Sero-Epidemiological Study of Hepatitis B and C Viruses among HIV-Positive Individuals Attending State Hospital Asaba, Nigeria

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Abstract

Hepatitis B and C virus infections are common among HIV-infected individuals. This study aimed at determining dual and triple infection rates of these viruses and correlating with immune status ($CD4^+$ count) of HIV-infected individuals attending State Hospital Asaba, Nigeria. Blood samples and socio-demographic data were collected from 182 HIV-infected participants. HBsAg and anti-HCV antibody levels in blood samples were detected using Enzyme Linked Immunosorbent Assay (ELISA). Dual (HIV-HBV, HIV-HCV) and triple (HIV-HBV-HCV) infections were 6.6%, 6.6% and 0.6% respectively. HCV infection was strongly associated with gender, age and $CD4^+$ count of the participants. Baseline screening of HIV-infected persons is recommended for early detection and appropriate treatment options.

Keywords: HIV, HBsAg, anti-HCV, co-infection

1.0 Introduction

Human Immunodeficiency Virus (HIV) is undoubtedly one of the greatest pandemics that confront humans today [1]. Various efforts have been made to control and ultimately eradicate it. However, the problem of case management is being compounded by cases of co-infection with other dangerous viruses including Hepatitis B virus (HBV) and hepatitis C virus (HCV) [2]. Co-infection is common and is an emerging concern in the clinical management of HIV patients because of increased mortality and accelerated hepatic disease progression [2].

According to estimates from UNAIDS, there were 34.0 million people living with HIV worldwide in 2011, up from 29.4 million in 2001.The increase is attributed to new infections, people living longer with HIV and general population growth [3]. On the other hand, an estimated 320-350 million individuals are chronic carriers of HBV and about 1.5 million people die annually from HBV-related causes [4] while approximately 170 million people are chronically infected with HCV [5]. The rates of co-infection of hepatitis B and C viruses with HIV vary geographically and according to subpopulation studied. Prevalence of HIV co-infection with HBV ranges from 6.3% to about 39% [6,7] while HIV with HCV co-infection rate ranges from 8% to 30% [8,9]. Studies from different parts of Nigeria indicated that HIV/HBV and HIV/HCV co-infection rates are high in the country [7, 10-12].

Whether HBV and HCV infection affect the natural history of HIV infection remains debated. Studies have reported rapid decline in $CD4^+$ cell count, accelerated progression to AIDS and increased morbidity and mortality in HIV/HBV and HIV/HCV co-infected than HIV mono-infected individuals as well as reduced rate of survival among co-infected subjects [13,14]. Other studies also reported no effect of HBV infection on the risk of acquiring an AIDS-defining condition or on overall mortality [15,16]. However, HIV infection is well known to affect the clinical outcome of both HBV and HCV infections. HIV-infected persons are half as likely as HIV-uninfected persons to spontaneously clear HBV when infected with HBV at adulthood [17]. Similarly, HIV patients chronically infected with HBV have higher levels of HBV DNA and lower rates of clearance of the hepatitis B envelope antigen (HBeAg) [18]. Furthermore, HIV/HCV co-infected individuals are less likely to clear HCV without treatment, with an estimated 85% developing chronic HCV after acute infection [19]. This reduced rate of clearance of HBeAg and HCV is partly attributed to diminished cellular immune response characterized by weak $CD8^+$ T cell and $CD4^+$ T cell immune activity [20].

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Also in the presence of co-infection, the rate of HCV replication is enhanced resulting in higher serum and liver HCV RNA levels. Ultimately, HIV increases the risk of hepato-toxicity, liver cirrhosis and end-stage liver diseases in HBV and HCV infected individuals due to antiretroviral therapy [21]. The increased risk is more pronounced in HIV infected individuals with very low CD4 count [22]. Additionally, low CD4 count has been associated with loss of previously developed protective anti-HBs antibody and development of acute hepatitis B infection in HBV infected individuals co-infected with HIV [23].

In the United States and Europe, expert guidelines recommends screening of all HIV-infected persons for infection with HCV and HBV with appropriate management of those found to be chronically infected [24]. In Nigeria however, with high endemicity of both HBV and HCV infections, mandatory screening for both infections in HIV-infected individuals will ensure early detection and guide treatment options. This study was therefore conducted to determine the seroprevalence of hepatitis B and C virus infections, and correlate the findings with immune status ($CD4^+$ count) of HIV-infected persons and to provide useful data for appropriate health authorities.

2.0 Materials and Methods

2.1 Study Design and Subjects

The study was a cross-sectional, secondary health facility-based study conducted between April and September, 2012 at the State Hospital Asaba, Delta state, Nigeria. The subjects included 182 (36 males and 146 females: age range 3-75 years; mean age 36.6 years) confirmed HIV-infected individuals assessing healthcare in the hospital. The subjects were randomly selected irrespective of age, state of health and gender. Inclusion criteria include HIV infection while all those who are infected and declined consent were excluded from the study. Socio-demographic data were collected using interviewer-administered questionnaire forms. For the purpose of analysis, the participants were divided into two categories based on $CD4^+$ cell count (i.e. ≤ 200 and > 200 $CD4^+$ count categories). Approval to carry out the study was obtained from Delta State Hospital Management Board with reference number T^A/A.583/104.

2.2 Collection of blood samples

About 5 ml of blood samples were aseptically collected from each participant into ethylene diamine tetraacetic acid (EDTA) bottles. The blood was centrifuged and the plasma was aspirated into new Eppendorf tubes, appropriately labelled and stored at -20°C until assayed.

2.3 Serological Assay

Samples were tested for the presence of Hepatitis B surface antigen (HBsAg) and anti-HCV antibodies using commercially available AccuDiag™HBsAg ELISA and AccuDiag™ HCV Ab ELISA (manufactured by Diagnostic Automation/Cortez Diagnostics, Inc., Calabasas, CA, USA) respectively. All the assays were performed according to the kits manufacturer's instructions. Detection of HBsAg was based on the principle of antibody "sandwich" ELISA method while detection of anti-HCV antibodies was based on Indirect ELISA: Double Antigen Sandwich Coated Plate. For the HBsAg ELISA assay, cut-off optical density value was calculated by $Nc \times 2.1$, where Nc= the mean absorbance value for three negative controls. For the anti-HCV assay, cut-off optical density value was calculated by $Nc + 0.12$, where Nc = the mean absorbance value for three negative controls. Absorbance was read at 450 nm wavelength. Samples giving absorbance less than the cut-off value were considered negative while samples giving an absorbance greater than, or equal to the cut-off value are considered initially reactive. Samples with absorbance to cut-off ratio between 0.9 and 1.1 are considered borderline. All reactive samples were repeated in duplicate tests before accepting as positive.

2.4 Statistical Analysis

Data were analysed using Statistical Package for Social Sciences (SPSS version 21). Chi square was used to test association. A p value of <0.05 was considered significant.

3.0 Results

Overall seroprevalence of 6.6% (12/182) was recorded for both HIV/HBV and HIV/HCV dual infection among the study participants. Triple infection of HIV, hepatitis B and hepatitis C was found to be 0.6% (1/182) (Fig. 1).

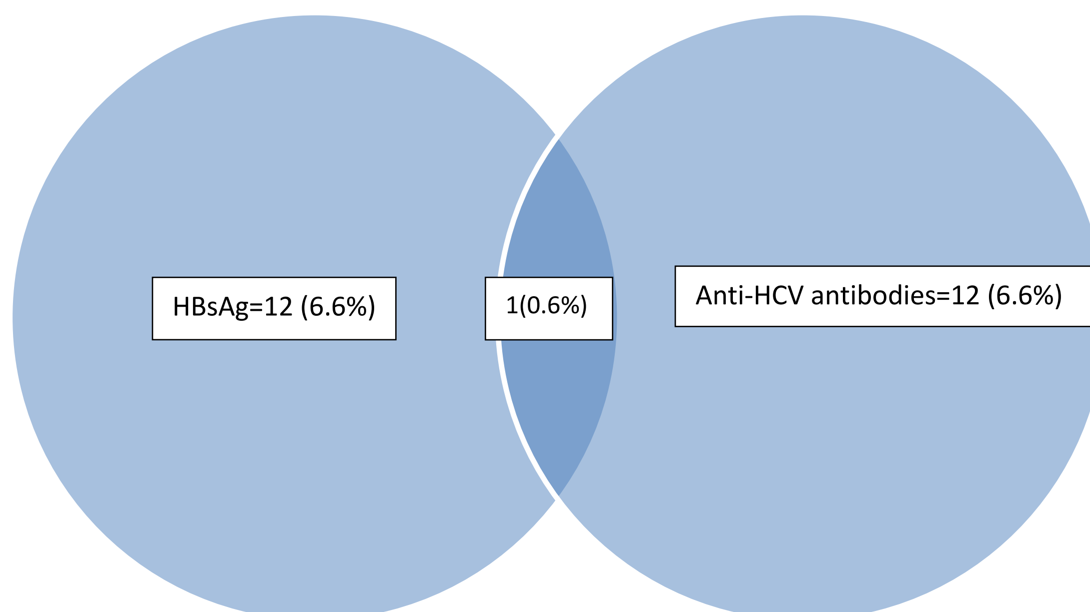

Fig.1: Distribution of HBsAg and anti-HCV antibodies among the HIV-infected study participants

Demographic variables of the participants showed that modal age group was 21-40 years with 63.7% (116) of the study participants while age group ≤ 20 years had the least, 2.2% (4) number of participants. The participants who declined disclosure of their age were classified as undetermined. Sex distribution of the participants shows that 19.8% (36) were males while 80.2% (146) were females. Analysis of the result by gender shows higher co-infection rates of 11.1% and 16.7% for HIV/HBV and HIV/HCV respectively among the male participants compared to female's 5.5% and 4.1% prevalence rates respectively for HIV/HBV and HIV/HCV co-infections (Table 1).

The result also shows a steady increase in the rate of both HIV/HBV and HIV/HCV co-infection with increasing age as shown in Table 1. Highest co-infection rate of 25% for both HIV/HBV and HIV/HCV were however recorded among participants whose ages were not determined (Table 1). It was also observed that participants with $CD4^+$ of ≤ 200 count/μL had higher prevalence rates for either HBsAg or anti-HCV compared to participants with $CD4^+$ of >200 count/μL as shown in Table 1.

Table 1: Relationship between Study Predictors and Serologic Outcomes among HIV-Infected Participants in State Hospital Asaba, Delta State

Predictors	Number (%) tested	Number (% positive) HBV	P values	Number (% positive) HCV	P values
Gender					
Male	36 (19.8)	4 (11.1)		6 (16.7)	
Female	146 (80.2)	8 (5.5)	P=0.3994	6 (4.1)	P=0.019
Age group (years)					
≤20	4 (2.2)	0 (0)		0 (0)	
21-40	116 (63.7)	6 (5.2)		2 (1.7)	
>40	54 (29.7)	4 (7.4)		8 (14.8)	
Undetermined	8 (4.4)	2 (25)	P=0.162	2 (25)	P=0.0017
CD4+ count					
≤ 200	52 (28.6)	6 (11.5)		8 (15.4)	
> 200	130 (71.4)	6 (4.6)	P=0.1703	4 (3.1)	P=0.0071

4.0 Discussion

Hepatitis B surface antigen sero-prevalence of 6.6% recorded among this cohort of HIV-infected individuals is comparable to 6.0% prevalence recorded in Nairobi, Kenya [25]. The result is however a clear departure from the trend that have been observed in different parts of Nigeria [11,26] where relatively higher prevalence were reported. The departure from high to relatively low prevalence is therefore a welcome development and calls for sustenance of efforts towards further reduction in the prevalence.

The observed anti-HCV antibodies prevalence of 6.6% in this study is however high when compared with other similar studies conducted in the country. Prevalence rates of 2.3% [12] and 3.9% [11] have earlier been reported from Abuja and Ilorin respectively. As expected, the prevalence obtained in this study is significantly lower when compared with results from more developed countries. Prevalence of 35% [27] and 36.2% [28] were reported from USA/Europe and Brazil studies respectively. The relatively low prevalence in Nigeria versus high prevalence in more advanced countries have been attributed to low level of indulgence of Nigerians in intravenous drug use (IDU), which has been observed as a major driving force for the transmission of HCV [29]. This study also showed that 0.6% of the study participants had evidence of both hepatitis B and C viruses. This result is however not surprising considering the shared route (sexual) of transmission of these viruses. Similar finding have earlier been reported [30].

Grouping the study participants by age expectedly showed the modal age group as 21-40 years (sexually active group) with 63.7% (116) of the study participants while age group ≤20 years had the least [2.2% (4)] number of participants. The higher percentage (80.2%) of female participants in the study is similar to a trend reported in Benin [30]. This is consistent with earlier observation that more Nigerian adult females seek medical care than men [7].The finding was however in sharp contrast with studies in western countries of Spain [8] and Italy [31] which reported higher participation of males.

Although greater percentage of males was infected by HBV, the difference is insignificant ($p>0.05$). On the other hand, HCV infections is significantly ($p<0.05$) higher in male participants. This is also consistent with pattern earlier reported by Udeze *et al*. [11] among similar study group in Ilorin, North-Central Nigeria. The suggested reasons for this pattern include predilection of males for aggressive sports and activities that may result in injury with bleeding, societal acceptance of multiple sexual partners for men than women [32], and mandatory circumcision of the males compared to females [33].

Analysis also showed significant association ($X^2=15.08$, $p=0.0017$) between HIV/HCV co-infection with advancing age. Although there is an increase in prevalence of HIV/HBV co-infection with increasing age, the difference was not significant ($X^2=5.12$, $p=0.162$). This result is consistent with earlier report from Jos [7] in which highest prevalence of HIV/HBV co-infection was reported among age group 51-60 years but differs from report of Udeze *et al*. [11]. Higher prevalence among older participants in this study suggests that acquisition of the infections in this locality occurs predominantly at adulthood rather than vertical transmission (mother to child). Furthermore, Hepatitis B vaccine was first introduced as part of the National Program on Immunization in Nigeria in 2004. Children born prior to that time therefore were not protected against the infection hence higher prevalence among older participants.

Since $CD4^+$ count is acceptable as indicator of immunologic deficiency or proficiency; we investigated differences between HBV and HCV in relation to $CD4^+$ count of the HIV-1 infected participants. Higher prevalence of both HBV and HCV were found among participants with CD4 of ≤ 200 count/µL compared with their counterparts with CD4 of >200 counts/µL. This finding supports earlier suggestion of possible detrimental effect of viral hepatitis on HIV infection [34–36] and may partly explain the decline in $CD4^+$ count of subjects co-infected with HBV and HCV. Similar findings have earlier been reported [37].

5.0 Conclusion

Significantly high rate of co-infection of HBV and HCV with HIV were recorded in this study. Although the observed prevalence of HBV is lower than reported rates in different parts of the country, the rate for HCV is significantly higher and strongly associated with immune status of the patients. This has the potential of complicating treatment and prognosis in these patients. Routine screening of all HIV-infected persons is therefore advocated to enable early detection and guide treatment options.

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