Fulminant hepatic failure resulting from coexistent Wilson’s disease and hepatitis E

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Fulminant Wilson’s disease is characterised by severe derangement of liver function, encephalopathy, and haemolysis in patients with previously undiagnosed liver disease. Early diagnosis is essential as without orthotopic liver transplantation, the mortality is virtually 100%. Although the basic defect, a failure of biliary copper excretion, has been known for many years, the exact pathophysiology of the defective excretory mechanism has remained elusive. It is known that Wilson’s disease may run a varied course, ranging from fulminant Wilson’s disease to an insidious progression to cirrhosis over 20–30 years. The factors that dictate disease tempo in any particular patient remain unknown. Many patients with fulminant Wilson’s disease have grossly raised serum copper concentrations, and it has been postulated that an acute insult damages the hepatocytes, which release free copper in toxic concentrations, which in turn damage cell membranes both directly and by free radical mediated damage. It is therefore possible that in some patients a hepatic injury unrelated to the metabolic abnormalities of Wilson’s disease in itself may be the primary process that triggers fulminant Wilson’s disease. This study reports on a case of fulminant hepatic failure occurring in a six year old girl with biochemical and histopathological evidence for underlying Wilson’s disease complicated by hepatitis E virus (HEV) infection. The diagnosis of HEV was made by nested polymerase chain reaction and confirmatory DNA sequencing. The patient required emergency orthotopic liver transplantation. The histopathology of HEV, the time course of viral shedding in stool, the viral titre in serum, and the relation to Wilson’s disease are discussed.

Case report
A six year old girl, born and resident in Birmingham, England to unrelated Indian parents was well except for severe vitiligo (Fig 1A), which started at four and a half years of age. Twelve weeks before presentation, the patient left for a six week holiday to Bombay, India. Before leaving the United Kingdom she was given anti-typhoid and anti-cholera vaccinations and normal immunoglobulin prophylaxis. She was well and without symptoms while in India. Two weeks after her return to the UK, she developed diarrhoea, and a flu like illness with arthralgia and a non-pruritic rash. Other family members were asymptomatic. Severe jaundice developed and the patient was referred three weeks after the onset of symptoms. Dietary copper intake was normal and there was no history of copper intoxication. On physical examination the liver was of normal size and the spleen impalpable. She was not encephalopathic. Serum biochemistry on admission suggested severely deranged liver function (bilirubin 550 μmol/l, normal (n) <20 <μmol/l; aspartate aminotransferase 640 IU/l, n<50 IU/l; alkaline phosphatase 1081 IU/l, n<350 IU/l; albumin 29 g/l, n=35–45 g/l). International normalised ratio (INR) increased from 2.9 to 4.9 in the first 12 hours after admission. Haematological investigations were compatible with Coombs’ negative haemolytic anaemia: HB 7·9 (n=13–4–16·5 gm/dl), reticulocyte count 220×10⁶/l (n=50–100×10⁶/l), white cell count 9·4×10⁹/l (n=4–11×10⁹/l), platelets 220×10⁹/l (n=200–400×10⁹/l). Serological tests for hepatitis B, hepatitis A, Ebstein-Barr virus, human immunodeficiency virus, herpes viruses, and leptospirosis were negative while cytomegalovirus titre (1:16) was unremarkable. There were no serological features of autoimmune hepatitis (anti-smooth muscle antibody, anti-nuclear antibody, anti-liver/kidney microsomal antibody, and anti-gastrointestinal cell antibody negative) and serum immunoglobulins were normal. Anti-liver specific lipoprotein was positive at a titre of 1:1050 and anti-asaialoglycoprotein receptor was also
Progressive deterioration, including coagulopathy for Kayser-Fleischer positive at a titre of 1:50. Slit lamp examination for Kayser-Fleischer rings was negative. During the patient’s stay in hospital, liver function progressively deteriorated, she became encephalopathic with asterixis and confusion and worsening coagulopathy (maximum INR 8.3 despite replacement treatment). Copper studies were consistent with the diagnosis of Wilson’s disease, and a decision to undertake orthotopic liver transplantation was taken 10 days after admission.

Methods

At the time of liver transplantation, serum, the resected liver, and bile obtained at direct puncture of the removed gall bladder were examined for hepatitis C and E virus nucleic acid by nested polymerase chain reaction (PCR). Ribonucleic acid was extracted from serum, bile, and liver by solubilisation 50:50 in 8 molar guanidinium isothiocyanate as described by Chomczynski et al., before phenol/chloroform extraction and overnight ethanol precipitation. Complementary DNA (cDNA) was synthesised using avian myeloblastosis virus (AMV, Promega, UK) mediated reverse transcription using primer RS HEV2 5’-TCTACTTCAACTTCAAGCC-3’ to initiate the reverse transcription reaction. Polymerase chain reaction was carried out on the cDNA thus synthesised using RS HEV1 5’-ACAGGTGAGACCATGCGCC-3’ as its primer pair, and cycling parameters of 95 degrees for one minute, 55 degrees for one minute, and 72 degrees for one minute for 25 cycles. These primers theoretically produce an amplicon of about 573 base pairs in size. Five μl of the resultant first round reaction were then used as a template for a second round PCR using RS HEV3 5’-TTGATGACACCCTCTTGCC-3’ and RS HEV4 5’-CAGTATTTCCATAGAAGATGCC-3’, which produce an amplicon of 266 base pairs when HEV is correctly amplified. Albumin mRNA was amplified as an internal control. Appropriate positive and negative controls for PCR amplification were used for all experiments, which were performed in triplicate. Hepatitis C virus (HCV) was also sought by nested PCR using the primers described by Ulrich et al. to examine the temporal changes in viral excretion.

Results

Figure 2 shows the initial PCR findings in liver, serum, and bile and show a positive nested PCR signal for HEV in the patient’s serum, bile, and liver tissue. Although a distinct band at the appropriate molecular weight was obtained, the specificity of this product was confirmed by direct, bidirectional sequencing of the PCR product by Sanger chain termination sequencing.
chemistry using the Femptomole (sequencing kit; Promega, UK) (data not shown). Both positive and negative stranded HEV RNA was detected in liver tissue at transplantation, indicating active viral replication at the time of grafting (Fig 3). Selective PCR of both strands, however, from the two subsequent biopsy specimens (performed to investigate deteriorating liver function) showed only the presence of positive stranded forms. Serial examinations of serum and stools by nested PCR confirmed continued viremia and excretion of HEV in stools for four weeks after orthotopic liver transplantation, while viral titres, as defined by serial end point dilution of the cDNA synthesised from undiluted serum, showed an early biphasic fluctuation. No evidence of HCV infection was found.

Biochemical estimation of tissue copper was consistent with homozygous Wilson's disease and showed massively raised copper stores with substantial regional variation of copper concentration within the resected liver (134, 403, 536, 707 μg/g dry weight, mean 445 μg/g dry weight, n<50 μg/g dry weight), with areas of regeneration being least overloaded. Serum caeruloplasmin was low (0.09 g/l, n 0.2–0.6 g/l), total serum copper was normal (12–20 μmol/l, n 11–20 μmol/l), free serum copper was not measured. Urinary 24 hour copper excretion was also grossly raised (15 μmol/24 hours, n<1–2.5 μmol/24 hours) while urinary copper excretion post D-penicillamine challenge (500 mg at a 12 hour interval orally) was also increased (28–7 μmol/24 hours) to a value typical of Wilson's disease.12 Investigation of the patient's immediate family (both parents and a younger brother) was uninformative as serum copper, caeruloplasmin, and urinary copper excretion were normal in all.

HISTOPATHOLOGY

Macroscopically, the excised liver was small (weight=310 g) with broad areas of multinaicolar collapse and scattered foci of nodular and cholestatic parenchyma. Histological examination (Fig 4) showed that in the collapsed areas there was extensive cell loss and mixed inflammatory cell infiltrate, with periporal ductular structures and evidence of hepatic venulitis (Fig 4A, B). In less affected areas the parenchyma showed features of regeneration with large cholangiolar bile casts and areas of fatty infiltration (Fig 4C). Prominent areas in periporal distribution showed hepatocyte ballooning and cytoplasmic vesculation with single cell necrosis and shrunkened, irregular shaped eosinophilic cells. Oecine staining showed random deposits of fine copper associated protein granules, which gave positive, yet weaker staining with rhodamine (Fig 4D). There was no evidence of cirrhotic transformation, but pre-existing fibrosis could not be excluded because of the overall derangement of the liver architecture.

CLINICAL OUTCOME

After orthotopic liver transplantation, performed with a 'cut down' left lobe graft, the patient required a further laparotomy for intra-abdominal bleeding six hours later. In the next 24 hours, the coagulopathy improved and the bleeding settled the day after liver grafting. Abdominal sepsis and intestinal obstruction required two further laparotomies and drainage of subhepatic collections at 12 and 35 days respectively. After grafting, aspartate aminotransferase activity was initially grossly raised, reflecting preservation injury and reductive surgery but apart from two episodes of mild acute rejection, liver function tests progressively returned towards normal (Fig 3). The patient was discharged from hospital after two months and by six months both the extent and the severity of the vitiligol had dramatically improved (Fig 1B). At 19 months after initial presentation the patient remains well, with normal graft function on a maintenance immunosuppressive regimen of cyclosporin 170 mg/day (4 mg/g body weight), azathioprine 25 mg/day, and prednisolone 3 mg/day.

Discussion

The clinical presentation of this patient is
copper excretions before and after penicillamine challenge were at the values seen in Wilson's disease and the liver copper content was 3–15 times the upper limit of normal, well within the range seen in fulminant Wilson's disease. Because of the ethnic origin of our patient, Indian childhood cirrhosis, which is also characterised by grossly raised tissue copper concentrations, should be considered. Indian childhood cirrhosis, however, is exceptionally rare outside India, generally presents earlier in life with an insidious cirrhosis, rarely if ever produces fulminant hepatic failure, and has both a characteristic histological pattern of coarse granular deposits of copper associated protein, not seen in our patient, and a normal serum caeruloplasmin.

As recently described in the course of acute hepatitis E transmitted to a volunteer, our patient had detectable HEV RNA both in serum and stools at the time of acute symptomatic hepatitis. She continued to be serum and stool HEV RNA positive for about one month after transplant. As Figure 3 shows, viral titres showed a biphasic pattern early in the patient's hospital course, probably reflecting the severity of hepatic necrosis and release of virions into the serum rather than a true variation in the rate of viral replication. Antigenomic or negative stranded HEV RNA was detected in the resected liver, confirming the presence of active viral replication, whereas attempted selective amplification of both genomic and antigenomic RNA from two subsequent biopsy specimens showed only the presence of genomic strands, suggesting the cessation of active viral replication, or the amplification of viral RNA in blood trapped within the liver biopsy specimen.

The initial early rise in serum transaminases seen after transplantation in this patient provoked concern about recurrence of hepatitis, but the biopsy features were those of mild cellular rejection, and no clinical evidence of recurrent hepatitis was seen. We do not know why hepatitis E did not recur. The clinical course of hepatitis E, however, is similar to that of hepatitis A, the recurrence of which after transplant is exceptional.

The epidemiological evidence of faecal-oral transmission of HEV, in addition to the finding of viral like particles in stools and bile ducts, and its cloning from the bile of experimentally infected cynomologus macaques show that viral particles are excreted in bile or by biliary cells. In the few cases of fulminant hepatitis E with well recorded pathology in English language medical publications, cholestasis seems a prominent feature of the disease. It is conceivable that the excretory defect in Wilson's disease may result in defective viral excretion with failure to clear the virus and consequent particularly severe hepatocellular damage. If this hypothesis is correct, the oestrogen induced bile secretion impairment seen in some women during pregnancy and the comparatively immature bile secretory apparatus in neonates may explain the high death rate for HEV seen in these groups of patients.

Finally, although the severe viltigo of this case made us suspect an autoimmune pathogenesis for
her liver disease, she did not have non-organ specific autoantibodies or increased immunoglobulin G, typically present in autoimmune hepatitis. The increased titre of anti-liver specific lipoprotein and the low titre of anti-asialo-glycoprotein protein antibodies are compatible with severe Wilson’s disease (unpublished data). The dramatic improvement of the vitiligo after immunosuppressive treatment/liver grafting was unexpected. Before orthotopic liver transplantation the patient’s vitiligo had been a disfiguring social handicap. By six months after transplantation, however, both the extent and the severity of depigmentation had greatly improved. While we would not advocate immunosuppressive treatment for vitiligo, the clinical response in this patient was certainly impressive and emphasises the probable autoimmune pathogenesis of this condition.

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