Seroepidemiology of hepatitis E virus infection in 2–25-year-olds in Sari district, Islamic Republic of Iran

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الوبائيات السيرولوجية للعدوى بفيروس التهاب الكبدي "ئي" في من تتـراوح أعمارهم بين 2 و25 في مقاطعة ساري في جمهورية إيران الإسلامية

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الخلاصـة: درس الباحثون معدَّل الانتشار السيرولوجي لفيروس التهاب الكبد "ئي" E بين الأطفال وصغار البالغين، في مسح مجتمعي المرتكز في إحدى المناطق شمال جمهورية إيران الإسلامية. وقد أخذت عينات المصل من 1080 شخص تم اختيارهم عشوائياً، ممن تظهر عليهم علامات الصحة، وتتـراوح أعمارهم بين سنتين وخمس وعشرين سنة من مناطق حضرية وأخرى ريفية من مقاطعة ساري. وقد وُجدت أضداد الغلوبولين المناعي المضاد لفيروس التهاب الكبد "ئي" E لدى خمس وعشرين شخصاً (2.3٪)، وكان الانتشار المصلي يزداد بشكل ملحوظ مع تقدُّم العمر من 3 من أصل 255 (1.2٪) في الأطفال دون سن العاشرة إلى 18 من أصل 110 (7.3٪) فيمن تتـراوح أعمارهم بين 20 و25 عاماً. ولم تلاحظ أية فروق في وضع فيروس التهاب الكبد "ئي" E بين الجنسين. ولوحظ أن سكان المناطق الريفية يتعرّضون للعدوى في سن أصغر وبمعدَّل أعلى من سكان المناطق الحضرية.

ABSTRACT The seroprevalence of hepatitis E virus infection (HEV) in children and young adults was determined in a community-based survey in an area of northern Islamic Republic of Iran. Serum samples were taken from 1080 randomly selected apparently healthy 2–25-year-olds from urban and rural regions of Sari district. Anti-HEV IgG antibodies were detected in 25 individuals (2.3%). Seroprevalence increased significantly with age from 3/255 (1.2%) in children < 10 years to 8/110 (7.3%) in those aged 20–25 years. No differences in HEV status were noted between the sexes. Earlier age at exposure to infection and a higher infection rate were found in people residing in rural areas than in urban areas.

Séroépidémiologie de l'infection par le virus de l'hépatite E chez les enfants et les jeunes de 2 à 25 ans dans le district de Sari (République islamique d'Iran)

RÉSUMÉ La séroprévalence de l'infection par le virus de l'hépatite E (VHE) chez les enfants et les jeunes adultes a été déterminée grâce à une enquête communautaire réalisée dans une région du nord de la République islamique d'Iran. Des échantillons de sérum ont été prélevés sur 1 080 enfants et jeunes âgés de 2 à 25 ans sélectionnés au hasard, apparemment en bonne santé, qui venaient de régions urbaines et rurales du district de Sari. Des anticorps anti-VHE de type IgG ont été détectés chez 25 sujets (2,3 %). La séroprévalence augmentait significativement avec l'âge, de 3 sur 255 (1,2 %) chez les enfants de moins de 10 ans à 8 sur 110 (7,3 %) chez les jeunes âgés de 20 à 25 ans. Aucune différence n'a été observée entre les deux sexes quant à la sérologie VHE. L'enquête a montré que les personnes vivant en zone rurale étaient exposées plus jeunes à l'infection et connaissaient un taux d'infection plus élevé que celles vivant en zone urbaine.

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Received: 13/04/06; accepted: 27/07/06

Introduction

Hepatitis E virus (HEV) infection, also called enterically-transmitted non-A non-B hepatitis, is a major cause of epidemic and acute sporadic hepatitis in many areas of Asia, Africa and Mexico, where HEV is considered endemic. In countries where the virus is endemic, HEV is associated with greater than 50% of cases of sporadic acute hepatitis. The disease is self-limiting but sometimes has severe complications and a high case-fatality rate, particularly among pregnant women (about 20%) [*1–4*].

Epidemics of HEV have the highest attack rate in the age group 15–40 years. However, studies on children show that they are susceptible to HEV infection and many cases of sporadic acute viral hepatitis are caused by HEV [*5–7*]. Results of studies on children from Sudan [*8*] and Hong Kong [*9*] have demonstrated that 59.0% and 11.7% of cases of acute sporadic hepatitis cared for in hospital had evidence of recently acquired HEV infection. Mathur et al. from India, a high-endemic country for HEV infection, showed in their study of 1–10-year-old children that 23.8% of rural and 28.7% of urban children were serologically positive for anti-HEV immunoglobulin G (IgG) [*10*]. The rates of positivity increased with increasing age.

In 2 previously reported studies from the Islamic Republic of Iran it was indicated that enterically-transmitted non-A non-B hepatitis was found to be the most common cause of epidemic hepatitis in Iranian adults and children, and was associated with a high mortality rate (about 20%) among pregnant women [*11,12*]. Epidemic non-A non-B hepatitis was diagnosed in those cases by lack of parenteral risk factors and exclusion of acute hepatitis A and B. Also, other studies have described the prevalence of HEV infection as 1.6% to 8.8% in selected groups among adult subjects [*13,14*]. However, community-based studies and information about HEV infection in children and young adults are lacking in the Islamic Republic of Iran.

The present study was designed to determine the prevalence of HEV infection (anti-HEV IgG positive status) among children and young adults aged 2–25 years in rural and urban areas of one city in the Islamic Republic of Iran. Demographic features that are potential risk factors for acquiring HEV infection were compared between the 2 areas.

Methods

This study was a cross-sectional community-based survey conducted from June 2003 to December 2003. The study group was 1080 apparently healthy children and young adults aged 2–25 years. They were selected by randomized age-stratified sampling from urban and rural areas of Sari district, the capital of Mazandaran province, northern Islamic Republic of Iran (population 450 000 and 110 000–120 000 in the range 2–25 years). The sample size (1152) was calculated based on studies from other countries [*10,15–21*] and probable prevalence of 25%. Individuals with acute infection, pregnancy, immunodeficiency, receiving blood or blood products within the last 3 months and chronic renal and liver diseases were excluded.

The study was approved by the university medical ethics committee and informed written consent was obtained from the adult subjects or the parents of children. Sampling was primarily based on the proportion of the population in rural and urban areas and a random sampling method was used to recruit the actual households to be included in the study. All selected participants were

visited at their own home by a health-care worker and one of the coauthors (RF, AA), where a blood sample was drawn and a questionnaire answered. When a subject was not contactable or refused to participate, another subject of similar status was substituted from his or her neighbourhood. A specially developed questionnaire was used to collect data about: age, sex, education level of subject's mother and father, education level of subjects aged ≥ 20 years, number of siblings, area of residence, presence or absence of toilet facilities in the home, method of sewage and waste disposal, type of water supply and history of clinical hepatitis in the subjects and their family members.

From eligible subjects, a 3 mL blood sample was drawn and serum was obtained by centrifugation and stored at –20 ºC until further processing. Specific IgG antibody (anti-HEV) was measured using a 3rd generation enzyme immunoassay for the determination of IgG antibody for HEV (Diapro, Milan, Italy) with a sensitivity, specificity and accuracy of 100%, 99.3%–99.6% and 99.4%–99.6% respectively. The cut-off was defined with positive and negative control sera that were included in each assay according to the manufacturer's instructions. Samples were considered positive if the optical density value was above the cut-off value and all positive samples were confirmed with a 2nd assay using the same kit.

The associations between seropositivity and demographic and socioeconomic characteristics were described as percentages and odds ratio (OR) with 95% confidence intervals (CI) and analysed using a bivariate model with *z*- and chi-squared tests. For statistical analysis *SPSS*, version 11 software was used and $P < 0.05$ was considered statitically significant.

Results

Of the 1102 participants, 22 were excluded as the serum samples were haemolysed or lipoid. Therefore 1080 participants aged 2–25 years were enrolled; 515 (47.7%) were female.

There were 587 subjects residing in Sari city (urban) and 493 in the areas around Sari city (rural). The demographic characteristics of the subjects in urban and rural areas are compared in Table 1. There were significant differences between the 2 groups in parents' education level ($\chi^2 = 49.94$, $P < 0.001$) and number of household members ($\chi^2 = 7.99$, $P < 0.05$). No differences were noted for drinking-water supply systems (100% in both areas had piped supplies) and defaecation habits (indoor or closed system outside the house), but the sewage and solid waste disposal systems were different in urban and rural areas, being central systematic in the former and with no apparent system in the latter (i.e. dumped directly into the environment).

Anti-HEV IgG antibodies were detected in 25 individuals (2.3%). A clinical history of jaundice was detected in 1/25 (4.0%) of the seropositive and in 5/1055 (0.5%) of the seronegative subjects ($\chi^2 = 5.49$, $P = 0.05$).

Seroprevalence increased significantly with age from 3/255 (1.2%) in children < 10 years to 8/110 (7.3%) in those aged 20–25 years ($z = 2.61$, $P = 0.009$). No differences were noted between the sexes regarding the prevalence of HEV: 12 females and 13 males (OR = 1.01; 95% CI: 0.43–2.39).

As shown in Table 2, 11/587 (1.9%) subjects in urban areas were anti-HEV positive compared with 14/493 (2.8%) in rural areas ($z = 13.04$, $P < 0.001$). Although in both areas anti-HEV prevalence rates increased with age, exposure to HEV occurred earlier in rural than urban areas, as no infection

Table 1 **Demographic characteristics of study subjects from urban and rural areas of Sari, Islamic Republic of Iran**

Variable	Urban area (*n* = 587)		Rural area (*n* = 493)		Statistical tests
	No.	%	No.	%	
Sex					
Female	264	45.0	251	50.9	
No. of household members					
≤ 2	11	1.9	8	1.6	$\chi^2 = 7.99$, $P < 0.05$
3–5	439	74.8	329	66.7	
6	137	23.3	156	31.6	
Mother's education level					
University	30	5.1	6	1.2	$\chi^2 = 4.57$, $P = 0.03$
High school	184	31.3	93	18.9	$\chi^2 = 30.45$, $P < 0.001$
Primary school	286	48.7	203	41.2	$\chi^2 = 6.67$, $P = 0.009$
Illiterate	87	14.8	191	38.7	$\chi^2 = 76.65$, $P < 0.001$
Father's education level					
University	57	9.7	9	1.8	$\chi^2 = 27.6$, $P < 0.001$
High school	286	48.7	121	24.5	$\chi^2 = 65.68$, $P < 0.001$
Primary school	206	35.1	263	53.3	$\chi^2 = 35.59$, $P < 0.001$
Illiterate	38	6.5	100	20.3	$\chi^2 = 44.62$, $P < 0.001$
Subject's education level[a]					
University	19	3.2	2	0.4	Fisher exact test, $P = 0.001$
High school	26	4.4	12	2.4	$\chi^2 = 6.09$, $P = 0.01$
Primary school	9	1.5	33	6.7	$\chi^2 = 21.75$, $P < 0.001$
Illiterate	2	0.3	7	1.4	Fisher exact test, $P = 0.09$

[a]*For subjects ≥ 20 years.*
n = *total number of subjects.*

Table 2 **Anti-hepatitis E virus immunoglobulin G (IgG) status in urban and rural areas by age of subjects in Sari, Islamic Republic of Iran**

Age group (years)	Urban area		Rural area		Mean prevalence (95% CI)
	No. positive/ no. tested	% positive	No. positive/ no. tested	% positive	
2–4	0/208	0.0	0/138	0.0	0.0
5–9	0/138	0.0	3//117	2.6	1.2 (0.02–1.98)
10–14	5/139	3.6	4/99	4.0	3.8 (1.04–4.96)
15–19	2/46	4.3	3/85	3.5	3.8 (1.04–4.96)
20–25	4/56	7.1	4/54	7.4	7.0 (3.00–11.00)
Total	11/587	1.9	14/493	2.8	2.3 (1.20–2.80)[a]

[a]z = *13.04,* P < *0.001.*
CI = confidence interval.

was noted in the age group < 10 years in urban areas whereas a 2.6% infection rate was observed in the age group 5–9 years in rural areas.

Discussion

To our knowledge, the present work is the largest population-based age-specific seroepidemiological study of HEV infection in the Islamic Republic of Iran. The results of this study on a population aged 2–25 years old in Sari in the north of the Islamic Republic of Iran confirm earlier reports about HEV infection in the Islamic Republic of Iran [*11,12*]. The results showed that HEV infection is endemic, acquired early in life and its seroprevalence rate increased steadily with age, reaching 7.3% in young adults.

The patterns of increase are similar to those reported in many other studies from different endemic countries [*10,15–21*]. In north India, a region with high endemicity for HEV infection, exposure to HEV was shown to occur in early life and seropositivity for anti-HEV IgG increased progressively from 7.2%–14.2% in infancy to 33.3%–38.0% by 10 years of age in rural and urban children respectively [*10*]. A similar epidemiological picture was also seen in other studies from Turkey [*17,18*], Saudi Arabia [*19*], Egypt [*20*] and Mexico [*21*]. A study by Kamel et al. on the seroepidemiology of HEV infection in an entire village population located in the Egyptian Nile delta showed seropositivity of 5.19% in the age group < 5 years, increasing progressively and peaking at 33.33% in the age group 20–24 years [*20*]. This pattern of increasing anti-HEV IgG levels might be explained by the cohort effect [*15,21*], or by failure of young children to mount a brisk anti-HEV response [*4,15,22*], rapid decay of anti-HEV antibodies occurring after the initial acquisition of infection [*10,22,23*] or if a large dose of virus is required to cause infection [*15*].

The main route of HEV transmission is faecal–oral via contaminated water. Many studies indicate that poor sanitation, crowding, low levels of education and poverty are risk factors for HEV infection [*1–4,19–21,24*]. Our study demonstrated that the anti-HEV prevalence was significantly higher and occurred earlier in rural compared with urban communities (2.6% in age group < 10 years old in rural areas versus 0% in the same age group in urban areas). When comparisons were made between the characteristics of the population in rural and urban areas, the most significant differences were in parental education levels ($P < 0.001$), numbers of household members ($P < 0.05$) and systematic collection of sewage and solid waste material. Arif et al. studied the rate of exposure to HEV infection in 2 areas of Saudi Arabia on the basis of the quality of water sanitation and sewage disposal in each area and showed that HEV infection was endemic in both areas [*19*]. However, acquisition of the infection started earlier in areas with poor sewage disposal and water sanitation systems and the prevalence of infection increased with age. Similar findings were also reported by Alvarez-Munoz et al. from Mexico, which showed that age, type of community (rural versus urban) and educational level were risk factors for infection [*21*].

In contrast to the above-mentioned studies about the role of residential area (rural versus urban) on the risk of exposure to HEV, Mathur et al. demonstrated that exposure to the HEV occurred earlier, and overall seroprevalence rates of IgG antibodies against HEV were significantly higher in urban compared to those in rural subjects [*10*]. They postulated that these differences may be due to longer duration of breastfeeding in rural areas, which in turn would

reduce the risk of waterborne infections. They also concluded that in a heavily contaminated environment and prevailing poor hygienic conditions, demographic features that could be potential risk factors to acquire anti-HEV antibody might not be related to the anti-HEV status of the children.

As the results of this study indicate, serologically anti-HEV positive subjects rarely had a history of any particular signs and symptoms related to acute clinical hepatitis. Similar to reports by Mathur et al. on 6–120-month-old children (by assessing anti-HEV IgM antibody in IgG seropositive subjects) [*10*] and Shields et al. [*25*], the HEV infection in our studied sample was subclinical or mild as only 1 of 25 seropositive subjects had a past history of jaundice. Although, historically most of the seropositive individuals were clinically asymptomatic, a fulminant course of hepatitis E has been reported for pregnant women. Therefore, precautions should be taken for pregnant women residing in the area.

The major limitation of the study was its sampling procedure. The cross-sectional design did not allow determination of the age-specific attack rates for HEV. Also the study frame could not differentiate acute or recent infections from old ones. Further community-based, age-stratified studies with larger sample sizes and assessment of IgM and alanine aminotransferase levels is recommended to detect recent infection in those with anti-HEV IgG antibodies, to evaluate the role of HEV in clinical hepatitis in the community and to determine the role of different factors in exposure to HEV.

In conclusion, our study showed that HEV infection is endemic in the Sari area. Earlier age at exposure to infection and a higher infection rate was found in people residing in rural areas than urban areas. Lower education levels, greater household crowding and inadequate sewage disposal systems were more common in rural areas. Public health measures such as improvement of mass education in personal and public hygiene are effective measures for controlling the spread of HEV infection.

Acknowledgements

This project was funded by the deputy for research of Mazandaran University of Medical Sciences. Many thanks are due to M.R. Parsaei and J. Shojaei for their help in sample collection. Also special thanks go to Dr Enayati for critical review of the manuscript.

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