Hepatitis E Virus Infection in Selected Brazilian Populations

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A retrospective study on the prevalence of hepatitis E virus (HEV) infection was conducted in selected populations in Rio de Janeiro, Brazil. A total of 1,115 subjects were tested including 146 patients with acute Non-A Non-B Non-C (NANBNC) viral hepatitis, 65 hemodialysis patients, 93 blood donors, 102 intravenous drug users (IVDUs), 304 pregnant women, 145 individuals living in the rural area and 260 individuals living in the urban area. In order to characterize a favorable epidemiological set for enterically transmitted infection in the studied populations we also evaluated the prevalence of anti-HAV IgG (hepatitis A virus) antibodies. Specific antibodies to HEV (anti-HEV IgG) were detected by a commercial EIA and specific antibodies to HAV (anti-HAV IgG) were detected using a competitive "in house" EIA. We found a high prevalence of anti-HAV IgG in these populations, that could indicate some risk for infections transmitted via the fecal-oral route. The anti-HEV IgG prevalence among the different groups were: 2.1% in patients with acute NANBNC viral hepatitis, 6.2% in hemodialysis patients, 4.3% in blood donors, 11.8% in IVDUs, 1% in pregnant women, and 2.1% in individuals form the rural area. Among individuals living in the urban area we did not find a single positive serum sample. Our results demonstrated the presence of anti-HEV IgG in almost all studied populations; however, further studies are necessary to establish the real situation of HEV epidemiology in Rio de Janeiro, Brazil.

Key words: hepatitis E virus - hepatitis A virus - seroepidemiology - Rio de Janeiro - Brazil

Hepatitis E virus (HEV) infection is the major cause of enterically transmitted Non-A Non-B (NANB) hepatitis in many parts of the world. The infection has been shown to occur in both epidemic and sporadic forms and to be primarily associated with the ingestion of fecally contaminated drinking water (Balayan 1997). HEV and hepatitis A virus (HAV) infections share some clinical and epidemiological proprieties: both are transmitted via fecal-oral route, the diseases are self limited, and the prevalence of antibodies is closely related to the level of sanitation. However, HEV infection has a few distinct epidemiological features that differentiates it from hepatitis A. Unlike HAV, HEV appears to affect mainly young adults, which is unusual for a virus transmitted via the fecal-oral route. Also unusual is that secondary cases of HEV infection are much less frequent than with HAV, and finally, a major difference is the high mortality rate seen in pregnant women infected with HEV (Bradley 1992).

Outbreaks of HEV infection have been reported from Central Asia, Southeast Asia, Middle East, Northern Africa, Sub-Saharan Africa, and Mexico. In develop countries outbreaks have not been recognized, however, sporadic cases have been identified in Mediterranean countries and in the United States, mostly imported from endemic areas (Balayan 1997). In endemic areas, seroepidemiological studies have shown that anti-HEV antibodies in normal human populations appear to be unexpectedly low. A low but constant presence of anti-HEV antibodies has been also observed in normal human populations in non-endemic areas and in some risk groups, such as: intravenous drug users (IVDUs), blood donors rejected from blood donation, hemodialysis patients and health-care workers (Isrshad 1999). The pathobiological meaning of these findings remains obscure. In fact, the precise worldwide distribution of HEV infection has not been determined, mainly due to the lack of well standardized and readily available serodiagnostic tests.

To add some data to the epidemiology of HEV infection we selected different population groups in Rio de Janeiro, Brazil to evaluate the prevalence of anti-HEV IgG antibodies.

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SUBJECTS AND METHODS

Study population - All serum samples analyzed were collected between January 1994 and December 1998 by the National Reference Center for Viral Hepatitis (NRCVH), IOC/Fiocruz, Rio de Janeiro, as part of several studies on viral hepatitis. These samples represent different segments of the Rio de Janeiro population including individuals from several regions, and mostly with low income. A total of 1,115 serum samples were tested: 146 patients with acute Non-A Non-B Non-C (NANB NC)viral hepatitis, 65 hemodialysis patients, 93 blood donors, 102 IVDUs, 304 pregnant women, 145 individuals living in the rural area and 260 individuals living in the urban area.

Patients with acute NANBNC viral hepatitis - Between January 1994 and December 1998, 1,057 subjects were attended at NRCVH with sings and symptoms compatible with acute viral hepatitis. After specific serological tests for hepatitis A, B and C, 146 NANBNC patients were selected and tested for anti-HEV IgG and anti-HAV IgG. Of the 146 NANBNC patients investigated, 77 were males (52.7%) and 69 were females (47.3%). The mean age was 30.6 years.

Individuals living in urban area - Blood samples were collected in 1995 from subjects attending public day-care centers, primary and secondary schools who lived in the Palmares neighborhood, a suburb of the western area of Rio de Janeiro. This population was composed by 133 males (51.2%) and 127 females (48.8%) and the mean age was 10.8 years.

Individuals living in rural area - Blood samples were collected from residents of a small highland city (Sumidouro) of Rio de Janeiro, between September 1996 and January 1997. This population was composed by 76 males (52.4%) and 69 females (47.6%) and the mean age was 31.3 years.

IVDUs - The blood samples analyzed here were part of an epidemiological multicenter project about hepatitis risk among IVDUs (Oliveira et al. 1999). This survey was carried out on volunteers selected at drug use treatment centers and from the "drug scene" (streets) of the city, between August 1994 and August 1996. Out of the 102 IVDUs investigated, 88 were males (86.3%), 15 were females (14.7%), and the mean age was 33.7 years.

Hemodialysis patients - The blood samples were collected at a private hospital (Hospital Evangélico), between March 1996 and September 1997, from patients who underwent hemodialysis irrespective of the nature of the original pathology. This population was composed by 31 males (48%) and 34 females (52%), and the mean age was 65.1 years.

Blood donors - Blood samples were collected from healthy blood donors in the blood unit at the National Institute of Cancer, during 1995. This population was composed by 93 males with the mean age of 34.6 years.

Pregnant women - Blood samples were taken from women at various stages of pregnancy which were attended at the Carmela Dutra County Maternity, between April and May 1998. Among this population the mean age was 23.5 years.

Laboratory tests and statistical analysis - All sera were stored at -20ºC until use. Selected samples were tested for anti-HEV IgG and anti-HAV IgG. Anti-HEV antibodies were detected by a commercial EIA (Abbott Lab., Chicago) using two recombinant antigens (S-G-3 and 8-5). These are derived from the structural region of Burmese HEV strain expressed in *Escherichia coli*, and are used as solid phase antigens. Samples were considered positive only if repeatedly positive on at least two occasions. Anti-HAV antibodies were determined using a competitive "in-house" EIA (Vitral et al. 1991). Subjects with acute viral hepatitis were tested for specific antibodies to hepatitis A (anti-HAV IgM), hepatitis B (HBsAg and anti-HBc IgM) and hepatitis C (anti-HCV) using a commercial EIA (Organon Teknika). Statistical significance was assessed by X^2 test with Yate's correction, according to Epi Info program, version 6.0 (Centers for Disease Control and Prevention, Atlanta, GA).

RESULTS

Table I shows the distribution of specific serological markers for hepatitis E and hepatitis A among NANBNC patients selected from NRCVH between January 1994 and December 1996. In this population we found that 14% of the individuals had no serological markers for hepatitis A, B and C, but when tested for anti-HEV IgG and anti-HAV IgG antibodies they showed prevalences of 2.1% and 86%, respectively. Table II shows the age-specific prevalence of anti-HEV and anti-HAV IgG antibodies in subjects from the rural and urban areas. Among individuals from the rural area we found a prevalence of 2.1% of anti-HEV IgG, but among individuals from the urban area we did not find any positive serum sample. Our results also showed a high prevalence of anti-HAV IgG antibodies in the rural area (79%), however, a low value was observed in the urban area (31.2%). The highest prevalence of anti-HEV IgG antibodies was observed in subjects under 10 years of age. Table III shows the prevalence of hepatitis A and E IgG antibodies in subjects with risk of parenteral exposure. Both hemodialysis patients and IVDUs showed high val-

TABLE I

Non-A, Non-B, Non-C (NANBNC) patients selected between January 1994 and December 1996

Years	Subjects	NANBNC patients Positive number (%)	Specific serological markers	
			Anti-HEV IgG Positive number (%)	Anti-HAV IgG Positive number (%)
1994	358	49 (13.7)	00 (0)	44 (89.8)
1995	344	43 (12.5)	03 (7)	40 (93)
1996	355	54 (15.2)	00 (0)	41 (76)
Total	1057	146 (14)	03 (2.1)	125 (86)

HEV: hepatitis E virus; HAV: hepatitis A virus

TABLE II

Age-specific prevalence of hepatitis E virus (HEV) and hepatitis A virus (HAV) IgG antibodies in subjects from rural and urban area

Age	Anti-HEV IgG		Anti-HAV IgG	
	Urban area no.pos./no.test.(%)	Rural area no.pos./no.test.(%)	Urban area no.pos./no.test.(%)	Rural area no.pos./no.test.(%)
< 10	0/137 (0)	2/21 (9.5)	9/137 (7)	8/21 (38.1)
10-20	0/103 (0)	0/30 (0)	53/103 (51.5)	19/30 (63.3)
>20	0/20 (0)	1/94 (1.1)	19/20 (95)	87/94 (93)
Total	0/260 (0)	3/145 (2.1)	81/260 (31.2)	114/145 (79)

HEV: hepatitis E virus; HAV: hepatitis A virus

TABLE III

Prevalence of hepatitis A and E in subjects with risk of parenteral exposure

Population groups	Number of subjects	Mean age	Anti-HEV IgG no. pos. (%)	Anti-HAV IgG no. pos. (%)
Hemodialysis patients	65	65.1	4 (6.2)	59 (91)
Intravenous drug users	102	33.7	12 (11.8)	82 (80.4)

HEV: hepatitis E virus; HAV: hepatitis A virus

TABLE IV

Prevalence of hepatitis A and E infection according to sex

Sex (population group)	Number of subjects	Mean age	Anti-HEV IgG no. pos. (%)	Anti-HAV IgG no. pos. (%)
Male (blood donors)	93	34.6	4 (4.3)	86 (92.5)
Female (pregnant women)	304	23.5	3 (1.0)	257 (84.5)

HEV: hepatitis E virus; HAV: hepatitis A virus

ues of anti-HAV and anti-HEV IgG antibodies. Table IV shows the prevalence of hepatitis A and E according to sex. Our results showed a slightly higher values of anti-HEV and anti-HAV IgG antibodies prevalence in males.

DISCUSSION

Like other enterically transmitted disease such as hepatitis A, hepatitis E is a great public health problem in many developing countries where the poor socio-economic and hygiene conditions and the high population density contribute to maintain these infections.

Studies on HEV seroepidemiology in many parts of the world have shown some disagreement. It is not known why the overall seroprevalence of anti-HEV in normal human populations of endemic areas are low or why a low but constant presence of anti-HEV is observed in normal human populations of non-endemic industrialized countries (Balayan 1997). It is also unknown why in endemic and non-endemic areas, individuals with risk of parenteral

exposure have high rates of anti-HEV IgG antibodies (Thomas et al. 1997). Another striking feature of the epidemiology of HEV infection is that, even in populations with rates of HAV exposure approaching 100%, the rate of detection of anti-HEV IgG is rarely greater than 50% in any age group (Seow et at. 1999). These results, observed for two enterically transmitted diseases, were both unexpected and difficult to explain. The high seroprevalence values of HAV infection observed in our study could mean a favorable environmental set for enterically transmitted disease. However, when we compared the high levels of anti-HAV IgG antibodies with the anti-HEV IgG values, we observed a significant difference (p=0.01).

In spite of favorable environmental conditions, outbreaks of hepatitis E have never been reported in Brazil (Souto et al. 1997). Nevertheless, a few reports have demonstrated the evidence of anti-HEV antibodies in some Brazilian regions. Seroprevalence studies in the Brazilian Amazon showed that 6% of miners from Peixoto Azevedo had anti-HEV IgG antibodies (Pang et al. 1995). Also in the Brazilian Amazon, Souto et al. (1997) showed that two out of 16 individuals with acute hepatitis had IgG antibodies against HEV. A report from Parana et al. (1997) showed prevalence rates of 29% among NANBNC hepatitis patients and 2% in blood donor from Salvador, Bahia.

Data published until now have shown that IgG antibodies against HEV appear early in the course of infection and range from 10.9 ± 6.4 days to 4 years after the onset of the symptoms (Arankalle et al. 1995, DeTam et al. 1995, Purcell & Tsarev 1996). Arif et al. (1994) showed that 86.7% of all patients with acute hepatitis and anti-HEV IgG positive also had anti-HEV IgM positive. Our results showed that 2.1% of the cases of NANBNC acute hepatitis registered at NRCVH in Rio de Janeiro between January 1994 and December 1996 were anti-HEV IgG antibodies positive. This value is in accordance with reports from non-endemic areas (Zaaijer et al. 1995, Balayan et al. 1996). Further studies using other tools are needed to precise the real situation of HEV infection in this population.

Among individuals living in urban area we did not find a single positive serum sample. We also found a low seroprevalence among subjects living in rural area (2.1%). These values are not surprising due to the marked difference in the living conditions of the two groups. The major transmission route of HEV is by contaminated water. Subjects from rural areas do not have adequate environmental sanitation conditions; they use water from rivers for washing and drinking purposes as well as for their personal hygiene. Another possible fact that could explain this difference is the role of animals, including rodents, pigs and monkeys as possible reservoirs for HEV (Seow et al. 1999).

In epidemic situations, as well as in sporadic cases, the group between 15 and 40 years of age shows the highest attack rate, however, pediatric cases have been reported in some parts of the world (Balayan 1997). In areas where almost 100% of children are infected with HAV by 5 years of age, very few are infected with HEV until adolescence and young adulthood (Purcell 1996). The reason for this unusual age distribution for an enterically transmitted infection, is still unclear and may suggest a sexual transmission. Our results showed a high prevalence of anti-HEV IgG (9.5%) among subjects under 10 years of age. Such result could be explained by the poor health and hygiene conditions in this population.

The seroprevalence values found in hemodialysis patients (6.2%) and in IVDUs (11.8%) were high in comparison with the other groups studied. These high values were also found in other studies with similar population from non-endemic areas: 10.9% in France (Halfon et al. 1996) for hemodialysis patients and 23% in Baltimore, USA for IVDUs (Thomas et al. 1997). These results suggest the possibility of an alternative, non- fecal-oral route of HEV transmission. However, more convincing data is needed to support this assumption.

The distribution of HEV infection by sex has pointed to higher values in males than in females; this is probably the result of more involvement of males in risky situation (Balayan 1997). Our seroprevalence results from males blood donors were higher (4%) than those found in pregnant women (1%), although the difference was not statistically significant (p=0.05).

According to Arif et al. (1994), some facts must be considered when studying the prevalence of HEV infection: (i) HEV excretion is limited to low concentrations which may restrict the spread of the virus (Ticehurst et al. 1992); (ii) we do not know the persistence of antibodies to HEV. Some reports showed that anti-HEV IgG disappears 6 to 12 months post-infection (Goldsmith et al. 1992), whereas others claim that these antibodies can persist for 1 to 5 years (Dawson et al. 1992, Rapicetta et al. 1999); and (iii) the tests available for the detection of HEV antibodies seem to lack sensitivity or specificity to fully characterize HEV infection (Arif et al. 1994). Many of the unexpected results and discrepancies found in HEV epidemiologic studies in Brazil and in other countries can be ascribed to differences in assays for anti-HEV. Specifically, the choice and size of antigen appear to make significant difference in the results (Ghabrah et al. 1998).

As a general conclusion, the overall prevalence of anti-HEV in Rio de Janeiro was much lower than

expected. However, until the natural history of the antibody response to HEV infection is defined or a well standardized test is available, it is difficult to determine the true frequency of HEV infection in any population. Therefore, further investigations are needed to establish the real situation of HEV infection in Brazil.

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