Neuroimaging Findings in Hyperargininemia

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ABSTRACT

In hyperarginenemia, there is a defect in argininase enzyme, which is a catalyzer of urea cycle. Though the pathogenesis of neuronal damage in hyperargininemia is not clear, high serum and cerebrospinal fluid arginine levels can be directly related with neuronal damage. In this study, our aim was to assess brain magnetic resonance images and magnetic resonance spectroscopy (MRS) patterns of two siblings with hyperarginenemia. We acquired single voxel MRS from the white matter to show the myelination pattern and to figure out any abnormal peak of metabolite stored due to enzymatic defect. We observed mild cerebral and cerebellar atrophy and infarct at bilateral posterior putamen and insular cortex localization on conventional images and elevated choline/creatine ratios and abnormal peak at 3.8 ppm, most likely representing arginine deposition. To the best of our knowledge, this is the first article revealing the brain MRS pattern of hyperargininemia. We reported the clinical and imaging findings of patients and discuss the correlation.

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Introduction

The urea cycle is composed of a series of reaction in which arginase is the final enzyme. The first three enzymes: N-acetyl-glutamate synthase, carbomoyl phosphate synthase, and ornithine transcarbamylase function inside the mitochondria whereas the latter three: argininosuccinic acid synthase, argininosuccinic acid lyase, and arginase, act in the cytosol. Arginase catalyzes the hydrolysis of arginine to ornithine and urea. Its deficiency results in an autosomal disorder named hyperargininemia.[1,2] The clinical features of this rare disease differ from other urea cyclus enzyme deficiencies. The most prominent physical findings are spastic paraparesis or paraplegia with lesser effects on the upper extremities, increased deep tendon reflexes, scissoring and cross adductor responses, toe walking, ataxia, choreoathetosis, retardation in motor and mental developmental milestones, poor growth, seizures, and EEG abnormalities. Severe neurologic disease leads to secondary skeletal abnormalities.[1-3]

In vivo brain metabolism can be noninvasively evaluated by means of magnetic resonance spectroscopy (MRS). It can be used to identify distinct metabolic patterns that are characteristic of specific disease processes.[4] In the relevant literature, there are some articles reporting the conventional brain magnetic resonance imaging (MRI) findings of hyperargininemia,[1,2] however, to the best of our knowledge MRS findings of this metabolic disease were not described yet. We aimed to emphasize the MRI and MRS pattern of this rare disorder and assess the influence of imaging findings on clinical stage.

Neuroimaging Methods

MRI examination was conducted on 1.5-T scanner (Gyroscan Intera Master, Philips, Best, The Netherlands). First, conventional MR images T1-weighted image (TR/TE: 450/10 ms) and FLAIR (TI: 2,000 ms, TR: 6,000 ms, TE: 1,110 ms), T2-weighted images (TR/TE: 5,304, 110 ms) were obtained. Subsequently, we selected point-resolved spectroscopy sequence (PRESS) with single voxel proton spectroscopy and used fast speen echo T2-weighted images (TR/TE: 5,304 ms/110 ms/1, 256×192 matrix, 3 mm slice thickness with 1 mm slice intersection gap) for the placement of voxel for ^{1}H MRS. Acquisition parameters were TR: 2,000 ms TE: 31 and 136 ms. Spectral sweep width of 1,000 Hz was used. Water suppression was obtained by three chemical shift selective radiofrequency wave pulses each followed by dephasing gradients pulses. The volume of interest (VOI) was $20 \times 20 \times 20$ mm^3. Voxel size and location was determined by using axial, coronal, sagital plane T2- weighted images. The voxels were positioned on the parietal white matter. The magnitude spectra were processed automatically using baseline correction and curve fitting procedures.

As we decided to compare the MRS patterns of both hyperargininemia patients, we planned to position voxels to the same localization. In the first case, there was increased T2 signal both in posterior putamen and insular cortex representing ischemic changes. But in the second case there was no similar abnormal finding. So we thought that it is not appropriate to compare the MRS patterns of this localization and decided to acquire MRS of normal appearing parietal white matter.

Case 1

Three years old male patient admitted to our outpatient clinic with the complaint of retardation both in developmental milestones and speech. He spoke 5-6 words and could not have created sentences. He was vivacious and had attention deficit. He was born from the third and uncomplicated pregnancy of 35 years old mother as the second living child. His parents were second-degree relative. No problem was faced at birth but there was delay in the developmental milestones. He could sit with support at 9th and started to walk at 18th months. On physical examination: body weight was 14.5 kg (50-75%), height

was 94 cm (25-50%), and his head circumference was 48.5 cm (3-10%). There was moderate-to-mild retardation in the fine and coarse motor functions and speech performance was consistent at 15 months. Cranial nerves examination was normal, muscle strength was normal, no spasticity was noted, deep tendon reflexes were normal, no pathologic reflexes were noted. Pes planus was noted. Intermediate degree mental retardation was detected in psychometric tests. Biochemical findings were normal except AST (116 U/L), ALT (308 U/L) and ammonia (172 μg/dL) (normal: 11-35 μg/dL). Thyroid function test was normal. In amino acid chromatography, 1,557.25 μmol/L (23-86 μmol/L), glutamine level was 192 μmol/L (normal: 333-809 μmol/L) in serum. Arginine level was 238.8 mmol/molcre (0-9 mmol/molcre) in urine and orotic acid level in urine was 194 μmol/mmolcre (<6 μmol/mmolcre). Arginase activity could not be measured in red blood cells. Brainstem auditory-evoked potentials (BAEP) and visual-evoked potentials (VEP) were normal. Mild cerebral and cerebellar atrophy, hyperintense signal at bilateral posterior putamen, and insular cortex localization were detected on T2-weighted series of cranial MRI (Fig 1A–B). In brain single voxel proton spectroscopy (SVS) with TE: 136 ms, N-acetylaspartate (NAA)/creatine (Cr): 2.44, choline (Cho)/Cr: 2.58 and arginine/Cr: .9 were obtained (Fig 1C). (NAA)/creatine (Cr): 1.19, Cho/Cr: .67 and arginine/Cr: .66 were noted in SVS with TE: 31ms (Fig 1D).

Case 2

The senior brother of the first case had been born after an uncomplicated pregnancy. He had an uneventful perinatal, neonatal, and infancy period with normal development until 2 years of age (head control at second, sit without support at 7th, walk at 12th month), and afterward he started to regress in motor

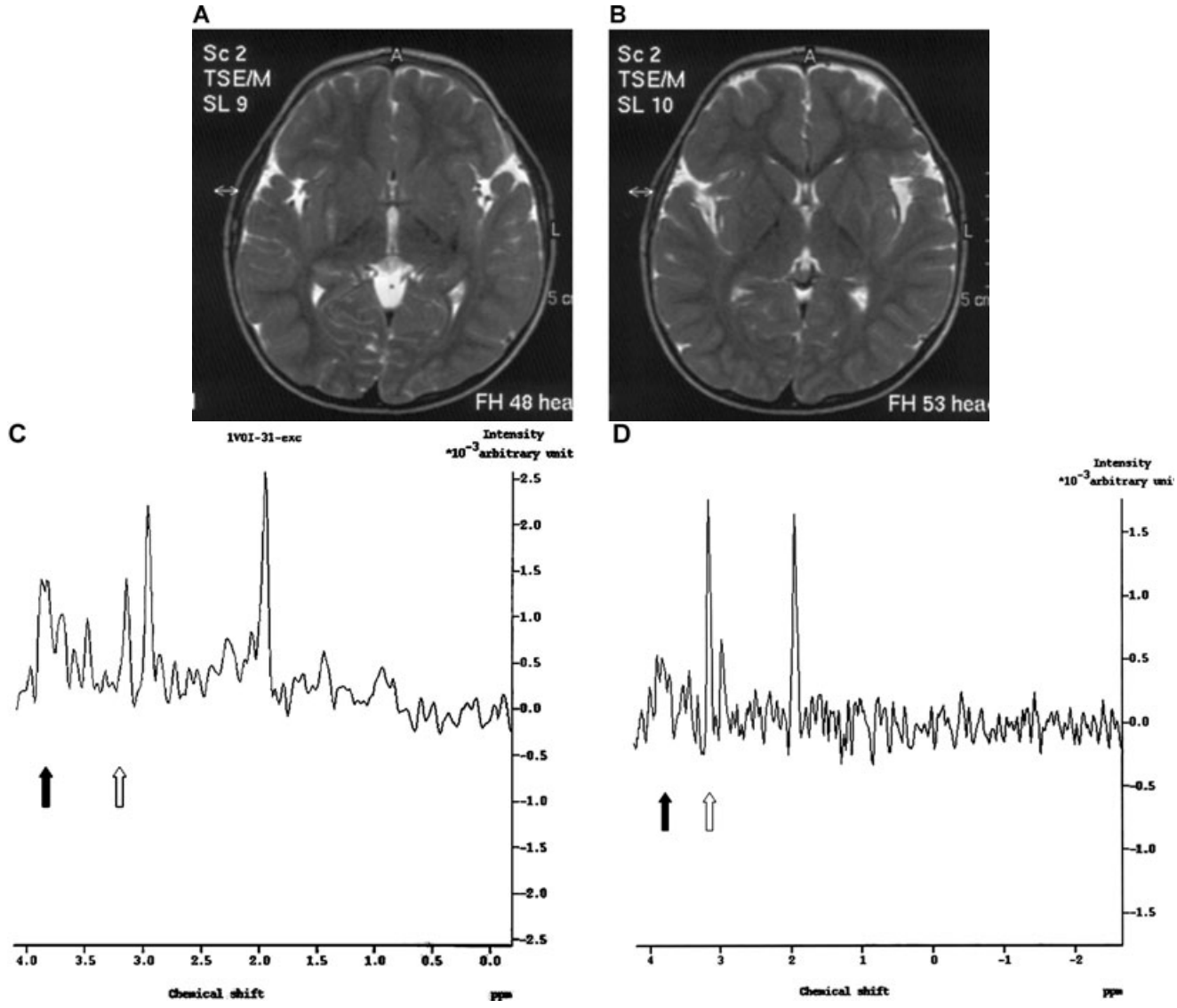

Fig 1. Axial T2-weighted images of 3 years old case. Significant T2 hyperintensity was noted at the posterior-lateral aspect of putamen (A) and at the bilateral insular cortex (B). Magnetic resonance spectra of normal appearing parietal white matter with short-echo time (C) and long-echo time (D). Arginine peak localized at 3.8 ppm (black arrows) and Choline peak (3.2 ppm) (white arrows) were noted.

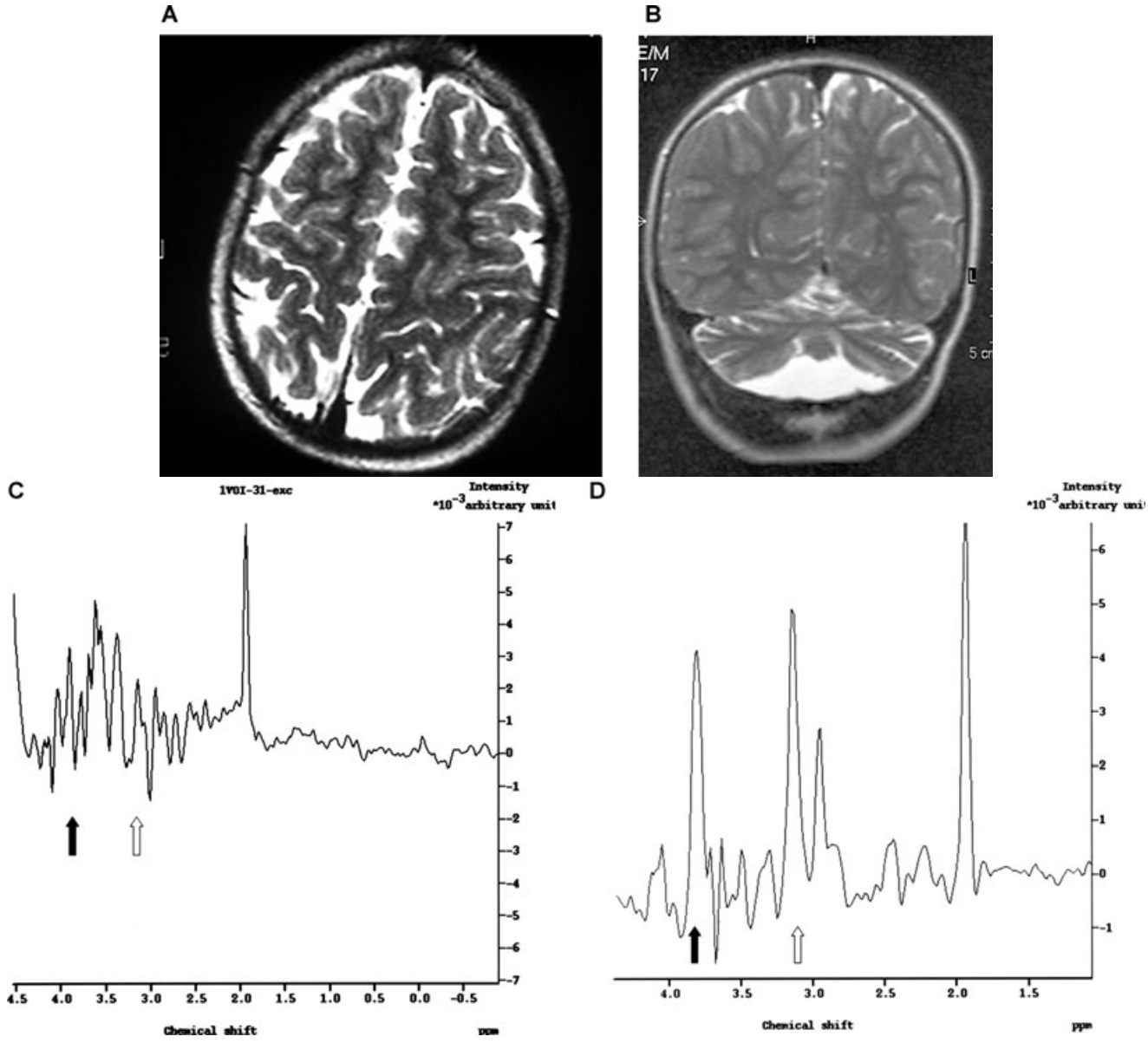

Fig 2. Axial and coronal T2-weighted images of 9 years old case. Cerebral (A) and cerebellar atrophy with associated mega cisterna manga (B) were noted. On magnetic resonance spectra of parietal white matter with short- echo time (C) and long-echo time (D) significant arginine and choline peaks (3.2 ppm) (white arrows) were shown.

functions. Now, he is speaking two or three words and cannot walk. We detected failure to thrive, microcephaly (head circumference was 45 cm, <3%), motor and mental retardation, spasticity, scissoring, strained achille tendon versus toe walking with support, increase in deep tendon reflexes, clonus, athetoid movements, pes equino varus deformity on physical examination. In laboratory examinations: AST 66 U/L, ALT 104 U/L, ammonia 162 μg/dL were noted. BAEP was normal but there was cortical unresponsiveness in VEP. In amino acid chromatography, arginine level was 897.5 μmol/L in serum, 54.2 mmol/molcre in urine, and 113.5 μmol/L (13.1-35.1 μmol/L) in cerebro spinal fluid (CSF). Glutamine level was 348.7 μmol/L (normal: 333-809 μmol/L) in serum and 262.5 μmol/L (normal: 356-680 μmol/L) in CSF. Excretion of orotic acid in urine was elevated. Cerebral and cerebellar atrophy and mega cisterna magna were noted (Figs 2A–B). In brain SVS with TE: 136 ms, NAA/Cr: 2.61, Cho/Cr: 1.84 and arginine/Cr: 1.55 were measured (Fig 2C). (NAA)/creatine (Cr): 359, choline (Cho)/Cr: 1,1 and arginine/Cr:1.61 were noted in SVS with TE: 31ms (Fig 2D).

Discussion

The urea cycle disorders are group of inborn errors of hepatic metabolism that affect the transfer of waste nitrogen into urea. The urea cycle (Fig 3) has two main functions: the detoxification of waste nitrogen into excretable urea and the de novo biosynthesis of arginine.[5] Deficiencies of different enzymes of urea

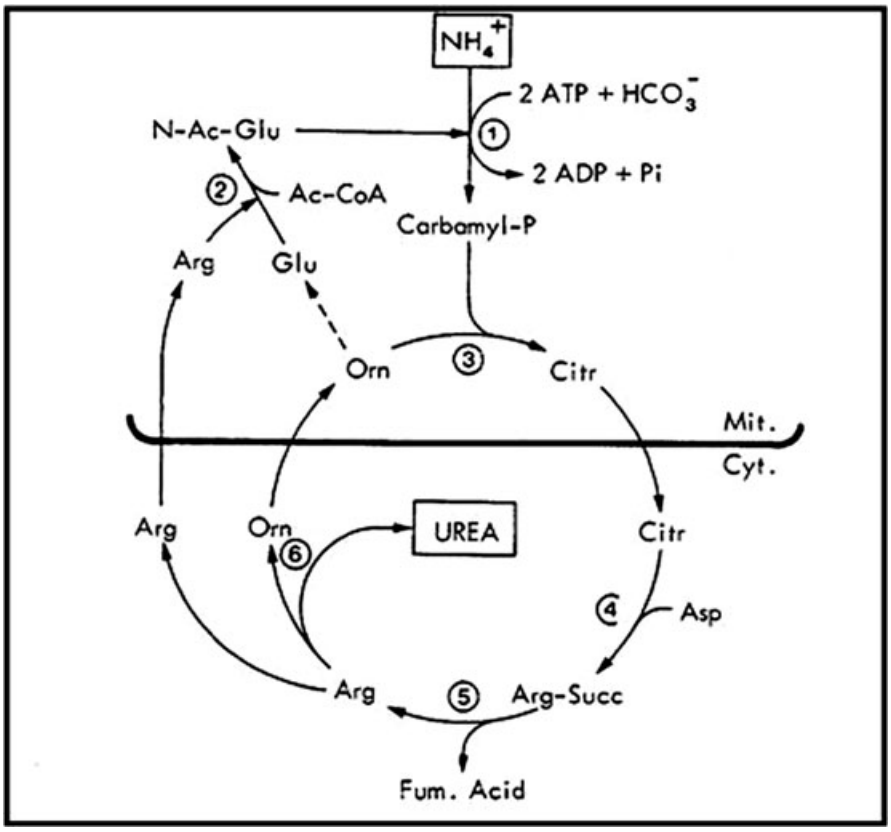

Fig 3. Metabolic pathway of urea cycle (1.N-acetyl-glutamate synthase, 2.Carbomoyl phosphate synthase, 3.Ornithine transcarbamylase, 4.Argininosuccinic acid synthase, 5.Argininosuccinic acid lyase, 6.Arginase).

cycle have been identified, and each specific disorder results in accumulation of different metabolites.[2] Hyperargininemia is a metabolic disorder biochemically characterized by accumulation of arginine in tissues. Seizures, lethargy, and psychomotor delay or cognitive deterioration are predominant clinical features of this disease.[1,3] Arginase deficiency was inherited autosomal recessively and its real incidence is unknown but estimated as 1 in 2,000,000 living birth.[2] Although neurological symptoms predominate in this disorder, their pathophysiology is still unknown.[2]

In hyperargininemia; elevated serum, urine, and CSF arginine levels,[6,7] excessive orotic aciduria, increased serum urea, and urinary urea excretion were related to increased protein intake.[6] In intermediate hyperammonemic cases increased serum and CSF glutamine levels can be observed.[7] In the hyperammonemic state, alanine, glutamine, glutamate, and sometimes lysine are increased in plasma and urine of the affected patients. Ammonia induces glutamate dehydrogenize to form glutamic acid from α-ketoglutaric acid, and induces glutamine synthetase to form glutamine from glutamic acid. On the other hand, arginine is an activator of N-acetyl glutamate synthase (NAGS), providing a mechanism for arginine to produce a positive feedback on ureagenesis.[7] These metabolic pathways (Fig 3) contribute to hyperargininemia and increased glutamine levels in arginase deficiency state.[5] The chronic presence of high levels of glutamine in CSF is believed to cause the fatal neurotoxicity.[8] But, CSF and serum glutamine levels were normal in our patients.

It is unlikely that elevated plasma ammonia is the main neurotoxic compound in hyperargininemia because hyperammonemia rarely occurs in this condition. In most hyperargininemia cases the neurological complications could result from the accumulation of arginine and its metabolites. In hyperargininemia several guanidine compounds, which are known to be in vitro and in vivo neurotoxins increases. Some guanidine compounds inhibit the activity of transketolase and they may produce demyelination with consequent upper motor neuron signs. Accumulation of guanidine compounds may affect GABAergic neurotransmission, resulting in epileptogenic properties. These compounds may induce seizures by decreasing the fluidity of the plasma membrane.[2]

Arginine may act as a precursor to glutamate or GABA and thus cause damage on an excitotoxic basis, and is also a precursor of nitric oxide and elevated levels of arginine may cause greater synthesis of nitric oxide and by that way oxidative damage via reduction of energy metabolism.[1-3,9] Arginine, N-acetylarginine, homoarginine, and argininic acid induced free radical defenses and decreased antioxidant defenses in vitro. These compounds inhibited the activities of catalase, superoxide dismutase, and glutathione peroxidase, the main enzymatic defenses in the brain against damage, at concentrations reminiscent of those observed in patients with hyperargininemia.[3] On

the other hand direct accumulation of chronically elevated levels of arginine on the neuronal tissue can be responsible from neuropathology.[2,3] In our patients elevated levels of arginine noted in CSF and serum were thought to be responsible for severe neurologic problems.

Neuroradiological imaging findings related with clinical findings in urea cycle defects are described by Takanashi et al[10]: (1) Type 1: severe cerebral edema following diffuse atrophy, (2) Type 2: infarcts like condition, (3) Type 3: watershed ischemic lesions, and (4) Type 4: reversible symmetric involvement of cingulate gyrus, temporal lobes, and insular cortex.

On MRI cerebral atrophy and increased signal intensity at T2-weighted images are seen. These T2 hyperintensities are consistent with abnormal myelinization at white matter[1,2] and cerebral edema.[11] In addition to the above mentioned findings, we have detected cerebellar atrophy in both of our cases, which has been reported to occur rarely in hyperargininemia cases.[9] Mega cisterna manga presented in the second case was accepted as a coincidental finding.

Brain MRS provides significant information about the metabolism and energy of cell membrane and status of neuroaxonal structure. Short-echo SVS was reported to depict most of the prominent resonances like: NAA, Cho, Cr, MI, glutamate, glutamine, lactate, and lipid. The decrease in the NAA/Cr ratio could reflect neuronal loss and the increase in the Cho/Cr ratio possibly suggests demyelination and inflammation.[12] In the literature, MRS study has not been reported yet in hyperargininemia, but in another urea cycle defect, ornithine transcarbamylase (OTC) deficiency; elevated levels of brain glutamine have been reported.[13,14] Arginine, glutamate, and glutamine produce mainly the common peak between 3.77 and3.79 ppm in brain MRS.[15] Kojic et al[13] had accepted this peak between 3.65 and 3.8 ppm as glutamate/glutamine complex. We observed high serum and CSF arginine with normal glutamate and glutamine in our patients and we thought that this peak noted at 3.8 ppm was formed mostly by arginine and to some extent by other metabolites: glutamine and glutamate.

It was well known that MRS with short-echo time was better than long-echo time in the examinations of glutamate and glutamine peaks.[12] When we compared the short- and long-echo time MRS patterns, we saw that the peaks between 3.5 and 3.8 ppm were more prominent at short-echo time spectra. However, the peak at 3.8 ppm was constantly seen prominent on all echo times. These peaks between 3.5 and 3.8 ppm were most likely representing glutamate and glutamine and myoinositol whereas significant peak at 3.8 ppm was representing mostly arginine deposition, which was supporting our prior assumption. We recommended acquiring both short- and long-echo MRS together in patients with urea cycle defects in order to differentiate arginine from glutamate-glutamine complex.

In late-onset ornithine transcarbamylase deficiency patients when compared with age-matched controls, NAA and Cr concentrations were normal in all patients and a decreased Cho concentration was detected only in two clinically severe patients.[14] We observed normal Naa/Cr ratio in both siblings as in the reports of Takanashi et al.[14] Increase in Cho/Cr ratios on SVS with TE: 136 ms of our patients were compatible with demyelinization in white matter areas. In these localizations, conventional MRI did not demonstrate any abnormality. These findings reveal that MRS demonstrates the metabolic changes due to hyperargininemia, initial finding of neurotoxic injury prior to end-stage changes like edema, cerebral atrophy, ischemic disease. As a result, with the aid of MRS we can investigate the amount of neurotoxic agent like arginine, glutamine, and glutamate deposited in normal-appearing cerebral tissue. Additionally, high arginine, glutamate-glutamine peaks noted in MRS showed the importance of direct toxic effect of these metabolites on neuronal tissue.

In metabolic diseases mental and motor function of patients and imaging findings deteriorate correlated to duration of disease. In our patients clinical findings was better in younger child. Additionally, Arginine/Cr ratio was lower compared to elder sibling, representing lower deposition. But interestingly, ischemic changes were noted in younger sibling. With above-mentioned data, we saw that ischemic changes were not directly correlated with the duration of disease and neurologic stage. We concluded that ischemic changes had separate etiopathologic mechanisms, it was not directly related to neurotoxic metabolite accumulation. We realized that arginine/Cr ratios were well correlating with the duration and stage of the disease.

In conclusion, cerebral and cerebellar atrophy, ischemic changes and edema could be seen in the conventional MRI of hyperargininemia patients. But, these findings may not constantly be seen on all patients. MRS has an important role in urea cycle defect by revealing the accumulation of metabolites. According to our findings MRS is a valuable tool as an adjunct to conventional MRI in the diagnosis of urea cycle defects and in the evaluation of neurologic involvement.

References

1. Crombez EA, Cederbaum SD. Hyperargininemia due to liver arginase deficiency. *Mol Genet Metab* 2005;84:243-251.
2. Scaglia F, Lee B. Clinical, biochemical, and molecular spectrum of hyperargininemia due to arginase I deficiency. *Am J Med Genet C Semin Med Genet* 2006;142:113-120.
3. Delwing D, Tagliari B, Streck EL, Wannamacher CM, Wajner M, Wyse AT. Reduction of energy metabolism in rat hippocampus by arginine administration. *Brain Res* 2003;983:58-63.
4. Cecil KM, Jones BV. Magnetic resonance spectroscopy of the pediatric brain. *Topic Magn Reson Imaging* 2001;12:435-452.
5. Bloom W, Huijmans JGM. Differential diagnosis of (inherited) amino acid metabolism or transport disorders. *Amino Acids* 1992;2:25-67.
6. Cederbaum SD, Shaw KN, Spector EB, Verity MA, Snodgrass PJ, Sugarman GI. Hyperargininemia with arginase deficiency. *Pediatr Res* 1979;3:827-833.
7. Grody WW, Kern RM, Klein D, et al. Arginase deficiency manifesting delayed clinical sequelae and induction of a kidney arginase isozyme. *Hum Genet* 1993;91:1-5.
8. Picker JD, Puga AC, Levy HL, et al. Arginase deficiency with lethal neonatal expression: evidence for the glutamine hypothesis of cerebral edema. *J Pediatr* 2003; 42:349-352.
9. Wyse AT, Stefanello FM, Chiarani F, Delwing D, Wannmacher CM, Wajner M. Arginine administration decreases cerebral cortex acetylcholinesterase and serum butyrylcholinesterase probably by oxidative stress induction. *Neurochem Res* 2004; 29:385-389.

10. Takanashi JI, Barkovich AJ, Cheng SF, et al. Brain MR imaging in neonatal hyperammonemic encephalopathy resulting from proximal urea cycle disorders. *AJNR Am Neuroradiol* 2003;24:1184-1187.
11. Harrington JW, Stiefel M, Gianos E. Arginase deficiency presenting with cerebral oedema and failure to thrive. *J Inherit Metab Dis* 2000;23:517-518.
12. Choi CG, Yoo HW. Localized proton MR spectroscopy in infants with urea cycle defect. *Am J Neuroradiol* 2001;22:834-837.
13. Kojic J, Robertson PL, Quint DJ, Martin DM, Pang Y, Sundgren PC. Brain glutamine by MRS in a patient with urea cycle disorder and coma. *Pediatr Neurol* 2005;32:143-146.
14. Takanashi J, Kurihara A, Tomita M, et al. Distinctly abnormal brain metabolism in late-onset ornithine transcarbamylase deficiency. *Neurology* 2002;59:210-214.
15. Tugnoli V, Mucci A, Schenetti L, et al. Molecular characterization of human gastric mucosa by HR-MAS magnetic resonance spectroscopy. *Int J Mol Med* 2004;14:1065-1071.