ORIGINAL ARTICLE

Anti Hepatitis E virus seropositivity in a group of male blood donors in Makkah, Saudi Arabia

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Abstract

Objectives: To determine the seropositivity of Immunoglobulin-G and Immunoglobulin-M to hepatitis E virus in male blood donors in Makkah, Saudi Arabia.

Methods: The study was carried out from March to August 2009, in which 900 blood samples were collected from 4 different hospital blood banks in Makkah City: AL-Noor Hospital, Central Blood Bank, Maternity and Children Hospital, and Herra Hospital. All the samples were tested for Immunoglobulin-G and Immunoglobulin-M antibodies specific to hepatitis E virus using the enzyme-linked immunosorbent assay test.

Results: Hepatitis E virus-specific Immunoglobulin-G antibodies were detected in 168/900 (18.7%), and IgM in 39/900 (4.3%) samples. Prevalence of the former was found to be higher in non-Saudi donors. In addition, its prevalence increased with age. Moreover, its prevalence was found to be higher in uneducated donors and in donors who drank well-water.

Conclusion: Exposure to hepatitis E virus among blood donors in Makkah City was high in comparison to the neighbouring areas in the region. Further studies are warranted to determine the true seroprevalence of the virus in the society at large.

Keywords: Makkah, Saudi Arabia, Blood donors, HEV, ELISA IgG, IgM. (JPMA 63: 185; 2013)

Introduction

Hepatitis E is an important public health concern in many developing countries of Asia and Africa where environmental sanitation facilities are poor.[1] In addition, a high incidence of sporadic hepatitis E has been observed in several countries in which outbreaks have not been reported.[2] In non-endemic areas, travellers to endemic regions are at major risk of hepatitis E virus (HEV) infection, but sporadic cases of acute hepatitis E without an implicated travel history have also been reported in Europe and the United States.[3] HEV is an unclassified non-enveloped virus which is transmitted by the faecal-oral route. HEV infection generally causes self-limited acute hepatitis associated with high mortality in pregnant woman where the mortality rate can be as high as 20%.[4] Although transmission of HEV is generally via the faecal-oral route, person-to-person transmission, and transmission via the parenteral route or blood transfusion, has also been suggested.[5]

The gold standard for diagnosis of HEV infection is by the detection of virus-like particles in faecal specimens by immuno-electron microscopy (IEM). Although IEM is a superior technique for specificity, the sensitivity of the assay is insufficient for routine analysis. IEM is difficult to perform and most clinical specimens do not contain sufficient virus-like particles to be detected. Enzyme-linked immunosorbent assay (ELISA) is the preferred screening format for large-scale sero-surveys. The assay is formatted for both Immunoglobulin-G and M (IgG and IgM) detection and is useful in diagnosing acute and past HEV infection.[6] The specificity of the HEV IgG ELISA is 99%; the specificity of the IgM ELISA is 97%.

Infection with HEV may produce asymptomatic to clinical disease with varying degrees of severity. Hepatitis E has been one of the common types of adult's acute hepatitis in the hyper-endemic parts of Asia, and an important human pathogen in the Central and Southeast Asia, Middle East, and Central and North Africa.[7] Anti-HEV seroprevalence of 1%-5% in blood donors has been reported from several countries where HEV is not endemic using commercially available recombinant protein-based tests.[8] It has been reported that a substantial proportion of blood donors (1.5%) were positive for HEV ribonucleic acid (RNA), and viraemic blood donors are potentially able to cause transfusion-associated hepatitis E in areas of high endemicity.[9] Thus the importance of HEV in endemic areas cannot easily be estimated, and must be measured specifically.

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Most studies have looked into the prevalence of HEV infection in patients with hepatitis or in selected settings.[10] Community-based surveys are limited, and information on HEV infection in populations is scant. To our knowledge, only a few epidemiological reports on the prevalence of hepatitis E antibodies in Saudi blood donors have been published,[11] but no data exists on the prevalence in the community. This study, therefore, aimed at assessing anti-HEV prevalence in the city of Makkah, Saudi Arabia. The possible contribution of socio-demographic factors on this prevalence was also examined.

Subjects and Methods

The population-based prevalence study was carried out from March to August 2009, during which 900 blood samples were collected from 900 blood donors >18 years, selected by simple randomisation method, from 4 different hospital blood banks in Makkah city: AL-Noor Hospital, Central Blood Bank, Maternity and Children Hospital and Herra Hospital. The sample size of a minimum of 665 blood donors was needed to meet the objectives of the study, with a 99% confidence interval, 5% error rate and around 1.6million population of the city.

Informed consent was obtained from each individual before inclusion in the study. Every subject had been informed about the procedure before the blood sample was taken, making sure that he understood the procedure to be carried out. A 10ml sample of blood was collected from each donor after informed consent was obtained. All samples after clotting were transported immediately to the Virology Research Laboratory at the Faculty of Medicine of Umm Al-Qura University in cooler bags with ice packs. On arrival at the laboratory, all samples were centrifuged and the serum was separated, aliquoted into two Eppendorf tubes and stored at -20°C and -80°C until they were tested. The ELISA test (Bioelisa, Barcelona, Spain) for IgG and IgM antibodies was carried out according to the manufacturer's instructions in the package insert.

The results were statistically analysed by calculating the mean, median, mode, standard deviation, range and p value, and distributed according to age, nationality and gender differences using SPSS version 20. P values were calculated using Fisher Exact test (GraphPad Instat programme), and were considered significant if less than 0.05.

Results

The age range of the donors was from 18 to 66 years, with a mean age of 30±7.8 years, median age of 29 years, and mode age of 30 years.

Frequency of HEV-specific-IgG antibodies detected in 900 blood donors was 168 (18.7%), and 39 (4.3%) in case of IgM antibodies (Table-1). With regards to age, 102/534 (19%) in the age group 24-35 years were positive for IgG antibodies, while 25/488 (5%) were positive with the IgM ELISA test (Table-2). Of the 900

Table-1: Prevalence of the HEV specific IgG and IgM antibodies.

Class of Immunoglobulin	Number of Positive Samples (%)	Number of Negative Samples (%)	Total Number of Samples tested
IgG	168 (18.7%)	732 (81.3%)	900
IgM	39 (4.3%)	861 (95.7%)	900

Table-2: Distribution of the prevalence of the HEV-specific IgG antibody.

Age (years)	Number of Positive Samples (%)	Number of Negative Samples (%)	Number of Samples tested
18 - 23	20 (11.49 %)	154 (88.51 %)	174
24 - 29	54 (19.01%)	230 (80.99%)	284
30 - 35	48 (19.20%)	202 (80.80%)	250
36 - 41	25 (23.80%)	80 (76.20%)	105
42 - 47	11 (22.00%)	39 (78.00%)	50
48 - 53	7 (27.33%)	23 (72.67%)	30
54 - 59	3 (50%)	3 (50%)	6
60 - 66	0 (0%)	1 (100%)	1
Total	168 (18.67%)	732 (81.33%)	900

HEV: Hepatitis E virus. IgG: Immunoglobulin G.

Table-3: Distribution of the Prevalence of the HEV-specific IgG and IgM antibodies according to nationality differences.

n = 900	Elisa Test			
	IgG		IgM	
Nationality	Positive	Negative	Positive	Negative
Saudi (514)	78 (15.2%)	436 (84.8%)	22 (4.3%)	492(95.7%)
Non-Saudi (386)	90 (23.3%)	296 (76.7%)	17(4.4%)	369(95.65%)

HEV: Hepatitis E virus. IgG: Immunoglobulin G. IgM: Immunoglobulin M.

blood donors, 78/514 (15.18%) of the Saudi nationals were IgG positive, while 90/386 (23.32%) of non-Saudis were positive for IgG antibodies. This was very significant (p= 0.0024). With regard to IgM antibodies, 22/514 (4.3%) of Saudi donors were positive, while 17/386 (4.4%) of non-Saudi donors were positive. However, there was no significant difference between the two groups of patients with regard to IgM positivity (p= 1.0) (Table-3).

On analysing the donors with regard to the level of education and ELISA IgG positive results, 300 (33.3%) were uneducated and 158 (17.5%) had received education to high school level and beyond. There was a significant difference between these two groups (p= 0.02). However, there was no significant difference for IgM positivity in the same group of donors (p= 0.4). On analysing results of ELISA IgG and IgM with regards to drinking water habits, only 12.9% of donors who drank Zam-Zam water were IgG positive when compared to those who drank well-water (20.68%). This result was not significant (p= 0.3). With regard to IgM positivity, 5.1% of those who drank Zam-Zam were positive, while 7.4% who drank well-water were positive. This result was also not significant (p= 0.6).

Among the donors' place of residence in the greater Makkah area, 21.3% who lived in the eastern part of Makkah were IgG positive, while 14% in the northern area were IgG positive. The result was significant (p= 0.04). With regard to IgM, 6.1% were IgM positive from the eastern area, and 3.5% positive IgM from the northern part. There was no significant difference between the two groups (p= 0.2).

Discussion

Hepatitis E, previously known as enterically transmitted, enteric, or epidemic hepatitis,[12] is a worldwide public health problem. The viral agent causing the disease is known as the HEV. Cyclic outbreaks have been documented in the tropics of Asia and Africa. In these endemic regions, hepatitis E continues to occur between epidemics in the form of sporadic hepatitis. In the disease-endemic countries of India, China, Sudan, Somalia and Mexico, HEV is implicated in causing more than 50% of the sporadic cases of acute viral hepatitis. HEV has also been shown to be the causative agent for sporadic acute hepatitis in parts of South America and Europe. HEV is not a prevalent infectious agent in the United States and Western Europe, though the presence of the virus in these countries has been firmly established in 'imported cases' among travellers to HEV-endemic regions.[13]

Documented direct evidence for transfusion-transmitted HEV infection has been reported in several countries.[14] Indirect evidence implicating HEV as a potential transfusion risk has been reported by many investigators worldwide. These include a high prevalence of anti-HEV IgG among volunteer blood donors in industrialised countries, indicating past sub-clinical infection.[15] Detection of HEV-RNA in a significantly larger number of multi-transfused patients compared to controls[16] and identification of HEV viraemic subjects among symptom-free blood donors with elevated alanine transminase (ALT) levels, as well as the demonstration of ongoing sub-clinical infection of HEV in 0.1-3% of blood donors.[17]

The prevalence of anti-HEV IgG antibodies among our blood donors was 18.7%, which is almost similar to a previous study done in Jeddah (16.4%),[18] but higher than figures reported from developed countries (0.4% to 3.9%),[19] although lower than those from other countries of the Eastern Mediterranean Region where reports of up to 52% seroprevalence for anti-HEV have been reported.[20] Regarding anti-HEV IgM antibodies, 4.3% of our donors were positive. Others have reported prevalence rates of 0.94% in China to 3.6% in Hong Kong.[21] However, the presence of anti-HEV is not a measure of infectivity, and no tests are available that would be appropriate for hepatitis E. Even the use of IgM anti-HEV EIA kits has its limitations. It has been reported that antibody responses are detected in only 21% of patients in whom viraemia or faecal shedding (or both) were detected.[22] This suggests that some persons do not produce an IgM antibody response to infection with HEV. Although the HEV-specific polymerase chain reaction test is sensitive and specific, screening of the blood supply for HEV infection would not be cost-effective. We found the greatest number of positive individuals for IgG antibodies in the age group 24-35 years, followed by a decline in the older age groups. This is in keeping with one study which reported similar findings, but in contrast to another in

which seroprevalence increased significantly with age.[23,24]

With regard to nationality, 15.18% of Saudi blood donors were IgG positive, while 23.32% of non-Saudi donors were positive for IgG antibodies. This was a very significant difference between the two groups (p= 0.002). With IgM positivity there was no significant difference between the two groups (p= 1.0). Uneducated individuals were at higher risk for infection than educated people. This is similar to other countries in which education level is associated with risk for HEV infection. In outbreak settings, HEV transmission is most often associated with faecally contaminated drinking water. However, risk factors for infection among sporadic cases of hepatitis E in both HEV-endemic and non-endemic regions have not been determined or clearly defined. In our study there was a significant difference in the prevalence of HEV positivity between those who drank Zam-Zam water when compared to well-water.

In terms of area of residence, 21.3% of the donors who lived in the eastern part of Makkah were positive for IgG antibodies to HEV, while 14% were positive who lived in the northern part (p= 0.04). There was no difference in IgM seropositivity in donors between the two areas of residence (p= 0.2).

HEV causes epidemics, especially in developing countries where hygiene is poor and many affected pregnant women suffer from fulminant hepatitis.[25] Healthy blood donors in the pre-icteric phase of the disease and presumably infectious, could transmit HEV to recipients of their blood. The importance of HEV in relation to blood transfusion practices stems from the possibility that there is evidence that the virus could be transmitted parenterally. The blood products that theoretically carry the risk of transmission of HEV are packed red cells, whole blood or platelet concentrate collected from an asymptomatic donor during the viraemic phase. Studies on HEV sero-epidemiology in many parts of the world have found conflicting results. It is not known why the overall seroprevalence of anti-HEV in normal populations of endemic areas is low or why a low but constant presence of anti-HEV is observed in normal human populations of non-endemic industrialised countries.[26]

Regarding seroprevalence studies on HEV infection, the degree of HEV excretion by infected people is not very high, and this can limit the transmission and distribution of infection in the community. The duration of anti-HEV seropositivity in not well-known. Therefore, previously infected people might be ignored. However, several studies denote duration of months to years. Sensitivity and specificity of the available screening tests may be variable. Therefore, it is imperative to select tests with good sensitivity, specificity and accuracy in carrying out seroprevalence studies.[27]

Conclusion

The results emphasise the need to initiate more studies on the prevalence of HEV in other parts of Saudi Arabia. Besides, they also highlight the dangers of using blood donor data in lieu of epidemiologically sound, population-based observations.

Acknowledgments

We are grateful to the Medical Research Centre of the Institute of Scientific Research and Islamic Heritage Revival of Umm Al-Qura University, Makkah, Saudi Arabia, for funding the project. We would also like to thank Mr. Abdullah Qadamany, Mr. Muhammed Al-Zahrany, Mr. Muhammed Al-Taezi and Mr. Nwaf Bugary for their assistance.

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