IMPACT OF GENDER AND AGE ON CD4+T LYMPHOCYTE COUNT AMONG HIV- HBV CO-INFECTED SUBJECTSOF HIV COUNSELING AND TESTING, AMINU KANO TEACHING HOSPITAL, KANO

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ABSTRACT

Background: Hepatitis B virus (HBV) infection is a dynamic disease and co-infection with HIV impacts directly on the outcome of HBV infection, considerably complicating its natural history, diagnosis, and management. There is a heavy burden of Human Immunodeficiency Virus (HIV) and HBV co infections in many regions of the developing world, Nigeria inclusive, co-infection with HIV accelerates disease progression in both HCV and HBV and also increases the risk of antiretroviral drug associated hepatotoxicity. With an increase use and accessibility of highly active antiretroviral therapy among HIV positive patients in sub Saharan Africa, co-infection with these viruses could contribute significantly to continuing morbidity and mortality among this group of patients over the coming years.

Aim: The aim of the study was to determine the impact of gender and age on CD4+ cell count of HIV and HBV co-infected clients in Aminu Kano Teaching Hospital Kano(AKTH).

Methodology: Two hundred (200) consented adults were selected for this study. The HIV status of the clients was confirmed using serial algorithm, while HBV was tested using Skytec HBsAg test kit. The study population comprised of males 73 and females 127 with ages ranging from 15 to 75 years.

Result: The results showed that 17 out of the 200 clients were positive for HIV and HBV co-infection (8.5%). The incidence of co-infection was found to be higher in the age group 26-35 years with 8 HIV and HBV co-infected clients (47%) while the 15-25 years age group had 3 clients (18%). The 36-45 year age groups had 4 clients (23%) and the 46-55 years age group had 2 clients (12%).

Conclusion: The prevalence of HIV-HBV co-infection was higher than reports from general population. Lowered CD4 counts were seen in HIV-HBV co-infections. These findings underscore the importance of screening all HIV positive individuals before initiating antiretroviral treatment.

Keywords: HIV, HBV, Co-infection, CD4+ Lymphocyte count.

INTRODUCTION

Hepatitis B (Serum hepatitis) is caused by the hepatitis B virus (HBV), a double stranded circular DNA virus of complex structure. HBV is classified as an *Orthohepadnavirus* within the family *Hepadnaviridae* (Mboto and Edet, 2012). Globally more than two billion persons have been infected at some time with the hepatitis B virus (HBV) (Te and Jessen, 2010) and approximately 3.5 million refugees have chronic HBV infection (Rossi *et al*., 2012). Recent estimates suggest that HBV infection caused 686 000 deaths in 2013 (MacLachlan *et al.,* 2015). HBV is transmitted by percutaneous and mucosal inoculation in blood and body luids. The virus remains viable on environmental surfaces for at least 7 days (Noele*et al*., 2014).

The endemicity of HBV varies by region (WHO, 2014). Most countries in Africa have a high HBV endemicity, with the exception of Morocco and Tunisia, which have intermediate endemicity (Bahal*et al.,* 2013). A prevalence rate of 9.1 % of HBV was found among pregnant women in Hong Kong, (WHO, 2014), 10% in Taiwan (Cheng and Chang 2010) and18% in Burkina Faso (Tao *et al.,* 2014*).*Nigeria is classified among the group of countries endemic for HBV infection. Currently about 18 million Nigerians are infected (Yakasai*et al.,* 2012). Prevalence varies greatly in different parts of the world, but is higher in tropical regions causing both acute and chronic liver disease. In Nigeria, 21% prevalence rate has been reported in Maiduguri (Goni*et al*., 2011), 7.3 % in Lagos (Omilabu*et al.,*2015), 4.7% in Port Harcourt (Iklaki*et al.,*2015), 10.9 % in Ilorin (Shittu*et al.,*2014) and 8.3% in Zaria (Okwuraiwe*et al.,* 2011*).*

The HIV is an RNA virus belonging to the genus *Lentivirus* of the *Retroviridae*family (Roche, 2003). There are two types of HIV virus, HIV-1 and HIV-2. The most widespread type is HIV-1 which is further classified into subtypes or clades (A, B, C, D, F, G, H, J, K and several circulating recombinants) (Roche, 2003). The HIV prevalence is increasing worldwide because people on antiretroviral therapy are living longer, although new infections decreased from 3·3 million in 2002, to 2·3 million in 2012(Priyangaa and Ezhilarasan 2015). Global AIDS-related deaths peaked at 2·3 million in 2005, and decreased to 1·6 million by 2012 (Priyangaaand Ezhilarasan 2015).

The co-infection between HBV and HIV is a state in which an individual is infected with both the Human immunodeficiency virus and the Hepatitis B or C virus. In other words there is a co-existence of both viruses in an individual (Omonkheli*et al.,* 2010).There is a heavy burden of HIV and HBV co infections in many regions of the developing world, Nigeria inclusive, co-infection with HIV accelerates disease progression in both HCV and HBV and also increases the risk of antiretroviral drug associated hepatotoxicity. With an increase use and accessibility of highly active antiretroviral therapy among HIV positive patients in sub Saharan Africa, co-infection with these viruses could contribute significantly to continuing morbidity and mortality among this group of patients over the coming years (Balogun *et al.,* 2012).

MATERIALS AND METHODS

Study Area

The study area for this research was Aminu Kano Teaching Hospital (AKTH) which is a 500- bed tertiary health facility located in Tarauni Local Government of Kano state, the largest commercial center of Northern Nigeria, with a population of over9 million people as reported during the 2006 census (NPC, 2006). The hospital receives majority of its clients from within Kano and neighboring states of Jigawa, Katsina, Kaduna, Bauchi and Zamfara states.

Study Population

The study population comprised of all HIV positive clients that attended HIV counseling and testing (HCT) clinic at AKTH and were not placed on ARVs treatment. The consented clients were randomly selected from the study population that attended the HCT clinic at AKTH over a period of 8 months. The selected clients for the study comprised of males (73) and females (127) with ages ranging from 15 to 75 years.

Sample Size

Two hundred consented adults were selected for this study. The sample size was calculated as 166 (rounded up to 200) using the software OpenEpi version 2.3 based on 12.5% prevalence rate from study conducted in AKTH Kano (Hamza *et al.,* 2013).

Sample Collection and Storage

The patient's identification was confirmed prior to collection, EDTA vacutainer (tube) was labeled with clients identifiers, date and time of blood drawn.

Five (5) mls of whole blood was collected from each informed and consenting subject by venipuncture. The blood sample was dispensed into the labeled EDTA container. The plasma was used for HIV and HBsAg screening tests while the blood in the EDTA container was used for CD4+ T cell count (CDC, 1997).

Specimen Handling/Processing

Each specimen and batch was accompanied by appropriate documentation. The plasma was separated from whole blood by centrifugation and stored in 2ml micro tubes (CDC, 1997).

HIV Rapid Diagnostic Kit

Total of 200 newly enrolled consented HIV positive clients that attended the HCT of AKTH from March to October 2014 were re-screened by serial algorithm using HIV rapid diagnostic technique (Determine, Unigold and Statpak) for the confirmation of HIV infection. The clients were further screened for the presence of Hepatitis B surface antigen (HBsAg). The CD4+ cell count of HIV and HBV co-infected clients was also carried out before and after commencement of ARVs therapy (covering a period of three months).

The test strip (Determine) is an *in vitro,* visually read, qualitative immunoassay for the detection of antibodies to HIV-1 and HIV-2 in human serum, plasma or whole blood. The test is intended as an aid to detect antibodies from an infected individuals. (Alere, 2013). The client identification number was labeled and brought to room temperature (25^0c). The protective foil paper cover was removed and 50ul of plasma was added using Pasteur pipette. The specimen was applied to the absorbent pad on the strip and the specimen was allowed to run for 15 minutes before reading the result. Positive results were indicated by colored line in the specimen (T) regions. Valid results must indicate a colored line in the control (C) region (Alere, 2013).

The test kit Uni-gold is a cassette an *in vitro,* visually read, qualitative immunoassay for the detection of antibodies to HIV-1 and HIV-2 in human serum, plasma or whole blood. Specimen were brought to room temperature. The test kit was labeled with appropriate patient's number. Using disposable pipettes, 60µl of sample was added into kit's sample port followed by 60µl of wash reagent. It was allowed to stand for 10 minutes. A line of any intensity forming in the test region plus a line in the control region indicates positive result while if only control line appears indicates negative result (Trinity, 2013).

The test kit STAT-PAK is a cassette an *in vitro,* visually read, qualitative immunoassay for the detection of antibodies to HIV-1 and HIV-2 in human serum, plasma or whole blood. The test kits were brought to room temperature. The test kits were labeled with appropriate patient's number. Using sample loop, 5 µl of sample was added into kit's sample port followed by 3 drops of buffer. It was allowed to stand for 10 minutes. A line of any intensity forming in the test region plus a line in the control region indicates positive result while if only control line appears indicates negative result (Chembio, 2011)

Screening for Hepatitis B Surface Antigen

The HBsAg one step Hepatitis B surface antigen test strip (Serum or Plasma) is a rapid chromatographic immunoassay for the qualitative detection of Hepatitis B surface antigen in serum or plasma. The test device from the sealed pouch was brought to room temperature before opening it. The test device was placed on a clean and level surface then 60µl of plasma was added onto the specimen well (S) of the test device. Results were read after 15 minutes. Positive results were indicated by colored line in the specimen (S) regions. Valid results must indicate a colored line in the control (C) region. Invalid test, control line failed to indicate red color (ABON, 2010).

CD4+ T cells count

A 10 µl of CD4+ partec antibody was added into partec test tubes, followed by 10µl of well mixed whole blood (EDTA) was added into each partec test tubes and mixed(Partec,2001).

The mixed samples were incubated in the dark for 15minute at room temperature. The samples were mixed after every 5 minute, and then 800µl of $CD4^+$ buffer was added into partec tubes, it was gently mixed and plugged on to the cyflow machine (Partec, 2001). After counting, the CD4+ cells were separated from monocytes and gated using Flomax software(Pattanapanyasat K. *et al.,*2005). Results were displayed on the screen and printed.

RESULTS

The result of the study revealed that of the 200 confirmed HIV clients 47% were aged from 26-35 years while 17.6% and 23.5% were aged from 15-25 years and 36-45 years respectively.

Table 1 further revealed that 17 (8.5%) of the 200 confirmed HIV clients were co-infected with HBV infection while 183 (91.5%) were not infected with HBV. The highest co-infection rate of 47% was noted among clients aged from 26-35 years. The least co-infection rate of 11.7% was among clients aged 46-55 years. None of the 200 patients was aged 55-65 years or 66-75 years.

Table 1: Distribution of HIV and HBV Co-infection among HIV Clients of HCT, AKTH, Kano by Kano by Their Age Groups

	HBV Infection Status		
Age Groups	Positive (%)	Negative (%)	Total
15-25	3 (17.6%)	30 (15%)	33 (16.5%)
26-35	8 (47 %)	81 (40.5 %)	89 (44.5%)
36-45	4 (23.5%)	41 (20.5 %)	45 (22.5%)
46-55	2 (11.8 %)	19 (9.5 %)	21 (10.5%)
>56	0 0	12 (6%)	12 (6 %)
66	17 (8.5%)	183 91.5 %	200 100 %

Table 2 Illustrated that of the 17 co-infected HIV and HBV clients of the study, females had the highest co-infection rate of 70.6 % with the 12 female having both infections, while only 5 (29.4%) of the males were co-infected . The table further revealed that females aged from 26-35 years had the highest co-infection rate of 41.2% whereas males aged from 15-25 years had the highest co-infection rate of 17.6%. However the least co-infection rate of 5.9% was recorded among both males and females of age group 46-55 years.

Table 2 Distribution of HIV and HBV co-infection among HIV Clients of HCT, AKTH, Kano with Regards to their age and Sex.

Age group	HIV/HBV Males (%)	Positive (n-17) Females (%)	Total
15-25	3 (17.6%)	0	3
26-35	1 (5.9%)	7 (41.2%)	8
36-45	0 (0%)	4 (23.5%)	4
46-55	1(5.9 %)	1 (5.9%)	2
>56	0	0	0
Total	5 (29.4%)	12 (70.6%)	17

Table 3 shows the highest CD4+ cell count of >350 cell/µl was recorded in 3 (17.6%) of the 17 HIV and HBV co-infected clients. While the lowest count of 0-50 cells /µl was recorded in only 1 (5.9%) of the co-infected clients. Majority of co-infected clients (23.5%) had a CD4+ counts of 101-150 cells/µl and 3 (17.6) had CD4+ count of 201-250 cells/µl.

Table 3 CD4+T Lymphocyte Count (cells/µl) Among HIV and HBV Co-infected Clients of HCT, AKTH, Kano

CD+ T cell count (cells/µl)	No of clients
0-50	1 (5.9%)
51-100	2(11.8%)
101-150	4(23.5%)
151-200	0
201-250	3(17.6%)
251-300	2(11.8%)
301-350	2(11.8%)
>350	3(17.6%)
Total	17

DISCUSSION

There is a heavy burden of HIV and HBV co infections in many regions of the developing world, Nigeria inclusive. Co-infection with HIV accelerates disease progression in HBV and also increases the risk of antiretroviral drug associated hepatotoxicity (Balogun *et al.,* 2012). With an increase use and accessibility of highly active antiretroviral therapy among HIV positive clients in sub Saharan Africa, co-infection with these viruses could contribute significantly to continuing morbidity and mortality among this group of clients over the coming years (Balogun *et al.,* 2012).

The population prevalence of HIV and HBV co-infection in Africa is thought to reflect the population prevalence of hepatitis B surface antigen (HBsAg) (Puoti*et al.,* 2008). In this study, the prevalence of HBV infection among HIV infected individual was observed to be 8.5% (17 of 200). This is relatively low compare to a prevalence of 12.5% reported by Hamza *et al*., (2013). A prevalence rate of 9.1% of HBV was found among pregnant women in Hong Kong (WHO, 2014), 10% in Taiwan (Cheng and Chong 2014), and 18% in Burkina Faso(Tao *et al.,* 2014*).*Nigeria is classified among the group of countries endemic for HBV infection. (Yakasai*et al.,* 2012).

In Nigeria, 21% prevalence rate has been reported from Maiduguri (Goni*et al.,* 2011), 7.38% from Lagos (Omilabu*et al.,*2015), 4.7% from Port Harcourt (Iklaki*et al*., 2015), 10.9% from Ilorin (Shittu*et al.,*2014), 8.3% from Zaria (Okwuraiwe*et al.,* 2009*).*

The result of this study showed that the prevalence of HIV and HBV co-infection among, study population was 8.5% and was found to be higher when compared with studies of other state in Nigeria such as Port Harcourt and Lagos. The result was relatively lower than that of other state such as Maiduguri and Ilorin but similar with studies conducted in Zaria. The reasons for decrease in the prevalence in Kano could be as a result of increased awareness by Non-Governmental Organizations on the use of condom, HBV vaccine and educating clients on HBV mode of transmission and preventive measures. The accessibility to health care facilities has improved life of people living with HIV infection. Forty seven (47%) of the HIV positive clients in this study were aged from 26-35 years and this is not surprising as Hakim et al. 2012 stated that these are the most sexually active age group – heterosexual intercourse being the major mode of transmission in Africa.

However since children were largely excluded from this study, the age profile is obviously skewed in favor of adults, and accentuates the prominence of the sexually active age group.

About half (41.2%) of the HIV and HBV co-infected clients in this study had AIDS as defined by CD4+ cell count < 200 cells/µl according to WHO (2009) and this is higher than the 32% reported inIlorin, Nigerian (Afolabi*et al.,* 2014). Obviously the major reason for this high proportion of AIDS could be attributed to clients late presentation to the HCT center, largely due to the fact that in this part of the country people are yet to imbibe the culture of voluntary screening for early detection and treatment. Fear of stigmatization, lack of awareness and inadequate trained counseling personnel are some of the factors militating against voluntary screening also many clients only tend to seek medical attention for other causes and in the cause may be diagnosed with HIV infection which becomes complicated by AIDS defining illnesses. Burns (2007) made a similar observation where Blacks in South Africa similarly presented late with advanced disease.

The mean CD4+ cell count of the HIV and HBV co-infected clients in this study before commencement of ARVs was 264cells/µl, and this is lower than 286 cells/µl reported in Ilorin Nigeria (Adekunle*et al.,* 2011). A similar research was carried out in AKTH Kano, by Nwokedi (Nwokedi*et al.,*2007) and reported a higher count of 307 CD4+ cells/ml when compare to this study.

The WHO/CDC have recommended ART at higher CD4+ cell count the new guideline has improve the life of people living with HIV/AIDS. According to CDC (2013) all clients with CD4 count less than 350 were placed on ARV drugs, while those with CD4 counts above 350cells/ml were only placed on ARVs if they develop clinical complications. The clients with CD4 count from 500 to 1000 cells/ml were 20 which are considered asymptomatic (CDC 2013).

CONCLUSION

The study revealed that the prevalence of HBV among the 200 studied HIV clients attending HCT, AKTH was 8.5% indicating that 17 of the 200 HIV clients were co-infected with HIV and HBV. The highest co-infection rate of 47 % was recorded among clients aged 26-35 years, and more females (70.6%) were infected than males (29.4%). Majority of the co-infected clients (23.5%) had a CD4+cell count of 101-150 cell/µl. The highest CD4+cell counts of 301-350 cells/µland lowest was 0-50 cell/µl. Hence, routine screening of HBV in HIV positive clients should be considered before initiation of antiretroviral treatment so as to proffer proper treatment to the co infected individuals and regular evaluation of CD4 status that will improve quality of life and reduce morbidity/mortality.

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