ABSTRACT

**Aim:** Hepatitis B and C viruses and HIV infections remain major global health concerns as causes of high morbidity and mortality in developing countries. This study aimed at determining the prevalence of HBsAg antibody, anti-Hepatitis C and HIV 1 and 2 antibodies in antenatal pregnant women in Nsukka, Nigeria.

**Study Design, Area and Duration of Study:** The study utilized both experimental and survey designs. Blood samples were collected from women attending antenatal clinics in two prominent hospitals in Nsukka, Enugu State, Nigeria from April to August 2016.

**Methods:** Blood samples from 200 pregnant women were collected into plain vacutainers and allowed to clot. The serum was separated and used to test for antibodies to the viral infections using rapid test kits with immobilized antigens following the manufacturer's instructions. Additional information was collected using standard questionnaire.
Results: From the results, 11 (5.5%), 9 (4.5%) and 5 (2.5%) women tested positive for HBsAg antibody, anti-HCV and HIV 1 and 2 antibodies, respectively. None of the patients was co-infected with HBV/HCV, HBV/HIV, HCV/HIV or triple infections with HBV/HCV/HIV. The mean age distribution was 28.2 years (Standard Deviation = 6.46). Marital status, age distribution and occupation were found to be statistically insignificant to the prevalence of HBsAg antibody, anti-HCV and HIV 1 and 2 antibodies (P > 0.05). Blood transfusion and history of previous surgeries were the risk factors for HBsAg and HIV 1 and 2 antibodies (P < 0.05), but not for anti-HCV (P > 0.05) among the study population. None of the respondents had body tattoo or admitted to have multiple sexual partners.

Conclusion: There are still high seroprevalence of HBV, HCV and HIV among antenatal pregnant women in the study area. Hence, there is need for integration of hepatitis screening tests among the antenatal pregnant women in the town.

Keywords: Hepatitis B surface antigen (HBsAg); anti-HCV; HIV 1&2; antibodies; antenatal; pregnant women.

1. INTRODUCTION

There has been an improvement in the treatment of Human Immunodeficiency Virus (HIV) with Highly Active Antiretroviral Therapy (HAAT) among pregnant women. However, the co-infection of Hepatitis B virus (HBV) or Hepatitis C virus (HCV) or both with HIV has been brought to the fore in recent years [1,2]. Hepatitis B virus and HCV infections are known to be the common cause of chronic hepatitis and leading causes of liver-related deaths [3,4]. Both HBV and HCV have been implicated in liver cirrhosis and hepatocellular carcinoma [3-6], while HIV infection has been widely established to target and decrease the CD4+ cell, a glycoprotein found abundantly on the surfaces of regulatory T cells, T-helper cells, dendritic cells, monocytes and macrophages [7].

The three viral infections have become so endemic in Africa countries [8], such that Perinatal and horizontal transmission of HBV and HCV is now common in many countries in the continent [1,9], where 8-10% of the population are chronic carriers [1]. Hepatitis B virus and HCV infections are of global concern, with about 350 million persons and 190 million persons being chronically infected with HBV and HCV respectively [1]. However, a more recent study [10], reported a lower global infection for HBV of about 257 million persons. About 3% of the world population is reported to be infected with HCV [3,4]. Many risk factors have been documented to be associated with single, double and triple co-infection of these viruses [1,5,7,9].

The natural causes of HBV and HCV infections show variability among individuals [11] and all three viruses have been identified to share common routes of transmission [7,11,12]. Some of the factors that predispose an individual to infection with these viruses are: transfusion of infected blood sample, tattooing, intravenous drug use, hemodialysis and abortion [1,7,11,12]. Others are unprotected sex, infants born by HBV, HCV and HIV infected mothers, reuse of non-disposable needle without sterilization and frequent dental procedures [4,5,11].

A study that was carried out among pregnant women in Umuahia, a capital city of another state in the same South East zone of Nigeria reported a prevalence of 1.7% HBsAg [13]. Another study carried out in Benue state in North Central zone of Nigeria reported a prevalence of 7.5% of HBV [14] and 10.3% of HIV [15] among women seeking antenatal care. A study carried out in Nsukka, the same study area as this study, but among prisoners and rural dwellers reported a prevalence of 9.1% HBV [16]. To the best of our knowledge, there is no published study on the prevalence of these three viruses together among pregnant women in Nsukka. Hence, this study aims at determining the seroprevalence of HBV, HCV and HIV 1/2 viruses infection among pregnant women who attend antenatal care clinics at two referral hospitals in Nsukka, Enugu State, Nigeria.

2. MATERIALS AND METHODS

2.1 Study Design and Population

A total of 200 apparently healthy pregnant women (i.e., not seeking for any other medical attention) with age range of 20-43 years, attending antenatal care from the month of April to August 2016, were enrolled into the study. The study was a cross-sectional survey carried out in two selected hospitals in Nsukka, Enugu State, Nigeria. Bishop Shanahan’s hospital Nsukka and Renaissance hospital and Maternity Nsukka are...
two hospitals used for this study; both are located in the urban area of Nsukka.

2.2 Data Collection

A structured questionnaire was administered to the participant after informed permission was obtained. The information obtained includes demographic data, previous history of blood transfusion or surgery, presence of body tattoo or body piercing among others.

2.3 Sample Collection and Testing

About 4 ml of blood sample was collected from agreed participant by venipuncture using standard procedure. Collected blood sample was emptied into plain vacutainer bottle and allowed to clot. The samples were centrifuged at 3000 round per minute (rpm) for 10 min. The serum obtained was tested for HBV using HBsAg Ultra Rapid test strips (Nicecare Diagnostics, Japan); for HCV using HCV Ultra Rapid test strips (Nicecare Diagnostics, Japan); screened for HIV-1/2 using HIV-1/2 Determine (Alere Medical, Chiba, Japan) and positive HIV-1/2 confirmed using Uni-Gold HIV Rapid Tests (Trinity Biotech, Bray, Ireland). The testing was done according to the manufacturer’s directives. Briefly, the kits were brought to room temperature, 2 drops of serum were added into the sample well of each strip and the respective buffer added. The results were read within 15 min. Test strips with band in the control region were considered valid; when bands appeared in both the test and control regions, the sample is considered positive; if a band appeared only in the control region, the sample is considered negative, when there was no band in the control region, the test is considered invalid and the sample repeated.

2.4 Data Analysis

The prevalence of HBV, HCV and HIV 1/2 were calculated as percentages of the study population. Comparison of variables was done using cross tabulations and differences in proportion were compared using Chi-square on Statistical Package for Social Sciences (SPSS) (IBM SPSS version 23). P value less than 0.05 was considered statistically significant.

3. RESULTS

In this study, a cross-sectional survey to determine the seroprevalence of HBsAg antibody, anti-HCV and HIV 1/2 antibodies among antenatal pregnant women in Nsukka was carried out. About 315 pregnant women attending antenatal care in the two clinics were approached and 200 of them agreed and participated in the study. The laboratory analysis of the samples showed that 11 (5.50%) women were HBsAg seropositive, 9 (4.50%) women were anti-HCV seropositive and 5 (2.5%) were HIV 1/2 seropositive. According to the sociodemographic analysis, the marital status of the women showed that 156 (78%) were married, and 44 (22%) were unmarried. While the percentage prevalence of HBsAg was higher in married women, the percentage prevalence of anti-HCV was almost equal in the two groups and prevalence of HIV 1/2 antibodies were higher in single women. However, the prevalence of the viral infections does not depend on the marital status (P > 0.05) as shown in Table 1.

The mean age distribution is 28.2 years (Standard Deviation (SD) = 6.46). Highest number of respondents 108 (54%) were in the age group 26-31 years and lowest numbers of respondents 2 (1%) were in the age group 38-43 years. Based on age distribution, the highest prevalence of the HBsAg antibody and anti-HCV was seen among patients between the ages of 26-31 years, while HIV 1/2 antibodies occurred highest among young adults between the ages of 20-25 (Table 1).

Again, the seroprevalence of HBV, HCV and HIV were independent of age groups (P > 0.05) (Table 1). According to occupation, HBsAg antibody was highest among civil servants at 36.36% prevalence, while HIV occurred highest among traders and students at 40% prevalence each. Data for seroprevalence of anti-HCV according to occupations were not complete and so the parameter for HCV could not be calculated. The seroprevalence of HBsAg antibody, and HIV 1/2 antibodies did not depend on occupation (P > 0.05) as shown in Table 2.

In this study, blood transfusion and previous surgery were found to be a risk factors for HBV and HIV (P < 0.05), but not for HCV (P > 0.05) (Table 3). None of the respondents had body tattoo and none admitted to having multiple sexual partners.

4. DISCUSSION

This study was carried out to determine the seroprevalence of HBV, HCV and HIV in pregnant women in Nsukka, Enugu State, Nigeria. The results of the study showed the HBsAg antibody, anti-HCV and HIV 1/2 antibodies seroprevalence among antenatal
### Table 1. Sociodemographic data and seroprevalence of HBsAg antibody, anti-HCV and HIV 1/2 antibodies among the antenatal pregnant women

| Marital status | Respondents (%) N = 200 | HBsAg +ve (%) N = 11 | HCV +ve (%) N = 9 | HIV 1 & 2 +ve (%) N = 5 | $\chi^2$ (CI= 0.05) P-value |
|----------------|-------------------------|----------------------|------------------|------------------------|--------------------------|
| Married        | 156 (78)                | 9 (81.82)            | 7 (77.78)        | 3 (60)                 | 1.067                    | 0.957                    |
| Single         | 44 (22)                 | 2 (18.18)            | 2 (22.22)        | 2 (40)                 |                          |                          |
| Age groups     |                         |                      |                  |                        |                          |                          |
| 20-25          | 52 (26)                 | 2 (18.18)            | 0 (0)            | 3 (60)                 | 16.939                   | 0.323                    |
| 26-31          | 108 (54)                | 5 (45.45)            | 8 (88.89)        | 2 (40)                 |                          |                          |
| 32-37          | 38 (19)                 | 3 (27.27)            | 1 (11.11)        | 0 (0)                  |                          |                          |
| 38-43          | 2 (1)                   | 1 (9.09)             | 0 (0)            | 0 (0)                  |                          |                          |

### Table 2. Percentage prevalence of HBsAg antibody and HIV 1/2 antibodies among antenatal pregnant women according to occupations

| Occupations | Respondents (%) N = 200 | HBsAg +ve (%) N = 11 | HIV 1 & 2 +ve (%) N = 5 | $\chi^2$ (CI= 0.05) P-value |
|-------------|-------------------------|----------------------|------------------------|--------------------------|
| Civil servant | 34 (17)                 | 4 (36.36)            | 1 (20)                 | 7.452 0.826             |
| Traders     | 34 (17)                 | 2 (18.18)            | 2 (40)                 |                          |                          |
| Farmers     | 24 (12)                 | 2 (18.18)            | 0 (0)                  |                          |                          |
| Students    | 80 (40)                 | 2 (18.18)            | 2 (40)                 |                          |                          |
| others      | 28 (14)                 | 1 (9.09)             | 0 (0)                  |                          |                          |

### Table 3. Risk factors of HBV, HCV and HIV in antenatal pregnant women

| Blood Transfusion (HBV) | Respondents (%) N = 200 | +ve (%) N = 11 | -ve (%) N = 189 | $\chi^2$ (CI= 0.05) P-values |
|-------------------------|-------------------------|----------------|-----------------|-----------------------------|
| Yes                     | 21 (10.50)              | 4 (36.36)      | 17 (8.99)       | 8.285 0.00399               |
| No                      | 179 (89.5)              | 7 (63.64)      | 172 (91.01)     |                            |
| Blood Transfusion (HCV) | Respondents (%) N = 200 |                 |                 |                            |
| Yes                     | 21 (10.50)              | 2 (22.22)      | 19 (9.95)       | 1.3780 0.240               |
| No                      | 179 (89.5)              | 7 (77.78)      | 172 (90.01)     |                            |
| Blood Transfusion (HIV 1 & 2) | Respondents (%) N = 200 |                 |                 |                            |
| Yes                     | 21 (10.50)              | 4 (80)         | 17 (8.72)       | 26.3586 0.00001           |
| No                      | 179 (89.5)              | 1 (20)         | 178 (91.28)     |                            |
| Previous History of surgery (HBV) | Respondents (%) N = 200 |                 |                 |                            |
| Yes                     | 21 (10.50)              | 4 (36.36)      | 17 (8.99)       | 8.285 0.00399               |
| No                      | 179 (89.5)              | 7 (63.64)      | 172 (91.01)     |                            |
| Blood Transfusion (HBV)                          | Respondents (%) N = 200 | +ve (%) N = 11 | -ve (%) N = 189 | $\chi^2$ | (CI= 0.05) | P-values |
|------------------------------------------------|-------------------------|---------------|-----------------|---------|-----------|----------|
| Previous History of surgery (HCV)              |                         |               |                 |         |           |          |
| Yes                                            | 21(10.50)               | 2 (22.22)     | 19(99.5)        | 1.3780  | 0.240     |          |
| No                                             | 179(89.5)               | 172(90.01)    | 4(80)           |         |           |          |
| Previous History of surgery (HIV 1 & 2)        |                         |               |                 |         |           |          |
| Yes                                            | 21(10.50)               | 20(10.58)     | 175(92.59)      | 0.4925  | 0.0364    |          |
| No                                             | 179(89.5)               | 172(90.01)    | 4(80)           |         |           |          |
| Body Tattoo/piercing (HBV)                     |                         |               |                 |         |           |          |
| Yes                                            | 0(0)                    | 0(0)          | 0(0)            | 0       | 0         |          |
| No                                             | 200(100)                | 189(100)      | 11(100)         |         |           |          |
| Body Tattoo/piercing (HCV)                     |                         |               |                 |         |           |          |
| Yes                                            | 0(0)                    | 0(0)          | 0(0)            | 0       | 0         |          |
| No                                             | 200(100)                | 191(100)      | 9(100)          |         |           |          |
| Body Tattoo/piercing (HIV 1 & 2)               |                         |               |                 |         |           |          |
| Yes                                            | 0(0)                    | 0(0)          | 0(0)            | 0       | 0         |          |
| No                                             | 200(100)                | 195(100)      | 5(100)          |         |           |          |
| Multiple sexual partners (HBV)                  |                         |               |                 |         |           |          |
| Yes                                            | 0(0)                    | 0(0)          | 0(0)            | 0       | 0         |          |
| No                                             | 200(100)                | 195(100)      | 5(100)          |         |           |          |
| Multiple sexual partners (HCV)                  |                         |               |                 |         |           |          |
| Yes                                            | 0(0)                    | 0(0)          | 0(0)            | 0       | 0         |          |
| No                                             | 200(100)                | 191(100)      | 9(100)          |         |           |          |
| Multiple sexual partners (HIV 1 & 2)            |                         |               |                 |         |           |          |
| Yes                                            | 0(0)                    | 0(0)          | 0(0)            | 0       | 0         |          |
| No                                             | 200(100)                | 195(100)      | 5(100)          |         |           |          |
pregnant women in the study area to be 5.5%, 4.5% and 2.5% respectively. In a study carried out in Yenagoa, Bayelsa State, Nigeria [17], similar prevalence rates of HBsAg, and HIV antibodies at 5.5% and 5.25% respectively were recorded, with different anti-HCV prevalence rate of 0.42% when they screened apparently healthy pregnant women attending antenatal care. Also, a related study carried out to determine the seroprevalence of viral infections (HBV, HCV and HIV) and HTLV-1 among antenatal pregnant women at Ladoke Akintola University Teaching Hospital Osogbo Southwestern Nigeria, recorded 7.1%, 2.7% and 4.9% for the prevalence rate of anti-HBsAg, anti-HCV and HIV antibodies, respectively [9]. However, a lesser prevalence rates for HBsAg and HCV antigenemia of 1.0% and 0.5% respectively, but higher prevalence rate for HIV antibodies of 8.5% were obtained after screening for the three viral infections in pregnant women attending antenatal clinic at Kogi State University Teaching Hospital, Anyibba, North-central Nigeria [18]. In another study, the same anti-HBsAg seroprevalence rate of 5.5% was obtained after screening for HBsAg seromarker among pregnant women attending antenatal clinic at Ade Oyo State Hospital and University College Hospital Ibadan, South west Nigeria [19]. When compared to the result obtained from this study, very high HBsAg seroprevalence rates of 7.5% [15], 10.2% [20], 8.3% [21], 16.5% [22], 6.6% [23], 12.5% [24], 6.08% [25] and 8.2% [26], have been recorded in different regions in Nigeria, thus demonstrating the high endemicity of anti-HBsAg among pregnant women in Nigeria.

This study and that of another [27] that screened and recorded 3.9% for the seroprevalence of anti-HCV in pregnant women at the antenatal clinic of Irrua Specialist Teaching Hospital, Edo State, have shown recent increase in HCV infection among pregnant women. This increase has become worrisome as most hospitals in the country have not integrated the screening of anti-HCV as one of the compulsory routine tests during antenatal care. In contrast to this study, other studies carried out on antenatal pregnant women at University of Port Harcourt Teaching Hospital, Port Harcourt [28], Imo State Teaching Hospital, Orlu [29] and Benue State [15], recorded 7.3%, 11.5% and 10.3% anti-HIV antibodies seroprevalence respectively, all higher than the 2.5% prevalence recorded in this study. However, another study [22] reported 2.4% HIV antibodies seroprevalence rate, which is almost the same rate with this study. Thus, considering the timeline of these reports, it may be suggested that there has been an improvement in the screening and management of HIV infection such as the use of HAART in HIV treatment. The differences observed in the seroprevalence of HBsAg, anti-HCV and HIV 1/2 antibodies among the pregnant women could be associated with demographic, sociocultural and life style differences among the interest populations.

Even though this study did not record HBV/HCV, HBV/HIV, HCV/HIV or HBV/HCV/HIV triple infections among antenatal pregnant women, the fight against any of these viral infections have been highly challenged by recent findings of their co-infections. To this end, different studies have recorded 0.5% for both HBV/HCV and HBV/HIV co-infections [2], 0.5% HCV/HIV co-infection [18], 1% HBV/HCV co-infection and 12.5% HCV/HIV co-infection [27]. Another study reported that 33% of HCV positive pregnant women were co-infected with HIV [30], while another recorded 0.08% HBV/HCV/HIV triple infection among antenatal pregnant women at Nigeria Institute of Medical Research Lagos, Nigeria [1]. Therefore, because of confirmed vertical transmission to foetus especially for hepatitis [20], more stringent measures for screening and management of these viral infections need to be instituted in most antenatal care clinics to reduce mother-to-child transmission.

This study showed that 156 (78%) of the women screened were married, while 44(22%) were single and the prevalence rate of the three viral infections were highest among the married pregnant women. However, there was no statistically significant relationship between prevalence rate of these infections and marital status (p > 0.05), which is similar to what was reported in another study [18]. By contrast, a statistically significant relationship between marital status and HBsAg antibody seroprevalence (p < 0.05) was recorded in another study [2], but the same study reported that seroprevalence of anti-HCV and HIV antibodies and marital status are statistically independent.

According to age groups, this study recorded the highest prevalence rate of HBsAg antibody and anti-HCV among pregnant women between the age of 26-31 years while the highest HIV 1&2 antibodies occurred in young adult pregnant women within the age of 20-25 years, which is similar to the results of other studies that recorded highest HBsAg antegenemia among
pregnant women in the age group 25-29 years [26,31] and highest seroprevalence of anti-HCV within the age group 25-29 years in antenatal pregnant women [27]. However, other studies had recorded higher HIV antibodies seroprevalence in higher age groups of 26-30 years [29] and 31-35 years [32]. The finding that HIV is highest among young adults between the age group 20-25 years in this study, may suggest that women at their peak of sexual activeness are more likely to be infected with HIV infection.

Occupationally, most respondents in this study were students 80(40%), but seroprevalence of HBsAg antibody occurred highest among civil servants while HIV antibodies occurred highest among the students and traders, but there were no statistically significant relationship between HBV and HIV infection with occupation. In contrast to the findings of this study, two other studies had reported that HBsAg antegenemia was highest among pregnant women who engaged in one form of trade or the other [18, 22], while another had recorded highest HIV infection among public servants [30].

Blood transfusion was found to be a possible risk factor for HBV infection (p = 0.0399), which was also recorded by another study [21], but different from what others [20,25,33,34] reported where blood transfusion and seroprevalence of HBsAg antibody among antenatal pregnant women were statistically independent. In this study, blood transfusion was also found to be a possible risk factor for HIV infection (p < 0.001) as had been reported in a different study [1], but was not in tandem with what others reported [28,35]. This survey also showed that previous surgery was found to be a possible risk factor for both HBV and HIV infections (P< 0.05) but this differed with the findings of another study [1]. This survey has it that both blood transfusion and history of previous surgeries may not increase the prevalence rate of HCV (p > 0.05) among pregnant women as had also been earlier reported [27]. None of the pregnant women in this study had body tattoo and none accepted to having multiple sexual partners.

5. CONCLUSION

This study reported relatively high seroprevalence of 5.5% for HBV, 4.5% for HCV and 2.5% for HIV 1/2 infections in the study population. The results also showed that blood transfusion and previous history of surgery are significant risk factors of HBV and HIV in women attending antenatal clinics in the study area. It is recommended that routine screening of all pregnant women for HBsAg, ant-HCV and HIV 1/2 antibodies and quick referral and treatment of these viral infections becomes paramount important to reduce chronic hepatitis in pregnant mothers and prevent perinatal transmission to children born by these women. Sequel to this, in rural and semi-urban areas with limited facilities, high index of seroprevalence of HBV and HIV viral infections should be anticipated in pregnant women and those with history of blood transfusion and history of surgery should be quickly referred to regions with higher facilities.

6. LIMITATIONS OF THE STUDY

This study had the limitation that the testing was carried out with rapid test strips. Future studies should aim at using more sensitive and specific methods like ELISA or nucleic acid based tests such as polymerase chain reaction (PCR).

CONSENT AND ETHICAL APPROVAL

Approvals for the study were obtained from each of the Hospital Research Ethics Committee. Written informed consents were obtained from participants before enrollment into the study. The inclusion criteria included women who have been confirmed to be pregnant and are attending antenatal clinic, gave consent and supplied the information contained in the questionnaire. Women who were not confirmed pregnant or who did not give consent or failed to supply the information in the questionnaire were excluded from the study. To ensure the confidentiality of the patients and other ethical implications, samples were processed without participant’s name, hospital number and other personal identification information. All data were handled with utmost confidentiality in accordance with World Medical Association (WMA) declaration of Helsinki.

ACKNOWLEDGEMENTS

We thank the Medical Directors and management of the two hospitals, Bishop Shanahan’s hospital and Renaissance hospital & Maternity, both in Nsukka for allowing us to collect samples for this study from their patients.

COMPETING INTERESTS

Authors have declared that no competing interests exist.
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