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ORIGINAL ARTICLE

Hepatitis E Virus Infection among Antenatal Attendees in Ekiti State, Nigeria: Awareness and Prevalence

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Abstract

Background: Hepatitis E virus (HEV) infection is an enteric viral infection and a global problem of increasing threats among pregnant women with an estimated annual burden of 20 million cases, 70,000 deaths, and 3,000 stillbirths. This study aims to determine the level of awareness of HEV infection, its prevalence, pregnancy risks, and transmission mode among antenatal attendees in Ekiti State.

Methods: This is a dual-center study performed at the antenatal clinics of the study sites over a period of fifteen months following ethical approval. Socio-demographic, pregnancy-related, and HEV awareness data of 300 antenatal attendees were collected during counseling sessions at the clinics. A Biopanda rapid test device was used to test for HEV antibodies. Data were analyzed using the descriptive statistics of the SPSS version 21 software, and the association between hospital and HEV awareness and HEV mode of transmission variables was tested with Chi-square analysis.

Results: The 300 enrollees had a mean age of 31.2 ± 0.3 years and consisted of 161 (53.7%) and 139 (46.3%) from study sites 1 and 2, respectively. Nearly 90% (89.3%) were in their second to third trimesters, and 89% had a hematocrit of 31-39%. The prevalence of HEV antibodies reported in this study was 2.0% and consisted of anti-HEV IgM (0.3%), anti-HEV IgG (1.0%), and anti-HEV antibody total (0.7%). Irrespective of HEV awareness variables or associated health facility, HEV level of awareness was poor, with only awareness of mode of transmission showing a statistically significant difference between hospitals (P =0.025, $\gamma 2 = 5.027$, OR = 2.371; 95 CI=1.096-5.131). Acute liver disease (50.0%), feto-maternal death (15.8%), and death of a live baby soon after birth (15.8%) were the three most reported HEV infection risks in pregnancy.

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Conclusion: Low level of awareness of HEV infection despite counselling at clinics reflected the gaps in current national policy on hepatitis and the need for urgent review and update of the same to include result-oriented and all-inclusive antenatal patients' education on HEV infection, its risk to pregnancy and routine testing for HEV to prevent inherent dangers of risks of acute liver failure, feto-maternal death and still birth.
Key words: Awareness, Hepatitis E Virus, Mode of transmission, Antenatal attendees, Ekiti

Background

Hepatitis E virus (HEV) is the causative agent of non-A, non-B hepatitis, and acute viral hepatitis worldwide (1). It is highly endemic in tropical and subtropical Africa, Asia, and Central America (1). HEV is the only member of the genus *Hepevirus* with four genotypes (2). Genotypes 1 and 2 cause human disease and are responsible for outbreaks in developing countries, while genotypes 3 and 4 are zoonotic (3). It is a small, spherical, single-stranded RNA virus without an envelope but surrounded by an icosahedral capsid (2, 4).

It is estimated that the global annual burden of HEV infection is 20 million cases, with 70,000 deaths and 3,000 stillbirths (5-6). The infection is mainly acquired via the oral route and transmitted through water. Other transmission routes include food, blood transfusion and from mother to fetus (1, 7). HEV infection is mostly asymptomatic with few developing jaundice while most clear it spontaneously (5, 8), although sudden and severe or rapidly progressing hepatitis leading to death within days or weeks (otherwise known as fulminant hepatitis) may occur particularly in pregnant women or those with immunosuppression where mortality may be up to 30-100% (9-11). When it occurs in pregnancy, especially during the second or third trimester, hepatitis E carries a high risk of acute maternal hepatic failure with a very high fatality rate, as well as high neonatal mortality and morbidity rates (12-13). The mother-to-infant transmission of HEV of 100% was reported in a study in the United Arab Emirates, while a small percentage of babies born to those mothers with active disease either developed anicteric hepatitis or were born preterm[14]. Laboratory confirmation is based on isolating HEV RNA in stool or serum or detecting anti-HEV antibodies by serological tests. The detection of anti-HEV IgM indicates acute infection, while the presence of anti-HEV IgG signifies previous exposure to HEV (15).

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The reported seroprevalence of HEV IgG antibody in Central Nigeria (16) was 31.5% and 29.13% among pregnant women in Africa, with the highest found in Egypt (84.3%) and the lowest in Gabon (6.6%) (17). An anti-HEV IgG positivity rate of 8.4% was reported in Argentina (18), while 16.19% were positive for anti-HEV IgG and 1.44% for anti-HEV IgM in Benin Republic (9). The prevalence rates of 0.4% (2) and 15% (19) respectively have been reported in other regions of Nigeria. A cross-sectional study in South East Nigeria showed low HEV prevalence (2.2%) and poor knowledge of HEV infection (98.6%) among the study population (20).

Routine antenatal policy in Nigeria prescribes the screening for hepatitis B and C viruses as part of antenatal care protocol during clinics following general counseling on hepatitis. Despite the potential danger HEV has on pregnant women, especially during the third trimester, whether or not the counseling sessions in health facilities during clinics adequately capture hepatitis E virus awareness, testing, and treatment is not clearly understood, and that might explain the cause of reported cases of fulminant hepatitis, unexplained abortion, feto-maternal deaths to mention a few. Although several studies have been conducted globally, there is a paucity of data in Nigeria, especially on awareness of HEV infection among antenatal attendees (2, 24, 21). This study focuses on this grey area and assesses the level of awareness of HEV infection among antenatal subjects, including its risk to pregnancy, vaccine availability, and mode of transmission during antenatal clinics in clinical settings in Ekiti state. Previous data concentrated more on prevalence studies (2, 19, 21). This study seeks to bridge these gaps among antenatal clinic attendees in Nigeria. This research aimed to determine the antenatal attendees' level of awareness of HEV infection, HEV prevalence, its risks to pregnancy, vaccine availability, and possible risks of HEV infection in pregnancy and assess their knowledge of the mode of transmission of the virus in Ekiti state, Nigeria

Materials and Methods

Study Design

This hospital-based descriptive cross-sectional study was carried out on all would-be mothers attending the antenatal clinics of the Federal Teaching Hospital, Ido Ekiti (FETHI) and Ekiti State University Teaching Hospital (EKSUTH), Ado Ekiti, Nigeria, between 15th June 2020 and 30th September 2021 and tested their levels of HEV awareness, its risks to pregnancy, vaccine availability, HEV infection risks on pregnancy, mode of transmission and

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determined the prevalence of HEV antibodies. The study was carried out at the departments of Obstetrics and Gynecology and Haematology departments at Federal Teaching Hospital, Ido Ekiti (FETHI), Ekiti State, and Ekiti State University Teaching Hospital (EKSUTH). Ekiti State is one of the Southwestern states in Nigeria with three senatorial districts (Ekiti North, Ekiti South, and Ekiti Central), 16 local government areas, and an estimated population of 3,592,200 inhabitants. Federal Teaching Hospital, Ido Ekiti, is one of the two tertiary health institutions in the state and is located in the Ido Osi local government Area, a rural setting when compared to the Ekiti State University Teaching Hospital, which is located at an urban and industrial center of the state, Ado Ekiti, the capital of the state, Ado Ekiti which is the urban and industrial centre of the region. These two health facilities provide tertiary healthcare in Ekiti State and take referrals from other public and private health facilities in the state and parts of Ondo, Osun, and Kogi States. STROBE cross-sectional reporting guidelines were used to carry out this study 22.

Data Collection

An estimated total number of 347 antenatal patients presented at the antenatal clinics, out of whom 300 eligible participants (FETHI-161; EKSUTH-139 participants) that met the inclusion criteria such as age bracket of 16 - 49 years, not known HEV patients, registered booked patients who consented and were non-alcoholic had questionnaires administered to them. All consecutively consenting pregnant women in the first, second, and third trimesters were included in the study. Structured self-administered and provider-administered questionnaires containing closed-ended questions relating to HEV variables under study were used to collect information from the respondents. The questionnaire was divided into three sections. Section 1 included questions on the antenatal patients' social demographic and pregnancy characteristics covering the age, marital status, educational status, gravidity (the number of times a woman has been pregnant), gestational age of pregnancy, and antenatal attendee's packed cell volume (a measure of the space occupied by the red blood cells in a whole volume of blood). The ages of antenatal attendees were grouped into seven categories to enable authors to capture the age group range with the highest antenatal booking. The packed cell volume (PCV) was grouped into four to enable authors to assess outcomes of antenatal care with respect to nutrition and compliance with medications. Section 2 covered their awareness of hepatitis E virus infection and its possible risks to pregnancy (including acute hepatitis, acute liver disease, feto-maternal death, premature delivery, abortion,

death of a live-born baby soon after birth, renal failure and fulminant hepatic failure), vaccine availability and awareness of its mode of transmission. Section 3 included questions on HEV transmission route variables such as sharing of infected sharp objects, transfusion of infected blood and blood products (whole blood, packed red cells, platelets, fresh frozen plasma et cetera), poor sanitation, oral exposure to shed HEV in feces, sneezing and coughing, HEV infected partner, vertical transmission (also known as mother-to-child transmission), occupational exposure to domestic swine, wild boar and deer, sharing of unsterilized needles and syringes and weak public health system.

Bias, Confounders, and Missing Data Handling

Non-consenting pregnant women, those with ectopic pregnancy, pregnant minors, and known hepatitis B or C virus-infected patients (estimated to be 47) were excluded from the study to eliminate potential bias in study outcomes. Missing data were handled through data cleaning, assessment of patients' medical records during antenatal clinics to include missed data, and phone contact with antenatal attendees.

Calculation of Sample Size

One of the available studies in Southwestern Nigeria with HEV seroprevalence of 15% was used to calculate the sample size for this study[19]. The study size was calculated with the formula: $\mathbf{n=z^2pq/d^2}$

Where n=desired study size

z=level of confidence (the standard normal deviate set at 1.96, which corresponds to the 95% confidence level

p =prevalence (the proportion in the target population estimated to have a particular characteristics) = 15 % (0.15)

q= 1-p =1 - 0.15 = 0.85

d= degree of accuracy desired usually set at 0.05

Therefore;

$$n=\frac{(1.96)^2 \times 0.15 \times 0.85}{0.05^2} = 196.0$$

Therefore, n = 196

N = the estimate of population size, which is = 8550 (that was the estimated total number of registered study participants at the antenatal clinics of both FETHI and EKSUTH in 2020).

Since N = 8550, which is less than 10,000 over a given period of one year (year 2020),

Therefore,

nf = n / (1+ n/N) will be applied. Where;
nf = The desired sample size when the population is less than 10,000
i.e., nf= n / (1+ n/N)
= 196/ (1 + 196/ 8550)
= 196/ (1+0.023) = 196/1.023
nf = ≈ 192

This was 230, with an attrition rate of 20%. To give more representation, the study used a total of 300 consenting antenatal patients who were attending the antenatal clinics.

Sample collection

Five milliliters (5ml) of EDTA anticoagulated sample were collected by venipuncture from each antenatal attendee during the clinic visit following informed consent. If the sample was not run immediately, it was spun at 3000rpm for 5 minutes, and the plasma was separated into another plain container for HEV IgM/ IgG antibody testing.

Hepatitis E Virus Testing

Biopanda rapid immunochromatographic test device (Biopanda Diagnostics, UK) was used to screen for HEV IgM and IgG antibodies. With the aid of the supplied dropper, a drop (approximately 35 µL) of whole blood was

placed on the sample pot, and 2 drops (70 µL) were added and left for 15 minutes. Plasma was used where the sample was not run immediately. Approximately 70 - 105µL of plasma was placed on the sample pot. Results were read at 15 minutes but over 20 minutes to avoid false positive results.

Data Entry and Statistical Analysis

The data collected from 300 antenatal attendees divided into the three sections of the questionnaire were coded into Statistical Package for Social Sciences (SPSS) software, version 21.0 (IBM, IL, USA). A total of 68 and 93 (161) questionnaires were administered to FETHI antenatal attendees in 2020 and 2021, respectively, while 58 and 81 (139) questionnaires were administered to EKSUTH antenatal attendees in 2020 and 2021, respectively. The data were entered and cross-checked for wrong and missing entries through the descriptive statistics of the SPSS. The dependent variables (main outcomes) in this study were the HEV awareness variables (awareness of HEV infection, HEV risks to pregnancy, mode of transmission, and vaccine availability, which were coded as 1 = 'yes' and 2 = 'no') and mode of transmission variables which were coded as 1= 'yes', 2 = 'no' and 3 = I don't know. The independent variables identified in the literature included age, marital status, gravidity, educational status, and packed cell volume. Age and gestational period in trimesters were continuous variables. The frequency and percentage of the antenatal attendees who responded on the awareness of HEV infection risk in pregnancy were presented as a chart. Descriptive statistics were computed using frequency, percentage, mean, and standard deviation. By cross-tabulation, chi-square analysis was also computed to determine the association between hospital and HEV awareness variables and hospital and HEV transmission routes. A P value < 0.05 is statistically significant.

Results

An estimated three hundred and forty-seven antenatal attendees were seen during antenatal clinic. Of these, 47 (13.5%) were excluded based on non-informed consent, ectopic pregnancy, age below or above study eligibility criteria, and known hepatitis B or C patients. The actual number of antenatal attendees enrolled for the study was 300 (86.5%). Some of the participants could not consent or participate due to socioeconomic factors and the negative impacts of the COVID pandemic due to travel restrictions. Figure 1 is the flowchart showing the data of recruited participants.

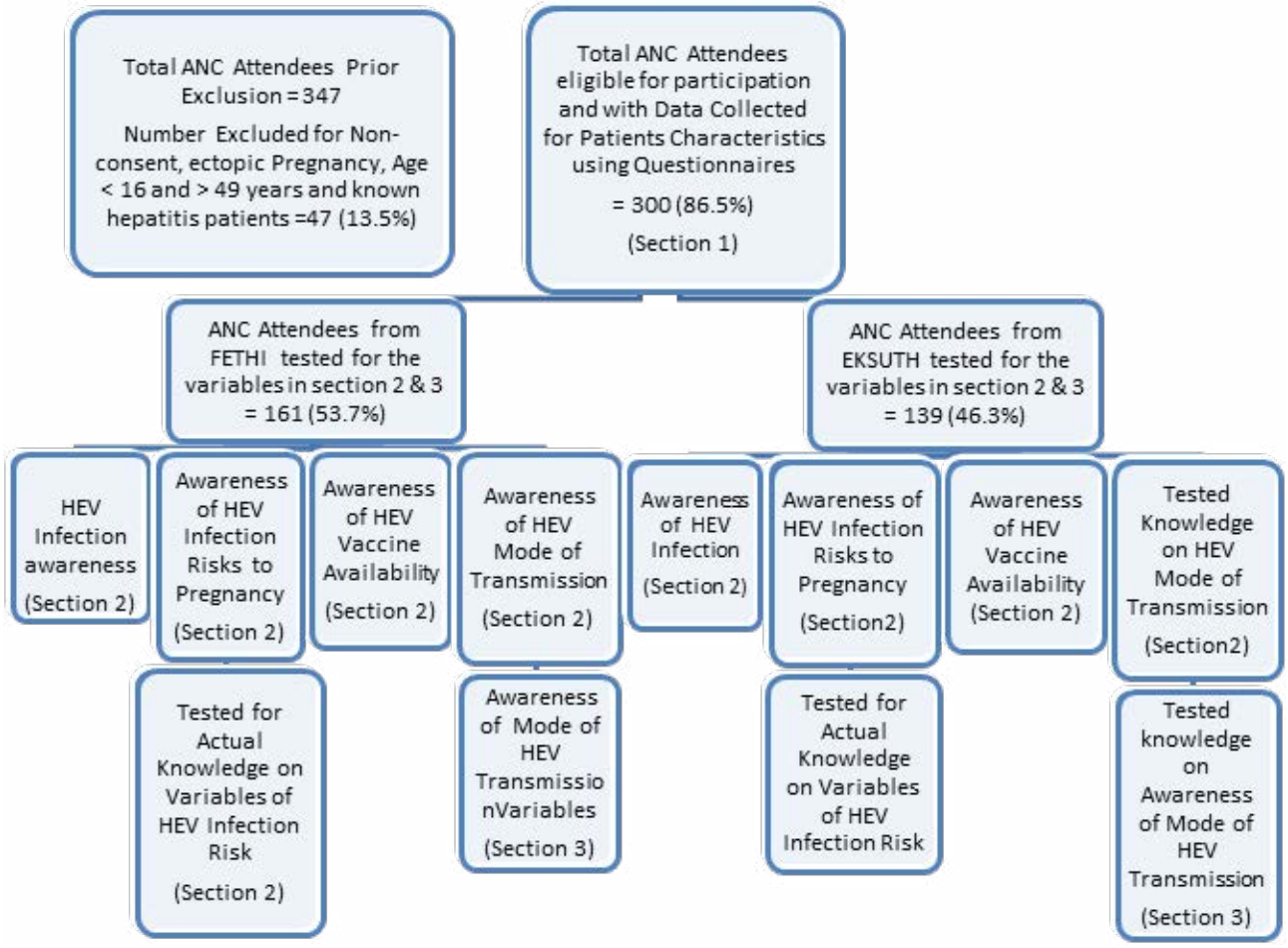

Figure 3: Flowchart for Selection of Participants. This figure shows the flowchart of frequency of total antenatal attendees recruited, division according to health facilities involved (Section 1) and HEV awareness and HEV mode of transmission variables tested for according to health facilities (Section 2 and 3)

Antenatal Patients' Social Demographic and Pregnancy Characteristics

Table 1 shows the demographic and pregnancy characteristics of the study population. The mean age of the respondents was 31.2 ± 0.3 years while the respondents were fairly equally distributed among the two health facilities (46.3% for EKSUTH and 53.7% for FETHI). Only two (0.7%) fell within the minimum threshold age limit 16-19 (< 20) years. Antenatal attendees within the 25-39yrs age group constituted the larger population and highest percentage (263 or 87.6%) while 233 (77.7%) were married, 289 (96.5%) had at least secondary education, 255 (85.0%) were multigravida, 268 (89.3%) were in their second to third trimester, 21 (7.0%) of them had packed cell volume ≤ 30% while 267 (89.0%) had PCV of 31-39%.

Prevalence of HEV Antibodies among Antenatal Attendees

According to Table 2, the overall HEV antibodies seroprevalence among antenatal attendees was 2.0%. HEV Anti-IgG, anti-IgM, and both anti-IgG and anti-IgM seroprevalence were 1.0%, 0.3% and 0.7 % respectively.

Table 1: Antenatal Patients Social Demographic and Pregnancy Characteristics

Variable	Mean (± SD) in Years	
Total number of Attendees at Health Facilities	31.2 ± 0.3	300 (100.0)
EKSUTH		139 (46.3)
FETHI		161 (53.7)
Age Group of Enrollees:		
< 20		2 (0.7)
20 – 24		20 (6.7)
25 – 29		94 (31.3)
30 – 34		115 (38.3)
35 – 39		54 (18.0)
40 – 44		13 (4.3)
45 – 49		2 (0.7)
Marital Status		
Single		65 (21.7)
Married		233 (77.7)
Divorced/Separated		1 (0.3)
Widowed		1 (0.3)
Educational Status:		
At most Primary Education		11 (3.7)
At least Secondary Education		289 (96.3)
Gravidity		
Primigravida:		45 (15.0)
Multigravida:		255 (85.0)
Gestational Age in Trimesters:		
First trimester		32 (10.7)
Second trimester		97 (32.3)
Third trimester		171 (57.0)
Packed Cell Volume (%)		
≤ 30		21 (7.0)
31 – 34		135 (45.0)
35 – 39		132 (44.0)
≥40		12 (4.0)

KEY: SD = Standard deviation; % = Percentage; † = Approximate percentage

EKSUTH = Ekiti State University Teaching Hospital; FETHI = Federal Teaching Hospital, Ido Ekiti; Gravidity: A term that defines the number of times a woman has been pregnant; Primigravida = A woman who conceives for the first time; Multigravida = A woman who has given birth more than once; Primary education= Six years of education; At least Secondary education= Minimum of 12 years of education. Packed cell volume = A measure of the space occupied by the red blood cells in a whole volume of blood.

Table 2: Seroprevalence of Hepatitis E Virus Antibodies among ANC Attendees Screened

ANC Attendees tested: Frequency (%)	**Biopanda Rapid One-step HEV Antibody test**			
	HEV Antibody Markers Screening Results			
	HEV IgM Positive: Frequency (%)	HEV IgG Positive: Frequency (%)	HEV Antibody Total Positive: Frequency (%)	HEV Antibody Negative: Frequency (%)
300 (100.0)	1 (0.3)	3 (1.0)	2 (0.7)	294 (98.0)

Key: HEV = Hepatitis E Virus; **HEV IgM** = HEV immunoglobulin M; **HEV IgG** = HEV immunoglobulin G; **ANC** = Antenatal clinic; % = Percentage

Association between Hospital and Awareness of HEV Infection, Its Possible Risk to Pregnancy, Mode of Transmission and Vaccine Availability

The antenatal patients' (respondents') awareness of hepatitis E virus, the possible risks of hepatitis E virus infection to pregnancy, its mode of transmission and vaccine availability is shown in Table 3. The awareness was poor as less than 20% of respondents from each health facility ever heard of HEV infection with no statistically significant difference (P =0.059, γ^2 = 3.552, OR = 1.953; 95 CI=0.965-3.952). Nearly one-fifth of respondents knew about risks of HEV infection to their pregnancy from each health facility with no statistically significant difference (P =0.847, γ^2 = 0.037, OR = 0.945; 95 CI=0.534-1.672). A paltry sum of 11.7% of the respondents knew about both vaccine availability and mode of transmission of HEV but with no statistically difference with respect to health facilities for the former (P =0.060, γ^2 = 3.540, OR = 2.038; 95 CI=1.096-4.329) and significant difference for the latter (P =0.025, γ^2 = 5.027, OR = 2.371; 95 CI=1.096-5.131).

Antenatal Patients' Level of Awareness of the Possible Risks of Hepatitis E Virus Infection on Pregnancy

Figure 2 showed the antenatal patients' level of awareness of the possible risks HEV infection on pregnancy among the 38 respondents who claimed to be aware of the HEV infection risks. Half of the respondents, based on their experiences, believed that acute liver disease could result from HEV infection; while 15.8% each believed that HEV infection could induce feto-maternal deaths and early neonatal deaths.

Table 3: Association between Hospital and Awareness of HEV Infection, It's Possible Risk to Pregnancy, Mode of Transmission and Vaccine Availability

HEV Awareness Variables		Hospital		*p*-value (χ^2)	OR (95%CI)
		FETHI Frequency (%)	EKSUTH Frequency (%)		
Have you heard of HEV infection	Yes	27(16.8)	13(9.4)	0.059 (3.552)	1.953 (0.965-3.952)
	No	134(83.2)	126(90.6)		
Are you aware of HEV infection risk to your pregnancy?	Yes	21(13.0)	17 (12.2)	0.967 (0.931)	1.001 (0.491-1.952)
	No	140 (87.0)	111(87.8)		
HEV Mode of Transmission Awareness	Yes	25(15.5)	10(7.2)	**0.025** (5.027)	2.371 (1.096-5.131)
	No	136(84.5)	129(92.8)		
Are you aware of hepatitis E virus vaccine availability?	Yes	24(14.9)	11(7.9)	0.060 (3.540)	2.038 (0.960-4.329)
	No	137(85.1)	128(92.1)		

Key: OR = Odds Ratio; CI= Confidence Interval

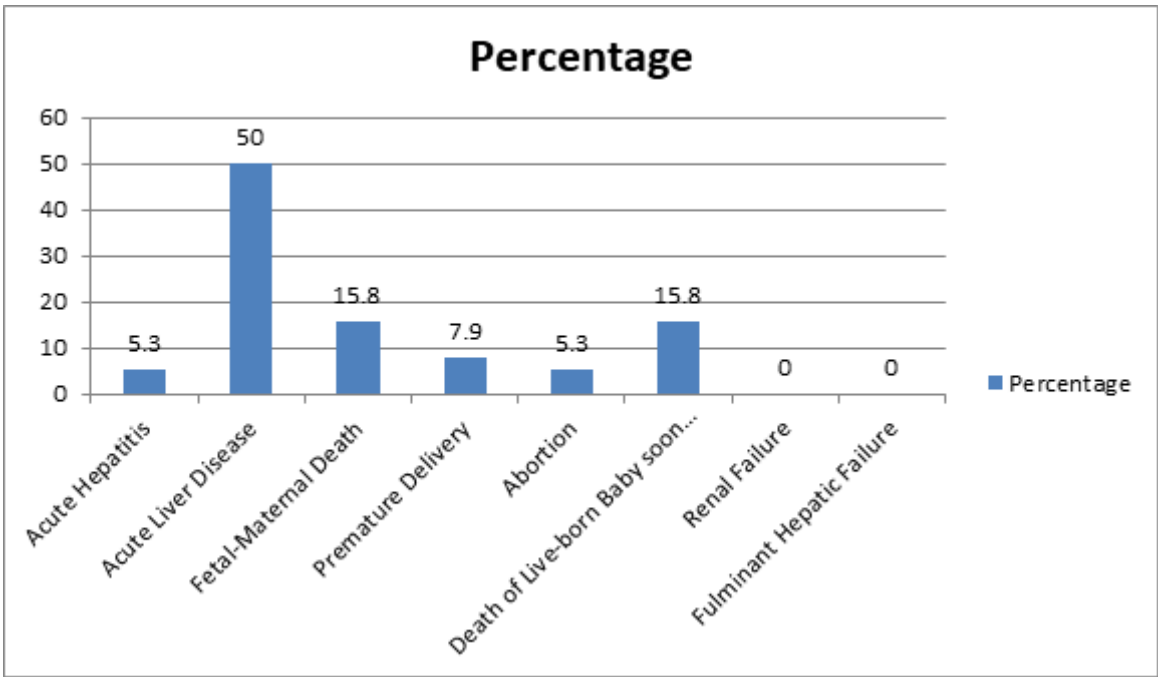

Figure 2: Antenatal Patients' Awareness of the Possible Risks of Hepatitis E Virus Infection on Pregnancy.

Association between Hospital and HEV Mode of Transmission Variables

Table 4 showed the association between hospital and HEV mode of transmission variables (i.e. HEV transmission routes) among antenatal attendees. Out of the antenatal population studied from FETHI and EKSUTH, 30 (18.6%) and 11 (7.9%) respectively considered sharing of infected sharp objects as a route of HEV transmission while 34 (21.1%) and 16 (11.5%), 21 (13.3%) and 14 (10.1%), 23 (14.3%) and 11 (7.9%) from FETHI and EKSUTH respectively considered transfusion of infected blood products, poor sanitation and exposure to shed HEV in feces as transmission routes. Moreover, for sneezing and coughing, intravenous drug abuse, HEV infected partner, vertical transmission, oral exposure to domestic swine, wild boar or deer, sharing of unsterilized needles and syringes, and weak public health system, 15 (9.4%) and 12 (8.6%), 25 (15.8%) and 11 (7.9%), 31 (19.3%) and 9 (6.5%), 23 (14.3% and 12 (8.6%), 20 (12.4%) and 12 (10.1%), 31 (19.3%) and 12 (8.6%), and 23 (14.3%) and 15 (10.8%) respectively from FETHI and EKSUTH considered the variables as HEV transmission routes. Knowledge of antenatal patients attending the clinics of the two health facilities differed significantly on the contributions of sharing of infected sharp objects ($p= 0.026$, $\chi^2 = 7.271$), transfusion of infected blood and blood products ($p=0.011$, $\chi^2 = 8.939$), HEV infected partner ($p=0.005$, $\chi^2 = 10.588$) and sharing of unsterilized needles and syringes ($p=0.029$, $\chi^2 = 7.094$) to HEV transmission but no statistically significant difference observed for other HEV transmission routes.

Table 4: Association between Hospital and HEV Modes of Transmission

HEV Transmission Routes Variables	Hospital	Yes: Frequency (%)	No: Frequency (%)	I don't know Frequency (%)	*p*-value χ^2
Sharing of infected sharp objects	FETHI	30(18.6)	16(9.9)	115(71.4)	**0.026**
	EKSUTH	11(7.9)	16(11.5)	112(80.6)	7.270
Use of infected blood and blood products	FETHI	34(21.1)	11(6.8)	116(72.0)	**0.011**
	EKSUTH	16(11.5)	21(15.1)	102(73.4)	8.939
Poor Sanitation	FETHI	21(13.0)	18(11.2)	122(75.8)	0.599
	EKSUTH	14(10.1)	13(9.4)	112(80.6)	1.026
Oral Exposure to Shed HEV in Faeces	FETHI	23(14.3)	13(8.1)	125(77.6)	0.217
	EKSUTH	11(7.9)	13(9.4)	115(82.7)	3.055
Sneezing and Coughing	FETHI	15(9.4)	22(13.8)	123(76.9)	0.587
	EKSUTH	12(8.6)	14(10.1)	113(81.3)	1.065
Intravenous Drug Abuse	FETHI	25(15.8)	14(8.9)	119(75.3)	0.118
	EKSUTH	11(8.0)	14(10.1)	113(81.9)	4.268
HEV Infected Partner	FETHI	31(19.3)	13(8.1)	117(72.7)	**0.005**
	EKSUTH	9(6.5)	12(8.6)	118(84.9)	10.588
Vertical Transmission	FETHI	23(14.3)	17(10.6)	121(75.2)	0.154
	EKSUTH	12(8.6)	10(7.2)	117(84.2)	3.746
Occupational exposure to domestic swine, wild boar or deer	FETHI	20(12.4)	16(9.9)	125(77.6)	0.069
	EKSUTH	14(10.1)	5(3.6)	120(86.3)	5.338
Sharing of Unsterilized Needles and Syringes	FETHI	31(19.3)	11(6.8)	119(73.9)	**0.029**
	EKSUTH	12(8.6)	13(9.4)	114(82.0)	7.094
Weak Public Health System	FETHI	23(14.3)	15(9.3)	123(76.4)	0.076
	EKSUTH	15(10.8)	5(3.6)	119(85.6)	5.165

4.1 Discussion

Nearly 14% of the antenatal attendees were ineligible for participation in the study. Ectopic pregnancy (defined as the implantation and development of a fertilized ovum anywhere outside the uterine cavity) is one of the most feared conditions in women of reproductive age due to its potentially fatal nature, as ruptured ectopic pregnancy contributes to 10-15% of maternal death and usually ectopic pregnancy does not survive beyond first trimester (23, 24). Thus, the inclusion of antenatal attendees with a history of ectopic pregnancy would represent

a confounding factor and has the potential to influence outcomes of participants' awareness of risks of HEV infection during pregnancy. Similarly, based on global data, pregnant minors were excluded due to a higher risk of preterm deliveries, feto-maternal deaths, and other neonatal outcomes that could affect participants' views (25-28). This study showed that women in the active reproductive age group constituted the larger percentage of enrollees, and that called for intensive enlightenment among the study population. Most antenatal patients were married, multigravida, had at least secondary education, were in their second and third trimesters, and had packed cell volume or hematocrit greater than 30%. We deduced from this study that the majority of the antenatal patients were elites and received optimal maternal-fetal care, but that only positively impacted their nutritional status and promoted normal pregnancy through proper dietary intakes and possible proper adherence to prescribed medications during antenatal visits but not their knowledge on HEV awareness, its risks to pregnancy, vaccine availability or mode of transmission (29). Moreover, it could connote the concentration of the healthcare providers during antenatal visits which did not cover these all-important subjects. That called for inclusion of HEV infection awareness and prevention counselling during antenatal visits.

The overall HEV antibodies seroprevalence in this study is low and similar to 2.2% prevalence reported in the South-eastern part of Nigeria by Mbachu *et al* (20). Anti-HEV IgG antibody seroprevalence reported in the study is 1.0% and that is approximately 16 times lower than the seroprevalence reported in Benin Republic (9). Anti-HEV IgG indicates past exposure or ongoing chronic infection among the research subjects. This study showed 0.7% anti-HEV antibodies (total) seroprevalence, which is extremely lower than the 15.0% reported by Osundare et al. (19). Similarly, anti-HEV IgM antibody prevalence, evidence of acute infection, reported in this study was 0.3%, and that was similar to 0.4% reported by Ifeorah *et al* in 2017 (7) nearly 13-fold lower than another study from South-western region (19). At first look, the low prevalence may be thought of as not requiring any urgent attention. However, 2.0% prevalence translates into 200 per 10,000 pregnant women infected with HEV. That calls for a more robust counselling session during antenatal clinics covering HEV as an emerging viral infection, discussion on its risks to pregnancy, vaccine availability, HEV prevention, testing and treatment of pregnant women that are positive for the HEV and prevention of mother-to-child transmission.

Moreover, irrespective of health facility for antenatal attendees' recruitment, this present study showed poor awareness of HEV, its risk to pregnancy, its mode of transmission and vaccine availability among the pregnant population studied. This may not be unconnected with the emerging nature of the HEV infection as not much is talked about it in the antenatal clinics. However, there was a statistically significant difference in the association between hospital and awareness of HEV mode of transmission. Study showed the need for more enlightenment program during antenatal clinic at EKSUTH. Hepatitis E virus screening is not part of the routine antenatal screening protocol in most of Nigerian tertiary health facilities and possibly in African clinical settings with accompanied increased risk of maternal mortality compared to other parts of the world. The incidence of HEV infection in pregnancy is high in developing countries contributing to about 30 to 100% maternal mortality (21). This is one of the very few studies that evaluate awareness of HEV among antenatal population. Previous studies have focused only on sero-prevalence of HEV among the pregnant women (3,17,18) Findings in this study were consistent with the findings from previous studies, though these studies evaluated for the awareness of hepatitis B and C virus (30-35). Only Mbachu *et al* studied

the prevalence of HEV, its knowledge and practice of preventive measures in Nigeria, though among adolescents in rural areas (20). The majority of their respondents (98.6%) had poor knowledge of HEV, similar to these study outcomes. Nearly two-fold (13.3%) of the study population compared to 7.0% found by Mbachu *et al* was aware of the mode of transmission of HEV (20). Moreover, the antenatal patients had poor awareness of the risks of HEV infection to pregnancy based on the variables assessed when compared to a prospective study by Kumar et al in 2017 (36).

Except for acute liver disease, followed by feto-maternal deaths and death of live-born baby soon after birth, the rest were less than 10%. Previous reports have showed the significant association of negative impacts of HEV infection with pregnancy (37-38). A study by Kumar *et al* in 2017 showed that HEV infection especially in the third trimester of pregnancy was the cause of approximately 23% of abortion, 72% premature delivery, 38% intrauterine demise, 22.0% maternal deaths and fulminant hepatic failure which was the cause of 100% maternal deaths reported in the study (20). More critical assessment by the authors revealed that most pregnant women with worse feto-maternal outcomes did not have adequate antenatal care nor registered anywhere prior admission.

Besides, antenatal attendees' poor knowledge of all HEV mode of transmission variables as revealed by Chi square analysis was evident as less than 20% of research participants knew the role of each HEV transmission route. Study outcomes on hepatitis E virus mode of transmission can be explained from two different angles. On the one hand, literatures, have discussed hepatitis E as an emerging viral infection with high risk of feto-maternal mortality thus requiring proactive and proper education of antenatal attendees on the major contributions of vertical or transplacental transmission (14), poor sanitation and fecal-oral routes such as exposure to shed HEV virus from feces and occupational exposure to domestic swine, wild boar or deer (7, 12, 13). Incidentally, there was no statistically significant difference in the knowledge level of antenatal attendees on these HEV modes of transmission reported in this study That again pictured, besides proper education, the need for identification of HEV-positive mothers during antenatal clinics through routine testing, viral load monitoring, initiation of antiviral therapy where antenatal patients are eligible, emphasis on proper hygiene and possible vaccination of eligible and high-risk subjects. A previous study reported that transplacental or vertical transmission is the most important HEV transmission route in pregnant women and probably the major cause of high incidence of preterm delivery, intrauterine deaths and abortion (20). On the other hand, as a blood borne infection with increasing threats to blood safety, proper understanding of the roles of transfusion of infected blood and blood products and related routes such as sharing of infected sharp objects, sharing of unsterilized needles and syringes and weak public health system in HEV transmission cannot be over-emphasized (39). Study did not only show low level of awareness among antenatal patients attending the two health facilities, it also revealed that significant difference existed in their level of awareness on the contributions of transfusion of infected blood and blood products, sharing of infected sharp objects, HEV infected partner and sharing of unsterilized needles and syringes to HEV transmission. Infection with HEV through transfusion of blood or blood products especially in the third trimester can be very dangerous and lead to fulminant hepatitis and consequent maternal death (38-39). Therefore, there must be a holistic national policy on blood safety that includes stringent screening of blood to be transfused to antenatal attendees using fourth-generation enzyme-linked immunosorbent assay (ELISA) where HEV RNA quantification (viral load) test is not available in resource-limited settings (40).

Few studies showed intravenous drug abuse and HEV-infected partners also contribute to HEV transmission (41-42). In contrast, sneezing and coughing does not. Similar reports of limited knowledge of hepatitis B virus (HBV) and hepatitis C virus (HCV) transmission routes among antenatal patients have been published (30-33), with only a few studies presenting contrasting findings of high levels of awareness of HBV and HCV (34-35). Inferentially, that emphasizes the need to re-strategize on antenatal enlightenment approaches. Therefore, there is need for regular and periodic antenatal health education to improve the awareness of HEV infection. Also, factors or contributors to enhanced awareness of HEV (and by extension HBV and HCV) modes of transmission such as grassroots outreaches to antenatal patients attending primary healthcare centers (PHCs) or traditional birth attendants (TBAs), use of public enlightenment materials like flyers and leaflets, encouragements of antenatal subjects to access care at tertiary health facilities for optimal management and prevention of maternal-to-child-transmission, linkage to support organizations, and creation of facility-based viral hepatitis enlightenment team must all be identified and used as regular means of educating antenatal attendees.

Strengths and Limitations of Study

This study was the first to be carried out in Ekiti state and exemplified the importance of holistic enlightenment of antenatal patients on HEV infection as it gave answers to the knowledge gaps. Though the study has some limitations with respect to the sample size, study still gave significant baseline outcomes of relevance during the COVID-19 pandemic era. Future research should focus on multi-center studies involving more population of antenatal patients to give the bigger picture.

Conclusion

A low level of knowledge of HEV infection, its modes of transmission, risks to pregnancy and vaccine availability was recorded in our study among pregnant women, despite the risks it poses to pregnancy. Women of reproductive age group showed by the study should be particularly targeted for routine antenatal care enlightenment programme to improve their knowledge on HEV infection awareness and prevention. Educational status should not be used as a yardstick for assessing HEV awareness status. Study outcomes reflected the gaps in current national policy on hepatitis and the need for urgent review and update of the same to include result-oriented and all-inclusive antenatal patients' education on HEV infection.

Declarations

Ethical Approval and Consent to Participate

Ethical approval was obtained from the Human Research and Ethical Committee and Ethical Review Board of Federal Teaching Hospital and Ekiti State University Teaching Hospital, respectively, with these identities: ERC/2020/06/378A and EKSUTH/A67/2020/06/006. After explaining the purpose of the study, written informed consent was obtained from participating antenatal attendees.

Conflicts of Interest

The authors declare we have no competing interests.

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Author Contributions

The manuscript was conceptualized and initially designed by KAF, AAA, and AWA. The initial manuscript draft was done by OPA and KAF and edited by ZAJ, OAA, YM, OBB, and AIO. Data collection was

done by MOA, ASA, AOB, AAA and EOO. KAF and ODA contributed to data entry and analysis while all co-authors contributed to data entry assessment and the statistical analysis review. AAI, MAM, ODA, YO MIO, AA and ZAJ provided expert contributions to blood safety content of the manuscript. ASA, BAO, EOO, AOB, BSA, AB, OTA, OEA, OMA. MOA monitored antenatal attendees' recruitment, group, counselling, and inclusion and exclusion criteria during clinics being in-charge of the patients.

Table and Figure Legends

SD = Standard deviation
% = Percentage
† = Approximate percentage
EKSUTH = Ekiti State University Teaching Hospital
FETHI = Federal Teaching Hospital, Ido Ekiti
Gravidity: A term that defines the number of times a woman has been pregnant
Primigravida = A woman who conceives for the first time
Multigravida = A woman who has given birth more than once

Primary education= Six years of education

At least Secondary education = Minimum of 12 years of education.

PCV = Packed cell volume defined as a measure of the space occupied by the red blood cells in a whole blood volume.

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