

# Seroprevalence of hepatitis-E virus infection among pregnant women at a secondary healthcare facility in Abeokuta, Southwest, Nigeria

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## ABSTRACT

**Background:** Recent reports show that approximately 939 million individuals globally have experienced Hepatitis E virus infection, and 15 to 110 million have recent or ongoing HEV infection. This study aimed to evaluate the seroprevalence of Hepatitis E virus infection among pregnant women in Abeokuta, Ogun State.

**Methods:** Blood samples were collected from a cross-section of 230 consenting healthy pregnant women with no history of immuno-suppressive diseases attending Oba-Ademola Maternity Hospital, Abeokuta, Nigeria. A structured questionnaire was administered to participants containing socio-demographic information such as age, education, marital status, occupation, parity, and pregnancy trimester. Collected plasma was subjected to the analysis of Hepatitis E Immunoglobulin G (IgG) and M (IgM) using an Enzyme-Linked Immunosorbent Assay (ELISA) kit. Data were subjected to statistical analysis using SPSS version 20.0.

**Results:** Seroprevalence for anti-HEV IgG and anti-HEV IgM were 7.8% and 4.3% respectively. Seroprevalence of age group 15-19 years was 33.3% for HEV IgM and 0.0% for HEV IgG, 20-24 years was 7.5% for HEV IgG and 2.5% for HEV IgM, 25-29 years was 3.8% and 2.6% for both HEV IgG and IgM respectively. Ages 30-34 was 5.8% for both HEV IgG and IgM while ages 35-<40 was 57.3% for anti-HEV IgG and 6.5% for HEV IgM. Based on education status, those with primary education were 15.0% for HEV IgG and 10.0% for HEV IgM, secondary education was 9.3% for HEV IgG and 4.1% for HEV IgM and tertiary education was 5.3%, and 3.5% for both HEV IgG and IgM. With regards to trimester, in those in their first trimester, 18.5% seroprevalence was recorded for HEV IgG and 11.1% for HEV IgM, second trimester 8.3% for HEV IgG and 6.4% for HEV IgM, also for third trimester 4.3% for HEV IgG, however, no prevalence was recorded for anti-HEV IgM. This study shows a high rate of seroprevalence observed for both anti-HEV IgG and IgM in pregnant women who were in their first trimester.

**Conclusion:** This study reveals that a significant number of cases in the study area have had exposure to HEV in their life time.

## 1. Introduction

Hepatitis is an inflammation of the liver. The liver is a vital organ that processes nutrients, filters the blood, and fights infections, when the liver is inflamed or damaged, its functions can be affected. However, Hepatitis can be a result of viral infection though there are other possible causes of it. Such causes include autoimmune hepatitis and hepatitis that occurs as a secondary result of medications, drugs, toxins, and alcohol<sup>1</sup>. Viruses are the smallest known

infective agents and are perhaps the simplest known form of life. Viruses do not possess a cellular organization and they do not fall strictly into the category of unicellular microorganisms. They contain only one type of nucleic acid, either DNA or RNA but never both. They are obligate intracellular parasites that lack the enzymes necessary for protein and nucleic acid synthesis and depend on the host cells for replication. There are five main viral classifications of hepatitis. They are Hepatitis virus type-A, B, C, D, and E.

Different viruses are responsible for each type of viral hepatitis<sup>1</sup>.

In a recent report, approximately 939 million of the global population have at one time, experienced Hepatitis E Virus (HEV) infection, and 15-110 million individuals have recent or ongoing HEV infection<sup>2,3</sup>. These findings indicate that the Hepatitis E Virus has emerged as a global health burden requiring the implementation of specific control measures<sup>4</sup>. Hepatitis E Virus (HEV) has an interesting course in pregnant women in certain geographical regions of the world. Reports from various developing countries have shown that the incidence of HEV infection especially genotype 1, in pregnancy is high<sup>5</sup>. A significant proportion of pregnant women especially those in their third trimester can progress to fulminant hepatic failure and maternal death with a mortality rate varying from 30–100%<sup>6,7</sup>. Although the mechanism of liver injury is not yet clear, it is possible that the interplay of hormonal and immunologic changes during pregnancy, along with a high viral load of HEV, renders the woman more vulnerable<sup>8</sup>. Also, there is a very high risk of preterm delivery in pregnant women with HEV infection, with poor neonatal survival rates. The global annual burden of infection is estimated at 20 million cases, with 3.4 million clinical cases, 70,000 deaths (0.35 % mortality rate), and 3000 stillbirths<sup>6</sup>.

Hepatitis E Virus (HEV) is generally considered a waterborne disease in developing countries, transmitted by poor sanitation and faecal contamination of water supplies<sup>9</sup>. In developed countries, it is primarily a zoonotic disease and transmission is by consumption of undercooked infected meat, especially pork. Over the past 30 years, clinical cases of HEV have been identified more frequently, in developing countries. This has manifested as large outbreaks of increasing frequency due to HEV 1 and 2. The majority of these outbreaks occur in Asia (59%) and Africa (39%) while Latin America accounts for the remaining 2%<sup>10</sup>. Epidemiology has shifted from travelers returning from endemic areas with acute HEV 1 and 2 to local zoonotic transmission of HEV 3 and 4, associated with eating undercooked or raw pork, wild boar, or deer<sup>11</sup>. A recent surveillance report recorded an increase in the number of confirmed HEV cases in Europe from 514 cases in 2005 to 5617 cases in 2015<sup>12</sup>.

Hepatitis E Virus (HEV) has been isolated from humans and several animal species and, hence has been considered a zoonosis agent. Generally, HEV infection mostly causes a self-limited disease with a low case fatality rate of no more than 0.1% in adult men or non-pregnant women<sup>13</sup>. Nevertheless, HEV infection is also considered

characteristically associated with a high occurrence of symptomatic presentations of clinical syndrome in pregnant women, with a mortality rate varying from 30% to 100%<sup>14</sup>. Though the exact reasons are still unknown, hormonal changes were found in pregnant women with fulminant hepatic failure caused by HEV infection. Evidence indicates that HEV infections may pose serious threats to pregnant women resulting in congenital defects, spontaneous abortion, and even death<sup>13</sup>.

Therefore, this study aimed to evaluate the seroprevalence of Hepatitis E virus infection among pregnant women in Abeokuta, Ogun State, Nigeria.

## 2. Materials and Methods

### 2.1 Materials

Blood samples, Centrifuge, Refrigerator, Sample bottles, ELISA kit (WANTAI-China), Anti-HEV IgM ELISA kit, Anti-HEV IgG ELISA kit

### 2.2 Methods

#### 2.2.1 Study Area

The study was carried out at Oba-Ademola Maternity Hospital, Abeokuta, Ogun State in the tropical belt of Abeokuta, Southern part of Nigeria (Africa). Its coordinates are 7°9'30"N and 3°21'20"E in DMS (Degrees Minutes Seconds) (fig. 2.1). This Facility is a Government-owned Hospital that caters to the medical needs of the populace in Abeokuta –South Local Government Area of Ogun State. It was chosen for this study because it is the Hospital of first choice in the area.

#### 2.2.2 Study Population

Two hundred and thirty (230) consenting healthy pregnant women with no history of immuno-suppressive diseases, such as human immunodeficiency viruses (HIV), cancers, chronic liver diseases, and use of corticosteroids attending Oba-Ademola Maternity Hospital for antenatal care residing in different areas of Abeokuta, Ogun state, in the age range 15 to <40 years were enrolled for this study.

#### 2.2.3 Sample Size

According to Yamane's formula,  $n = N/(1+N(e)^2)$ ; where N (population size) =540, (Population of pregnant women enrolled for antenatal programmes in the Hospital within the period of this study), confidence = 95%,  $e = 0.05$  (5%) (Margin of error). The calculated sample size for the research is 230.

## 2.2.4 Ethical Consideration

Ethical approval was obtained from the Ministry of Health, Ogun State Health Research Ethic Committee with number OGHREC/467/15. In addition, individuals were provided with an informed consent form and they gave both verbal and written consent before they were interviewed. Participant data was kept confidential and protected except in cases where the researcher is legally obligated to report specific incidents.

## 2.2.5 Data and Sample Collection

Structured questionnaires about socio-demographic characteristics and other relevant information which includes age, education, marital status, occupation, and pregnancy trimester were administered to all participating subjects as well as verbal and informed consent prior to blood sample collection. Any individual who refused to give consent as well as provide necessary information on the questionnaire was excluded from the study.

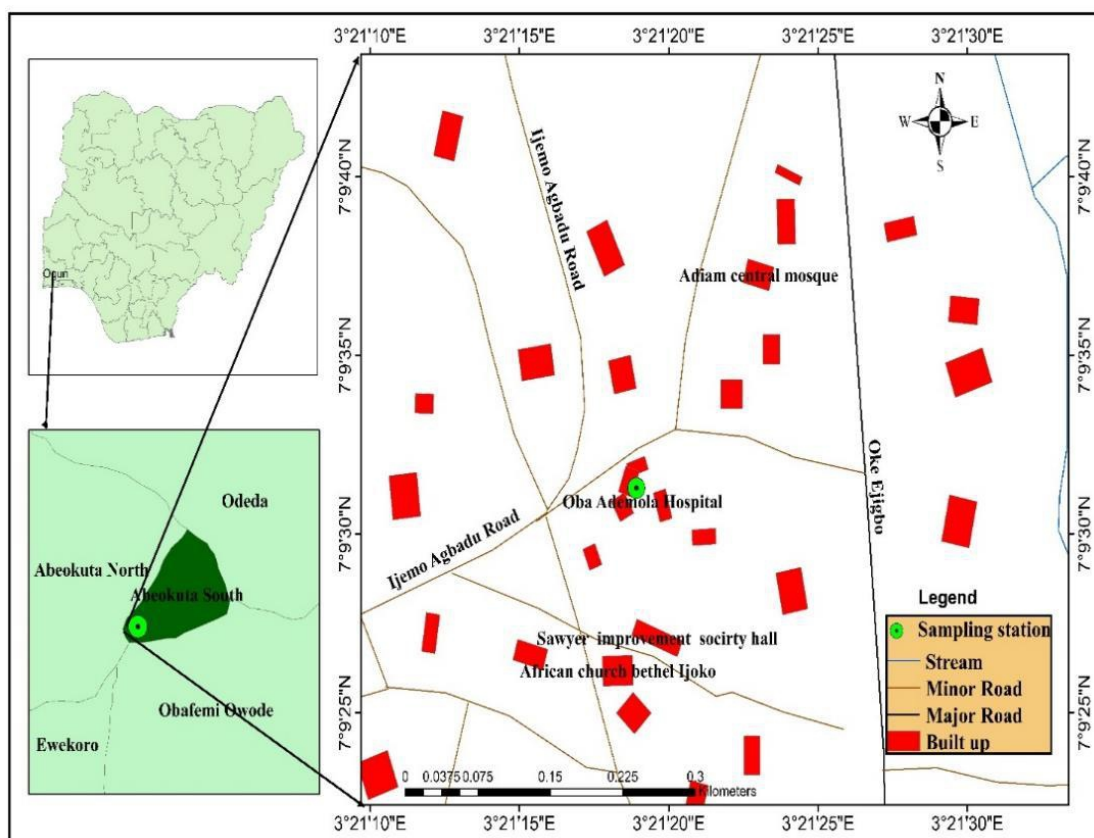


Figure 2.1 Geographical map of sample collection site (A), Oba-Ademola Maternity Hospital.

### 2.3.1 Extraction of Plasma from Blood Samples

Blood samples were collected from each participant into EDTA bottles. The blood samples were centrifuged at 1500rpm for 15 minutes. The plasma was separated and stored at -20°C for further analysis. All the plasma was screened for anti-HEV IgM and anti-HEV IgG using Enzyme enzyme-linked immunosorbent Assay (ELISA) commercial kit with high sensitivity and specificity. Assays were performed according to the manufacturer's instructions. Optical density was read using an ELISA microplate reader. The results were recorded and

interpreted accordingly<sup>15</sup>.

### 2.3.2 Serological Analysis of the Plasma using ELISA.

Ten (10) µl of Positive control, Negative control, and Specimen (plasma) was added into their respective wells using different disposable pipette tips (Note: 3 negative control wells, 1 positive control well, and 92 wells containing plasma for a plate). Specimen diluent of 100µl was added into each of the wells. The plates were covered with the lid and incubated at 37°C for 30 minutes. The wells

were washed 5 times with diluted wash buffer while allowing the micro-wells to stand for 30-60 seconds. The surfaces were blotted with blotting paper to remove excess fluid. HRP-conjugate of 100µl was added into each well. The plates were incubated the second time for 30 minutes at 37°C and washed. To each well, 50µl of Chromogen Solution A and Chromogen Solution B were added and mixed gently. The plates were incubated at 37°C for 15 minutes in the absence of light. The appearance of blue color indicates positive. Using a multichannel pipette, 50µl of Stop Solution was dispensed into each well and mixed gently. An intensive yellow color indicates a positive for HEV presence. The absorbances of the plates were read at 450nm using an ELISA microplate reader<sup>16</sup>.

#### 2.4 Statistical Analysis

- The overall mean age of pregnant women was calculated.
- The percentage of pregnant women positive for anti-HEV IgM and anti-HEV IgG was calculated.
- The mean age of pregnant women positive for anti-HEV IgM and anti-HEV IgG was calculated.
- The seroprevalence of anti-HEV IgM and anti-HEV IgG were determined among positive participants in the studied population and expressed in percentages.
- The seroprevalence of anti-HEV IgM and anti-HEV IgG were expressed in pie charts.
- Statistical associations between sociodemographic characteristics such as age, education status, trimester, and seroprevalence of HEV infection were calculated.
- The data obtained was analyzed using a Statistical Package for Social Sciences (SPSS version 20.0). Values were organized and summarized in terms of frequencies and the results of the study were

presented in tables and figures.

- The chi-square ( $\chi^2$ ) test was utilized to assess the association between sociodemographic variables and HEV status.
- Statistical significance was set at  $P \leq 0.05$ .

#### 1. Results

Based on the questionnaire, the socio-demography of the 230 participants (Table 3.1) revealed age range from 15- <40 years with mean age at  $29.36 \pm 5.87$  years. The age range 25-29 years (33.9%) represents the highest participants while the lowest age range stands at 15-19 years (1.3%). Educational status shows that 8.7%, 42.2%, and 49.1% had primary, secondary, and tertiary education respectively. Data from marital status demonstrated that 8.3%, 91.3%, and 0.4% were single, married, and separated respectively. Business owners, artisans, civil servants, and students among participants recorded 53.5%, 4.8%, 24.8% and 17.0% respectively. In terms of family type, monogamous women showed a significant value of 80.4% while polygamous women were at 19.6%. However, no report of polyandry women was observed. According to the response on risk factors (Table 3.2), values showed that portable water was accessible to 32.2% while 67.8% had no access to portable water. The incidence of jaundice was reported in 0.8% as 99.1% never had it. Data on parity revealed 24.3% in their prime, 33.0% as having one child, and 42.7% as having more than one child previously. Trimester values showed the highest in the second trimester (47.4%) followed by the third trimester at 40.9% and the least in the first trimester at 11.7%. Data from ELISA showed that 18 participants (7.8%) were positive for anti-HEV IgG, while 10 participants (4.3%) were positive for anti-HEV IgM as shown in Figure 3.3.

**Table 3.1: Socio-demographic Characterization of Pregnant Women Attending Antenatal at Oba-Ademola Maternity Hospital.**

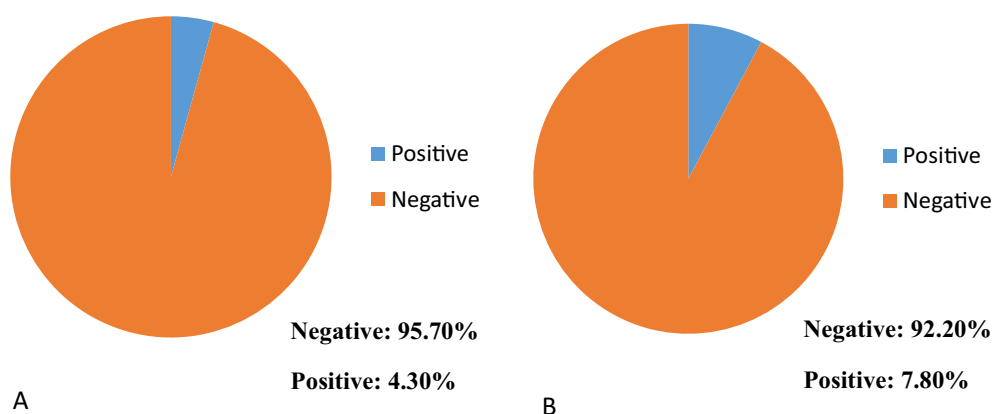
Variables	Frequency	Percentage (%)
<b>Age group</b>		
15 -19	3	1.3
20 -24	40	17.4
25 -29	78	33.9
30 -34	69	30.0
35 -<40	40	17.4

<b>Educational Status</b>		
Primary	20	8.7
Secondary	97	42.2
Tertiary	113	49.1
<b>Marital Status</b>		
Single	19	8.3
Married	210	91.3
Separated	1	0.4
<b>Occupation</b>		
Business	123	53.5
Artisan	11	4.8
Civil servant	57	24.8
Student	39	17.0
<b>Family type</b>		
Monogamous	185	80.4
Polygamous	45	19.6

Mean age: 29.36 ± 5.87 years

**Table 3.2: Risk factors Associated with the Studied Population**

Variables	Frequency	Percentage (%)
<b>Good source of water</b>		
Yes	74	32.2
No	156	67.8
<b>Parity</b>		
Once	76	33.0
Twice	62	27.0
3times	36	15.7
Prime	56	24.3
<b>Trimester</b>		
First	27	11.7
Second	109	47.4
Third	94	40.9
<b>Previous Jaundice</b>		
Yes	2	0.8
No	228	99.1



**Figure 3.1: Results for IgM (A), and IgG (B)**

### 3.1 Seroprevalence of Anti-HEV IgM and Anti-HEV IgG in relation to Socio-demographic Characteristics

The distribution of seropositive samples according to Age, Educational status, and Pregnancy trimester of the study participants are shown in Table 3.3. **Note:** There was no statistical significance ( $p > 0.05$ ) between seropositivity and the variables

#### 3.1.1 Age

Prevalence of anti-HEV IgM tend to have increased with age, from, 2.5% in the age range 20-24 years, 2.6% in the age range 25-29 years, 5.8% in the age range 30-34 years, to 6.5% in the age range 35->40 years.

A similar pattern was observed in the prevalence of anti-HEV IgG, where 0.0% prevalence was recorded in the age range 15-19 years, and 57.3% in the age range 35- <40years.

#### 3.1.2 Educational Status

According to educational status, the highest prevalence of anti-HEV IgM was recorded among those who had primary education (10.0%), while 4.1% was recorded among those with secondary education, and 3.5% was also recorded among those who had tertiary education.

Furthermore, anti-HEV IgG also had the highest prevalence among those that had primary education (15.0%), 9.3% was recorded among participants that had secondary education while 5.3% was recorded among participants that had tertiary education.

#### 3.1.3 Trimester

For anti-HEV IgM, the highest prevalence was observed in women in their first trimester (11.1%), then 6.4% was observed among those in their second trimester, while no prevalence was recorded in the population that was in their third trimester.

Prevalence of anti-HEV IgG decreased with pregnancy trimester with a prevalence of 18.5% in the first trimester, 8.3% in the second trimester, and 4.3% prevalence in the third trimester.

**Table 3.3: Association between Age, Education status, Trimester and Seroprevalence of HEV infection Among Participants.**

Variable characteristics	Seroprevalence IgM		Total	Seroprevalence IgG		Total
	Positive	Negative		Positive	Negative	
	N=10, n (%)	N=220, n (%)		N=18, n (%)	N=212, n (%)	
<b>Age Group</b>						
15 -19	1(33.3%)	2(66.7%)	3(100%)	0(0.0%)	3(100%)	3(100%)
20 -24	1(2.5%)	39(97.5%)	40(100%)	3(7.5%)	37(92.5%)	40(100%)
25 -29	2(2.6%)	76(97.4%)	78(100%)	3(3.8%)	75(96.2%)	78(100%)
30 -34	4(5.8%)	65(94.2%)	69(100%)	4(5.8%)	65(94.2%)	69(100%)
35 -39	2(6.5%)	29(93.5%)	31(100%)	4(12.9%)	27(87.1%)	31(100%)
<40	0(0.0%)	9(100%)	9(100%)	4(44.4%)	5(55.6%)	9(100%)
<b>Total</b>	10(4.3%)	220(95.7%)	230(100%)	18(7.8%)	212(92.2%)	230(100%)
<b>P-value</b>	0.96		0.01			
<b>x<sup>2</sup></b>	0.15		0.01			
<b>Odd ratio</b>	0.98		0.54			
<b>Educational Status</b>						
Primary	2(10.0%)	18(90.0%)	20(100%)	3(15.0%)	48(85.0%)	51(100%)
Secondary	4(4.1%)	93(95.9%)	97(100%)	9(9.3%)	57(90.7%)	66(100%)
Tertiary	4(3.5%)	109(96.5%)	113(100%)	6(5.3%)	107(94.7%)	113(100%)
<b>Total</b>	10(4.3%)	220(95.7%)	230(100%)	18(7.8%)	212(92.2%)	230(100%)
<b>P-value</b>	0.30		0.10			
<b>x<sup>2</sup></b>	0.42		0.25			
<b>Odd ratio</b>	1.61		1.78			
<b>Trimester</b>						
First	3(11.1%)	24(88.9%)	27(100%)	5(18.5%)	22(81.5%)	27(100%)
Second	7(6.4%)	102(93.6%)	109(100%)	9(8.3%)	100(91.7%)	109(100%)
Third	0(0.0%)	94(100%)	94(100%)	4(4.3%)	90(95.7%)	94(100%)
<b>Total</b>	10(4.3%)	220(95.7%)	230(100%)	18(7.8%)	212(92.2%)	230(100%)
<b>P-value</b>	0.05		0.05			
<b>x<sup>2</sup></b>	0.05		0.96			
<b>Odd ratio</b>	0.98		0.54			

#### 4. Discussion

Seropositivity was seen from 33.3% in the age group 15-19 years for anti-HEV IgM and 0.0% for anti-HEV IgG, 2.5% in the age range 20-24 years for anti-HEV IgM and 7.5% for anti-HEV IgG then 2.6% for anti-HEV IgM and 3.8% for anti-HEV IgG in the age range of 25-29 years, 5.8% in the age range 30-34 years for both anti-HEV IgM and IgG and 6.5% for anti-HEV IgM and 57.3% for anti-HEV IgG for the age range 35-<40. This trend is similar to what<sup>17</sup> reported in a more expanded study in some communities within Osun State Nigeria.

The differences in seropositivity rates across age groups may reflect variations in exposure to HEV over time. Older individuals may have had more opportunities for exposure to the virus compared to younger individuals, leading to higher seropositivity rates<sup>18</sup>. Additionally, the immune response to HEV infection can vary depending on factors such as age, underlying health conditions, and immune status. Older individuals may have a more robust immune response to HEV infection, resulting in higher levels of IgG antibodies<sup>19</sup>.

Behavioural factors such as dietary habits, hygiene practices, and occupational exposure to contaminated environments can influence the risk of HEV infection. Older individuals may have different lifestyle factors or occupational exposures that increase their risk of HEV infection compared to younger individuals<sup>16</sup>. Also, environmental factors such as sanitation and access to clean water sources can also affect the transmission of HEV. Older individuals may be more likely to live in environments with poor sanitation, increasing their risk of exposure to the virus, an observation which agrees with the report of<sup>20</sup>. It could also be attributed to immune senescence, which is the gradual deterioration of the immune system associated with aging. This may have impacted the susceptibility to and severity of HEV infection in older individuals. While older individuals may have higher seropositivity rates, they may also be more prone to developing severe complications from HEV infection due to age-related changes in immune function<sup>21</sup>.

Biological factors such as hormonal changes, liver function, and genetic predisposition must also be put into consideration when interpreting the result of this study. These factors could also influence susceptibility to HEV infection and the immune response to the virus. These factors may vary across different age groups and contribute

to the observed differences in seropositivity rates<sup>22</sup>.

In summary, the observed differences in seropositivity rates of anti-HEV IgM and IgG across age groups are likely influenced by a combination of factors including exposure history, immune response, behavioural and environmental factors, immune senescence, and biological factors. Further research is needed to better understand the complex interplay of these factors and their impact on HEV infection rates across different age groups.

Considering educational background, individuals who had primary education and other form of education had higher prevalence rates of anti-HEV IgM and IgG (10.0% and 15.0%) as compared to those that had secondary education rate of anti-HEV IgM and IgG (4.1% and 9.3%) and the least prevalence was recorded in women who had tertiary education rate of anti-HEV IgM and IgG (3.5% and 5.3% respectively). This finding agrees with a much related study carried out at the Federal Medical Centre Keffi, North-Central Nigeria by<sup>23</sup>.

Low educational attainment is often associated with lower socioeconomic status. Individuals with lower educational backgrounds may have limited access to healthcare resources, lower health literacy, and less awareness of preventive measures against infectious diseases like HEV. Our finding on this count was in line with what was reported by<sup>15</sup>.

Limited knowledge about proper hygiene practices, sanitation, and food safety measures may also contribute to an increased risk of HEV infection among individuals with lower educational backgrounds according to the report of<sup>24</sup>. We also believed that socioeconomic factors such as living conditions, access to clean water, and sanitation facilities may also play a role in the transmission of HEV. Individuals with lower socioeconomic status may be more likely to live in environments with poor sanitation, which can increase the risk of exposure to HEV<sup>25</sup>.

Additionally, individuals with higher educational attainment may be more likely to have access to healthcare services and information, leading to better awareness and adoption of preventive measures against HEV infection<sup>15</sup>. It is important to note that socioeconomic status is a complex and multifaceted construct influenced by various factors beyond educational attainment alone. Further research exploring the relationship between socioeconomic status, knowledge about HEV infection risk factors, and



prevalence rates of anti-HEV IgM and IgG would provide a more comprehensive understanding of these associations. The seroprevalence of the anti-HEV IgG was highest in pregnant women, especially those in their first trimester with 18.5%, while 8.3% prevalence was recorded in pregnant women in their second trimester and the lowest was recorded for those in their third trimester with 4.3%<sup>26</sup>. However, this part of our finding is in contrast to that reported by<sup>23</sup>, who found seroprevalence highest in pregnant women in their third trimester.

One potential reason behind this observation could be related to the immunological changes that occur during pregnancy. Pregnancy is associated with significant alterations in the immune system to accommodate the developing fetus and prevent rejection of the semi-allogeneic fetus by the maternal immune system<sup>4</sup>. During the first trimester, there is a shift towards a Th2-type immune response, which is characterized by the production of antibodies such as IgG. This shift helps to suppress the maternal immune response to prevent rejection of the fetus. Therefore, pregnant women in their first trimester may have a higher prevalence of anti-HEV IgG antibodies due to this immunological modulation<sup>27</sup>.

As pregnancy progresses into the second and third trimesters, there is a gradual return to a more balanced immune state. This shift may lead to a decrease in the production of IgG antibodies against HEV, resulting in the observed decrease in seroprevalence in the second and third trimesters<sup>26</sup>.

Additionally, other factors such as hormonal changes, changes in liver function, and variations in behaviour or exposure to risk factors throughout pregnancy could also contribute to the observed differences in seroprevalence across trimesters. Further research would be needed to fully understand the underlying mechanisms behind this observation.

## 2. Conclusion

This study was designed to evaluate the seroprevalence of Hepatitis-E virus infection among pregnant women in part of Abeokuta, Ogun State, Nigeria. From the findings therefore, age, educational background, and pregnancy trimester appear to influence seropositivity and seroprevalence rates of anti-HEV antibodies, reflecting a complex interplay of factors including exposure history, immune response dynamics, socioeconomic status, and

physiological changes during pregnancy. Further research is required to elucidate the underlying mechanisms and implications of these findings for HEV infection control and prevention strategies.

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