

# Triple positivity of HBsAg, anti-HCV antibody, and HIV and their influence on CD4+ lymphocyte levels in the highly HIV infected population of Abeokuta, Nigeria

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## Abstract

**Background:** Few studies exist on hospital-based seroprevalence of triple positivity of HIV/HBV/HCV in Nigeria.

**Objectives:** The study aimed at determining the triple positivity of HIV, HBsAg and HCV among HIV-infected individuals in Abeokuta, Nigeria and defining the influence of these triple infections on CD4+ counts of HIV-infected individuals as antiretroviral therapy improves in Nigeria.

**Methods:** Enumeration of CD4+ levels in 183 HIV-infected persons was done with Partec Flow Cytometer. Seropositivity of HBsAg and anti-HCV antibody was detected with rapid kits.

**Results:** From the result obtained, significance variance ( $p < 0.05$ ) existed between HIV positive persons and persons who tested positive to HIV/HBV/HCV triple infection before and after the commencement of HAART. Of these infections, 31(16.9%) had HBV/HCV/HIV triple infection, while 152(83.1%) had HIV mono infection only, 56(30.6%) had HBV/HIV dual infection only and 43(23.5%) had HCV/HIV dual infection only. Significant variance ( $p < 0.05$ ) also existed between subjects with CD4 counts of  $< 200$  cells/ $\mu$ l, 200-499 cells/ $\mu$ l and  $> 500$  cells/ $\mu$ l. Highest seroprevalence of HIV (35.0%) was found in age groups 35-44 years and  $> 65$  years had the least (2.7%). Significant variance ( $p < 0.05$ ) also existed in the progression of CD4+ lymphocytes cells between subjects with persistent decrease (32.3%) in CD4+ lymphocytes cells and those with fluctuation in their CD4+ lymphocytes cells (12.9%) after the commencement of ART.

**Conclusion:** The study further confirms that triple positivity of HIV/HBV/HCV infection is common in Abeokuta, Nigeria. Testing of these triple infections should be a big concern in the best choice and commencement of ART. Also, the study showed that consistent and prolonged use of HAART had a positive impact on the CD4 count of HIV-infected individuals.

**Keywords:** AIDS, ART, HAART, CD4, HIV/HBV/HCV

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## Introduction

Dual or triple infections with human immunodeficiency virus (HIV), hepatitis B and C viruses (HBV and HCV) remain a major public health concerns as most

drugs have considerably enhanced the control and/or management of single infections<sup>1</sup>. There is increasing evidence that triple infections of HIV/HBV/HCV is a common unrestricted health issues<sup>2</sup> which affects the clinical course of the disease<sup>3,4</sup>. They are overwhelming disease agents that shared modes of transmission<sup>5-7</sup>, thus HIV infected individuals are at risk of dual or triple infections with HBV and HCV infections<sup>8</sup>. Long-lasting infections with HIV, HBV, and HCV are major public health problems<sup>6</sup> which may potentially be as a result of virological interactions and could have an underlying immunological mechanism<sup>4,9-10</sup>.

Dual infections with HIV/HBV or HIV/HCV and triple infections with HIV/HBV/HCV is also vastly prevailing among intravenous drug users (IDUs)<sup>11-13</sup>. Amongst the transmissible blood-borne viruses through

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the parenteral route (blood transfusion and sexual intercourse), HIV, HBV and HCV are significant and have numerous consequences<sup>6,13-15</sup>. However, epidemiology of HIV-HBV-HCV triple infections varies as a result of differences in background of hepatitis infections and routes of HIV transmission<sup>16</sup>. These viruses does not merely create asymptomatic tenacious infections with chance sequelae, nevertheless they similarly lead to major illness and death when spread by transfusion<sup>6,17</sup>.

Immunologically, the incursion of the human body by any of HIV, HBV or HCV is initially known to innate immunity, thereafter to the cellular and humoral immune reaction<sup>7,18-22</sup>. Furthermore, cellular and humoral immunity consist of cluster of differentiation-4+ (CD4+) T-helper cells-1 and cytotoxic CD8+T-cells which mark and fix endogenously treated viral proteins which are conveyed on the superficial of diseased hepatocytes, and are ultimately disintegrate on the long run<sup>7,23-24</sup>. This facilitates shedding of HIV, HBV and HCV from the body of immunocompetent ill persons. This leads to immune-intermediated hepatocytes (liver) impairment<sup>7,25</sup>. The explicit cellular and humoral immune reaction is consist of antibodies targeted at particular antigens of HIV, HBV and HCV<sup>7</sup>. The absence or presence of anti-HCV antibodies or HBsAg is measurable by particular laboratory investigations, besides, they consequently function as dependable markers of normal infection, which are valuable in epidemiology of HIV, HBV and HCV<sup>7,26-29</sup>.

In the case of CD4+, which is a glycoprotein conveyed on the exterior of regulatory T cells, T-helper cells, dendritic cells, monocytes and macrophages and; they are the primary target for HIV<sup>30</sup>. The CD4+ T-lymphocytes cells however, are used to measure disease progression and to decide the commencement of ART<sup>31</sup>. HIV leads to a consistent decrease in CD4+ T-lymphocytes cells. Majority of people with HIV have been observed to have fallen in the CD4+ T-lymphocytes cells over time. Persons with AIDS show T-cell lymphopenia, a forfeiture of CD4+ lymphocytes and comparative proliferation in CD8+subtype and in the CD3+CD4-CD8- subtype<sup>31</sup>. A clear-cut count of CD4+T cells is essential for dependable and well-ordered antiretroviral therapy (ART) and monitoring<sup>31</sup>. Therefore, the CD4+lymphocytes count is useful to monitor the immune system, when to start HIV treatment and effectiveness of HIV treatment<sup>32</sup>.

Highly active antiretroviral therapy (HAART) has distorted HIV and AIDS from a consistently deadly ailment into a controllable long-lasting infection and has been presented to reinstate CD4+ cells in HIV positive persons<sup>8,33,34</sup>. The achievements of HAART might be conceded by dual or triple infections with hepatitis viruses as they are recognized to have antagonistic consequences on the scenario of HIV and hepatitis infections<sup>8,34,35</sup>. Subsequently, improved attention has to be paid on dual or triple infections of hepatitis viruses and HIV particularly in the emerging countries such as Nigeria where these sets of viruses are prevalent<sup>8</sup>.

While the proportion of individuals with dual or triple positivity is lesser, the blend of HIV and HBV and/or HCV is a precarious co-existence<sup>6,36-38</sup> and might devise a damaging consequence on the infected persons and the outcome of treatment<sup>6</sup>. Dual or triple infections with HBV or HCV upsurges the menace for hepatotoxicity of HAART and possibility of inception of an AIDS-defining illness, likened with infection with HIV only<sup>8,34,35,39</sup>. Studies previously have proposed that dual positivity of HIV/HBV or HIV/HCV and triple positivity HIV/HBV/HCV have dampened immune reaction to HAART likened with those with only HIV<sup>40-42</sup>. While others studies have reported some degrees of immune reinstatement in individuals with HIV/HBV or HIV/HCV dual-infection<sup>42-48</sup>. Management of either hepatitis virus is multifaceted because of pharmacokinetic interfaces with constituents of HAART regimens. Hence, the marvel of HIV and hepatitis viruses' dual or triple infections is a cause for concern<sup>8</sup>.

Although the HIV dual or triple infections with HBV and/or HCV has been documented globally in persons prone to blood-borne diseases, restricted data are obtainable on the degree of dual or triple infection and consequence of these viruses on the immune system in emerging countries<sup>8</sup>. Nigeria fits to the set of countries vastly prevalent for viral hepatitis<sup>49</sup>. Limited studies have been completed on HIV, HBV, HCV individually in Nigeria nevertheless the information about the interrelationship between these viruses and their consequence on the immune system remains vague<sup>8</sup>.

Thus, the study aimed at determining the triple positivity of HIV/HBV/HCV in HIV-infected persons in Abeokuta, Nigeria. The study also aimed at defining the impact of these infections on CD4+ counts among HIV-infected persons as antiretroviral therapy improves in Nigeria.

## Methodology

### Study area and population

The present study was carried out at the Federal Medical Centre, Idi-Aba, Abeokuta, Ogun State, Nigeria. One hundred and eighty three (183) HIV-positive persons participated in the study, one hundred were females while 83 were males. Participants were confirmed HIV-positives, ages 15 years and above. Approval was obtained from the Ethics Committee of the hospital. Participants also gave their consents and pertinent confidentiality was kept all through the study.

### Sample collection and preparation

Venous blood from the 183 HIV-infected persons were obtained into plain tubes without any anti-coagulants. Sera from the venous blood was obtained by spinning for 5 min at 2000 resolution per minutes (rpm). The decanted sera were kept at -20oC for serological analysis. In the same vein, another four millilitres (4ml) of blood was collected into an EDTA anti-coagulated tube for enumeration of CD4+ cells.

### Serologic assay

The whole samples were retested using HIV rapid kits (Bioline Standard Diagnostic Inc, Korea). It is a quick immunochromatography technique for detecting IgA, IgG and IgM antibodies specific to HIV-1/2 concurrently in serum. A red colour line in the patient and control windows is indicative of seropositivity while the existence of the red-coloured line in the control and its non-appearance in the patient window is indicative of seronegativity.

The samples were also screened for HBsAg and an-

ti-HCV antibody using ACON HBsAg and anti-HCV antibody test kits respectively. This is a membrane-based immunoassay (manufactured by ACON Lab., Inc. San Diego, USA). In this kit, the test region had been previously coated with recombinant HBsAg and HCV antigen. Both kits are based on chromatographics vessel movement to form coloured lines. The existence of coloured line is indicative of seropositivity while its non-appearance is indicative of seronegativity.

Assay for the CD4+ lymphocytes cells were carried out using the Partec Flow Cytometer (CyFlow counter). This is a fluorometric method used to differentiate and count cells present in the sample. Cells in suppression pass through in flow curvette in a narrow stream. Finally fluorescence signal are segmentally generated and signal are detected and displayed. The CD4 levels of each patient were examined at initial visit and then three months and six months after commencement of ART treatment.

### Data analysis

The proportions of participants with triple infection of HIV/HBV/HCV were calculated. CD4+ counts of individuals infected with HIV/HBV/HCV were presented in figures with sex, age and the CD4+ count progression as well as their response to ART. Relevant chi-square statistics were calculated to complement each result using SPSS 20.0 window packages.

### Results

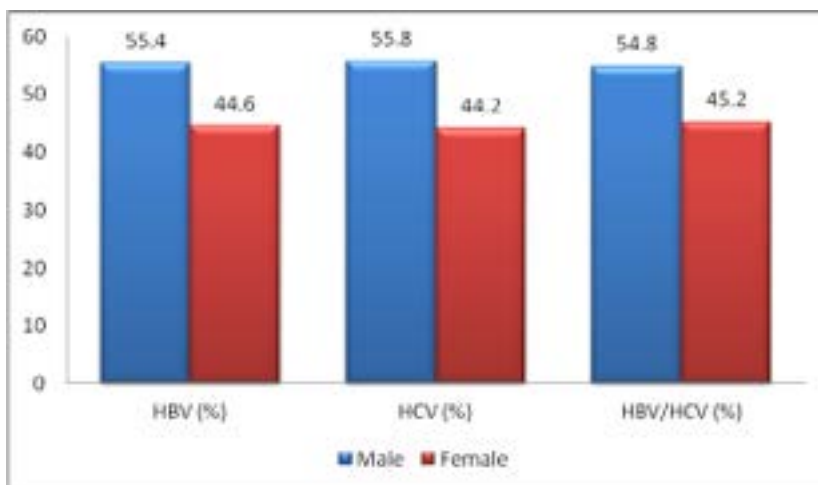
In the present study, 183 HIV-infected individuals were examined (Table 1). Of which, 100 (54.6%) were females and 83 (45.4%) were males.

**Table 1: Sex and Age Distribution of HIV-Infected Subjects**

<b>Risk factors</b>	<b>Number (%)</b>
<b>Sex</b>	
Males	83 (45.4)
Females	100 (54.6)
<b>Age</b>	
15-24	33(18.0)
25-34	42(23.0)
35-44	64(35.0)
45-54	29(15.8)
55-64	10(5.5)
65-74	5(2.7)
<b>Total</b>	<b>183 (100.0)</b>

Higher percentages of HIV positives were in age group 35-54 years (35.0%) while age group 65-74 years (2.7%) had the least as shown in Table 1. Significant difference ( $p < 0.05$ ) existed between sex, age and seropositivity of HIV.

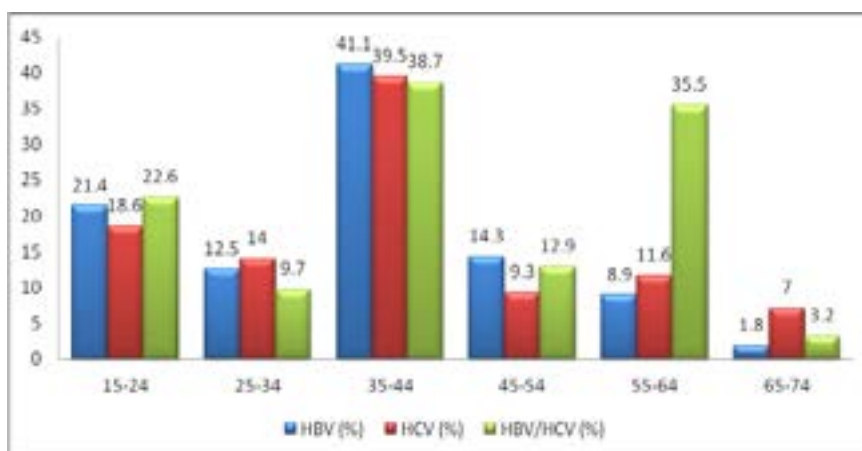
Of these 183 HIV-infected subjects, 152(83.1%) had HIV mono infection only, 56(30.6%) HBV/HIV dual infection only and 43(23.5%) had HCV/HIV dual infection only and 31 (16.9%) had HBV/HCV/HIV triple infection. Figure 1 shows the sex-related seroprevalence of triple infections of HIV/HBV/HCV.



**Figure 1: Sex- related seroprevalence of HIV/HBV/HCV triple infections**

The sex-related seropositivity revealed higher seropositivity of HIV/HBV/HCV in males (54.8%) compared

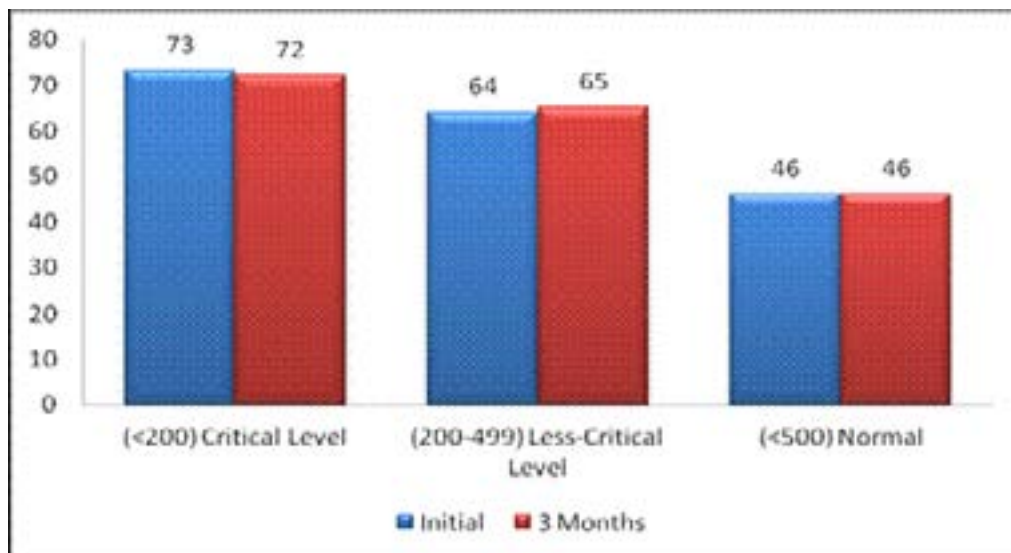
to females (45.2%). However, no significant variance ( $p > 0.05$ ) existed between sex and triple infections of HIV/HBV/HCV.



**Figure 2: Age-related seroprevalence of HIV/HBV/HCV triple infections**

The age-related seroprevalence of HIV/HBV/HCV is shown in Figure 2. Triple seropositivity of HIV/HBV/HCV (38.7%) was higher in age group 35-44 years than in others. However, no significant variance ( $p > 0.05$ ) existed between age and triple infections of HIV/HBV/HCV.

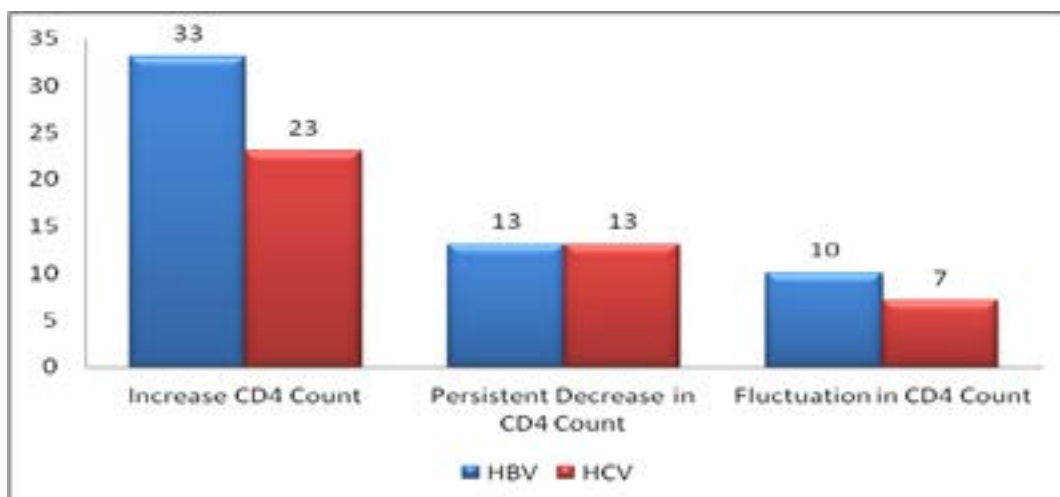
Figure 3 shows CD4+ lymphocytes cells of HIV-infected subjects before and after initiation of ART. It showed that CD4+ lymphocytes cells at the initial visit before commencement of the antiretroviral therapy (ART) with  $< 200$  cells/ $\mu$ l were 73 (39.9%) and subjects having CD4+ lymphocytes cells  $> 500$  cells/ $\mu$ l were 46(25.1%).



**Figure 3: CD4 Count of HIV-Infected subjects before and after Initiation of ART**

CD4+ lymphocytes cells after 3 months of initial visit to the ART clinic showed that there was a marginal decrease among individual with CD4+ lymphocytes cells [72(39.9%)] and marginal increase with those CD4+ lymphocytes cells between 200-499 cells/ $\mu$ l [65(35.5%)] and individual with CD4+ lymphocytes cells of >500 cells/ $\mu$ l were same as the initial visit [46(25.1%)]. Furthermore, CD4+ lymphocytes cells after 6 months

of initial visit to the ART clinic showed that there was a marginal decrease in subjects with CD4+ lymphocytes cells <200 cells/ $\mu$ l [70(38.3%)] and subjects having CD4+ lymphocytes cells of 200-499 cells/ $\mu$ l were 62(33.3%). Subjects with CD4+ lymphocytes cells of >500 cells/ $\mu$ l increased to 52(28.4%) showing a positive impact on the CD4+ lymphocytes cells on the basis of consistent rise in the use of ART (Figure 3).



**Figure 4: CD4 Count Progression in relation to seroprevalence of HIV/HBV/HCV triple infections**

This difference is also statistically associated ( $P < 0.05$ ). Figure 4 shows CD4+ lymphocytes cells progressions in relation to HIV-HBV-HCV triple infections among the subjects. It showed a progression of CD4+ lympho-

cytes cells among subjects with HIV-HBV-HCV triple infections [17 (55.8%)]. Significant difference ( $p < 0.05$ ) existed between CD4+ lymphocytes cells progressions of HIV-infected subjects and HIV/HBV/HCV triple infections (Figure 4).

## Discussion

Several studies have proposed that human immunodeficiency virus can fast-track the usual sequence of long-lasting HCV and/or HBV<sup>13</sup>. Earlier studies devise that persons with long-lasting HCV/HBV and HIV will experience swift advancement besides being prone to death in comparison with non-HIV infected persons<sup>13</sup>. Contrariwise, the consequence of HCV and/or HBV on HIV infection remains indistinct<sup>13,38,50-51</sup>. Dual or triple infections of HIV and hepatitis viruses have considerably amplified disease and death rates of HIV and AIDS infected-persons<sup>13,38,50-51</sup>.

Triple infections are thus rising menace in Nigeria and cautious attention is needed due to its adversarial consequences on HIV treatment reaction<sup>8</sup>. Thus, it became necessary to estimate the seroprevalence of triple infections with HIV/HBV/HCV, particularly amid groups known to be a high-risk populace of dual or triple infections. The present study aimed at determining the seroprevalence of HIV/HBV/HCV triple infections among HIV-infected individuals in Abeokuta, Nigeria. The study also aimed at defining the impact of these infections on CD4+ lymphocytes cells among HIV positive persons as access to antiretroviral therapy advances across the Nigerian nation.

Of the 183 HIV-infected subjects used in this study, 152(83.1%) had HIV mono infection and 31 (16.9%) had HIV/HBV/HCV triple infections. Previous studies in Nigeria had reported varying seroprevalences of HIV/HBV/HCV infections. Forbi et al.<sup>8</sup> had reported an overall HIV/HBV/HCV prevalence of 7.2%. A 3.9% seroprevalence of HIV/HBV/HCV was reported by Balogun et al.<sup>52</sup> and 6.5% seroprevalence was reported by Okeke et al.<sup>53</sup>.

Higher seroprevalence of HIV/HBV/HCV triple infections in males than their female colleagues. Higher seroprevalence was recorded among age groups 35-44 years than in other age groups. This is in consonance with what was reported previously in the Northern Nigeria<sup>16,54</sup> and elsewhere in the world<sup>32,55-56</sup>. This may be due to fact that males at age group are most sexually active. Elsewhere, the triple infection rates of 19.1% and 10.4% was reported in China and Myanmar respectively<sup>13</sup>. A seroprevalence of 1.83% was reported by Agarwal et al.<sup>57</sup>. Musyoki et al.<sup>58</sup> reported 29.4% prevalence of HIV/HBV/HCV infections in South Africa. Moreover, it has been indicated that the dual infections of HIV/HBV and HIV/HCV) or triple infections of

HIV/HBV/HCV is most common, though the dual or triple infections rates varies liable to risk groups, type of exposure involved and geographic regions<sup>13,59-61</sup>.

The usual antiquity of HBV is identified to be intricated by HIV dual infections nevertheless the influence of HBV on the aftermath of persons infected with HIV-1 is contentious<sup>8,63</sup>. There was flawless clue of amplified HCV infection in HIV positive persons in Nigeria. It is also well-known that HIV/HCV dual positivity fast-track patients rapidly to AIDS defining clinical event, end-stage liver disease and death<sup>8,39,63</sup>. Unfortunately at this time, only HBV vaccine is available, HIV vaccine is under way and no active vaccine against HCV<sup>8</sup>.

The CD4+ lymphocytes values of 183 confirmed HIV positive persons were counted by means of a superior fluorescence activated cell sorter system. The result indicates that most of the subjects were diagnosed when the CD4+ lymphocytes cells were <200 cells/ml. The CD4+ lymphocytes cells of HIV/HBV/HCV triple infected subjects were significantly lower ( $P < 0.05$ ) than those with HIV mono infection only. The details for the drop in CD4+ level is unclear however, it is recognised that there is disparity in outlying blood T-lymphocyte subsets and commotion in cellular immunity in the persons with long-lasting hepatitis B<sup>64</sup>. Also, HIV-HBV-HCV triple infected individuals responded poorly to the ART treatment than those with HIV mono infection only. This finding is in agreement with previous reports<sup>16,32,65-67</sup>.

This study also showed a progression of CD4+ lymphocytes cells among subjects with HIV-HBV-HCV triple infections [17(55.8%)]. The consistency and regular use of antiretroviral therapy as prescribed by the counselors improved the immunity of the HIV infected individual as indicated by progressive increase in the CD4+ lymphocytes cells which is in support with previous reports<sup>3,9,30,32,68-70</sup>. Forbi et al.<sup>8</sup> in a similar study, reported that rise in CD4+ cells after HAART does not alter in persons with triple infections of HIV/HBV/HCV however, HCV seems to deter virological response to ART.

## Conclusion

As far as we know, there are few reports on the triple positivity of HIV/HBV/HCV infections in the highly HIV infected populations in Nigeria. This study further endorses the possibility and endemicity of HBV and HCV dual or triple infections and increased infection in

HIV-infected individuals. Planned prevention, screening and treatment are needed to reduce further transmission and morbidity of these infections. Intensified HBV vaccination program is also advocated in Nigeria which is known to be able to substantially diminish the occurrence of HBV infections.

This study has also demonstrated that dual or triple infections of HIV and hepatitis viruses (HBV and/or HCV) are on the rise in Nigeria. It seems to decline the CD4+ lymphocytes cells of patients who have triple positivity of HIV, HBV, and HCV. It also showed that consistent and prolong use of antiretroviral therapy will influence positively CD4+ lymphocytes cells of HIV-infected individuals. Thus, we call for sustainable educational promotion programmes on the means of acquisition and spread of HIV, HBV and HCV, as well as increasing HBV vaccinations in order to curtail the spread of HIV, HBV and HCV in Abeokuta, Nigeria. However, further studies on the mechanism by which the HBV and HCV act as a cofactor for the HIV-infected individual is thus warranted in this part of the country.

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