Assessment of some Immunohaematological parameters of HIV Positive Patients Co-infected with Hepatitis B Virus in Osun State, Nigeria

ABSTRACT

Background: Increased morbidity and mortality rates have been reported among patients co-infected with Human Immunodeficiency Virus (HIV) and Hepatitis B Virus (HBV).
Aims: To determine the prevalence of HBV infection among HIV-infected anti-retroviral therapy (ART) naïve patients attending two Public Health Facilities in Osun State, Nigeria; as well as to determine the effect of HIV/HBV co-infections on some immunohaematological parameters.
Study design: This is a prospective, analytical and Institutional based study.
Place and Duration of Study: HIV Out-Patients Clinic, State Specialist Hospital Osogbo and State Hospital Iwo, Osun State, Nigeria, between February and August, 2015.
Methodology: A total of 321 blood samples were collected from 121 HIV HAART Naïve patients and 200 HIV negative patients, aged between 4 and 52 years. HIV antibodies were detected using 3 rapid diagnostic kits (Determine, Unigold and Stat Pak). Hepatitis B surface antigen (HbsAg) was detected using Clinotech Diagnostic test strips. CD4+ cells were counted using Partec® Cyflow Counter (Germany); while Haemoglobin concentration and Platelet Counts were determined using Sysmex auto-analyzer (Japan).
Results: The prevalence of HBV infection was found to be significantly higher ($P<0.05$) among HIV positive patients (16.5%) than in HIV negative patients (3.5%). HIV status was identified as a risk factor for acquiring HBV infection. There were no significant differences between the immunohaematological parameters of HIV/HBV co-infected and mono-infected group on the basis of age, except for gender. The mean CD4 Count of HIV/HBV co-infected males (327±200.64 Cells/µL) was found to be significantly higher ($P<0.05$) than their mono-infected counterparts (274.5±81.33 Cells/µL), whereas, their Platelet Count and haemoglobin concentration did not differ significantly ($P>0.05$). Also, the mean CD4 cell count of co-infected females (408.4±331.28 Cell/µL) was significantly ($P<0.05$) higher than that of their male counterparts (327.6±81.33 Cell/µL). However, there were no significant differences in their mean platelet count and haemoglobin concentration.
Conclusion: HBV exist among HIV positive patients living in Osun State, Nigeria, with a prevalence rate of 16.5%. Although, no significant difference was observed in the Platelet count and Haemoglobin concentration of HIV/HBV co-infected when compared to those of HIV mono-infected patients, regular monitoring of their immunohaematological parameters is encouraged to prevent associated sequalea.

Keywords: *HIV/HBV Co-infection, CD4 Count, Platelet Count, Haemoglobin Concentration*

1. INTRODUCTION

One of the most dreaded viral pathogen with global impact is the Human immunodeficiency virus (HIV), the etiological agent of acquired immunodeficiency syndrome (AIDS) which belongs to the family *Retroviridae*, subgroup Lentivirus. It is a spherical enveloped virus, about 90-120 nm in size, surrounded by a lipoprotein membrane [1]. Infection with the virus is associated with increased mortality and morbidity worldwide, with prevalence rates that varies from region to region. It is known to affect people of all ethnicity, gender, age and sexual orientation [2]. Sub-Saharan Africa remains by far the most affected region with 24.5 million people living with HIV, representing a little below two-thirds of all people living with HIV in the world [3]. HIV infection is a major health concern in Nigeria, where it is estimated that about 2.9 million people are living with the virus [4]. Global estimate of HIV/AIDS put Nigeria only behind South Africa in the list of HIV/AIDS most endemic countries of the world [5].

Hepatitis B virus (HBV) on the other hand, is a major cause of liver disease morbidity worldwide, accounting for over 360 million cases of chronic hepatitis and 620,000 deaths per year [6]. It is hyper-endemic (*i.e.*, >8% of the population infected) in Sub-Sahara Africa and a major cause of chronic liver

disease [7]. Palella *et al.* [8] estimated that 44% of cirrhotic liver disease and 47% of heptocellular carcinoma cases in Sub-Saharan Africa are attributed to HBV. Researchers have reported varying national and risk group-specific in Nigeria. Prior reports suggest a prevalence rate of 10-15% in the average risk Nigerian population [9]. Nigerian Researchers have found varying HBV prevalence rates among different groups. Sadoh and Ofili [10] reported a prevalence rate of 16.3% among infants, while Oladeinde *et al.* [11], documented 5.6% among pregnant women.

Due to shared routes of transmission, HBV infection is common among HIV infected patients [12], [13]. Worldwide, it is estimated that 10% of the 40 million HIV-infected individuals have chronic Hepatitis B. Since the introduction of the Highly Active Anti-Retroviral Therapy (HAART) in the United States and other industrialized countries, deaths from AIDS-related causes have declined, but liver disease has emerged as one of the leading causes of morbidity and mortality [8].

As HAART is introduced into areas of the world with high HBV endemicity, Hepatitis B-related liver disease has increased in the HIV-infected population [12]. Co-infection of HIV and HBV has been associated with increased morbidity and mortality. As a matter of concern, HIV has been reported to increase the infectivity of HBV, the rate of HBV reactivation and the risk of cirrhosis. However; some conflicting reports, also indicate that HBV accelerates HIV disease progression [14] - [17].

Early detection of disease is critical to its effective management and treatment. However in Nigeria, reports indicate that HIV infected patients are not routinely screened for HBV [18]. This increases the risk for the development of end-term liver diseases and hepatocellular carcinoma and even death [15]. HIV/HBV co-infected patients therefore may present more quickly with AIDS defining illness than HIV mono-infected patients, a situation that can hamper response to anti-retroviral therapy and cause death. It is therefore important to understand the interaction of these two chronic viral infections.

A major laboratory indicator of immune function in patients who have HIV infection is the CD4 T-cell [19]. According to Gottlieb *et al.* [20], it a reliable marker of the progression of HIV infection. Count $CD4^+$ cells orchestrate the immune response to attack by HIV, but HIV invades the $CD4^+$ cells and uses them to replicate itself. Soon after infection with HIV, CD4+ cells count decrease by approximately one-quarter and then decrease slowly thereafter [21]. When the $CD4^+$ cell count reaches 350 cells/µL; HIV-related opportunistic infections become evident and at 200 cells/µL, the person is classified as having AIDS regardless of the appearance of other opportunistic infections; and at 50 cells/µL, patients are prone to die.

Furthermore, one of the most important clinical problems in HIV patients and those with AIDS is anaemia: lowered oxygen carrying capacity of blood [22]. A lowered hemoglobin concentration (in addition to red blood cell count) is a major marker [23] and diagnosis in men is based on an hemoglobin level of less than 13 to 14 g/dl, while in women it is less than 12 to 13 g/dl [24].

Still, another clinical problem in HIV/AIDS patients is thrombocytopenia, characterized with a platelet count $<100 \times 10^9$ cells/L. It is a known complication of HIV infection, primarily in patients with AIDS, low CD4 cell counts and advanced stages of the disease [25], [26]. Platelets play a central role in normal hemostasis, as well as in cardiovascular diseases, inflammation and tumor metastasis. As a part of frontline defensive duties in the innate immune response to acute HIV infection, platelets can bind to, and be sequestered by specific inflammatory monocytes subsets [27].

There is inadequate information on the prevalence of HBV among HIV infected patients in Osun State, of Nigeria. Against this backdrop, this study was therefore designed to determine the prevalence of HBV infection among HIV-infected anti-retroviral therapy (ART) naïve patients attending two Public Health Facilities in Osun State, Nigeria; as well as to determine the effect of HBV on some of the immunohaematological parameters of such patients. This is necessary as such information will help in the diagnosis and prognosis of affected patients.

2. MATERIAL AND METHODS

2.1 Study Area

This prospective cohort study was carried out at the HIV Out-Patients Clinic, State Specialist Hospital, Osogbo and State Hospital, Iwo: two major public health facilities in Osun State. Osun is an inland state in South-western Nigeria, co-ordinates: 7°30'N 4°30'E. It is bounded in the north by Kwara State, in the east partly by Ekiti State and partly by Ondo State, in the south by Ogun State and in the west by Oyo State. It has a total land area of about 9, 251 km^2 with a population of about 4, 137, 627 (2005, estimate). Its capital city is Osogbo, which seats the Headquarters of both Osogbo Local Government Area and Olorunda Local Government Area. Osogbo shares boundary with Ikirun, Ilesa, Ede, Egbedore and Iragbiji and is easily accessible from any part of the state because of its central nature. It has a population of about 156,694 people, based on the 2006 Census [28]. Majority of the population (60%) engage in farming as their primary occupation, about 10% are traders and about 30% are civil servants. Iwo on the other hand is one of the ancient towns of Osun State, in Iwo Local Government Area of Osun State. The major sub-ethnic groups in Ọsun State are Ife, Ijesha, Oyo, Ibolo and Igbomina of the Yoruba people, although there are also people from other parts of Nigeria. Yoruba and English are the official languages. People of Osun State practice Islam, Christianity and paganism called traditional faith [29].

2.2 Duration of study: The study was carried between February and August, 2015.

2.3 Determination of Sample Size

The minimum sample size (n) required was estimated using the single population proportion formula:

$N = Z^2 * P(1-P)/d^2$ [30]

where:

N = Minimum sample size required
Z = Confidence Interval (1.96)
P = 0.027 (2.7%), Prevalence rate in a target population from a previous study [31],
d = Desired level of significance (set at 0.05)

The above formula, gave 40 as the minimum sample size required. However, in other to make our conclusions robust and all encompassing, we decided to screen a total of 121 HIV infected patients.

2.4 STUDY DESIGN

A cohort of 321 patients which consist of 121 HIV HAART Naïve patients (17 males and 104 males) and 200 HIV negative patients (50 males and 50 females) which serve as control, aged between 4 and 52 years) attending the HIV Out-Patients Clinic in State Specialist Hospital Osogbo and State Hospital Iwo, Osun State, Nigeria were randomly recruited for the study. Pre-test counseling was instituted in which the purpose, benefit and procedures of the study were explained to the participants. A brief structured questionnaire was used to obtain demographic information from consenting subjects. Interpreter was provided for translation in local dialect where necessary. Informed consent was obtained from each patient and all participants were requested to voluntarily sign the consent forms in their own handwriting as proof of willingness to provide samples for the tests. The study groups were stratified by sex and age. For reasons of privacy, all data were kept confidential in accordance with World Medical Association declaration of Helsinki [32].

2.5 Collection of Specimen

Ten (10) milliliters of blood was collected from each participant and 5 ml was dispensed into a container having ethylene diamine tetra-acetic acid (EDTA) and the remaining 5 ml was placed in a plain container and allowed to clot. The sera obtained were used for the serological diagnosis of HIV and HBV using a previously described method [11], [33]. The anti-coagulated blood was used for the determination of CD4 cell count, Platelet count and haemoglobin concentration.

2.6 Laboratory analysis

2.6.1 HIV Detection

HIV detection was carried out using the current National algorithm for HIV sero-diagnosis. This involved the use of 3 rapid diagnostic kits, following their manufacturer's instructions. Briefly, each patient's serum was screened for the presence of HIV antibodies using Determine (Abbott Laboratories, Tokyo, Japan) and Unigold HIV (Trinity Biotech Plc Bray, Co. Wicklow, Ireland). When both kits showed positivity, the patient was regarded as positive for HIV infection and vice versa. However, when test results were discordant, a third kit, which is the Tie breaker, $^{1}/_{2}$ Stat Pak (Chembio Diagnostic Systems, New York, USA) was used. The HIV serostatus of the patient was taken as the result of either of the first two kits that agree with that of the third kit.

2.6.2 Hepatitis B Virus (HbsAg) Detection

HBV was detected by immunochromatograhic method using HbsAg test strip supplied by Clinotech Diagnostic (Richmond, Canada) according to the manufacture's instruction. Briefly, sera obtained from patients were gently applied to the sample area on test strips and observed for the emergence of line bands on the test strips. Positive and negative control sera were also run alongside test. Interpretation of results was done according to manufacturer's instruction.

2.6.3 $CD4^+$ Cell Count Evaluation

$CD4^+$ cell count was evaluated using Partec® Cyflow Counter (Germany), as described by PCC [34]. The Cyflow Counter was operated as instructed in the user's operational manual. Briefly, to a 20 µL of CD4 MAb (monoclonal antibody) already pipette into a Partec test tube, 20 µL of well mixed whole EDTA blood collected within 6 hours was added, properly mixed by gentle tapping and incubated in the dark for 15 minutes. The mixture was agitated during incubation every 5 minutes. Afterwards, 800 µL of CD4 buffer was added and mixed thoroughly to avoid bubbles. This was then plugged to the counter for CD4 cell counting.

2.6.4 Platelet Count and Haemoglobin Concentration Estimation

Platelet count and haemoglobin concentration estimation were determined using the Sysmex® Automated Haematology Analyzer KX-21N, Sysmex Corporation, (Kobe-Japan) as described by Samuel *et al.* [35].

2.7 Statistical Analysis

All numerical results were collated from laboratory analysis of the blood and sera of the 121 HIV positive and 200 HIV negative patients recruited for the study. Data generated are presented as mean±SD and analyzed with one way analysis of variance (ANOVA) using SPSS-18.0 (Statistical packages for social Scientists – version 18.0) statistical program. P values<0.05 were considered statistically significant. Odd ratio (OR) analysis was determined with GraphPad INSTAT® Software. An association was established between two variables when an *OR* value ≥1.00 was obtained [36].

3. RESULTS AND DISCUSSION

The prevalence of HBV infection among the study participants is presented in Table 1. Twenty (16.5%) out of the 121 HIV Positive patients were infected with HBV, while the remaining 101 patients (83.5%) were HBV negative (*i.e.,* HIV mono-infected). Only 7 (3.5%) out of the 200 HIV Negative individuals were infected with HBV, while the remaining 193 (96.5%) were HBV negative. Overall, 27 (8.4%) participants were found to be infected with HBV in the study irrespective of their HIV status. The number of HIV positive patients co-infected with HBV was significantly higher (P<0.05) than those that were HIV negative. An association between HIV positive and HIV negative status was established with an odd ratio ≥1.00. Thus, HIV status was identified as a risk factor for acquiring HBV infection.

Table 1: Prevalence of Hepatitis B virus infection among the study participants

HIV Status	Number examined (N)	Number HBV Positive N (%)	Number HBV Negative N (%)	*P-value*
Positive	121	20 (16.5)	101 (83.5)	0.001
Negative	200	7 (3.5)	193 (96.5)	
Total	321	27 (8.4)	294 (91.6)	

HIV Positive vs HIV Negative: 16.5% vs 3.5%, OR= 5.460

Table 2 represents the age and sex distribution of HBV infection among HIV infected patients. No HBV infection was recorded among age group 4-10 years and 11-17 years. The highest HBV prevalence (33.3%) was found among patients within the age group 18-24 years; however there was no significance difference (P>0.05) in the prevalence of HBV infection among HIV infected patients with respect to age. Although, HBV infection was more prevalent (29.4%) among the HIV infected male participants than in their female counterparts (14.4%), the difference was not statistically significant (P>0.05).

Table 2: Age and sex distribution of HBV among HIV infected patients

Characteristics	Category	Number examined N	Number HBV Positive N (%)	Number HBV Negative N (%)	*P-value*
Age range	4-10	6	0 (0.0)	6 (100.0)	1.000
(Years)	11-17	4	0 (0.0)	4 (100.0)	1.000
	18-24	12	4 (33.3)	8 (66.7)	0.631
	25-31	40	6 (15.0)	34 (85.0)	0.579
	32-38	35	6 (17.1)	29 (82.9)	0.593
	39-45	15	2 (13.3)	13 (86.7)	0.489
	46-52	9	2 (22.2)	7 (77.8)	0.298
	Total	121	20 (16.5)	101 (83.5)	
Gender	Male	17	5 (29.4)	12 (70.6)	0.125
	Female	104	15 (14.4)	89 (85.6)	0.156
	Total	121	20 (16.5)	101 (83.5)	

P value>0.05 is considered statistically not significant.

The effect of Hepatitis B virus on the $CD4^+$ Cell Count of the HIV infected patients is presented in Table 3. The highest mean $CD4^+$ Cell Count in the HIV/HBV co-infected group, was observed among the age group 18-24 years (644.75±289.10 Cells/µL) and female (408.4±331.28 Cells/µL) category; whereas in the HIV mono-infected group, the highest mean CD4 Cell Count was observed among the age group 4-10 years (584.2±452.40 Cells/µL) and female (391.96±282.84 Cells/µL) category. The difference in the mean $CD4^+$ Cell Count between the co-infected and mono-infected groups was not statistically significant (P>0.05) on the basis of age. However, with regard to gender, the values of the co-infected males (327±200.64 Cells/µL) were significantly higher (P<0.05) than those of mono-infected group (274.5±81.33 Cells/µL). Howbeit, in the female category, the difference was not statistically significant (P>0.05).

Furthermore, the effect of Hepatitis B virus on Platelet Counts of the HIV infected patients is presented in Table 4. The mean platelet count varied significantly (P<0.05) among and within the HIV/HBV co-infected group on the basis of age, but not in the HIV mono-infected group (P>0.05). Also, although, the female participants in both HIV/HBV and HIV mono-infected groups had a higher mean platelet count (286.70±124.40X10^3/µL and 279±114.30X10^3/µL, respectively) than their male counterparts (180.20±82.98X10^3/µL and 265.46±109.13X10^3/µL, respectively), the difference was not statistically significant (P>0.05). Still there was no significant difference (P>0.05) when the mean platelet count of the

co-infected patients was compared to those of the mono-infected patients in the same age group and on the basis of gender as well.

Table 3: Effect of Hepatitis B virus on CD4+ Cell Count of HIV infected patients

		HIV/HBV Co-infected		HIV mono-infected		
Variable	**Category**	**Number examined N**	**Mean±SD CD4 Count (Cells/µL)**	**Number examined N**	**Mean±SD CD4 Count (Cells/µL)**	***P-value***
Age range	4-10	0	-	5	584.2±452.40	*ND*
(Years)	11-17	0	-	4	472.25±283.49	*ND*
	18-24	4	644.75±289.10	9	411.0±189.59	0.161
	25-31	6	349.5±288.66	33	397.5±288.79	0.579
	32-38	6	294.17±349.76	29	348.75±263.53	0.154
	39-45	2	285±106.77	14	289.71±90.00	0.405
	46-52	2	326±62.25	7	196.35±155.42	0.299
	Total	20		101		
Gender	Male	5	327±200.64	12	274.5±81.33	0.048*
	Female	15	408.4±331.28	89	391.96±282.84	0.184
	Total	20		101		

KEY: *ND = Not determined, SD = Standard Deviation, *P value<0.05 is considered statistically significant*

Table 4: Effect of Hepatitis B virus on Platelet Counts of HIV infected patients

		HIV/HBV Co-infected		HIV mono-infected		
Variable	**Category**	**Number examined N**	**Mean±SD Platelet Count ($X10^3$/µL)**	**Number examined N**	**Mean±SD Platelet Count ($X10^3$/µL)**	***P-value***
Age range	4-10	0	-	5	314.00±94.71	*ND*
(Years)	11-17	0	-	4	294.75±85.46	*ND*
	18-24	4	347.25±106.05	9	240.75±141.39	0.294
	25-31	6	338.66±119.70	33	273.82±101.98	0.259
	32-38	6	161.67±98.43	29	279.79±132.87	0.262
	39-45	2	193.00±59.39	14	296.36±121.26	0.367
	46-52	2	237.00±46.67	7	243.85±113.04	0.305
	Total	20		101		
Gender	Male	5	180.20±82.98	12	265.46±109.13	0.537
	Female	15	286.70±124.40	89	279±114.30	0.316
	Total	20		101		

KEY: *ND = Not determined, SD = Standard Deviation, P value>0.05 is considered statistically not significant*

Finally, the effect of Hepatitis B virus on the mean haemoglobin concentration of the HIV infected patients is presented in Table 5. Statistically analysis, shows that there was no significant difference (P>0.05) between the mean haemoglobin concentration of the co-infected and mono-infected patients between and within the same age category. Still the mean haemoglobin concentration of the male category in both co-infected and mono-infected groups (12.58±2.75g/dL and 11.6±2.67 g/dL, respectively) was not

statistically difference (P>0.05) from those of their female counterparts (9.81±2.12 g/dL and 10.01±2.03 g/dL, respectively). The mean haemoglobin concentration of the HIV/HBV co-infected males (12.58±2.75g/dL) was observed to be higher (statistically not significant) than those of their HIV-mono-infected male counterparts (11.6±2.67 g/dL); whereas, reverse was the case with the female category, in which the HIV/HBV co-infected females (9.81±2.12 g/dL)) had a lower mean haemoglobin concentration then their HIV mono-infected female participants (10.01±2.03 g/dL)).

Table 5: Effect of Hepatitis B virus on haemoglobin concentration of HIV infected patients

		HIV/HBV Co-infected		HIV mono-infected		
Variable	**Category**	**Number examined N**	**Mean±SD Hb Conc. (g/dL)**	**Number examined N**	**Mean±SD Hb Conc. (g/dL)**	***P-value***
Age range	4-10	0	-	5	8.95±1.59	*ND*
(Years)	11-17	0	-	4	10.42±1.17	*ND*
	18-24	4	8.87±2.08	9	10.58±1.24	0.107
	25-31	6	9.31±1.17	33	9.94±1.72	0.291
	32-38	6	10.78±2.61	29	9.84±1.97	0.157
	39-45	2	14.55±0.49	14	10.18±2.28	0.167
	46-52	2	12.4±3.11	7	9.25±2.24	0.214
	Total	20		101		
Gender	Male	5	12.58±2.75	12	11.6±2.67	0.415
	Female	15	9.81±2.12	89	10.01±2.03	0.374
	Total	20		101		

KEY: *ND = Not determined, SD = Standard Deviation, P value>0.05 is considered statistically not significant*

Increased morbidity and mortality rates have been reported among HIV positive patients co-infected with Hepatitis B Virus [11]. The prevalence of HIV/HBV co-infection is known to vary from place to place and even within the same place over a period of time and depends on a complex mix of behavioral, environmental and host factors [37]. In this current study, the prevalence of HBV infection among HIV infected patients was found to be 16.5%. This is lower than the 28.7% and 20.6% reported by Irisena *et al.* [38] and Forbi *et al.*, [39], respectively. It is however, higher than the 0.4%, 2.2% and 12.3% reported by Egah *et al.* [40], Diwe *et al.* [41] and Hamza *et al.* [42], respectively in other Nigerian studies.

The observed variation in the prevalence of HBV infection among HIV infected people may be due to differences in the geographical regions. The study by Egah *et al.* [40] and Hamza *et al.* [42] were both conducted in North Central Nigeria, Diwe *et al.* [41], in South Eastern Nigeria, whereas, ours was conducted in South Western Nigeria.

Of all the 200 HIV negative control patients screened in this study, only 7 (3.5%) were infected with HBV. The prevalence of HBV infection among HIV infected patients was observed to be significantly higher than value recorded among HIV negative cohort in this study (P = 0.001). Indeed, the HIV infected patients had a 1-6 folds (*OR= 5.460*) increased risk of being infected with HBV than HIV negative subjects. A report by Cheng *et al.* [43] indicated that clearance of HBV in HIV infected patients is slower than in their HIV negatives counterparts. Thus, the observed significantly higher prevalence of HBV infection among the HIV infected patients in comparison with the HIV-negative group may be due to differences in HBV clearance rate among them. In addition, HIV infected individuals are more likely to lose previously developed protective anti-HBs antibody and develop acute hepatitis B infection [44]. This may also explain the higher prevalence of HBV infection observed among the HIV infected cohort in this study.

Furthermore, no HBV infection was observed among children (4-10 years) and adolescent (11-17 years) HIV infected patients, while the highest prevalence was recorded among young adults (18-24 years). The low prevalence of HBV infection in children and adolescent HIV infected patients in this study is in contrast to the findings from another African study, where the highest prevalence of HBV infection was recorded among patients less than 20 years old [45].

In 2004, Nigeria commenced her universal HBV immunization program [10]. Study participants within the age group of 4-10 years and 11-17 years are more likely to be beneficiaries of such program than older participants, thus present with lower risk for HBV infection. On the other hand, the age range, 18-24 years is a period of greatest sexual activity and those in this group tend to be promiscuous and are therefore prone to sexually transmitted diseases like HIV infection. And since HBV shared the same routes of transmission with HIV, this could have precipitated the higher prevalence rate (33.3%) recorded among young adults in this study. Besides, increase in poverty and hard economic situation in the country appear to have expose young women to see sex as a business and means of using what they have to get what they lack: including getting money and obtaining what they desire from wealthy men and men in authority. This is more especially among students of higher institutions where sex is exchanged for better academic performance and also among junior workers in both public and private sectors for promotion and other favors. Nevertheless, the outcome of this current study fails to show age as a risk factor for HBV sero-positivity ($P>0.05$). This agrees with the findings from a Nigerian study by Akyala *et al.* [46], but disagrees with another by Nakwagala and Kagimu [45].

Several reports have indicated that the rate of HBV clearance is higher in females than males leading to a generally higher prevalence of HBV among male population [47]. This is consistent with findings from this present work, where a higher prevalence of HBV infection was observed among HIV infected male patients (29.4%) than their female counterparts (14.4%). Although the prevalence rate recorded did not differ significantly ($P=0.156$) with respect to gender. A similar finding has been reported elsewhere by Lesi *et al.* [48].

Immunosuppression which is characterized by a drastic reduction in CD4 cell count is a hall mark of HIV infection [49]. Research from animal models and human subjects suggest that with increasing age, there are several important changes in the innate and adaptive immune responses, a phenomenon termed "immunosenescene" [50]. In this study, there were no significant differences ($P>0.05$) in the mean CD4 cell counts of the HIV/HBV co-infected and HIV mono-infected patients of the same age. This does not agree with the findings from other Nigerian based studies, which reported statistically significant differences in their CD4 cell counts [44], [51]. It is important to mention here that the study conducted by Obi *et al.* [51], did not take into consideration the effect of age on CD4 cell count between HIV/HBV co-infected patients and HIV mono-infected patients, Rather, their mean CD4 cell count were compared and deductions made irrespective of their age differences. This does not provide a level ground for the assessment of the effect of HBV on CD4 cell count in HIV infected patients, and may account for the variation observed in our study.

The CD4 cell count of our HIV/HBV co-infected patients was generally observed to decrease steadily with increase in age of study participants; howbeit, the difference was not significant ($P>0.05$). Multiple cohort studies involving untreated HIV-infected persons have established that older persons have a more rapid progression to AIDS and shortened survival when compared with younger persons [52] - [55].

Although studies have reported that HBV increases HIV proliferation, a situation that should naturally be associated with reduced CD4 cell count. In our study, the mean CD4 cell count among male HIV positive patient co-infected with HBV was observed to be significantly ($P=0.048$) higher than those of HIV mono-infected group. The reason for this is not clear. Further research is therefore necessary to elucidate this finding. However, with respect to gender, females in the HIV/HBV co-infected and HIV mono-infected groups were observed to have higher mean CD4 count than their male counterparts, howbeit, the difference was not statistically significance. Several studies conducted among mono-infected groups in Africa, have reported a generally higher CD4 cell count among females than males [56] - [58].

With regard to the development of thrombocytopenia in HIV infection, several mechanisms have been proposed to contribute to the drop of platelet count, including platelet destruction mediated by platelet-associated immunoglobulin G (IgG) or platelet-leukocyte aggregation, possibly leading to sequestration by macrophages, sequestration of platelets in the enlarged spleen, impaired thrombopoiesis, and direct effect of viruses on platelets [25], [59]. While more platelets are ordinarily expected to be destroyed by a possible combination of action of HIV and HBV in co-infected than HIV mono-infected patients, the pattern of result obtained in our study did not indicate that. Indeed, young adults (18-24 years and 25-31 years) that were co-infected with HIV and HBV were observed to have a higher mean platelet count than HIV mono-infected patients in the same age group category, while the converse was the case in older subjects, suggesting that young age may confer some form of protection against the action of HIV and HBV on platelets. However, the difference in platelet count between HIV/HBV co-infected patients and HIV mono-infected patients did not differ significantly ($P>0.05$) for the same age group studied. This contradicts the work of Obi *et al.* [51], who reported that HIV mono-infected patients have significantly higher platelet count than HIV/HBV co-infected patients.

In this study, age was identified to significantly affect the mean platelet count among HIV/HBV co-infected patients; with young adult participants (18-24 years) having the highest mean platelet count and those within the age group category of 32-38 years having the least. Analyses of small groups of healthy subjects initially suggested that platelet count is higher among youths than the elderly ones [60], with larger studies later confirming these findings [61], [62]. However, the reason for the sharp decline in the mean platelet count of middle age subjects as compared to much older participants is not entirely clear. Perhaps, they may harbor higher viral loads of both viruses than older subjects which may facilitate the destruction of platelet in them. The disparity in mean platelet count could also be due to the genetic disposition of the study participants, as platelet count has been reported to be influenced by host genetics factors [63]. However, to confirm this, further studies will be required.

With respect to gender, females in both HIV/HBV co-infected and HIV mono-infected groups had higher mean platelet counts than their male counterparts. This agrees with other several previous studies which reported a generally higher mean platelet count in females than males [61] - [65].

Finally, the association between HIV and anaemia is well documented in literature [22]. It is common knowledge that HIV infection is associated with anaemia (which is usually normocytic and normochronic, but can also be macrocytic in late HIV disease), necessitating frequent blood transfusion. Report indicates that most blood used for transfusion in developing countries do not undergo standard screening recommending by WHO [66]. Frequent receipt of such blood by HIV infected persons, places them at higher risk of contracting other blood borne related diseases such as Hepatitis B. This may well explain the higher prevalence of HBV in HIV infected persons. Studies have also reported that infection with hepatitis viruses can lead to bone marrow failure and pancytopenia causing anaemia [59].

In this study, though varying mean hemoglobin concentration was recorded among HIV/HBV co-infected and HIV mono-infected patients across all age groups, HBV infection was found not to significantly affect their haemoglobin concentration. This is however; contradict the report of Obi *et al.* [51]. The observed variation may be due to differences in the nature of the study population. The cohort in Obi *et al.* [51]'s work were HIV infected patients on HAART, whereas, ours were HAART naïve HIV infected patients.

4. CONCLUSION

HBV exist among HIV positive patients living in Osun State, Nigeria, with a prevalence rate of 16.5%. Although, no significant difference was observed in the Platelet count and Haemoglobin concentration of HIV/HBV co-infected when compared to those of HIV mono-infected patients, regular monitoring of their immunohaematological parameters is encouraged to prevent associated sequalea.

CONSENT

All authors declare that 'written' informed consent was obtained from the participants and pre-test counseling was instituted in the course of the conduct of this study.

ETHICAL APPROVAL

Ethical approval was obtained from the Igbinedion University Health Research Ethics Committee, and also from the Ethical Committee, Ministry of Health, Osun State, Nigeria.

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