

Prevalence of Hepatitis E Virus in Egyptian Children Presented with Minor Hepatic Disorders

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Hepatitis E virus (HEV) is considered as one of the common causes of particular hepatitis in developing countries. It is transmitted in a fecal-oral manner. It causes sporadic infections and large epidemics. To estimate the prevalence of anti-HEV IgG and IgM antibodies to ORF3 peptide of Hepatitis E virus genome in an age of children, study subjects (100 children) between 6 months and 10 years with minor, hepatic illnesses were recruited for the study during the period from September 2004 to September 2005. Serum anti-HEV IgG and anti-HEV IgM antibodies were screened in all subjects, anti-HEV IgM antibodies were assayed as an indicator of recent infection. Serum transaminases (AST and ALT) were estimated in positive subjects. Out of 100 subjects recruited, 26 subjects (26%) demonstrated anti-HEV IgG and 6 (6%) were anti-HEV IgM and IgG positive. Anti-HEV IgG were present since the first year of age till 10 years of age and increased with advancing age. Serum transaminases were raised in one (17%) of subjects with anti-HEV IgM antibodies. **Conclusions:** Children are susceptible to HEV infection since early infancy. Seropositivity to HEV antibodies increased by over 2 times beyond 4 years of age as compared to younger age.

Enterically transmitted non A, non B hepatitis has been known to occur in both sporadic and epidemic forms in developing countries. The virus is transmitted in a faecal-oral manner and is a major cause of viral hepatitis in much of the developing world (Bradley, 1999). Infection is presumed to be initiated via cells lining the alimentary tract. Virus then spreads to liver, eventually infecting a large proportion of the hepatocyte population but without causing direct cytolytic damage. After an incubation period of 5 to 6 weeks post exposure to the virus, liver damage results and is thought to be mediated by the cellular immune response to the virus infections in young children generally swing a benign course (Li et al., 1994). Patients with fulminant hepatitis E infection demonstrate necrosis of parenchyma with collapse of liver lobules, swelling of hepatocytes (which have a foamy appearance), arrangement of hepatocytes into an acinar pattern, proliferation of small bile ductules, phlebitis of portal and central veins, and portal inflammation with lymphocytic and

polymorphonuclear leukocyte infiltration (Krawczynski, 1993). The first epidemic was reported retrospectively from New Delhi in 1955 (Khuroo, 1980) and since then many more have been documented from different parts of the world (Belabbes et al., 1985 and Naik et al., 2002). Epidemics of hepatitis E virus (HEV) have a high attack rate in young adults in the age group of 15-40 years (Krawczynski, 1999). But studies in children from Sudan (Hyams et al., 2002) and Hong Kong (Lok et al., 2003) have demonstrated that 12-66% cases of acute sporadic hepatitis seeking care in hospital had IgM anti-HEV antibodies indicating recently acquired infection. Thus, it is clear that children are susceptible to HEV infection although exposure and the immune profile may be different from that of adults. HAV and HEV are both spread through orofecal route and thus the exposure probabilities are likely to be similar. Recently, Arankalle et al., 2001, showed that although most of the children acquire anti-HAV IgG antibodies by 5 years of age, the anti-HEV antibody positivity

ranged between 0-9% in pediatric population in and around Pune, Western India. On the other hand, HEV seroprevalence in adults was between 16-40%. It was not possible to assess the susceptibility of children to HEV infection at various ages and determine the changing prevalence of sero-positivity with increasing age.

The present study was undertaken to determine the prevalence of HEV infection (anti-HEV IgG antibody status) along with recent (anti-HEV IgM antibody status) infection among children in different age groups.

Subjects and Methods

Recruitment of study subject

The study was carried out in the out-patient clinics at the Department of Pediatrics, El-Hussein University Hospital, Faculty of Medicine, Al-Azhar University, Cairo, Egypt. In our preliminary seroprevalence study among 100 children, IgG and IgM antibodies to ORF-3 peptide of HEV were studied. Children between 6 months to 10 years of age attending the outpatient pediatric clinics for minor hepatic ailments were recruited during the period from September, 2004 to September, 2005. A questionnaire was developed which included details about subject identification, age, sex and demographic details (including defecating habits, sewage and waste disposal, water supply and area of residence), clinical examination for liver, spleen and presence of jaundice.

From each eligible subject, after a written concerned consent from the mother, 2 ml blood was withdrawn aseptically into a plain test tube. After clotting, the serum was obtained by centrifugation which was then labeled and stored at -20°C till further processing.

Anti HEV IgG and IgM Antibody Assay

An in-house developed enzyme immuno-assay system with a synthetic ORF-3 HEV peptide was used to determine anti-HEV IgG and IgM antibodies (Nanda et al., 2002). An immunoreactive ORF-3HEV peptide (comprising amino acids 91-123) was synthesized using an automated peptide synthesizer (Milligo, Millipore, Bedford, MA). After cleaving and

deprotection, the peptide was purified by reverse phase high performance liquid chromatography. One microgram of this peptide was coated to each well of a micro-titre plate (NUNC, Kamstrup, Denmark) in 100 μL of carbonate buffer (pH 9.6) at 4°C and incubated at 37°C for 6-12 hours. The un-coated sites were blocked with 5% fat free milk powder (Everyday; Nestle, Mumbai, India) in phosphate-buffered saline (0.05 mmol/L, pH 7.4) for 1 hour at 45°C . After incubation, the plates were washed three times with wash buffer (Phosphate buffered saline, pH 7.4, 0.05 mmol/L with 250 mmol/L NaCl and 0.1% Tween-20 – BDH, Mumbai, India) The patient sera, diluted 1:20 in phosphate-buffered saline containing Tween-20 (0.1%) and skimmed milk powder (10%) were incubated in peptide coated wells for 1 hour at 45°C . After thorough washing with the wash buffer, 100 microlitre of secondary antibody, affinity purified Anti-Human IgM (μ chain specific)/IgG peroxidase-conjugate (DAKO, the Netherlands) was allowed to react at a dilution of 1:1000 for 45 minutes at 45°C , respectively. The wells were thoroughly washed, and the color was developed using orthophenylene diamine dihydrochloride (Sigma, St. Louis, MO) in citrate phosphate buffer (0.1 mmol/L, pH 5.5) with hydrogen peroxide (0.0006%). The reaction was allowed to continue for 20 minutes and stopped with 50 μL of 2 mmol/L sulfuric acid. The color intensity was read at 490 nm using a Flow-Titrek enzyme-linked immuno-sorbent assay reader (Flow Labs, Scotland). The cut-off value was calculated as half of mean summated positive and negative control. The usual IgM positive samples gave an optical density (OD) value of more than 0.5 and the negative value was below 0.3.

To assess presence of anicteric hepatitis, serum ALT and AST levels were estimated in all positive subjects. Serum ALT and AST levels were assessed 8-12 weeks after collection of samples. It was stored at -20°C till processing. More than two folds the standard upper normal limit for the laboratory (12 IU/L) was considered as evidence of anicteric hepatitis.

Data Analysis

"Stata" statistical package was used to analyze the results. The 95% Confidence Intervals (CI) for proportions, means and medians were also determined. Comparisons were made between various categories using χ^2 test, Fisher's exact test, Student 't' test and Mann Whitney U Rank Sum test as appropriate for proportions, parametric or non-parametric continuous data. Differences were considered significant at an alpha of 5% or less. Logistic regression modelling was done for multivariate analysis.

Results

(i) Baseline Characteristics

The baseline features and demographic details of the children (6 months-10 years) attending the urban health facilities is presented in (Table 1).

Table 1. Baseline Characteristics of Study Subjects

Parameter (N: n)	N	n	%
Sex: Female	100	31	31
Residence#: Slums	100	11	11
Defecation: Child# Open	100	34	34
Drinking water source: Outside house#	100	20	20

Blood samples were available for analysis in all recruited children.

(ii) Anti-HEV IgG antibody status (table 2)

IgG antibodies to HEV were present in all age categories and showed an increasing prevalence rate from one year (10%; 95% CI,

5.3-14.4) till 10 years (36%; 95% CI 30.1-42.1) of age. The prevalence was significantly higher among those who were more than 48 months than those less than 48 months. The study design incorporated demographic features that could be potential risk factors to acquire HEV infection. However, none of these were related to anti-HEV status of the child.

(iii) Anti-HEV IgM antibody status (table 2)

Anti-HEV IgM positivity ranged between 17-33 % in different age groups of 25 months and above. Recently, acquired infection (anti-HEV IgM positive) was observed in 23% (6/26; 95% CI 20-26.6) in those older than 24 months. As a proportion of total subjects, evidence of recently acquired infection was 25% (1/4), 17% (1/6), 33% (2/6) and 25% (2/8) in 25-48, 49-72, 73-96 and 97-120 months age categories, respectively. No evidence of recently acquired infection was detected in 6-24 months age categories. Overall, 6% (6 out of 100) children had anti-HEV IgM antibodies.

Table 2. Anti-HEV IgG and Ig M antibody status in relation to age.

Age (months)	Total no of subjects	Anti-HEV IgG positivity		Anti-HEV IgM positivity	
		n	(%; 95% CI)	n	(%; 95% CI)
6-12	10	1	(10; 5.3-14.4)	0	0
13-24	9	1	(11; 5.6-16.4)	0	0
25-48	19	4	(21; 15.8-26.2)	1	(25; 16.6-33.2)
49-72	20	6	(30; 24.1-35.9)	1	(17; 12.4-21.2)
73-96	20	6	(30; 24.1-35.9)	2	(33; 25.3-40.6)
97-120	22	8	(36; 30.1-42.1)	2	(25; 16.6-33.2)
Total	100	26	(26; 23.3-28.8)	6	(23; 20-26.6)

(iv) Serum ALT and AST levels

Median serum ALT and AST levels were 12 IU/L (95% CI 12-12) in 8 children with anti HEV IgG and IgM antibodies in whom sera were available. In one child (4%; 95% CI 1.4-6.5) both ALT and AST were more than twice the upper normal limit for the laboratory (>24 IU/L). None of these children had clinical evidence of jaundice.

Discussion

In the present study, 100 children between the age of 6 months and 10 years were screened for anti-HEV IgG and IgM antibodies. Both anti-HEV IgG and IgM antibodies are present in recently acquired HEV infections, anti-HEV IgM antibodies were estimated as an evidence of new/recent infection. The study

demonstrated that infection with HEV started occurring in the first year of life and that anti-HEV IgG antibody positivity progressively increased till 10 years.

The probability of carrying anti-HEV IgG antibody increased by over two folds in those above 48 months of age as compared to younger children. This indicated increased probability of exposure to HEV during school age group. It was particularly true in areas where prevalence of IgM antibodies was significantly higher in >24 months age group. The possibility of some children below 12 months having anti-HEV IgG antibodies of maternal origin cannot be ruled out. There were no infants <24 months with anti-HEV IgM antibodies. This may be due to longer duration of breast feeding which in turn would reduce the risk of all water borne infections (Perera et al., 2002). During the study period, no hepatitis (A, B, C or E) epidemics occurred, but sporadic cases of acute hepatitis (A) continued to attend the pediatric outpatient of hospital.

In all, 6 out of 26 children with IgG antibodies (23; 95 % CI 20-26.6) had evidence of recent infection (i.e., anti-HEV IgM antibodies). This constituted 6% (95 % CI 5.2-6.6) of the total study population. In a recent study by Wei et al., 2001, individuals with acute HEV infection had IgM antibodies without concurrent IgG antibodies.

Native HEV infection has been described in almost all parts of the world, including industrialized nations. Seroprevalence of HEV infection was low and varied between 1-7% in children in countries like Australia (Moaven et al., 1999), Germany (Trautwein et al., 2000), France (Coursaget et al., 2001) and The Netherlands (Zaaijer et al., 2001). In contrast, the seroprevalence rates in Asia, Africa and South America are higher and ranged between 5.5% to 71% in various studies (Arankalle et al., 2001, Khuroo et al., 2000, Koshy et al., 1924, Tan et al., 2001, Grabow et al., 2001, Mushahwar et al., 2002,

Paul et al., 2001, Pujol et al., 2001 and Ibarra et al., 2002). The seroprevalence in children in our study was similar to that observed in other asian countries. The socio-economic status and sanitary conditions prevailing in the community may explain the major differences in the prevalence patterns seen across the regions. There are only a few studies estimating serological evidence of exposure to HEV IgG in non-icteric children. Report from Pune, Western India (Arankall et al., 2001), Kathmandu, Nepal (Clayson et al., 1999), Egypt (Goldsmith et al., 1992 and Hyams et al., 1992), Chile (Ibarra et al., 2002), Sudan (Hyams et al., 2002) and Lucknow, North India (Aggarwal et al., 2000) showed a wide range (0-44%) of seroprevalence rates to HEV infection. These studies are from regions endemic for HEV and where epidemics have also been described. In Pune, Western India (Arankalle et al., 2001), the prevalence of anti-HEV IgG antibodies was 0-9% in the first decade of life; none of the 20 children below 18 months had these antibodies. Similarly, neither IgG nor IgM antibodies were detected in 99 children in Kathmandu, Nepal (Clayson et al., 1999). Aggarwal et al. (2000) from Lucknow, North India, detected HEV IgG antibodies in 64% (95% CI 30.6-69.3) of children below 5 years and in 59% (95% CI; 36.3-79.2) between 6-10 year of age.

HAV and HEV are both feco-orally transmitted infections which cause similar acute self limiting illnesses and no chronic sequelae. In the present study, the seropositivity to anti-HEV IgG increased progressively by 10 years of age. This pattern of increasing seropositivity was very similar to HAV epidemiology published (Arankalle et al., 2001). In this study, recently acquired HEV-infection was indicated by the detection of anti-HEV IgM antibodies. Hyams et al., 1992 detected anti HEV IgM in 12% of children living in Cairo, the difference between our study and Hyams et al., study results is due to the difference in methodology

as they used Western blot methodology. The study design incorporated demographic features that could be potential risk factors to acquire HEV infection. However, none of these were related to anti-HEV status of the child. This may be either due to wide distribution of virus in a heavily contaminated environment and prevailing poor hygienic conditions or related to other un-identified risk factors. Continuous efforts to improve sanitation, and personal hygiene along with provision of clean drinking water to the communities are necessary interventions to control exposure to HEV and other water borne infections.

In conclusion, children are exposed to HEV infection early in life and seropositivity increased with age. Anti HEV IgG antibodies against ORF3 peptide were present in 26% children at Cairo by the age of 10 years. Recent HEV infection as indicated by the presence of anti HEV IgM antibodies was present in 8%.

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