CASE REPORT

Hyperinsulinemic Hypoglycemia of Infancy due to Novel *HADH* Mutation in Two Siblings

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Background: Hyperinsulinemia is the commonest cause of persistent hypoglycemia in infancy. Inactivating mutations in the genes *ABCC8* and *KCNJ11* are the commonest cause. Mutation in the *HADH* gene, which encodes the short-chain-L-3-hydroxyacyl-CoA dehydrogenase, is a rare cause. **Case characteristics:** Two Indian sisters who presented with hyperinsulinemic hypoglycemia of infancy. **Observation/Intervention:** A novel homozygous missense mutation in the *HADH* gene was identified in both the sisters, while the parents were found to be heterozygous carriers. **Outcome:** Establishment of molecular diagnosis, optimization of therapy and counseling of parents regarding risk of recurrence in future pregnancy. **Messages:** *HADH* mutations are rare causes of hypoglycemia and can be mitigated with diazoxide and appropriate dietary therapy if identified early.

Keywords: *Diagnosis, Hyperinsulinemia, Genetics, Treatment.*

Congenital hyperinsulinism (CHI) is characterized by dysregulated and inappropriate release of insulin from pancreatic beta cells leading to persistent hypoglycemia. CHI typically presents in the newborn period, although it may present later. The incidence is 1:50,000 live births, although in consanguineous families, the incidence goes up to 1:2500. At a molecular level, mutations in nine different genes have been identified to cause CHI [1]. Mutations in *ABCC8* and *KCNJ11* genes that encode for ATP-sensitive potassium channels in the beta cells of pancreas are the commonest. The other mutations are in *GLUD1*, *GCK*, *HNF4A*, *HNF1A*, *SLC16A1*, *UCP2* and *HADH*. *HADH* encodes the enzyme short chain 3-hydroxyacyl-CoA dehydro-genase, which catalyzes the third (penultimate) step in mitochondrial fatty acid oxidation, and recessively inherited loss of function mutations in *HADH* cause protein-induced hyperinsulinemic hypoglycemia. Here, we report a novel homozygous mutation in *HADH* presenting as recurrent hypoglycemia in early infancy in two siblings.

Case Report

This female infant was born to a non-consanguineous Hindu couple by normal vaginal delivery, without any adverse perinatal event with a birth weight of 3100 g. She was on exclusive breast feeding and gaining milestones till 3 months of age when she developed episodes of tonic seizures. On evaluation at a private hospital, the infant was noted to have hypoglycemia without ketonuria. Critical sampling during the episode of hypoglycemia was suggestive of hyperinsulinemia with serum insulin level of 8.5 mIU/ml (normal: undetectable or <2mIU/mL during hypoglycemia) with thyroid, cortisol and growth hormone levels being within normal limits. The baby was started on oral diazoxide at a dose of 5 mg/kg/day with regular blood sugar monitoring. Compliance to therapy was inadequate and the baby continued to have intermittent hypoglycemia and seizures, but she gained milestones normally and had normal vision. The child was brought to our attention at 15 months of age with these complaints. There was history of similar complaints in a 3-year-old elder sister who had intermittent episodes of seizures from 4 months of age with documented hypoglycemia. Following evaluation in another center she was also confirmed to have hyperinsulinemic hypoglycemia, and was treated with diazoxide. She was gaining developmental milestones appropriately, except for mild motor delay.

A repeat critical sample during an episode of hypoglycemia for the younger sister at our institute revealed serum insulin of 6.9 mIU/mL and absence of ketonuria. Glycemic response to glucagon could not be assessed. Serum ketones and free fatty acids also could not be measured. Fasting serum ammonia was mildly elevated at 150 µg/mL (normal range 50-80 µg/mL). Mutation analysis for congenital hyperinsulinism was under taken for both the siblings and parents. DNA was extracted from peripheral leukocytes, and mutation testing (University of Exeter Medical School, UK). by

Sanger sequencing identified a novel homozygous missense mutation, p.I184F (c.550A>T), in exon 5 of *HADH* gene in the two sisters. Both parents were heterozygous for the mutation.The isoleucine residue at codon 184 is highly conserved across species and current *in silico* evidence suggests that the mutation is likely to be pathogenic [2]. The result was consistent with a diagnosis of autosomal recessive congenital hyperinslinismdue to homozygous *HADH* mutation. Both the siblings were advised to continue diazoxide treatment at a dose of 5 mg/kg/day with frequent blood sugar monitoring. Appropriate dietary advice was also provided. On follow-up, both the sisters are maintaining blood sugar within acceptable range.

DISCUSSION

Deficiency of short - chain - L-3 - hydroxyacyl - CoA dehydro-genase enzyme as a result of a mutation in the *HADH* gene is a rare cause of hyperinsulinemic hypoglycemia in infancy. In our case, two sisters presented with recurrent episodes of seizures and hypoglycemia from 3-4 months of age, without documented urinary ketones or metabolic acidosis. Critical samples during hypoglycemia revealed hyperinsulinemia and mild hyperammonemia. At this stage, we thought of the possibilities of mutations in either *GLUD1* or *HADH* gene. *GLUD1* encodes for the mitochondrial enzyme glutamate dehydrogenase (GDH), which is expressed in liver, kidney, brain and pancreatic beta cells. Mutations in this gene are responsible for hyperinsulinism/ hyperammonemia (HI/HA) syndrome, the second most common type of CHI. Affected infants manifest with fasting as well as protein sensitive hypoglycemia, along with persistently elevated serum ammonia. The hyperammonemia in our case was milder than that typically observed in HI/HA. Mutation analysis identified a novel homozygous missense mutation in the *HADH* gene. Children with *HADH* gene mutations have fasting and protein-induced hypoglycemia similar to patients with HI/HA and respond well to diazoxide therapy. In our cases, since the family was vegetarian, no clear suggestion of induction of hypoglycemia by high protein diet was present.

The *HADH* gene encodes for the enzyme short-chain 3-hydroxyacyl-CoA (SCHAD) which catalyzes the penultimate step in the fatty acid oxidation cycle. Though fatty acid oxidation defects causing hypoglycemia are well understood phenomena, the basis for its association with hyperinsulinemia has been elucidated more recently. It has been noted that SCHAD is an inhibitory regulator of the enzyme GDH, which catalyzes the oxidative deamination of glutamate to α-ketoglutarate and ammonia, and is involved in amino-acid stimulated insulin secretion. Loss of GDH's inhibition due to SCHAD deficiency results in insulin dysregulation [3], and protein-sensitive hyperinslinemic hypoglycemia. SCHAD also negatively regulates insulin secretion through a mechanism independent of the KATP channel[4].

Biochemical markers of congenital hyperinsulinism due to *HADH* gene mutation include increased concentration of 3-hydroxybutyrylcarnitine in plasma and 3-hydroxyglutaric acid in urine. These children do not exhibit the cardiac, skeletal or hepatic dysfunction associated with fatty acid oxidation disorders, and usually show good response to diazoxide and maintain blood sugar with minimum dose [5], as in our cases.

In conclusion, one should suspect *HADH* gene mutation in infants with hyperinsulinemic hypoglycemia with a relatively late onset (after 2-3 months), especially if precipitated by protein-rich foods. Although rare, this is an important cause of autosomal recessive form of CHI. Molecular diagnosis not only confirms the diagnosis but also helps in explaining to the parents the nature of the condition, need for avoiding high protein diet, continuing diazoxide, and chances of recurrence in subsequent offspring.

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