Haemolytic uraemic syndrome in India due to Shiga toxigenic Escherichia coli

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The emergence of Shiga toxigenic Escherichia coli (STEC) as a causative agent of diarrhoea, haemorrhagic colitis and haemolytic uraemic syndrome (HUS) in humans is a significant public health concern worldwide. Here we describe a case of HUS following dysentery due to STEC. Though STEC is not a major cause of diarrhoea in India, we recommend that STEC should be looked for in all cases of bloody diarrhoea. To our knowledge, this is the first case of HUS caused by STEC in India.

Introduction

Shiga toxigenic Escherichia coli (STEC) is the major causative agent of haemorrhagic colitis (HC) and haemolytic uraemic syndrome (HUS), leading to acute renal failure and death. HUS occurs more often in children of less than 10 years of age, as compared with adults (Bae et al., 2006). Although O157 : H7 is the main serotype associated with the majority of outbreaks and sporadic cases of HUS in humans, non-O157 serogroups also pose a significant public health problem (Schimmer et al., 2008; Wahl et al., 2011). The recent outbreak of HUS that occurred in Germany is the largest ever reported and was due to STEC O104 : H4, a non-O157 serotype (WHO, 2011). The isolation of Shigella and non-typhoidal Salmonella from HUS patients has been reported. In 2005, we reported, what we believe to be, the first case of HUS caused by ciprofloxacin-resistant Shigella dysenteriae serotype 1 in India (Taneja et al., 2005). STEC is not a major cause of diarrhoea in India and there have been no reports of its isolation from HUS patients.

Here, we report a case of HUS in India associated with STEC. We also characterized the isolated strain phenotypically for haemolysin and Shiga toxin production, to determine its serotype, and also genotypically for major virulence genes.

Case report

An 8-month-old male infant, a resident of Mandi district, (Himachal Pradesh, India) presented to the paediatrics emergency department of the Advanced Paediatrics Centre in the Postgraduate Institute of Medical Education & Research (PGIMER), Chandigarh, a tertiary care referral centre, with a clinical diagnosis of acute renal failure on 15 February 2011. The patient’s illness started with fever, vomiting and loose stools with blood and mucus 2–5 days, almost a week, prior to admission to the PGIMER. The carer of the patient also reported decreased urine output in the 4 days before admission. On physical examination, the patient’s body was swollen, he had pallor and was anuric. The blood pressure was 84/62 mm Hg, weight 7 kg, length 72 cm and heart rate 110 min \(^{-1}\). In a peripheral blood smear, schistocytes were detected. Results from haematological tests revealed a haemoglobin level of 6.4 g dl \(^{-1}\), a platelet count of 99 \(\times 10^9\) l \(^{-1}\), plasma LDH (4509 U l \(^{-1}\)). Table 1 shows the biochemistry and haematological results from tests carried out during the patient’s hospitalization. As evident, the patient had anaemia, thrombocytopenia, high creatinine and blood urea levels. The reticulocyte count was 2.5%. The differential diagnosis included HUS, acute gastroenteritis with acute tubular necrosis and bilateral renal vein thrombosis. The patient received the following antibiotics for possible sepsis: ceftriaxone, cloxacillin, piperacillin–tazobactam, meropenem and vancomycin, and developed status epilepticus on 22 February 2011 (6 days post-admission) (Fig. 1). The patient was put on peritoneal dialysis and later died on 1 March 2011. The cause of death was refractory shock/nosocomial sepsis and pulmonary haemorrhage. The C-reactive protein level was 31.2 and 35 mg l \(^{-1}\) on day 2 and 4 post-admission, respectively (normal range is less than 10 mg l \(^{-1}\)). Cerebrospinal fluid culture was performed post-mortem and was sterile. Electrolyte levels were tested frequently (Na varied from 132 to 149 mEq l \(^{-1}\) and K from 5.9 to 3.2 mEq l \(^{-1}\) (Table 1).
On stool culture examination, a pure growth of *E. coli* was observed and found to be negative for other pathogenic bacteria, such as *Shigella*, *Salmonella*, *Aeromonas*, *Yersinia* and *Vibrio* species. For the detection of STEC, a stool sample was inoculated into EC medium (Difco Laboratories) and incubated overnight at 37 °C. From 1 ml broth culture, DNA was extracted by boiling at 100 °C for 10 min and centrifuged at 5000 r.p.m. (700 g) for 5 min. The presence of STEC was tested for by using PCR for *stx1* and *stx2* genes, as described previously by Paton & Paton (2002). PCR-positive broth culture was serially diluted in 10 mM PBS (pH 7.0). About 100 μl each dilution was streaked on sorbitol MacConkey agar (SMAC; Difco Laboratories). Ten to 15 colonies were selected and inoculated into EC broth for overnight incubation. Again, 1 ml broth was boiled for 10 min and the supernatant was used as a template for the screening of STEC, as described above. After confirmation of the presence of STEC, the strain was characterized for both chromosomal (*stx1*, *stx2* and *eae*) and plasmid-encoded (*hly*, *etpD*, *espP* and *katP*) virulence factors (Brunder et al., 1996,1997; Paton & Paton, 2002). The strain was tested for four putative adhesion genes (*saa*, *iha*, *toxB* and *efa*) by using primer sequences as described previously by Paton & Paton (2002). The *stx* subtypes *stx1c*, *stx1d*, *stx2d*, *stx2e* and *stx2f* were also screened for by using PCR, as described.
previously by Beutin et al. (2007), Wang et al. (2002) and Zhang et al. (2002). The STEC strain was positive for the virulence genes stxl, stx2, eae and iha. Molecular subtyping of the stx gene revealed the presence of an stxl gene variant. We performed the serotyping of this strain on the basis of the O antigen of E. coli by using the slide agglutination test with the available O antisera (Denka Seiken). However, the serotype was untypeable with the tested antisera. The isolate produced haemolysin on 5% sheep blood agar and we detected the production of Shiga toxin by using the RIDASCREEN enzyme immunoassay kit for verotoxin (R-Biopharm).

Discussion

HUS is characterized by haemolytic anaemia (with fragmented erythrocytes), thrombocytopenia and acute renal failure (Kaplan et al., 1998). In North America and Western Europe, E. coli O157 : H7 is associated with 70% of HUS cases (Noris & Remuzzi, 2005). After STEC infection, 38–61% of patients develop HC, and in 3–20% of these patients the disease progresses to HUS (Banatvala et al., 2001). In 55–75% of HUS cases, renal failure occurs (Milford, 1992). In the developing countries of Asia and Africa, HUS can also be caused by S. dysenteriae serotype 1 (Taneja et al., 2005). Recently, the largest outbreak of HUS due to STEC O104 : H4 was reported in Germany, where 4000 people became infected and 40 died (WHO, 2011). However, HUS associated with STEC has not yet been reported in India. To the best of our knowledge, this is the first description of HUS caused by STEC in India. In the current case study, the patient developed pulmonary haemorrhage and status epilepticus. Pulmonary haemorrhage in typical post-diarrhoeal HUS is rare, but it is associated with a poor prognosis (Piastra et al., 2004; Chang-Poon et al., 1985). The pathogenesis is not clear but microvascular damage with a loss of capillary integrity and necrosis of the alveolar wall have been proposed as possible mechanisms (Piastra et al., 2004).

Cytokines such as tumour necrosis factor alpha and interleukin-1 that are produced in response to Shiga toxin mediate cell damage and can contribute to the pathogenesis of renal and central nervous system (CNS) vascular lesions in HUS (Ramegowda et al., 1999). The management of HUS involves correcting electrolyte and fluid imbalances, managing haematological complications, monitoring for extra-renal involvement in HUS and possibly avoiding antibiotic therapy. Administering antibiotic therapy for STEC-induced diarrhoea is controversial. It has been shown that children who developed HC associated with STEC infection and received antibiotic therapy were more likely to develop HUS, as compared with patients who did not receive antibiotic therapy (Scheiring et al., 2008). However, a meta-analysis study has contradicted this conclusion (Saferd et al., 2002) and states that of the 26 studies, only 9 met the inclusion criteria. Four retrospective studies and one prospective study did not find any association with HUS and antibiotic treatment. One prospective and one retrospective study each reported a protective effect of fosfomycin, whereas only one prospective and one retrospective study reported a significant risk of HUS after antibiotic therapy. Similarly, in vitro studies in experimental animals have shown conflicting results (Saferd et al., 2002). A study by Kurioka et al. (1999) showed that trimethoprim–sulfamethoxazole increased the mortality rate, whereas norfloxacin, kanamycin, ampicillin and clarithromycin reduced the risk of complications, decreased the levels of toxins in blood and stools, and also reduced the excretion of O157:H7 STEC in stools. Conflicting reports may be related to strain variation, the class of antibiotic used and the time and duration of the antibiotic therapy (Saferd et al., 2002). They concluded that the major limitation of their analysis was their inability to take into account the severity of the illness and to analyse the risk of HUS with various classes and treatment durations of antibiotics, the timing of therapy and the lack of multiple representative strains of E. coli O157:H7. Our patient received four classes of β-lactam agents and one glycopeptide to cover suspected sepsis by multi-drug-resistant, Gram-negative organisms and Enterococcus species. Wong et al. (2000) in their prospective cohort study of 71 children with E. coli O157:H7 enteritis showed that both trimethoprim–sulfamethoxazole and β-lactams appeared to be associated with an increased risk of developing HUS. It is difficult for us to speculate the role of extensive antibiotic treatment on the outcome of the patient. In our geographical region, bloody diarrhoea and HUS may be caused by shigellae, for which antibiotic treatment is recommended (Taneja et al., 2005). Therefore, we recommend that STEC should be looked for in all cases of bloody diarrhoea. Diagnosing STEC infections early and treating them aggressively with fluids, but not antibiotics, may be the key to preventing a patient from developing HUS.

The mortality rate of HUS caused by STEC has been described as between 3 and 5% (Milford, 1992) and death is nearly always associated with severe extra-renal disease, such as severe CNS involvement and intestinal necrosis (Siegel, 1995). In 60% of HUS cases mortality occurs due to the duration of renal failure and renal cortical necrosis (Srivastava et al., 1991).

In the current study, we characterized the STEC strain isolated from the patient’s stools for various virulence and adhesion genes. The genes stxl and stx2 are the major virulence factors of STEC and the presence of one or both of them defines the STEC pathotype. Ostroff et al. (1989) have hypothesized that the type of toxin produced by STEC determines the risk of developing microangiopathic sequelae. They reported that strains with the stx2 gene were more likely to cause HUS than other genotypes. The current isolate possessed both the stxl and stx2 genes, and was also positive for the eae gene, which encodes intimin (intimate attachment to epithelial cells) that causes attaching and effacing lesions in the intestinal epithelial cells (Paton & Paton, 2002). In addition to this, the iha gene, which is reported to be the most common factor for adherence, was
also detected. On subtyping the stx1 and stx2 variants, stx1c was found to be present. Zhang et al. (2002) reported the presence of the stx1c gene in 36 STEC (17 %) strains, one of which was recovered from an HUS patient.

In conclusion, we have reported a case of HUS associated with diarrhoea due to STEC. To the best of our knowledge, this is the first reported case of HUS caused by STEC infection in India. Though many selective media are available to isolate O157 STEC, which include SMAC, cefixime–tellurite SMAC and CHROMagar O157, no differential media are available to differentiate non-O157 STEC from other non-pathogenic E. coli strains.

References


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