Abstract

Haemolytic uraemic syndrome is characterized by the triad of microangiopathic haemolytic anaemia, thrombocytopenia and acute renal failure. It is a common cause of acute renal failure in infants and young children. Diarrhoea-associated haemolytic uraemic syndrome is most commonly caused by \textit{E. coli 0157:H7}. The epidemiology, pathophysiology and treatment options will be reviewed. Nondiarrhoea-associated haemolytic uraemic syndrome accounts for only 10\% of all cases. It usually has a poorer outcome. Neuraminidase-producing \textit{Streptococcus pneumoniae} is a common causative agent. The neuraminidase exposes the T (or Thomsen-Friedenreich) antigen in the cell membrane of erythrocytes, platelets and glomerular endothelial cells. Anti-T IgM in normal sera will react with the exposed T-antigen, resulting in agglutination and haemolysis of red blood cells, thrombocytopenia and thrombotic renal microangiopathy. The cautious use of blood and plasma products may improve the prognosis. Long-term follow up of the renal function is warranted in all patients with HUS as renal failure may develop more than 10 years after the initial insult.

Key words

Haemolytic uraemic syndrome; Pathogenesis; Treatment

Introduction

Haemolytic uraemic syndrome (HUS) is a heterogeneous group of disorder characterized by the triad of microangiopathic haemolytic anaemia, thrombocytopenia and acute renal failure. Characteristic findings of the renal biopsy on light microscopy include endothelial oedema, with endothelial degeneration and destruction, thickening of the glomerular capillary walls, intraluminal thrombi consisting of fibrin and platelets, and fragmented red blood cells. Endothelial swelling and separation from the basement membrane may also occur in arterioles and arteries. HUS is the commonest cause of acute renal failure in infants and young children in the western countries. It is mostly diarrhoea-related although many other causes are involved. Thrombotic thrombocytopenic purpura (TTP) is another clinical entity, which shares very much similar clinical and pathological features with HUS. Both conditions have been considered as different forms of thrombotic microangiopathy but affecting different major target organs. In fact, Kaplan believed that HUS and TTP are distinctly different syndromes. TTP usually has an insidious onset with predominant neurological features and is not associated with bloody diarrhoea. Contrary to HUS, TTP rarely affects children and the prognosis is guarded.

Over the years, there are numerous publications highlighting new and evolving understanding on the epidemiology, pathophysiology and treatment of HUS. Without a proper registry, it is difficult to determine the true incidence of HUS in the local population that is understandably low (personal communication). We had two isolated cases of HUS (one was associated with \textit{E.coli 0157:H7} and the other following a severe pneumonia with unknown pathogen) in Queen Mary Hospital in Hong Kong in 1998. We encountered approximately one patient per year in the past ten years in our hospital (unpublished observation). Though our experience is limited, a literature review will help to consolidate the state-of-the-art understanding of HUS, which is essential for optimal care and improved outcome of the patients.
Definition and Classification of HUS

The classical diagnostic triad of HUS includes microangiopathic haemolytic anaemia, thrombocytopenia and acute renal failure. HUS is mainly divided into two sub-groups: diarrhoea-associated (D+HUS), which accounts for 90% of all cases and nondiarrhoea-associated (D-HUS) for the remaining 10% of the cases (Table 1). The post-diarrhoea HUS is typically related to verotoxin-producing E. coli O157:H7. Shigatoxin-producing Shigella dysenteriae is another common organism. The nondiarrhoea-associated HUS is found to be related to infection with neuraminidase-producing Streptococcus pneumoniae or other infectious agents. Other non-infectious causes of HUS include drugs, post-transplant, malignancy, familial, pregnancy (rarely in children) or idiopathic.

Etiology and Epidemiology

HUS occurs throughout the world with certain countries reporting a higher incidence. The prevalence of HUS in Argentina is estimated to be 30 cases per 100,000 children and in United States range from 0.3 to 10 cases per 100,000 children. Diarrhoea-related HUS is found to be less common in black than in white people. D+HUS is more common in summer and early fall while D-HUS does not have a seasonal variation. There is no published literature on the prevalence of HUS in Hong Kong or China.

The commonest causative agent for diarrhoea-associated HUS is E. coli O157:H7. It is one of the enterohemorrhagic E. coli (EHEC) that causes hemorrhagic colitis and produces potent cytotoxins known as Shiga-like toxin (identical to toxin produced by Shigella dysenteriae type 1) or verotoxin (extremely toxic to cultured Vero cells from African green monkey kidneys). EHEC generally produces more than one type of verotoxin (VT) and most strains produce both VT-1 and VT-2.

The source of the E. coli O157:H7 is mainly from the infected cattle. It has been shown that the lower incidence of D+HUS in winter and spring corresponds to the decrease in fecal shedding of E. coli O157:H7 from cattle in that period. The rate of isolation of E. coli O157:H7 in cattle also correlates with the different prevalence of D+HUS in different countries. The route of transmission is from contaminated undercooked minced beef in hamburger, unpasteurized cow’s milk and even goat’s milk and deer meat. Contaminated water is also responsible for the outbreak of D+HUS, as reported by Keene and associates. There was a simultaneous outbreaks of bloody diarrhoea and HUS caused by E. coli O157:H7 and bloody diarrhoea caused by Shigella sonnei in the summer of 1991 at a lakeside park in Oregon, USA. The outbreak was shown to be due to fecal contamination of the lake water by swimmers. Person-to-person transmission has also been demonstrated in day-care centres and institutes for mentally retarded persons.

Diarrhoea-associated HUS

Clinical Features

The diarrhoea may be watery or bloody and precedes the onset of HUS symptoms by three to twelve days. Vomiting and crampy abdominal pain are often present. Rarely, patients may present with features of bowel infarction, intestinal perforation, rectal prolapse or intussusception. There is usually a sudden onset of pallor, generalized deterioration and oliguria. Complications of acute renal failure may develop subsequently which include hyperkalemia, severe acidosis, fluid overload and hypertension. Central nervous system (CNS) may be involved and varies from irritability and lethargy to a more severe state with coma, seizures, cerebral swelling, hemiparesis and ataxia.

The characteristic laboratory findings include Coombs’ negative haemolytic anaemia with microangiopathic RBC morphology, thrombocytopenia and elevated creatinine.

Table 1  Classification of Haemolytic Uraemic Syndrome

<table>
<thead>
<tr>
<th>Diarrhoea-associated (D+HUS): 90%</th>
<th>Nondiarrhoea-associated (D-HUS): 10%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Typical, related to E. coli O157:H7</td>
<td>related to Streptococcus pneumoniae (neuraminidase-associated)</td>
</tr>
<tr>
<td>Related to Shigella dysenteriae</td>
<td>related to other infectious agents</td>
</tr>
<tr>
<td>Related to other bacteria or viruses</td>
<td>familial: autosomal dominant</td>
</tr>
<tr>
<td>idiopathic</td>
<td>autosomal recessive</td>
</tr>
<tr>
<td></td>
<td>pregnancy-associated</td>
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<tr>
<td></td>
<td>drug-associated: cyclosporin A, oral contraceptives, chemotherapy</td>
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<tr>
<td></td>
<td>post-transplant</td>
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<tr>
<td></td>
<td>autoimmune diseases, e.g. SLE</td>
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<tr>
<td></td>
<td>malignancy-associated</td>
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<td></td>
<td>idiopathic</td>
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Other hematological findings include increased reticulocyte count, decreased haptoglobin and elevated white cell count with left shift. The prothrombin time and partial thromboplastin time are usually normal. Other associated biochemistry changes include elevated urea, increased bilirubin and liver enzymes, decreased serum protein, increased uric acid, decreased bicarbonate and variable potassium level. Urinalysis usually shows proteinuria, dysmorphic red cells, white cells, cellular or granular casts and sometimes bilirubin. Oliguria usually continues for four to twelve days. Frequently, platelet count returns to normal before oliguria resolves and hematocrit returns to normal slowly.

**Risk Factors for Developing D+HUS after E. coli O157: H7 Infection**

The risk for developing HUS after *E. coli* O157:H7 infection is still controversial. Data from outbreak investigation from United States suggested that 5% to 10% of infected children developed HUS with 3% to 5% mortality. Moreover, the proportion of patients with D+HUS was 8% in a nationwide study in the United States. 8.1% in the study in Alberta in Canada and 14% during an outbreak of *E. coli* O157:H7 infection at Washington, USA in 1993. The highest age-specific risk of HUS in Alberta was 12.9% in those children with age less than five years. In contrast, the age and sex differences were not significantly associated with HUS in Bell’s study. Vomiting within three days of onset of diarrhoea and vomiting in children less than five and a half years old were identified as factors associated with development of HUS. Bloody diarrhoea and fever were not additional risk factors in Bell’s series. High white cell counts or polymorph counts and C-reactive protein at the initial state were also shown to be related to the progression to HUS.

Shiga-like Toxin type 2 seemed to increase the likelihood of developing HUS but Slutsker and associates could not identify any association between the different types of Shiga toxin and the development of HUS. Anti-motility agents in the early course of diarrhoea was believed to increase the risk of HUS by increasing toxin contact time in the intestine although Slutsker’s series again did not show such association.

The risk of HUS after treating the EHEC diarrhoea with antibiotics was controversial. An increased risk of HUS after antibiotics was noticed in a United State nationwide study but a Japanese group showed a decrease risk of developing HUS with antibiotics (fosfomycin) during the 1996 outbreak in Sakai, Japan. A retrospective analysis of the 1993 Washington outbreak failed to demonstrate any difference. A recent prospective cohort study in Seattle concluded that antibiotic treatment (with sulfadimethoxine and β-lactam antibiotics) in children with *E. coli* O157:H7 infection increases the risk of haemolytic ureamic syndrome. Ito et al from Japan reported in 1997 an interesting experiment on the effect of ten antibiotics on extracellular release of verotoxin from eleven EHEC O157 clinical strains. It was found that antibiotics which were inhibitors of cell wall biosynthesis (ampicillin, cefdinir, cefaclor and fosfomycin) showed an increase in verotoxin release. Inhibitors of protein synthesis (kanamycin, minocycline, doxycycline and tetracycline) were the safe antibiotics that did not cause the release of verotoxin from the cells.

**Pathophysiology**

It is important to understand the pathophysiology of the disease to develop appropriate treatment strategies. The proposed pathogenic cascade starts from the ingestion of EHEC and binding to the intestinal epithelial cells. The toxins produced most probably damage the microvasculature of the intestinal wall resulting in the haemorrhagic and ulcerative intestinal lesions. The toxins then enter the circulation through the damaged gut-blood barrier. Direct toxin invasion to the glomerular and arteriolar endothelial cells is believed to be the primary event of renal damage. Swelling of the injured endothelial cells promotes fibrin and platelet deposition resulting in a "localized" intravascular coagulopathy. Glomerular capillary lumens are occluded by the swollen cells and microthrombi, with subsequent reduction in glomerular filtration, fragmentation of red cells and the classical microangiopathic haemolytic anaemia. Lipopolysaccharide of the *E. coli* has an independent role on renal endothelial cell damage. In addition to the glomerular endothelial cells, renal tubular cells may be another site of renal damage. Hughes et al showed that cultured human proximal tubular cells are extremely sensitive to the cytotoxic effect of Shiga toxins and some inflammatory factors, e.g. interleukin-1 and lipopolysaccharide, can increase toxin responsiveness.

Inflammatory mediators also play an important role in the pathogenesis of the thrombotic microangiopathy. D+HUS is associated with significant changes in the circulating and tissue levels of cytokines including tumour necrosis factor (TNF)-α, interleukin (IL)-1α, IL-1β, IL-6 and IL-8. In 19 children with D+HUS in Birmingham, IL-1β and IL-8 were most commonly elevated in the plasma (8/19). Urinary IL-8 was detected in seven cases and four of them had plasma level below the limit of detection, suggesting renal secretion of this cytokine. Karpman also demonstrated an increase in IL-6 levels in the serum in 94% (33/35) of patients with HUS and a much higher level in those with anuria and extrarenal involvement. Sequential serum and urine samples showed that IL-6 levels varied with disease activity, suggesting that urine IL-6 might be used to monitor disease activity.
**Figure 1** Pathogenesis of diarrhoea-associated Haemolytic Uraemic Syndrome. (Modified from “Siegler RL. The hemolytic uremic syndrome.”)²

**Diagnosis**

The diagnosis of HUS depends on microangiopathic haemolytic anaemia with hemoglobin less that 10.5 g/dl, decreased platelet count (<150x10⁸/L) and increased creatinine (>95th percentile for age).¹⁰ Diagnosis of verotoxin-producing E. coli is based on stool culture¹⁸ and serotype identification. Acute and convalescent sera can be tested for anti-O157 lipopolysaccharide antibodies with an indirect haemagglutination assay.¹⁹ Anti-O157 antibody titre of more than 1:500 suggests recent infection. Two enzyme immunoassays (EIA) have been developed for the rapid diagnostic tests to detect verotoxin-producing E. coli and the Shiga toxins. The Premier E. coli O157 EIA detects E. coli O157 lipopolysaccharide in stool in 30 minutes. The specificity and specificity are 81% and 98% as compare to the gold standard of diagnosis by stool culture and serum antibody detection.²⁰ The Premier enterohemorrhagic E. coli EIA detects verotoxins 1 and 2 in stool after overnight incubation of the specimen in MacConkey broth at 37°C. The sensitivity is 91%.²⁰ It has an additional advantage to detect non-O157 E. coli that produce verotoxins.²⁰ The development of rapid diagnostic tests aids the trial of anti-toxin therapy at an earlier stage of the E. coli O157:H7 infection.

**Treatment**

The mainstay of treatment for D+HUS at present is supportive therapy. This includes careful attention to fluid and electrolytes balance, nutritional support by total parenteral nutrition and nasogastric feeding, treatment of severe anaemia and symptomatic thrombocytopenia, control of hypertension, seizures and azotemia.² Renal replacement therapy, either by peritoneal dialysis, haemodialysis or continuous veno-venous haemofiltration, is indicated when there is uncontrollable hypertension and fluid overload, hyperkalemia, acidosis or significant uremic symptoms.² Azotemia alone may not be an indication for dialysis.²¹ 38% of D+HUS patients from Alberta, Canada required dialysis with an average duration of 11.6 days.²⁰ The use of antibiotics is controversial as discussed previously when considering the risk factors for developing HUS from E. coli affected patients. However, recent study by Wong and colleagues in Seattle advised against the use of antibiotics.¹³ Specific therapies have been tried to reverse or ameliorate the microangiopathic process in D+HUS. These included, alone or in combination, infusion of fresh frozen plasma, plasmapheresis, aspirin, dipyridamole, heparin, warfarin, streptokinase, urokinase, vitamin E (as an anti-
oxidant) and intravenous immunoglobulin G. There is no convincing evidence for the therapeutic efficacy of any of these therapies in typical D+HUS.

Newer therapeutic approach includes the development of verotoxin-binding agent and immunization. Synsorb-Pk is a synthetic molecule that binds verotoxin with high avidity in vitro. It is administered orally. A phase II trial of Synsorb-Pk\(^2\) has been conducted in children with documented verotoxin-producing E. coli (VTEC) infection or close contact with an individual with HUS or VTEC infection or symptoms consistent with VTEC infection. The data suggested that there was a 54% reduction in the risk of developing HUS if the drug was started within three days of the onset of diarrhoea. A phase III trial, incorporating the rapid diagnostic test for the early detection of E. coli O157:H7 and verotoxins, is in progress. The possibility of immunization against verotoxin-producing E. coli and Shigella species is being investigated in animal studies.\(^6\)

As D+HUS is caused by an infective agent, mostly E. coli O157:H7, prevention of the infection is of utmost importance. Unpasteurized milk should not be consumed and ground beef should be well cooked. Prevention for the spread of the infection through contaminated water in swimming pools and within institutes should be carried out by early detection and isolation of the index case. Change in veterinary practice is also proposed. A recent study reported that switching cattle from a grain-based to a hay-based feed for as short as five days markedly reduced the fecal shedding of acid-resistant strains of the E. coli, which could survive the acidic pH in the stomach. This may have profound importance in the prevention of verotoxin-producing E. coli infection and D+HUS.\(^6\)

**Long Term Outcome and Prognostic Features**

The short-term outcome in D+HUS was better than HUS resulting from other causes and acute mortality ranged from 5% to 10% in most centres.\(^2\) Concerning the long-term outcome, around 10% of the survivors progressed to end stage renal disease (ESRD).\(^2\) Less severe renal sequelae included hypertension, proteinuria and impaired renal function, with variable reported incidence up to 65%.\(^2\) Reports on risk factors associated with adverse long-term outcome were variable. Many authors agreed that prolonged period of anuria (>7 days) or oliguria (>14 days) is a bad prognostic indicator.\(^1,2,26\) Other poor prognostic indicators include severe hypertension,\(^1,2,23,24,26\) central nervous system involvement,\(^2,23,26\) duration of dialysis (>7 days),\(^2,26\) and elevated white cell count\(^1,27\) at presentation. Renal histology at presentation was also considered to be a major outcome indicator. Arteriolar involvement in addition to glomerular pathology was shown to have a poorer outcome.\(^26\) Patients with patchy cortical necrosis from renal histology shortly after recovery have a poorer prognosis than those with glomerular form of thrombotic microangiopathy.\(^25\) In a long-term (15 to 25 years) report on 28 patients with classic HUS from Gagnadoux in France,\(^25\) only half of the patients had same outcome status during follow up at one year and more than 10 years later. 23% of the remaining half (three patients) improved and 77% (11 patients) had secondary worsening after 10 years. ESRD eventually developed in four patients, 16 to 24 years after typical HUS. Two of them remained symptoms-free during follow up at 10 years after initial renal recovery. Recurrence is rare in the diarrhoea-associated HUS and renal transplantation is the treatment of choice for ESRD.\(^2\) As renal failure can progress slowly over years, long-term follow up is therefore necessary for all patients with D+HUS.

**Non-diarrhoea-associated HUS due to Streptococcus Pneumoniae Infection**

HUS associated with invasive *Streptococcus pneumoniae* infection is worth mentioning among the different etiology of D-HUS. HUS is more commonly reported in children with infection of the lungs and rarely, the meninges.\(^2\) Difference in the pathogenesis may influence the treatment strategies, especially in the use of blood products. Renal pathology shows more involvement of the thrombotic microangiopathy (TMA) in the arterioles\(^4\) as different from the predominant glomerular TMA in D+HUS.

The production of the enzyme neuraminidase by Streptococcus pneumoniae is thought to be involved in the pathogenesis of the thrombotic process.\(^26\) Circulating neuraminidase removes N-acetylneuraminic acid and exposes the T (or Thomsen-Friedenreich) antigen in the cell membrane of erythrocytes, platelets and glomerular endothelial cells. Anti-T IgM is usually present in normal sera and antigen-antibody reaction can lead to the agglutination and haemolysis of red blood cells, thrombocytopenia and thrombotic renal microangiopathy. Infusion of blood and plasma products may aggravate the haemolytic process as most adult sera contain anti-T antibody. The presence of erythrocyte T-antigen activation can be detected by the peanut lectin agglutination test.\(^2,26-31\)

Plasma infusion as a routine therapy is therefore contraindicated in neuraminidase-associated HUS with positive T-antigen exposure. If blood transfusion is indicated, washed red blood cells should be given. If fresh frozen plasma is essential for coagulation support, only low titre anti-T plasma should be used.\(^32\) Appropriate antibiotics should be given and other supportive measures are essentially the same as D-HUS. Exchange transfusion has been suggested as a specific therapy, aiming at the
removal of circulating neuraminidase and red blood cells with exposed T-antigen. However, some cases treated with exchange transfusion subsequently died, while other patients made a complete recovery with supportive therapy alone. McCaggart and Burke therefore did not recommend exchange transfusion as a routine therapy. The mortality rate for pneumococcal-associated HUS is much higher than D+HUS. A review in the United States reported a mortality of 50%.

Early detection of the presence of T-antigen exposure and appropriate use of blood products may have provided a better outcome.

**Conclusion**

Haemolytic uraemic syndrome is a common cause of acute renal failure in children. Advances in the pathogenesis and treatment approach are continuously developing. Frequent updates on these information are essential to improve the outcome of the patients. Long-term follow up of possible renal sequelae is necessary for all patients with haemolytic uremic syndrome, including those with apparent full recovery of renal function shortly after the disease.

**References**