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Seroprevalence of Hepatitis E Virus (HEV) Infection in Pregnant Women in Sokoto State, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author BRA designed the study. Authors MB and ABS performed the statistical analysis and wrote the protocol. Authors BRA and MB wrote the first draft of the manuscript. Author MK managed the analyses of the study. Authors BIA, AF, NMB and OFA managed the literature searches. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

The seroprevalence of hepatitis E virus infection among pregnant women attending Antenatal Clinic at specialist hospital, Sokoto, Nigeria was investigated. One hundred and eighty two (182) serum samples from pregnant women aged between 18-45 years were screened for the presence of anti-HEV specific IgG antibody using a commercially available ELISA kits obtained from EUROIMMUN Medical Laboratory Diagnostics AG. Information was obtained from the subjects using a well structured interviewer-administered Questionnaire. Data were entered into SPSS software version

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20.0 and analyzed using Chi-square test. An overall prevalence of 18/182(9.9%) was obtained, while the age specific prevalence rates of 11.8%, 17.1%, 4.5%, and 6.2% among the age groups 18-23, 24-29, 30-35, and 36-41 years were recorded respectively. The prevalence rates of the infection at various gestational period of pregnancy were 14.9% and 5.4% at second and third trimester respectively. However, Chi-square test has shown that there was no statistically significant association between HEV infection with age and trimester of pregnancy. Education about environmental and personal hygiene before and during pregnancy may be useful for decreasing the rate of infection in this high risk population.

Keywords: Prevalence; Enzyme Linked Immunosorbent Assay (ELISA); trimester.

1. INTRODUCTION

Hepatitis E refers to liver disease caused by hepatitis E virus (HEV). HEV is a small, spherical, non-enveloped virus that measures 32 to 34 nm in diameter. It is a major public health concern in developing countries [1], with a mortality rate of up to 30% in infected pregnant women [2]. Hepatitis E virus (HEV) is a causative agent of enterically transmitted acute hepatitis in humans [3]. It is one of the five known human hepatitis viruses: A, B, C, D and E. It is a positive-sense single-stranded RNA icosahedral virus with a 7.5 kilo base genome. HEV has a faecal-oral transmission route. Infection with this virus was first documented in 1955 during an outbreak in New Delhi, India [4].

The virus is classified into the *Hepeviridae* family in the Hepevirus genus [1]. There are four genotypes of HEV, namely; genotypes 1, 2, 3, and 4. Genotypes 1 and 2 affect humans only while genotypes 3 and 4 affect both humans and animals [5].

ELISA can be used in the laboratory to detect IgM and IgG antibodies against HEV that is specific for current and old infection. In addition, viral detection can be done by RT-PCR [6].

Studies on HEV infection are scanty in Nigeria, including Sokoto State. The study was therefore designed to determine the seroprevalence of HEV infection among pregnant women in Sokoto state.

2. MATERIALS AND METHODS

2.1 Study Area and Population

Between the month of May and August, 2016, one hundred and eighty two (182) pregnant women within the age ranges 18-45 years, attending Antenatal Clinic (ANC) at Specialist Hospital, Sokoto (located in the Sudan Savannah belt of North–West zone of Nigeria; Lying between longitude 050111 to 130031 East and latitude 130001 to 130061 North, covering an area of 60.33 km^2), who willingly consented to participate in this study were recruited. Data form was used to record the age of the participants and gestational age of pregnancy. Ethical approval was obtained from Sokoto State Ministry of Health.

2.2 Samples Collection

Five millilitres (5 mL) of venous blood was collected from each subject into sterile labelled plane Vacutainer test tubes. Each blood sample was allowed to clot. The serum was separated from the clot by centrifugation at 3000 rpm for five minutes to avoid haemolysis of the red blood cells. The serum sample was then transferred safely into 2 mL cryovial and stored at -20℃ until tested.

2.3 Serological Test

The serum samples were screened for the presence of Hepatitis E Virus IgG antibodies using Hepatitis E Virus (HEV) ELISA kit, obtained from EUROIMMUN Medical Laboratory Diagnostics AG. The test was carried out based on the manufacturer's instructions.

The general principle of the test was based on antigen-antibody reaction. The kit contains microtiter strips each with 8 break-off reagent wells coated with recombinant antigens of hepatitis E virus. In the first reaction step, diluted patient samples were incubated in the wells. In the case of positive samples, specific IgG antibodies bound to the antigens. To detect the bound antibodies, a second incubation was carried out using an enzyme-labelled anti-human IgG (enzyme conjugate) catalysing a colour reaction.

2.4 Procedure

One hundred microliters of the calibrators, positive and negative controls and diluted patient samples were transferred into the individual microplate wells and incubated for 30 minutes at room temperature. The wells were emptied and subsequently washed 3 times using 300 µl of working strength wash buffer for each wash. After washing, all liquid was thoroughly disposed from the microplate by tapping it on absorbent paper with the openings facing downwards to remove all residual wash buffers. One hundred microliters of enzyme conjugate was pipetted (peroxidise-labelled anti-human IgG) into each of the microplate wells, and Incubated for 30 minutes at room temperature. The wells were emptied, and washed as described above. One hundred microliters of chromogen/substrate solution was pipetted into each of the microplate wells, and incubated for 15 minutes at room temperature (protected from direct sunlight. One hundred microliters of stop solution was pipetted into each of the microplate wells in the same order and at the same speed as the chromogen/substrate solution was introduced.

Photometric measurement of the colour intensity was made at a wavelength of 450 nm and within 30 minutes of adding the stop solution. Prior to measuring, the microplate was slightly shaken to ensure a homogeneous distribution of the solution.

Results were evaluated by calculating a ratio of the extinction value of the control or patient sample over the extinction value of calibrator 3. The ratio was calculated according to the following formula:

Extinction value of the control or patient sample / extinction value of calibrator 3 = Ratio

Ratio <0.8: negative
Ratio ≥0.8 to <1.1: borderline
Ratio ≥1.1: positive

2.5 Statistical Analysis

The data obtained was subjected to chi square test of association between the seroprevalence of HEV with age and trimester of pregnancy using the Statistical Package for Social Sciences (SPSS) version 20 statistical software (SPSS, Inc., Chicago, IL, USA). P≤0.05 was considered significant.

3. RESULTS

3.1 Seroprevalence of HEV Infection among Pregnant Women Attending ANC in Specialist Hospital, Sokoto

Of the 182 serum samples analyzed, Anti-HEV IgG antibody was detected in 18(9.9%) pregnant women attending ANC at specialist hospital, Sokoto as shown in Table 1.

Table 1. Seroprevalence of HEV infection among pregnant women attending ANC at Specialist Hospital, Sokoto

Results	Frequency	Percentage
Positive	18	9.9
Negative	164	90.1
Total	182	100

Based on age group of the studied subjects, this study recorded the highest prevalence of 6(17.1%) in the age group 24-29 years, followed by age groups 18-23 and 36-41 years with prevalence rates of 8(11.8%) and 1(6.2%) respectively. Lowest prevalence of 3(4.9%) was observed in the age group 30-35 years (Table 2). There was no statistically significant association between Hepatitis E Virus infection and age of the pregnant women (p>0.05).

Table 2. Age specific seroprevalence of HEV infection among pregnant women in Sokoto state

Age group (years)	Number examined	Positive n (%)	Negative n (%)
18-23	68	8(11.8)	60(88.2)
24-29	35	6(17.1)	29(31.5)
30-35	61	3(4.9)	58(95.1)
36-41	16	1(6.2)	15(93.8)
>41	2	0(0.0)	2(100.0)
Total	182	18(9.9)	164(90.1)

Chi-Square= 4.84, p value = 0.345 (p>0.05); Not significant
n=Number, %=Percentage, HEV= Hepatitis E Virus, ANC=Antenatal Clinic

Table 3. Seroprevalence of HEV infection based on gestational age of pregnancy among pregnant women in Sokoto state

Trimester	Number examined	Positive n (%)	Negative n (%)
First	2	0(0.0)	2(100.0)
Second	87	13(14.9)	74(85.1)
Third	93	5(5.4)	88(94.6)
Total	182	18(9.9)	164(90.1)

Ch-Square=4.838, P value = 0.089 (p>0.05); Not significant
n=Number, %=Percentage, HEV= Hepatitis E Virus

Based on trimester of pregnancy, results from this study showed that the highest prevalence of 13(14.9%) was observed in the second trimester of pregnancy, followed by third trimester 5(5.4%) and there was no positive sample in the first trimester. This is summarized in Table 3 above. Chi square test has shown that, there was no statistically significant association between Hepatitis E Virus infection and trimester of pregnancy (p>0.05).

4. DISCUSSION

Hepatitis E is one of the important hygienic infectious problems of the world, causes about 20 million infections a year resulting to about three million acute illnesses and 57 thousand deaths annually in developing countries, mainly Asia and Africa [7]. Significant mortality rate of up to 30% heave been reported among infected pregnant women primarily those in their third trimester [8].

Of the 182 serum samples from pregnant women attending ANC at Specialist Hospital, Sokoto analyzed, Anti-HEV IgG was detected in 18(9.9%). Findings from this study are comparable to that of Tabarraei et al. [9] who reported a prevalence rate of 7.36% among pregnant women in Gorgan, Iran, Hannachi et al. [10] reported 12% seroprevalence of anti-HEV antibodies among pregnant women in Tunisia, and Taremi et al. [11] that reported a prevalence of 7.8% in their study among healthy blood donors in Tabriz, Iran.

However, lower prevalence of 3.6% was reported by Khameneh et al. [12] in their study among pregnant women in Urmia, Iran. Extremely higher prevalence rate than our findings of 45% were reported by Singh et al. [13] among pregnant women in New Delhi, India. The disparity could be due to difference in level of hygiene, water supplies, endemicity of the virus and use of different test system with varying sensitivity.

Highest prevalence of 17.1% was recorded in the age group 24-29 years, followed by age groups 18-23 and 36-41 years with prevalence rates of 11.8% and 6.2% respectively.

Lowest prevalence of 4.9% was observed in the age group 36-41 years. Results of our study indicated that, prevalence is higher in younger age groups. This could be due to lack of knowledge regarding the effect and mode of transmission of HEV infection. Our finding is in accordance with that of Adjei et al. [14] who reported that pregnant women of age groups 21-25 and ≤20 years had the high prevalence rates in Accra, Ghana, but in contrary to that of Tadesse et al. [15] and Adesina et al. [16] who reported that seroprevalence of HEV increases significantly with age. Chi square test has shown that there was no significant association between age and HEV infection (P=0.345).

From our findings, highest prevalence of 14.9% was observed among pregnant women in their second trimester of pregnancy, than 5.4% prevalence recorded in the third trimester. This is in agreement with that of Tabarraei et al. [9] who reported the highest prevalence of HEV among pregnant women in second trimester, in Gorgan, Iran, but not in line with that of Adjei et al. [14] who reported highest prevalence in the third trimester of pregnancy. The reason for the difference could be due to the fact that we did not take equal proportions of the study participants from all trimesters and this has effect on data analysis. However, Chi-Square test showed no statistically significant association between HEV infection and trimester of pregnancy (p=0.089).

5. CONCLUSION AND RECOMMENDATION

In conclusion, a considerable number of pregnant women attending ANC at Specialist Hospital Sokoto had been exposed to Hepatitis E virus. The prevalence was greater in the age group 24-29 years and in second trimester of

pregnancy. However, there was no statistically significant association between HEV infection with age and trimester of pregnancy ($p>0.05$). A careful surveillance in the general population and further appropriate investigations in order to identify the exact mode of transmission and risk groups in Sokoto state is suggested.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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