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Original Article

Clinic Analysis on 4 Infantile Citrin Deficiency Cases

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Abstract ***Objective:*** To understand infant Citrin deficiency as a type of infantile cholestasis, and further improve the understanding of infantile cholestasis and Citrin deficiency, provide more effective treatment and prognosis for children. ***Method:*** 4 cases of infantile cholestasis hospitalised patients finally diagnosed infantile baby Citrin deficiency in the Department of Endocrine and Genetic Metabolism in Children of Hubei Maternal and Child Health Care Hospital, analyses the characteristics of medical history, the relevant test results, after treatment and after follow-up. ***Results:*** Infant with simple Citrin deficiency syndrome are treated with symptomatic treatment, the clinical symptoms are obviously relieved, and the indexes of liver function can be gradually restored to normal. ***Conclusion:*** To infants without cholestasis caused by the surgical condition and clear infection, early haematuria, tandem mass spectrometry, and related gene examination should be perfected to identify the cause. Infants with Citrin deficiency usually have a good prognosis.

Key words *Cholestasis; Citrin deficiency; Infant; Prognosis; Treatment*

Introduction

Citrin protein deficiency is a kind of autosomal recessive disease that elucidates the cause of disease in recent years, including two types: neonatal intrahepatic cholestasis (NICCD) caused by Citrin protein deficiency, and type 2 citrullinaemia (CTLN2). Especially in infancy mainly for slow retrogression of jaundice, cholestasis, hepatitis, if a not timely diagnosis of cholestasis, it may influence the prognosis of the infant with delayed treatment. But in general, if diagnosed infant Citrin deficiency, it can achieve most good prognosis. We summarise and analyse the clinical data of 4 cases which diagnosed "infant Citrin protein deficiency" in Hubei Province Maternal and Child Health Hospital from July 2016 to October 2017. It helps us to improve the understanding of neonatal cholestasis and Citrin protein deficiency, adopt more effective treatment and judge prognosis of children.

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Materials and Methods

Patients. Four boys, diagnosed "infantile cholestasis" originally, were investigated in this study. Blood samples of 4 infants were taken on an empty stomach with the informed consent of parents. After completing blood biochemistry, blood gas analysis, blood ammonia, coagulation function, haematuria tandem mass spectrometry, and related gene examination, they were

diagnosed with "infant Citrin protein deficiency" finally. Detailed information of 4 infants is shown in Table 1 below.

Various blood biochemical indexes. The blood biochemical indexes of 4 cases on empty stomach with 3 hours were shown in Table 2. Serum total bilirubin increased obvious, the ration of direct bilirubin reached 40%-79%, bile acid was significantly increased, biliary duct enzymes GGT and ALP increased. Two infants had slight impairment of liver function, and 2 infants had hyperlipidemia. Some infants had abnormal coagulation function, hypoglycemia, high blood ammonia and hyperglycemia. All the infants completed the liver and gallbladder colour Doppler ultrasound and biliary MRCP (Magnetic Resonance Cholangiopancreatography) to eliminate the situation of surgery, such as biliary atresia and related pathogenic examination to eliminate the infection of cytomegalovirus and septicemia.

Blood tandem mass spectrometry and gene diagnosis. The 4 infants tandem mass spectrometry results showed that the citrulline and many acylcarnitine (lauroyl carnitine, myristoyl carnitine, palmitoyl carnitine, etc.) were significantly increased. Citrulline ranges from 100 to 300 (reference value 5.5 to 45 μM), two of them also had elevated levels of multiple amino acids (methionine, threonine, and arginine). 4-hydroxybenzoate and other organic acid were increased in the urine of 3 infants, one had normal urine. All the 4 infants had completed gene examination, and the SLC25A13 heterozygous mutation was confirmed in 3 cases, and the gene examination results of patient 2 were negative. The Blood tandem mass spectrometry and gene diagnosis of 4 cases were shown in Table 3. Gene sequencing diagram of patient 1 was shown in Figure 1.

Treatment and follow-up. Four infants were hospitalised for several days. After the results of blood tandem mass spectrometry were reported, 4 infants were fed with lactose-free and strengthening MCT treatment of milk, supplemented with fat-soluble vitamins, ursodeoxycholic cholic acid, and other treatments. The review results of a liver function of 4 infants were shown in Table 4. During the hospitalisation period, cholestasis symptoms of patient 1, 2, 4 improved significantly, direct bilirubin and bile acid decreased significantly, aminotransferase improved, hypoproteinemia improved

Table 1 Detailed information of 4 infants

	Patient 1	Patient 2	Patient 3	Patient 4
Gestation age (years old)	26	29	35	33
Gender	Boy	Boy	Boy	Boy
Mode of delivery	Natural	Caesarean	Caesarean	Caesarean
Birth weight (kg)	3.45	4	3	3
Antenatal complications	–	Gestational diabetes	Threatened abortion	–
Consanguinity	Non consanguineous	Non consanguineous	Non consanguineous	Non consanguineous
Family history	–	–	–	Elder sister has jaundice delay
Mode of feeding	Breast feeding	Mixed feeding	Artificial feeding	Breast feeding
Age at presentation	1 month 12 days	2 months 2 days	5 months	4 months 23 days
Clinical features at presentation	Skin and icteric sclera, pale yellow stool	Skin and icteric sclera, yellow-white stool	Only icteric sclera, white stool, varicose veins of abdominal wall	Skin and icteric sclera, yellow-white stool
Age at diagnosis (Gene diagnosis)	3 months	–	7 months	6 months
Age when treatment commenced (After the results of blood tandem mass spectrometry)	1 month 12 days	2 months 10 days	5 months 7days	5 months
Time to normalisation of liver indices	6 months	4 months	No improvement	9 months

Table 2 Preliminary blood biochemistry results of 4 infants

	Patient 1	Patient 2	Patient 3	Patient 4	Reference ranges
Alanine transaminase (ALT U/L)	16.2	17.7	160.5	47.1	0-50
Aspartate transaminase (AST U/L)	75.2	117.9	277	68.7	0-40
Total bilirubin (TB μmol/L)	252.9	145.2	98.8	135.8	0-24
Direct bilirubin (DB μmol/L)	109.3	83.3	49	107.6	<5
Alkaline phosphatase (ALP U/L)	969	645	481	993	<449
Gamma glutamyl transpeptidase (GGT U/L)	239	201	109	191	10-60
Total bile acid (TBA μmol/L)	290.8	321.8	132.1	599.4	0.5-10
Albumin (g/L)	31	29	30	32.2	35-52
Blood ammonia (μmol/L)	61.3	37.3	138.5	82.6	10-47
Blood glucose (mmol/L)	2.8	3.2	3.0	3.9	3.9-6.1
Lactic acid (mmol/L)	3.9	9.6	2.8	2.7	1-1.7
Triglyceride (mmol/L)	1.09	2.78	3.95	0.89	0-2.26
Cholesterol (mmol/L)	5.88	4.8	3.52	4.2	<5.2
Prothrombin time (PT S)	11.5	15.8	18.9	17.4	10-14
Activated partial thromboplastin time (APTT S)	47.5	48.7	43.6	56.9	20-40

Table 3 Blood tandem mass spectrometry and gene diagnosis results

Patient	Time of first diagnosis	Gene mutation region	Gene mutation type	Nucleotides change	Amino acid change
Patient 1	42 days	exon6	Splice	c.615+5G>A	Splicing
	exon9	Frameshift	c.852_855del	p.M285Pf*2	
Patient 2	2 months and 2 days	–	–	–	–
Patient 3	5 months	Intron16	Insertion	g.IVS16ins3kb	
		exon6	Splice	c.615+5G>A	Splicing
Patient 4	4 months and 23 days	exon9	Frameshift	c.851_854delGTAT	p.Met284fs
		exon11	Missense	c.G1157G>T	p.Gly385Val

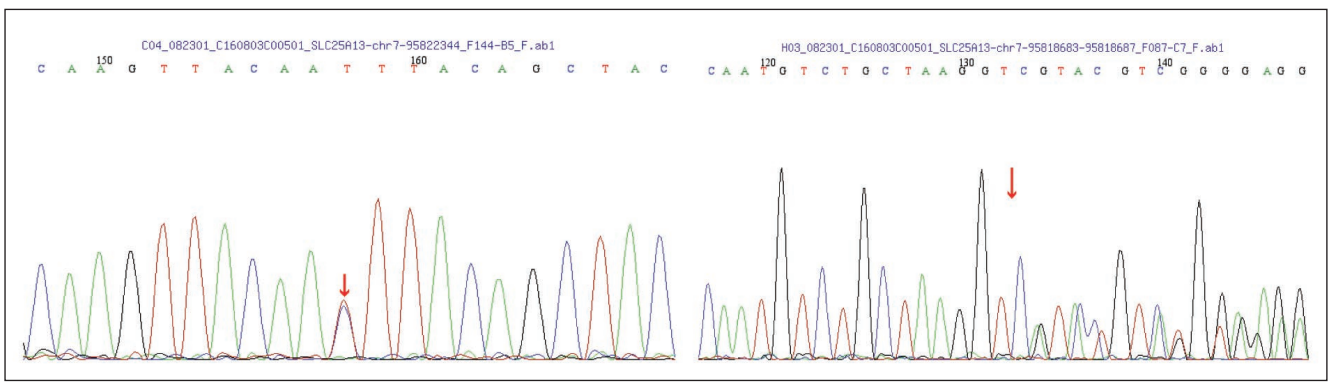

Figure 1 Gene sequencing diagram of patient 1.

significantly. After a few months, the indicators basically recovered, no obvious abnormality was found in the growth and development. Patient 3 was not effective in hospitalisation. One month after hospitalisation, the liver continued to increase. After review, blood lipids increased significantly, liver biopsy showed severe fatty liver.

Discussion

The cause of infant cholestasis and clinical features are complex. Along with the development of genetic and metabolic diseases examination, genetic testing and liver biopsy in recent years, the understanding of this kind of disease increased among medical staff, the detection rate of Citrin protein deficiency is higher and higher, the positive rate of NICCD in high risk hereditary metabolic disease patients in China ranks second only after methylmalonic aciduria,[1] the morbidity in China is 1/79. It is one of the important reasons of infants with intrahepatic cholestasis in China with jaundice as the first presentation,[2] delayed healing of disease and treatment.[3]

Because of SLC25A13 gene mutation,[4] the disease is autosomal recessive. It causes Citrin protein insufficiency in liver mitochondria. Aspartic acid produced in the mitochondria cannot be transported to the cytoplasm to participate in the urea cycle so that it produces a series of biochemical reactions, the pathways of malic acid, citric acid shuttle, urea cycle, protein synthesis, glycolysis, and sugar isogenesis are changed leading to liver metabolic abnormalities and complex biochemical metabolic disorders.[5] It can cause liver dysfunction, abnormal coagulation function, abnormal glucose metabolism, and galactose accumulation, promote fat synthesis, inhibit ketone body synthesis, and cause the increase of short-chain acylcarnitine and long-chain acyl carnitine.[6] The laboratory examinations have the characteristics of hypoglycemia, hyperlactacidemia, hyperammonemia, hypoproteinemia, coagulation dysfunction, galactosemia, hyperlipidemia, etc.[7]

With the first diagnosis of jaundice, the liver function of the patient 1 was examined, and his total bilirubin, direct bilirubin, and bile acid were found to be significantly higher. After hospitalised, he was given the general anti-infection, jaundice fadeaway, and liver preservation treatment, his transcutaneous bilirubin jaundice fadeaway, but the inspections still suggest that direct bilirubin and bile acid level was high, and albumin and blood-glucose were low. The result of tandem mass spectrometry showed Citrin protein deficiency of the infant. After the supplementation of fat-soluble vitamins and lactose-free milk symptomatic treatment, the indicators were better than before. The disease was confirmed after gene diagnose. It is found that the abnormality of direct bilirubin and bile acid cannot be seen through simple percutaneous detection of jaundice, especially for infants with longer jaundice. The period of blood urine tandem mass spectrometry is generally 1 week, and the gene examination period, from one month to one and a half month, is longer. The liver function and impaired glucose metabolism of NICCD infants are more severe than the infants with biliary atresia and other idiopathic cholestasis.[8] The infants with cholestasis whose albumin and blood-glucose level are low can be supplemented with fat-soluble vitamins and lactose-free milk powder before tandem mass spectrometry and gene diagnose results are reported, which is helpful for early recovery of the disease.

For patient 3, sclerotic jaundice was the first symptom. His transcutaneous bilirubin (TCB) and aminotransferase index of liver function were not increased, but cholestasis and blood lipid were abnormal. During the follow-up period, the liver and blood lipids of the infant increased. Liver biopsy results of light microscope showed hepatocytes diffused water-like degeneration and balloon-like change,

Table 4 Review and follow-up results of 4 infants

Patient	TB (Range value: 0-24 μmol/L)	DB (Range value: <5 μmol/L)	ALT (Range value: 0-50 U/L)	GGT (Range value: 10-60 U/L)	TBA (Range value: 0.5-10 μmol/L)
Patient 1	37.6	10.8	35.7	62	16.7
Patient 2	11.7	<5	12.1	47	5.2
Patient 3	85	69.3	297.5	126	174.8
Patient 4	42.6	13.8	56	75	59.4

For the explain of TB, DB, ALT, GGT, TBA, please see Table 2.

severe and large vesicles mixed fatty degeneration, cholestasis was found in mild hepatocytes and capillary bile duct, scattered focal was necrosis, liver sinus was infiltrated by small amounts of inflammatory cells, portal area was expanded, fibrous tissue was hyperplasia, fibrous septa formed, and severe fatty liver was suggested. His gene diagnoses confirmed Citrin protein deficiency and suggested that the defects of Citrin protein lead to cholestasis and fat deposition. The serious patients can have liver fibrosis and poor prognosis.[9] Fat soluble vitamins and fatty acid malabsorption of the infants can be induced due to cholestasis. The gastric emptying speed of medium chain fatty acids (MCT) is faster than long chain fatty acids (LCT) without bile and steapsin digestion, so it is easier to absorb and able to provide energy quickly. The infants can be fed with lactose-free and strengthening MCT treatment of milk.[10] At present, the child is still following up.

But genetic examination also has some limitations. Although the results of patient 2 were negative, the disease could still be clinically diagnosed with the results of liver function and tandem mass spectrometry examinations. After adjusting diet and symptomatic treatment, liver function returned to normal after several weeks. The possibility of finding new mutation sites or deletions by other gene sequencing methods remains to be further verified.

With the opening of the two - child policy in China, it is particularly important to examine the family history and the history of birth in detail. For patient 4, it was found that the old sister of the infant had a similar disease history before 1 year old, and she was naturally relieved without treatment, which provided important clues for our gene detection. SLC25A13 gene mutation was confirmed later. Although the screening of newborns diseases has been carried out in China for many years, it has expanded from 2 diseases to 5 diseases screening in recent years, there are still a large number of congenital inherited metabolic diseases that cannot be diagnosed early. The reason may be that the grassroots medical staffs do not know enough about this kind of disease, the tandem mass spectrometry and gene detection period are long and the cost is high, parents are not highly compliant in grassroots hospitals which lead to the ratio of missed diagnosis is high.[11] The disease is closely associated with clinical early diagnosis and diet control, clinical medical staffs should develop ideas. To infants with cholestasis, etiology, biliary imaging, and tandem mass spectrometry examinations are required, if condition permitting, gene diagnose is also suggested, which are important to early diagnose, improvement of clinical outcome, the prognosis of children determination and the next birth of couple who had a NICCD child before.

Through this study, it is found that most of the infants with this disease can be cured clinically through dietary adjustment and symptomatic treatment. Most of the people think the diagnosis of congenital, hereditary and metabolic diseases cannot be cured, but this study not only provides a sense of achievement for clinicians but also increases the confidence of the majority of children's families in treatment.

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Declaration of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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