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CASE REPORT

Hemolytic Uremic Syndrome Caused by Enteroviral Infection

Ming-Dar Lee [a,b], Chin-Yuan Tzen [c,d,e], Chun-Chen Lin [b,f], Fu-Yuan Huang [b,f], Hsi-Che Liu [b], Jeng-Daw Tsai [b,g,*]

[a] Department of Pediatrics, Hsinchu Mackay Memorial Hospital, Hsinchu, Taiwan
[b] Department of Pediatrics, Mackay Memorial Hospital, Taipei, Taiwan
[c] Department of Pathology and Laboratory Medicine, Cathay General Hospital, Taipei, Taiwan
[d] College of Medicine, Fu Jen Catholic University, Taipei, Taiwan
[e] National Taipei College of Nursing, Taipei, Taiwan
[f] Mackay Medicine, Nursing and Management College, Taipei, Taiwan
[g] Department of Pediatrics, Taipei Medical University, Taipei, Taiwan

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A 4-year-old boy presented with enteroviral infection complicated with atypical hemolytic uremic syndrome (aHUS). Enterovirus RNA was detected by reverse transcription polymerase chain reaction (RT-PCR) of both blood and kidney biopsy specimens. A survey of the complement system did not reveal a specific complement defect. Supportive therapy with blood components transfusion, plasma therapy, and immunosuppressants was administered, however, renal function did not recover. The results of this report demonstrate that the enterovirus is the cause of aHUS.

1. Introduction

Hemolytic uremic syndrome (HUS) is a common cause of acute renal failure in children. The presentations include microangiopathic hemolytic anemia, thrombocytopenia, and progressive renal failure. It can be classified according to the etiology and clinical associations.[1] Approximately 10% of HUS cases are classified as atypical HUS (aHUS), which has a poor prognosis, with death rates of up to 25% in the acute phase, and 50% of patients requiring ongoing renal replacement therapy.[2] Atypical HUS is a heterogeneous disease that is caused by defective complement regulation in >50% of cases. The association between viral infections and aHUS has been reported, however, reports

* Corresponding author. Department of Pediatrics, Mackay Memorial Hospital, Number 92, Section 2, Chungshan North Road, Taipei, Taiwan.
E-mail address: tsaijd@yahoo.com.tw (J.-D. Tsai).

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on its association with enteroviral infection are limited. Viral infections were assumed to be the trigger of aHUS in patients with underlying complement defects. This report describes the case of a 4-year-old boy who developed HUS after clinical presentations of enteroviral infection. Renal pathology confirmed the diagnosis of HUS, and enterovirus RNA was identified in the kidney and blood specimens. The possible pathogenesis of enteroviral infection-induced aHUS is discussed.

2. Case Report

A previously healthy 4-year-old boy developed a sore throat, oral ulcers, and skin vesicles on his limbs, 9 days prior to admission. Hand-foot-mouth disease was diagnosed by his primary physician, and symptomatic medicines were administered. Jaundice and skin petechiae developed after 2 days, and he was transferred to hospital. During this period, the patient did not have diarrhea, nor did he exhibit symptoms of upper respiratory tract infection. None of his family members exhibited the same symptoms. Anemia, thrombocytopenia, and acute renal failure were detected at the hospital. The initial laboratory data revealed hemoglobin (Hb) = 6.2 g/dL, platelet = $46 \times 10^3/\mu L$, white blood cell count (WBC) = 3190/uL, serum urea = 82 mg/dL, creatinine = 1.6 mg/dL, lactic dehydrogenase (LDH) = 1662 U/L, and total/direct bilirubin = 1.7/0.2 mg/dL. The direct and indirect Coombs tests were both negative. The urinalysis revealed proteinuria (>300 mg/dL) and microscopic hematuria, but no pyuria. Fragmented red blood cells (RBC) were found on peripheral blood (PB) smear, and HUS was highly suspected. His anemia and thrombocytopenia progressed after a washed packed RBC and platelet concentrate transfusion. Impaired renal function progressed and hypertension developed. Consequently, the patient was transferred to our hospital.

He was afebrile (36.4°C) on arrival, and his blood pressure was 160/103 mmHg. A physical examination revealed petechiae over his face, neck, and anterior chest, and pale conjunctiva and icteric sclera were found. He also had hepatomegaly (3 cm below right costal margin (RCM)). Supportive treatments with antihypertensive drugs (hydralazine and amlodipine) and diuretics (furosemide) were initially administered, which stabilized his condition. The throat bacterial culture and antistreptolysin titers were negative, as was screening for antinuclear factor, anti-ds DNA, anti-neutrophil cytoplasmic antibody (ANCA), and anti-glomerular basement membrane (GBM) antibodies. The complements C3 and C4 were both normal. The viral culture via the throat and rectum resulted in no growth after 30 days' incubation. Because no *Escherichia coli* serotype O157:H7, *Shigella*, or *Salmonella* was isolated from the stool specimen, pneumococcal infection was excluded by negative chest roentgenologic finding, urine pneumococcal antigen, blood culture, and Thomsen-Friedenreich antigen. Atypical HUS caused by enterovirus was highly suspected. A renal biopsy was performed 8 days after admission. Swelling and hyperplasia of the endothelium of arterioles with narrowing of the lumen were observed under light microscopy. The glomeruli exhibited swollen endothelia and were bloodless. Both the renal tubules and interstitium were unremarkable. No glomeruli were identified in an immunofluorescent study (IgG, IgA, IgM, C3, C1q, kappa, and lamda). Electron microscopy revealed the swollen endothelium and a widened subendothelial space. The pathology was consistent with thrombotic microangiopathy. Furthermore, diagnostic reverse transcription polymerase chain reaction (RT-PCR)[3] was used to detect enterovirus RNA. We extracted the viral RNA from the blood and renal biopsy samples of the patient. A 236-bp gene fragment in the second half of the 5′ noncoding region (NCR) was amplified through one-step RT-PCR using 10 μL viral RNA template and two 5′ NCR-specific primers, E4KB-F (AAGGTGYGAAGAGYCTATTGAGCTA) and E1-R (CACCGGATGGCCAATCCA). The results of RT-PCR were strongly positive in the renal biopsy sample and weakly positive in the blood sample (Figure 1). Identification of enterovirus RNA in the kidney and blood, combined with the characteristic renal histopathologic finding, established enterovirus as the cause of HUS. Immune investigation, including complement C3, factor H (CFH), factor I (CFI), membrane cofactor protein (MCP), and ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; also known as von Willebrand factor-cleaving protease) revealed normal findings. Plasmapheresis (1.5 × plasma volume) was performed according to the hemolytic indexes (Hb, platelet count, LDH, and fragmented RBC on PB smear). Immunosuppressants with steroid, and rituximab were subsequently administered (Figure 2). The patient received >40 courses of plasmapheresis and subsequent plasma infusion. The hemolytic process ceased gradually; however, the renal function continued to deteriorate. The patient was initially placed on hemodialysis and shifted to peritoneal dialysis for renal failure. He was discharged, and was under regular outpatient follow-up for end-stage renal disease (ESRD).

3. Discussion

More than 50% of aHUS cases are caused by defective complement regulation. Complement control is provided by a multilayered system of soluble (e.g., complement factor H and complement factor I) and membrane-anchored proteins (e.g., MCP/CD46; thrombomodulin, THBD/CD 141). Mutations in these regulators result in loss of control

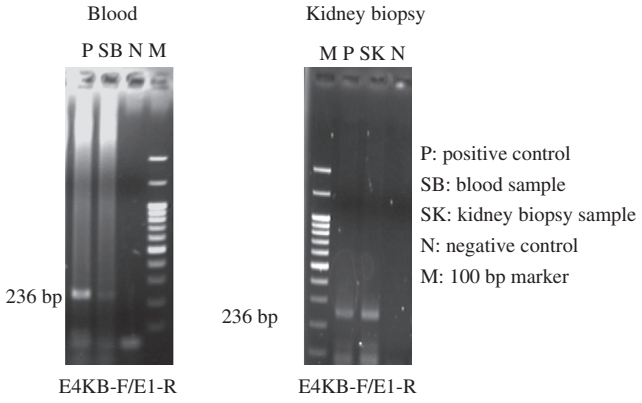

Figure 1 Reverse transcription—polymerase chain reaction of blood and kidney biopsy specimens.

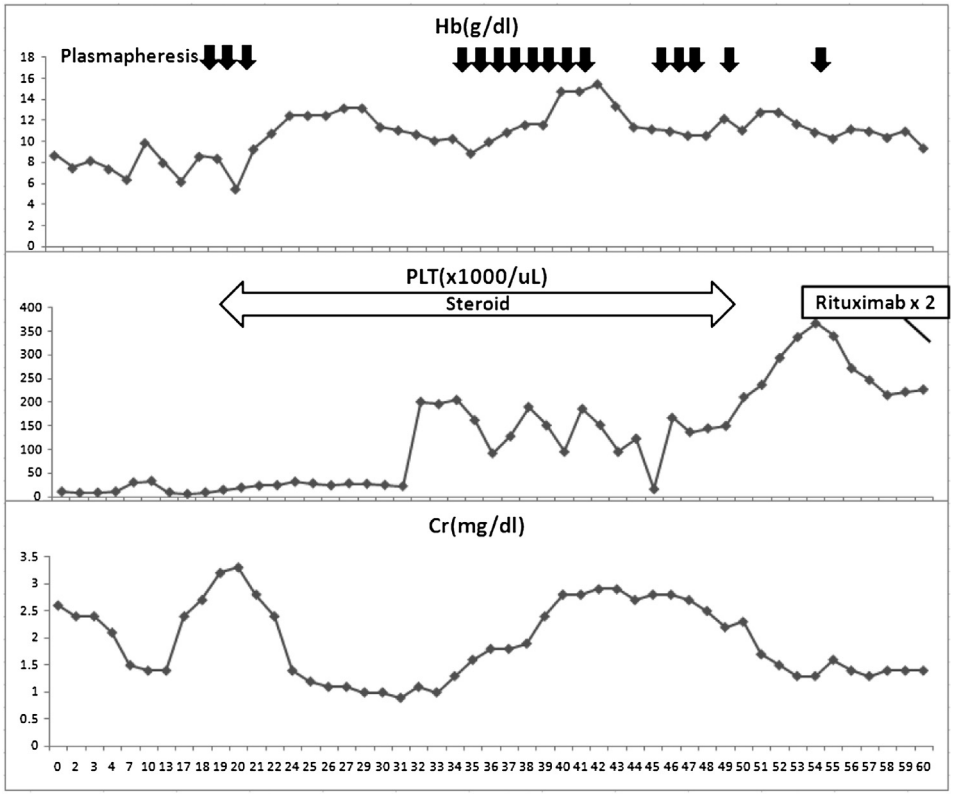

Figure 2 Hemoglobin (Hb), platelet (PLT) and creatinine (Cr) concentrations, and treatments during hospitalization.

of the alternative C3 convertase, C3bBb, whereas mutations in complement factor B or C3 result in gain of the function of this enzyme.[4] Among the patients with aHUS, 50% had mutations of the complement regulatory proteins CFH, MCP, or CFI. In addition, 5—10% of children have factor H dysfunction caused by anti-factor H antibodies.[5] The remaining 40% of aHUS cases are currently unaccounted for, thereby necessitating the investigation of additional susceptibility factors. Our patient had a normal serum C3 level; however, a normal C3 level does not exclude an underlying complement disorder. Moreover, although our investigations did not identify abnormalities in CFH, CFI, MCP, or ADAMTS 13, an underlying complement defect could not be excluded.

Reported viruses associated with aHUS include human immunodeficiency virus, Epstein-Barr virus, influenza A, and parvovirus B19.[6] Enterovirus-associated HUS was first described by Glasgow and Balduzzi in 1965.[7] Since then, >60 patients were reported in seven subsequent studies, including with Coxsackie virus and ECHO virus.[8—14] The diagnoses of enteroviral infection were mostly based on serology, viral culture from the throat, rectum and feces, and viral particle found through electron microscopy in feces (Table 1). Only two patients exhibited evidence by isolation of a Coxsackie virus A-like agent[8] and detection of Coxsackie virus B antigen from a kidney biopsy specimen.[9] Nine familial cases were reported,[8,12—14] however, none of these reports mentioned the underlying complement defect. We do not agree with De Petris et al that enteroviral infections must not be considered a cause of HUS.[14] Our patient exhibited symptoms and signs of enteroviral infection, and other possible etiologies, such as Stx-toxin producing *E. coli*, *Shigella*, and pneumococcal infection were excluded. HUS was confirmed by clinical presentations and renal biopsy. Diagnostic RT-PCR, which targeted a 236-bp gene containing enterovirus RNA using fragment in the 5′ NCR, was used to confirm this virus as the cause of HUS. The 5′ NCR seems to be extremely conserved among enteroviruses and the primers targeted to the 5′ NCR are pan-reactive with the human enteroviruses. Therefore, the 5′ NCR has been used extensively in diagnostic RT-PCR assays for enterovirus infection. The reported sensitivity and specificity of RT-PCR using serum for detection of the enteroviruses range from 81% to 92% and 98% to 100%, respectively. No reports evaluated the use of RT-PCR using renal biopsy, but by combining the RT-PCR results from specimens collected from multiple nonpermissive sites, it may be possible to improve the ability to establish enteroviral causality.[15] The low sensitivity of the viral culture for identification of enteroviruses from clinical specimens (only 35—75%) explained the negative results of our patient.

The pathogenesis of viral infection-induced aHUS is not fully understood. Most authors suggested that viral infection may be the trigger event of aHUS in patients with underlying genetic predispositions. Direct or cytokine storm-mediated endothelial injury is also a possible mechanism.[6] Evidence suggests that persistent infection of Coxsackie B virus in human kidney cells and vascular endothelial cells induces an increased expression of adhesion molecules, which may contribute to injury of the endothelial cells and lead to thrombotic microangiopathy.[16] The demonstration of enteroviral RNA sequence on RT-PCR in the renal tissue supports a direct viral effect. However, we did not identify

Table 1 Reported cases of hemolytic uremic syndrome associated with enterovirus.

Reference	Virus	Method	Patient number	Familial case
Glasgow and Balduzzi[7]	Coxsackie virus A4	Serology	1	Nil
Ray et al[8]	Coxsackie virus A and B	Serology Viral culture from tissue, pharyngeal secretion, stools and urine	7	2
Ray et al[9]	Coxsackie virus B	Serology Viral culture	2	Nil
Austin and Ray[10]	Coxsackie virus B	Serology Viral culture from throat and rectum	15	Nil
O'Regan et al[11]	ECHO virus 22	Serology Viral culture from stool	8	Nil
Larke et al[12]	Coxsackie virus A4, B2, B4 Echo virus 11	Serology Culture from pharyngeal secretions and feces Viral particle on electron microscopy	10	4
Vecilla et al[13]	Coxsackie virus B	Viral culture	2	2
De Petris et al[14]	Non-polio enterovirus	Serology	33	1

the enterovirus type by using the 5′ NCR-based RT-PCR assays. Further studies may be required to clarify the exact mechanism of enterovirus-induced aHUS.

The management of HUS was based on the etiology. Anemia, thrombocytopenia, acute renal failure, and hypertension require supportive therapy. Dialysis may be necessary. Patients with aHUS, such as complement factor H or von Willebrand protease deficiency, benefit from plasma therapy[17]; however, plasma therapy is contraindicated for pneumococcal-HUS. The interval between disease onset and plasma therapy may be too long in this patient, and the initial plasma therapy may require higher intensity (only 8 times in 3 weeks).[17] Plasma therapy was not arranged immediately after diagnosis, because the patient exhibited improvement after the supportive treatments and blood products transfusion. Subsequently, plasmapheresis was arranged according to the hemolytic indexes and clinical conditions of the patient. However, our patient progressed to ESRD, which may have been caused by the treatment delay or the destructive nature of the disease, because 50—60% of patients with aHUS progress to ESRD.

In conclusion, our report suggests that enteroviruses must be considered one of the causes of aHUS. The pathogenesis of enterovirus-caused HUS includes direct endothelial injury, or a trigger in patients with underlying complement dysfunctions. Plasma therapy must be administered as soon as possible after the diagnosis of aHUS.

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