



ISSN: 0067-2904

GIF: 0.851

Prevalence of Hepatitis E Virus in AL-Muthanna Province (Iraq) Patients

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Abstract

Hepatitis E (HE) is an inflammation of the liver caused by hepatitis E virus (HEV) infection. Iraq is one of the Asian countries with high incidence and prevalence of hepatitis. In this paper, HE prevalence will be determined in AL-Muthanna province /Iraq. Commercially available Micro-ELISA for marker of hepatitis E (HEV IgM, Foresight, USA) kit was used to test (270) patients for HEV IgM antibodies. Also (10) blood samples from normal healthy individuals were used as normal control in this study. Among the (270) analyzed serum samples, a total of (72) samples (26.66 %) were found to be positive for anti-HEV IgM antibodies, and all these patients were tested for confirmatory test at central public health laboratories (CPHL) in Baghdad province. These patients consist of (45) females and (27) males were all negative for routinely screened markers of Hepatitis A, Hepatitis B and Hepatitis C. The positive sera anti-HEV IgM also were tested for total serum bilirubin (TSB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) colorimetrically. The age ranged between (4-74) years old. Highly significant differences ($\chi^2 = 10.271$, $p \leq 0.01$) appeared among age groups. This study showed that the HEV IgM is more common among younger age group (15-24), with a percentage of (41.67%). And higher in females (63%) than in males (37%).

Keywords: HE; HEV; prevalence; outbreak.

انتشار التهاب الكبد الفيروسي نمط إي في مرضى محافظة المثنى (العراق)

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الخلاصة

التهاب الكبد نمط إي هو التهاب الكبد الناتج عن الإصابة بفيروس التهاب الكبد نمط إي. العراق هو إحدى الدول الآسيوية التي تعاني من ارتفاع نسبة حدوث وانتشار التهاب الكبد. في هذه البحث سنعرف مدى انتشار هذا المرض في محافظة المتشي / العراق. باستخدام تقنية الامتزاز المناعي المرتبط بالإنزيم (ELISA)، تم الكشف عن الأجسام المضادة: Anti-HEV IgM antibodies في مصل (270) مريضاً. كما وشملت الدراسة أيضاً (10) من الأفراد الأصحاء كمجموعة سيطرة. (72) مريضاً (26.66 %) من أصل (270) مريضاً اظهروا نتائج موجبة لفحص الأجسام المضادة Anti-HEV IgM antibodies . وتم اختبار جميع هؤلاء المرضى لاختبار تأكيدي في مختبرات الصحة العامة المركزية (CPHL) في محافظة بغداد. يتكون هؤلاء المرضى من (45) إناث و (27) ذكور اظهروا جميعاً نتائج سالبة للفحص الروتيني لالتهاب الكبد الفيروسي نوع (أي، ب، ج). تم الكشف عن مستويات إنزيمات وظائف الكبد في الأمصال الموجبة لفحص الأجسام المضادة: Anti-HEV IgM antibodies. تراوحت الفئات العمرية للمرضى بين (4-74) سنة. ظهرت بين الفئات العمرية فروقا معنوية عالية ($\chi^2 = 10.271, p \leq 0.01$). هذه الدراسة بينت إن HEV IgM هو أكثر شيوعاً بين الفئة العمرية (15-24) بنسبة (41.67%) . وأعلى في الإناث (63%) منها في الذكور (37%) .

Introduction

Hepatitis E virus (HEV), the etiological agent of hepatitis E (HE), was described for the first time by using electron microscopy in 1983 as a spherical viral particle non-enveloped being 27 to 30 nm in size. The HEV genome comprises a 7.2 kb non-segmented single-stranded positive-sense RNA chain. Encoding three open reading frames (ORFs), with ORF 3 overlapping both ORF 1 and ORF [1] .

The non-structural proteins, including the putative methyltransferase, helicase and RNA polymerase are encoded by ORF 1, while the major structural protein (PORF2) is encoded by ORF 2 [2] . A hydrophobic stretch of 22 amino acids is present at the amino terminus of PORF2 and it has been suggested that this functions as a signal peptide [2,3]. And ORF3 encodes a small multifunctional protein. The ORF2 and ORF3 proteins are translated from a single, bicistronic mRNA [4]. HEV was suggested to be classified in the Picornaviridae family [1,5]. However, later studies showed that it does not belong to members of this family. Between 1988 and 1998, HEV was tentatively classified in the Caliciviridae family, based on virion morphology. This classification also was rejected after a phylogeny analysis of the HEV genome, and HEV was newly classified as an independent genus HEV-like virus, unassigned to any family [6,7]. At present, HEV is the only member of the Hepevirus genus, Hepeviridae family [1,8].

HEV causes acute sporadic and epidemic viral hepatitis worldwide. HEV infections are spread mainly by the faecal-oral route and large epidemics, due to this virus which is often associated with contaminated water [9 ,10 ,11]. The incubation period after exposure ranges from 3 to 8 weeks (mean 40 days) and it is dose dependent [12]. There is also a possibility of zoonotic transmission of the virus. Seroepidemiological studies revealed that anti- HEV antibodies are present in numerous animal species including pigs, rodents, chickens, dogs, cows, sheep and goats from developing and industrialized countries [13]. There are four major genotypes of HEV: I, II, III and IV . Homology of members of the same genotype is presumed not to be less than 81% [1]. The phylogenetic analysis divided HEV genotype I into five subtypes, genotype II into 2 subtypes, whereas genotypes III and IV were divided into 10 and 7 subtypes, respectively [14]. Genotypes I and II have been identified exclusively in humans, and genotypes III and IV have been found in humans and several animal species. Genotypes I and II have been isolated in Asia, Africa, North America; genotype IV has been identified only in Asia; and genotype III has been found in almost every country [15]. Various clinical manifestations of the disease have been observed, from more frequent subclinical forms to fulminant

forms of hepatitis. HEV infection is most often seen in children, young to middle aged adults (15 to 40 years old) and might be serious in pregnant women. In most cases, the signs and symptoms of the disease include moderately severe hepatitis with concurrent signs of influenza-like symptoms, abdominal pain, fever tenderness, nausea, vomiting and with concurrent jaundice and dark urine, liver enzyme elevations, antibody seroconversion and clearing of the virus [1]. Prolonged viraemia and viral shedding are unusual and chronic infection does not occur. Fulminant hepatitis occurs more frequently in pregnancy and induces a mortality rate of 20% and can also cause premature births [16]. Hence the aim of this research is to identify the prevalence of HEV in AL-Muthanna patients.

Materials And Methods

A total of (270) blood samples from jaundiced patients of both sexes from Al-Muthanna province were enrolled in this study, and (10) samples from normal healthy individuals were used as normal control in this study. Serum specimens were separated from all the samples.

Serology

Serum specimens were stored at -20°C until tested for HEV IgM Antibody using commercially available Micro-ELISA for markers of hepatitis E (HEV IgM, Foresight, USA). This assay is based on synthetic immunodominant antigens derived from ORF2 and ORF3. Specimens were tested according to the manufacturer's instruction, those with absorbance (S) value less than cut-off (CO) value were considered negative, whereas those with absorbance (S) value greater than or equal to (CO) value were considered positive; thereafter, they were re-tested in duplicate to confirm the result at central public health laboratories (CPHL) in Baghdad province.

Biochemical Tests

Sera of positive HEV IgM Antibodies also were tested for total serum bilirubin (TSB), serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST), and serum alkaline phosphatase (ALP) by using a colorimetric method according to the guidelines mentioned in the leaflet supplied by the manufacturer (BioMerieux, France).

Statistical Analysis

Chi-square test was used to significant comparison between percentage, and Least significant difference (LSD) test was used to significant compare between means in this study. The statistical analysis were performed using The statistical analysis system- SAS (2012) program. Values were regarded as significantly different at the $p < 0.05$ level [17].

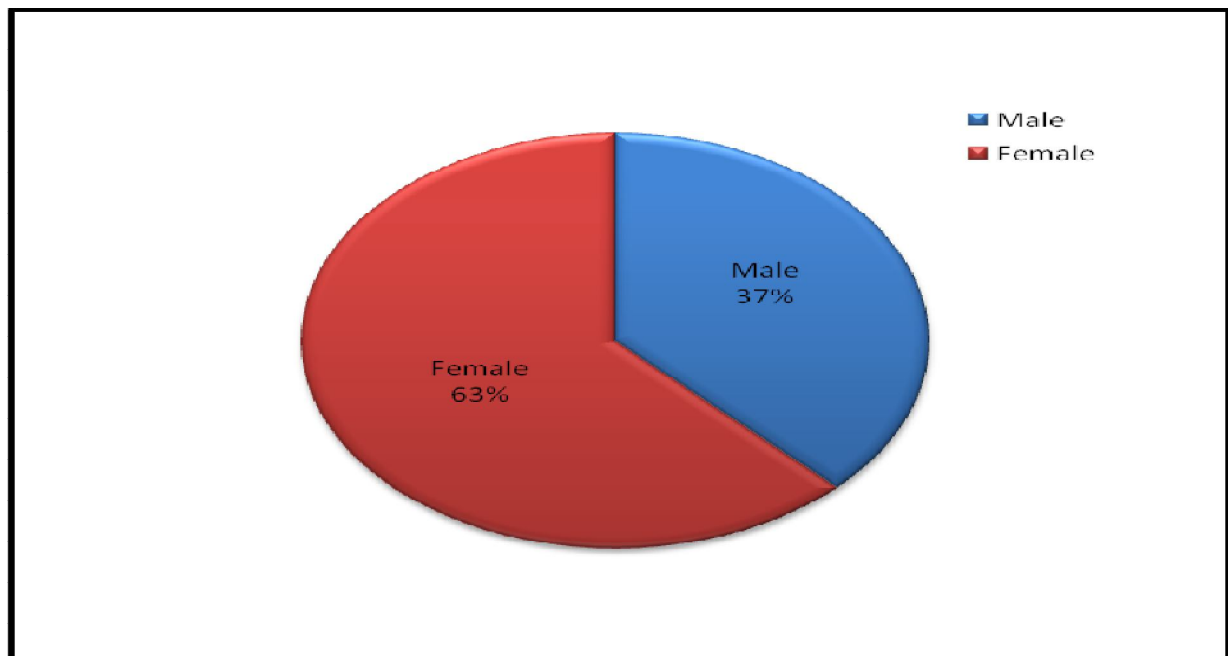
Results and discussion

Out of (270) patients, (72) patients (26.66 %) (ranged between 4-74 years old) marked with appearance of anti-HEV IgM antibodies, and all these patients were tested for confirmatory test, these patients consist of (45) females and (27) males were all negative for routinely screened markers of Hepatitis A, Hepatitis B and Hepatitis C. This study demonstrates that the age groups of patients with HEV infection showed a wide spread and it is more common among younger age group (15-24), with a percentage of 41.67% (table 1).

Table 1- Distribution of HEV patients according to age groups

Age groups (years)	No.	Percentage (%)
4-14	11	15.28
15-24	30	41.67
25-34	20	27.77
35-44	8	11.11
45-54	1	1.39
55-64	1	1.39
65-74	1	1.39
Chi-square (χ^2)	---	10.271 **
** (P<0.01).		

The statistical results showed Highly significant differences ($\chi^2 = 10.271$, $p \leq 0.01$) among age groups. This result coincided with Chandra *et al.* (2012) in India who found the most common HEV infection among younger age group [18]. Due to that this age group are the most contact with the environment and more tendencies to eat and drink at the outside of the home. The prevalence of HEV-IgM Antibodies between patient groups from AL-Muthanna province in Iraq was higher in females (63%) than in males (37%)(figure-1).

**Figure 1-** Distribution of HEV patients according to gender

Suggesting that exposure to HEV might be more frequent in females than males. This result coincided with Teshaleet *al.* (2010) in Ugandawho reported that the number of symptomatic cases was higher for women (28%) than for men (22%; $p < 0.001$) [19].Ghezeldashtet *al.* (2013) in Iran reported that 13.2% (95/718) of males and 15.0% (130/864) of female were HEV positive [20]. This was probably due to poor nutrition and pregnancy converged, and work up the energy. The influence of each of these problems in public health for women, and strain the body becomes more susceptible to the disease. Elevated levels of (TSB), (ALT), (AST), and (ALP) (table 2) show the significant differences (mean \pm SE, $P \leq 0.05$) between patients and healthy controls.

Table 2- A comparison between HEV patients and healthy control for TSB, GOT, GPT & ALP tests

Group	No.	Mean \pm SE			
		TSB Mg/dl	GOT U/l	GPT U/l	ALP U/l
Patients	72	23.41 \pm 12.38	887.17 \pm 9.87	859.91 \pm 13.28	206.56 \pm 5.04
Healthy	10	0.884 \pm 0.05	37.27 \pm 4.58	34.14 \pm 7.63	48.09 \pm 15.42
LSD value	---	4.706 *	28.661 *	25.782 *	64.935 *
* ($P \leq 0.05$).					

The increase in bilirubin levels reflect the deficiencies in bilirubin metabolism caused by viral hepatitis. Several studies suggested that elevated serum (ALT), (AST), and (ALP) may be marker of HEV infection [18]. This result coincided with Hoofnagleet *al.* (2012), who found that the symptomatic phase coincides with elevated hepatic aminotransferase levels [21]. Teshaleet *al.* (2010) in Uganda reported that markedly elevated levels of liver enzymes in HEV patients [19]. Lhommeet *al.* (2012) in France reported that elevated levels of ALT, AST and ALP in HEV patients [22]. From this study it is concluded that the HEV is highly endemic in Al-Muthanna province /Iraq. The reasons for the high HEV prevalence in this population are uncertain but may be due, at least partially, to the contaminated water with virus and to the culinary culture of the local community. Thorough cooking of meat would help minimize the risk for HEV infection and could form part of a public health initiative in this area. This may imply that HEV can be transmitted from one person to another nearby through a certain route, such as faecal contamination and people movement, this may be caused by non-viral factors, such as sanitary conditions, hosts, facilities. Increased migration, tourism, and international trade, particularly of food products, may all contribute to the spread of HEV into new regions.

Conclusions

HEV is highly endemic in Al-Muthanna province /Iraq. Elevated levels of liver function enzymes in patients with acute hepatitis E were found to be higher than in healthy control which emphasizes their important role in hepatitis E patients.

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