

Reviewer #1: Thank you for taking your valuable time to evaluate our article and for your criticisms because of the contributions to our paper.

1. Manuscript is important and instructive because this disease is an orphan disease. But, the title is not appropriate with the subject of the article. Because, the findings which are mentioned in article are seen in most of patients with argininemia. This cases weren't presented with Different Clinical Manifestations. So, the title should be corrected.

1. Answer: The title was changed to “A rare cause of spasticity and microcephaly: Argininemia”

2. The introduction and discussion sections should be revised with new and updated articles. For examples, while genetic section is discussed, recent articles should be used (Diez-Fernandez C, et al. Mutations and common variants in the human arginase 1 (ARG1) gene: Impact on patients, diagnostics, and protein structure considerations. Hum Mutat. 2018 Aug;39(8):1029-1050.

2. Answer: Introduction and discussion sections revised with new and updated articles. It is shown underlined in red color in the manuscript.

Recurrent nonconvulsive status epilepticus and liver failure are rarely defined clinical findings of the disorder (1-4) Arginase I mRNA therapy is a novel promising therapeutic approach to replace deficient proteins (6).

Hyperargininemia is one of the few treatable causes of spastic paraparesis and can be confused with cerebral palsy. Jichlinski et al. (2) reported a case of an 11-year-old girl who presented with a diagnosis of cerebral palsy, seizure, and fatigue.

Zhang et al. (10) reported two patients diagnosed by neonatal screening at the age of 13 days and 30 days who did not show obvious clinical features during follow-up period.

Diez-Fernandes et al. (11) reported 66 mutations, and showed most common mutations are p.Thr134Ile, p.Gly235Arg and p.Arg21* in Brazil, China and Turkey.

Added references;

2. Jichlinski A, Clarke L, Whitehead MT, Gropman A. "Cerebral Palsy" in a Patient With Arginase Deficiency. *Semin Pediatr Neurol*. 2018;26:110-114.
3. Bakhiet M, AlAwadi AMI, AlHammadi MM, Ali MF, Butti N. A case report of neurological complications owing to lately diagnosed hyperargininemia emphasizing the role of national neonatal screening policies in the kingdom of Bahrain. *Medicine*. 2018 ;97:e10780.
4. Yucel H, Kasapkara ÇS, Akcaboy M, Aksoy E, Sahin GE, Derinkuyu BE, et al. Recurrent hepatic failure and status epilepticus: an uncommon presentation of hyperargininemia. *Metab Brain Dis*. 2018;33:1775-8.
6. Asrani KH, Cheng L, Cheng CJ, Subramanian RR. Arginase I mRNA therapy - a novel approach to rescue arginase 1 enzyme deficiency. *RNA Biol*. 2018;15:914-22.
10. Zhang T, Yang J, Yin X, Yu P, Mooney R, Huang X, et al. Three novel mutations of ARG1 identified in Chinese patients with argininemia detected by newborn screening. *Clin Chim Acta*. 2017;466:68-71.
11. Diez-Fernandez C, Rüfenacht V, Gemperle C, Fingerhut R, Häberle J. Mutations and common variants in the human arginase 1 (ARG1) gene: Impact on patients, diagnostics, and protein structure considerations. *Hum Mutat*. 2018;39:1029-50.

3. The spelling mistakes of reference section should be corrected.

3. Answer: Spelling mistakes of reference section corrected. All references are written according to the author guidelines of Gazi Medical Journal.

Reviewer #2: Thank you for taking your valuable time to evaluate our article and for your criticisms because of the contributions to our paper.

This is a nice interesting case report deserving publication. Please rewrite according to the following minor comments. According to my opinion this article can be published after these changes are made.

1. For case 2; Is there a consanguineous marriage between parents? Medical history should be detailed The information should be given about the follow-up period of the patient.

1. Answer: He was the first child of a nonconsanguineous Turkish couple. He has not any seizures or encephalopathy, he has minimal spasticity. Our patient is being followed in another metabolism department in Turkey.

2. There are few typographical errors. which should be corrected. For examples; remove the points used at the end of the sentence in the table ‘ the mutation analyses’ as mutation analysis

2. Answer: Requested changes made.

Table 1 The Clinical Findings, Mutation Analysis, Motor and Mental Development of Our Patients

Current report	Ethnicity	Gender	Mutation	Clinical findings at diagnosis	Neurological status at this time
Case 1	Turkish	Male	c.231C>A	Psychomotor retardation, difficulty of walking and progressive tiptoeing	Moderate spasticity, he can walk without help and his intelligence is mildly impaired
Case 2	Turkish	Male	c.703G>C	Microcephaly and hypotonia	Minimal spasticity and can walk without any help. Intelligence is normal

Title page

Manuscript title: A Rare Cause of Spasticity and Microcephaly: Argininemia

Running head: Argininemia

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Abstract

Argininemia is an autosomal recessive urea cycle disorder caused by the deficiency of arginase. Our first case presented with psychomotor retardation, difficulty of walking and progressive tiptoeing. Laboratory investigations revealed mildly elevated hepatic enzymes, elevated plasma arginine concentration and increased blood ammonia level. Molecular genetic analysis was made for suspected argininemia and a novel homozygous mutation [p. S77R (c.231C>A)] was detected in *ARG1* gene.

Second case was admitted because of poor head control when he was six months old. Microcephaly was detected in his physical examination, and basic metabolic tests were studied.

Elevated levels of plasma arginine and orotic acid in urine organic acid analysis were compatible with argininemia. A homozygous mutation [p. G235R (c.703G>C)] was detected in *ARG1* gene and the diagnosis was confirmed.

Neurological symptoms such as ataxia, epilepsy, spastic paraparesis/ plegia and mental retardation can be seen in most of the patients with argininemia. Rarely, microcephaly can be the initial symptom.

Key words: Arginase deficiency, microcephaly, spastic paraparesis, urea cycle defect

Introduction

Argininemia is a rare, autosomal recessive, inborn error of metabolism due to arginase deficiency. Estimated prevalence is 1/363.000-2000000 individuals. It is a rare pan ethnic disease differs from other urea cycle defects and it is caused by mutations in *ARG1* gene (1).

The majority of the patients present with progressive neurological signs such as spastic paraparesis or plegia, failure to thrive, mental retardation, and seizures. Encephalopathy due to hyperammonemia, ataxia and athetosis, **recurrent nonconvulsive status epilepticus and liver failure are rarely defined clinical findings of the disorder (1-4)** Low protein diet, lowering regimens of ammonia and supportive care are the treatment methods of the disease (5). **Arginase I mRNA therapy is a novel promising therapeutic approach to replace deficient proteins (6).**

Case 1

Four-year-old male, second child of a consanguineous Turkish couple, presented with psychomotor retardation, difficulty of walking and progressive tiptoeing. He was born by spontaneous vaginal delivery with a weight of 3800 g after a 40-week of gestation. There was not any history of an acute encephalopathic event in newborn period. He was referred to our hospital with the diagnosis “cerebral palsy”.

His body weight was 12 kg (below the %3 percentile), height was 90 cm (below the %3 percentile) and head circumference was 48 cm (-2SD). Physical examination revealed increased tonus of lower extremities, tiptoeing and psychomotor retardation.

In laboratory investigations; aspartate aminotransferase level was 126 IU / L (N: 0- 34), alanine aminotransferase level was 153 IU / L (N:10-49), plasma arginine level was 1072 nmol / ml (N: 38-98), ammonia level was 38.4 μ mol / L (N: 11.2-35.4) and urinary orotic acid level was 182 mmol/mol creatinine (N: 0–11).

The diagnosis was confirmed by molecular **genetic** analysis. A homozygous novel mutation [p. S77R (c.231C>A)] was found in *ARG1* gene.

Low-protein diet with essential amino acid supplementation and sodium benzoate was started. Although the patient has moderate spasticity, he can walk without help and his intelligence is mildly impaired. Cranial magnetic resonance imaging (MRI) revealed no abnormal findings and he has not any seizures or encephalopathy. Plasma arginine levels remained around 300 nmol / ml after treatment.

Case 2

Six-month-old patient was admitted because of poor head control. He was born by caesarean section after 39 weeks of gestation with a weight of 3000 g and his head circumference was 33 cm (%3-10 percentile) at birth. **He was the first child of a nonconsanguineous Turkish couple.**

His body weight was 7.5 kg (25-50 % percentile), his height was 70cm (75-90 % percentile) and head diameter was 41.2 cm (below the %3 percentile). Hypotonia and microcephaly were major clinical findings of the patient.

In laboratory investigations; complete blood count, blood glucose, alanine aminotransferase, aspartate aminotransferase and creatine kinase levels were within normal limits. TORCH antibodies, cranial MRI, thyroid function and cytogenetic tests were also normal.

Basic metabolic tests were studied for inborn error of metabolism. Elevated plasma levels of arginine (840 nmol / ml, range: 38-98) and orotic acid level (150 mmol / mol creatinine (range: 0–11) in organic acid analysis were compatible with argininemia.

A homozygous mutation [p. G235R (c.703G>C)] was detected in ARG1 gene. Low-protein diet (1.5g / kg / day) with essential amino acid supplementation and sodium benzoate (250 mg / kg / day) was started. He has minimal spasticity and can walk without any help after therapy. His intelligence is normal. Plasma arginine levels remained around 200-300 nmol / ml during follow-up and head circumference was %3-10 percentile after treatment. Genetic counseling was given to these families and they were referred to an appropriate genetic center for preimplantation genetic diagnoses in Turkey.

The clinical findings, mutation analysis, motor and mental developments of the patients are shown in Table 1. These case reports were written after receiving informed consent from the families.

Discussion

The arginase is the final enzyme of the urea cycle and catalyzes the conversion of arginine to urea and ornithine.

Carvallo et al. (5) reported 16 patients with argininemia, and 6/16 (37%) of the patients have microcephaly. Lower limb spasticity was the first neurologic manifestation in twelve patients. Three individuals have seizure as their first clinical sign and one patient presented with ataxic tremor of the upper limbs. Although microcephaly was detected in six patients, it was not described as the first symptom.

Episodes of irritability, feeding difficulties and lethargy can be rarely seen in argininemia due to hyperammonemia (7). Although our first case had mildly elevated ammonia level, he has not any history of an acute encephalopathic event.

Hyperargininemia is more closely linked to neurological damage than hyperammonemia in argininemia. The neuropathogenic mechanism of hyperargininemia is not clearly understood. Some metabolites of arginine, such as guanidine compounds and elevated levels of nitric oxide were shown to damage to the brain because of their neurotoxic effects (8).

Hyperargininemia is one of the few treatable causes of spastic paraparesis and can be confused with “cerebral palsy”. Jichlinski et al. (2) reported a case of an 11-year-old girl who presented with a diagnosis of cerebral palsy, seizure, and fatigue. Our first case presented with progressive spasticity similar to this case and confused with cerebral palsy before the diagnosis.

Edwards et al. (9) reported that spasticity wasn't developed in a 6-year-old patient with argininemia who was diagnosed with neonatal screening program. Zhang et al. (10) reported two patients diagnosed by neonatal screening at the age of 13 days and 30 days who did not show obvious clinical features during follow-up period. Although our first case was diagnosed at four years of age, he has moderate spasticity with minimal impairment of intelligence. Second case has minimal spasticity and can walk without any help. His intelligence is also normal with appropriate treatment. Neonatal screening program combined with genetic analysis is important for early diagnosis and treatment.

Diez-Fernandes et al. (11) reported 66 mutations, and showed most common mutations are p.Thr134Ile, p.Gly235Arg and p.Arg21* in Brazil, China and Turkey. We found a novel homozygous [p. S77R (c.231C>A)] mutation in the first case and a common homozygous mutation [p. G235R (c.703G>C)] in the second one.

In conclusion; argininemia should be considered in differential diagnosis in the presence of progressive neurological signs such as, progressive spastic paraparesis/plegia, mental retardation and epilepsy. Although, microcephaly is an almost frequent clinical finding of argininemia, rarely it can be the initial symptom and may require investigation for inborn error of metabolism.

Acknowledgments

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Conflict of interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical approval

Our case reports do not require ethics committee approval. Written consent was obtained from their parents.

References

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11. Diez-Fernandez C, Rüfenacht V, Gemperle C, Fingerhut R, Häberle J. Mutations and common variants in the human arginase 1 (ARG1) gene: Impact on patients, diagnostics, and protein structure considerations. *Hum Mutat.* 2018;39:1029-50.

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