Original Research Article

PREVALENCE OF HEPATITIS E VIRUS INFECTION AMONG PREGNANT WOMEN ATTENDING THE ANTE-NATAL CLINIC OF A TERTIARY HEALTH CARE CENTRE

ABSTRACT

Hepatitis E virus (HEV) is the most common cause of viral hepatitis in developing countries. HEV is particularly problematic in pregnancy due to the vulnerability of pregnant women to infections and often causes spontaneous abortion, premature delivery, maternal (≤ 20%), and infant mortality. While pregnant women attending ante-natal clinics are mandatorily screened for some infections, Hepatitis E, except in endemic areas, is not included in the protocol, even though the prevalence of Hepatitis E globally is increasing. This study aimed to determine the presence and prevalence of Hepatitis E among ante-natal clinic attendees at the Ladoke Akintola Teaching Hospital, Osogbo, Osun State. A total of 100 consenting individuals were enrolled in the study between ages 20 and 45. Twenty-one percent of the participants enrolled were in the first trimester of their pregnancy, 33% in the second trimester, and 46% were in the third trimester of pregnancy. A structured questionnaire was administered to extract information on the participants' demography and clinical history. A volume of 5ml of blood sample was collected from each consenting participant, and the obtained serum, stored at -20°C until ready for analysis. A double antibody sandwich HEV-ELISA technique was performed to analyze the sera for HEV antigen, using an ELISA Kit. Of the 100 samples analyzed, 8% ($\chi 2$=12.164, df=1, P=0.019) were positive for HEV Ag. The study only showed an association between the number of positive cases and a history of blood transfusion whereas, there was no such association with age, pregnancy stage, and educational status of the participants. Being an index study in Osun State, this study showed an 8% HEV prevalence rate, which is considered statistically significant.

Keywords: Pregnant women, Hepatitis E Screening, ELISA, LAUTECH

Introduction

Hepatitis E virus (HEV) is one of the most common causes of viral hepatitis in the world (Webb and Dalton, 2019) and the principal etiological agent of enterically transmitted, non-A, and non-C hepatitis. It is highly endemic in the tropical and subtropical countries of Asia, Africa, and South America (Aggarwal, 2010). The infection is mainly transmitted through contaminated water, but may also be transmitted via food or blood transfusion, or vertically from mother to fetus (Aggarwal and Jameel, 2011). In most cases, the infection is asymptomatic as the virus is spontaneously cleared, and only a few cases develop jaundice (Zhu *et al.*, 2010; Rein *et al.*, 2012). However, such cases could be severe and accompanied by acute fulminant hepatitis (De Paschale *et al.*, 2016).

While hepatitis E virus (HEV) is often considered an emerging infectious agent, it has been reported worldwide as a significant cause of acute viral hepatitis (AVH) (Abebe, 2017). Approximately one-third of the world's population are HEV positive (Kim *et al.*, 2014). Of the more than 20 million cases annually, ~70,000 instances lead to death. The majority of these deaths occur in developing Asian, African, and Latin American countries. These estimates, however, may not give an accurate picture of the entire burden of disease, because many areas were either not comprehensively covered adequately in some regions; use of assays which are now known to have inferior sensitivity, or the reports themselves being outdated. The currently perceived global burden of the disease may be a massive underestimation (Webb and Dalton, 2019).

The average seroprevalence of HEV in many developed western countries like the USA and Canada is <5% (Bouwknegt *et al.*, 2008), with HEV-induced acute hepatitis being sporadic and attributed to travelers from endemic regions. Comparatively, areas of high HEV endemicity like Asia, Africa, the Middle East, and South America, where widespread

epidemics involving hundreds of thousands of people have been reported (Echevarría *et al.,* 2013; Kmush *et al.,* 2013; Kim *et al.,* 2014), with mortality rates varying from 0.2% to 4%, for some unexplained reasons, possibly hormonal or immunological, the mortality rate of HEV is more frequent in pregnant women, especially those in the third trimester of gestation (Aggarwal, 2011).

The geographical distribution and severity of HEV genotypes (1-4) appear to show variations (Aggarwal, 2010; WHO, 2015), with genotypes 1 and 2 generally prevalent in developing countries, causing epidemic outbreaks (De Paschale *et al.,* 2016). Genotype 1 is associated with vertical transmission (Kar *et al.,* 2008), while genotype 3 is usually seen in developed countries where it does not cause outbreaks, and the infection resolves without transmission to the infant (Tabatabai *et al.,* 2014). Genotype 4 has been found to occur in sporadic cases of acute hepatitis E in China, Taiwan, Japan, and Vietnam (WHO, 2015).

The first serologically confirmed HEV epidemic occurred in New Delhi (India) in 1955–56 (Balayan *et al.,*1983). The first confirmed outbreak in Africa occurred in Côte d'Ivoire in 1986 (Kim *et al.,* 2014). The reported prevalence rates in Africa range from 0% to 94% (De Paschale *et al.,* 2016). Among pregnant women, HEV antibody prevalence is 11.6% in Burkina Faso and 84.3% in Egypt (Hannachi *et al.,* 2011; Traoré *et al.,* 2012). Although there has never been any significant HEV outbreak in Nigeria, we cannot remain complacent, as there have been sporadic cases of HEV infection in the past. A report by Buisson (2000) showed that HEV infection was present in some middle-aged adults residing in Port-Harcourt (South-South Nigeria) (Buisson *et al.,* 2000). A study carried out in Ekiti State reported the presence of HEV antibodies in 13.4% of participants (Adesina *et al.,* 2009). A study carried out in two geographical locations of Nigeria (Oyo and Anambra States) reported a prevalence of HEV in 0.4% of the cohort (Ifeorah *et al.,* 2017). Oladipo *et al.,* 2017, who carried out a study at Ogbomosho, Oyo State, reported an HEV sera positivity in 2.7% among 186

participants. Another research carried out in Ibadan, Oyo State in 2018, reported the presence of HEV IgM antibodies in 9.0% of the participants (Fowotade *et al.*, 2018). A review carried out in 2019 to determine the prevalence of HEV in 1178 Nigerian individuals reported a prevalence of 10.8% in the population sampled (Okagbue *et al.*, 2019).

Hepatitis E (HEV) is a significant public health concern in developing countries because people with viral hepatitis infection are at risk of developing liver disease. For pregnant women, HEV infection is more frequent and most frequently lethal in the third trimester, with mortality rates around 15-20% of all cases (Webb and Dalton, 2019). HEV infection contributes to adverse maternal and fetal experiences during pregnancy (Abebe *et al.*, 2017), which may also induce premature births. Additionally, the death of infants shortly after birth is also common. Transplacental transmission of HEV in the third trimester of pregnancy has been established while acute hepatitis is reported to cause vertically transmitted HEV infection in neonates, which correlates with a high perinatal mortality of infected neonates (Abebe *et al.*, 2017). A study carried in two geographical regions of Nigeria (Anambra and Oyo) on 272 pregnant women showed a prevalence of HEV in 0.4% of the study population (Ifeorah *et al.*, 2017).

Presently, the prevalence of hepatitis E among pregnant women at the target facility in this study is unknown. Sequel to this information gap, pregnant women attending ante-natal clinics at the LAUTECH Teaching Hospital, Osogbo, Osun State are not screened for hepatitis E, and because of the possible effects on the fetus and pregnancy, it is pertinent that the prevalence of this pathogen is determined. This will help to create more awareness of hepatitis E infection and to ultimately generate adequate prevention and control measures to manage the disease.

Methodology

The ethical approval to carry out this study was obtained from the Research Ethics Committee of Ladoke Akintola University of Technology Teaching Hospital (LAUTECH), Osogbo, Osun State, Nigeria. A total of 100 pregnant women attending the ante-natal clinic (ANC) at the Ladoke Akintola University of Technology Teaching Hospital (LAUTECH), Osogbo, Osun State, Nigeria, were enrolled in the study. Consenting pregnant women visiting the ante-natal clinic for their routine screening were enrolled in the study, taking into consideration some inclusion and exclusion criteria. Participants were subjected to a face-to-face interview with the data collector, whereby pre-test counseling on hepatitis E was done before sample collection. Sample collection was done on-site, and the sterile plain bottles in which the blood was collected were duly labeled. Questionnaires were filled to obtain information on relevant medical, obstetrical, and socio-demographic characteristics of all participants enrolled in the study.

A volume of 5 ml of blood sample was collected in plain bottles with no anticoagulant. The blood samples were then separated by centrifugation at 3000 rpm for 10 minutes to obtain serum. Serum was obtained and stored at -20 °C until ready for analysis. HEV ELISA technique was performed at the Ladoke Akintola University of Technology Teaching Hospital's blood bank laboratory. All of the sera were analyzed in duplicate for HEV antigen, using ELISA Kit [Melsin Medical Corporation, China - sensitivity (97%) and specificity (97%)]. The assay employs a double-antibody sandwich technique to analyze the presence of HEV Ag in human serum. The double antibody sandwich ELISA test was carried out following the instructions contained in the manufacturer's manual.

Results

Table 1: Socio-demographic factors of the study participants, including distribution of HEV samples

Parameter	Participants' variables	Frequency (%)	Distribution (%) of positive participants n=8
Age	20-25	9	-
	26-30	35	5 (62.5%)
	31-35	32	3 (37.5%)
	36-40	20	-
	41-45	4	-
Pregnancy Stage	First trimester	21	3 (37.5%)
	Second trimester	33	2 (25%)
	Third trimester	46	3 (25%)
Source of drinking water	Tap/well-water	66	4 (50%)
	Bottled/sachet water	17	2 (25%)
	Others	17	2 (25%)
Contact with animals	Yes	27	4 (50%)
	No	73	4 (50%)
Occupation	Trader	27	-
	Artisans	27	3 (37.5%)
	Civil servant	24	2 (25%)
	Healthcare worker	5	1 (12.5%)
	Unemployed	9	1 (12.5%)
	Others	8	1 (12.5%)
Education status	Graduate	71	4 (50%)
	Undergraduate	9	-
	Secondary education	17	3 (37.5%)
	Others	1	-
	No formal education	2	1 (12.5%)

Association of possible risk factors with HEV seropositivity

The distribution of seropositive samples according to age, pregnancy stage, source of drinking water, contact with animals, occupation, and education status of the study participants are shown in Table 4. Statistical analysis showed that there was no significant correlation (P> 0.05) between the HEV and the possible risk factors evaluated. The study revealed that there was a seroprevalence of HEV Ag in 13.3% of the test participants with a prior history of blood transfusion relative to those with no previous history of blood transfusion. Blood transfusion history showed statistical significance ($\chi 2$=12.164, df=1, P=0.019). The source of drinking water, however, did not show statistical significance ($\chi 2$=11.817, df=6, P=0.58). There is a 2-fold risk of getting infected with HEV among those who had a previous history of blood transfusion (95% C.I. = 0.135-9.303, OR = 2.03). Likewise, there is a one-fold risk of getting infected with HEV among those whose source of water was bottled/sachet water (95% C.I. = 0.368-11.138, OR = 1.0), and those who picked other water sources than those listed (95% C.I. = 0.368-11.138, OR = 1.71). Multivariate logistic regression analysis showed no statistical correlation between the tested parameters and HEV seropositivity in the individuals enrolled into the study (P> 0.05). Linear regression analysis showed no correlation between the parameters tested and the presence of HEV Ag in the study population (P> 0.05).

Table 2: HEV Seroprevalence infection in comparison to socio-demographic characteristics

Parameter	Participants' variables	HEV Status		df	Chi-square	P-value
		Positive	Negative			
Age	20-25	0(0.0%)	9(100%)	19	18.478	0.491
	26-30	5(14.3%)	30(85.7%)			
	31-35	3(9.4%)	29(91%)			
	36-40	0(0.0%)	20(100%)			
	41-45	0(0.0%)	4(100%)			
Pregnancy Stage	First trimester	3(14.3%)	17(85.7%)	2	1.433	0.489
	Second trimester	3(6.1%)	31(93.9%)			
	Third trimester	3(6.5%)	43(93.5%)			
Source of drinking water	Tap/well-water	4(6.1%)	62(93.9%)	2	0.990	0.609
	Bottled/sachet water	2(11.8%)	15(88.2%)			
	Others	2(11.8%)	15(88.2%)			
Contact with animals	Yes	4(14.8%)	23(85.2%)	1	2.603	0.107
	No	4(5.5%)	69(94.5%)			
Occupation	Trader	0(0.0%)	27(100%)	5	4.023	0.55
	Artisans	3(11.1%)	24(88.9%)			
	Civil servant	2(8.3%)	22(91.7%)			
	Healthcare worker	1(20%)	4(80%)			
	Unemployed	1(11.1%)	8(88.9%)			
	Others	1(12.5%)	7(87.5%)			
Education status	Graduate	4(5.6%)	67(94.4%)	4	5.866	0.139
	Undergraduate	0(0.0%)	9(100%)			
	Secondary education	3(17.6%)	14(82.4%)			
	Others	0(0.0%)	1(100%)			
	No formal education	1(50%)	1(50%)			

Table 3: Possible risk factors' correlation and HEV

Variable	Participants' variables	Positive	Negative	OR	95% C.I.	P-value
Source of drinking water	Tap/wellwater	4(6.1%)	62(93.9%)	0.48		
	Bottled/sachet water	2(11.8%)	15(88.2%)	1.0	0.368-11.138	0.58
	Others	2(11.8%)	15(88.2%)	1.71		
History of blood transfusion	Yes	2(13.3%)	13(86.7%)		0.135-9.303	0.019
	No	6(40%)	79(0.0%)	2.03		

Discussion

Hepatitis E virus is a leading cause of liver disease globally, and while long-term complications are rare, it causes substantial mortality($\leq 20\%$) in infected pregnant women (Abebe, 2017). HEV is particularly problematic in pregnancy due to the vulnerability of pregnant women to infections, which often causes spontaneous abortion, premature delivery, including maternal and infant mortality. The symptoms of the disease are more commonly pronounced during the third trimester of pregnancy (De Paschale *et al.*, 2016). Although some research has been carried out in various locations in Nigeria, the burden of the infection among pregnant women is still largely unknown. While pregnant women attending ante-natal clinics are mandatorily screened for HIV, Hepatitis B and C, Hepatitis E, except in endemic areas, is not included in the protocol, even though the prevalence of Hepatitis E globally is on the increase.

This study was carried out to determine the prevalence of hepatitis E among pregnant women attending the ante-natal clinic at the LAUTECH Teaching Hospital, Osogbo. The results of this study showed a seroprevalence of 8% among the study population, a figure which is consistent with those reported in studies carried out to determine the seroprevalence of HEV

among pregnant women in Sokoto (9.9%) (Bello *et al.,* 2017) and Nasarrawa (12.1%) (Adamu *et al.,* 2018), and some African countries like Burkina Faso (11.6%) (Traoré *et al.,* 2012), and Gabon (14.1%) (Caron and Kazanji, 2008).

The result obtained in this study is, however, much lower than the results of related studies carried out among pregnant women in most developing countries. For example, a study carried out in Addis Ababa, Ethiopia, to determine HEV seroprevalence among pregnant women showed a seroprevalence of 31.6% (Abebe *et al.,* 2017), while a similar research carried out in Darfur, Sudan reported a seroprevalence of hepatitis E virus among pregnant women in 31.1% of the study participants. Another study carried out among pregnant women in Benin Republic reported a seroprevalence of 17.63%, while studies carried out in other African countries also reported as follows: Ghana (28.6%), Sudan (41%), and Egypt (84.3%) (Kim *et al.,* 2014). The observed seroprevalence in this study is lower than that of other similar studies because of the small sample size, delayed sample analysis because of the lockdown of activities due to the Covid-19 pandemic, and also because hepatitis E might still be an emerging disease in Nigeria.

Conclusion

In this study, the HEV seroprevalence (8%) among pregnant women is significant ($p<0.05$), considering the small sample size. History of blood transfusion has a particularly significant association with HEV infection. At the same time, age may also be a risk factor of HEV infection, but it was not statistically supported in this study. There was no correlation between the HEV Ag prevalence rates among the study participants in terms of occupation, source of drinking water, contact with animals, and educational status.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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