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The First Iranian Case of N-acetyl-glutamate Synthase (NAGS) Deficiency Treated with N-carbamylglutamate

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Abstract Background: N-acetyl-glutamate synthase (NAGS) deficiency is a rare cause of severe neonatal hyperammonemia. Case report: An 8-day old boy, who was born of non-consanguineous Iranian parents by cesarean section, was admitted to the neonatal intensive care unit due to poor feeding, unconsciousness, and seizures. High Ammonia (920 µmol/L, ref. < 100), high plasma glutamine (1628.6 µmol/L, ref. 410-960) and alanine (1151.5 µmol/L, ref. 200-600), low plasma citrulline (6.6 µmol/L, ref. 8-47) and arginine (26.7 µmol/L, ref. 20-160), without orotic aciduria (orotic acid in urine below detection limit) was revealed in metabolic work-up. Based on these results carbamoyl-phosphate synthetase 1 (CPS1) or NAGS deficiency were suspected. The infant was treated by peritoneal dialysis, intravenous sodium benzoate, L-arginine, and oral sodium phenylbutyrate and ammonia declined to 390 µmol/Lafter 10 days. Results: The genetic analysis in the patient and parents confirmed the NAGS deficiency with a novel heterozygous maternal missense mutation in exon 5 c.1172T>G (p.Leu391Arg) and the known change in exon 6c.1450T>C (p.Trp484Arg) on the paternal allele. Carglumic acid (Carbaglu®, Orphan Europe Recordati, Paris, France) was started and ammonia declined to normal (55 µmol/l) after 24 hours, for the first time ever in the patient. Based on the severe neurological impairment due to the initial hyperammonemic crisis and difficulties to access to the drug in Iran, a decision was made with the parents to stop treatment with carglumic acid (while sodium benzoate and sodium phenylbutyrate were continued) and the patient died five days later due to hyperammonemic decompensation. Conclusion: NAGS deficiency, although rare, seems to be panethnic. Thus, in case of hyperammonemia without orotic aciduria but with low plasma citrulline, NAGS deficiency should be considered and a trial with carglumic acid started as early as possible. Our case demonstrates that the prognosis of neonatal onset NAGS deficiency largely depends on early recognition and start of therapy.

Keywords: *Urea cycle disorder, NAGS deficiency, N-carbamylglutamate, hyperammonemia*

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1. Introduction

N-acetyl-glutamate synthase (NAGS) deficiency (MIM#237310) is the rarest form of urea cycle disorders (UCDs) [1]. This enzyme produces N-acetyl-glutamate (NAG), the essential activator of the first urea cycle reaction, catalyzed by carbamoyl-phosphate synthetase 1 (CPS1), from glutamate and acetyl-CoA [2].

NAGS is located in the mitochondrial matrix mainly of periportal hepatocytes in the liver and in small intestinal cells, as well as with lower expression in few other tissues [3,4].

The age of clinical presentations can vary from early and severe neonatal hyperammonemia to mild adult-onset form depending on the severity of enzyme deficiency [1,2,5-11]. Near to half of the patients hitherto described show an early onset form of disease. Neonatal patients can present in the first days of life with poor feeding, vomiting, unconsciousness, and seizures [6,7,9,13]. Patients with late-onset NAGS deficiency, defined as an onset outside the neonatal period, may present at any age in life with chronic headaches and nausea [6,9,13]. The clinical picture of NAGS deficiency is indistinguishable from CPS1 deficiency because in both conditions plasma citrulline is low, and urinary orotic acid is low normal or decreased [5,7,9,14,15,16]. The discrimination between these two disorders can only be achieved through enzymatic studies [17] or mutation analyses [3].

To date, 34 patients from 28 families with NAGS deficiency have been reported [18]. Here we describe the first Iranian case of NAGS deficiency that presented with severe neonatal hyperammonemia in the first week of his life. This case report underlines the panethnic character of NAGS deficiency and the utmost importance of an early start of treatment to avoid severe neurological disease.

2. Case

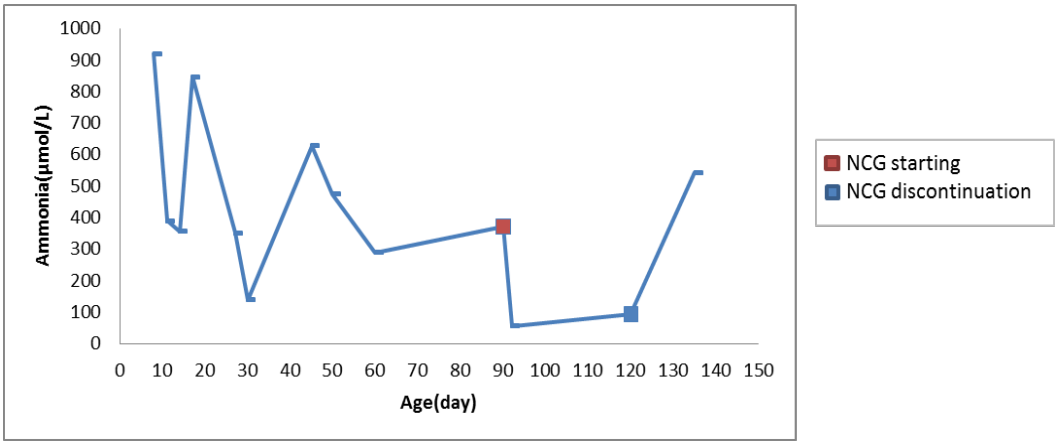
A.M. was born at 39 weeks of gestation by cesarean section without any prenatal or perinatal complications. He was the second child of Iranian non-related healthy parents. His parents lost another child at 13 months of age due to hyperammonemia without an exact diagnosis.

Birth weight and head circumference were 3400 grams and 35 cm respectively. The Apgar score was 8/9 and physical examinations were completely normal. He was on breastfeeding and did not have any problem during the first five days of his life. Due to the family history, a full metabolic work-up including plasma amino acid and acyl carnitine profiles and urinary organic acids assessment has been done on the third day of life which was all completely normal.

On the 6th day of life, poor feeding and sleepiness was obvious and on the 8th day of life, he developed vomiting, lethargy, and respiratory distress and was admitted to the neonatal intensive care unit (NICU). On admission, he was resuscitated, intubated, and mechanical ventilation was started. At that time, ammonia was extremely high (920µmol/L, ref. < 100) and metabolic acidosis (pH7.21, HCO38.6mmol/L, BE-13.5) was revealed. The metabolic acidosis disappeared after 24 hours (pH 7.36, HCO324mmol/L) without any intervention apart from fluid replacement. Electrolytes, blood glucose and liver function tests were normal, and urine ketone bodies were negative.

Oral feeding was stopped on the day of hospitalization and 12.5% glucose infusion (120 kcal/kg per day) was started. On physical examination at this time, liver was palpable (1.5 cm below the costal margin) with truncal hypotonia and spasticity in extremities.

Since a UCD was suspected, intravenous L-arginine hydrochloride (600 mg/kg/d) and sodium benzoate as well as oral sodium phenyl butyrate (both with loading doses 500 mg/kg followed 500 mg/kg/d) by nasogastric tube was started, but there was no improvement in the clinical condition and the plasma ammonia level stayed high (701µmol/L). Therefore peritoneal dialysis (PD) was initiated on the day of admission. After six days dialysis, ammonia decreased to 356 µmol/L and the clinical situation improved allowing to stop PD and mechanical ventilation on the 6th day after admission. Oral feeding with UCD formula was started on the 7th day of admission. After three days ammonia levels raised again so the neonate became nil per mouth and PD started again accompanied by the sameregime of drugs as before. Under this regime, ammonia unfortunately never normalized (Figure 1 and Table 1). During hospitalization seizures occurred which were treated with anti-convulsant drugs, however the EEG reported no epileptic discharges. Brain ultrasound showed brain edema.

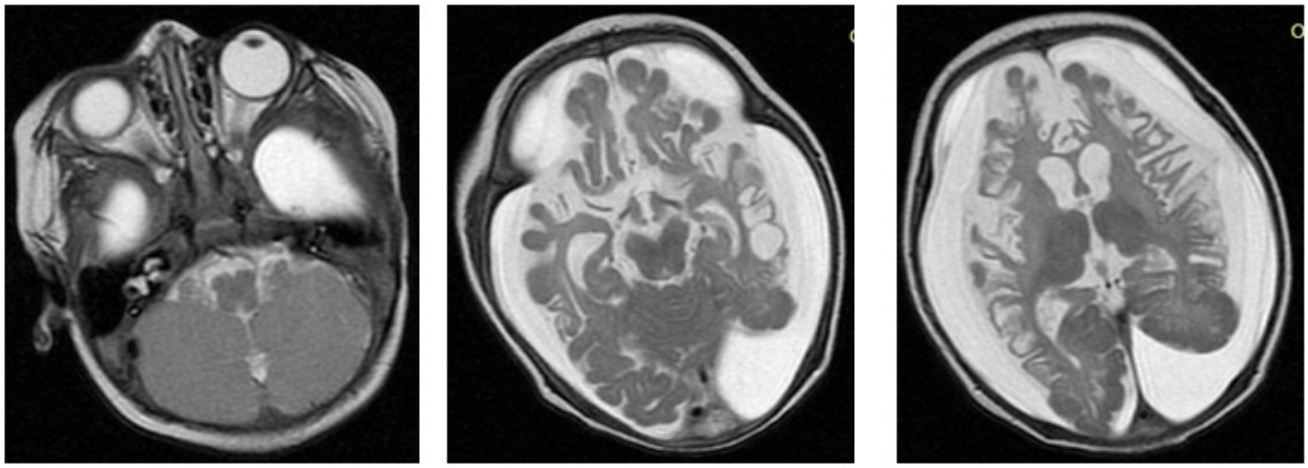


This graph shows the summary of all ammonia levels obtained in the patient and the variation of ammonia level during treatment. Unfortunately, parents did not check ammonia between days 90 and 120 due to good clinical situation.

Figure 1. The variation of ammonia level during treatment

Table 1. Ammonia and amino acid levels in plasma of patient A. M

Variable	Control	Age												
		8 d	*11 d*	*14 d*	*17 d*	*27 d*	*30 d*	*45 d*	*50 d*	*60 d*	*90 d*	*92d*	*120 d*	*135 d*
Ammonia	17-60 (µmol/L)	920	390	3556	847	352	139	627	475	288	371	55	93	542
Lactate	0.9-20(mg/dl)	80	15	17	16	16.8	20	29	18	16	-	-	14.2	35.3
Glutamatic acid	10-190(µmol/L)	312	103	254	-	57	66	123	-	315	87	-	87	66
Glutamine	410-960(µmol/L)	1628	1002	989	-	629.7	674	932	-	504	728	-	414	832
Citrulline	8-47(µmol/L)	6.6	10	6.9	-	5.2	7	4.3	-	2.7	3.8	-	33	5.9
Arginine	20-160(µmol/L)	26	28	180§	-	20	83§	32	-	33	33	-	41	54
Ornithine	20-135(µmol/L)	27	18	61	-	49	15	39	-	41.7	27	-	40	32
Alanine	200-600(µmol/L)	1151	600	687	-	249	413	470	-	297	445	-	364	423
Peritoneal Dialysis		+	+	-	+	-	-	+	+	-	-	-	-	-
Protein intake (g/kg/d)		-	-	0.5	-	0.25	0.5	-	-	1	1-1.5	2	2.5	-
Carglumic acid (mg/kg/d divided in three doses)		-	-	-	-	-	-	-	-	-	NCG started	100	100	-



Axial T2 weighted MR images from the patient taken at age 4 months illustrate the severe supratentorial brain damage as sequelae from longstanding hyperammonemic encephalopathy. While infratentorial structures (Figure 1A) appear normal, both hemispheres are atrophic (Figure 1B,C) but basal ganglia are spared from this with only minor atrophy of caudate nuclei (Figure 1C).

Figure 2. Brain MRI at 4 months of age

In the metabolic screening, profiles of blood acylcarnitines and urinary organic acids were normal and orotic acid in urine was below detection limit. Based on high levels of plasma glutamate (312.9 µmol/L, ref. 10-190), glutamine (1628.6 µmol/L, ref. 410-960), and alanine (1151.5 µmol/L, ref. 200-600) but low citrulline (6.6 µmol/L, ref. 8-47), an enzymatic block before ornithine trans-carbamylase (OTC), including CPS1 or NAGS deficiency were the most probable diagnoses. Genetic analysis confirmed NAGS deficiency with a heterozygous novel missense mutation in exon 5 of the NAGS genec.1172T>G (p.Leu391Arg), and in exon 6 the known change c.1450T>C (p.Trp484Arg). The analysis of the parents for carrier status confirmed the mutation c.1172T>G in exon 5 in the mother and c.1450T>C in exon 6 in the father.

As N-carbamylglutamate(NCG) was not available in Iran at that time, treatment with high doses of oral sodium phenylbutyrate, sodium benzoate, and L-arginine with UCD formula were continued. By this regimen the ammonia levels were between 140-370 µmol/Lbut never normalized.

At three months of age, carglumic acid (Carbaglu®, Orphan Europe Recordati, Paris, France) was started with 100 mg/kg/d divided in three doses. After 24 hours, plasma ammonia decreased for the first time in this patient to a normal level (55 µmol/L) (Figure 1). Growth parameters at that time showed failure to thrive with weight4800 g (<5%), head circumference37 cm (<5%), and length 54 cm (<5%). In the physical exam, he had truncal hypotonia with spasticity in extremities, could not control his head and did not have normal gaze. Brain CT scan and MRI showed marked brain atrophy, bilateral low attenuated white matter of centrum of semi-oval and peri-ventricular areas (Figure 2).

As the ammonia reached to the normal level when carglumic acid was introduced, the protein content of the diet was increased gradually and he was fed with half breast milk and half UCD formula. This therapy continued for one month and the child did not have any problem. Unfortunately, at 4.5 months of age carglumic acid was discontinued according to the parents' decision because of severe neurological impairment secondary to the initial hyperammonemic crisis, the high costs of the treatment and the difficulties to establish this as a long-term therapy. When carglumic acid was stopped, ammonia increased dramatically to 542 µmol/L and the clinical situation worsened rapidly. The child died at4.5months of age after five days of discontinuation of carglumic acid due to cardiopulmonary arrest despite of high dose sodium benzoate, sodium phenylbutyrate, L-arginine hydrochloride and protein restriction.

3. Discussion

The first case of NAGS deficiency was described in 1981 by Bachmann et al. [1]. The human NAGS gene is located on chromosome 17q21.31 and consists of 7 exons and 6 introns [3,4,5]. Cloning of the human NAGS gene was achieved in 2002 by Caldovic et al. [4] which allowed the molecular testing to become the best method of diagnosis [5,6,13,18]. Up to now 22 pathogenic mutations in the NAGS gene, including 15 missense, 1 nonsense, 4 frame-shift and 2 splice site mutations have been reported [9,18]. In addition, one disease causing mutation has been found in the regulatory region [20], but no amino acid substitution has been reported either in the mitochondrial targeting signal or the variable segment of theNAGS gene [21].

In our case, we found a heterozygous mutation in exon 5 c.1172T>G (p.Leu391Arg), and in exon 6 c.1450T>C (p.Trp484Arg). To our knowledge, the mutation in exon 5 is a novel mutation, while the mutation c.1450T>C was found before in neonatal onset NAGS deficiency.

Nearly half of reported patients were homozygous and the others were compound heterozygotes [8,23,24]. Homozygosity for nonsense or frame-shift mutations will cause complete absence of NAGS enzyme or residual enzyme activity less than 5%, and most likely results in a severe neonatal-onset form [3,4,6,9,16,17,25]. Missense mutations, depending on the effect of the single amino-acid substitution, may result in either complete or reduced residual activity of NAGS, which can cause later and milder forms of disease [3,6,16]. However, there is a poor genotype-phenotype correlation [3,6,9].

The correct diagnosis of NAGS deficiency has a significant clinical impact because, unlike in other UCDs

requiring intensive treatments including protein-restricted diet, medications, dialysis and liver transplantation, NAGS deficiency can be effectively treated with N-carbamylglutamate, which completely restores ureagenesis [14]. This has been also clearly demonstrated in our patient, who rapidly showed normal ammonia levels under treatment with carglumic acid. Therefore, to confirm the diagnosis by mutation analysis and the use of carglumic acidin every patient with a high suspicion of this disorder are highly recommended [19,27].

As we did not have access to this drug in Iran, we could not treat our patient immediately after the diagnosis was made (or even earlier upon suspicion of the diagnosis) so he experienced long time hyperammonemia which caused severe brain damage and neurological sequels. As soon as the drug became available, it was started, and ammonia levels decreased dramatically. He did not have any hyperammonemic episode while under carglumic acid, whereas before he never had normal ammonia levels despite full doses of sodium benzoate, sodium phenylbutyrate, L-arginine, and restricted protein diet. Unfortunately, severe brain damage secondary to hyperammonemic crises indicated a poor outcome thus limiting the benefits from this therapy which was also difficult to establish as long-term therapy and very costly, so the parents decided to withdraw the treatment despite of the good biochemical response. As expected, ammonia levels increased rapidly after discontinuation of carglumic acid and the patient died at 4.5 months of age due to cardiopulmonary arrest after hyperammonemic crisis.

It is very likely that if the patient was diagnosed and treated in his first episode of hyperammonemia in neonatal period, the outcome would have been entirely different. Following the diagnosis of this patient, the drug has recently been registered by the ministry of health (MOH) and we hope to not confront a similar situations in next cases. To prevent similar conditions and to achieve an improved outcome it is required to arrange international or regional collaborations in the Middle East to ensure early diagnosis and treatment of patients with rare diseases that require immediate management.

Although the treatment of our patient was started too late, we fortunately will be able to help this family in a future pregnancy by offering prenatal diagnosis (PND), and hereby prevent a similar catastrophic situation.

In conclusion NAGS deficiency is an exceedingly rare disease, but it is the only UCD that can be effectively treated with a drug. Therefore, it has been suggested that all hyperammonemic newborns with a suspected diagnosis of a UCD especially with low plasma citrulline and absent or low urinary orotic acid, should receive a therapeutic trial of NCG, which may provide a life-saving therapeutic option for patients with NAGS deficiency [8,24,27].

Abbreviations

Urea cycle disorders (UCD), N-acetyl-glutamate synthase (NAGS), carbamoyl-phosphate synthetase 1 (CPS1), intensive neonatal care unit (NICU), N-carbamylglutamate (NCG), peritoneal dialysis (PD), ornithine transcarbamylase (OTC),ministry of health (MOH), prenatal diagnosis (PND).

Conflict of Interest

There is no conflict of interest for this paper.

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