Age-specific Antibody to Hepatitis E Virus Stays Constant during the Past 20 Years in Japan

Short title: Silent HEV infection in Japan

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Summary

We examined antibodies to hepatitis E virus (anti-HEV) and hepatitis A virus (anti-HAV) by enzyme immunoassays in sera from 1,015 individuals collected in 1974, 1984 and 1994. Age-specific profiles of anti-HEV remained unchanged with a peak at 40-49 years, while those of anti-HAV started to increase in individuals with ages of 20-29 years in 1974, those with 30-39 years in 1984, and 40-49 years in 1994. These results suggest that the silent HEV infection occurred in the past 20 years in Japan, while HAV infection terminated at least since 1974.

Key Words

Hepatitis A virus, hepatitis A virus antibody, hepatitis E virus, hepatitis E virus antibody, seroepidemiology

Introduction

Hepatitis E virus (HEV) transmits mainly by a fecal-oral route, and causes waterborne outbreaks and sporadic cases of acute hepatitis in developing countries with poor sanitary conditions [1]. Outbreaks and endemics of HEV have been primarily noted in developing countries, whereas cases in developed countries were considered to be infected in foreign countries. However, cases of acute hepatitis due to indigenous HEV strains were reported in patients in the United States, Europe and Japan who had never traveled abroad [2-4]. Recently, HEV strains have been isolated from pigs in developed countries, which are closely related to local swine HEV strains, suggesting its zoonotic infection [5-7]. Due to these lines of evidence, HEV has attracted increasing attention even in developed countries where HEV is not endemic. For example in Japan, more than 20% of acute, sporadic hepatitis cases are non-A, non-B, non-C [8].

Impact of HEV infection in developed countries, however, has not been fully explored. The past exposure to HEV can be examined by enzyme-linked immunosorbent assay (ELISA) by detecting antibody to HEV (anti-HEV). In the present study, we surveyed the extent and changes of HEV infection in Japan by testing sex- and age-specific prevalence of anti-HEV in serum samples collected in 1974, 1984, and 1994. Using the same samples, we also tested antibodies to hepatitis A virus (anti-HAV), and compared them with anti-HEV.

Materials and Methods

Serum samples

A total of 1,015 samples were selected at random from the Serum Reference Bank of the National Institute of Infectious Diseases, Tokyo. They were obtained from healthy volunteers aged from 0 to 89 years (median: 35.6 years) living in seven prefectures in the central part of Japan. Of them, 349 were collected in 1974, 324 in 1984, and 342 in 1994. The present study was reviewed by the ethical committee of the National Institute of Infectious Diseases.

Hepatitis viral markers

Anti-HAV (total antibody) was determined by radioimmunoassay with commercial kit (HAV-AB RIA kit; Dainabot Co., Ltd, Tokyo, Japan). Positive and negative results were judged according to the manufacturer's instruction with intermediate results recorded as negatives. Anti-HEV was determined by ELISA by the method of Li et al. [9]. Briefly, wells of microplates were coated with purified virus-like particles of HEV expressed by a recombinant baculovirus. The test serum (100 μl) was delivered to each well coated with virus-like particles and the plate was incubated at 37 °C for 1 hr. The plate was washed 6 times, and then 100 μl of buffer containing goat anti-human IgG conjugated with horseradish peroxidase were delivered to each well. The plate was incubated for 1 hr at 37 °C, washed 6 times, and thereafter, each well received 100 μl of buffer containing thophenylenediamine. After the plate was incubated at room temperature for 30 min, 50 μl of 4 N H_2SO_4 was delivered to each well. Absorbance at 492 nm of colored substrate was recorded, and positive and negative results were judged by the method of Li et al [9].

Statistical analyses

Statistical analyses were performed using the $\chi 2$ test, and a p value less than 0.05 was considered significant.

Results

Age-specific prevalence of anti-HEV and anti-HAV.

We examined the serum samples collected from healthy Japanese volunteers in 1974, 1984, and 1994 for anti-HEV. Using the same samples, we also examined anti-HAV. Basic patterns of age-specific prevalence of anti-HEV were similar in the three examination years (Figure 1). The prevalence of anti-HEV increased gradually until 40–49 years, and then decreased slowly with age. In contrast, the prevalence of anti-HAV was almost nil in younger people younger than 20years, increased steeply at a certain age range, and then reached 80%–90% in older people in all the three years of examination. The age range at which the prevalence of anti-HAV started to increase sharply was 20–29

years in 1974, 30–39 years in 1984 and 40–49 years in 1994. Thus, it shifted by 10 years at each examination year. Anti-HAV was significantly more prevalent than anti-HEV in all age ranges over 30 years in 1974 ($p < 0.001$ in all). Similarly, anti-HAV was significantly more prevalent in all age ranges over 40 years in 1984 ($p < 0.001$ in all), and in all age ranges over 50 years in 1994 ($p < 0.001$ in all).

Sex- and age-specific prevalence of anti-HEV

Figure 2 illustrates prevalence of anti-HEV in serum samples from different age groups of healthy Japanese volunteers collected in 1974, 1984, and 1994 that are stratified by the sex and age. Although basic profiles of the prevalence of anti-HEV did not differ between men and women, anti-HEV in men was significantly more frequent in 1974 (21.6%) than in 1984 (11.1%, $p = 0.012$) and 1994 (10.4%, $p = 0.013$); the difference is attributed to a high frequency of anti-HEV in age groups older than 30 in 1974. In a sharp contrast, the age-specific prevalence of anti-HEV in women stayed unchanged in the three different years examined (11.0% in 1974, 10.6% in 1984, and 11.9% in 1994). Differences in the prevalence of anti-HEV between men and women were significant only in the year 1974 ($p = 0.008$). Age-specific prevalence of anti-HAV was quite similar between males and females in each year of the examination (data not shown).

Discussion

Many immunolgical methods have been developed for the determination of anti-HEV utilizing natural and recombinant viral proteins as antigens. They are, however, disappointingly non-specific and created a wide discrepancy of results on the same panel of sera with or without anti-HEV [10]. Lack of reliable serological assays for anti-HEV has hampered the accurate examination of exposure to HEV in various epidemiological and clinical settings.

Recently, Li et al. succeeded in producing virus like particles (VLPs) using a recombinant baculovirus containing HEV gene coding for its capsid protein [11]. The structure of the VLPs was examined and it has a similar antigenicity with

authentic HEV particles [12]. Using this VLPs, they developed a novel ELISA for anti-HEV that is sensitive and specific in seroepidemiological surveys for HEV infection. Considerable Japanese adults (around 10%–20%) were demonstrated to have the previous exposure to HEV, even though Japan is not endemic for hepatitis E [9, 13].

The clinical features of HAV infection are similar to those of HEV infection, in that they both are transmitted by a fecal-oral route and cause acute hepatitis without chronic sequelae. In the present study, serological markers of HAV and HEV infections were determined and compared among healthy Japanese volunteers at three different time points (1974, 1984 and 1994). Age-specific prevalence of anti-HAV increased steeply and reached 90% at a certain age range dependent on the year of examination. The age range at which anti-HAV increased shifted in three examinations separated by 10 years, indicating HAV infection endemic several decades ago in Japan and has been contained thereafter. This would be mainly due to improvement of sanitation in Japan since the 1950s.

Age-specific profiles distinct between anti-HAV and anti-HEV during the recent 20 years in Japan would have not only an epidemiological but also a clinical relevance. The prevalence of anti-HAV increasing with age involving by far the most aged individuals represents a life-long immunoprotection against HAV. By a sharp contrast, the prevalence of anti-HEV did not increase linearly with age, and peaked in age groups of 40–49 years. Furthermore, unlike anti-HAV that has become increasingly absent in younger age groups, anti-HEV was detected in younger individuals aged 20–29 years in both men and women, and among women aged less than 20 years, in the three examination years. Based on these epidemiological profiles, anti-HEV would not persist for long, and therefore, people who have been exposed to HEV and seroconverted to anti-HEV would be able to contract a second or third HEV infection when they are exposed after they lost anti-HEV. A similar age-specific profiles of anti-HEV has been reported in India [14]. Prevalence of anti-HEV in Japanese individuals younger than 30 years old was somewhat higher in the present study than that in the previous study at nearly zero by Li et al. [9]. Although there was some

difference in percentages, the basic pattern of age-specific prevalence of anti-HEV was almost similar between the two studies.

Based on the age-specific distribution of anti-HEV the exposure to HEV would not have decreased during the past 20 years in Japan, unlike that to HAV. This implies that the principal transmission route of HEV may be different from that of HAV and would not be prevented only by improved sanitary conditions, despite the lower infectivity and transmissibility of HEV than HAV [14]. A zoonotic transmission of HEV through domestic and wild animals may give an account on the perpetuation of HEV infection, and deserves to be examined in future studies. Eventually, transmission of HEV from pigs and deer to human beings has been recently reported in Japan [15, 16].

In conclusion, the exposure to HEV has stayed unchanged during recent 20 years in Japan, contrasting with that to HAV, which has been terminated since decades ago. These results warrant closer attention to infection with HEV, especially because it can induce fulminant hepatitis not only in pregnant women in developing countries [14], but also in sporadic cases in developed countries [15].

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Figure Legends

Figure 1. Age-specific prevalence of anti-HEV and anti-HAV in Japan at three different times. Number of individual tested in each age group and year is indicated below in parentheses.

Figure 2. Sex and age-specific prevalence of anti-HEV in Japan at three different times. Numbers of men/women tested in each age group and year are indicated below in parentheses.

Figure 1.

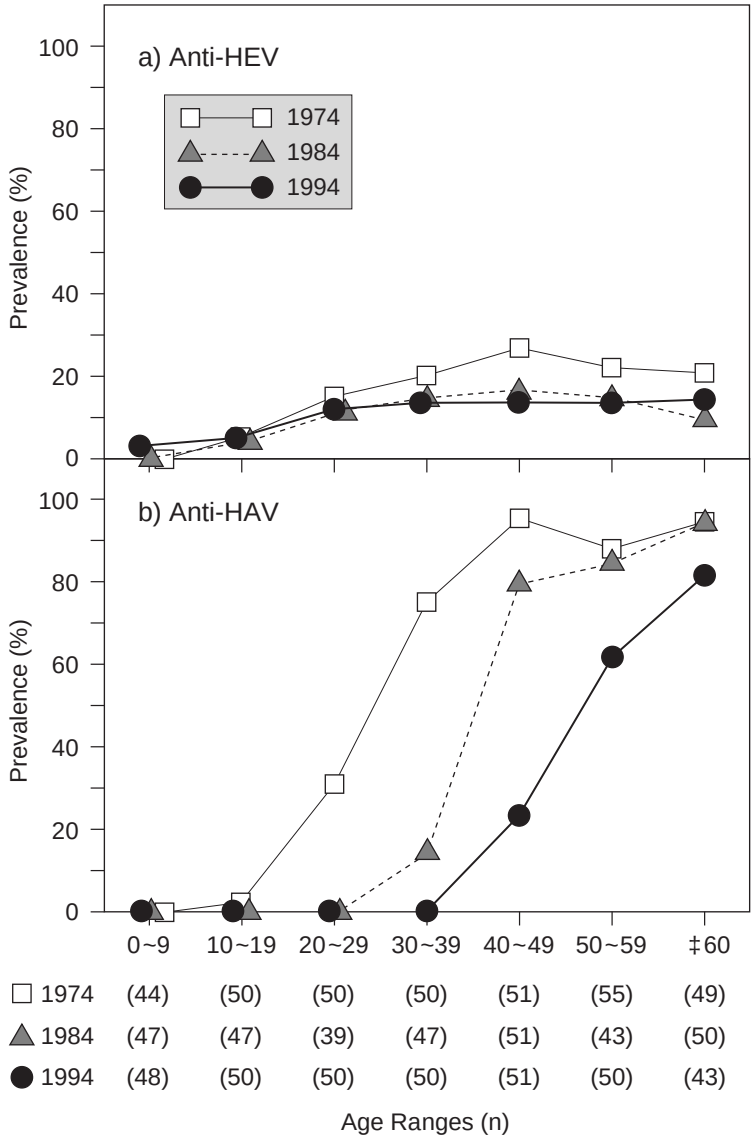
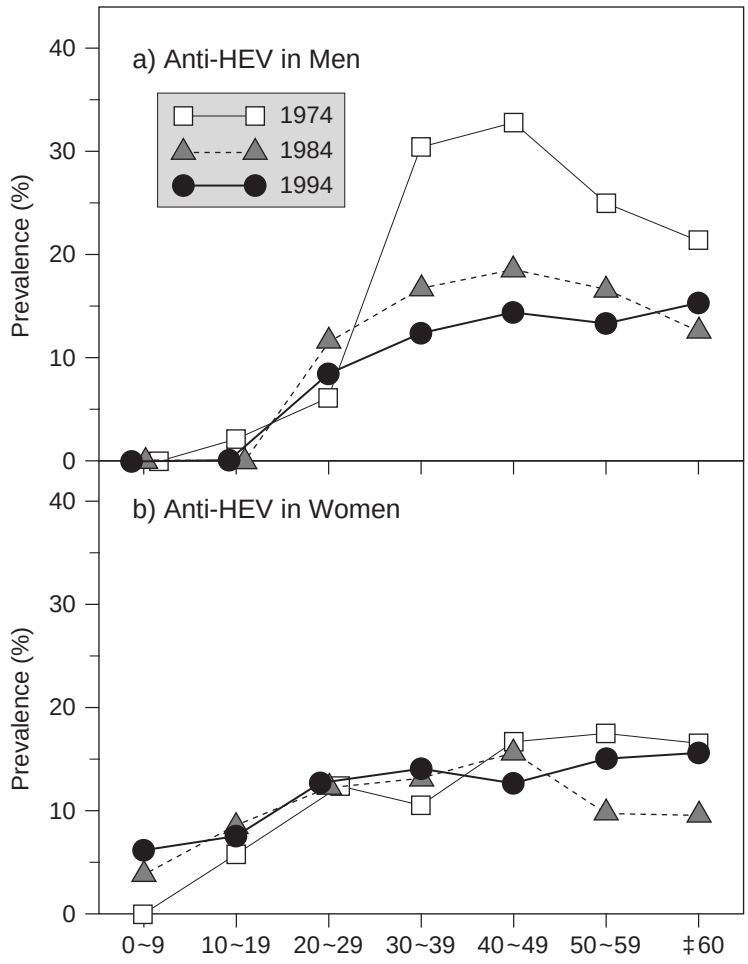

a) Anti-HEV
1974
1984
1994
Prevalence (%)
100
80
60
40
20
0
b) Anti-HAV
Prevalence (%)
0~9
10~19
20~29
30~39
40~49
50~59
‡60
1974 (44) (50) (50) (50) (51) (55) (49)
1984 (47) (47) (39) (47) (51) (43) (50)
1994 (48) (50) (50) (50) (51) (50) (43)
Age Ranges (n)

Figure 2.


a) Anti-HEV in Men
1974
1984
1994
Prevalence (%)
40
30
20
10
0
b) Anti-HEV in Women
Prevalence (%)
40
30
20
10
0
0~9
10~19
20~29
30~39
40~49
50~59
‡60
□ 1974 (23/21) (19/31) (12/38) (23/27) (39/12) (32/23) (37/12)
▲ 1984 (21/26) (23/24) (16/23) (18/29) (27/24) (24/19) (15/35)
● 1994 (15/33) (11/39) (13/37) (8/42) (21/30) (23/27) (24/19)
Age Ranges (n: Men/Women)