100.0-200.0 μ mol/L), usually asymptomatic, may be prominent early but may disappear later in childhood; often leucine-sensitive |
| Exercise-induced hyperinsulinemic hypoglycemia | <i>SLC16A1</i> promoter mutations | AD | Children/adults with syncopal episodes after exercise |
| SCHAD deficiency | <i>HADH</i> gene | AR | Intermittent unpredictable hypoglycemia with seizures; ↑ C4-OH-carnitine, ↑ 3-OH-glutarate (urine) |
| Beckwith-Wiedemann syndrome | Chromosomal imbalance 11p15 | e.g., paternal UPD | Hyperinsulinism (disappears in most patients within weeks); typical facial characteristics (macroglossia, ear creases, omphalocele, visceromegaly, hemihypertrophy) |

PHHI: persistent hyperinsulinemic hypoglycemia of infancy; AR: autosomal recessive; AD: autosomal dominant; ↑: elevated; ↓: low/decreased; FFA: free fatty acids; IGFBP-1: insulin-like growth factor-binding protein; HI/HA: hyperinsulinism-hyperammonemia; SCHAD: short-chain hydroxyacylCoA dehydrogenase; CA-OH-carnitine: 3-hydroxy-butyril-carnitine; 3-OH-glutarate: 3-hydroxy-glutarate.

via voltage gated calcium channels and insulin exocytosis [14] (Figure 1).

The *GLUD1* gene mutations lead to an increased enzyme activity, causing increased insulin secretion by pancreatic β -cells, increased ammonia production and its decreased removal by the hepatocytes. Leucine stimulates insulin secretion by allosterically activating GDH activity [15]. Typically, hypoglycemia follows meals with a high protein content [12,15,17,18] (Figure 1).

In our patient, the autosomal dominant syndrome, a mutation on the *GLUD1* gene (p.Arg274Cys), was confirmed with genetic analysis. The mutation (p.Arg 274Cys) has previously been reported as p.Arg221Cys in patients with HI/HA, who also had epileptic seizures and mild mental retardation [3,4]. Our patient had no epileptic seizures and his neurocognitive development was within normal ranges at the age of 2 years.

We also performed genetic analyses on our patient's father who had hypoglycemia episodes in his childhood. Initially, we suspected the father to have the same mutation, as the boy displayed autosomal dominant syndrome and the father had hypoglycemia episodes in his childhood. However, the father's (as well as his mother's) genetic analyses results were normal. *De novo* mutations of the

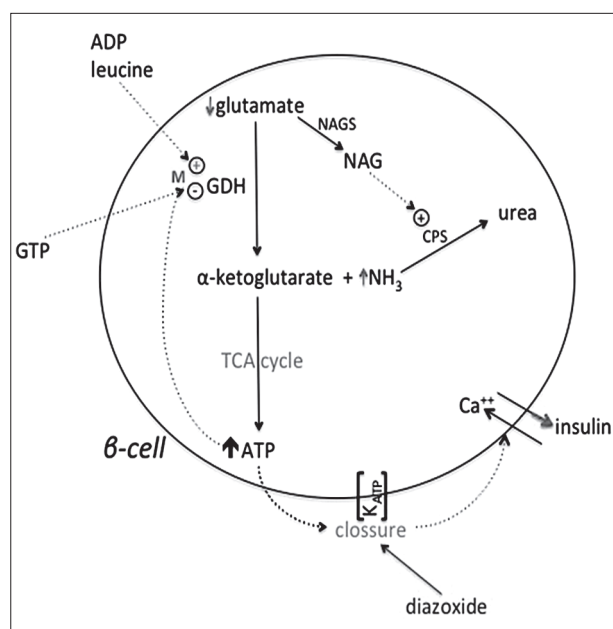


Figure 1. Patophysiological mechanism of hyperammonemia in GDH deficiency.

M: mutation; GDH: glutamate dehydrogenase; TCA: tricarboxylic acid; ATP: aden- osine triphosphate; ADP: adenosine diphosphate; GTP: guanosine triphosphate; NAG: N-Acetylglutamate; NAGS: N-Acetylglutamate synthase; CPS: carbamoyl-phosphate synthetase.

gene *GLUD1* account for 80.0% of cases [16], as happened in our patient.

Normally, protein intake stimulates insulin release without causing hypoglycemia because glucagon is also secreted to neutralize the effect of insulin on glucose production in the liver [15,20]. Protein-induced hypoglycemia in HI/HA might be a consequence of impaired regulation of pancreatic α -cells, as well as β -cells [15]. Patients with HI/HA are susceptible to hypoglycemia in response to both fasting and protein feeding, but in most instances hypoglycemia occurs within a few hours after a meal [15]. The same was seen in our patient who had the hypoglycemia episode during his afternoon rest (probably caused by protein load in his meal half an hour before afternoon rest) and only after 11 hours of fasting. Thus, HI/HA must be considered in the differential diagnosis of postprandial or reactive hypoglycemia [15].

In HI/HA, plasma ammonia levels are increased 3-5 times normal as a consequence of the hyperactivity of GDH, which causes increased ammonia release from glutamate and its diminished elimination [10] (Figure 1). Hyperammonemia in patients with HI/HA syndrome tends to be asymptomatic and ammonium lowering therapy is not considered to provide any benefits in HI/HA syndrome. Ammonia levels in HI/HA syndrome are also not shown to depend on fasting, protein intake or on blood glucose levels [10]. All the above mentioned clinical and biochemical features were present in our patient.

Therapy with diazoxide is shown to be effective in PHHI, binding to the intact SUR1 component of the ATP-sensitive potassium (K_{ATP}) channels in the pancreatic β -cell, preventing the cell membrane depolarization and insulin secretion [6]. The HI/HA patients with a *GLUD1* mutation usually respond to diazoxide therapy, only very rarely is a pancreatectomy needed [21]. Our patient has now been on therapy for more than 1 year, without documented hypoglycemia or seizures and with normal growth and development.

Conclusions. It is important to always consider hypoglycemia and hyperammonemia when a baby “is not well,” as symptoms may not be specific. If transient hypoglycemia in an infant is ruled out, metabolic disorders must be taken into account. In HI/HA syndrome, diazoxide is the first line of treatment; hypoglycemia is well controlled and hyperammonemia appears to be asymptomatic.

Declaration of Interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

Funding. This study was supported by the Slovenian Research Agency [grant number P3-0343].

REFERENCES

1. Potter M, Hammond JW, Sim KG, Green AK, Wilcken B. Ornithine carbamoyltransferase deficiency: Improved sensitivity of testing for protein tolerance in the diagnosis of heterozygotes. *J Inher Metab Dis*. 2001; 24(1): 5-14.
2. Santer R, Kinner M, Passarge M, Superti-Furga A, Mayatepek E, Meissner T, *et al*. Novel missense mutations outside the allosteric domain of glutamate dehydrogenase are prevalent in European patients with the congenital hyperinsulinism-hyperammonemia syndrome. *Hum Genet*. 2001; 108(1): 66-71.
3. Sarajlija A, Milenkovic M, Djordjevic K, Mitrovic S, Todorovic B, Kecman Khalid H. Early presentation of hyperinsulinism/hyperammonemia syndrome in three Serbian patients. *J Clin Res Pediatr Endocrinol*. 2015; 8(2): 228-231.
4. Stanley CA. Hyperinsulinism in infants and children. *Pediatr Clin North Am*. 1997; 44(2): 363-374.
5. Senniappan S, Shanti B, James C, Hussain K. Hyperinsulinaemichypoglycaemia: Genetic mechanisms, diagnosis and management. *J Inher Metab Dis*. 2012; 35(4): 589-601.
6. Zschocke J, Hoffmann GF. *Vademecum Metabolicum. Diagnoses and Treatment of Inborn Errors of Metabolism*, 3rd ed. Friedrichsdorf (Hesse, Germany): Schattauer GmbH, 2011.
7. Mathew PM, Young JM, Abu-Osba YK, Mulhern BD, Hammoudi S, Hamdan JA, *et al*. Persistent neonatal hyperinsulinism. *Clin Pediatr (Philadelphia)*. 1988; 27(3): 148-151.
8. Kapoor RR, Flanagan SE, James C, Shield J, Ellard S, Hussain K. Hyperinsulinaemichypoglycaemia. *Arch Dis Child*. 2009; 94(6): 450-457.
9. Senniappan S, Arya VB, Hussain K. The molecular mechanisms, diagnosis and management of congenital hyperinsulinism. *Indian J Endocrinol Metab*. 2013; 17(1): 19-30.
10. Stanley CA. Hyperinsulinism/hyperammonemia syndrome: Insights into the regulatory role of glutamate dehydrogenase in ammonia metabolism. *Mol Genet Metab*. 2004; 81(1): 45-51.
11. MacMullen C, Fang J, Hsu BY, Kelly A, de Lonlay-Debeney P, Saudubray JM, *et al*. Hyperinsulinism-hyperammonemia syndrome in children with regulatory mutations in the inhibitory guanosine triphos-

- phate-binding domain of glutamate dehydrogenase. *J Clin Endocrinol Metab.* 2001; 86(4): 1782-1787.
12. Kelly A, Ng D, Ferry RJ Jr, Grimberg A, Koo-McCoy S, Thornton PS, *et al.* Acute insulin responses to leucine in children with the hyperinsulinism/hyperammonemia syndrome. *J Clin Endocrinol Metab.* 2001; 86(8): 3724-3728.
 13. Stanley CA, Fang J, Kutyna K, Hsu BY, Ming JE, Glaser B, *et al.*; HI/HA Contributing Investigators. Molecular basis and characterization of the hyperinsulinism/hyperammonemia syndrome: Predominance of mutations in exons 11 and 12 of the glutamate dehydrogenase gene. *Diabetes.* 2000; 49(4): 667-673.
 14. Ashcroft FM. ATP-sensitive potassium channelopathies: Focus on insulin secretion. *J Clin Invest.* 2005; 115(8): 2047-2058.
 15. Hsu BYL, Kelly A, Thornton PS, Greenberg CR, Dilling LA, Stanley CA. Protein-sensitive and fasting hypoglycemia in children with the hyperinsulinism/hyperammonemia syndrome. *J Pediatr.* 2001; 138(3): 383-389.
 16. Stanley CA, Matschinsky FM. Historical perspective on the genetic forms of congenital hyperinsulinism. In: Stanley CA, De León DD, Eds. *Monogenic Hyperinsulinemic Hypoglycemia Disorders*. *Frontiers in Diabetes*, Vol. 21. Basel (Basel-Stadt, Switzerland): S. Karger Ag, 2012: 1-6.
 17. Sener A, Malaisse-Lagae F, Malaisse WJ. Stimulation of pancreatic islet metabolism and insulin release by a nonmetabolizable amino acid. *Proc Natl Acad Sci USA.* 1981; 78(9): 5460-5464 (<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC348765/>).
 18. Gao Z, Li G, Najafi H, Wolf BA, Matschinsky FM. Glucose regulation of glutaminolysis and its role in insulin secretion. *Diabetes.* 1999; 48(8): 1535-1542.
 19. Maechler P, Wollheim CB. Mitochondrial glutamate acts as a messenger in glucose-induced insulin exocytosis. *Nature.* 1999; 402(6762): 685-689.
 20. Aoki TT, Muller WA, Brennan MF, Cahill Jr GF. Blood cell and plasma amino acid levels across forearm muscle during a protein meal. *Diabetes.* 1973; 22(10): 768-775.
 21. Flanagan SE, Patch AM, Locke JM, Akcay T, Simsek E, Alaei M, *et al.* Genome-wide homozygosity analysis reveals HADH mutations as a common cause of diazoxide-responsive hyperinsulinemic-hypoglycemia in consanguineous pedigrees. *J Clin Endocrinol Metab.* 2011; 96(3): 498-502.

