Hemolytic Uremic Syndrome

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Context: Hemolytic uremic syndrome (HUS) is a severe acute disease, sometimes with long-term sequelae. The diarrhoea-unrelated forms are particularly associated with a poor prognosis. The aim of this paper is to review current evidence regarding etiology and management, and explore methods by which the outcome may be optimized.

Evidence acquisition: An internet search of Medline, Medscape, MDConsult and Cochrane databases for publications related to HUS from 1998 onwards was performed. A review of articles pertaining to etiopathogenesis and management was undertaken.

Results: HUS is now classified according to cause. New assays and gene studies allow exact diagnosis of many of the atypical forms. Post-exposure prevention of diarrhoea associated HUS with vaccines and toxin-binding agents, remains in the experimental stages. Specific directed therapies aimed at replacing deficient factors can improve the outcome of atypical HUS.

Conclusions: Supportive care remains the cornerstone of management of HUS. The infection-unrelated forms should in addition be treated rapidly with plasma therapy. Efforts should be made to make an exact etiological diagnosis in all patients, as long-term treatment and prognosis is affected. Prevention of diarrhea-associated HUS by improving sanitation and proper attention to food hygiene is a practical goal.

Key words: ADAMTS13, Complement, Hemolytic uremic syndrome, Shiga toxin, Thrombotic thrombocytopenic purpura.

Hemolytic uremic syndrome (HUS) is a relatively rare disease that can have devastating consequences. It classically includes the triad of microangiopathic hemolytic anemia (MHA), thrombocytopenia and renal failure. The hallmark histopathological lesion is thrombotic microangiopathy (TMA), characterized by capillary endothelial damage and microvascular formation of platelet/fibrin plugs. This induces tissue ischemia, erythrocyte damage and consumptive thrombocytopenia(1,2). Other than the gut and kidney, different organs like the brain, liver and pancreas may be affected. Thrombotic thrombocytopenic purpura (TTP) is a similar disease, which occurs more frequently in adults, and affects the nervous system more than kidneys(3).

In children, diarrhea related “typical” HUS (tHUS) is commonest (80-90%), occurs sporadically or in epidemics, rarely recurs, and has a relatively better prognosis(1). Atypical or diarrhea-unrelated HUS (aHUS) is more severe, difficult to treat, and can occur with diverse conditions, like pneumococcal infections, autoimmune disease, HIV, transplantation, irradiation and certain drugs(4,5). Genetic forms of aHUS, may be familial, relapsing or recurrent, and are more commonly associated with progression to end stage renal disease (ESRD), with high risks of recurrence in transplants(6-8). An etiopathological classification was recently proposed(9) and a simplified version is presented in Table I.

Etiopathogenesis

Typical/Diarrhea associated/Shiga Toxin associated HUS

Worldwide, the commonest cause of pediatric HUS
is diarrhea causing enterohaemorrhagic \textit{E. coli} (EHEC) infection\cite{10}. In developing countries, HUS is also associated with \textit{Shigella dysenteriae} type 1 infection; however, its incidence in India has fallen along with a reduction in incidence of shigella dysentery\cite{11}. Rarely, HUS can occur with \textit{E. coli} urinary tract infection\cite{10}.

Several serotypes of \textit{E. coli} are known to cause HUS, the commonest being the serotype: 0157:H7\cite{7}. However, only about 10-15\% patients with \textit{E. coli} 0157:H7 infection will develop HUS\cite{12}. Sources of infection are milk and animal products (incompletely cooked beef, pork, poultry, lamb), and human feco-oral transmission\cite{2}. Vegetables, salads and drinking water may be contaminated by bacteria shed in animal wastes.

HUS occurs due to bacterial toxin production in the colon. EHEC release verotoxin or verocytotoxin, which is structurally and functionally homologous to Shiga toxin (Stx) released by HUS producing strains of Shigella, and the two terms are used synonymously. Two types of Stx are known, Stx1 and Stx2, the latter being 400 times more virulent. EHEC adhere to and efface intestinal cells and release Stx, which enters the blood stream and is transported by neutrophils. Stx binds to globotriaosyl ceramide (GB3) membrane receptors presented on endothelial cells of kidney and other target organs. At these sites, Stx disrupts protein synthesis, causes endothelial cell death and damage, induces inflammatory and procoagulant cascades that promote microvascular thrombosis\cite{7,13,14}. Despite producing similar toxins, Shigella infections are associated with higher incidence of fever, bacteremia, endotoxemia, leukemoid reactions, severe hemolysis, pseudomembranous colitis, and a higher fatality rate, indicating their enteroinvasive-ness and additional ability to cause direct cellular injury\cite{2}.

\textbf{Atypical/Non-Diarrhea Related HUS}

\textbf{Pneumococcal HUS}

5\% of all HUS and 38-43\% non-diarrheal HUS are reported in association with invasive \textit{Streptococcus pneumoniae} infection (commonly pneumonia, empyema, meningitis, and more rarely, pericarditis, peritonitis, bacteremia, mastoiditis, otitis media)\cite{5}. Renal endothelial cells, erythrocytes and platelets

\begin{table}[h]
\centering
\caption{Classification of HUS / TTP according to etiopathogenesis}
\begin{tabular}{|l|l|l|}
\hline
Type of HUS / TTP & Specific Cause & \hline
Infection related & Shiga toxin producing \textit{E. coli} / \textit{Shigella} infection & Diarrhea or typical \hline
& Pneumococcal infection & \hline
& HIV & \hline
& Other viral or bacterial infections & \hline
Complement factor abnormality & Factor H deficiency & Genetic mutation (AD) \hline
& Factor I deficiency & Acquired antibody \hline
& Membrane cofactor protein deficiency & \hline
& Factor B excessive activity & \hline
& Complement 3 excessive activity & \hline
ADAMTS13 deficiency & Genetic mutation (AR), acquired antibody & \hline
Cobalamin metabolism defect & Genetic mutation (AR) & \hline
Miscellaneous & Connective tissue disease & \hline
& Transplantation & Radiation \hline
& Drugs & Pregnancy \hline
& Malignancy & Unknown \hline
\end{tabular}
\end{table}

\textit{AD}: autosomal dominant inheritance; \textit{AR}: autosomal inheritance.
have a structure on their surface called Thomsen-Friedenreich antigen (TAg). This is normally obscured by neuraminic acid. Pneumococci containing the enzyme-neuraminidase are able to cleave this neuraminic acid from the cell surface thus exposing the TAg to pre-formed anti-TAg IgM (normally present in plasma from 6 months of age). This leads to antigen-antibody binding, activation of an immune cascade, with resultant, glomerular endothelial cell damage, hemolytic anemia, platelet aggregation and consumption, and a fall in GFR(15,16). This TAg is also present on hepatocytes, and hepatic dysfunction may co-exist(17).

HUS due to Complement abnormalities

The majority of non-infection related HUS in children is due to complement dysregulation. Complement gene mutations are found in 30-50% patients with aHUS(8), with 14-33% having abnormalities in the Factor H (FH) gene, 10-15% in membrane co-factor protein (MCP) gene and 2-13% in Factor I (FI) gene (18-20). These genes code for proteins that inhibit activity of complement C3b. Deficiency causes unregulated amplification of the alternative pathway and deposition of activated complement on the surface of invading bacteria or damaged self-tissue, such as apoptosed or inflamed renal endothelial cells(18,21,22). A minority of patients have gain in function mutations of factor B or C3 that accelerate the activity of the alternative pathway(23).

The majority of these genes are situated in a cluster of complement regulatory genes on chromosome 1q32. The mutations are generally heterozygous, patients having reduced (but not absent) activity of the factor, with autosomal dominant inheritance and 50% penetration. Homozygous and compound heterozygous mutations have also been described, usually having a more fulminant course(24). Autoantibodies to FH have been identified in a few patients, some of whom in addition have genetic deficiency of complement factor H related proteins, CFHR1 and CFHR3.

**ADAMTS13 deficiency and HUS/ TTP**

ADAMTS-13 (a disintegrin-like and metallopro-
tease with thrombospondin type 1 repeats, number 13) is an enzyme produced by stellate cells in the liver. It acts as a von Willebrand factor (VWF) cleaving protease, and degrades large multimeric forms of VWF by cleaving peptide bonds. In the deficiency of this enzyme, ultralarge multimeric form of VWF (ULVWF) that are released by stimulated endothelial cells circulate in plasma. Circulating platelets spontaneously and preferentially bind to ULVWF strings (rather than to smaller VWF). Continuing platelet aggregation, ensuing TMA and embolisation of ULVWF-platelet strings causes tissue ischemia.

The **ADAMTS13** gene is located on chromosome 9q34. The autosomal recessive, familial form of the disease usually seen in children, is rare (2-3%), and occurs due to homozygous or double heterozygous mutations of this gene. Acquired forms of ADAMTS13 deficiency, often associated with the presence of anti-ADAMTS13 antibodies, are more common in adults and older children. The manifestations are more classically of frank TTP (pentad of fever, neurological manifestations, TMA, severe thrombocytopenia, and relatively less severe renal dysfunction). There is a high risk of recurrence, particularly when there is persistence of low ADAMTS13 levels and circulating autoantibodies during remission(3,4).

**Miscellaneous Causes of HUS / TTP**

Abnormalities in intracellular vitamin B12 metabolism, caused by mutations of the cobalamin genes cause a severe HUS usually presenting in infancy, associated with neurological manifestations, leukopenia and megaloblastic anemia(25). HUS/TTP has been reported in association with HIV, systemic lupus erythromatosus, and/or the antiphospholipid syndrome, malignancies, radiation and certain drugs (e.g. cyclosporine, quinine, oral contraceptives etc.)(26). Post transplant HUS/TTP can occur due to recurrent disease, however de-novo disease is also seen in both solid organ and stem cell transplantation. In these conditions, endothelial damage leading to TMA is postulated as the inciting factor. ADAMTS13 deficiency has been detected in some cases. Other infections associated with HUS include viruses like influenza, cytomegalovirus and...
infectious mononucleosis, and bacteria like streptococcii and salmonella(5).

**Clinical Features**

The commonest clinical presentation of HUS is with acute pallor and oliguria, following diarrhea or dysentery. It occurs commonly in children between 1-5 years of age. Hematuria and hypertension are common. Complications of fluid overload may present with pulmonary edema and/ or hypertensive encephalopathy. Despite thrombocytopenia, bleeding manifestations are rare. Neurological symptoms like irritability, encephalopathy and seizures may occur. Other extra-renal manifestations include pancreatitis, jaundice and necrosis of gut mucosa. Incomplete or partial forms may exist(1,2,7).

Patients with aHUS have more insidious and sometimes fluctuating symptoms at onset, that may be preceded by viral or bacterial illness, connective tissue disease or history of drug intake. Family history may be present. The degree of hypertension and duration of oligo-anuria is greater than in tHUS. Extrarenal complications like cerebrovascular events and pulmonary hemorrhages, occurring due to multiorgan TMA, are more common. Patients with genetic forms of HUS due to complement or ADAMTS13 gene mutations, can present in infancy, or later after a precipitating “second hit”. ESRD may ensue in the first episode or progressive chronic kidney disease can develop with subsequent relapses. Partial forms may occur, with varying degrees of hemolysis, jaundice or thrombocytopenia(4,18).

**Investigations**

Peripheral blood smears reveal the presence of MHA by fragmented RBCs (schistocytes, burr cells and helmet cells), caused by their passage through damaged blood vessels. Platelet counts drop due to increased consumption. The degree of leukocytosis present has been related to a poor outcome(27). Reticulocyte levels are high. Lactate dehydrogenase levels are also high reflecting increased breakdown and turnover of RBCs. Unconjugated hyperbilirubinemia is present due to hemolysis. Serum haptoglobin levels are low due to binding with released hemoglobin. The degree of renal involvement varies and determines the increase in blood urea, creatinine, potassium and phosphate. In early stages, PT and APTT are normal or only mildly deranged, differentiating from disseminated intravascular coagulation (DIC). In some cases, liver transaminases, pancreatic enzymes and glucose levels may be affected. Urinalysis reveals hemoglobinuria, hematuria and proteinuria(2,3). A summary of investigations is given Fig.1.

**Investigations to Identify Cause**

In patients with diarrhea, the identification of pathogenic EHEC or *Shigella* is performed by stool culture and further serotyping by agglutination or enzyme immunoassay(10). Shiga toxin assays and PCR-assays have also been used for bacteriological identification. Rarely HUS can occur with *E. coli* urinary tract infection, and urine cultures are indicated in non-diarrheal patients. Bacteriological cultures of body fluids (sputum/CSF/blood/pus) are indicated in suspected pneumococcal disease.

C3 levels may be transiently low in tHUS and persistently low in aHUS due to complement factor deficiency. Persistently low C3 levels are commonly associated with FH or FI gene mutations(47); however, this is not universal, and up to 50-60% patients with demonstrable mutations have normal C3 levels. Serum C4 levels are usually normal(18, 19).

The direct Coombs test is positive in over 90% of patients with pneumococcal HUS(5); however, its specificity has not been tested. Aminoacid chromatography of serum and urine revealing homocystinuria, hyperhomocystinemia and methy-malonic aciduria with low serum levels of methionine indicates cobalamin metabolism defects. Total serum vitamin B12 levels are normal(25). Autoimmune serology (ANA, anti-dsDNA, anti-phospholipid antibodies) and HIV screening may be indicated(26).

In patients with no history of diarrhea, blood samples for the assay of specific complement factors (FH, FI and MCP), ADAMTS13 levels, and antibody to FH/ADAMTS13 may be taken prior to infusion of plasma or blood products and frozen for
later analysis. Genetic studies for mutations in complement factor genes, ADAMTS13 gene or cobalamin genes provide definitive diagnosis. Although these studies are not available in India, samples can be sent abroad for analysis(28).

Renal Biopsy

Renal biopsy is not essential for diagnosis, and often cannot be performed in the acute stage due to thrombocytopenia. It may be indicated in partial forms where the diagnosis is in doubt, or in recurrent or severe disease, to confirm the diagnosis before starting aggressive therapies. The pathognomonic finding of TMA is common to all types (Fig. 2). In hUS, predominantly fibrin thrombi are found in glomerular capillaries. In contrast, in aHUS, the thrombi are made up of a combination of fibrin, platelet and VWF clumps that involve larger renal and interlobular arterioles, thus causing ischemia and inflammation of larger volumes of renal
parenchyma(1). Glomerular capillary wall thickening, occlusion or narrowing of capillary lumens, inflammation and necrosis of endothelial cells and their detachment from the basement membrane may be observed. Infiltration of inflammatory cells (macrophages and neutrophils) is seen. Tubular epithelial injury, mesangial expansion and mesangiolysis may also occur. Cortical necrosis is present in severe cases, and indicates a poor outcome(2).

MANAGEMENT

Supportive Therapy

In all patients, supportive treatment is primary. Close clinical monitoring of fluid status, blood pressure, and neurological and ventilatory parameters is required. Blood levels of glucose, electrolytes, creatinine and hemogram need frequent monitoring. The aim in early diarrhea associated disease is to prevent dehydration and maintain intravascular volume. The use of antimitility therapy for diarrhea has been associated with a higher risk of developing HUS(7).

With the onset of acute renal failure, fluid restriction and diuretics may be required along with salt and potassium limitation. Antihypertensives are used to control blood pressure. Packed cell transfusions are given to correct significant anemia. Nutrition needs to be maintained and parenteral nutrition may be indicated where there is severe gut involvement. Platelet transfusions are reserved for patients with active bleeding, or prior to surgical procedures (like dialysis catheter placement). Early dialytic support is indicated if there is worsening uremia, if electrolyte or fluid homeostasis cannot be controlled conservatively, or if space is required for transfusions, drugs or nutrition(1,2,7). Further specific treatment varies according to type of HUS (Fig.1).
**Antibiotics**

The use of antibiotics in *E. coli* associated HUS is controversial. Several reports claimed a worse outcome with antibiotic use, however, a meta-analysis did not support any effect of antibiotics on the occurrence of HUS(7,29). Antibiotics are essential for the management of shigellosis to treat its complications and prevent transmission.

In pneumococcal HUS, aggressive antibiotic treatment of the primary infection is essential. In countries where penicillin resistance is high, vancomycin (dose adjusted for renal involvement) should be used in addition to a 3rd generation cephalosporin, until sensitivity results return.

**Plasma Therapy**

In aHUS due to complement factor abnormality or ADAMTS13 deficiency, there is a rational role for the replacement of the deficient factor with FFP. In ADAMTS13 deficiency HUS, the early institution of plasma therapy or cryosupernatant, greatly improves recovery rates(30). The use of plasma is more controversial in HUS due to complement dysregulation. No randomized controlled studies are available, but recent series report a 32-72% response(8,18).

Since the specific cause of aHUS is rarely known in the acute stage, the early use of FFP is recommended in all non-diarrheal/non-pneumococcal cases. Daily plasma infusions (10 to 20 mL/kg/day) have been effective in some case reports, whereas in others, plasma exchange which can deliver higher volumes of FFP has shown better response(24,28,31). The volume of FFP that can be infused is limited in patients with oligo-anuria. Additionally, plasma exchange may be more useful than infusion, particularly in acquired forms as removal of autoantibodies, ULVWF strings and cytokines is facilitated.

The European Pediatric Study Group for HUS has just published guidelines based on ‘opinion rather than evidence’, which advocates the early use of intensive plasma exchange as primary therapy in all patients with aHUS(28). Exchange of 1.5 times plasma volume (*i.e.* 60 to 75 mL/kg/day) using FFP as replacement fluid has been recommended in this guideline. Plasma therapy is generally continued on a daily basis until hematological and biochemical recovery, and then weaned gradually. Plasma infusions at 3-weekly intervals or at the onset of any possible precipitating illness, has been used to prevent relapses(31).

No definite role of plasma therapy has been documented in tHUS, although it has occasionally been used in severe cases particularly with neurological involvement (7,32). Plasma and blood products may worsen the outcome in pneumococcal HUS by providing more anti-TAg IgM(5). In this situation, blood products should only be used if unavoidable, RBCs should be washed with dextran which removes 95% of plasma and FFP should only be used if there is severe bleeding.

**Miscellaneous**

In infants with HUS associated with cobalamin abnormalities, treatment with hydroxycobalamin, oral betaine and folic acid normalizes the metabolic abnormalities and can help prevent further episodes(25). Removal of the offending drug, and appropriate management of the primary disorder is required in patients with HUS due to drugs/HIV/ connective tissue disease/malignancy. In patients with persistent ADAMTS13 antibodies and poor response to plasma exchange, immunosuppressive therapy with high dose steroids/cyclophosphamide/ cyclosporin/rituximab and splenectomy have been tried(4,26).

**Outcome**

The early outcome of tHUS is relatively good in children with <5% failing to regain renal function. In epidemics, in the acute stage, children do better than adults. There is a 5%-10% acute mortality, mainly due to extra-renal complications. Although the long term outcome is better than in other forms of HUS, upto 10-30% develop chronic kidney disease and nearly 5-10% of these patients develop ESRD in the next 10 years(1,2). Long term follow-up is therefore, indicated in all patients as hypertension and proteinuria may develop after several years. Recurrence in transplanted patients is extremely rare.
In pneumococcal HUS, outcome depends on degree of associated infection, and is worst with meningitis where the mortality may be up to 37%. The combined overall acute mortality in 73 patients, reviewed in 2007, was 12% with 75% requiring dialysis in the acute stage and 10% developing ESRD on follow-up(5).

In non-infection related HUS, up to 25% patients die in the acute phase, and 50% progress to end stage renal failure(8). FH and FI disease is more severe than MCP disease which may resolve even without plasma therapy. The failure rate of renal transplantation in FH and FI mutations is high, with approximately 80% graft loss due to thrombosis or recurrence(33). Combined liver and kidney transplants have been successful in 4 patients who received intensive pre- and peri-operative plasma exchange. In contrast, with MCP mutations, the outcome of renal transplantation is relatively good as MCP levels are partially replenished within the donor kidney. The outcome of HUS due to ADAMTS13 deficiency has improved with the advent of plasma therapy with mortality figures dropping from 80-90% to 10-20%(4,30).

FUTURE DEVELOPMENTS

Ideally, the transmission of tHUS should be prevented by improving standards of sanitation, hygiene and food handling. Vaccines utilizing recombinant forms of the B subunit of Stx are under study in animals. Compounds that mimic the structure of GB3 receptors have been synthesised (Synsorb/Starfish) and shown to avidly bind to Stx in vitro, and in the gut of experimental animals. Clinical trials have not yet confirmed their efficacy in affected children, probably because early diagnosis of Stx production and administration of the drug would be required to prevent the translocation of Stx to extraintestinal sites. The use of chimeric monoclonal antibodies to neutralize Stx and provide passive postexposure protection are also being studied(7).

Pneumococcal serotypes reported to cause HUS are 1, 2F, 3, 6A, 6B, 8, 9V, 14, 19, and 23F(15,16). The 7-valent conjugate pneumococcal vaccine currently available in India, and included in the universal immunization programmes of several developed countries, contains serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F. It remains to be seen whether there will be a reduction in pneumococcal HUS prevalence in these countries. Plasma concentrates of Factor H and ADAMTS13, complement inhibitors and recombinant active forms of ADAMTS13 are being developed and may soon be available for clinical use(31).

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