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Case Report

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Case Report: 2 Hereditary Spherocytosis Cases Featuring Liver Damage Related to Ank1 Mutations

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Abstract

Background: *ANK1 mutations are usually related to hereditary spherocytosis [HS]. Reports of HS patients with liver failure are limited. Here we report two liver damage cases with ANK1 mutations. Based on liver manifestations and ANK1 gene mutations in these two patients, we suggest liver damage is related to ANK1, hoping it can expand the phenotypic spectrum.*

Case Description: *In both 2 patients, recurrent jaundice was the main symptom. Before and during hospitalization, hepatomegaly and abnormal liver functions were found. Genetic tests confirmed de novo heterozygous mutations in ANK1 gene. Liver biopsy of one child indicated iron accumulation. Based on these clinical performances, these two patients were diagnosed with liver failure and hereditary spherocytosis. Conservative treatment can improve the condition of one patient while the other patient responded poorly and liver transplant was conducted.*

Conclusions: *We believe liver damage is a new manifestation of ANK1 mutations. ANK1 mutations may decrease the level of hepcidin, which may cause iron overload and then ferroptosis, resulting in liver damage.*

Keywords: *ANK1*, Liver Damage, Pediatrics, Hepcidin, Iron Overload, Ferroptosis.

Background

ANK1 is the coding gene for ankyrin-1 protein which is mostly expressed in red blood cells [1]. Mutations in *ANK1* are related to about 50% of hereditary spherocytosis [HS] cases [2]. HS is a type of hemolytic anemia disease that usually leads to jaundice and enlarged spleen [3]. Other complications of HS include gallstones and aplastic crisis.

Based on previous reports, no case showed severe liver damage in HS patients with *ANK1* mutations. Recently, a study showed HS patient with *SPTB* gene mutations may suffer liver damage or cirrhosis, indicating HS patients with different pathogenic gene mutations may have a larger phenotypic spectrum [4]. But studies on the direct relationship between *ANK1* mutations and liver failure are limited. One animal experiment showed potential association between *ANK1* gene mutations and liver damage in which iron overload and ferroptosis are important factors [5]. Here we report the clinical features, diagnosis, and treatment of two pediatric cases with *ANK1* mutations featuring liver dysfunction, hoping to expand the phenotypic spectrum of *ANK1* gene mutations.

Also, literature reviews connect *ANK1* mutation and liver damage with iron overload. When similar cases emerge in the future, attention can be paid to iron metastasis and hopefully can provide

some insights on early recognition and timely treatment. We present the following case in accordance with the CARE reporting checklist.

Case Description

Patient 1 was a 6-year-and-11-month-old Chinese boy presented with jaundice for more than a year. The boy was born healthy via normal delivery [G1P1] with a birth weight of 3200g. There was no special perinatal history and family history. He has been diagnosed with liver failure, cholestatic liver disease, coagulopathy, spherocytosis type 1, abdominal pain with unknown cause and low T3 syndrome at the age of 5 at local hospital. The genetic test in local hospital showed a novel heterozygous mutation in *ANK1* gene which was pathogenic. Unfortunately, we cannot get the original genetic test report to clarify the detailed information. He experienced partial splenic embolization surgery for spherocytosis. But the recurrent jaundice and abnormal liver function urged him to come to our hospital for further medical advice.

On admission, the boy showed mild yellowing of skin and hepatomegaly were found. The vital signs were unremarkable. During hospitalization, the whole blood cells analysis and liver function tests were normal. Pathogens including Epstein Barr virus [EBV], TORCH, hepatitis A, B, C, and We were all negative. Liver biopsy indicated liver damage, with swelling of sinusoidal endothelial cells, increased number of lymphocytes, presence of Kupffer cells and the Scheuer score was G2S1. Prussian blue staining revealed slight iron deposition. The biopsy also found some enlarged mitochondria with irregular shape. Genetic test confirmed a *de novo* heterozygous mutation in *ANK1* gene, which is usually associated with HS. Multiple abdominal ultrasonography discovered an enlarged spleen.

Based on above signs and findings, the boy was diagnosed with cholestatic hepatitis, hereditary spherocytosis, and post splenic embolization. After admission and until the liver biopsy, treatment given included oral ursodeoxycholic acid for cholestasis, Intramuscular injection of vitamin K for coagulation abnormalities. The boy also received plasma and albumin transfusion to further stabilize his condition. The boy's condition was significantly improved with no signs or symptoms and thus he was discharged. Up to now, follow-ups show a good outcome.

Patient 2 was a 4-month-and-21-day-old boy brought to the hospital due to yellowing of skin and sclera for more than four months. The boy suffered low birth weight [caesarean section, 2400 g] and was a premature infant [35 weeks and 3 days] with unremarkable family history. He was mainly diagnosed with neonatal hyperbilirubinemia, neonatal anemia, and neonatal thrombocytopenia. He was hospitalized for two weeks after birth because of hyperbilirubinemia and was discharged when the conditions improved. However, the yellowing of skin and sclera persisted despite the use of ursodeoxycholic acid. He was then hospitalized several times for recurrent jaundice. The treatment including ursodeoxycholic acid glutathione did not work well. His liver function and jaundice became worse which led to his hospitalization in our hospital.

On physical examination, the boy's vital signs were unremarkable and there were no significant abnormalities except for the yellowing of sclera and skin. The whole blood cell analysis showed increased white blood cells and moderate anemia [WBC 18.18*109/L, hemoglobin 65g/L] liver function tests showed liver damage [DB 496μmol/L, TB 842.78μmol/L, ALT 1211 U/L, AST 1655/L]. Also, liver damaging pathogen tests [EBV, TORCH, hepatitis A, B, C, and E] were all negative. Radiological examinations found hepatomegaly, splenomegaly, peritoneal effusion in the lower abdomen, and mild inflammation in both lungs. Hepatic biopsy showed edema of hepatic cells and iron deposition [similar to Patient 1]. To figure out the exact etiology, whole-exome sequencing [WES] confirmed a *de novo* heterozygous mutation c.3630-1G>C [p.?] in *ANK1* gene which was considered to be pathogenic. This mutation does not exist in either parent. Following above examinations, the boy was diagnosed with infant liver syndrome, acute liver failure, hyperbilirubinemia, hepatic encephalopathy, coagulopathy, hyperammonemia, hyperlactic acidemia, anemia, and bronchopneumonia. Several medications including ursodiol and Transmittal were administered. But conditions were not improved well. After interventions including but not limited to vitamin K and transfusion of blood components, despite minor improvements, the boy was still facing a predicted poor outcome. Therefore, continuing treatment at that time was no longer an option and he was transferred to another hospital for liver transplantation. Now the transplantation has finished, and the liver function is significantly improved while his anemia is still worrying which was considered to be related to HS. The condition is still in progress and to be followed-up. Detailed laboratory test could be seen in Table 1, Table 2 and Table 3.

Discussion

Compared to classic HS scenarios, these two cases are associated with liver damage accompanied by *ANK1* mutations. Anemia, jaundice, hepatomegaly, and splenomegaly are the main features of HS [6]. Based on above clinical appearances and the finding of *ANK1* mutations, Patient 1 was previously diagnosed with HS and had taken partial splenic embolization surgery. The condition of HS was corrected mostly. However, his liver function did not improve, implying that HS may not be the only driving factor of jaundice. As for Patient 2, genetic diagnosis confirmed *ANK1* mutation and HS was suspected. Given the young age and the severity and urgency of his liver condition, liver transplantation was of higher priority. Since the liver condition of our cases cannot be solely explained by HS and other origins, we speculate that liver damage may also be a manifestation of *ANK1* mutations. To investigate if other connections between *ANK1* mutation and liver damage exist, some literature reviews were conducted. We believe iron is a key factor in this underlying association between abnormal liver function and *ANK1* mutation.

Iron is an essential element in the body playing a key role in many biological metabolism routes, of which liver is an important site

for maintaining its homeostasis. When dysregulated, iron-related diseases, such as anemia and iron overload, will be caused.[7] To regulate iron metabolism, hepcidin [a hepatic hormone] is of vital importance [8]. Its malfunction may result in excessive iron, producing toxicity of which the liver is the main target organ [9]. It is reported that in mice models, *ANK1* mutations may decrease the hepcidin expression and lead to iron overload especially in kidney and liver [5]. In addition, there are evidences that hepcidin level positively corelates to the prognosis of acute liver failure patients and hepcidin can be used as a biomarker of some liver disorders [10, 11].

Some researchers illustrated that *ANK1* mutations may cause hepcidin deficiency, then iron overload.[5] Liver, as the main source of hepcidin, is also the main target of iron toxicity [9]. Milic et al. have revealed the underlying mechanism, that the combination of excessive iron and reactive oxygen increases hydroxyl radical level, thus influences the normal properties of phospholipids, amino acid side chains, proteins and DNA strains [12]. Recent studies also showed that iron overload can trigger ferroptosis, which is an iron overload-induced nonapoptotic cell death [13]. According to Wang et al., mitochondria, the center of cellular metabolism, also serves as the core organelle of iron regulation [14]. Although the exact mechanism of ferroptosis is still uncertain, ferroptosis plays an important role in chronic liver damage especially [15]. The findings of iron deposition and swelled mitochondria of one patient during biopsy further aroused our suspicion of iron dysregulation. This may provide us with an insight into what happened to our cases, especially why Patient 1, after splenic embolization, still suffered from worsening liver condition. Additionally, it is now clear that hepcidin has protective role against ferroptosis [16]. For these two patients, both liver biopsy showed iron deposition, hinting that *ANK1* may be related to iron overload and liver damage. Therefore, further tests on iron metabolism are needed to see if the hypothesis is valid and try treatment for iron overload through adjusting hepcidin level.

Our two cases indicated that the clinical features of HS and liver manifestations are closely linked. These hepatic manifestations may be a rare performance in HS patients with *ANK1* gene mutations. Some presentations [e.g., jaundice and hepatomegaly] can attribute to both HS and iron metabolism pathway. Problems in our analysis include the lack of adequate cases, the halt of disease progression in one case, and limited evidence found during diagnosis and treatment. Therefore, *ANK1* mutation and iron metabolism situation, especially hepcidin level, deserve being taken into consideration when facing similar cases in future practice. In addition, due to COVID-19 pandemic, we encountered extreme difficulties in the follow-up procedure. We lost valuable opportunities to do further tests and examinations on both patients. We meant to further study the iron metabolism conditions in both patients during follow-up. For further investigation, we will continue to acquire more information through follow-ups and try diagnostic treatment if necessary.

List of abbreviations

ALT: Alanine Aminotransferase.
AST: Aspartate Aminotransferases.
HS: Hereditary Spherocytosis.

Ethics approval and consent to participate

Written consents were obtained from the parents of the patients for the study and the publication of this case report. All personal information of both patients was excluded in this report.

Consent for publication

Written consent forms for this case report publication were obtained from parents of both patients.

Availability of data and materials

The data supporting the conclusion of this report is included within the article.

Competing interests

The authors declare no competing interests.

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Authors' contributions

HH, TZ and YX acquired the clinical data, LY and DL drafted the manuscript, YW edited the manuscript. All authors read and approved the final manuscript.

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