SEROLOGICAL DIAGNOSIS OF JAUNDICE EPIDEMICS IN INDIA

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Abstract. Enterically transmitted non-A, nonB- hepatitis (ET-NANBH) is a major public health problem in India, where the endemicity of this disease is high and poor public sanitation coupled with compromised quality of drinking water leads to major and minor outbreaks. Sophisticated technics for characterization of hepatitis E virus (HEV) are not easily available/affordable, resulting in continuation of the diagnosis of NANBH for most epidemics. This study attempts to serologically determine the etiology of epidemics of NANBH in India. Eighteen outbreaks of jaundice occurring in various regions of India over a period of twenty months were selected for this laboratory based study. Representative cases of each outbreak were subjected to detailed serological investigation for immunological markers of viral hepatitis. Each serum sample was tested for the immunological markers of acute or recent infection with hepatitis A or B viruses (anti-HAV-IgM, HBsAg and anti-HBc-IgM) by Macro ELISA (Abbott).The sera found to be negative for these three markers *ie* non-A, non-B hepatitis (NANBH) sera were further tested for anti-HEV by Macro ELISA (anti-HEV EIA, Abbott). A highly significant number of NANBH sera were reactive for anti-HEV in case of almost all the outbreaks. The lowest figure for anti-HEV positivity in NANBH sera of outbreak was compared with anti-HEV positivity in the controls and found to be significantly high. It was concluded that anti-HEV is an important marker revealing probability of the NANBH outbreak being due to HEV.

INTRODUCTION

Recognition of a clearly demarcated group with nomenclature of non-A, non-B hepatitis (NANBH), came as a corollary to serodiagnostic ability for detection of immunological markers of hepatitis A virus (HAV) and hepatitis B virus (HBV) (Bradley *et al,* 1991a; Purcell and Ticehurst, 1988; Reyes *et al*, 1990). This diagnosis of exclusion has been broken up further, on the basis of epidemiological data, into two distinct groups, namely, parenterally transmitted NANBH (PT-NANBH) and enterically transmitted NANBH (ET-NANBH). Molecular biological technics have identified two distinct viruses, one in each group: Hepatitis C virus (HCV) which causes PT-NANBH and hepatitis E virus (HEV) which causes ET-NANBH (Arankalle *et al*, 1988; Bradley *et al*, 1991b; Purcell and Ticehurst, 1988; Reyes *et al*, 1990).

ET-NANBH is a major public health problem in the developing countries where the endemicity of this disease is high and compromised quality of public sanitation coupled with inadequate supply of safe drinking water leads to major and minor epidemics (Arankalle *et al*, 1988; Balayan *et al*, 1983; Bradley *et al,* 1991b; Hillis *et al*, 1973; Naidu and Viswanathan, 1957; Purcell and Ticehurst, 1988; Reyes *et al*, 1990).

Most of the epidemics of jaundice in India, as in other developing countries, are due to ET-NANBH (Bradley, 1990; Khuroo, 1980, 1991; Naik *et al*, 1992; Ramalingaswami and Purcell, 1988; Vishwanathan, 1957) especially as almost the entire population in these countries is already infected with HAV by age 5-10 years (Gust *et al*, 1979; Nath *et al*, 1980; Purcell and Ticehurst, 1988; Szmuness *et al*, 1977; Wong *et al*, 1979).

The epidemics of ET-NANBH have been characterized as due to HEV, once in a while, using PCR technic to demonstrate the HEVgenome in stool (Reyes *et al,* 1990) or by immune electron microscopy to detect HEV like particles in stool (Arankalle *et al,* 1988; Balayan *et al,* 1983). But such technics are not easily available, resulting in continuation of the diagnosis of NANBH for most epidemics. This study attempts to serologically determine the etiology of epidemics of NANBH in India.

MATERIALS AND METHODS

Source and study period

Eighteen outbreaks of jaundice occurring in various regions of India over a period of twenty months (from January 1994 to August 1995) were selected

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for this laboratory based study.

Study group

A large number of people were affected in each of these outbreaks, as was confirmed by clinical and/or biochemical profile of cases as well as by epidemiological investigations. Only a few representative cases of each outbreak were subjected to detailed serological investigation for immunological markers of viral hepatitis.

Each outbreak was epidemiologically investigated by the local public health authorities and the epidemiology teams from the NICD. The cases of jaundice were identified on the basis of clinical and/or biochemical criteria and the representative cases were sampled for their blood.

Sample collection, transportation and storage

3-5 ml of blood was collected from the selected cases in clean screw-capped glass vials and allowed to clot at room temperature. Serum was separated using disposable pasteur pipettes, after centrifugation at 400*g* for 5 minutes. When samples were from remote areas without minimal laboratory facilities, serum separation was carried out by decantation with appropriate care. The clotted blood/serum samples were brought to the Hepatitis Laboratory at NICD, either at room temperature or at low temperature using a thermos flask. All hemolyzed samples as well as dripping or leaking samples were rejected. Sera were stored in disposable screw capped plastic vials at-20°C till tested.

Tests performed

Each serum sample was tested for the immunological markers of acute or recent infection with hepatitis A or B viruses (anti-HAV-IgM, HBsAg and anti-HBc-IgM) by Macro ELISA or Bead ELISA of Abbott Laboratories, USA. The diagnostic kits for the same (Havab M, Auszyme Monoclonal, and Corzyme M respectively) were supplied by the WHO.

The sera found to be negative for these three markers, *ie* non-A, non-B hepatitis (NANBH) sera were further tested for anti-HEV by Macro (Bead) ELISA (anti-HEV EIA, Abbott Labs).

Controls

100 medical/paramedical personnel from all over Delhi were tested, as the control group, for anti-HEV. This group consisted of individuals who had come for prevaccination sero testing for hepatitis B vaccination, 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. Only those sera were considered as non-B which were negative for both HBsAg and anti-HBc-IgM. NANBH sera were the sera that were non reactive for anti-HAV-IgM, HBsAg and anti-HBc-IgM. Anti-HEV reactivity was interpreted as exposure to HEV.

Statistical analysis

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

RESULTS

Serum samples of representative cases from the eighteen outbreaks of jaundice (Table 1), were tested for anti-HAV-IgM, HBsAg and anti-HBc-IgM. Most of the samples were found to be non reactive for all the three markers of acute viral hepatitis A and B (Table 2). All these outbreaks were considered to be due to non-A, non-B hepatitis (NANBH), on the basis of serological testing; and due to ET-NANBH, on the basis of epidemiological data.

One sample each from the outbreaks at CRPF Training Center, Madras (Tamil Nadu), Surat (Gujarat), Govindpuri (South Delhi), and Kailash Nagar (East Delhi) were found to be reactive for anti-HAV-IgM, thus revealing one representative patient in each of these outbreaks to be suffering from hepatitis A. This is possible because hepatitis A is also enterically transmitted, and HAV contamination along with susceptibility of a person would lead to hepatitis A in that person.

Table 1

Outbreaks of jaundice : where and when.

Outbreak No.	Place of occurrence (State)	When
1	Avadi (Tamil Nadu)	October 1993
2	Surat (Gujarat)	January 1994
3	Govind Puri (Delhi)	January 1994
4	Kalkaji (Delhi)	January 1994
5	Sukhdev Vihar (Delhi)	March 1994
6	Sri Ganganagar (Rajasthan)	April 1994
7	Pitampura (Delhi)	June 1994
8	Jodhpur (Rajasthan)	July 1994
9	Fatehabad (Haryana)	August 1994
10	LHMC (Delhi)	November 1994
11	Kailash Nagar (Delhi)	February 1995
12	Hanumangarh (Rajasthan)	March 1995
13	Mehsana (Gujarat)	April 1995
14	Mhow (Madhya Pradesh)	April 1995
15	Calcutta (West Bengal)	May 1995
16	Kalkaji (Delhi)	July 1995
17	E. Patel Nagar (Delhi)	July 1995
18	Dilshad Garden (Delhi)	August 1995

Table 2

Seroinvestigation of outbreaks (for Hepatitis A and B markers).

Outbreak No.	Total sera	HBsAg +ve	Anti-HBc-IgM +ve	Anti-HAV-IgM +ve	Negative for all three (%)
1	26	0	0	1	25 (96.2)
2	65	5	1	1	59 (90.8)
3	5	0	0	1	4 (80.0)
4	17	0	0	0	17 (100.0)
5	4	0	0	0	4 (100.0)
6	45	6	2	0	39 (86.7)
7	5	0	0	0	5 (100.0)
8	42	4	0	0	38 (90.5)
9	4	0	0	0	4 (100.0)
10	5	0	0	0	5 (100.0)
11	11	0	0	1	10 (90.9)
12	25	4	3	0	20 (80.0)
13	5	0	0	0	5 (100.0)
14	5	0	0	0	5 (100.0)
15	11	1	0	0	10 (90.9)
16	5	1	0	0	4 (80.0)
17	10	0	0	0	10 (100.0)
18	6	0	0	0	6 (100.0)

Certain serum samples from outbreaks at Surat (Gujarat), Sri Ganga Nagar (Rajasthan) and Hanumangarh (Rajasthan) were reactive for anti-HBc-IgM, showing these cases to be suffering from hepatitis B. This could be due to wrong selection of cases which is possible when the occurrence of a non-outbreak jaundice case is temporarily coincident with the occurrence of a jaundice outbreak in that spatial entity (*ie* village or township), without being etiologically related. These outbreaks were still considered to be due to NANBH as the percentage of NANBH sera was 90.8%, 86.7% and 80.0% respectively.

The sera which were reactive for HBsAg and non reactive for anti-HBc-IgM were ignored when computing the number of NANBH sera, for the purpose of this study. These sera could be from patients of early acute hepatitis B or from patients who were carriers of hepatitis B but were now suffering from infection with another hepatitis virus which is responsible for the outbreak.

The NANBH sera from 17 of these 18 outbreaks were further subjected to testing for anti-HEV. A highly significant number of NANBH sera were reactive for anti-HEV in case of almost all the outbreaks (Table 3).

DISCUSSION

Eighteen epidemiologically proven outbreaks of jaundice from various parts of India, majority from the northern region, that had been detected over a period of twenty months, from January 1994 to August 1995, were studied serologically for etiodiagnosis. The sera from clinically and/or biochemically confirmed representative cases were tested in two phases.

In phase I, the serum samples were tested for anti-HAV-IgM, HBsAg, and anti-HBc-IgM, the three well established markers for acute/recent hepatitis A and B (Hollinger 1990; Hollinger *et al* 1990; Jawetz *et al,* 1987). Based on this the outbreaks under study were considered to be as due to non-A, non-B hepatitis (NANBH) (Reyes *et al,* 1990).

Anti-HAV-IgM being the IgM variety of antibody against hepatitis A virus, increases in titer for 4-6 weeks after clinical onset of disease and declines to non detectable levels in 3-6 months (Hollinger *et al,* 1990; Jawetz *et al,* 1987). The absence in serum of this marker is definite evidence of no infection with HAV in the past 3-6 months.

Anti-HBc-IgM is the IgM variety of antibody against core antigen of hepatitis B virus and is indicative of recent/acute infection with HBV or of acute activity in a chronic hepatitis B patient (Hollinger, 1990; Jawetz *et al,* 1987). The clinical and epidemiological evidence in case of these outbreaks supports the former possibility of recent hepatitis B infection rather than acute on chronic hepatitis B.

HBsAg or hepatitis B surface antigen is positive in acute as well as chronic hepatitis B (Hollinger, 1990; Jawetz *et al,* 1987). When considered along with anti-HBc-IgM, there can be various interpretations regarding the presence of HBsAg. For the purpose of this study, only those sera were considered as non-B which were negative for both HBsAg and anti-HBc-IgM.

A very high percentage (80-100%) of representative cases being serotested as NANBH, these outbreaks were diagnosed as due to non-A, non-B hepatitis.

In phase II of our sero-investigation the NANBH sera from 17 of these 18 outbreaks were further subjected to screening for anti-HEV (Table 3). A high positivity of the NANBH sera for anti-HEV was revealed for almost all the outbreaks: 100% anti-HEV positivity was seen in 7 out of 17 outbreaks (41.2%). Anti-HEV positivity was 75-99% in 6 (35.3%) outbreaks, and 50-74% in 3 (17.7%) outbreaks. Only one outbreak had less than 50% anti-HEV positivity of the NANBH sera.

Anti-HEV is the sole immunological marker of hepatitis E for which standardized test kits have become commercially available recently. Anti-HEV being total antibody (predominantly IgG variety) against hepatitis E virus, is a marker indicative of exposure to the virus rather than recent infection. A highly significant presence of this marker in the representative NANBH sera in an outbreak situation can be considered as indirect evidence for etiodiagnosis of hepatitis E for the outbreak.

HEV has orofecal transmission with known epidemic potential. In an epidemiologically proven outbreak, when clinically and/or biochemically suspected acute viral hepatitis cases are found exposed to HEV in significant numbers along with serological evidence of absence of hepatitis A and B, it goes in favor of HEV being the etiological agent responsible for the outbreak.

There are various limitations to this conclusion:
(a) The evidence is indirect.

Table 3

Seroinvestigation of outbreaks (for anti-HEV).

Outbreak No.	Total sera	NANBH sera	NANBH sera tested	Anti-HEV positive	Percentage
1	26	25	21	16	76.2
2	66	59	46	27	58.7
3	5	4	4	3	75.0
4	17	17	17	13	76.5
5	4	4	4	3	75.0
6	45	39	34	17	50.0
7	5	5	NS	NS	-
8	42	38	38	28	73.7
9	4	4	4	3	75.0
10	5	5	5	5	100.0
11	11	10	10	10	100.0
12	25	20	19	8	42.1
13	5	5	5	4	80.0
14	5	5	5	5	100.0
15	11	10	8	8	100.0
16	5	4	4	4	100.0
17	10	10	10	10	100.0
18	6	6	6	6	100.0

(b) The jaundice case testing as NANBH serologically may not be suffering from viral hepatitis at all and the jaundice may be due to some other cause. But the probability of such a happening is low in an outbreak situation.

(c) Anti-HEV positivity could be unrelated to the outbreak. As in case of HAV, possibly most of the population is exposed early to HEV with consequent anti-HEV positivity. The NANBH outbreak could be due to some other agent.

A small study was undertaken to get some idea regarding the anti-HEV positivity in normal population so as to have more information regarding limitation (c). For this, sera from 100 normal healthy adults were tested for anti-HEV using the same technic and kit. These controls were the medical and paramedical personnel from all over Delhi who had come for prevaccination testing for hepatitis B vaccination and had no significant history in terms of jaundice. It was found that out of 100 control sera tested, 11 were positive for anti-HEV (Table 4).

Therefore, even if the lowest figure for anti-HEV positivity in NANBH sera of outbreak is compared with anti-HEV positivity in normal population, it is found to be significantly high (Table 4).

This is in consonance with other studies where person-to-person spread in case of HEV is considered to be relatively inefficient as compared to HAV (Bradley *et al,* 1991; Purcell and Ticehurst, 1988) or where a relatively low incidence of clinical disease is observed in contacts in case of HEV, unlike HAV (Bradley *et al,* 1991a,b).

This study covers various regions of India over

Table 4

Lowest anti-HEV positivity among NANBH outbreaks: comparison with control group.

Sera tested from	Positivity for anti-HEV
Outbreak	42.1%
Controls	11.0%
Significance	$p < 0.001$

a long period of time and examines a large number of outbreaks serologically for etiological diagnosis. Anti-HEV is not the best or a flawless marker for this purpose. But with other technics (virus isolation studies in animals, molecular biology technics, immuno electron microscopy, etc) being cumbersome, difficult, expensive and inaccessible (Arankalle *et al,* 1988; Reyes *et al,* 1990), anti-HEV is an important marker revealing high probability of the NANBH outbreak for being caused by HEV.

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