Age-Specific Prevalence of Antibodies to Hepatitis A and E Viruses in Pune, India, 1982 and 1992

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The age-specific seroprevalence of antibody to hepatitis A virus (HAV) and antibody to hepatitis E virus (HEV) were studied in persons in Pune, India, where both viruses are endemic. The data showed that HAV infected the majority of persons by age 3 years and virtually 100% by late childhood. In contrast, infection with HEV was rare in children and did not reach peak prevalence (33%–40%) until early adulthood. The reason for the differences in infection rates between HAV and HEV is not known. Age-specific antibody patterns in serum samples obtained 10 years apart show that neither HAV nor HEV has diminished in medical importance in this Indian community.

Hepatitis A and hepatitis E are enterically transmitted viral diseases that are endemic in many developing countries, including India. Hepatitis A virus (HAV) was identified in 1973, and as a result of molecular studies was classified as a picornavirus. The epidemiology of hepatitis A was previously defined by applying sensitive and specific serologic tests to large populations to determine the prevalence and age distribution of specific antibodies (anti-HAV). Hepatitis E virus (HEV) was identified 10 years after HAV, and much less is known about it. HEV appears to be unique and not closely related to any other recognized virus. HEV has been identified as a major cause of waterborne epidemics in India [1] and as a significant cause of enterically transmitted sporadic hepatitis [2].

Although HAV and HEV coexist in India, the epidemiology of the diseases they cause appears quite distinct. Previous serologic studies showed that the vast majority of the Indian population was infected with HAV early in childhood [3]. Therefore, since seroconversion to HAV apparently confers lifelong immunity, clinical hepatitis A is relatively rare in the adult Indian population. However, although HAV infections of the young can be asymptomatic, they cause 60%–70% of clinical hepatitis in children <15 years old [4]. In contrast, hepatitis E has been reported infrequently in young children and is more common in older children and adults. The relative paucity of hepatitis E in the young could be explained if HEV infections of the young are either predominantly asymptomatic or if they are simply less common than those of older persons. Serologic studies should be able to discriminate between these two possibilities.

The recent development of a sensitive ELISA for antibodies to HEV (anti-HEV) has enabled a more extensive analysis of the seroepidemiology of HEV over time. Here we compare the age-specific prevalence of anti-HAV and anti-HEV in Pune, India, over a decade.

Materials and Methods

Study population. Serum samples were obtained in 1981–1982 and in 1992 from male students (3–20 years old) who attended a local school and a college. Serum samples were also collected from boys and girls <3 years old seen at a local hospital for nonhepatic disorders in 1982 and 1992. In addition, in 1982 and 1992, blood samples were collected from volunteer male blood donors (>18 years of age) at a Red Cross blood bank in Pune. All persons in this study were of lower or lower-middle socioeconomic classes by income level. History of jaundice, if any, was recorded for all persons from whom blood was obtained.

Serology. All serum samples were stored at −20°C until tested. Anti-HAV was detected by RIA (HAVAB; Abbott Laboratories, Abbott Park, IL). Anti-HAV–positive sera from patients <1 year old were also tested for anti-HAV IgM (HAVAB-M; Abbott). All samples were tested at the same time for anti-HEV IgG by ELISA as described [5]. Titers of anti-HAV and anti-HEV were determined in all positive sera.

Determination of ELISA cutoff. Serum samples from a normal population (apparently healthy persons) were tested in duplicate for anti-HEV IgG. Duplicate optical densities (ODs) from 1127 of these sera were averaged, and the resulting set of mean ODs was shown as a frequency distribution. The latter was unimodal and markedly skewed to the right with a highly significant departure from normality (Shapiro-Wilk test, $P < .0001$).

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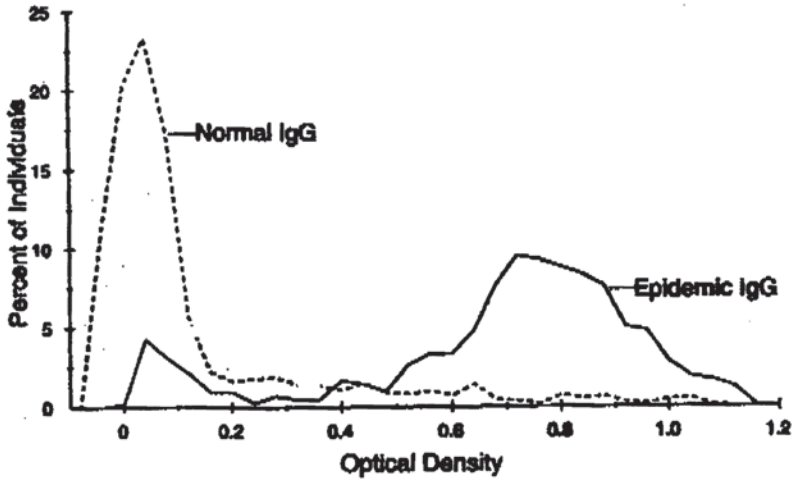

Figure 1. Plot of mean optical densities of sera from Pune tested for anti-HEV IgG by ELISA. Sera were from normal populations (normal) or hepatitis patients identified in epidemics of waterborne hepatitis (epidemics).

Skewness was reduced by shifting the mean ODs upward by 0.1 and then taking logarithms. The resulting frequency distribution was bimodal with peaks of unequal height (the highest was at an OD of 0.058) (figure 1). Data were smoothed with a three-point moving average; the lowest point of the trough between the two peaks was −0.55, which corresponds to an OD of 0.182. A similar analysis was done of 420 sera from 17 Indian epidemics of waterborne hepatitis that were caused primarily by HEV (figure 1) [1]. For the present study, serum samples giving an OD (cut-off) value of ≥0.2 in the ELISA were considered positive for anti-HEV.

Results

Four hundred eighty-five sera collected in 1982 were tested for both anti-HAV and anti-HEV. As seen in table 1, by age 3 years, 73% of the children had seroconverted to HAV and virtually the entire population was anti-HAV–positive by 15 years of age. In all groups of subjects ≥4 years old, significantly fewer persons had seroconverted to HEV ($P < .001$). The peak anti-HEV prevalence (33%) was in the 26- to 35-year-old age group and remained at about the same level in older age groups.

In 1992, we obtained 664 sera from a similar population in the same area and tested 39–109 sera from each group with subjects >3 years old for anti-HAV and anti-HEV. Sera from children <6 months old were available only from the 1992 collection. Six of the 9 sera tested were positive for anti-HAV IgG but the sample to negative control (S:N) ratio in 5 of the 6 samples was low (2.7–9.7) and IgM anti-HAV could not be detected, suggesting that the anti-HAV mea-

Table 1. Age-stratified IgG antibody to HAV and HEV in Pune, India.

	1982		1992	
Age group	anti-HAV	anti-HEV	anti-HAV	anti-HEV
<6 months	NA	NA	6/9 (67)	0/9
7 months–1.5 years	2/11 (18)	0/11	3/11 (27)	0/11
1.6–3 years	8/11 (73)	1/11 (9)	23/27[a] (85)	2/30 (7)
4–5 years	32/37 (86)	0/37	35/39 (90)	3/39 (8)
6–10 years	72/76 (95)	4/76 (5)	101/103 (98)	5/103[b] (5)
11–15 years	87/88 (99)	14/88 (16)	105/106 (99)	13/1064[b] (12)
16–25 years	96/98 (98)	14/98 (14)	106/109 (97)	44/109[b] (40)
26–35 years	71/72 (99)	24/72 (33)	91/91 (100)	35/91 (38)
36–45 years	60/60 (100)	18/60 (30)	79/79 (100)	31/79 (39)
>45 years	32/32 (100)	10/32 (31)	87/87 (100)	35/87 (40)
Total	460/485 (95)	85/485 (18)	636/661 (96)	168/664 (25)

NOTE. Data are no. positive/no. tested (%). Same sera were tested for both anti-HAV and anti-HEV. NA, not available.

[a] There was insufficient sera to test 3 samples for anti-HAV.

[b] In a study not included here, anti-HEV prevalences for 200 females in Pune were for age groups 6–10 years, 5/80 (6%); 11–15 years, 8/73 (11%); and 16–25 years, 20/47 (43%).

sured was probably passively transferred maternal antibody (table 1). The serum sample from the child with a high S:N ratio (38.6) for IgG anti-HAV was also positive for IgM anti-HAV, indicating that the child's HAV infection was recent (data not shown).

An obvious difference between levels of anti-HAV and anti-HEV seropositivity was again noted (table 1). The pattern of anti-HAV positivity was indistinguishable from that found in 1982; maximum seroconversion occurred at ages 1.5–3.0 years. In contrast, among infants and younger children (ages 7 months–10 years), HEV infection was infrequent (3.7% in 1982, 5.5% in 1992) but began to rise in those 11–15 years old (16% in 1982 and 12% in 1992). HEV infection leveled (30%–33%) in those ages 26–35 years (tested in 1982) and was only slightly higher (38%–40%) in those 16–25 years old (tested in 1992; table 1). The mean difference in the percentage of subjects HEV-positive in 1982 and in 1992 was significantly greater in those from 16 to ≥45 years old (13.9%) than in those ages 6 months to 15 years (0.6%) [6]. Thus, the degree of exposure to the two viruses differed considerably. There was consistently more exposure to HAV than to HEV in this population at both time points. Therefore, the apparent rate of infection by the two enteric hepatitis viruses was quite different.

To investigate if reexposure to either virus resulted in reinfection, anti-HAV and anti-HEV titers were determined in all seropositive sera. Overall the geometric mean titer (GMT) for anti-HEV titers were low and remained constant regardless of age group (data not shown). The highest anti-HAV titers were in children ≤5 years (GMTs for 1982 and 1992, respectively, 858.3 and 1389.4). A decline in GMT, with a half-life of ~4 years, was noted up to age 15 years. Thereafter, the anti-HAV GMT steadily declined with a half-life of ~20 years.

Discussion

In this study, we compared the seroepidemiology of two enteric hepatitis viruses, HAV and HEV, in the same population over 10 years. During this period, no hepatitis epidemics were recorded in the area, but endemic cases were identified. It is evident from table 1 that the epidemiology of HAV is distinct from that of HEV in the same population. By age 5 years, ~85% of the children in 1982 and 1992 had been exposed to HAV, mainly through subclinical infections. The steady decline in age-specific anti-HAV GMTs is consistent with early and universal seroconversion followed by the absence of significant anamnestic responses. This pattern is suggestive of efficient protective immunity, possibly against reinfection as well as disease.

In contrast, seroconversion to HEV was infrequent among young children. The lower prevalence of anti-HEV among children than in adults in Pune is similar to that reported for population-based studies in Somalia, where epidemics of hepatitis E have occurred [7] and in Hong Kong, where such epidemics have not been seen [8]. Similarly, anti-HEV was found in only 2.3% of Turkish residents <26 years old but in 6.2%–8.5% of older persons [9]. In Taiwan, where HAV infections have diminished over the past 20 years, the prevalence of anti-HEV paralleled that of anti-HAV, with peak prevalences of both antibodies after age 20 [10]. In the present study of a region where HAV and HEV remain endemic, the prevalence of antibodies to both viruses was higher than in Taiwan, but exposure to HEV was detected significantly less frequently than was exposure to HAV in all age groups studied. However, the age-specific pattern of anti-HEV was similar to that in the Taiwanese study [10]. Thus, these two enterically transmitted viruses appear to have quite different epidemiologies.

There are several possible explanations for the age-specific prevalence of anti-HEV that we and others have observed. First, the lower prevalence of antibody could be artifactual, resulting from the rapid decay of anti-HEV after infection. The half-life of anti-HEV IgG appears to be only 1 month when measured during early convalescence and just 0.5–4.0 years when measured at later times [11, 12]. It is not yet clear how long anti-HEV can be detected after infection with the virus, but in a study of HEV disease in children in Egypt, the seropositivity rate fell from 38% at time of hospital admission to 17% 1 year later [13]. Our experience was somewhat different: 100% of young adults with serologically documented HEV infection were still positive for anti-HEV IgG 20 months later [11], and we and others have found anti-HEV in patients with documented HEV infection several years after onset ([12], unpublished data). If a short half-life for anti-HEV IgG were the explanation of our findings, we would have expected to detect antibody in a higher proportion of very young children, since the sampling intervals were shorter. Instead, there was virtually no anti-HEV detectable in these sera.

Second, failure of young children to mount a brisk anti-HEV response when compared with adults could lead to lower prevalences of anti-HEV in the youngest age groups. However, serologic analysis of an epidemic of HEV among schoolchildren in Talegaon, India, [14] showed that at least some children develop high titers of anti-HEV that are comparable to those found in adults after infection (unpublished data). Furthermore, we found a very high prevalence of anti-HEV IgM in adults with HEV infection [11]. The finding of IgM antibodies in acute, self-limiting infections is accepted as evidence of a primary infection.

Third, our findings could result from serologic differences among strains of HEV. However, we have shown that strains from multiple outbreaks of HEV in different regions of India are closely related [1]. Furthermore, we have found that the serologic test used in this study to detect anti-HEV can identify anti-HEV with equal sensitivity after infection with the

most genetically divergent strains of the virus [5]. For these reasons, we believe that the age-specific antibody profile that we describe here is a true representation of the antibody status of the population studied and that HEV does not infect a significant proportion of infants and children, despite its enteric route of transmission.

In this study, the peak incidence of HEV infection, as measured by anti-HEV IgG, occurred in young adults. Increased exposure to HEV in this age group could result from sexual contact, increased exposure to high-risk environments through work and travel, or by increased exposure secondary to increased volumes of ingested food and water (compared with infants and children). We have not attempted to determine which (if any) of these premises is correct.

Only 10.0% and 5.2% of persons positive for anti-HAV and anti-HEV, respectively, reported a history of jaundice. Thus, HEV, like HAV, appears to cause inapparent infections in most cases.

The antibody prevalence patterns showed that the epidemiology of HAV and HEV in Pune basically remained unchanged over 10 years and suggested that factors involved in the spread of HAV and HEV in 1982 were unchanged a decade later. In contrast, in some countries the anti-HAV prevalence has been declining, presumably because of improvements in the water supply and in socioeconomic status, hygiene, and sanitary conditions [15]. Such may also be the case for anti-HEV [10].

In conclusion, our study shows that over 10 years, both HAV and HEV remained endemic in Pune. The results suggest that HAV is constantly present in Pune in concentrations high enough to infect most children by age 5 years. In contrast, HEV appears to be less common or less infectious and only infrequently causes infection in nonepidemic situations. However, after gross contamination of the drinking water supply, HEV can become epidemic, likely because most of the population lacks protective immunity. Public health measures, such as provision of pure drinking water, improvement of sanitation, and mass education in personal and public hygiene, are needed to control HAV and HEV and other enteric infections.

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