Seroprevalence of Hepatitis E virus in human and animals in Southwestern Saudi Arabia

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Abstract: The seroprevalence of hepatitis E virus (HEV) infection in Najran, a rural community's province in the southwestern of Saudi Arabia was investigated. Blood samples from 1080 person (360 hepatic patients and 720 apparently healthy volunteers) were collected from October 2011 to March 2012. Serum samples were tested for anti-HEV IgG antibodies by enzyme linked immunosorbent assay (ELISA) method and anti-HEV IgM antibodies by rapid diagnostic strips. Anti-HEV IgG antibodies were detected in a total of 88 cases out of 360 hepatic samples with percent of 25% while anti-HEV IgM antibodies were detected in 9 cases with percent of 2.5 %. None of the apparently healthy volunteers were positive for HEV. To identify the possible source of infection, 630 and 540 blood samples from sheep and chicken were tested for presence of IgG antibodies against HEV. All sheep and chicken samples were negative for HEV.


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Key words: HEV, blood samples, Najran, ELISA

1. Introduction

Hepatitis E is a common cause of acute hepatitis in areas with poor sanitation (Aggarwal and Naik, 2009). Hepatitis E virus is a spherical, non-enveloped with an icosahedral symmetry virus belongs to the Herpesviridae family. Viral particles are approximately 32 to 34 nm in diameter. HEV is a single-strand, positive-sense RNA virus with at least four known main genotypes of mammalian HEV and one avian HEV. HEV genotypes 1 and 2 are found exclusively in humans while genotypes 3 and 4 are found both in humans and other mammals (Purcell, 1996; Xing et al., 1999; Purcell and Emerson, 2008). Avian isolates of HEV are genetically distinct with a shorter (6.6 Kb) genome and only about 50% sequence homology with the mammalian isolates. The avian HEV constitute a fifth HEV genotype, these isolates are now considered as belonging to a separate genus (Huang et al., 2002). Hepatitis E is transmitted by the fecal-oral route, usually via the consumption of contaminated water or food, human-to-human transmission of HEV is rare. Incubation time ranges from 2 weeks to 2 months with an average of 40 days. From a clinical point of view, hepatitis E is acute self-limiting and symptomatic disease that varies in severity from subclinical to fulminant. The mortality rate associated with HEV infection is 1 to 4% and can reach up to 20% during pregnancy (Purcell, 1996; Emerson and Purcell 2003; Purcell and Emerson, 2008). The acute hepatitis E virus has the highest attack rates in young adults and the disease is particularly severe among pregnant women. HEV superinfection can occur among persons with pre-existing chronic liver disease. In recent years, an increasing number of cases, mostly due to genotype 3 or 4 HEV, have been recognized (Aggarwal and Naik, 2009; Aggarwal and Jameel, 2011).

HEV is unique among the known hepatitis viruses, in which it has an animal reservoir. Domestic pigs and wild boars are the main animal reservoir for the genotypes 3 and 4 strains of HEV worldwide. Besides pigs, anti-HEV antibodies have also been detected in many other animal species including deer, rats, dogs, cats, mongooses, cows, sheep, goats, avian species, rabbits and horses (Saad et al., 2007; Meng, 2009; Reuter et al., 2009; Pavio et al., 2010). Zoonotic transmission of hepatitis E raises an important public health concern over food safety and zoonotic risk. Several lines of evidence indicate that, in some cases involving HEV genotypes 3 and 4, animal to human transmissions occur. Individuals with direct contact with animals are at higher risk of HEV infection (Tien et al., 1997; Favorov et al., 1998; Meng 2009). Cross-species infections with HEV genotypes 3 and 4 have been demonstrated experimentally (Mancera et al., 1996; Goens and Perdue 2004; Lu et al., 2006). Epidemics of hepatitis E have been reported primarily in developing regions of Africa, the Middle East, and Southeast and Central Asia; one epidemic occurred in North America (Mexico) (Lu et al., 2006; Purcell and Emerson 2008). Several studies have been done on HEV in different regions in Saudi Arabia such as Jeddah, Riyadh and Giza (Arif et al., 1994; Arif 1996; Arif et al., 1996; Al-Knawy et al., 1997; Abdelaal et al., 1998; Ayola et al 2001) but no work has been done in

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Najran. Here, this study was carried out to evaluate anti-HEV seroprevalence in Najran Province.

2. Material and Methods

A cross-sectional study was carried out to determine the seroprevalence of HEV infection in hepatic and non hepatic persons in Najran. The individuals were informed in detail about the research and the protocol was approved by the Institutional Research Ethics Committee of Najran University.

Blood samples:

Blood samples from a total of 1080 persons (844 males and 236 females), over the age of 20 years with a mean age of 38.4 years, were evaluated. Of these, 360 had chronic hepatitis and 720 were apparently healthy volunteer. The most common causes of chronic liver disease were hepatitis B virus (HBV) infection present in (58.3%), hepatitis C virus (HCV) infection present in (33.4%), and HCV/HBV co-infection present in (8.3%) of the persons. A total of 630 and 540 blood samples were collected from sheep and chicken from Najran abattoirs. All sera (human, sheep and chicken) were separated and stored at -20 °C until used.

Serological assays for anti-HEV IgG:

All human, sheep and chicken sera samples were tested for specific anti-HEV IgG antibodies by ELISA, according to the method described by the manufacturer, using commercially available reagents (HEV ELISA, DIAGNOSTIC AUTAMATION, INC, CA, USA). According to the manufacturer, this assay presents 99.8% sensitivity and 99.8% specificity. The results were scored as positive or negative according to standard procedures recommended by the manufacturers. The individuals were considered to be seropositive when they showed two repeated positive reactions. Positive and negative controls were included in all the ELISA microplates assayed.

Serological assays for anti-HEV IgM:

All human, sheep and chicken sera samples were tested by rapid diagnostic strips for detection of anti-HEV IgM (Blue Cross Bio-Medical (Beijing) Co., Ltd.) according to the manufacturer instruction.

Table 1: Characteristics of 1080 persons evaluated in study

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No.</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>844</td>
<td>78.9</td>
</tr>
<tr>
<td>Female</td>
<td>236</td>
<td>21.1</td>
</tr>
<tr>
<td>Chronic liver disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>360</td>
<td>33.33</td>
</tr>
<tr>
<td>No</td>
<td>720</td>
<td>66.66</td>
</tr>
<tr>
<td>Hepatitis infection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HBV</td>
<td>210</td>
<td>58.3</td>
</tr>
<tr>
<td>HCV</td>
<td>120</td>
<td>33.4</td>
</tr>
<tr>
<td>HBV/HCV</td>
<td>30</td>
<td>8.3</td>
</tr>
</tbody>
</table>

Statistical analysis

The statistical analysis was performed using the Chi-square (x2) test. The level of significance adopted for all tests was 5% (p < 0.05).

3. Results

Seroprevalence of HEV in human and sheep samples:

The results showed that anti-HEV IgG and anti-HEV IgM were detected in patients suffering from hepatitis only. No positive HEV samples were detected in apparently healthy volunteers. The overall anti-HEV IgG seroprevalence rate was (88 out of 1080) with percent of 8.1% and anti-HEV IgM seroprevalence rate was (9 out of 1080) with percent of 0.8%. In 360 samples of hepatic patients, 88 developed HEV IgG antibodies and 9 developed HEV IgM antibodies, therefore, the prevalence of IgG and IgM HEV antibody in this group was 25% and 2.5%, respectively. Of these positive sera samples, 21 out of 30 from patients coinfected with HBV/HCV with percent of 66%, 30 out of 120 from patient infected with HCV with percent of 25% and 37 out of 210 from patient infected with hepatitis B with percent of 17.6%. There was no significant difference in HEV seropositivity between the subjects grouped according to gender or age (p > 0.05). However, the infection rate was always higher in male than female and the infection rate was higher in group aged from 30 to 40 years old than other two groups. All sheep and chicken samples were negative for HEV. The prevalence of HEV IgG seropositivity is showed in Tables 2 and 3.

4. Discussion:

This study was carried out to determine the seroprevalence of HEV in Najran, and to evaluate whether the rate of seroprevalence of IgG anti-HEV antibodies is associated with sociodemographic variables and with seropositivity for hepatitis B, C virus infection. The region was chosen for the study because Najran is considered rural, based on sewage disposal and water sanitation systems in this area. Previous studies indicated that tests based upon open reading frame (ORF) 3 of HEV are of limited value for seroepidemiologic studies, whereas ORF2-based antigens have broad utility and yield data that are reproducible in more than one laboratory (Ghabrah et
al., 1998). So, ELISA method based on antigens derived from open reading frame (ORF2) was used in our study.

Table 2: Prevalence of hepatitis E virus IgG seropositivity

<table>
<thead>
<tr>
<th>Age (Year)</th>
<th>Male NO</th>
<th>%</th>
<th>Female NO</th>
<th>%</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 30</td>
<td>9/150</td>
<td>(6%)</td>
<td>2/48</td>
<td>(4.1%)</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>30 - 39</td>
<td>38/388</td>
<td>(9.8%)</td>
<td>5/86</td>
<td>(7.3%)</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>≥ 40</td>
<td>21/306</td>
<td>(6.9%)</td>
<td>5/102</td>
<td>(4.9%)</td>
<td>&gt; 0.05</td>
</tr>
</tbody>
</table>

Table 3: Relationship between hepatitis E virus infection and hepatitis B virus, hepatitis C virus or co-infection HBC/HCV

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>HEV positive IgG</th>
<th>Percentage</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBV antibody positive</td>
<td>37/210</td>
<td>17.6%</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>HCV antibody positive</td>
<td>30/120</td>
<td>25%</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>HCV and HBV positive</td>
<td>21/30</td>
<td>66%</td>
<td>&gt; 0.05</td>
</tr>
</tbody>
</table>

The HEV overall seropositivity rate (8.1%) detected in Najran was lower than the average of other rural areas in Saudi Arabia as Gizan areas (14.9%) and was similar to the mean rates of exposure HEV in urban area as Riyadh 9.1% (Arif et al., 1994; Arif 1996; Al-Knaawy et al., 1997; Abdelaal et al., 1998). Our results showed that the infection rate was always higher in male than female and in patient's ages 30-40 years than other ages. Males are at higher risk of acquiring the infection than females and this is probably because of social habits rather than genetic factors. However, the difference in infection rate according to age or gender was insignificant. Our results were consistent with others (Ayoola et al., 2001) who mentioned that HEV seropositivity did not significantly affected with age or gender. Our results were inconsistent with others who mentioned that HEV seropositivity was significantly affected with age or gender (Fix et al., 2000; Pavio et al., 2010; Cheng et al., 2012; Houcine et al., 2012). Significant association was observed between HEV seropositivity and HBV and HCV. It seems that HEV highly infect the patients infected with HBV, HCV or both viruses. However, the reasons for these results need to be investigated. Similar results were mentioned by others who mentioned that a significant association between anti-HEV and anti-HCV with donors who were positive to anti-HCV having about 5 times the risk of HEV than those who were anti-HCV negative (Abdelaal et al., 1998).

Contact with animals and living in a rural habitat were the main risk factors for transmission of the disease (Eker et al., 2009; Favorov et al., 1998; Meng 2009). In this study the virus was not detected in the sheep or chicken samples which may refers to other possible sources of infection. The contaminated water due to sewage system in Najran could be the possible source of infection. However, there was no statistically significant difference in anti-HEV prevalence when the presence of a sewage system and the source of water were taken into consideration (Cheng et al., 2012).

Our study has limitations that should be noted. As is the case in many retrospective analyses, we were unable to collect all risk factors and characteristics of persons in the study. To our knowledge, the present study is the first seroepidemiological study on HEV infection in this unique population. The study showed that this virus is prevailed among the population and point out the need of further studies to define the clinical and epidemiological importance of HEV infection and to identify the risk factors involved in the epidemiology and pathogenesis of this infection.

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