Original Article

Seroprevalence of subclinical HEV infection in asymptomatic, apparently healthy, pregnant women in Dakahlya Governorate, Egypt

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Abstract:

Background and Aim: Hepatitis E virus (HEV) is a major public health problem in the developing countries. HEV infection in pregnant women is more common and fatal in the third trimester. The present study was designed to determine the seroprevalence of subclinical HEV infection in asymptomatic pregnant women. **Materials and Methods:** A total of 116 asymptomatic pregnant women divided into 2 groups: Group 1 included 56 pregnant women with HCV positive serology and group 2 included 60 pregnant women with negative HCV serology were included in this study. Prevalence of anti-HEV antibodies and anti-HCV were determined by an enzyme linked immunosorbent assay (ELISA) kit. **Results:** The overall prevalence of anti-HEV IgG was highly significant among pregnant women with chronic HCV infection 40/56 (71.42%) than pregnant women free from chronic HCV infection 28/60 (46.7%) ($P = 0.006$). Chronic HCV infection in pregnant women appeared to be a risk factor associated with HEV IgG seropositivity (OR = 2.86, CI = 1.24-6.6). The seropositivity of anti-HEV IgG was significantly high in rural areas than urban areas (62.5% vs. 37.5%) in group 1 and (78.58% vs. 21.42%) in group 2 ($P = 0.15$) and OR = 2.2, CI = 0.65-7.7). A decrease in albumin level ($P = 0.047$) and an increase in bilirubin ($P = 0.025$), ALT ($P = 0.032$), and AST ($P = 0.044$) in pregnant women with positive HCV and IgG anti-HEV than the second group with negative HCV serology. **Conclusions:** The seroprevalence of anti-HEV IgG in pregnant women is high in Egypt especially in rural areas. With chronic HCV coinfection, a marked increase in anti-HEV IgG seropositivity and significant worsening of the biochemical liver indices were noted. Increased public awareness about the sound hygienic measures for a less prevalence of HEV is strongly advised. The need for HEV vaccination for those at risk, especially pregnant ladies, should be considered.

Key words:

Chronic HCV infection, hepatitis E virus, pregnancy

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Introduction

Hepatitis E virus (HEV) is a major public health problem in the developing countries. The disease occurs either in the form of large epidemics which are related to contamination of drinking water supplies, or in the form of sporadic cases in the absence of a conspicuous outbreak.[1,2] In countries with poor sanitation, HEV is endemic and typically causes explosive outbreaks of acute hepatitis, usually associated with fecal contamination of the water supply. The disease is generally mild, yet pregnant women suffer significant morbidity and mortality.[3-5] In contrast, in countries with high standards of sanitation, HEV occurs sporadically, initially identified as an imported disease in travelers from highly endemic regions, but subsequently diagnosed in patients with no travel history as well; this latter form has been named "hepatitis E indigenous to developed countries."[6,7]

HEV infection represents a significant public health and economic burden particularly in countries where the absence of sanitation infrastructures, or their breakdown as a consequence of wars or natural disasters, brings the hygienic conditions below a safe level.[8-10]

Phylogenetic analysis of HEV genome from different isolates has led to the identification of our main genotypes, with genotypes 1 and 2 circulating in Africa and Asia, genotype 3 showing a broad distribution world wide, and genotype 4 being restricted to Asia. Genotypes 3 and 4 are enzootic in a variety of wild and domestic animals, particularly pigs,[11,12] which gave rise to the question of whether human HEV infection is a zoonosis. Evidence from Japan[13-15] and China[16] now confirms that humans can acquire HEV infection from animals.

HEV infection can be diagnosed by either detection of viral particles in stool using electron microscopy or detection of anti-HEV antibodies in serum. Similar to hepatitis A virus, HEV occurs in high concentrations in stool in the weeks immediately prior to the onset of symptoms. Viral shedding in the stool usually continues about two weeks after the onset of jaundice, although in a few persons viral

shedding has persisted as long as four weeks. Antibodies to HEV are detectable in nearly all infected patients upon presentation of their illness.[17] Enzyme immunoassays based on recombinant proteins of HEV have been used for most seroprevalence studies. The recombinant proteins contain immunodominant epitopes encoded by ORF2 and ORF3 of the HEV genome from different strains.[18] The IgM antibody to HEV is used as an acute phase marker of HEV infection and HEV IgG is used to study the exposure to HEV in a given population. Evidence of exposure and/or positive IgG serology has been demonstrated in varying proportions (9.3–26%) in the healthy populations of developed countries.[1,2] This work was designed to study the seroprevalence of subclinical HEV in asymptomatic pregnant women with or without chronic HCV infection.

Materials and Methods

This study includes 116 asymptomatic pregnant women attending antenatal clinic of Mansoura Univesity Hospital for routine antenatal care during 2009. They were divided into two groups:

A – group 1 includes 56 pregnant women with HCV-positive serology for more than six months as defined by positive viremia for hepatitis C virus (HCV) RNA by reverse transcriptase-polymerase chain reaction (RT-PCR) and have no other causes of acute or chronic liver diseases. B – group 2 includes 60 pregnant women free from chronic HCV infection as defined by negative anti-HCV antibody and negative PCR.

All other causes of chronic liver diseases were excluded on the basis of analytical, clinical, and epidemiological data; autoimmunity, metabolic and genetic disorders, nonalcoholic steatohepatitis, alcohol intake, and drug toxicity and all cases were negative for anti-HIV antibodies. Personal, family, and socioeconomic history was recorded in detail. All females included in the study were subjected to full medical history and thorough clinical examination included obstetric examination. Laboratory investigations were performed, including liver function tests by Synchron autoanalyzer (Beckman Coulter, Fullerton, CA, USA). Informed consent was obtained from all patients.

IgG anti-HEV enzyme-linked immunosorbent assay

All serum samples from pregnant women were tested with IgG anti-HEV enzyme-linked immunosorbent assay (ELISA) kits (Genelabs Diagnostics, Singapore). Fusion proteins M 3-2, B 6-1-4, and M 4-2, corresponding to the immunodominant epitopes found in ORF2 and ORF3, were used to coat the solid phase of the ELISA to detect IgG anti-HEV. The ELISA was performed according to the protocols provided by the manufacturer.

Statistical analysis

The statistical analysis of data was done by using *Excel* program and *SPSS* program (statistical package for social science) version 10. The description of the data done in the form of mean ± SD for quantitative data and frequency and proportion for qualitative data. The analysis of the data was done to test statistical significant difference between groups. For quantitative data Student's *t*-test was used to compare between two groups and a paired sample *t*-test was used to compare one group at different measurements. Significant data in univarate analysis were entered in multivariate logistic regression to detect predictable data. A *P*-value <0.05 was considered to be significant.

Results

A total of 116 pregnant women were enrolled in this study. They were divided into two groups: Group 1; 56 pregnant women with chronic HCV infection with the mean age of 32.5 ± 12.3 years and group 2; 60 pregnant women free from such infection with the mean age of 33.6 ± 7.8 years. Table 1 shows that 40/56 patients (71.42%) were tested positive for IgG anti-HEV antibodies among pregnant women with chronic HCV, while 28/60 patients (46.7%) tested positive for IgG anti-HEV antibodies among pregnant women free from chronic HCV infection and the OR (2.86; CI, 1.24-6.6).

The seropositivity of IgG anti-HEV when compared with the place of residence was found to be significantly higher in rural areas than in urban areas (62.5% vs. 37.5%) in group 1 and (78.58% vs. 21.42%) in group 2 ($P = 0.15$), the OR (2.2; CI 0.65-7.7). The majority of pregnant women in our study were primipara, 80.35% in pregnant women with chronic HCV infection and 66.67% in pregnant women free from chronic HCV infection. Most were pregnant women in both groups above 30 years 67.84% in group 1 and 53.3% in group 2.

Table 2 shows a significant decrease in albumin ($P = 0.047$) and an increase in bilirubin ($P = 0.025$), ALT ($P = 0.032$), and AST ($P = 0.044$) in patients with positive HCV serology and IgG anti-HEV than patients with negative serology for HCV with positive anti-HEV IgG.

Discussion

Studies reported very high levels of anti-HEV prevalence among healthy adults and pregnant females in rural areas in Egypt (67.7% and 84.3%, respectively).[19,20] The authors hypothesize that both zoonotic and anthroponotic transmission of a virulent (possibly genotype-3) HEV is occurring extensively in these rural villages and that the rate of seropositivity increases with age.

Table 1: Characteristics of studied groups

	Group (1) HCV +VE *N* = 56 (%)	Group (2) HCV –VE *N* = 60 (%)	OR (95% CI)	*P* value
Age in years (mean ± SD)	(32.5 ± 12.3)	(33.6 ± 7.8)		
20–30 years	18 (32.14)	28 (46.7)	0.54 (0.24–1.23)	0.11
30–40 years	38 (67.84)	32 (53.3)		
Primipara	45 (80.35)	40 (66.67)		
Multipara	11 (19.64)	20 (33.33)	2.05 (0.81–5.2)	0. 09
Positive anti-HEV IgG	40/56 (71.42)	28/60 (46.7)		
Urban	15/40 (37.5)	6/28 (21.42)	2.86 (1.24–6.6)	0.006
Rural	25/40 (62.5)	22/28 (78.58)	2.2 (0.65–7.7)	0.15

Table 2: Comparison of biochemical liver indices between hepatitis E virus IgG positive pregnant females with or without chronic HCV infection

	HCV-ve with positive IgG anti-HEV (*N* = 37)	HCV+ve with positive IgG anti-HEV (*N* = 40)	*P* value
Albumin	4.3 ± 0.46	3.8 ± 0.5	0.047
Bilirubin	0.93 ± 0.3	1.4 ± 0.3	0.025
ALT	35.1 ± 11.15	58.27 ± 17.5	0.032
AST	28.6 ± 9.8	52.6 ± 13.2	0.044

In our study we found that the seroprevalence of anti-HEV IgG was 71.42% among pregnant females with chronic HCV infection and 46.7% among pregnant females free from chronic HCV infection. Compared with other studies,[19,20] our better results may be explained by the continuous official efforts that resulted in remarkable improvement of sewage disposal and more sanitary water supply in both urban and rural areas during the last few years.

On the other hand in our findings when compared with the international studies, pregnant women showed a higher prevalence of anti-HEV IgG than those in other countries (2–13%).[21-23] This finding could indicate that the journey is still at its beginning and more mutual efforts among the people and the health authorities are required.

In the present work, the seroprevalence of anti-HEV IgG among pregnant females with chronic HCV infection is 71.42%. A striking association between HEV and HCV infection was reported from southern Italy[24] and from Greece[25] (27.0%, 10.7%, respectively). This association may be explained by the fact that transmission of HEV occurs predominantly by the fecal-oral route which is an easier route for transmission in areas endemic for both viruses and doubtfully sanitary health conditions. However, the parenteral route has also been implicated.[26]

In this study when our patients with positive anti-HEV IgG were compared, we found that a significant increase in AST, ALT, bilirubin, and a significant decrease in the albumin level in patients with chronic HCV infection than those with negative HCV serology. This result may be explained by the superadded hepatic necro-inflammation induced by chronic HCV infection.[27]

In our study, the seroprevalence of anti-HEV IgG was high in rural areas than urban areas (62.5% vs. 37.5%) in group 1 and (78.58% vs. 21.42%) in group 2; this result is in agreement with Begum *et al.* (2009) who found that the lowered socio-economic status appeared to be the major risk factor for increased prevalence HEV infection among pregnant women. Health measures such as health directions to improve the personal as well as the public hygiene are known to be the most effective available measures for controlling the spread of HEV infection.[28]

In conclusion, the seroprevalence of anti-HEV IgG in pregnant women is high in Egypt especially in rural areas. With chronic HCV coinfection, a marked increase in anti-HEV IgG seropositivity and significant worsening of the biochemical liver indices were noted. Increased public awareness about the sound hygienic measures for a less prevalence of HEV is strongly advised. The need for HEV vaccination for those at risk, especially pregnant ladies, should be considered.

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