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Seroprevalence of hepatitis e among food handlers in central Khartoum Sudan

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Abstract
Background and Objectives: Hepatitis E virus (HEV) causes acute viral hepatitis. Majority of the documented studies on hepatitis E virus have been focused on the incidence of this disease in Khartoum. Limited data are available on HEV infection among food handlers cases in central Khartoum. The present study was undertaken to investigate the contribution of HEV infection in food handlers cases in Khartoum state.
Methods: Two hundred workers suspected to have viral hepatitis were screened for HEV anti- IgM by using commercial Enzyme-Linked Immuno-Sorbent Assay (ELISA) to detect the virus kit with high sensitivity and specify.
Results: The samples that collected were from males and females that worked in restaurants of Khartoum state to detection HEV among food handlers. Hepatitis E virus was found to be the major cause HEV among food handlers (15.5%) in this region was in Khartoum state, from 200 samples. 81samples were females and 119 samples were males, with ages range between 15 to 53 years.
Conclusion: It is important to enterically transmitted for HEV among food handlers. There is a need to additional serological tests to identify the etiological agent in the cases of hepatitis.

Keywords: HEV, Hepatitis, Khartoum, food handlers, ELISA

Introduction

Hepatitis E virus (HEV), the causative agent of hepatitis E in humans, is an important public health disease in many parts of the World [1–4].

Hepatitis E Virus (HEV) belongs to the genus Hepevirus in the family Hepeviridae and consists of four recognized genotypes and at least two putative new genotypes [5]. HEV is non-enveloped, positive sense, single-stranded ribonucleic acid (RNA) virus, is recognized as the principal cause of enterically transmitted, non-A, non-B (NANB) hepatitis, which occurs worldwide although rarely in industrial countries [6]. There is also a possibility of zoonotic transmission of the virus. Hepatitis E is now a recognized zoonotic disease with swine and likely other animals serving as the reservoir for human infections [1, 7]. Food safety associated with HEV contamination is an important public health concern with the recent identification of infectious HEV in meat and meat products and resultant sporadic cases of foodborne hepatitis E in the human population [3, 8–11]. HEV causes an acute icteric disease that varies in symptoms from subclinical to fulminant hepatitis [4].

The asymptomatic patient typically clears the virus rapidly, while the symptomatic patient experiences clinical signs including anorexia, hepatomegaly, myalgia, jaundice and sometimes abdominal discomfort, nausea, vomiting, and fever [5, 12].

In immune compromised patients such as organ transplant recipients, lymphoma and leukemia patients, or patients with HIV infection, the course of disease may progress to a chronic state with cirrhosis of the liver and persistence of viral shedding [13–17]. Of particular concern is the ability for HEV-infected immune compromised individuals to develop clinical disease well after the initial exposure [15–18]. Currently, chronic HEV infection in immune compromised individuals is an emerging and significant clinical problem. Future studies are warranted to identify the immunological correlates and host factors leading to chronicity.

HEV is considered hyperendemic in many developing countries such as India, Bangladesh, Egypt, Mexico, and China. Hyperendemic countries carry an HEV prevalence of 25% of all non-A, non-B, acute hepatitis cases or have experienced a major waterborne outbreak of hepatitis E according to the Centers for Disease Control and Prevention [19]. HEV is considered endemic where there is a prevalence of less than 25% of all reported non-A, non-B acute hepatitis [19]. Endemic countries include much of Western Europe, the United States, New Zealand, many countries in South America, much of Asia, and the Middle East [19–21]. Trends throughout the World point to continued high anti-HEV seroprevalence and HEV infection likely due to increases in interest, awareness and surveillance efforts as well as increased spread among known animal reservoirs and hosts [20, 19–24]. Seroprevalence reports vary dramatically from country to country and study to study with some studies reporting overall declines in seroprevalence over time, while other yield continued high levels of seroprevalence [24, 26, 27].

Prevalence of anti-HEV IgG tends to increase with age, especially in men [24, 28–32]. Humans and other animals excrete a considerable amount of virus early in the acute phase of HEV infection and likely contribute to maintain the cycle of endemicity [25]. The lack of a standardized serological assay further complicated the interpretation of the sero-epidemiological data. Therefore, development of a FDA-approved diagnostic assay for HEV should be a priority in the future.

HEV is recognized as a common source of waterborne outbreaks, involving fecally contaminated water [33]. In June 2004, a large hepatitis E outbreak occurred in western Darfur, Sudan. A total of 2621 cases were reported between 26 June and 31 December 2004 in Mornay Internally Displaced Persons Camp (78,800 inhabitants) [34].

The epidemiological investigation suggested an increased risk of HEV infection with drinking water from chlorinated sources [35].

Materials and Methods

Blood Samples

Tow hundred (200) blood samples were collected from Khartoum State; the samples were collected during month of February to April 2015. The samples were collected from both males and females work as food handlers in Khartoum's restaurants.

Data about food handlers including (gender, age, resident and work site) also collected, and then a 5 ml venous blood sample was drawn from each subject and centrifuged at 2000 rpm for 5 minutes. Serum was separated, and a 1 ml serum sample was stored at –80°C until used. The serum samples were thawed and tested for anti-HEV IgM by commercial ELISA test (Globe Diagnostics, Milan, Italy). All positive samples were confirmed with a second assay, using the same ELISA test.

This kit is two-steps incubation, solid phase antibody capture ELISA assay in which polystyrene micro-well strips are pre-coated with antibodies directed to human immunoglobulin M proteins (anti-μ chain).The patient's serum/plasma sample is added and during the first incubation step, any IgM-class antibodies will be capture in the wells. After washing out all the other components of the sample and in particular IgG-class antibodies, the specific HEV IgM captured on the solid phase is detected by the adding recombinant HEV ORF2 antigens conjugated to horseradish peroxidase (HRP-Conjugate).During the second incubation, the HRP-Conjugated antigens will specifically react only HEV IgM antibodies and after washing to remove the unbound HRP-Conjugate, Chromogen solution are added into the wells. In presence of (anti-μ) – (anti-HEV-IgM) – (HEV Ag-HRP) immunocomplex, the colorless Chromogens are hydrolyzed by the bound HRP-Conjugate to blue-colored product. The blue color turns yellow after stopping the reaction with sulfuric acid. The amount of antibody captured in the wells, and to the sample respectively. Wells containing samples negative for HEV IgM remain colorless.

Detection of anti-HEV IgM by enzyme-linkedimmunosorbent assay (ELISA)

All serum samples were tested for anti-HEV IgM using commercially available enzyme-linkedimmunosorbent assay (ELISA) kits according to the protocols provided by the manufacturer (MP Diagnostics, Singapore, previously Gene-labs Diagnostics). Briefly, samples were diluted 1/21 in supplied diluent buffer (Tris-base saline containing goat sera and bovine serum Albumin) and 200 μl of the diluted samples were added to the corresponding wells of the ELISA plate coated with HEV antigens. Positive and negative controls were added to the corresponding wells. The plates were incubated at 37C. After 30minutes, the plates were washed 5 times with washing buffer (PBS + 0.1% Tween 20) and 100 μl of mouse monoclonal anti-human IgM labeled with horseradish peroxidase conjugate at the recommended concentrations was added and the plates were incubated at 37C. After 30 minutes, the plates were washed 5times with washing buffer and 100 μl of tetramethyl benzidine (TMB) substrate solution was added to each well and incubated at room temperature. After 10 minutes, the reaction was stopped by adding100 μl of stop solution (1 N HCl) and read with automatic ELISA reader at 450nm absorbance. The test was considered valid if the blank values had an absorbance of ≤ 0.100, and the negative control values had an absorbance ≤ 0.100 and the positive control values had absorbance ≥ 0.500 after subtracting the blank. Additionally, the assay to be valid, the difference between the mean absorbance of the positive controls and the negative controls should be ≥ 0.400.Cut off value was calculated according to the manufacturer's instruction as the average negative control values + 0.4. Signal/Cut off value (S/C) > 1.2was considered positive. To reduce the cost for the study, samples were tested as mini pools. Each pool consists of 20 μl of serum from each of 10 blood donors. Positive pools with the S/C ratio ≥ 3 were then retested as individual samples as described herein.

Statistical analysis

SPSS 10.0 was used for data analysis. The descriptive data are given here as mean ± standard deviation (SD). Logistic regression was used for statistical analysis.

Results

Two hundred samples were tested for HEV anti-IgM among food handlers in Khartoum State. 170(85%) samples were from the Arabian Market in Khartoum, and 30(15%) samples from different restaurant in Khartoum. Two hundred samples, founded 119 (59.5%) samples for males and 81(40.5%) samples for females. Positive samples among males were 20 (10%) and among females were 11 (5.5%).

Table 1. Number of persons and ratio of gender among food handlers in center of Khartoum State.

Gender	Number	%
Female	81	40.5
Male	119	59.5
Total	200	100

Table 2. Distribution of the food handlers according to Age

Age range	Number	Percentage %	Average ± SD
15-25	65	32.5%	20.13±0.23
26-35	81	40.5%	26.24±1.03
36-45	36	18%	36.12±0.81
46-53	18	9%	45.17±3.02
Total	200	100%	

Number of positive sample and ratio of gender among food handlers

Gender	Number	Positive	Percentage %
Female	81	11	13.58
Male	119	20	16.8
Total	200	31	15.5

Discussion

HEV is recognized as a common source of waterborne outbreaks, involving focally contaminated water, cause of enterically transmitted non-A, non-B (NANB) hepatitis, which occurs worldwide. Viral transmission is via fecal-oral rout and rarely through person-to-person transmission. In study in Darfur-Sudan, Hyams 1991 reported 119 patients, 88 (73.9%) with acute HEV, while in this study 200 patients collected from two different health center in Khartoum state, founded 31 (15.5%) with HEV. IgM anti-HEV was done in this study which showed thirty one positive sample (15.5%) among 200 samples from food handlers tested randomly for HEV in Khartoum state.
In Japan Hashimoto 2003 [36]. studied 87 patients samples for also IgM anti-HEV he found that there was 11 (13%) patients positive for IgM ant-HEV.
Arif and Rania, 1996 [37]. in Saudi Arabia were studied 69 patients with acute non-A non-C (NANC) hepatitis, they found that there are seven positive for IgM and IgG anti-HEV, while in this study for only IgM ant-HEV study there were Thirty one positive sample for IgM anti-HEV.
In this study both males and females were tested for HEV anti-IgM, in similar study, El-Gohry 1994 [38]. in Egypt, studied 140 males and females patients with acute sporadic viral hepatitis. He found that Four (2.8%) showed positive for anti-HEV.
Hyams 1992 showed that 39 samples were studied for HEV for both male (498%) and female (36%), the result was the 23 (59%) male positive for HEV anti-IgM, but in the female do not any positive sample. In this study males were (85%) and female were (15%) and both were tested for HEV anti-IgM and contrast the only eighty one positive results were for females (13%) and male (27.5%).
In early 2011 Trico 2006 [39]. in Australia studied serology test for HEV IgM among food handlers and noted positive HEV IgM and negative for IgG for who was diagnosed with HEV among the food handlers. The similar result in this research showed that there were eighty one positive samples for HEV anti-IgM among food handlers in Khartoum state.
Bandoypadhyay 1993 [40]. studies that a focal outbreak of jaundice in Delhi during February-March 1992, 97%) of the population had acute hepatitis E with no age or gender predication, in this study which done during February-April 2015 and 200 samples were tested for HEV and we found that there were thirty one samples positive for HEV anti-IgM among food handlers in Khartoum state.

Conclusion

The present study is the first report on the Seroprevalence of HEV in food handlers in Khartoum. We found a low proportion of IgM of HEV among food handlers pigs suggesting that the risk of HEV transmission to humans in this geographical area was substantial. Hepatitis E is not included as a notifiable disease in the Khartoum, and laboratory testing for acute hepatitis is not routinely performed in the country. Since only pig population from a small geographic area were investigated in the present study, further studies are required to define the genotype distribution in other areas, genetic relationship between HEV strains from swine and human and human health impact of HEV in the Khartoum.

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