SEROPREVALENCE OF HEPATITIS C VIRUS (HCV) ANTIBODIES IN PREGNANT WOMEN IN ANYIGBA, KOGI STATE, NORTH CENTRAL NIGERIA

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ABSTRACT
Background: Hepatitis C virus (HCV) infection is a major public health concern. The aim of this study was to ascertain the seroprevalence and risk factors of HCV antibodies among pregnant women in Anyigba, Kogi State North Central Nigeria.

Materials and methods: Blood samples (5mls) were collected from one hundred and thirty consenting pregnant women attending antenatal clinic in Grimad hospital and eighty non-pregnant women from the out-patient clinic were screened for the presence of HCV antibodies. This was done by Skytech diagnostic laboratory rapid strip (USA) and confirmed by ELISA method.

Results: Out of 130 pregnant women 9(6.9%) were confirmed to be positive to HCV antibodies. Also out of 80 non-pregnant women of the same age group, used as control, 4(5.0%) were positive.

Conclusion: A prevalence rate of 6.9% calls for urgent attention by policy makers and healthcare providers to begin massive enlightenment of this problem. All pregnant women should be screened for early detection.

Keywords: Hepatitis C Virus, Pregnancy, Screening, Transmission.

INTRODUCTION
Following the identification of the hepatitis C virus (HCV) in 1989 (1) and the development of specific diagnostic assay (2), hepatitis C infection is now recognized as a worldwide public health concern (3) and accounts for 15–20% of all cases of viral hepatitis (4). Hepatitis C virus progresses slowly causing liver problems, including hepatocellular carcinoma (HCC) (5).
Hepatitis C virus infection among pregnant women in the UK is not known but only an estimate is provided to be 1% or less (6, 7). Prevalence rates in Europe have been reported to be between 1.7% and 2.5% (8, 9). The difficulty of obtaining accurate measurement of vertical transmission risk is as a result of inability to identify all infected mothers at follow-up and loss of infants born to HCV positive mothers (11). Robinson et al (12) reported that the mode of delivery (Caesarean section/normal delivery) by HCV infected mother did not appear to alter the rate of HCV transmission. However, membrane rupture is a source of HCV mother to child transmission (13, 14). Despite the fact that HCV RNA has been detected in milk, breastfeeding does not appear to play an important role in the mother-to-infant transmission of HCV (14). Since gastric acid rapidly destroys HCV transmission through breast milk has not been reported (13).

MATERIALS AND METHODS
The study was conducted at Grimad Hospital Anyigba, a secondary healthcare facility between June and December 2013 after obtaining ethical approval. A questionnaire was used to obtain information from the patients. The inclusion criteria such as personal consent, pregnant woman, HIV and hepatitis B virus negative were used while exclusion criteria included non-consent, non-pregnant and HIV, HBV negative. A total of 210 comprising 130 test samples and 80 control blood samples were collected into 5ml neat containers and allowed to clot. This was later centrifuged to collect the serum samples for analysis. These were stored at -20°C until required for analysis.

Ethical consideration
Consent of those enlisted for the research and the approval of the appropriate ethical committee had been obtained.

HCV antibody detection
Strip method: One step ANTI-HCV rapid screen test is a lateral flow, immunochromatographic screening test. Two purified recombinant antigens of HCV are used in test line as capture materials and gold conjugates. If the antibody of anti-HCV is present in the sample in concentration above the labeled, complex will be formed. This complex is then captured by the membrane, producing a visible pink-rose color band on the membrane. The color intensity will depend on the concentration of the anti-HCV present in the sample. This one step test is very sensitive and only takes about 15-20 minutes. Test results are read visually without any instrument. Procedure was according to manufacturer's instructions.

ELISA method: Enzyme-linked immunosorbent assay (Biotech Laboratories, UK). The procedure according to the manufacturer was as follows: Specimens (100µl) were pipetted into all tests well leaving 5 wells for control and blank. Negative control (100µl) was pipetted into duplicate wells that contain no diluents. Positive control (100µl) was pipetted into duplicate wells that contain no diluents. Specimen diluents (100µl) were pipetted into the first well and were left as blank. Test samples (10µl) were added in assigned wells and mixed. The plates were sealed and incubated at 37°C for 30 minutes. Each well was washed 5 times by filling with diluted wash buffer, then the plates were inverted vigorously to get water out and blotting the rim of wells absorbent paper for 30 seconds. Enzyme conjugate (Horseradish peroxides) (100µl) were added to each well except the blank and were mixed by swirling the microtitre plates. The plates were sealed and washed 5 times as in number 7 above. Substrate solution (100µl) was pipetted to each well and was incubated at 37°C for 10 minutes. Stopping solution (50µl) was added to each well to stop the color of reaction. The intensity of the reaction was photometrically quantitated with a dual filter enzyme immunoassay reader (sigma diagnostics EIA Multi-well Reader 11) immediately using O.D at 450nm, 630nm.

Statistical analysis
Data obtained from the study were analyzed using EPI INFO Version 6 for chi-squared test. Significance was accepted at p<0.05. Comparison of other parameters were done by simple percentages.

RESULTS
Table 1 assessed the awareness of the presence of HCV infection among pregnant women and the control subjects. They were observed to be largely unaware of this infection.

Table 2 showed the age distribution of HCV antibodies among pregnant women. It was observed that the prevalence of 2(7.7%) and 7(7.6%) occurred in the sexually active group of (11-20)yrs and (21-30)yrs respectively.

Table 3 showed the association of some risk factors with HCV infection among pregnant women. There was no significant difference observed.
Table 1: Awareness of HCV among pregnant women in the study

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Awareness of HCV infection</td>
<td>2</td>
<td>128</td>
</tr>
<tr>
<td>Knowledge of mode of transmission</td>
<td>0</td>
<td>130</td>
</tr>
<tr>
<td>History of any previous liver disease</td>
<td>0</td>
<td>130</td>
</tr>
</tbody>
</table>

Table 2: Age distribution of HCV antibodies among pregnant women in Grimad hospital

<table>
<thead>
<tr>
<th>Age group</th>
<th>No. of sample</th>
<th>Positive</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>11-20</td>
<td>26</td>
<td>2</td>
<td>7.7</td>
</tr>
<tr>
<td>21-30</td>
<td>92</td>
<td>7</td>
<td>7.6</td>
</tr>
<tr>
<td>31-40</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>41-50</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>TOTAL</td>
<td>130</td>
<td>9</td>
<td>6.9</td>
</tr>
</tbody>
</table>

χ² = 0.98 df = 3 P-value = 0.805

Table 3: Comparison in infection rate between pregnant and non-pregnant women

<table>
<thead>
<tr>
<th></th>
<th>No. of sample</th>
<th>No. Positive</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females (Pregnant)</td>
<td>130</td>
<td>9</td>
<td>6.9</td>
</tr>
<tr>
<td>Control (Non Pregnant)</td>
<td>80</td>
<td>4</td>
<td>5.0</td>
</tr>
</tbody>
</table>

χ² = 0.07 df = 1 P-value = 0.40 (Fisher exact)

Table 4: Possible risk factors of HCV infection among pregnant women

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Anti HCV Positive n = 9</th>
<th>HCV negative n = 121</th>
<th>Relative Risk</th>
<th>Odds ratio</th>
<th>χ²</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Previous Blood transfusion</td>
<td>2</td>
<td>6</td>
<td>4.48</td>
<td>5.48</td>
<td>1.85</td>
<td>0.096</td>
</tr>
<tr>
<td>Intravenous drug use</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Tattooing</td>
<td>1</td>
<td>5</td>
<td>2.69</td>
<td>2.90</td>
<td>0.02</td>
<td>0.355</td>
</tr>
<tr>
<td>Multiple sexual partners</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Discussion

The prevalence of 6.9% HCV infection among pregnant women observed in this study is higher than the 2.5% reported in Maiduguri (15) and 4.5% reported in Kaduna among pregnant women. It is also higher than the worldwide prevalence range 0.1% - 4.5% reported by Steven (11). According to Robinson et al, (12) 5% of pregnant women with chronic HCV infection will transmit the infection to their infants. It is therefore envisaged that 5% of 6.9% seropositive pregnant women in this study will vertically transmit the infection to their infants. In the present study no significant difference was observed. This is in agreement with other reports (16). In the present study, our observation that the risk factors of HCV seropositivity are largely obscure agrees with a report from Nigeria (16). They also observed that none of the putative risk factors
evaluated in their study showed any significant association with HCV seropositivity. This also agrees with our findings.

In Nigeria, the low risk of transmission through blood transfusion may be related to the low prevalence in the general population and also the activities of safe blood for Africa foundation (17)(SBFA) to fight the spread of HIV/AIDS and other blood borne infections.

The high incidence of seropositivity of HCV in the age group of 11-20yrs and 21-30yrs which is regarded as very sexually active group was reported in some other studies (18).

Further studies will be required to ascertain the exact relationship between this infection and high sexual activity since their partners were not screened and the number of other wives married by their husbands were not ascertained.

It has been reported that antenatal HCV testing provides an opportunity to identify asymptomatic women with a chronic disease who are likely to benefit from modern therapy with interferon and ribavirin which cures nearly 40% of patients with chronic HCV (19) and we strongly advocate this in our locality in view of the increasing incidence of HCV antibodies in pregnant women.

The most efficient transmission of HCV is through large or repeated direct percutaneous exposure to blood in case of transfusion or transplantation from infectious donors and injecting drug use (20).

There is lack of awareness of HCV infection among the educated people. The fact that the risk factors are obscure makes intervention strategies rather difficult. However, enlightenment campaigns by infection control practitioners, further research on factors that facilitate its spread are advocated. This will reduce the prevalence rates observed in Nigeria when compared with what obtains in Europe and the United States and may possibly reduce the vertical transmission from infected mothers to their infants.

REFERENCES
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11. Steven KH. Mother to child transmission of HCV. Medical writer circle 2006; 18: 1-5.