HEPATITIS C AND HEPATITIS E IN ACUTE SPORADIC NON-A NON-B HEPATITIS IN HOSPITAL PATIENTS OF DELHI (INDIA)

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Abstract. Non-A, non-B hepatitis (NANBH) has been considered to be the commonest cause of acute sporadic viral hepatitis in India. Serological studies (Macro ELISA) were conducted on 477 such patients from 9 hospitals of Delhi for markers of hepatitis A and acute hepatitis B (Anti-HAV-IgM,HBsAg and anti-HBc-IgM) and 49.7% of these were found to be due to NANBH. On further testing of NANBH sera it was found that both hepatitis C and hepatitis E contribute significantly to acute sporadic jaundice in Delhi, the latter more than the former.

INTRODUCTION

Availability of sensitive and specific serological tests enables detection of markers of hepatitis A and of acute hepatitis B in patients of acute sporadic viral hepatitis; the absence of these markers being taken as a diagnosis of non-A, non-B hepatitis (NANBH)(Alter, 1989; Francis and Maynard, 1979; Sebastian *et al*, 1990; Tandon *et al*, 1984; Zuckermann, 1978). Various groups of workers have found NANBH to be the major cause of acute sporadic viral hepatitis in India (Ichhupujani *et al*, 1991; Jain *et al*, 1992; Malik *et al*, 1994; Sebastian *et al*, 1990; Tandon *et al*, 1984, 1985).

Two types of NANBH are known till date, hepatitis C and hepatitis E (Bradley *et al*, 1991). Though NANBH is a major public health problem in India both in the sporadic as well as the epidemic situation (Khuroo *et al*, 1983; Tandon *et al*, 1984, 1985), the involvement of HCV and HEV in acute sporadic NANBH in India has not been studied very extensively. The presented work attempts to serologically study this issue, using specific immunological markers for hepatitis C and hepatitis E.

MATERIALS AND METHODS

Study group

Four hundred and seventy-seven (477) patients of suspected acute sporadic viral hepatitis from 10 major hospitals of Delhi (India) were selected for sero-investigation on the basis of clinical and/or biochemical criteria. These patients were from all age groups and both sexes.

Source and study period

Outdoor patients from OPDs as well as admitted patients from wards of Medicine, Gastroenterology, and Pediatrics were included during the period January 1, 1994 to December 31, 1994.

Sample collection and transportation

3-5 ml of blood was collected in a clean screw-capped glass vial and allowed to clot at room temperature. Sample collection was done mostly by the clinician in the respective OPD/ward, but some patients were bled at the NICD laboratory also. The clotted blood was brought to the laboratory either at low temperature in a thermos flask or a polythene bag with ice, or at room temperature, by a relative of the patient, along with a case summary or a proforma filled by the clinician. Serum separation in the hospitals was actively discouraged. All hemolysed blood samples as well as dripping or leaking samples were rejected.

Serum separation and storage

Serum was separated by centrifugation at 400*g* for 5 minutes and removed using disposable pasteur pippets. Collected sera was stored at -20°C in screw capped plastic vials.

Tests performed

The serum samples were subjected to Macro ELISA (Bead ELISA) tests using diagnostic kits

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supplied through the WHO by the Abbott Laboratories.

All the 477 sera were screened for markers of acute hepatitis A and B to diagnose active infection due to these two viruses: IgM antibody to hepatitis A virus (Anti-HAV-IgM) by Havab M EIA, hepatitis B surface antigen (HBsAg) by Auszyme Monoclonal EIA, IgM antibody to core antigen of hepatitis B virus (Anti-HBc-IgM) by Corzyme M EIA.

235 and 236 of these NANBH sera (sera tested as non reactive by the above mentioned three markers) were further tested for anti-HCV and anti-HEV respectively. The diagnostic kits (Anti-HCV EIA 3.0 and anti-HEV EIA) based on Macro (Bead) ELISA were obtained from Abbott Labs, USA through the WHO.

Control groups

235 voluntary donors (from blood donation camps in schools and colleges) were tested, as the control group, for anti-HCV. This group had no relevant history of acute or chronic hepatitis, or exposure, and every individual was negative for HBsAg. 236 medical and paramedical personnel from all over Delhi were tested, as the control group, for anti-HEV. This group consisted of individuals who had come for pre-vaccination/post-vaccination sero testing for hepatitis B, and had no relevant history of acute or chronic hepatitis.

Interpretation of tests

Anti-HAV-IgM reactivity was taken as indicative of recent/acute hepatitis A infection. HBsAg and/or anti-HBc-IgM reactivity was taken as indicative of recent/acute hepatitis B infection. NANBH sera was the sera that was non reactive for anti-HAV-IgM, HBsAg and anti-HBc-IgM. Anti-HCV reactivity was taken as indicative of exposure to hepatitis C virus. Anti-HEV reactivity was taken as indicative of exposure to hepatitis E virus.

Statistical analysis

Chi-square test was used for calculation of statistical significance with an alpha of 0.001.

RESULTS

Out of the 477 cases sero-tested for the markers of acute hepatitis A and B, 167(35.2%) were found reactive for anti-HAV-IgM and thus diagnosed as hepatitis A(Table 1). 50 cases (10.4%) were reactive for anti-HBc-IgM and were taken as suffering from acute hepatitis B. Seventy-three cases(15.3%) were reactive for HBsAg.

NANBH cases were 260 (54.5% of the total) when only the two IgM markers were taken into consideration, irrespective of HBsAg status of a case (Table 2). But when all the three markers were considered, the number of NANBH cases was 237 (49.7%). For the purpose of this study the latter was kept in focus.

235 NANBH sera were tested for presence of anti-HCV and 26 (11.1%) were found reactive (Table 3). Out of the 235 sera of the control group tested for anti-HCV, positivity was recorded in 3 (1.28%).

236 NANBH sera were serotested for anti-HEV out of which 95 (40.3%) were found reactive. In the control group, 27 (11.4%) of the 236 sera tested positive.

Reactivity of the NANBH sera for anti-HCV and for anti-HEV was significantly higher than that of the respective control groups ($p< 0.001$).

DISCUSSION

Non-A, non-B hepatitis is responsible for the highest percentage of acute sporadic viral hepatitis cases in India. In this study 49.7% of clinically and/or biochemically suspected acute sporadic viral hepatitis cases were found to be due to NANBH (considering all three markers negative), hepatitis A and B were responisble for 35.2% and 10.4% of cases respectively (Table 2). This is in consonance with the figures reported by various groups of Indian workers where etioliability of NANBH in sporadic viral hepatitis was serodiagnosed to be more than 50%: Ichhpujani *et al*, (1991) (68.8%), Jain *et al*, (1992) (57%), Khuroo *et al*, (1983) (53%), Malik *et al*, (1994) (55.6%), Tandon *et al*, (1984) (58%), and Tandon *et al*, (1985) (55%).

In the presented study, hepatitis A was excluded by screening the sera for anti-HAV-IgM, the marker for recent/acute hepatitis A infection. Hepatitis B was excluded by screening for HBsAg as well as for anti-HBc-IgM (Table 1).

The presence of anti-HBc-IgM is indicative of recent/present activity of hepatitis B infection, which means that anti-HBc-IgM is present not only in recent/acute hepatitis B but also in acute on

Table 1

Hepatitis markers in acute sporadic jaundice from Delhi hospitals.

Name of the hospital	Samples tested	Positivity of hepatitis markers		
		HBsAg	Anti-HBc-lgM	Anti-HAV-lgM
1. Dr Ram Manohar Lohia Hospital	142	20	10	40
2. Safdarjung Hospital	38	8	5	13
3. Lok Nayak Jai Prakash Narain Hospital	88	14	9	23
4. Guru Teg Bahadur Hospital	10	1	1	8
5. Kalawati Saran Children's Hospital	79	11	10	37
6. Govind Ballabh Pant Hospital	4	1	1	1
7. Ganga Ram Hospital	2	1	1	Nil
8. Hindu Rao Hospital	43	9	7	15
9. Smt Sucheta Kripalani Hospital	9	3	2	Nil
10. Referred cases (NICD)	62	5	4	30
Total	477	73 (15.3%)	50 (10.4%)	167 (35.2%)

Table 2

Etioliability of various types of viral hepatitis in acute sporadic jaundice cases from Delhi hospitals.

Type of disease	No. of cases	Etioliability
Hepatitis A	167	35.2%
Hepatitis B	50	10.4%
NANBH *	260	54.5%
NANBH **	237	49.7%

* Considering two markers (anti-HAV-lgM, and anti-HBc-lgM) for exclusion of hepatitis A and hepatitis B.
** Considering three markers (anti-HAV-lgM, anti-HBc-lgM, and HBsAg) for exclusion of hepatits A and hepatitis B.

Table 3

Positivity of anti-HCV and anti-HEV : comparison of NANBH group with controls.

	Anti-HCV	Anti-HEV
NANBH		
Number tested	235	236
Number positive	26	95
Percentage	11.1	40.3
Control		
Number tested	235	236
Number positive	3	27
Percentage	1.3	11.4
Significance	p<0.001	p<0.001

chronic hepatitis B. The latter was excluded in this study at the point of selection of cases by stringent history taking and detailed clinical examination by the clinician. HBsAg can be present both in acute and chronic hepatitis B. It was included in the battery of tests for NANBH so that early acute hepatitis B cases (prior to rise of IgM antibody) are also picked up and screened out. It is possible to have a situation where a non active carrier of hepatitis B (HBsAg positive, anti-HBc-IgM negative) acquires an infection of NANBH leading to jaundice due to NANBH. But for the purpose of this study NANBH cases were taken as only those that were negative for all the three markers *ie*, anti-HAV-IgM,HBsAg, and anti-HBc-IgM.

Using only anti-HAV-IgM and anti-HBc-IgM for exclusion of hepatitis A and B (irrespective of HBsAg status) revealed etioliability of NANBH as 54.5% whereas the use of all the three markers for this purpose gave a figure of 49.7% (Table 2). Both figures show NANBH to be the largest contributor to acute sporadic viral hepatitis in Delhi hospitals.

NANBH is an enormous public health problem not only in India but also in other economically developing countries due to inadequacy associated with provision of safe drinking water and proper sewage disposal (Bradley *et al,* 1991; Tandon *et al,* 1985; Viswanathan,1957; Wong *et al,* 1980). NANBH is a major contributor to sporadic viral hepatitis incidence in the western world too, but its contribution is many times greater in the former group of countries (Bradley *et al*, 1991; Khuroo *et al*, 1983; Tandon *et al*, 1984; Trepo and Lindberg, 1982; Wong *et al*, 1980). NANBH is caused by at least 2 distinct viruses (HCV and HEV); in the western world the problem is more due to HCV (post transfusion NANBH) whereas in the developing countries it is more due to HEV (enterically transmitted NANBH) (Alter, 1989; Bradley *et al*, 1991; Jawetz *et al*, 1989).

There are different strategies for control of HCV and HEV due to difference in their public health importance (chronicity potential of HCV and epidemic potential of HEV) and due to different modes of transmission of these two viruses [Alter, (1989; Jawetz *et al*, (1989)]. Thus, it is important to know which of the two viruses is significant for causing NANBH in any politico-geographical entity.

Though the etioliability of NANBH towards acute sporadic viral hepatitis is well recognized and documented, there is a paucity of data regarding the contribution of HCV and HEV in these cases. This hospital based study was undertaken to study the situation in Delhi.

The NANBH sera (non reactive for anti-HAV-IgM, HBsAg and anti-HBc-IgM) were tested for anti-HCV and anti-HEV (Table 3). Both the markers represent total antibody, predominantly IgG, and not IgM. Hence both are indicative of exposure to HCV and HEV respectively, and not of acute infection. But in this study, the positivity of anti-HCV and anti-HEV in the NANBH sera was compared with that in the respective control groups and was found to be significantly higher (Table 3) indicating that these viruses were definitely responsible for causation of NANBH cases in the population.

There are certain lacunae in this study. Firstly, the markers anti-HCV and anti-HEV are indicative of exposure, not recent infection. So they reveal probabilities and constitute indirect evidence. Secondly, despite best efforts at selection of cases of suspected acute viral hepatitis, many cases of other causes of jaundice must be creeping in, as happens under the best of conditions (Alter, 1989), thus swelling the numbers of NANBH. Of course this is more than offset by the gross under reporting of jaundice cases which is seen all over the world (Jawetz *et al*, 1989).

Despite the lacunae, certain conclusions can still be drawn.

1. Both hepatitis C and hepatitis E contribute substantially to the causation of acute sporadic viral hepatitis in Delhi (India).

2. Hepatitis E is not only responsible for most outbreaks of jaundice, it probably is also the largest single contributor to the sporadic cases of viral hepatitis.

3. Both hepatitis C and hepatitis E are endemic in Delhi and there is a baseline level of prevalence of exposure in the healthy population.

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REFERENCES

Alter MJ. Non-A, non-B hepatitis: sorting through a diagnosis of exclusion. *Ann Intern Med* 1989; 110: 583-5.

Bradley DW, Krawczynski K, Beach MJ, Purdy MA. Non-A, non-B hepatitis: Toward the discovery of hepatitis C and E viruses. *Sem Liver Dis* 1991; 11: 128-46.

Francis DP, Maynard JE. The transmission and outcome of hepatitis A, B and non A, non B : A review. *Epidemiol Rev* 1979; 1: 17-31.

Ichhpujani RL, Riley LW, Lalit D, Kumari S, Gupta PS, Sehgal S. Demographic features of sporadic acute hepatitis as determined by viral hepatitis markers. *J Commun Dis* 1991; 23: 138-43.

Jain DC, Singh J, Sebastian M, Sharma RS. Viral hepatitis in Alwar during 1986-1988. *J Commun Dis* 1992; 24: 247-8.

Jawetz E, Melnick JL, Adelberg EA, Brooks GF, Butel JS, Ornston LN. Hepatitis viruses. In: Medical Microbiology. 18th ed. USA: Prentice-Hall International, 1989: 419-34.

Khuroo MS, Duermeyer W, Zargar SA, Ahanger MA, Shah MA. Acute sporadic non A, non B hepatitis in India. *Am J Epidemiol* 1983; 118: 360-4.

Malik A, Randhawa VS, Prakash C, Kumari S. Hepatitis in and around Aligarh: a sero-epidemiologic study. *Indian Med Gaz* 1994; CXXVIII: 112-4.

Sebastian M, Ichhpujani RL, Kumari S. Incidence of different types of viral hepatitis in Delhi, Uttar Pradesh and Rajasthan areas. *J Commun Dis* 1990; 22: 129-33.

Tandon BN, Gandhi BM, Joshi YK. Etiological spectrum of viral hepatitis and prevalence of markers of hepatitis A and B virus infection in north India. *Bull WHO* 1984; 62: 67-73.

Tandon BN, Gandhi BM, Joshi YK, Irshad M, Gupta H. Hepatitis virus non-A, non-B: The cause of major public health problem in India. *Bull WHO* 1985; 63: 931-4.

Trepo C, Lindberg J. Non-A, non-B hepatitis. *Scand J Gastroenterology* 1982; 17 (suppl 77): 75-92.

Vishwanathan R. Infectious hepatitis in Delhi (1955-56): a critical study; epidemiology. *Indian J Med Res* 1957; 45 (suppl): 1-30.

Wong DC, Purcell RH, Sreenivasan MA, Prasad SR, Pavri KM. Epidemic and endemic hepatitis in India: Evidence for a non-A, non-B virus etiology. *Lancet* 1980; 2: 876-9.

Zuckermann AJ. The three types of human viral hepatitis. *Bull WHO* 1978; 56: 1-20.