

Liver-transplantation-corrected coagulation dysfunction and neurological impairment in patients with argininemia

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Research

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Abstract

Background

Argininemia is a rare disease caused by inborn errors of metabolism. Advances in diagnosis and treatment have increased the number of patients receiving effective management. Argininemia is characterized by progressive spastic paraplegia, mental retardation, failure to thrive and irritability, and patients with coagulopathy are paid more attention. We studied the changes in coagulation dysfunctions and neurodevelopment in patients with argininemia before and after liver transplantation(LT).

Methods

The data in this study were obtained from the Liver Transplantation Center of Beijing Friendship Hospital, Capital Medical University between January 2015 and November 2019. We identified data related to argininemia in all patients diagnosed with urea cycle disorder, and extracted the details on coagulation, liver function, neurodevelopmental outcomes, histopathological and morphological examination, and other clinical presentations. The patients were followed up by telephone and/or in the clinic.

Results

Nine patients with argininemia diagnosed by tandem mass spectrometry and symptoms, and confirmed by gene sequencing. The symptoms deteriorated after dietary restriction in all patients with argininemia. Coagulopathy manifested before surgery, and no significant correlation was detected with plasma ammonia concentration. The coagulation dysfunction was completely resolved and progressive neurological impairment was prevented in seven patients with LT.

Conclusions

Coagulation dysfunctions are common manifestations of patients with

Introduction

Argininemia (also known as arginase deficiency or hyperargininemia; OMIM 207800) is an autosomal recessive inheritance disease caused by deficiency of arginase 1(ARG1), which is a rare type in urea cycle disorders (UCDs). The disease is panethnic, with an estimated incidence of 1 in 2 million live births¹⁻³. Compared with other UCDs, patients with argininemia are characterized by complicated manifestations and lack of specificity. Clinical presentations include progressive spastic paraplegia, growth deficit, liver damage, mental retardation, microcephaly, irritability, seizures, lethargy, nausea, reduced appetite and ataxia¹. Argininemia is often misdiagnosed as cerebral palsy (CP), cerebellar ataxia, and neurodegenerative disease. The progressive neurological impairment is an important feature that distinguishes argininemia from other UCDs, amino acid metabolism disorders and CP^{2,3}.

The accumulation of arginine in blood and other fluids is the hallmark of argininemia and presents in all patients, while hyperammonemic encephalopathy is rarely observed in contrast to other UCDs. Liver damage ranging from mild elevation of transaminases to liver failure have been reported in previous studies⁴. Additionally, coagulation dysfunction is the characteristic of patients with argininemia, which is not accompanied by life-threatening hemorrhagic complications, and the mechanism is still unclear⁵. Studies have suggested that elevated plasma ammonia is an

important cause of clotting disorders in other UCDs⁶; however, there is no consensus on whether coagulopathy in patients with argininemia is related to elevated plasma ammonia.

Dietary protein restriction, supplementation of essential amino acids, sodium benzoate and sodium phenylbutyrate play an important role in the treatment of hyperargininemia. Liver transplantation (LT) has also been approved for argininemia, which can significantly improve symptoms⁷. However, there are few reports about the coagulation dysfunction and the changes after LT in patients with argininemia. We reported nine patients with argininemia in this study; all of whom showed coagulation dysfunction and hyperammonemia. Seven patients had been treated with LT in our center, and the coagulation dysfunctions and plasma ammonia returned to normal within 1 month after LT.

Methods

Data Collection

We conducted a retrospective analysis of nine patients (six female, three male) with argininemia admitted to the Liver Transplantation Center of Beijing Friendship Hospital, Capital Medical University between January 2015 and November 2019. Blood arginine levels were analyzed by tandem mass spectrometry, and gene sequencing was performed in all patients to confirm mutation in the *ARG1* gene. Seven patients had undergone LT and two were waiting for the operation. The operation was approved by the Ethics Committee of Beijing Friendship Hospital. Age at diagnosis/onset/operation, sex, gene mutations, and neurological presentations are listed in Table 1. Laboratory data included ammonia level, platelet count, liver alanine aminotransferase and aspartate aminotransferase, total bilirubin, albumin, prothrombin time(PT), international normalized ratio(INR), activated partial thromboplastin time (APTT), and blood arginine concentration (Table 2).

Table 1
Clinical characteristics of nine patients with argininemia

	Gender	Age at presentation (months)	Age at diagnosis (months)	Age at operation (years)	Gene Mutation	Neurological presentations
Case1	Female	9	18	2	c.32T > C c.232dupG	Spastic paraplegia; Mental retardation; Language deficits
Case2	Female	12	18	2	c.603delT c.756-757insACAT	Spastic paraplegia; Seizure; Mental retardation
Case3	Male	4	Neonatal screening	1	c.603delT c.703G > A	Seizure; Mental retardation; Language deficits
Case4	Male	18	Neonatal screening	2	c.263-266delAAGA c.374C>T	Spastic paraplegia; Mental retardation; Language deficits; Ataxic tremor of the upper limbs
Case5	Female	22	34	3.5	c.703G > A c.295G > A	Spastic paraplegia; Mental retardation; Language deficits
Case6	Female	8	Neonatal screening	1.2	c.262-265delAAGA c.23T > C	Seizure; Mental retardation; Language deficits
Case7	Male	108	156	14.5	c.263-266delAGAA c.647T > C	Spastic paraplegia; Language deficits
Case8	Female	6	37		c.53G > A c.703G > A	Spastic paraplegia; Mental retardation; Language deficits
Case9	Female	24	60		c.51delG c.826 + 2T > C	Seizure; Spastic paraplegia; Mental retardation; Language deficits; Ataxic tremor of the upper limbs

Table 2
Laboratory data of nine patients in the group

	Case1	Case2	Case3	Case4	Case5	Case6	Case7	Case8	Case9	Reference range
Ammonia (μmol/L)	157	67	126	55	56	52	57	49	67	0–45
PT(s)	26.4	18.3	36.4	24.7	14.5	51	16.5	26.5	15	9.6–13.5
INR	2.68	1.58	3.14	2.19	1.27	4.6	1.51	2.39	1.38	0.8–1.2
APTT(s)	53.7	57.7	63.8	39.4	27.6	83.9	34.2	53.9	34.1	21–34
Platelet(*10 ⁹ /L)	283	333	200	384	350	339	201	350	173	100–300
AST (IU/L)	58	64	120	214	136	173	39	87	78	0–40
ALT (IU/L)	48	78	210	195	277	227	39	142	158	0–40
Albumin(g/L)	38.6	41.1	37	32.7	31.1	32.4	38.1	43.6	34.3	40–55
Total Bilirubin(μmol/L)	6	4.47	5.61	17.52	7.22	6.65	13.36	12.67	9.49	3.42–17.1
Blood arginine concentration# (μmol/L)	349.8	810	732.4	716.3	488.7	459.9	289.1	353.8	420	5–25
PT:Prothrombin Time; INR:International Normalized Ratio; APTT:Activated Partial Thromboplastin Time; AST:Aspartate aminotransferase; ALT:Alanine aminotransferase; #,maximum concentration of blood arginine.										

Statistical analysis

SPSS version 17.0 was used for statistical analysis. Correlation studies between plasma ammonia levels, INR values and APTT in patients were performed by calculating Pearson's correlation coefficient and significance was tested by the two-tailed test and $p < 0.05$ considered significant.

Results

Genetic analysis revealed two allele mutations in *ARG1* gene of all patients, which derived from their parents respectively, which was consistent with the characteristics of autosomal recessive inheritance. Three patients were found by neonatal screening, and the other six were confirmed by gene sequencing and plasma amino acid analysis after symptoms appeared. The maximum blood arginine concentration was 289.1–810 μmol/L.

Seven patients presented with typical spastic paraplegia of the lower extremities and progressive aggravation, but the upper extremities were not involved. Physical examination showed increased muscle tension and muscle strength decreased in bilateral lower limbs, ankle clonus and positive pathological signs. Three patients (Cases 1, 8 and 9) could not walk independently without help during the preoperative evaluation. Cases 8 and 9 were initially misdiagnosed with CP, and received rehabilitation training, but the symptoms became increasingly severe. Two patients (Cases 3 and 6) did not show obvious dyskinesia due to their younger age. Initial symptoms of three patients (Cases 3, 6 and 9) were generalized tonic–clonic seizures, which could be controlled by oral administration of topiramate and levetiracetam, but the drugs were withdrawn by the patients. Language deficits were observed in eight patients, with the exception of case 2. Only the late-onset Case 7 showed slow speech, while the rest lost the ability to speak sentences. All but Case 7 showed mental retardation, and ataxic tremor of the upper extremities was seen in Case 4 and 9. Growth deficit was observed in all patients, except Case 7. All patients manifested emotional instability and irritability.

All of seven patients who had done LT were emotionally stable and their irritable state completely disappeared. Muscle tone was decreased and growth deficit was corrected, while there was no improvement in ability to walk and speak and mental retardation in Case 1. The symptoms were alleviated in the other patients who had received LT; muscle tone was significantly decreased and gait improved concomitantly; and ability to speak was better than before the operation. Compared with normal children, the gap between the patients' behavior and intelligence gradually narrowed. Intellectual development in Case 2 was 2 years later than normal for that age. This means that postoperative mental development was normal. The postoperative follow-up period ranged from 6 to 59 months and patients are still being followed up.

Coagulation analysis indicated that PT was prolonged and INR was increased in nine patients, and APTT was significantly prolonged in five (prolonged > 10 s). At the same time, hyperammonemia was found in the blood of all patients. There was a positive Pearson's correlation between APTT and INR, but there was no significant correlation between them and plasma ammonia. All patients had elevated liver transaminases, while albumin and total bilirubin showed no significant changes, and platelet count was normal. No significant bleeding was found in any of the patients. Abdominal computed tomography (CT) showed mild hepatomegaly in two patients (Cases 2 and 9) and ultrasound examination showed enhancement of liver echogenicity in four patients (Cases 3, 6, 8 and 9).

Seven patients had been treated with LT and two were awaiting the operation (Table 2). Nonspecific findings revealed by histopathological and morphological examination included swollen hepatocytes, focal necrosis, mild inflammation characterized by lymphocytic infiltration, and increased cellular glycogen, without liver fibrosis and cirrhosis. Plasma ammonia and coagulation dysfunctions returned to normal within 1 month after the operation.

Discussion

ARG was discovered in mammalian liver tissue in 1904. Two isoenzymes, ARG1 and ARG2, are encoded by different genes in mammals^{8,9}, ARG1 is expressed highest in the liver cell cytosol, while ARG2 is mainly expressed in the prostate and kidneys. ARG1 is the final enzyme of the urea cycle, which converts arginine to urea and ornithine⁹, and deficiency of ARG1 leading to impaired ureagenesis is characterized by hyperargininemia². The *ARG1* gene is located on chromosome 6 (6q23) and comprises eight exons, and mutations in the gene cause changes in the enzyme structure that prevent it from functioning properly¹⁰. Although the functions of the two enzymes are not exactly the same, functional overlap between them may explain why severe hyperammonia was absent in patients with argininemia^{4,11}.

Blood and urine amino acid assays indicating high levels of arginine is the foremost clue to diagnosis. Analysis of *ARG1* gene is essential to confirm the diagnosis. Measurement of cytosolic ARG1 activity in red blood cells or liver biopsy is important for diagnosis. With the application of tandem mass spectrometry and genetic sequencing in neonatal and high-risk population screening, a growing number of patients with argininemia are diagnosed at the asymptomatic or early stage of the disease, and prognosis is improved by early treatment.

Dietary protein restriction, citrulline, sodium benzoate, and sodium phenylbutyrate are traditional treatment choices for argininemia, and can lower the levels of plasma arginine as well as prevent progression of symptoms³. A growing body of evidence supports LT for argininemia. Although LT does not reverse all neurological symptoms, it can prevent progressive aggravation of neurological symptoms⁷, and the idea was further confirmed in our study, and intellectual development was completely normal after LT in some patients.

The pathological mechanism of argininemia in nervous system damage is still unknown, and it may be related to direct damage by arginine or its metabolites, guanidino compounds and nitric oxide(NO)⁴. The plasma concentration and metabolites of arginine can be decreased to normal by LT, which is not achieved by traditional treatment⁷. Some studies have suggested that patients with two severe mutant alleles may be an indication for LT because they are less effective

at rigorous dietary control¹². The genetic analysis performed on nine patients admitted to our center with hyperargininemia revealed that they were compound heterozygotes, and the mutations in six patients were considered to be severe¹² (Cases 3–8), so this group of patients had poor response to dietary therapy. Age of onset, duration of arginine effect, peak plasma arginine, sensitivity of the nervous system to arginine and its metabolites, and genotype may be related to recovery, but the underlying mechanism and correlation need to be further studied³.

In addition to nervous system damage, the effect on liver is often manifested as mildly elevated ammonia and transaminase. Coagulation disorders with hyperammonemia episodes have been described previously, although there is no consensus on their relationship^{2,5}. All the patients in our study presented with marked coagulation dysfunction, and elevated liver transaminase and plasma ammonia were found in the same blood samples. CT and ultrasound examination showed mild hepatomegaly or echogenicity enhancement in five patients.

Although the mechanism of liver injury in UCD patients is still unclear, hyperammonemia is considered to be an important cause of liver damage^{5,6}. Fortunately, we got liver specimen by LT in seven patients with argininemia. Swollen hepatocytes, focal necrosis, mild inflammation characterized by lymphocytic infiltration, and increased cellular glycogen were observed in seven patients, which was consistent with previous reports¹³. No specificity was found in the histopathology of liver damage of argininemia patients, but these pathological changes were consistent with the tendency towards edema and reversible changes in hepatocytes caused by hyperammonemia¹⁴. Combined with imaging results that showed hepatomegaly and elevated liver transaminases during hyperammonemia, the findings suggest that liver dysfunction might be caused by hyperammonemia in patients with argininemia⁴.

PT was prolonged and INR was increased in all patients. APTT was prolonged for > 10 s in five patients with hyperammonemia, which is different from a previous report⁵. This maybe related to the severity of different groups of patients. None of the nine patients had life-threatening hemorrhage, despite the serious clotting disorder, which may be related to the decrease in clotting and anticlotting factors^{6,15}. Clotting factor V is considered to be a sensitive indicator of liver synthetic function, but levels are within the normal reference in patients with argininemia⁵. Measurement of coagulation factors was not included in the preoperative evaluation, so patients in this group were not further checked for coagulation factor deficiency. The deficiency in clotting factors did not result from the hepatic involvement of arginase deficiency⁵. Some underlying metabolic abnormalities may be involved in those patients.

It has been claimed that hyperammonemia can restrict liver protein synthesis, especially of liver-derived proteins with short plasma half-life. Decreased clotting factor VII and increased INR have been observed in ornithine transcarbamylase deficiency (OTCD) patients during hyperammonemia episodes⁶. In patients affected by acute liver failure, the increased INR is positively correlated with plasma ammonia levels, suggesting it is a sensitive index to reflect liver dysfunction associated with plasma ammonia⁶. However, one case report showed that there was no correlation between coagulopathy and plasma ammonia or serum albumin levels, which indicated the heterogeneous etiology of liver damage in OTCD patients¹⁶. Patients with argininemia present with permanent coagulation dysfunction in the absence of hyperammonemia, which suggests that coagulation dysfunction is independent of the patient's metabolic status and unrelated to hyperammonemia⁵.

All the patients in our study showed an increase in INR and hyperammonemia, but there was no significant correlation between them. Combined with histopathological results, we suppose that hyperammonemia may cause hepatocyte damage in patients with argininemia, but it may be irrelevant to coagulation dysfunction, and other potential mechanisms may play an important role. The coagulation dysfunction in hyperargininemia may be caused by NO, because the cycle of arginine metabolism is disrupted by arginase deficiency, leading to greater production of NO^{2-4,11}, which has an anticoagulant effect¹⁷. The active form of coagulation factor XIII stabilizes blood clotting, while NO has an

inhibitory effect on it by S-nitrosylation of a cysteine residue, resulting in clot solubilization or suppression of clot formation¹⁷. L-arginine, the principal substrate for NO synthase, suppresses expression of tissue factors in human microvascular endothelial cells, which is a critical determinant of thrombin generation¹⁸. We hypothesized that clotting dysfunction is related to inhibition of tissue factor by high levels of arginine and clot suppression by overproduction of NO in patients caused by ARG1 deficiency.

In this study, the coagulation dysfunction of seven patients returned to normal within 1 month after LT, because the arginine cycle can be normalized by LT through arginase supplementation in ARG1 deficiency patients. The metabolites of arginine are not completely reduced by traditional treatment, so persistent clotting dysfunction may be associated with its metabolite NO^{4,5,7}. In future, research on NO may be helpful to explain the mechanism of coagulation dysfunction and nervous system damage in patients with argininemia.

At present, the mechanism of coagulation dysfunction in argininemia patients is still unclear. In this study, we only included nine patients who underwent preoperative evaluation and selection bias may have been unavoidable. However, due to the rarity of the disease, it is difficult to conduct large cohort studies to study the interaction of coagulation disorders, hyperammonemia, clotting factors, liver metabolism function and changes after LT. Advanced and detailed laboratory analyses, longer follow-up, research on transformation of arginase and arginine cycle before and after surgery, and establishment of animal models will be helpful to elucidate the exact mechanism of coagulation disorder in patients with argininemia.

Conclusion

This study showed that coagulopathy is an important feature of ARG1 deficiency patients. Although the exact mechanism needs further study, coagulation functional analysis should be a routine check in patients. LT, as an important treatment for patients with argininemia, not only prevents the progression of symptoms, but also corrects coagulation dysfunction completely.

Abbreviations

LT: Liver Transplantation; UCD:Urea Cycle Disorder; ARG:Arginase; CP:Cerebral Palsy; PT:Prothrombin Time; INR:International Normalized Ratio; APTT:Activated Partial Thromboplastin Time; CT:Computed Tomography; NO:Nitric Oxide; OTCD:Ornithine Transcarbamylase Deficiency.

Declarations

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Authors' contributions

Z-JZ, L-YS, Bin-Cui and Lin-Wei: study concept and design. Bin-Cui, Y-LT, Jun-Wang: data collecting; manuscript writing. Wei-Qu, Z-GZ and Ying-Liu: statistical analysis and analysis of data; Z-JZ and L-YS study supervision; critical revision of the manuscript for important intellectual content. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate.

The study was approved by the Ethical Committee of Beijing Friendship Hospital, Capital Medical University. All subjects included in this study signed informed consent documents.

Consent for publication

Consent for publication was obtained from all participants.

Competing interests

All authors declare that they have no competing interests.

References

1. Sin YY, Baron G, Schulze A, Funk CD. Arginase-1 deficiency. *J Mol Med*. 2015;93(12):1287–96.
2. Crombez EA, Cederbaum SD. Hyperargininemia due to liver arginase deficiency. *Mol Genet Metab*. 2005;84(3):243–51.
3. Carvalho DR, Brum JM, Speck-Martins CE, et al. Clinical Features and Neurologic Progression of Hyperargininemia. *Pediatr Neurol*. 2012;46(6):369–74.
4. Scaglia F, Lee B. Clinical, biochemical, and molecular spectrum of hyperargininemia due to arginase I deficiency. *American Journal of Medical Genetics Part C: Seminars in Medical Genetics*. 2006;142C(2):113–20.
5. Kiykim E, Zubarioglu T, Cansever MS, et al. Coagulation Disturbances in Patients with Argininemia. *Acta Haematol-Basel*. 2018;140(4):221–5.
6. Laemmle A, Gallagher RC, Keogh A, et al. Frequency and Pathophysiology of Acute Liver Failure in Ornithine Transcarbamylase Deficiency (OTCD). *Plos One*. 2016;11(4):e0153358.
7. Silva ES, Cardoso ML, Vilarinho L, et al. Liver transplantation prevents progressive neurological impairment in argininemia. *JIMD reports*. 2013;11:25.
8. Kossel A, Dakin H. Über die Arginase. *Z Physiol Chem*. 1904;41:321–31.
9. Cederbaum SD, Yu H, Grody WW, et al. Arginases I and II: do their functions overlap? *Mol Genet Metab*. 2004;81:38–44.
10. Ash DE, Scolnick LR, Kanyo ZF, et al. Molecular Basis of Hyperargininemia: Structure-Function Consequences of Mutations in Human Liver Arginase. *Mol Genet Metab*. 1998;64(4):243–9.
11. Caldwell RB, Toque HA, Narayanan SP, Caldwell RW. Arginase: an old enzyme with new tricks. *Trends Pharmacol Sci*. 2015;36(6):395–405.
12. Uchino T, Snyderman SE, Lambert M, et al. Molecular basis of phenotypic variation in patients with argininemia. *Hum Genet*. 1995;96(3):255 – 60.
13. CEDERBAUM SD. Hyperargininemia with arginase deficiency. *Mol Genet Metab*. 1979;84(3):827–33. KNFS, SUGARMAN GI.
14. Grody WW, Argyle C, Kern RM, et al. Differential expression of the two human arginase genes in hyperargininemia. Enzymatic, pathologic, and molecular analysis. *J Clin Investig*. 1989;83(2):602–9.

15. Northup PG, Caldwell SH. Coagulation in Liver Disease: A Guide for the Clinician. *Clin Gastroenterol H.* 2013;11(9):1064–74.
16. Samuel N, Politansky AK, Hoffman R, Itzkovich S, Mandel H. Coagulopathy unmasking hepatic failure in a child with ornithine transcarbamylase deficiency. *The Israel Medical Association journal: IMAJ.* 2013;15(12):777.
17. BERNASSOLA F, ROSSI A, MELINO G. Regulation of Transglutaminases by Nitric Oxide. *Ann Ny Acad Sci.* 1999;887(1):83–91.
18. Yang Y, Loscalzo J. Regulation of tissue factor expression in human microvascular endothelial cells by nitric oxide. *Circulation.* 2000;101(18):2144–8.