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RESEARCH **Open Access**

High Prevalence of Hepatitis E IgM Antibody among Pregnant Women in Their Second and Third Trimester in Southwest Nigeria

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ABSTRACT

Background: Pregnant women with Hepatitis E virus (HEV) infection, particularly those in the second or third trimester, are at increased risk of acute liver failure, fetal loss, and mortality; but unfortunately, routine HEV screening is yet to be introduced for pregnant women in Nigeria and many developing nations. This study was conducted to determine HEV prevalence and the associated risk factors among pregnant women unaware of their HEV status in three states of the southwestern geopolitical zone in Nigeria.

Methods: Two hundred and thirty-two consenting pregnant women aged 18 -55 years from Lagos, Oyo, and Osun state were recruited for the study and screened for HEV IgM antibodies using DIAPRO HEV IgM ELISA KIT (Milanno-Italy). Sociodemographic data and risk factors were obtained using a structured questionnaire, and data were analyzed using SPSS version 23.

Results: An overall HEV prevalence rate of 9.9% was observed among pregnant women in Southwest Nigeria. State-specific prevalence of 14.7%, 8.5%, and 6.7% was observed in Oyo, Lagos, and Osun, respectively. HEV prevalence increased with increase in trimester of pregnancy, with more than 80% of the HEV prevalence found among women in their second and third trimester. All the HEV prevalence occurring in women of active childbearing age (≤ 40 years) and among low-income earners. None of the risk factors considered was found to have a statistical correlation with HEV prevalence.

Conclusion: High HEV prevalence in the second and third trimesters was reported in southwest Nigeria. We advocate routine screening of pregnant women for HEV IgM antibody for prompt HEV detection and proper HEV management to forestall HEV complications in pregnancy.

Keywords: Hepatitis E Virus, Pregnant women, IgM, Southwest Nigeria, Trimester, risk factor

1.0 INTRODUCTION

Hepatitis E virus (HEV) is a single-stranded, non-enveloped RNA virus belonging to the family *Hepeviridae*. It is transmitted through zoonotic route by eating infected under-cooked meat, close contact with infected animals and faecal-orally from contaminated water [1]. World Health Organization (WHO) estimates that 20 million people have HEV infection globally, and 44,000 people died from HEV in 2015, accounting for 3.3% mortality due to viral hepatitis E [2]. The prevalence of HEV varies greatly worldwide, with the seroprevalence of anti-HEV antibodies ranging from 2.5% to 49.8% [3,4]. Hepatitis E virus is highly endemic in many African nations, with regional incidence rates ranging from 14 to 61 percent [5]. Primarily, HEV spread in Africa through polluted water sources, especially during floods or in unsanitary settings. This is because the virus can persist in water for extended periods and is expelled in large quantities in the faeces of infected people [6].

Hepatitis E infection has advanced significantly over the past decade, with the recognition of chronic infection, risk of progression to cirrhosis, risk factors for transmission, and treatment strategies [7]. Inflammation of the liver due to HEV has a higher prevalence and rigorous course in pregnant women, leading to high morbidity and maternal death rate of 20% compared to non-pregnant populations [8]. It poses risk to pregnant women and their newborns due to the vulnerability of pregnant women to the infection, which results in premature delivery, spontaneous abortion, and maternal and infant mortality [4,9]. Pregnancy is a unique clinical condition accompanied by several physiological changes that impact several body organs, including the liver. Due to the distinctive immunologic and physiological changes that occur before, during, and after the gestational period, pregnancy can impact every element of the viral agent, and the viral agent also has an extreme impact on pregnancy [10]. Hepatitis E virus is thus becoming a global health concern among pregnant women.

Few HEV studies have been carried out in Nigeria among pregnant women in different states [11–13], however, to the best of our knowledge, no single study reported HEV prevalence exclusively among pregnant women in more than one state in each geographical location, to give a true reflection of HEV prevalence among pregnant women in such geographical region. This study, therefore, aimed to focus on the prevalence of hepatitis E virus and the associated risk factors among pregnant women in three (Lagos, Oyo, and Osun) in the six States of the Southwestern geopolitical region in Nigeria.

2.0 METHODOLOGY

2.1 The Ethical Approval

This was part of a viral hepatitis study. Ethical approval to carry out the study was obtained from the ethics committee of the Obafemi Awolowo University Teaching Hospital Complex (OAUTHC), Obafemi Awolowo University (IRB/IEC/0004553).

2.2 Study Area

The study was conducted among pregnant women from Oyo state, Lagos, and Osun state in south-western Nigeria. These states have an estimated population of 30,468,410 people. (National Bureau of Statistics, 2019).

2.3 Study Population and Size

2.3.1 Sample Size Determination

The minimum sample size for this study was determined using the Leslie Kish formula for sample size calculation.

$$n = \frac{Z^2 P (1-P)}{e^2}$$

Where n = required sample size

Z= Standard deviation with normal deviate corresponding to a 95% confidence level set at 1.96

P= estimated prevalence of Hepatitis E infection in Southwest Nigeria. Since no study was available for this, we added the values from Lagos, Oyo, and Osun (5.3% + 4.8% + 8%=18.1%). (Lagos [14]; Oyo [15]; and Osun [16]).

e = Degree of accuracy set as 0.05

Hence,

$$n = \frac{1.96^2 \times 0.181 (1- 0.181)}{0.05^2}$$

$$n = \frac{3.8416 \times 0.181(0.819)}{0.0025}$$

$$n = \frac{0.5694}{0.0025}$$

n=227.8 (approximately 228)

This is the minimum sample size, so we used 232.

2.3.2. Study Population

The samples used in this study were obtained from healthcare facilities across three states in western Nigeria

(Osun, Oyo, and Lagos state). A total of 232 pregnant women participated in this study after informed consent.

2.4 Sample Collection

Sample collection was carried out between 24th of August and 30th of November 2021. Pregnant women attending antenatal clinics in the selected healthcare facilities in the study area had five milliliters of blood drawn from each of them by venipuncture. All the samples were centrifuged at 1500 revolutions per minute, and the serum was then put into cryovials with labels. Until tested, sera were kept at -20^{o}C . Socio-demographic factors, clinical factors, and medical history information were obtained from the pregnant women using structured questionnaires. All pregnant women who agreed to participate in the study and signed informed consent were eligible for the study. Pregnant women who did not sign informed consent were excluded from the study.

2.5 Assay Procedure

HEV IgM antibody was detected by a commercial Enzyme-linked immunosorbent assay (ELISA) kit (DIAPRO HEV IgM ELISA KIT (Milanno-Italy) according to the manufacturer's instruction. Appropriate wells were marked as one negative control, two positive controls and one blank. Fifty microliters (50µl) of neutralizing reagent were dispensed into all wells except for blank and controls. Thereafter, 100 µl of controls and diluted samples (1:100) were added into appropriate wells. A first incubation at 37°C for 60 minutes was followed by a second incubation at the same temperature and time after washing the plate. Addition of 100 µl enzyme conjugate into appropriate walls was done after the second plate washing. Afterward, 100µl of chromogen/substrate mixture (Chromogen A and B) was added into appropriate wells, followed by a final incubation at room temperature for 20 minutes. The reaction was stopped by adding 100µl of the stop solution (sulphuric acid), and results were read using a 450 nm filter microplate reader.

2.6 Statistical Analysis

The data obtained were analyzed using Statistical Package for Social Science (SPSS) version 23. Results were summarized using frequency tables, percentages, mean, and standard deviation. Hepatitis E virus-positive and negative samples were compared in terms of age group, trimester of pregnancy, sociodemographic, and associated risk factors using the Pearson Chi-square test with two -tailed significances. A p-value of less than 0.05 was considered to be statistically significant.

3.0 RESULTS

3.1 The Prevalence of HEV IgM

Of the 232 Pregnant women tested, HEV IgM antibody was found in 23 (9.9%). Oyo state had the highest prevalence of 14.7%, followed by Lagos state with 8.5 %, and the least HEV prevalence of 6.7% was observed in Osun state (Table 1)

Table 1. State Prevalence of Hepatitis E Virus infection among Pregnant Women in Southwestern Nigeria

States	Number tested	Positive (%)	Negative (%)
Oyo	75	11 (14.7)	64 (85.3%)
Lagos	82	7 (8.5)	5 (91.5)
Osun	75	5 (6.7)	70 (93.3%)
Total	232	23 (9.9%)	209 (90.1%)

3.2 HEV Prevalence by Age

The pregnant women in this study ranged from 20 to 53 years and were grouped into age categories. The highest HEV prevalence rate (33.3%) was recorded among pregnant women in age groups 20 years and below, followed by a prevalence of 12.9% recorded within the age range 31–40. The age range 21–30 had a prevalence of 8.2%, while no HEV IgM antibody was recorded in the age range 41–50 and 51-60. All the HEV prevalence rates were detected in women under or equal to 40 years (Figure 1). However, there was no statistical correlation between HEV and Age in this study (p= 0.121)

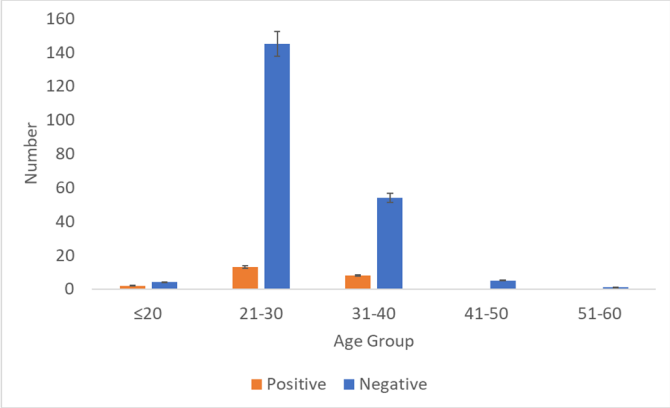

Figure 1. Hepatitis E virus Prevalence by Age

3.3 Association of HEV Prevalence with Trimester.

HEV prevalence was found to increase with increasing gestation period. Women in the first trimester of pregnancy recorded HEV prevalence of 4.8%, while a higher prevalence (11.5%) was observed in those in their second

trimester and the highest prevalence (12.3%) in the third trimester (Figure 2). More than 80% of the HEV occurred in the second and third trimester of pregnancy, but no statistical correlation (P= 0.766).

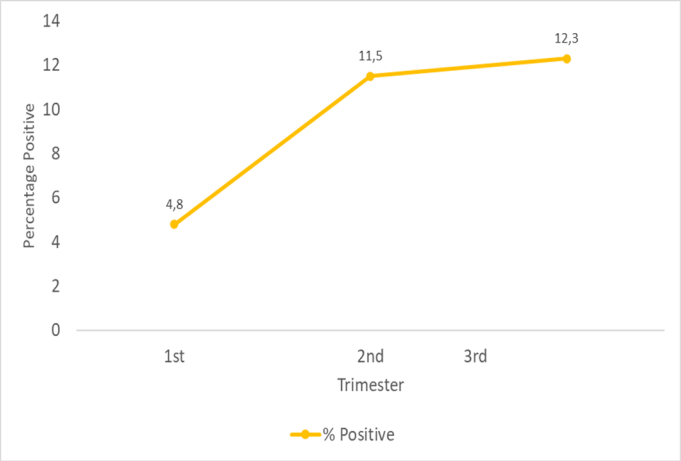

Figure 2: Hepatitis E virus Prevalence by Trimester

3.4 HEV Prevalence was inversely related to Socio-demographic factor (Income)

The higher the income of the pregnant women, the lower the HEV Prevalence. Higher HEV prevalence was found among low-income earners. A prevalence of 13.3% was observed for pregnant women who earned between 10-20 thousand naira, while a prevalence of 11.8% was recorded for women who earned 21-49 thousand naira. High income earners earning 50-100 thousand naira had a 3.0% prevalence, and no HEV was detected among the highest-income earners (Figure 3). Twenty-eight women chose not to answer the question, and two (7.1%) of these pregnant women were positive for HEV.

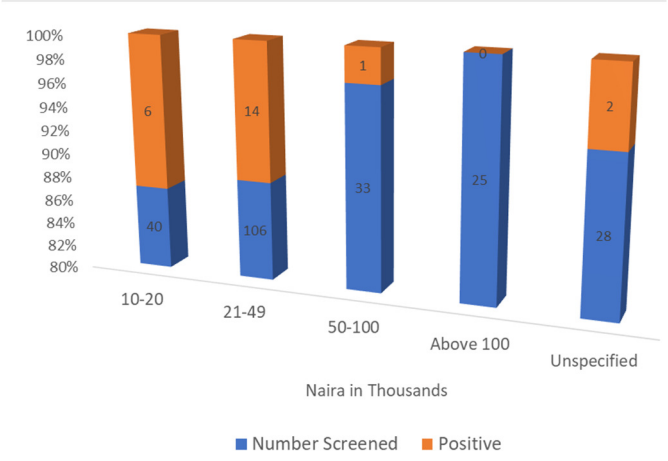

Figure 3. Hepatitis E Virus Prevalence and Income

3.5 Statistical Association between HEV and Risk Factors.

Table 2. Risk Factors and HEV Prevalence

	HEV		
Variable	**Negative**	**Positive**	**P value**
Sexual lifestyle			
Homesexual	2	0	(0.845)
Bisexual	1	0	
Heterosexual	196	23	
Total	199	23	
Sharing of Sharp Objects			
Yes	24	1	(0.268)
No	177	20	
Total	204	23	
Blood Transfusion			
Yes	27	3	(0.979)
No	177	20	
Total	204	23	
Contact or Relationship with the Infected Person			
Yes	9	1	(0.985)
No	194	22	
Total	203	23	
Surgical/Dental Procedure			
Yes	40	5	(0.853)
No	159	18	
Total	199	23	

Of all the risk factors considered, none was associated with HEV prevalence. There was no statistical correlation between the risk factors considered and HEV (P> 0.05), as shown in Table 2.

4.0 DISCUSSION

This study reports a recent HEV infection among pregnant women by detecting IgM antibodies. In the study, a high prevalence of Hepatitis E virus infection (9.9%) was recorded among pregnant women across three states in southwestern Nigeria. According to the classification for endemicity [8], this study shows that southwestern Nigeria is endemic for hepatitis E virus. Although our result shows that Nigeria is endemic for HEV, a systemic review of HEV among pregnant women in Africa showed that other African countries have reported higher HEV prevalence than Nigeria:45–84.3% in Egypt, 31.1–58% in Ethiopia, 12.5–61.2% in Sudan and 12.2%-28.7% in Ghana. North Africa was reported as the African region with the highest (50.01%) HEV seroprevalence [17]. The difference in HEV seroprevalence among pregnant women between countries has been attributed to the difference in sanitary conditions, geographical location difference, and time difference of study conduct-

ed.

To the best of our knowledge, our study was the first published data on a detailed HEV study solely among pregnant women in more than two cities in a geopolitical region in Nigeria. Previous reports on HEV prevalence among pregnant women from state surveillance from the geopolitical regions in Nigeria showed the highest prevalence (28.0%) in the southeastern part [18], Northeast HEV prevalence of 13.3% comes next [19] followed by 0.9- 12.1% in North central [20,21], although IgG report by Ahumibe *et al.,* [22] and Ehi Airiohuodion *et al.,* [23] was as high as 19% and 31.5% respectively in the region. The only HEV prevalence study among pregnant women in Northwest (Sokoto) is an IgG study with a 9.9% prevalence [24], no HEV prevalence study among pregnant women was found in the south-south region, while our study showed a 9.9% in Southwest Nigeria. Reasons that may be responsible for the difference in HEV seroprevalence among pregnant women within country include differences in sanitary conditions, socio-economic, cultural, and geographical location differences, and time difference of study conducted [17].

Regarding HEV prevalence in individual states in southwestern Nigeria, IgM prevalence rates of 0.2%, 0.9%, and 4.8% reported for Oyo state [15,25,26] were lower than the 14.7% in our study. To the best of our knowledge, no study has been conducted on HEV IgM solely among pregnant women in Lagos states, however, HEV IgM or IgG prevalence of 0.6% and 5.3% has been reported among blood donors and healthy population [14,27], which are lower than the values we obtained while in Osun state among pregnant women HEV IgM and HEV antigen prevalence of 0.2-8% have been reported [13,16,26] compared to 6.7% in this study. An increase in HEV IgM prevalence across all states was observed in this study compared with previous reports, indicating the need for urgent HEV prevention intervention programs among pregnant women across the three southwestern states.

In this study, HEV prevalence was highest among women under 20 years. All HEV pregnant women were under the age 40 years, as reported in previous studies [18,28]. The higher HEV IgM in this group could be because most women of childbearing age fall in this age group. This assertion is supported by Junaid et al., (2014), who observed that those in the age bracket ≤ 20 had the highest IgM seroprevalence but the least IgG prevalence, while the highest IgG prevalence was observed among older subjects in the age group >60 and the lowest in subjects ≤ 20, suggesting that those older women were exposed earlier in their childbearing age, hence now have IgG antibodies.

HEV prevalence was found to increase with increasing gestation period in this study. This corroborates findings that more than half of acute HEV infection occurs in the third trimester [8]. HEV infection in pregnant women has been shown to often progress rapidly into a more severe hepatitis and acute liver failure (ALF), particularly in women infected during the second or third trimester [29] and [4] reported that up to 20–25% of pregnant women can die if they get hepatitis E in the third trimester. HEV IgM surveillance should thus be introduced into routine pregnancy screening for prompt intervention.

Hepatitis E infection is common in low- and middle-income countries with limited access to essential water, sanitation, hygiene and health services [4]. Higher-income is expected to translate to a better standard of living. This could explain why pregnant women with higher income in our study had lower HEV prevalence than those with poorer remuneration. It is expected that better remuneration will translate to a better standard of living, which may include living in areas with better sanitation, and access to quality water and food, factors which have been said to influence HEV transmission [6], hence reduced exposure to the virus.

Our study showed that all the risk factors considered had no statistical correlation with HEV prevalence. This could be the setback in our study, possibly because the study was part of a more extensive study involving other viral hepatitis type, hence the questionnaire design might not have considered HEV-specific risks such as water source, animal contact, washing of hands after defaecation, method of faecal disposal etc.

The strength of this study is the detection of HEV IgM, which shows recent infection other than total antibody (IgG), which only shows previous exposure. WHO guidelines state that a definitive diagnosis of hepatitis E infection is usually based on the detection of specific anti -HEV immunoglobulin M (IgM) antibodies to the virus in a person's blood, and this is considered adequate in areas where the disease is common [2], hence our study is relevant.

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Conflicts of Interest

The authors declare that there is no conflict of interests.

Authors' Contributions

MOJ conceived and designed the study, contributed to data collection, data analysis tools, analysis of data and manuscript writing. **TUA** contributed to data collection, data analysis tools, analysis of data and manuscript writing. **APO** contributed to data collection, analysis of data and manuscript writing. **TIO** contributed to data analysis tools, analysis of data and manuscript writing. **AA, SOE** contributed to data collection, data analysis tools, review the manuscript draft and make vital resources available for the work. All authors approved the final copy of the manuscript.

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