

STUDY OF SEROEPIDEMIOLOGY OF HEV AND ITS ASSOCIATION IN NEW ENTRANTS AND FINAL YEAR MEDICAL STUDENTS--A STUDY ON SYLHET MAG OSMANI MEDICAL COLLEGE, BANGLADESH

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Abstract: An attempt was made to investigate the seroepidemiology of hepatitis E virus (HEV) infection in fresh entrants and final year medical (MBBS) students of Sylhet MAG Osmani Medical College, Bangladesh. The study was based on analysis of primary data taken from the students. A total of 80 students were selected as sample from each group. Relevant information was gathered using a pre-designed questionnaire. ELISA method was used to detect HEV IgG. In the first year, only 4 (5%) and in the final year 14 (17.5%) students were HEV IgG positive. The final year students were more affected by HEV than the first year entrants. Most of the students in MAG Osmani Medical College came from middle and high income families of urban areas of Bangladesh. Bivariate analysis revealed that in the fifth year, positive seroprevalence of HEV has significant ($p \leq 0.05$) association with taking meal during travel. On the other hand, the association of positive seroprevalence of HEV with past history of jaundice was found insignificant in both groups of students. Therefore further research is needed to assess the emerging epidemiological information of HEV and requires to be validated by a larger population-based study.

Keywords: Fulminant hepatitis, Open reading frame, ELISA, anti-HEV, seroprevalence

Introduction

Hepatitis has become a common health problem among the youth of Bangladesh. Early detection and treatment of ailments is imperative. In Bangladesh, enterically-transmitted acute viral hepatitis is common due to lack of proper sanitation and food hygiene. The environment is favourable for contracting HEV infection in such crowded places as hostels, hospitals, and restaurants. HEV is usually transmitted through faeco-oral route by contaminated drinking water. Hepatitis E virus is a single-stranded, non-enveloped, RNA virus, of the Genus Hepevirus, family-Hepeviridae [1]. HEV comprises a single serotype. Based on phylogenetic analysis HEV have been classified into four genotypes and nine groups; genotypes I, II, III, IV include the

Burmese isolates, Mexican isolates, US isolates and Chinese isolates, respectively [2].

Sheikh *et al.* [3] have reported that HEV infection is highly endemic in Bangladesh and some patients may develop fulminant hepatitis. A study conducted from 2001-2006 in the Matlab Health Research Centre Bangladesh [4] defined the unknown burden of HEV in rural Bangladesh and addressed the gaps in HEV epidemiology. That study revealed a 22.5% seroprevalence of anti-HEV; seroprevalence peaking in the second and third decades of life. This study by Labrique [4] also pointed out that outdoor employment, work outside the home, and travel are some of the risk factors; recent contact with a "jaundice" patient and injection exposures were also found to be significant. Medical professionals are more exposed to hepatitis E virus due to poor workplace environment plus contact with the patients during physical examinations and during various diagnostic and treatment-related

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manoeuvres. In many hospitals, the patients, attendants and caregivers share the same food court or restaurant in and around the hospitals. First year medical students (fresh entrants) have less chance of exposure to enterically transmitted microorganisms; hence are less likely to develop immunity against it. On the other hand, there is an increased chance of seropositivity of HEV in the final year students. During more than four years of their stay on campus with two years of clinical studentship, they come in close contact with patients. The extent to which their living conditions and food habit during their long stay at the campus may contribute to seroprevalence of HEV in them has remained a poorly explored area. The present study was designed to explore the impact of these factors on HEV seropositivity in the final year medical students. The first year medical students were taken as control. The study evaluated the effect of food habits and lifestyle in the seroconversion rate of HEV among the medical students. The difficulty in controlling the various confounding factors in population-based seroepidemiological studies was largely avoided in the present study due to the very nature of this defined educated population.

Materials and Methods

This cross-sectional and comparative study was conducted at the Department of Microbiology, MAG Osmani Medical College, Sylhet, Bangladesh. The study period was January 01, 2007 to December 31, 2007. The total study population was 324 (1st year 178 & 5th year 146) containing first year (fresh entrants, 18-20 yr old) (Group A) and final year MBBS students (Group B, aged 22-24 years) of this college. Group A serving as control had 178 students and from these 178 students, 80 (40 male and 40 female) were randomly selected as sample. Ten students from first year were excluded due to exclusion criteria (see below). Group B contained 146 students and from these 146 students, 80 (40 male and 40 female) were selected as sample and constituted

the test group. Since they fulfilled the inclusion criteria and all were enrolled. It is noteworthy here that at first we selected the first and final year students as a population and next we used simple random sampling to select our sample from the population. The total sample size was thus 160. The history of the students was recorded in a predesigned data collection sheet.

Enrollment criteria

Inclusion criteria:

Students staying at the hostels of the Medical College.

Exclusion criteria:

(i) History of blood transfusion during the month preceding the study, (ii) history of hospitalization during the last six months, (iii) students staying at home during the 5th year, and (iv) 1st year students who stayed at the hostel previously (some students might have previously stayed at residential high schools).

Sociodemographic variables

To evaluate the effects of socioeconomic condition on seropositivity of anti-HEV, history on socioeconomic background and parental occupation was recorded. The history of previous residence (rural, urban), gender, travel, eating habits, source of drinking water and sanitary facilities at home were taken to evaluate the impact on anti HEV seropositivity and to find out any association with seropositivity. History of jaundice of the subjects and their family members was also recorded.

Collection of specimen

Blood samples were collected aseptically in a sterile test tube with disposable syringes. Approximately 3 mL of blood was collected from each participant by venupuncture within 15 days of enrolment). The sample was allowed to clot at room temperature for 30 minutes; serum was

obtained by centrifugation (2000 rpm) for 20 minutes and transferred to microcentrifuge tubes. The tubes were capped, labelled and stored at -20°C .

Estimation of HEV antibody by ELISA

Estimation of anti HEV-IgG antibody in the blood was done using HEV kits manufactured by MBS SRL Milan, Italy; Lot No-RN 29124. Competitive ELISA was done.

Statistical analysis

For bivariate analysis, the well known *Pearson's Chi-square* (χ^2) test was used to identify the association between HEV IgG and other variables. In the analyses, only 5% level of significance was considered. The computational equation of *Pearson's Chi-square* (χ^2) test as follows was applied:

$$\chi^2 = \sum \frac{(\text{Observed frequency} - \text{Expected frequency})^2}{\text{Expected frequency}}$$

Results

Most of the medical students came from middle to high-middle income families in both groups (A, B). Nearly all of the first year and final year medical students came from urban residence. Most of the families of the students in both groups used drinking water from deep tube wells. Almost all of the respondents' families of both groups of students had access to latrines.

In Group A, 4 students and in Group B, 14 students were found HEV IgG positive. The figures in parenthesis in Tables 1 and 2 indicate percentage. The majority of the students' fathers were service holders or businessmen, while their mothers were housewives. Among the HEV IgG positive students in first year (Group A), 75% were female and in final year (Group B) 57% students were male.

Table 1 shows the association between Seroprevalence of HEV antibody and eating and drinking habits of the students during travel. Taking

Table 1. Association between seroprevalence of HEV (+ve, -ve), and eating and drinking habits of students during travel.

Name of the Variables	First Year			Fifth Year		
	HEV(+VE)	HEV (-VE)	P-value	HEV (+VE)	HEV (-VE)	P-value
Take meal during travel						
Home made	1(25) [¥]	34(45)	≥ 0.05	4(29)	43(65)	≤ 0.05 **
Restaurant	3(75)	42(55)	10(71)	23(35)		
Take bottled water during travel						
Yes	2(50)	37(49)	≥ 0.05	6(43)	44(67)	≥ 0.05
No	2(50)	39(51)	8(57)	22(33)		

** Significant at 5% level [¥]Figures in parenthesis indicate percent of the column

Table 2. Association between seroprevalence of HEV (+ve, -ve) and jaundice.

Name of the Variables	First Year			Fifth Year		
	HEV (+VE)	HEV (-VE)	P-value	HEV (+VE)	HEV (-VE)	P-value
Past history of Jaundice of students						
Yes	2(50)	13(17) [¥]	≥ 0.05	6(43)	18(27)	≥ 0.05
No	2(50)	63(83)	8(57)	48(73)		
Past history of Jaundice of students' household						
Yes	2(50)	45(59)	≥ 0.05	8(57)	41(62)	≥ 0.05
No	2(50)	31(41)	6(43)	25(38)		

[¥]Figures in parenthesis indicate percent of the column

meal during travel was an important factor associated with positive HEV seroprevalence. The results show that 75% HEV IgG positive students of first year took meal from restaurant during travel. On the other hand, in the case of final year HEV IgG positive students, 71 % students took meal from restaurant during travel. The values of chi-square test were 3.60 and 6.38 in first and final years, respectively. Although the percentage of those who took meal from restaurant during travel for first year was more than that of the fifth year students, the association between Seroprevalence of HEV antibody and meals during travel was significant ($p \leq 0.05$) only for the fifth year students. Among the HEV IgG positive students in both groups, about 50% of the first year and 43% of the fifth year students took bottled water during travel. Similarly, 50% of first year and 57% of fifth year students did not use bottled water during travel. The results also confirm that there was no significant ($p \geq 0.05$) association between Seroprevalence of HEV antibody and use of bottled water during travel in both groups. Table 2 reveals the association between Seroprevalence of HEV antibody and jaundice. The results show that students' past history of jaundice and past history of jaundice of the students' household had no significant (all $p \geq 0.05$) impact on the two groups of students.

Discussion

In the first year, only 4 (5% of the total sample of 80) students and in the final year 14 (17.5% of the total sample of 80) students were HEV-IgG positive. The prevalence of anti-HEV seropositivity was found to be higher in the final year students. Age specific seropositivity of HEV-IgG among both groups studied only matched with the final year students of the Matlab population study carried out in 2004-2005 [5]. The low positivity in that study was probably because the workers looked for HEV IgM, which generally disappears early from the blood. Moreover, there are some controversies

regarding concordance among many serological assays available for detecting HEV antibody [6,7]. The study performed in Matlab has shown that seroprevalence dramatically increases in adolescence [4].

The final year students (Group B) had greater positive seroprevalence for HEV-IgG than the new entrants (Group A). We have found only 5% positive seroprevalence in Group A, whose age range is 18-20 years. The Matlab study found 17.3% HEV-IgG positivity for a similar age group. The difference in results of the two studies is thus rather large. This may be because almost the entire study population in the present work came from urban areas of Bangladesh and from economically solvent families. Facilities for better housing, improved sanitation, drinking water supply, accessible health care are available in urban communities. In the final year (group B), the age ranged between 23-25 years, and positive seroprevalence for HEV IgG was 17.5%, which matches closely with result in the Matlab study (16.9% HEV-IgG seropositivity) in a similar age group. This compels one to think about the risk factors for HEV infection in medical students and in other people of the same age group in Bangladesh. In the first year, HEV-IgG seropositivity was very low in comparison with the Matlab rural population [5]. In the final year, on the other hand, the seropositivity we have found is quite similar to the rural population of the Matlab study involving the same age group [5]. Egypt is an exception as regards HEV seropositivity, where more than 60% become seropositive for HEV antibody before reaching 10th birthday, though it has environmental conditions similar to those in other developing countries which are conducive to faecal-oral transmission [8]. A recent study [9] has identified prolonged excretion and carrier state of HEV especially in immunocompromised patients. The ageing population with immunocompromised status is increasing in our country due to increase in life expectancy [10] and availability

and affordability of better medical care. The possibility that such a population is liable to act as chronic carrier and spreader of HEV to caregivers needs to be investigated. Traditionally, aged family members are supported and cared for in the family environment in our country, raising the possibility of carrier state and transmission potential.

This study shows that most of the students' family residence belonged to urban area where modern education facilities exist, and the families are conscious of the impact of higher education on their children's future. Only a few students fell in low socioeconomic groups in MAG Osmani medical college (the subjects were chosen randomly but this information was revealed when inquired). It may be due to the fact that education these days has become commercialized and requires high level of investment to keep an edge in a highly competitive admission process in government medical colleges which are less expensive than the private colleges. Relatively lower income families of rural origin cannot keep pace with this financial investment race for education. The present study also reveals that only a few students in the medical college studied came from high income families compared to the middle income group. It seems that students from well-off families opt to study in foreign countries or in private institutions.

The students residing in hostels sometimes took food in restaurants, but this habit had no significant effect on positive seroprevalence of HEV in both groups of students. It seems that the students' HEV antibody status was not affected by their food habit at the campus. Restaurants around the Sylhet MAG Osmani Medical College campus probably maintain safe drinking water and hygienic food. Thus safe drinking water, good standard of food, student health consciousness seem to contribute to negative association with seroprevalence.

The study also shows that there is significant

difference of HEV seroprevalence among the students of the final year and the new entrants. Thus, it is concluded that HEV spreads more easily among persons who came frequently in contact with hepatitis E patients and health care centres. Among the positive HEV IgG students of the final year, most of them took meal from way-side restaurants during travel, and the association of feeding habits during travel was found significant ($p \leq 0.05$). This finding supports the assumption that the food served during travel was not hygienic enough. First year students did not have to travel frequently, being new entrants, which in turn gave them less exposure to HEV (less exposure, less probability). About 57% of the final year students did not carry bottled water during travel and hence this seems to contribute to positive seroprevalence for HEV. First year students (50%) did not use bottled water, yet they did not show positive HEV seroprevalence. This may be due to the fact that their blood samples were collected soon after entry to the hostel and time for producing any effect was too short. The students' past history of jaundice had no significant (all $p \geq 0.05$) association with HEV IgG positivity impact in both the groups of students. It indicates that other causative agents, not investigated in the present study, were involved for past jaundice in HEV negative cases of the final year students.

Conclusion and Recommendations

This study showed the final year students were more affected by HEV than the 1st year students. Feeding habits during travel were found significant only in the final year students with positive HEV seroprevalence. Past history of jaundice had no significant association with positive seroprevalence of HEV in both groups of students. Only 17.5% of the final year students showed positive seroprevalence for HEV. This indicates that a large number of students would leave the institution with seronegative status, carrying the risk of future HEV hepatitis. The present results need to be viewed in the light of the

limitation of a rather small sample size and cross sectional study. For more reliable results, a cohort study would be more appropriate than a cross sectional study.

The Following recommendations are made on the basis of this study:

- In view of large number of students remaining unexposed to HEV in the final year and the high risk of contracting the disease in future, appropriate preventive strategy must be developed to minimize such risk
- The emerging epidemiological information from this study on HEV requires to be validated by a larger population-based study.
- Improvement of food hygiene in the hostel and hospital environment should be given due importance.
- Awareness building among medical students about risk of food and water borne diseases prevailing in the community is required.

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