

Seroprevalence and Molecular Detection of Hepatitis E Virus (HEV) Among Pregnant Women in Port Sudan State, Sudan

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ABSTRACT

Background: The hepatitis E virus, which occur in epidemic and sporadic form is one of the major public health problems encountered in developing countries Most outbreak are associated with poor personal hygiene and water borne infections. HEV infection in pregnant women is more common and more often fatal in third trimester.

Objective: This cross sectional study aimed to determine the seroreactivity, and molecular HEV RNA positivity among pregnant women in Port Sudan city, Sudan , during the period November December 2015.

Methods: Ninety serum samples were collected from pregnant women in obstetric clinics of Port Sudan . Samples were tested for IgG and IgM antibodies using Enzyme Linked Immunosorbent assay (ELISA) and HEV RNA using Real-time PCR.

Results: The results showed that 95.6% (86/90) of the samples had HEV IgG and 3.3% (3/90) had HEV IgM. HEV RNA was detected in 4.4% (4/90) by using Real time RT-PCR.

Conclusion: The high prevalence of HEV pregnant women in Port Sudan state was documented through detection of HEV-specific antibodies and viral RNA.

Keywords: RNA, RT-PCR, HEV, PCR, IgM, IgG, ELISA, CTK

I. INTRODUCTION

Hepatitis E virus (HEV) is a spherical shape virus, non-enveloped, with single stranded RNA genome^(1,2), that belongs to the Hepevirus genus in the family Hepeviridae⁽³⁾. The viral genome is a positive-sense RNA molecule organized into three open reading frames (ORF1, ORF2, and ORF3). HEV is divided into at least four genotypes, all belonging to a single serotype, which are further divided into a total of 24 subtypes^(4,5). Genotypes 1 and 2 are associated with human illness, while genotype 3 and 4 are animal strains which are occasionally transferred to humans⁽⁶⁾. A large outbreak of hepatitis E virus infection was reported in June 2004 in the internally displaced population camps of Darfur-Western-Sudan⁽⁷⁾. HEV primarily affects young adults and is generally mild⁽⁸⁾, however the mortality rate is higher among pregnant women,

especially during the second and third trimester of pregnancy^(9,10). In Sudan a case fatality ratio of 17.8 % was found during the outbreak in Darfur in 2004, with a higher ratio of 31.1 % among pregnant women⁽¹¹⁾. Prevalence rates of 84.3% and 60% were reported among pregnant women in Egypt and India , respectively^(12,13). Hepatitis E virus cases are mostly sporadic, but also large epidemics , usually transmitted through fecal-oral route due to contaminated food or water can occur⁽¹⁴⁾. Hepatitis E virus is categorized as an acute and self-limiting infection⁽¹⁵⁾, The course of infection has 2 phases, the prodromal phase and the icteric phase⁽¹⁶⁾⁽¹⁷⁾, Furthermore, in healthy individuals during the course of the of the infection (usually several weeks), hepatitis E occasionally develops into an acute, severe liver disease, which is fatal in about 2% of all cases⁽¹⁸⁾. In contrast, none of the other recognized

hepatitis viruses cause such severe hepatitis in pregnancy⁽¹⁹⁾.

Hepatitis E infection during pregnancy and in the third trimester, especially with genotype 1, is associated with more severe infection and might lead to fulminant hepatic failure and maternal death. Many studies on the cause of jaundice and other symptoms of liver disorder, during pregnancy revealed that HEV infection carries a mortality rate of 20- 30% among infected women, primarily those in their third trimester^(20,21)

hepatitis E virus infection is not associated with chronicity, but a fraction of the patients progress to fulminant hepatitis⁽²²⁾ the most severe form of acute hepatitis.

Pregnant women are not routinely screened for HEV antibodies. Here, we reported on the prevalence of hepatitis E in pregnant women Port Sudan in 2016.

II. MATERIAL AND METHOD

Study site

This is a Cross-sectional study, carried out between October and November 2015. The study was conducted on pregnant women who attended obstetrics clinics in Port Sudan City, Sudan.

Samples collection

Five ml blood samples were collected from 90 pregnant women, and centrifuged at 5,000rpm for five minutes. Obtained Sera were stored at -20 until used.

Serology

ELISA for HEV IgG

commercial ELISA kits were purchased from CTK.biotech, (San Diego, USA), the ELISA procedure was performed according to the manufacturers' instructions.

ELISA for IgM

Serum samples were tested for ELISA IgM antibodies using commercially available ELISA Kits (Anti-hepatitis E virus IgM). (EUROIMUN, Germany). ELISA procedure was conducted according to the manufacturer instructions.

RNA extraction

RNA was extracted from 200/ul serum by using viral RNA extraction kit (Vivantismalysia) according to the protocol of the manufacturer.

Real time PCR

The qualitative real time RT-PCR was done by using Real time RT-PCR kits (Hanghi biotechnology, China) according to the protocol of the manufacturer.

Ethical Review

Ethical approval for this study was obtained from the Ministry of Health, Khartoum State, AL-Neelain University ethical committee board and from sector of obstetrics clinics hospitals in Port Sudan State, Ministry of Health Sudan. Only patients who agreed to participate were enrolled in this study and informed consents were obtained regarding the data and collection of blood samples.

III. RESULT

A total 90 pregnant women presented at the hospital with total various symptoms,

including fever, headache, nausea, vomiting, and history of jaundice were enrolled in the study. Their age ranged between 15-45 years,

Detection of HEV antibodies

HEV IgG antibodies were present in the serum samples of 86 women (95.6%) while HEV IgM antibodies were demonstrable in serum samples of only three of the 90 pregnant women (3.3%) tested. (table 1)

HEV Real-Time PCR

HEV RNA was detected in 4/90 (4.4%) of pregnant women

Comparison of IgG and IgM with Real-Time PCR results

The cross-tabulation between real-time PCR and ELISA (IgM and IgG) is seen in (Table 2). A total of 86 (95.6%) serum samples showed IgG by ELISA of which 4 samples showed HEV RNA by Real-Time PCR. IgM was detectable by ELISA in three serum samples, of which one sample showed both IgG, IgM antibody and RNA by Real-Time PCR.

Comparison of IgG and IgM ELISA with Real Time PCR results are shown in tables 1 and 2

Table 1. Comparison between ELISA IgG and IgM for the diagnosis of HEV in serum samples collected from pregnant women in Port Sudan State, Sudan, during the period from November to December, 2016

ELISA	Positive	Negative	Total
IgG	86(95.6%)	4(4.4%)	90(100%)
IgM	3(3.3%)	87(96.7%)	90(100%)

Table 2. Cross-tabulation between Real-Time PCR and ELISA(IgG and IgM) results for the diagnosis of HEV in serum samples collected from pregnant women in Port Sudan, during the period from November to December, 2016

Real- Time PCR

		Real time PCR			
		Positive	Negative	Total	
ELISA	IgG	Positive	4	82	86
		Negative	0	4	4
		Total	4	86	90
	IgM	Positive	1	2	3
		Negative	0	87	87
		Total	1	8	90

IV. DISCUSSION

Hepatitis E is an emerging infection and a major cause of a acute and endemic hepatitis in the world, Significant morbidity and mortality are especially seen in pregnant women.

RNA is an important marker of acute HEV infection, especially during early stages before the antibody response becomes evident. Serological testing alone may fail to diagnosis acute infection. In this respect Real Time PCR shows good sensitivity for HEV diagnosis

This study was conducted for serological detection of HEV antibodies and detection of HEV RNA in pregnant women in Port Sudan State. The study revealed the

prevalence of HEV IgG and IgM antibodies among pregnant to be 95.6% (86/90) and 3.3% (3/90), respectively. Cases of hepatitis E virus were reported in Sudan in 1992, where acute sporadic cases were found among Sudanese children and. acute hepatitis E (positive for IgM anti-HEV) was found in 59% of study population⁽²⁴⁾. In 1992 and in Darfur, western Sudan, acute hepatitis E virus infection was confirmed in 95% of the suspected cases with hepatitis in 2004⁽²⁵⁾.

The present investigation showed that 4.4% (4/90) of pregnant women were positive for HEV RNA of which, only 1.1% (1/90) were positive for HEV IgM antibodies and 4(4.4%) women who were positive for IgG (one woman had both IgG and IgM antibodies) These results indicate that HEV viral RNA could still be detected in both IgM and IgG positive patients serum samples.

These findings also highlight the need for establishing sensitive and specific diagnostic techniques in Sudan, such as those used in this study, for better management of HEV infection, especially in groups at high risk.

In summary, the incidence and existence of HEV in Sudan was documented through the detection of HEV-specific antibodies (IgG and IgM in serum samples, indicating a low prevalence of IgM antibodies, and of viral RNA using using ELISA and Real Time RT-PCR but high prevalence of IgG antibodies as indicated by ELISA among pregnant women in Port Sudan, These findings could represent an addition to the little information available about HEV in Sudan.

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