Seroprevalence of hepatitis E and *Helicobacter pylori* in a low socioeconomic area of a metropolitan city in a developing country

W. JAFRI*, J. YAKOOB*, S. ABID*, S. AWAN*, S. SIDDIQUI*, F. JAFRI*, S. HAMID* and S. Q. NIZAMI†

*Departments of *Medicine and †Pediatrics, The Aga Khan University, Karachi, Pakistan*

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ABSTRACT

This study aims to determine the prevalence of co-infection of *H. pylori* and hepatitis E virus (HEV) in the paediatric age group in an urban slum area of Karachi and identify risk factors associated with co-infection. Five hundred and forty children aged one to 15 years were investigated. Blood samples were collected and questionnaires completed on socio-demographic characteristics. Anti-*H. pylori*, HEV IgG and IgM antibodies were analysed by enzyme immunoassays (EIAs). The seroprevalence of *H. pylori* antibody was 47.2%, while that of HEV IgG and IgM was 14.4% and 2.4%, respectively. 12.4% exhibited seroprevalence for both *H. pylori* and HEV (IgG). In 67 (26%) cases positive for *H. pylori* IgG, HEV IgG positivity was also seen ($P<0.001$). Only 13 (5%) positive for *H. pylori* were also positive for HEV IgM ($P<0.001$). Only 11 (4%) HEV IgG-positive cases were *H. pylori* antibody-negative ($P<0.001$). Hepatitis E virus was common in children who had access to municipal piped water ($P=0.025$). *H. pylori* was common in children who used a non-flush toilet system ($P<0.001$). Children exposed to *H. pylori* infection were also exposed to the risk of HEV.

KEY WORDS: Coinfection.
Hepatitis E virus.
Helicobacter pylori.
Water supply.
Social class.

Introduction

Water-related diseases remain a leading cause of morbidity and mortality worldwide. Several species of microorganism from human and animal faeces and from environmental sources, including water, have been confirmed as pathogens (e.g., *Cryptosporidium*, *Legionella*, *Escherichia coli* O157, rotavirus, hepatitis and norovirus). Studies have demonstrated that *Helicobacter pylori* survives in water although isolation from water systems is difficult.

H. pylori is a Gram-negative bacterium that colonises the human gastric mucosa.[1] *H. pylori* seroprevalence is common in populations with poor standards of sanitation and hygiene. In a previous study in Karachi in apparently healthy children aged six months to 10 years in 1999, the prevalence of anti-*H. pylori* antibody was 60%.[2] In a recent study, multivariate analysis showed that *H. pylori* seroprevalence was high in children in the 6–10 and 11–15 age groups (odds ratio [OR]: 1.5, 95% confidence interval [CI]: 1.2–1.9 and OR: 1.9, 95% CI: 1.56–2.47, respectively).[3]

Hepatitis E virus (HEV) is a self-limiting disease and occurs most frequently as an epidemic or sporadic hepatitis in developing countries with poor sewage disposal and unclean water supplies. It is enterically transmitted and it is thought to be the most common cause of acute sporadic hepatitis in Pakistan, where it has also caused major epidemics.[4] The seroprevalence of HEV in adults in Pakistan in 1995 was 62%.[5,6] The waterborne nature of the epidemic was suggested by a study of the case distribution according to water supply. The infection rate was significantly higher in the 11–30 age group (15.3%) compared with children less than 11 years of age (1.4%) and also relative to those aged over 30 years (10.5%).[6]

IgM anti-HEV was detected in specimens collected up to two weeks before and five to seven weeks after hospitalisation in 91% and 100%, respectively, of 122 HEV-infected patients.[7] IgG anti-HEV followed a similar pattern. Peak antibody titres appeared two to four weeks after hospitalisation.[7]

This study aims to determined whether or not seroprevalence of HEV and *H. pylori* correlate in terms of age and risk factors in a low socioeconomic area of a metropolitan city in a developing country. It assesses the contribution of source of water supply, types of latrine, number of individuals sharing accommodation, education, and occupation of parents in contributing to *H. pylori* and HEV seropositivity in the target population.

Correspondence to: Dr. Javed Yakoob
Department of Medicine, The Aga Khan University, Karachi-74800, Pakistan.
Email: yakoobjaved@hotmail.com

Materials and methods

Subjects and setting

This is a subgroup analysis of a study carried out between May 2008 and November 2009.[3] Serum samples from 540 children aged one to 15 years were tested for anti-*H. pylori*, anti-HEV IgG and IgM antibody titres. A family's socioeconomic status (SES) was based on family income,

parental education level, parental occupation, and status in the community. Hollingshead index (HI) of Social Position (ISP) was used to classify social class.[8,9] Crowding index (CI) was calculated by dividing the number of individuals by the number of rooms in the house. The study was conducted in Manzoor colony, a typical urban slum area in central Karachi. In a previous study, stored drinking water samples were discovered to be heavily contaminated with thermotolerant coliform organisms in studied households compared to control households.[11]

Sampling strategy

A two-stage cluster sampling technique was employed to draw the required samples. The Federal Bureau of Statistics divided Karachi into 5000 clusters, each of about 250 households. On average, each household contains seven individuals of which about 40% (three individuals per household) are aged between one and 15 years; thus, each cluster has about 700 children in this age group. Approximately 85% (4250) of the total 5000 clusters were in the lower- and middle-income group.

Community health workers were trained regarding the study before going into the field. They explained the purpose and objectives of the study questionnaire to the head of the family. The questionnaire was tested in a small number of interviews before conducting the main interviews in the study population. Written, informed consent was obtained from parents of the eligible children before administering the questionnaire and collecting a blood sample. A pretest questionnaire was filled out by the mother/father of each child to obtain information on socio-demography and potential risk factors for *H. pylori* and HEV transmission.

Fifty-six households were selected randomly in a single cluster for this study. Serum samples from 540 children in the 1–15 age group were collected after filling out a questionnaire for anti-*H. pylori* and HEV antibody titres.

Blood sample collection

Blood (5 mL) was obtained from each child. The collected samples were centrifuged and the separated serum was stored at –70°C.

Laboratory testing

Third-generation enzyme linked immunosorbent assay (ELISA) visual test kits for IgG anti-*H. pylori* (Novotec Immunodiagnostica, Germany) widely used locally were employed to detect the presence of anti-*H. pylori* in sera.[12] The diagnostic sensitivity and specificity of the test was 94% and 93%, respectively. Anti-*H. pylori* positivity or negativity was determined according to the positive and negative controls supplied in the kit. Tests for anti-HEV IgG and IgM were performed by ELISA (MP Biomedical Asia Pacific, Singapore) according to the manufacturer's instructions.[13]

Statistical method

Results were expressed as mean±standard deviation (SD) for continuous variables and number (percentage) for categorical data. The Statistical Package for Social Science (SPSS, release 16) was used for data analysis. Results were presented as mean±SD for quantitative variables and number (percentage) for qualitative variables. Differences in proportion were assessed using Pearson χ^2, Fisher exact or likelihood ratio tests where appropriated. $P<0.05$ was considered significant (all P values two sided).

Ethical issues

The purpose of the study was explained to a responsible adult in each participating household and written, informed consent was obtained to test for the presence of anti-*H. pylori* and HEV antibody titres. Formal ethical approval was obtained from the Aga Khan University Ethical Review Committee.

Results

H. pylori seroprevalence was positive in 47.2% (255/540) while HEV IgM was positive in 2.4% (13/540) and IgG in 14.4% (78/540); 12.4% (67) exhibited seroprevalence for both *H. pylori* and HEV. In children with positive *H. pylori* seroprevalence, HEV IgG was positive in 67 (26%) ($P<0.001$) compared to 11 (4%) in *H. pylori* seronegative children. Hepatitis E IgM was positive in only 13 (5%), all of which were *H. pylori* seropositive.

H. pylori ***seroprevalence according to age and gender***

There was no significant difference in the seroprevalence of *H. pylori* according to gender ($P=0.618$) or age group ($P=0.536$) (Table 1).

Hepatitis E IgM and IgG seroprevalence

There was no significant difference in the seroprevalence of HEV IgM according to gender ($P=0.78$). It was significantly positive in the 1–5 year age group compared to the other two groups ($P<0.001$).There was no significant difference in the seroprevalence of HEV IgG according to gender ($P=0.085$); 21% in the 11–15 age group, 12% in the 6–10 age group, and 9% in the 1–5 age group ($P=0.002$).

Comparison of HEV IgM and IgG seroprevalence

Hepatitis E IgM was significantly positive in the 1–5 age group ($P<0.001$) compared to the other two groups, while HEV IgG was significantly positive ($P=0.002$) in the 11–15 age group.

There was no difference in mean antibody titre of *H. pylori* IgG and HEV IgM/IgG (Table 1). However, there was a significant difference in the distribution of HEV IgG titre by gender (Table 1).

Comparison of **H. pylori** ***and HEV IgM and IgG seroprevalence***

Sixty-seven (26%) children who were positive for *H. pylori* IgG were also positive for HEV IgG ($P<0.001$), while only 13 (5%) who were positive for *H. pylori* were also positive for HEV IgM ($P<0.001$). Only 11 (4%) who were HEV IgG-positive were *H. pylori* antibody negative ($P<0.001$). Hepatitis E virus IgM was negative when *H. pylori* antibody was negative ($P<0.001$) (Table 1).

Discussion

This study shows that seroprevalence of *H. pylori* compared to HEV was greater in the 11–15 age group. Seroprevalence of *H. pylori* and HEV continued to increase with age,

Table 1. Risk factors associated with the seroprevalence of hepatitis E and *Helicobacter pylori* in children.

	H. pylori			HEV-IgG			*H. pylori* and HEV		
	Pos	Neg	*P*	Pos	Neg	*P*	Pos	Neg	*P*
Age									
1–5 years	65 (43)	87 (57)	0.296	13 (9)	139 (91)	0.002	5 (3)	147 (97)	<0.001
6–10 years	80 (46)	92 (54)		20 (12)	152 (88)		20 (12)	152 (88)	
11–15 years	110 (51)	106 (49)		45 (21)	171 (79)		42 (19)	174 (81)	
Gender									
Male	118 (46)	138 (54)	0.618	44 (56)	212 (46)	0.085	36 (14)	220 (86)	0.268
Female	137 (48)	147 (52)		34 (44)	250 (54)		31 (11)	253 (89)	
Crowding index (CRI)									
Low (0–1)	25 (49)	26 (51)	0.932	6 (12)	45 (88)	0.287	6 (12)	45 (88)	0.275
Moderate (2–4)	150 (48)	166 (52)		52 (17)	264 (83)		45 (14)	27 (86)	
Crowded (4–5)	80 (46)	93 (54)		20 (12)	153 (88)		16 (19)	157 (91)	
Hollingshead index (HI)									
Upper	6 (10)	51 (90)	0.134	24 (42)	33 (58)	0.138	6 (10)	51 (90)	0.332
Middle	3 (6)	45 (94)		17 (35)	31 (65)		3 (6)	45 (94)	
Lower	69 (16)	366 (84)		214(49)	221 (51)		58 (13)	377 (87)	
Source of water supply									
Municipal pipeline	170 (67)	194 (68)	0.728	44 (56)	320 (69)	0.025	41 (11)	323 (89)	0.246
Bore water	85 (33)	91 (32)		34 (44)	142 (31)		26 (15)	150 (85)	
Type of latrine									
With flush	60 (68)	28 (32)	<0.001	16 (18)	72 (82)	0.276	16(18)	72 (82)	0.072
Without flush	195 (43)	257 (57)		62 (14)	390 (86)		51(11)	401 (89)	
Mother's education									
Uneducated	110 (43)	122 (57)	0.938	32 (41)	200 (59)	0.709	25 (37)	207 (63)	0.318
Educated	145 (43)	163 (57)		46 (43)	262 (57)		42 (44)	266 (56)	
Father's education									
Uneducated	107 (45)	131 (55)	0.349	23(10)	215(90)	0.005	23 (10)	215 (90)	0.086
Educated	148 (49)	154 (51)		55(18)	247(82)		44 (85)	258 (15)	
Mother's occupation									
Employed	6 (50)	6 (50)	0.845	0(0)	12(100)	0.231	0 (0)	12 (100)	0.378
Unemployed	249 (47)	279 (53)		78(15)	450(85)		67 (13)	461 (87)	
Father's occupation									
Employed	146 (46)	169 (54)	0.631	42(13)	273(87)	0.385	39 (42)	276 (58)	0.982
Unemployed	109 (48)	116 (52)		36(16)	189(84)		28 (42)	197 (58)	

demonstrating a significant peak in the 11–15 age group (Table 1). Exposure to HEV was greater in boys than in girls but did not achieve significance. Person-to-person transmission is uncommon in cases of HEV, and in the cohort studied here both HEV and *H. pylori* did not demonstrate progressive increase in seroprevalence with crowding.

Hepatitis E virus seroprevalence was greater in children who used water supplied by the municipal pipeline, but this risk factor was not associated with *H. pylori* infection. In contrast, *H. pylori* was seen more commonly in children using bathrooms not provided with water flush systems. This is in keeping with a previous study that showed availability of a flushing toilet was protective against *H. pylori* carriage (OR: 0.51, 95% CI: 0.27–0.95, $P=0.031$).[14]

Both HEV and *H. pylori* did not show any relationship to maternal education, but HEV seroprevalence was greater in children whose fathers had received education (Table 1). Parent occupation was not associated with seroprevalence of *H. pylori* and HEV.

In this study the rate of co-infection of HEV and *H. pylori* was 19% in children in the 11–15 age group, but did not show gender distribution. Co-infection increased with crowded conditions. Water source of affected children was both by municipal water pipeline and bore water.

This study demonstrates that younger children are more vulnerable to co-infection with HEV and *H. pylori*. In the majority of cases the water used was obtained from municipal water pipelines. These water supply lines are generally old and tend to dry up during the hot season of the year. As sewage lines run in close proximity, leakage tends to seep into the municipal water pipelines, leading to contamination of the drinking water and a predisposition to various gastrointestinal tract infections caused by bacterial, parasitic and viral agents including HEV and *H. pylori*. This

is consistent with the finding in this study that HEV was significantly associated with municipal water pipelines in this low-income urban community.

A previous cross-sectional survey documented the prevalence and identified the risk factors of *H. pylori* and HEV infections in the paediatric age group of low socioeconomic urban communities of Karachi between April 2002 and December 2004.[15] Correlation between increasing age and seroconversion was seen for hepatotropic viruses. At aged 14 and above, 26% were positive for anti-HEV, while *H. pylori* infection did not show a significant increase with age.[16] In children, HEV (IgG) exposure of 19% was reported by Huma *et al*. in 2000, which indicated a high faecal contamination of drinking water and the need to re-address the issue of use of boiled water, the widespread supply of potable water, and the need to produce a vaccine.[17]

In many urban areas of developing countries, municipally supplied water is not microbiologically safe. In a previous study, although 193 of the 293 households (66%) reported using some method to purify their drinking water, including 169 (58%) who boiled their water, only 48 (16%) of the drinking water samples were free of coliforms.[11] In some slum areas, occupants refuse to use chlorine tablets to purify their water because their husbands feared it will make them impotent, and there is a misconception about the real intention behind the promotion of the practice of purifying drinking water.[18]

Unsafe water and sanitation are the source of HEV and *H. pylori*. Most people in developing countries acquire the hepatitis E virus as children, and *H. pylori* contaminate municipal water through untreated sewage. This reflects the importance of population education through projects aimed at improving environmental conditions and promoting health of the population at large. Furthermore, greater attention to sanitation associated with toilet use will lead to reduced faecal-oral transmission. □

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