

Comparison of etiology of sporadic acute and fulminant viral hepatitis in hospitalized patients in Pune, India during 1978-81 and 1994-97

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Objective: To determine and compare the etiology of sporadic acute and fulminant viral hepatitis in two groups of patients 16 years apart. **Methods:** Serologic diagnostic tests for hepatitis A, B, C, D and E, and cytomegalovirus infection were carried out in 276 patients during 1994-1997 (Group A) and 206 patients during 1978-1981 (Group B). **Results:** Among children, hepatitis A virus was the major etiologic agent (81.6% in Group A and 51.4% in Group B), followed by hepatitis E virus (12.2%, 46.4%) and hepatitis B virus (5.4%, none). Among adults, hepatitis E virus was the main causative agent (42.4% in Group A and 71.2% in Group B) followed by HBV (28%, 25.5%) and hepatitis A virus (10.6%, 3.5%). Delta hepatitis was found only in Group A. No viral cause was found in 25% of patients in Group A and 13.5% patients in Group B. **Conclusions:** Hepatitis E virus is a major cause of sporadic acute and fulminant hepatitis. There has been an increase in hepatitis A in adults who developed fulminant hepatic failure. Our data points to the emergence of hepatitis A in adults and emergence of delta virus infection. Hepatitis C virus was unimportant in causing sporadic hepatitis. [*Indian J Gastroenterol* 2003;22:11-15]

Key words: Fulminant hepatic failure, hepatitis A virus, hepatitis B virus, hepatitis E virus, non-A to E hepatitis

Disease burden and major etiological agents for acute viral hepatitis (AVH) vary geographically. In India, hepatitis E virus (HEV) is the major etiological agent in epidemic situations¹ and among sporadic cases.²

Knowledge about the differential serodiagnosis of AVH is important for management of patients and their contacts. Periodic monitoring of etiology among hospitalized AVH cases may provide an idea about relative disease burden of various infections among patients with severe AVH, which in turn is helpful for deciding preventive policies.

We studied the etiology in hospitalized patients with sporadic AVH and fulminant hepatic failure (FHF) during 1994-97 and compared the data with similar cases during 1978-81 with reference, among others, to transmission routes and risk factors.

Methods

Two hundred and seventy-six AVH patients admitted in four hospitals in Pune were included in the study between 1994 and 1997 (Group A; Table 1). Of these, 56 patients belonged to the upper socio-economic group and 220 to the lower socio-economic group.³ Stored sera of 206 patients admitted in the same hospitals during 1978-81 (Group B) were also tested; their case records were analyzed. These patients belonged mainly to the lower socio-economic group. Prior approval of the institutional ethics committee was obtained; patients in Group A signed a written informed consent.

The diagnosis of AVH was based on standard clinical and biochemical criteria. FHF was defined as onset of hepatic encephalopathy within 8 weeks of onset of AVH without apparent evidence of underlying chronic liver disease.⁴ Patients with apparent or proven chronic liver disease, alcohol abuse, exposure to hepatotoxic drugs and having other likely non-viral causes of hepato-cellular injury were excluded.

A questionnaire was filled for each patient in Group A with information provided by the patient, or by a family member in case of children (age <15 years) and fulminant cases. Personal data and information pertaining to the present illness and to any past episodes of jaundice were recorded. Exposure to risk factors relevant to parenterally and feco-orally transmitted hepatitis viruses during the last six months was recorded; this included history of transfusions, injections, opera-

Table 1: Study population

Categories	Group A (1994-97)				Group B (1978-81)				
	Males	Females	Total	Age	Males	Females	Total	Age	
Adults	Acute viral hepatitis	91	41	132	32 (12.1)	94	47	141	29.8 (11.2)
	Fulminant hepatic failure	10	8	18		15	15	30	
Children	Acute viral hepatitis	52	46	98	6.3 (3.4)	23	12	35	7.4 (4.1)
	Fulminant hepatic failure	16	12	28		-	-	0	
Total		168	107	276		132	74	266	

Age is as mean (SD) years

tions, tattooing, exposure to commercial sex workers, promiscuous sexual behavior, and intravenous drug abuse. Details about drinking water source, type of toilets used, travel, contact with patients with hepatitis in the family or neighborhood were also recorded. Serum specimens were stored at -20°C till tested.

Tests conducted

Group A: All specimens were tested for IgM antibodies against hepatitis A virus (IgM anti-HAV),⁵ IgM antibodies against hepatitis E virus (IgM anti-HEV)⁶ and hepatitis B surface antigen (HBsAg)⁷ using in-house assays. Antibodies to hepatitis C (anti-HCV) were tested using a recombinant immunoblot assay (RIBA-III, Chiron, USA). All HBsAg-positive patients (n=59), patients with FHF (n=46) and pregnant women (n=7) were tested for IgM antibodies to hepatitis B core antigen (IgM anti-HBc) (Corzyme-M; Abbott Laboratories, USA). Of the above, 37 patients with acute hepatitis B and 23 HBsAg carriers were tested for anti-delta antibodies using EIA (Abbott Laboratories, USA).

Samples negative for all the above markers (n=51) were tested for hepatitis C virus RNA (HCV RNA) with a nested reverse-transcription polymerase chain reaction (RT-PCR) assay using primers representing 5'-noncoding region,⁸ IgM antibodies to cytomegalovirus (IgM anti-CMV) using ELISA (Diamedix, USA), and IgM anti-HBc.

Group B: All 206 samples were tested for IgM anti-HAV using HAVAB-M (Abbott, USA), HBsAg, IgM anti-HBc; 75 samples (57 acute hepatitis B patients, 18 HBsAg carriers) were tested for anti-delta antibodies. 162 samples were screened for IgM anti-HEV and anti-HCV.

Statistical methods

Epi-info version 6 (Centers for Disease Control and Prevention, USA, 2001) was used for statistical analysis. χ^2 test and Fisher's exact test were used to compare frequencies. In both groups, univariate odds ratio analysis was carried out for various risk factors in relation to individual etiological agents, followed by multivariate regression analysis. Multivariate odds ratio (OR) was calculated with 95% confidence limit (CL) using logistic regression (SSPS version 6.1.4). Three risk factors, i.e., history of surgery, parenteral therapy and blood transfusion were considered cumulatively as one variable due to low frequency.

Results

Group A

In Group A, 81.6% of children with AVH (n=98) had HAV infection either alone or concomitant with one or more of other hepatitis viruses (Table 2). HEV and HBV

infections were found in 12.2% and 5.1% children, respectively. Fourteen children (14.3%) were infected with more than one hepatitis virus; these included hepatitis A and E in 7, and hepatitis A and/or E in HBsAg carriers in 7 (both HAV and HEV 2, HAV alone 4 and HEV alone 1). Three children with AVH were HBsAg carriers with no other serological markers.

Among children with FHF (n=28) too, HAV was the predominant cause (71.4%; Table 3), followed by HEV (17.9%), HBV (10.7%) and HDV (one child). Seven children had multiple infections: hepatitis A and E in 2, acute hepatitis B and hepatitis A in 3 (including delta co-infection in 1), hepatitis E in HBsAg carrier in 1 and hepatitis A and E in HBsAg carrier in one. One child was only HBsAg positive.

Among adults with AVH, in Group A, HEV was the predominant etiological agent (42.4%; Table 2), followed by HBV, HAV and HDV. Nineteen adults had multiple acute infections, the most frequent being hepatitis B and E in 5 cases, hepatitis D in 3, hepatitis A and E in two cases and triple infection with A, B and D in one patient. Seven HBsAg carriers had superadded hepatitis E and one hepatitis A.

Of the 18 adult patients with FHF (Table 3), one had hepatitis A and two had acute hepatitis B, of whom one had delta co-infection. One HBsAg carrier had delta superinfection. Six patients suffered from hepatitis E, three of whom were HBsAg carriers. Of the two pregnant women, one was IgM anti-HEV positive and the other negative for all markers. Seven patients had no serological markers.

None of the children or adults with AVH and FHF in Group A had anti-HCV, HCV RNA, or IgM anti-CMV.

Group B

In Group B, among children with AVH (n=35), HAV was the most frequent cause (51.4%; Table 2), followed by HEV (46.4%) and HBV (25.7%). Dual infections were found in seven cases (25%); four patients had hepatitis A with E, two had acute with E and one acute hepatitis B with A. One child was an HBsAg carrier.

In adult patients in Group B (Table 2), HEV was the main cause (71.2%), followed by HBV (25.5%) and HAV

Table 2: Comparative etiological studies in AVH cases

Virus	Adults			Children		
	Group A	Group B	p value	Group A	Group B	p value
HAV	14/132 (10.6)	5/141 (3.5)	<0.05	80/98 (81.6)	18/35 (51.4)	<0.01
HBV (acute)	37/132 (28)	36/141 (25.5)	NS	5/98 (5.1)	9/35 (25.7)	<0.01
HCV	0/132	0/111	-	0/98	0/28	-
HDV	4/132 (3)	0/141	NS	5/98 (5.1)	0/10	NS
HEV	56/132 (42.4)	79/111 (71.2)	<0.05	12/98 (12.2)	13/28 (46.4)	<0.01
HBV (carrier)	8/132 (6.1)	17/141 (12.1)	NS	10/98 (10.2)	1/35	NS
Non A-E	33/132 (25)	15/111 (13.5)	<0.05	7/98 (7.1)	1/35 (3.6)	NS

Figures in parentheses denote percentage

NS - Not significant

Table 3: Comparative etiological studies in FHF cases

Virus	Adults		p value	Children
	Group A	Group B		Group A
HAV	1/18 (5.5)	0/30	NS	20/28 (71.4)
HBV (acute)	2/18 (11.1)	12/30 (40)	<0.05	3/28 (10.7)
HCV	0/18	0/23		0
HDV	2/18 (11.1)	0/30	NS	1/6 (16.7)
HEV	6/18 (33.3)	13/23 (56.5)	NS	5/28 (17.9)
HBV (carrier)	4/18 (22.2)	1/30 (3.3)	NS	3/28 (10.7)
Non A-E	7/18 (38.9)	3/23 (13)	NS	5/28 (17.9)

Figures in parentheses denote percentage

NS - Not significant

(3.5%). Twenty-five (22.5%) patients had acute infection; of these, 8 had acute hepatitis B and E, two had hepatitis A and E, and 15 were HBsAg carriers with recent hepatitis E and A (14 and 1, respectively).

All 30 adult patients with FHF were tested for markers of hepatitis A and B; 23 available samples were also tested for IgM anti-HEV and anti-HCV (Table 3). Six of 30 patients had acute hepatitis B alone, another 6 had acute hepatitis B in association with hepatitis E; 7 of the 23 patients tested had HEV alone. Three samples were negative for all markers. Of the 9 pregnant women with FHF, 6 of 8 tested had hepatitis E, of which one had concurrent acute hepatitis B. Two others had acute HBV infection. These pregnant women were not followed up for outcome. No patient in Group B had anti-HCV or anti-delta antibody.

Comparison of serological markers in Groups A and B

In adults and children, hepatitis A was significantly more frequent in Group A than in Group B (Table 2). Age-wise analysis among children in Group B showed that HAV infection in the 0-4 years and 5-10 years age groups was significantly more frequent (69.2% and 77.8%) than among the 11-14 years age group (15.4%; $p < 0.05$ and $p < 0.01$, respectively). In Group A, the above age groups were equally affected by HAV.

The proportion of acute hepatitis B and of HBsAg carriers in adults remained unchanged. Hepatitis E showed a significantly lower frequency in Group A among adults as well as children ($p < 0.05$ and < 0.01 , respectively). The frequency of non-A to E patients was higher in Group A adults ($p < 0.05$).

Among patients with FHF, hepatitis B was significantly less frequent in Group A as compared to Group B ($p < 0.05$) whereas the HBsAg carrier rate was not different. Hepatitis E was comparable in the two groups.

The frequency of multiple infections among AVH and FHF patients was not different.

Risk factors

In Group A, a strong negative association of hepatitis A with age was recorded (OR 32.21, 95% CL 16.4 to

63.22; $p < 0.05$). Adults (OR 5.101, 95% CL 2.6 to 9.35; $p < 0.05$) and women (OR 1.806, 95% CL 1.01 to 3.21; $p < 0.05$) were more prone to hepatitis E. HBV infection affected more adults (OR 4.54, 95% CL 2.00 to 10.27; $p < 0.01$) than children. Men had hepatitis B more frequently (OR 3.895, 95% CL 1.60 to 9.00; $p < 0.01$) than children. History of exposure to parenteral sources of infection did not seem to affect HBV infections (OR 1.64, 95% CL 1.58 to 4.6; $p < 0.05$). Exposure to commercial sex workers was not a significant independent risk factor, perhaps due to the low frequency of such an observation. Three men having such contact, and one woman with promiscuous sexual behavior, and one man with IV drug abuse had hepatitis B. Adults from the higher socio-economic group had a five-fold higher risk of HAV infection (OR 4.98, 95% CL 1.3 to 19.6; $p < 0.05$) than those from the lower socio-economic group. In children, frequency of HAV infection was comparable in both socio-economic groups (OR 1.9, 95% CL 0.5 to 7.9; $p = ns$); this may be related to the small sample size from the higher socio-economic group. In univariate analysis, none of the other risk factors influenced the etiology of hepatitis. No specific risk factors could be identified for patients with dual infections or non-A to E infection. Similarly, patients with FHF did not have any identifiable risk factor.

In Group B, children were more frequently infected with HAV (OR 35.14, 95% CI 11.59 to 106.58; $p < 0.01$). No other associations could be identified for other viruses.

Discussion

We studied the etiologic distribution of hospitalized patients with AVH and FHF during two time periods. The two study populations were somewhat different in that 56 of 276 subjects in Group A and none in Group B belonged to the high socio-economic class.

In both groups, HAV was the predominant etiological agent of both AVH and FHF among children. The frequency of HAV infections was higher in Group A. In Group B, children <10 years of age had hepatitis A more frequently than those between 11 and 15 years. Lack of such a difference in Group A may indicate exposure to HAV at an older age in the more recent group. In contrast, frequency of HAV infection in the children from higher socio-economic groups in central India⁹ was significantly higher in younger children, indicating earlier infection with HAV.

Decline of hepatitis E among children of Group A may be due either to a real decline in frequency of hepatitis E or to an increase in the proportion of patients with hepatitis A.

Among adults, as shown previously,^{2,8} HEV was the predominant cause of AVH as well as FHF in both

Groups A and B, although the frequency of hepatitis E was lower in Group A.

Sero-surveys conducted by us in the 1980s and 1990s showed that exposure to HAV and HEV in the healthy population was comparable in 1982 and 1992,¹⁰ whereas in 1998 a marked decline in seropositivity was observed¹¹ and higher socio-economic groups were more susceptible to hepatitis A. A five-fold higher risk has also been seen in our series. In another Indian urban community¹² HAV was the predominant cause (40%) of disease among children and adults; hepatitis E (7.1%) was less frequent.

Among children in Group A, hepatitis B was less frequent than in Group B in AVH cases, which could be because of increased use of disposable needles. A comparable frequency of acute HBV infection in adults of both groups underlines the importance of sexual transmission among adults.

HBsAg carrier rate was higher among adult patients of viral hepatitis in comparison with the healthy population of Pune in the corresponding time periods.¹³ Concurrent HBsAg carrier state and acute infection with other hepatitis viruses may result in aggravated clinical manifestation, requiring hospitalization in such patients. This has also been recorded in Taiwan, an area hyper-endemic for hepatitis B.¹⁴

A small number of patients were HBsAg carriers with a clinical and biochemical picture of AVH. Exacerbation of chronic hepatitis B during clearance of the e antigen¹⁵ or spontaneous reactivation with appearance of HBV DNA in previously HBV DNA-negative asymptomatic carriers¹⁶ could mimic AVH in HBsAg carriers. Confirmation was not possible due to non-availability of serum samples for analysis. Superimposed non-A to E hepatitis on HBsAg carriers status also cannot be ruled out.

Emergence of delta infection in Group A is noteworthy. Delta infection has been reported previously from other parts of western India.¹⁷ Our results also confirm that HCV has a negligible role in the etiology of sporadic AVH and FHF in India. Earlier studies involving sporadic and epidemic cases of AVH² and FHF,⁸ and reports from central¹⁸ and north India¹⁹ indicate the same.

The most frequent dual acute infection among children in both Groups A and B was hepatitis A and E, indicating simultaneous enterically-transmitted infection. In contrast to an earlier study,²⁰ multiple infections did not occur in our series. Disease in these patients could be either overlapping, simulating a single protracted illness necessitating hospitalization, or one of the two was subclinical in nature. In adults multiple acute infections were most frequently caused by acute HBV with HEV/HAV. Parenteral route of transmission of

hepatitis E has been suggested earlier.²¹ In fact, 12 of 23 of the above cases had history of parenteral therapy, blood transfusion or operations. Superinfection by HEV during the incubation period of hepatitis B could have caused these concomitant infections.

Some serologically undiagnosed cases caused by known viruses could have remained undetected due to limitations of currently available assays. Our study demonstrates existence of additional, yet to be identified, viral hepatitis-causing agent(s) in the community. Likewise, 28% of cases in northern India¹⁹ and 20% of cases in central India⁹ remained undiagnosed; 13% of cases were attributed to a non-parenterally transmitted agent in Saudi Arabia,²² and 7% from Taiwan were labeled as non-A to E.¹⁴ In community studies from India, 55% and 67% of cases could not be serologically diagnosed.^{12,23}

No risk factor could be implicated for etiology except young age and high socio-economic status for hepatitis A and adulthood for hepatitis B and E. Similar to world literature,¹⁴ our study records male preponderance for HBV infection. The female preponderance for HEV cannot be explained.

Similar serological findings have been reported in India during corresponding study periods. In Chandigarh,²⁴ among children 72% had hepatitis A and 9% were HBsAg positive, whereas in Kashmir²⁵ 53% of patients had non-A non-B hepatitis and children mostly had hepatitis A, which is in concurrence with Group B (study period 1978-81). Group A is comparable to studies from central⁹ and northern India^{12,19} during the years 1994-1997.

The most important findings of our study are the emergence of hepatitis A in the adult population and significant increase of hepatitis A causing AVH and FHF among children. Decreased frequency of HBV among children is noteworthy, although delta infection both in adults and children of group A is cause for concern. These changes in the epidemiology of viral hepatitis need to be considered while planning preventive strategies.

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