

Incidences of Hepatitis B and Syphilis Co-Infection with HIV in Antiretroviral Treatment-Naïve Adult Patients Attending APIN Clinic at a University Teaching Hospital in Lagos, Nigeria

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Abstract

Hundred (37 males and 63 females) sera samples of HIV patients were screened for hepatitis B antigens and antibodies, and 80 (28 males and 52 females) of these samples were also screened for *Treponema* specific antibodies using ELISA. Seven percent (5% males and 2% females) were positive for hepatitis B surface antigen (HBsAg). Four percent (3 males and 1 female) of the latter were also positive for the hepatitis B envelope antigen (HBeAg). While 5% (1% male and 4% female) were positive for hepatitis B surface antibody, 7% (1% male and 6% female) were positive for hepatitis B envelope antibody (HBeAb). Twenty three percent (8% males and 15% females) were positive for hepatitis B core antibody (HBcAb). Thirty seven percent of the patients have had previous exposure to hepatitis B virus as shown by positive results obtained for HBsAg, HBcAb and HBeAb. The mean CD4⁺ counts for positive samples were 228 for HBsAg, 3278.5 for HBeAg, 199.5 for HBsAb, 194 for HBeAb and 232.5 HBcAb. Twenty percent (10% males and 10% females) of the patients were positive for the syphilis antibodies. The mean CD4⁺ counts for the positive samples for syphilis were 290.5. The 7% prevalence of hepatitis B virus observed in this study is within the rates that had been previously reported, but the 20% prevalence of syphilis is high. This calls for a review of screening regimen that would assay for these bloodstream infections alongside HIV, in order to mitigate any influence they may have in the progression of HIV to AIDS.

Keywords: Hepatitis B; Syphilis; Co-infection; HIV; ARV treatment-naïve; APIN clinic

Abbreviations: HIV: Human Immunodeficiency Virus; APIN: AIDS Prevention Initiative in Nigeria; ELISA: Enzyme Linked Immuno Sorbent Assay; CD4: Cluster of Differentiation 4; AIDS: Acquired Immune Deficiency Syndrome; ARV: Anti Retroviral; HBV: Hepatitis B Virus; DNA: Deoxyribonucleic Acid; STD: Sexually Transmitted Diseases; VCT: Voluntary Counseling and Testing; HAART: Highly Active Antiretroviral Therapy

Introduction

The association between HIV, HBV and sexually transmitted disease (STDs) has been established. The Hepatitis B Virus (HBV) and Human Immunodeficiency Virus (HIV) are viruses that share certain epidemiological characteristics such as risk populations and transmission routes with syphilis. This puts HIV positive individuals at risk of co-infection with hepatitis B or syphilis or both. For HIV and HBV co-infection (HIV/HBV), the sero prevalence ranges from 6.3% to as high as 39% [1-3] while values as high as 27.5% and 12.4% have been reported for men and women for syphilis respectively [4].

In areas of low endemicity, such as North America, Australia and Europe, HBV and HIV infection are usually acquired in adulthood through sexual or percutaneous transmission and the prevalence of chronic co-infection is around 5-7% [5]. In countries with intermediate and high HBV endemicity, the main routes of transmission of HBV are perinatal or in early childhood. In these countries, HBV co-infection rates are 10-20% [6-8]. The rate of progression and complications from viral hepatitis has been reported to be accelerated in patients with HIV co-infection [9,10]. HIV/HBV co-infected individuals are 6 times more likely to develop chronic hepatitis B than HIV negative individuals [11-

13] and this is more likely to occur in HIV infected men with lower CD4⁺ cells [12]. Decreased rates of clearance of HBeAg and increased HBV replication are also seen, with higher HBV DNA viral load [14-16]. In addition, HIV infected individuals are more likely to lose previously developed protective anti-HBs antibody and develop acute hepatitis B infection; this risk is also associated with lower CD4⁺ counts [17,18]. However, it has been established that hepatitis B infection does not hasten HIV disease progression or severity [19].

Studies have shown that the clinical manifestations of certain STDs such as syphilis facilitate the transmission of HIV [20,21]. With syphilis, there is an increase in the shedding of HIV in coinfecting individuals [22], because of the presence of a chancre which provides a mechanical break in the protective skin barrier, allowing access to the spread of HIV. Griemberg et al. [23] reported a prevalence of 8.7% of HIV/syphilis co-infection in a study conducted in Argentina. According to their report, the prevalence of syphilis among HIV infected persons was almost twofold higher in men than women (67.7% vs. 36.0%, p=0.020). In Nigeria, the prevalence of syphilis infection among HIV patients has

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Received December 03, 2012; **Accepted** January 17, 2013; **Published** January 22, 2013

Citation: Opara MI, Ogbebor VO, Fasasi MA, Akanmu SA, Bamiro BS, Ayolabi CI, et al. (2013) Incidences of Hepatitis B and Syphilis Co-Infection with HIV in Antiretroviral Treatment-Naïve Adult Patients Attending APIN Clinic at a University Teaching Hospital in Lagos, Nigeria. J AIDS Clin Res 4: 191. doi:10.4172/2155-6113.1000191

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been estimated to be in the range of 0.4%-2.7% depending on the risk population. The highest syphilis prevalence (2.7%) has been detected among the armed forces [24].

This research is therefore an attempt to further investigate the incidences of HBV and syphilis co-infection with HIV among antiretroviral treatment-naïve patients with a view to determine the prevalence of these bloodstream pathogens in these patients.

Materials and Method

Study centre

The research was carried out in a non-governmental AIDS clinic; Aids Prevention Initiative in Nigeria Clinic. The clinic is a referral centre saddled with the responsibility of counselling, testing and providing care and support for the HIV patients and has a yearly patients' enrolment of over 1000.

Ethical approval

The ethical issues pertaining to being reviewed by the Ethics and Research Committee of the Lagos University Teaching Hospital (LUTH).

Recruitment of study subjects

One hundred HIV patients comprising 37 males and 63 females, aged between 18-60 years, who were first time attendees at the clinic between April, 2011 and March, 2012 were recruited into the study. Some of these patients came for VCT, while others were on referral from various health facilities within the locality of the teaching hospital. Standardized questionnaire was administered to the patients after obtaining their informed consent. Information on other parameters such as CD4⁺ counts and viral loads were obtained from medical records of the clinic.

Inclusion/exclusion criteria

Male and female patients who had attained 18 years or above were tested and confirmed HIV positive by the screening protocols of the clinic. Those that were yet to commence anti-retroviral treatment at the time of blood sample collection were considered eligible for the study, while those already on antiretroviral treatment were excluded.

Statistical analysis

The Statistical Package for the Social Sciences (SPSS, version 10.0, Chicago, IL, USA) was used to calculate mean values and percentages.

Screening of patients

The patients were screened for HIV 1 and 2 by the clinic using the Alere™ HIV 1 and 2 Determine™ kits (Cat. NO. 7D2342, 7D2343; Alere Medical Co., Ltd, Chiba, Japan).

Presumptive positive samples were confirmed (double-checked) with the Uni-Gold™ HIV kits (Cat. No. 1206502N; Trinity Biotech PLC, Co Wicklow, Ireland).

Sample collection and processing

Five milliliters of venous blood were aseptically drawn into the plain vacutainer bottle and was allowed to stand undisturbed for 2 hr, after which it was spinned in the centrifuge at 15,000 rpm for 5 mins. The resulting sera were aseptically transferred into another plain vacutainer bottle, properly labelled and kept for the serological assay.

Assay for Hepatitis B virus antigens and antibodies and syphilis antibodies

The screening for HBV antigens (HBsAg and HBeAg), antibodies (HBsAb, HBeAb, HBcAb) and Syphilis antibodies were done using Enzyme Linked Immunosorbent Assay (ELISA) (Biotec Laboratories Soffolk,UK) and ELAgen Syphilis New Generation ELISA kits (Cat. No.081043/081044; Adaltis Italia S.p.A) respectively. Before running the assay, all samples and reagents were allowed to warm up to ambient temperature.

Procedure

Each sera sample was diluted to final concentration of 1:100 by adding 10 µl of the serum to 1 ml of the sample diluents. The microtitre plate was appropriately labelled and 100 µl of the diluted samples were then placed into the wells of the microtitre plates in duplicate, negative and positive controls were also set up by pipetting 100 µl into respective wells in duplicate. (The inside of the microtitre plates were already coated with the hepatitis B antigens or antibodies and syphilis reagents, which constitutes the solid phase, depending on which of the marker is being assayed for). The plate was then incubated for 30 min at 37°C, after which the wells were washed with the buffered washing solution (care being taken to avoid cross-contamination during flicking). The washing step was repeated up to 5 times in order to remove unbound antibody or antigen as the case may be. The plate was drained by inverting it over an absorbent tissue paper, after which 50 µl of the enzyme conjugate was added to all the wells. This was followed by another round of incubation at 37°C for 30 min, washing and draining as described above. Thereafter, 50 µl of the chromogen (enzyme substrate) was dispensed into each of the wells, and the plate was allowed to incubate at ambient temperature for 15 min, during which period, the color develops. The color development was aborted by the addition of 50 µl of the stopping reagent into each well; the plate was then marked for orientation and fed into the ELISA reader where the absorbance (optical density) of each well was read at 450 nm within 10 min of stopping the reaction.

All assays were run in duplicate. The Cut off value for each batch was calculated using the mean optical densities (M. O. D) of the negative control according to the manufacturer's instruction. This cut off value was then used to calculate the activity index for each sample by dividing the MOD of each sample with the cut off value. Samples with activity index values higher or equal to those of positive control were considered positive, while those with values below were reported as negative.

Results

Table 1 shows the distribution of the patients screened for HBV and syphilis according to sex and age range. Sixteen percent of the patients were within the age range 18-27, 47% within 28-37, 28% within 38-47, 8% within 48-57 and 1% was above 57. The results obtained for the

Age Range	Number screened for hepatitis B virus antigens and antibodies		Number screened for syphilis antibodies	
	Male (%)	Female (%)	Male (%)	Female (%)
18-27	1 (1%)	15 (15%)	1 (1.25%)	13 (16.25%)
28-37	11 (11%)	36 (36%)	10 (12.5%)	31 (38.75%)
38-47	17 (17%)	11 (11%)	11 (13.75%)	7 (8.75%)
48-57	8 (8%)	0 (0%)	6 (7.5%)	0 (0%)
≥58	0 (0%)	1 (1%)	0 (0%)	1 (1.25%)
TOTAL	37 (37%)	63 (63%)	28 (35%)	52 (65%)

Table 1: Distribution of HIV patients according to Age and Sex.

Assay carried out	Number of samples tested	Number of samples positive		Total	Mean CD4+ counts of positive samples
		Male (%)	Female (%)		
HBsAg	100	5 (5%)	2 (2%)	7 (7%)	228
HBeAg	100	3 (3%)	1 (1%)	4 (4%)	278.5
HBsAb	100	1 (1%)	4 (4%)	5 (5%)	199.5
HBeAb	100	1 (1%)	6 (6%)	7 (7%)	194
HBcAb	100	8 (8%)	15 (15%)	23 (23%)	232.5
Syphilis Ab	80	8 (10%)	8 (10%)	16 (20%)	290.5

Table 2: Prevalence of hepatitis B antigens and antibodies, syphilis antibodies and mean CD4+ counts of positive samples.

HBV (Table 2), showed that 7% of the patients comprising 5% males and 2% females were positive for HBsAg. Four percent of the HBsAg positive samples were also positive for HBeAg. Five percent comprising 4% females and 1% male were positive for HBsAb while 11% male and 6% female were positive for HBeAb. Nine percent male and 21% female were positive for HBcAb. Thirty seven percent of the patients were positive for at least one of the HBV antigens or antibodies, while 5% had been successfully immunized as shown by the positive result for HBsAb. More positive samples for the HBV antigen and antibodies were recorded among male and female within the age range 28-37 followed by those within the age range 38-47. While the mean CD4+ counts for HBV positive samples were 234 for HBsAg, 336 for HBeAg, 165 for HBsAb, 215 for HBcAb, and 153 for HBeAb, 16% of the male and 33% of the female patients tested had a CD4+ count of less than 200. The result (Table 1), obtained for syphilis antibodies revealed that 17.5% of the patients were within the age of 18-27, 51.25% were within 28-37, 22.5% were within, 7.5% were within 48-57 and 1.25% was over 57 years old. Sixteen (20%) patients comprising 8 (10%) males and 8 (10%) females were positive for syphilis antibodies (Table 2). However, the ratio of females to males (52:28) in the sample shows that the male had a higher percent rate of the infection (28.6%) as against 15.4% observed for female. The mean CD4+ count for the positive samples for syphilis was 290.5.

Discussion

The prevalence of HBsAg among HIV patients in this study was 7%. Positive test for HBsAg suggests late incubation period of acute hepatitis and incidences of acute hepatitis among HIV patients in Nigeria had been reported [1]. Four (57%) of the HBsAg positive samples were also positive for HBeAg. These patients may be suffering from active or chronic hepatitis depending on their HBcAb results. Five percent of the patients that were positive for HBsAb have either been successfully immunized against the virus or have developed a natural immunity as a result of previous exposure to the virus. Twenty three percent and 7% of the patients that were positive for HBcAb and HBeAb respectively have had previous exposure to the virus even though they did not progress to full case of hepatitis. However, they are not immune to the virus and may eventually go on to suffer hepatitis. In all, at least 37% of the patients have had contact with virus but not all of them had clinical evidence of the infection. The 7% prevalence was slightly higher than 6.3% prevalence reported by Uneke et al. [1] in Jos, Nigeria suggesting that the viral infection may be on the increase among the population at risk. HIV and hepatitis B virus, share common mode of transmission; through sex and infected blood or blood products and greater percentage of Nigerian population do not practice safe sex through consistent use of condom. The fact that most of the study subjects (over 98%) were categorized as heterosexual, the highest risk of the transmission for both infections among the HIV patients could be through unprotected sexual intercourse. This assertion could further be justified by the fact that 75% of patients positive for hepatitis aged

28-47, which is considered to be age of active sexual life among male and female. There was no co-relation between CD4+ values of positive samples and those negative for hepatitis B virus antigens and antibodies. The mean CD4+ counts obtained for the positive samples were above the threshold values of 200 except for HBsAb (165) and HBeAb (153) which do not stand for active hepatitis infection.

Untreated hepatitis may eventually lead to liver cirrhosis and cancer and subsequent death [25]. There is also an association between hepatitis and elevation of liver enzyme. This finding highlighted some challenges to be encountered in treating patients who were co-infected, especially regarding which HAART regimen to use, how to prevent further hepatic damage, and when to initiate HAART, especially in resource-limited settings with limited ARV options [26].

Twenty percent of the patients-8% male and 8% female were positive for syphilis antibodies. This rate was higher than the 2.7% projected by the National Behavioral Survey [24] for syphilis infection among Nigerians. Syphilis and HIV are both transmitted sexually and so it is no surprise that a substantial number of people are infected with both agents. HIV has several effects on the presentation, diagnosis, disease progression, and therapy of syphilis. Syphilis may increase the risk of HIV transmission and acquisition by causing genital ulcers. Controversy has surrounded the area of HIV and syphilis co-infection. Some have argued that syphilis have a different clinical presentation and is more aggressive in people with HIV; others suggest there is little difference. Men and women who are sexually active are at a more risk of infection with syphilis. As was observed in this study, positive results for syphilis antibodies were more for patients within the range of 18-57. There was no correlation between the CD4+ counts of positive samples and syphilis infection as the mean CD4+ count for positive samples were 290.5 which were above the threshold value of 200. Major risk factors identified for co-infection with HIV, HBV and syphilis were multiple and unprotected sexual exposure, multiple blood transfusions, and scarification marks.

We have shown that HIV/HBV and HIV/Syphilis co-infections occur in our environment at rates that are comparable with those of other communities. Because of the shared transmission pathways, synergistic effects of these viruses and the impact of HBV and syphilis on presentation, morbidity, and mortality of HIV, screening for HBV should be included in the armamentarium of investigations to be done pre-HAART. Screening for HBsAg must be ensured at the point of screening for HIV, especially in young/middle-aged persons, in widowed/divorced persons, in individuals with a previous history of blood transfusion or scarification marks, and in females.

Acknowledgement

We would like to acknowledge the support of the staff and management of the AIDS Prevention Initiative in Nigeria (APIN) Clinic, LUTH, Idi-Araba, Lagos, Nigeria.

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