Original Research Article

Co-infection of Hepatitis B virus (HBV) and Hepatitis C virus among Human Immunodeficiency Virus (HIV) infected people: case study of Nsukka

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A B S T R A C T

HIV, HBV and HCV are major public health concerns because of shared routes of transmission; HIV-HCV co-infection and HIV-HBV co-infection are common. HIV-positive individuals are at risk of co-infection with HBV and HCV infections. This study aimed to examine HBV and HCV co-infection serologically in HIV-positive patients. This descriptive case study was carried out on 350 patients, of which 100 were HIV-positive patients: 210 males and 140 females in Nsukka community, Enugu state, to survey coinfection with HBsAg and anti-HCV. The demographic data of the subjects was collected and the patients' serums were analyzed by Test Kit and ELISA kit for HIV, HBsAg and anti-HCV. The collected data was analyzed with ANOVA. Fisher's exact test with 1% error intervals was used to measure the correlation of variables and infection rates. The results of the study indicated that the prevalence of coinfection in HIV-positive patients with hepatitis viruses was 30% (30 in 100), out of whom 20 (20%) cases were HBsAg positive, 10 (10%) cases were anti-HCV positive, and 4 (4%) cases were both HBsAg and anti-HCV positive. There was a significant correlation between coinfection with HCV and HBV among HIV-positive patients depending on different variables including sex, age, occupation, marital status, CD4+cell count.

Keywords
HIV; Hepatitis B and C; Co-infection.

Introduction

With an estimated 3,459,363 people living with HIV in 2012, Nigeria was reported to carry the second heaviest burden of HIV, with 88,864 new infections occurring in the year ended 2011 and 217,148 AIDS related deaths (Global AIDS s response Country Progress Report Nigeria GARPR 2012). Inspite of limited information on the prevalence of the triple coinfection with HIV, HBV and HCV in Nigeria, some evidence-based reports have been documented on HBV - HIV co-infection (Halim et al., 1992; Baba et al., 1998) and HIV/HBV-HCV co-infection in low risk group (Egah et al., 2007). A high prevalence of hepatitis B and C coinfection among HIV positive pregnant women was similarly reported in Enugu
in 2012 (Okeke et al., 2012). These reports suggest that HIV and Hepatitis B and C co-infection in Nigeria has therefore become a common place. The phenomenon could be traced to a wide range of factors including mode of acquisition and the wide-spread incidence of immunosuppression from AIDS and related opportunistic infections. HIV-related immunosuppression increases the viral replication and viral load of both HBV and HCV. The preponderance of these infections with the associated high mortality rates of liver cirrhosis and damage could be attributed to both perinatal (mother to child horizontal transfer) as well as the daily increasing incidence of immunosuppression resulting from decline in CD4 cell count and antiretroviral drug use. The consequence is continued progression of HIV to advanced disease, AIDS and subsequently increased morbidity and mortality among people living with HIV/AIDS. HIV and Hepatitis B and C co-infection concurrently grossly affects both the diagnosis and management of HIV.

HBV is a DNA virus that forms stable circular covalently closed (ccc) DNA with capacity of high persistence in the liver cells. Individuals positive for the core antibody, particularly the immunocompromised, those in antiretroviral use as well as people exposed to long use of steroidal prescriptions stand at a very high risk of the viral reactivation and progression. Hepatitis C virus is the major cause of nonA, nonB hepatitis, and is a serious and more common co-infecting viral infection in HIV disease. Co-infection of hepatitis C virus and HIV was reported to be associated with rapid decline in the CD4 count, rapid progression of HIV infection and increased morbidity and mortality (Sungkanuparph et al., 2004). In spite of the similarity in the mode of transmission of HBV and HIV, HBV is however considered more efficient than HIV: 30% of transmission rate was observed to occur following a needle-stick injury compared with 0.25% for HIV. The paradigm and consequence of the co-infection are a higher risk of cirrhosis and end-stage liver disease, and a more frequent flares of hepatic transaminasases, which can occur with immune reconstitution inflammatory syndrome (IRIS) owing to ART, interruption of HIV/HBV treatment, or the development of resistance to HIV/HBV treatment, as well as an increased rate of HIV progression to AIDS among the dually infected.

This study investigated the seroprevalence of HIV and HBV and HCV co-infection in Nsukka area of Enugu State, Nigeria (using HBV surface antigen [HbsAg] as a marker for HBV), with the view to establish paradigm of the co-infection and the at risk group which would serve as evidence-based model in the epidemiologic surveillance and management of HIV and hepatitis co-infection in the local setting.

Materials and Methods

Study Location

This study was conducted at the District hospital Enugu-Ezike, and Bishop Shanahan Hospital, Nsukka, designated as Referral Centres in Nsukka district for the screening (diagnosis), treatment and care of people living with HIV/AIDS.

Participants’ Characteristics

The study participants consisted of 350 patients (210 males, and 140 females) at in
and Out-patients ward of the designated Referral Centres. One hundred patients confirmed to be positive for HIV 1 and 3 antibodies by the abridged Enzyme Linked Immunosorbent Assay (ELISA) were finally recruited into the study from August to June, 2012. Informed consent was obtained from each patient with the assurance of confidentiality. Information on demographic, clinical manifestation, blood transfusion, sexual behaviour and intravenous drug use was obtained from participants using structured, pre-evaluated questionnaire. Control group consisted of participants who had no detectable antigen or antibodies to Human Immunodeficiency Virus, Hepatitis B Virus, Hepatitis C Virus infections, HIV /HBV co-infection and HIV/HCV co-infection.

Sample collection

Five milliliters (5mL) of venous blood was collected from each patient using sterile syringes and needles and dispensed into sterile testtubes (universal containers) and coded appropriately. The blood was allowed to clot and then spun at 1000 xg for 10 minutes and the serum samples separated into 2mL cryorials containers and stored at -20°C until use.

Viral Assay

Detection of HIV 1 and 2 antibodies

Antibodies to HIV 1 and 2 were determined by abridged Enzyme Linked Immunosorbent Assay (ELISA) using commercially available abridged ELISA Kits: (ACON HIV ½, ACON Diagnostics’ USA REF HH - 401, Bio System, USA N0 098 KE) and confirmed by a second stage confirmatory tests of two - three rapid test kits with different principles (Capillus HIV ½ Assay, Trinity Biotech Ireland and Determine kit list No 7D 23-43, Abbot Japan Co. Ltd) of antibodies and antigen testing methods as recommended by WHO for resource low countries including Nigeria (CDC, 2004) at 99.7% Confidence Intervals.

Detection of HBsAg

The HBsAg one-step ultra hepatitis B surface antigen test strip a qualitative, solid phase, two-sites and wich immunoassay for the detection of HBsAg in serum or plasma (ACON Lab.Inc., IHBSGu-301,USA,2003) was used. The membrane was pre-coated with anti-HBsAg antibodies on the test line region of the strips. The testing protocols consisted of the serum or plasma specimen reacting with the particles coated with anti-HBsAg on the membrane, generating a coloured line.

Laboratory detection of anti-HCV

The ACONHCV one-step hepatitis C virus test strip, a qualitative, membrane based immunoassay for the detection of antibody to HCV in serum or plasma was used. The test procedure is similar to that of HBs Ag detection by ACON on estepultra package insert method. The relative sensitivity, specificity ,and accuracy of the test was greater than 99.9%, 98.6% and 99.3% respectively and the precision was 98%(ACONLab.INC. IHC-301,USA,2003).

Data Entry and Analysis

Chi- square contingency table was used for data analysis .Percentages and ratios were calculated and results presented as Tables and Figures. Odds ratio and P-values were used to assess the strength of
association of statistical significance at 99% confidence Interval (CI) (P < 0.01).

**Result and Discussion**

The demographic characteristics of patients co-infected with HIV and hepatitis B and C in the rural community of Nsukka, Enugu State are here presented. Out of the 350: 210 (60%) males, and 140 (40%) females screened for HIV, 100 (60 males, and 40 females) were HIV-seropositive. Significant at $F_{\text{cal}}$: 11.63 (P <0.01) (Table 1).

The preponderance of HIV infection was higher among those within the 31-40 age bracket (29%), than those in other age groups: <11 years (10/100 – 10%), 11 – 20 years (16/100), 21 – 30 years (29/100), 41 – 50 years (7/100) while those above 50 had (1/100) (Figure 1).

Highest prevalence of single infection with HIV (40%), was found among students engaged in different levels of education; farmers (20%) and Traders (20%). A significant relationship was therefore found between occupation and HIV infection in the study group as shown in (Figure 2). significant at $F_{\text{cal}}$ : 11.63 ( P <0.01).

A lower viral load expressed as CD4 cells occurred among a great number of people living with HIV/AIDS, evident from the larger number of HIV infected people with lower CD4 cell count, which was highest in the group within 250 – 350uL/cell CD4 range. This relationship was significant at $F_{\text{cal}}$: 11.63 (P <0.01) as elucidated in Table 2. Profile of individuals with single infection with HIV at different CD$^4$ count is similarly presented (Figure 3).

The distribution of HIV among the surveyed population according to their marital status is shown below. Preponderance of HIV infection was observed among the single persons than the married and the divorced groups in the order: 70% > 20% >10% (Figure 4).

The preponderance of single HBV (single infection) in HIV-positive patients, was higher than was observed in HIV sero-negative groups. The prevalence of Hepatitis B virus was however higher among Female HIV patients than the Males (Figure 5) Significant at $F_{\text{cal}}$: 15.28 (P <0.01). Co-infectivity was similarly higher among the HIV infected people within the 31-40 age group (35% ), than other age brackets infected with HIV (Figure 6). $F_{\text{cal}}$: 15.28 (P <0.01)

Paradigm of occupational distribution of single and multiple infection with HIV and HBV indicated a preponderance of both multiple and single infectivity among the Student population (Figure 7). A significant correlation was also established between the occupation of the screened patients and predisposing conditions for infection with HBV in HIV-positive patients at $F_{\text{cal}}$: 15.28 (P <0.01).

Available results also demonstrated that co-infection with HBV and HIV produced a significantly reduced CD$^{+}$ cell count and consequently, a much more reduced viral load among HIV positive patients. This is evident from the larger number of HIV infected people with lower CD4 cell count, which was remarkable within the 250 – 350uL/cell CD4 range. A significant relationship was established between the co-infection and increased CD4 count at $F_{\text{cal}}$: 15.28 (P <0.01) as elucidated in Figure 8.

An association was similarly observed between marital status and the dual infection; HIV and HBV. The co-
Table 1. Gender prevalence of HIV in surveyed population.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Total Patients (%)</th>
<th>HIV Positive</th>
<th>HIV Negative (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>210 (60)</td>
<td>60 (0.29)</td>
<td>150 (0.71)</td>
</tr>
<tr>
<td>Female</td>
<td>140 (40)</td>
<td>40 (0.28)</td>
<td>100 (0.72)</td>
</tr>
<tr>
<td>Total</td>
<td>350</td>
<td>100</td>
<td>250</td>
</tr>
</tbody>
</table>

Figure 1. Single HIV distribution among different age brackets

Figure 2. HIV distribution among different Occupational Groups
Table 2: Baseline CD4+ count with Patient HIV status

<table>
<thead>
<tr>
<th>Baseline CD4+ count (uL/cell)</th>
<th>Total Patients (%)</th>
<th>HIV Positive (%)</th>
<th>HIV Negative (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;250</td>
<td>100 (28.5)</td>
<td>25 (0.25)</td>
<td>75 (0.75)</td>
</tr>
<tr>
<td>250-350</td>
<td>200 (57.2)</td>
<td>60 (0.3)</td>
<td>140 (0.7)</td>
</tr>
<tr>
<td>&gt;350</td>
<td>50 (14.3)</td>
<td>15 (0.3)</td>
<td>35 (0.7)</td>
</tr>
<tr>
<td></td>
<td>350</td>
<td>100</td>
<td>250</td>
</tr>
</tbody>
</table>

Figure 3: Single infection of HIV at different CD4+ count

Figure 4: Prevalence of Single infection among different Marital status
Figure 5: Gender Prevalence of Hepatitis B Virus among HIV patients.

Figure 6: Age specific distribution of Hepatitis B Virus and HIV co-infection

Figure 7: Prevalence of Single HBV and HIV/HBV coinfection among different occupational groups
Figure 8: Prevalence of Hepatitis B Virus and HIV/HBV coinfection at different CD4+ count

Table 3: Distribution of Hepatitis B Virus and HIV/HBV co-infection among marital status

<table>
<thead>
<tr>
<th>Marital Status</th>
<th>HIV and HBV co-infected</th>
<th>HIV without HBV</th>
<th>Control with HBV</th>
<th>Control without HBV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single</td>
<td>12</td>
<td>58</td>
<td>8</td>
<td>167</td>
</tr>
<tr>
<td>Married</td>
<td>5</td>
<td>15</td>
<td>2</td>
<td>48</td>
</tr>
<tr>
<td>Divorced</td>
<td>3</td>
<td>7</td>
<td>-</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>80</td>
<td>10</td>
<td>240</td>
</tr>
</tbody>
</table>

Figure 9: Gender prevalence of HCV and HIV coinfection
infection of HIV and HBV was prevalent amongst the single, unmarried group (60%) than other marital status (Table 3).

There was nonetheless a significant relationship between marital status and the predisposing conditions for infection with HBV in both HIV-positive and HIV-negative patients, including multiple partnership and heterosexual relationships. Prevalence of multiple infections with HIV and HCV was also apparent in the study. Infection rate of HCV among HIV positive persons was 20% compared with 6% among HIV negative patients. The preponderance was nevertheless higher among males (80%) than females (20%). The gender difference was statistically significant at $F_{cal}: 58.52$ ($P < 0.01$) (Figure 9). The observed prevalence rate of the dual infections was however higher among the HIV infected within the 21-30 age bracket (25%) than the HIV infected in other age groups. Similarly, among the HIV negative control group, preponderance of the coinfection was observed among those aged 41-50 years (Figure 10) ($P < 0.01$).

In relation to occupational groups, the coinfection rate was higher in the student group (50%) (Table 4). No significant difference was observed in the rate of coinfectivity among the HIV without HCV group ($P < 0.01$). The multiple infection similarly resulted to a significant reduction in CD$^{+}$ cell count and a much more reduced viral load among HIV positive patient, predominantly observed in the group within 250–350uL/cell CD4 range. This relationship was significant at $F_{cal}: 58.52$ ($P < 0.01$) (Figure 11).

The distribution of HCV among screened groups shows that there is no statistical relationship between HCV single infection of HIV sero-negative patients and coinfection of HCV and HIV patient. Moreover, infection rate with HCV in HIV-positive patients was higher than that found among HIV negative patient. The single HIV patients had more cases of HCV than those who stated otherwise in their questionnaire, while among the HIV negative patients the single patient also lead. (Figure 12) significant at $F_{cal}: 58.52$ ($P < 0.01$)

Overall prevalence rates of infection, Out of the 350 patients screened: 210 (60%) males, and 140 (40%) females for HIV, 100 (60 males, and 40 females) were HIV-seropositive. While for co-infection of HIV with HBsAg and anti-HCV antibodies among patients the results thus: 20(20%) and 10(10%) respectively. The rate of the total HBsAg co-infection was 20% (20 in 100) in HIV-positive patients, while in the HIV negative population HBsAg was 4.2% (10 in 240). Among the males, HIV/HBV co-infection was seen in 7(1.4%) out of the 350 patients, while among the females, HIV/HBV co-infection was observed in 15 (4.2%) out of the 350 patients. The rate of the HCV co-infection was 10% (10 in 100) in HIV positive patients, However, single infection rate of HCV with HIV sero-negative patients was 2.5% (6 in 240). Among the males, HIV/HCV co-infection was seen in 8 (2.2%) out of the 350 patients while among the females, HIV/HCV co-infection was found in 2(0.5%) out of the 350 patients. (Figure 13)

Nigeria is amongst the countries with the highest number of people living with HIV with Enugu state topping the chart among the South-eastern state of the country (WHO, 2012; NACA, 2012). The reported
Figure.10 Age specific distribution of Hepatitis C Virus and HIV/ HCV coinfection

Table.4 Pattern of Co infectivity of HIV and HCV among occupational groups

<table>
<thead>
<tr>
<th>Occupation</th>
<th>HIV and HCV Patients</th>
<th>HIV without HCV</th>
<th>Control with HCV</th>
<th>Control without HCV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Student</td>
<td>5</td>
<td>35</td>
<td>2</td>
<td>98</td>
</tr>
<tr>
<td>Farmer</td>
<td>-</td>
<td>5</td>
<td>-</td>
<td>13</td>
</tr>
<tr>
<td>Civil Servant</td>
<td>3</td>
<td>17</td>
<td>2</td>
<td>48</td>
</tr>
<tr>
<td>Trader</td>
<td>-</td>
<td>15</td>
<td>-</td>
<td>37</td>
</tr>
<tr>
<td>Others</td>
<td>2</td>
<td>18</td>
<td>2</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>90</td>
<td>6</td>
<td>244</td>
</tr>
</tbody>
</table>

Figure.11 Different CD^4 count in Hepatitis C Virus and HIV/ HCV coinfection
co-infection rates of HBV and HCV in HIV patients have been variable worldwide depending on the geographic regions, risk groups and the type of exposure involved (NACO, 2005). However, the study indicated that HIV-infected patients are at a high-risk of viral co-infections, as evident from the high prevalence of HBV (20%) and HCV (10%), which is fairly higher than the HBV and HCV prevalence reported in the Nigerian general population (Chandra et al., 2003). The study showed that the case study group predominantly comprised of heterosexually acquired HIV infections than other mode of transmission and the male gender were significantly (60% vs. 40%) higher than females. This is in accordance with previous studies which reported that male subjects were significantly at a higher risk to develop HBV coinfection (Sudet et al., 2001). Higher prevalence of the HBV/HIV coinfection (35%) was similarly observed in the 31-40 age-group, which also represents the most sexually active age group with the highest HIV sero-positivity in Nigeria (Poudelet et al., 2006), which further confirms the sexual route as the common mode of transmission for both HBV and HIV.

This study observed that the incidence of HBV co-infection rises with HIV disease progression, and that significant rates of co-infection occurred among both the symptomatic and asymptomatic groups of HIV infected patients. The co-infection seems to have had pronounced effect on the natural history of these infections. The increased viral replication of HBV in AIDS patients indicates that HIV significantly affects the HBV life cycle and the host ability to clear HBV infection. If this holds true, more HBV infection and more chronic carriers would be expected as the AIDS epidemic expands in this part of the country. Such a profile would have worrisome public health implications since more chronic liver diseases, including HCC, would be expected as the mortality rate associated with HIV is reduced (Shinreet et al., 2003). Chronic HBV infection can be associated with severe liver damage in HIV positive drug abusers and homosexuals.

Figure. 13 Overall prevalence of HIV, HBV and HCV

![Graph showing overall prevalence of HIV, HBV, and HCV](image)

Study Cases

- Control patient
- HCV
- HBV
- HIV
- Total
HIV infection does not seem to attenuate and may even worsen HBV associated chronic liver damage (Wright et al., 1994). The long-term effect of immunodeficiency on the outcome of hepatitis B infection remains to be evaluated.

The reported HCV/HIV co-infection rate in this study is in conformity with other reports from South (Padmapriyadarsini et al., 2006). However, in contrast to the HIV/HBV co-infection observed among the sexually active age group viz. (31-40 years), the HIV/HCV coinfection was higher (50%) among the age group 21 – 30 years, which implies that HCV transmission could, in addition to the possibility of sexual transmission, have been non-sexual and/or parenteral. The frequency of anti-HCV among HIV subjects (1.1%) in this study was nonetheless much lower than that reported previously amongst HIV/HCV co-infected Nigerian subjects (Poudelet et al., 2006) The low frequency of the observed HCV could be due to the low incidence of intravenous drug use (IVD), and infrequent rates of blood transfusion in the study population, compared with other parts of Nigeria where IVDs and blood transfusion history were the main risk factors identified for HCV infection among HIV patients (Kumarasamy et al., 2003). In our present report, the 2' individuals co-infected with HIV/HCV neither had blood transfusion history, IVDs use, tattooing, piercing, nor sexual promiscuity with IVDs users, but nevertheless their history presented details of high risk sexual behaviors and prior exposure to STIs, suggestive that sexual intercourse could as well, have been the route of the dual infection. In line with this observation, this study therefore further suggests that prior exposure to sexually transmitted infections including HIV, facilitates HCV transmission, and proposes that, though sexual transmission of HIV/HCV multiple infection is not widely documented, however, studies with more cohort (larger population size) under various risk groups matched with controls should be embarked on.

In relation to the effect of multiple infection with HIV and hepatitis on CD4 count, a remarkable reduction in patients’ CD4 count was apparent. Several of the multiply infected patients had CD4 cell count less than 200cells/μL. This is to be expected since the multiple infections affect the immunocompetence of the patients; and HCV/HBV thus acting as cofactors for HIV disease progression with resultant reduction in CD4 cells. This study therefore underscore the fact that in a individual with the triple infection, there are higher levels of each of the viral particles in the blood, with resultant reduction in CD4 cell; both high HBV DNA levels and the presence of HBeAg were dramatically and separately associated with reduced CD4^+T cell counts in the triply infected patients. A possible explanation to this is that the replication paradigm of HCV and HBV is a double bullet which tends to increase the apoptosis of CD4^+ T cells by efficiently increasing T cell activation and thereby facilitating HIV RNA replication, with subsequent reduction in CD4^+T cell counts (as opined by Nakamura et al., 1996), and subsequently more rapid progression to hepatitis-related liver diseases, and increased risk for cirrhosis and liver cancer. The preponderance of reduced CD4^+ cell count and a much more reduced viral load among HIV positive patient is evident from the larger number of HIV infected people with lower CD4 cell count, which was highest in the CD4 range within 250 – 350μL/cell CD4 range.
This relationship was significant at $F_{cal} = 58.52$ (P <0.01).

The findings of this study strengthen the evidence for the significance of HIV infection on the natural history of chronic HBV/HCV infections, which, by prolonging the period of infectivity could have influenced the epidemiology of both infections, i.e HBV/HCV infection in Nigeria (Poudelet et al., 2006). It is thus far clear that apart from other infections, HIV infected individuals have a high probability of getting co-infected with HBV and/or HCV. HIV disease progression and enhanced immunosuppression has a direct bearing on the natural history and pathogenesis of these infections. Sexual transmission of both HBV and HCV also appears to be significant, and is of epidemiological importance in the light of high heterosexual transmission of HIV in Nigeria. The knowledge of co-infection in HIV positive patients is vital since these patients, as they live longer on antiretroviral treatment will also need to be managed for their co-infection with HBV and/or HCV. Hence, there is an urgent need to conduct detailed studies on the interplay of HIV and hepatotropic viruses in the rural Nigerian communities, especially in the Nsukka area of Enugu State, with a plethora of multifaceted approaches to investigate the paradigm of HIV/hepatotropic viral infection crisis at the earliest to efficiently control and manage these emerging public health threat.

Acknowledgements

The authors of this work sincerely appreciate the various Heads of Departments and Staff of the participating Hospitals and Clinics as well as individuals whose advise or critical criticisms made this study a huge success.

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