

Original Article

Prevalence of dengue infection in north Indian children with acute hepatic failure

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Abstract

Hepatic manifestations of dengue viral infection are well known and cases of acute hepatic failure (AHF) with evidence of dengue infection are reported. Objectives: To study the role of dengue infection in AHF presenting to hospital. **Methods:** Setting: Pediatric wards of a teaching hospital in northern India. Subjects: Consecutive children hospitalized with AHF over a 3 month period in 2006. Clinical and laboratory details of subjects were charted. ELISA tests for dengue IgM were done in all patients using commercial kits. Real time PCR assays for dengue genome were done in randomly chosen subjects from those testing positive and negative for IgM. A PCR positive case was considered as definite dengue infection, while those who were only IgM positive were considered as 'probable' dengue. **Results:** Between July and September 2006, 27 patients were enrolled. Thirteen were unequivocally positive for dengue IgM. A random sample of 7 IgM positive and 3 IgM negative patients was tested by PCR, of which 4 IgM positive and one IgM negative patients were PCR positive. Prevalence of definite dengue infection in AHF was therefore 5/27 or 18.5%. No significant differences were observed in clinical and laboratory features of dengue and nondengue aHF. **Conclusions:** Dengue infection should be considered in the etiology of AHF in this part of the world. Clinico-laboratory differentiating features of dengue AHF should be studied in a larger sample of patients.

Key words: Dengue infection, dengue hemorrhagic fever, fulminant hepatic failure, acute hepatic failure.

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Dengue viral infections are the most important arboviral infections of man and the most important vector borne infection after malaria, with a wide geographical distribution in the Americas, the Pacific Islands, sub Saharan Africa and Asia. Over the last 50 years more complicated forms of the infection – dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) have been recognized.¹ Dengue viral infections are known to present a diverse clinical spectrum, ranging from asymptomatic illness to fatal dengue shock syndrome. Unusual manifestations of dengue infections such as encephalitis, myocarditis, Guillian Barre syndrome, hemolytic uremic syndrome and hepatic manifestations are recognized.^{2,3}

An increasing trend of outbreaks of DF and its complicated forms have been recorded over the north Indian plains over the last decade^{2,3} and all 4 serotypes of the virus are found here. Lucknow faced a severe epidemic in the later part of 2003. Since then annual outbreaks occur in the monsoon and postmonsoon season. There are no studies of the prevalence of dengue antibodies in the general or child population. A study of acute undifferentiated febrile illness showed that of 298 children studied over a 1 year period in Lucknow, 56 (18.8%) were positive for dengue IgM in serum.⁴

Hepatic injury with dengue infections has been described since 1967.⁵ Hepatomegaly is commonly observed in dengue fever (DF) and DHF and an increase in aminotransferases is often seen. Acute hepatic failure (AHF) is a common presentation seen in children admitted to our wards. An ongoing study on prevalence of DF as a cause of acute undifferentiated febrile illness in Lucknow and our observation of raised aminotransferases in DF cases prompted us to look for evidence of dengue infection in patients presenting with AHF also. The objective of this study was to study the role of dengue infection in children hospitalized with AHF.

Subjects & methods

The study was conducted in the Childrens' wards of Chhatrapati Shahuji Maharaj Medical University, Lucknow, which is the capital city of the state of Uttar Pradesh (or the northern state). This state is India's most populous (with a population of a 160 million, about 1/6th of that of the whole country) and also one of its poorest

with lowest human development indices. It is divided into 70 districts or administrative units. This part of the country is the gangetic plain of northern India. The state's population density is 689 people/sq km and children below 12 years account for roughly 40 % of the population.⁶ About 79% of the people live in rural areas. There are 3 seasons – summer (March to June), rainy or monsoon season (July to October) and winter (November to February). Temperatures may soar up to 45 °C in summer and fall as low as 3 °C in winter.

Lucknow, the state capital has a population of 4 million. The Chhatrapati Shahuji Maharaj Medical University Hospital is a tertiary care teaching hospital which caters mostly to the poor and severely ill. The catchment area of the hospital includes the Lucknow city and district and surrounding districts. The pediatric services of this hospital which cater to medical problems from birth to 12 years of age, have been admitting about 70-80 cases of acute hepatic failure (AHF) annually. Over the last decade, the region is recognized as a high dengue transmission area and a severe epidemic of DF/DHF occurred in late 2003.

Inclusion criteria: Children between 1-12 years of age hospitalized with AHF (i.e. altered consciousness occurring within 4 weeks of onset of jaundice in a previously well child)⁷ were enrolled in the study. Exclusion criteria : consent not obtained. The enrolled children were worked up according to a predesigned protocol incorporating a detailed history and examination including general, systemic and neurological examination. Daily follow up was done till discharge or death. Investigations done were complete blood counts and platelet count, liver function tests including s. bilirubin, transaminases, serum alkaline phosphatase, serum proteins, albumen and prothrombin time. Tests for hepatitis B and C are routinely done here in patients with acute hepatic failure. Hepatitis B surface antigen is tested by ELISA using Surase B commercial kits marketed by General Biological Corporation, Taiwan. Anti HCV antibodies are tested for by 3rd generation ELISA for core, NS3, NS4 and NS5 antigens by SP NANA-BaseC kit also marketed by General Biological Corporation, Taiwan. Wherever possible, anti HAV IgM antibodies were tested for by ELISA. In addition, 5 ml blood was collected in Eppendorf tubes and transported to the Virology Laboratory of Chhatrapati Shahuji Maharaj Medical University, Lucknow for serological investigations for dengue. IgM estimation for dengue virus was done in all cases in acute serum by IgM antibody capture ELISA using commercial kits marketed by Panbio, Australia. A random sample of those testing positive and negative for IgM by ELISA were subjected to PCR assays.

Real time quantitative PCR

Serum samples were subjected to Real Time polymerase chain reaction (PCR) assays. Total RNA was extracted by column based extraction kits of Qiagen (Viral RNA Mini

kit). Real time PCR was performed on Rotor Gene 3000, (Corbett Research Australia) using TaqMan probes, and primers designed to pick up all the four types of dengue virus synthesized according to Drosten et al by Tibmoliol, Berlin, Germany⁸ (provided by Professional Biotech, New Delhi). Single tube RT-PCR was performed using Single tube RT/PCR premix obtained from Professional Biotech, Ltd. Standard curves were established with plasmids which were cloned PCR products obtained from clinical samples (Tibmoliol) and contained complete cDNA sequence of the gene. Serial dilutions were prepared, starting at 10⁵ copies of the specific plasmid, to interpolate the unknown copy number of specific RNA in each sample.

Ethical considerations

Guardians of patients were informed that the child's blood sample was being tested for dengue. Verbal consent was obtained and the treating physician was informed about the test results.

Results

Over a period of 10 weeks from early July to mid September 2006, a total of 27 children were admitted to our wards as AHF. Of these 13 (48.1%) were unequivocally positive for dengue IgM, while one was equivocal. Serum samples of 7 randomly selected IgM positive patients were subjected to real time PCR assay as described, of which 4 were positive. The number of DNA copies/mL ranged from 2233 to 26,996/mL. Serum samples of 3 randomly selected IgM negative patients were also subjected to PCR, of which only one was positive, the number of DNA copies/mL being 153. The clinical and laboratory profile of dengue IgM positive and PCR positive patients is shown in *Tables I & II* respectively. Fourteen (51.8%) patients died. There were no significant differences in clinical or laboratory features among dengue IgM positive and negative cases or between dengue PCR positive and other cases.

Of 21 tested for hepatitis B surface antigen, only one was positive. Of 21 tested for HCV antibody, all were negative. Anti Hepatitis A virus IgM was tested in 4 patients and was positive in three. Anti Hepatitis E Virus IgM was tested in 3 patients and was negative in all.

Discussion

The term acute hepatic failure is used to describe the development of coagulopathy, usually an international normalized ratio (INR) of greater than 1.5, and any degree of mental alteration (encephalopathy) in a patient without preexisting cirrhosis and with an illness of less than 4 weeks' duration. The most common causes in this region are believed to be the hepatitis viruses – especially hepatitis A, E and B. Other viruses which cause AHF include Eb-

Table I. Clinical features of dengue IgM positive and PCR positive cases.

Feature ¹	Dengue IgM +ve (14)	Dengue PCR +ve (5)
Age in months ²	46.4 (24.1)	30.4 (13.7)
Male Sex	9 (64.3)	2 (40)
Bleeding	8 (57.1)	3 (60)
Convulsions	7 (50.0)	1 (20)
Rash	2 (14.3)	0 (0)
Swelling	4 (28.6)	2 (40)
Headache	3 (21.4)	0 (0)
Vomiting	5 (35.7)	2 (40)
Diarrhea	0 (0)	0 (0)
GCS ²	9.16 (3.38)	11.0 (4.0)
Breathing abnormality	4 (28.6)	3 (60)
Hepatomegaly	9 (64.3)	3 (60)
Splenomegaly	1 (7.1)	1 (20)
Mortality	9 (64.3)	3 (60)

¹ Figures () denote Number of patients (%)² Figures () denote mean (SD)² GCS: Glasgow Coma Scale

stein Barr virus, Herpes simplex virus, adenovirus, enterovirus, cytomegalovirus, parvovirus B19, varicella zoster virus and flaviviruses. Besides, autoimmune hepatitis, drugs and chemicals like carbon tetrachloride, acetaminophen, halothane and sodium valproate, toxins such as *amanita phalloides*, and metabolic disorders such as Wilson's Disease, galactosemia, tyrosinemia, fructose intolerance and others can cause AHF.⁹ Other causes of acute liver dysfunction in these parts are severe complicated malaria, dengue infections, enteric fever, leptospirosis and Reye's syndrome. Although dengue infection is known to be increasingly common here over the last decade and the liver is one of the target organs of dengue, it was not hitherto causally related to AHF.

Liver injury is known to occur with dengue infection.¹⁰⁻¹⁶ Kalyanaroop et al (1996) studied early clinical and laboratory indicators of acute dengue illness in Thailand and reported higher plasma alanine and aspartate aminotransferases in dengue than other febrile illnesses.¹⁷ Souza et al (2004) analysed 1585 cases of serologically confirmed dengue infection in Brazil and found that 44.5% presented with alterations in liver transaminases, 16.9% having at least one of the enzymes increased to at least 3 times the reference values and 3.8% had elevations of more than 10 times normal values.¹⁸

Many workers have reported AHF and acute hepatitis in dengue as single case reports,¹⁹⁻²⁶ but a detailed literature search revealed only 2 case series of this kind – both recent. Previous studies have relied on serological diagnosis only. In a recent communication from Thailand, 12 of 35 children 1-15 years of age enrolled from 14 centres during February 2000 to December 2001, including 8 of the 24 deaths had a positive serology for dengue. These authors concluded that dengue virus infection is a major cause of AHF in Thai children.²⁷ Deepak & Patel (2006) in a recent

Table II. Laboratory investigations in dengue IgM positive and PCR +ve cases.

Investigation ¹	Dengue IgM +ve (14)	Dengue PCR +ve (5)
Hemoglobin (g/dL)	11.0 (1.3)	11.8 (1.0)
% polymorphs	58.9 (18.2)	66.5 (11.8)
Platelets (10 ⁹ /L)	160.0 (75.4)	167.5 (51.2)
PCV	34.3 (2.76)	33.0 (1.41)
S Bilirubin (mg/dL)	13.6 (5.3)	15.4 (2.7)
sALT (u/L)	932.0 (1034)	1406.0 (1564)
sAST (u/L)	1326.0 (1514)	1760.0 (1965)
SAP (u/L)	957.0 (535)	1231.0 (156.9)
s Proteins (g/L)	66.3 (17.7)	66.0 (13.3)
s Albumen (g/L)	31.4 (10.4)	30.2 (.8.4)
Albumen/globulin	0.7 (0.3)	0.8 (0.11)
INR	5.1 (3.87)	4.7 (2.64)
Creatinine (g/L)	5.5 (1.0)	5.2 (0.5)
s Sodium (mmol/L)	136.3 (0.73)	134.2 (2.2)
S Potassium (mmol/L)	4.5 (0.73)	4.6 (1.07)

¹ Mean (SD)

PCV- packed cell volume AST – Aspartate transaminase, ALT- alanine transaminase; SAP – serum alkaline phosphatase; INR- International Normalised ratio, PCV- packed cell volume

report from Mumbai, India found 5 cases of dengue infection by serology among 56 cases of acute liver failure.²⁸ The present independent investigation shows that IgM was positive in 13/27 (48.1%) children with AHF in northern India. In a highly endemic area like ours, subclinical transmission of dengue infection may be ongoing and IgM antibodies persist for 60-90 days.¹ A positive IgM may therefore be found be labeled only as 'probable dengue' and does not conclusively prove that the present illness was dengue. However, Real Time PCR for dengue genome was positive in 5/10 patients tested including 4/7 IgM +ve patients, showing that at least 5/27 (18.5%) cases of AHF here were caused by dengue infection.

Real Time PCR assays could be done in a minority of patients only because of high cost. Our approach in this study was therefore to initially screen the patients presenting as AHF with IgM, and subjecting those testing positive to Real Time PCR assay. However, IgM is likely to be positive at a later stage of the infection than PCR and therefore this approach may have actually underestimated the PCR positivity.

Our cases which were dengue negative probably had a mixed etiology. As all the children also had fever, infective causes are likely. An important cause is likely to be hepatitis A virus, which is held to be the commonest cause of AHF in Indian children. Only 4 of our patients could be tested for HAV IgM of which 3 were positive. Other tropical infections may have also been responsible for some of the cases. Deepak & Patel²⁸ suggested that various tropical infectious diseases can be complicated by hepatitis, coagulopathy and encephalopathy and mimic AHF due to viral hepatitis. As only one patient was positive for HbsAg while none of those tested had

indicators of Hepatitis C or E infection, these infections can be considered minor causes of AHF here. None of our cases had history of drug or poison ingestion or any previous liver disease suggesting a metabolic disease.

By and large, the cases seen by us did not have the classical findings expected in dengue fever or DHF such as rash, thrombocytopenia, or evidence of capillary leak, some of which have been reported in the earlier case reports. Mourao et al reported thrombocytopenia and raised hematocrit in their patient of DHF with acute hepatitis.²⁵ Souza et al reported rash, positive tourniquet test and thrombocytopenia in their patient with hepatitis and dengue shock syndrome.²³ Vinodh et al reported a hematocrit of 50% and platelet count of 120,000/cu mm in their patient with dengue shock syndrome (stage IV) and acute liver failure.²⁶ Lawn et al described a patient with DHF and AHF who was not jaundiced but had marked thrombocytopenia and petichiae.²⁴ Our cases presented just like any AHF with jaundice but without classical features suggestive of dengue or DHF. A larger study is required to delineate clinicolaboratory features, if any, which can differentiate AHF due to dengue from other causes.

This small pilot study spans only 3 months between July and October 2006, the monsoon and post monsoon season – a season for high mosquito breeding. The high prevalence of dengue seen by us may therefore be seasonal to some extent. An year round study therefore should be done to find the overall year round importance of dengue in causing AHF. Still, our study does show that even during this particular season such a high proportion of AHF was probably due to dengue.

Conflict of interest: None

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