IMMUNOLOGICAL AND VIROLOGICAL CHANGES IN HUMAN IMMUNODEFICIENCY VIRUS (HIV) PATIENTS WITH HEPATITIS C VIRUS (HCV) CO-INFECTION ON ANTIRETROVIRAL THERAPY (ART) IN NORTH CENTRAL, NIGERIA.

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ABSTRACT

The Human Immunodeficiency Virus (HIV) and Hepatitis C Virus (HCV) are two viruses with similar characteristics having in common diversities in numerous subtypes and capacities for mutation. A total of 2,322 infected patients with HIV and 109 co-infected with HCV on ART were reviewed after every three months for fifteen months from June, 2013 through February, 2015 at ART hospitals in North Central, Nigeria. The determination of CD4+ count and viral load estimation were carried out using Flow Cytometry (Partec-cyflow, Germany) and PCR based Amplicor HIV-1 monitor version 1.5 (Roche Diagnostic Systems, Branchburg, NJ, USA) according to manufactures instructions. The results of the blood samples showed that ART therapy increased CD4+ count from 231.7±1.9401 to 466.8±2.0285cells/µl and viral load was suppressed from 17786.59±3316.36 to 1371.86±131.04copies/ml for HIV mono-infected patients while the mean CD4+ cell counts of co-infected individuals increased from 157.4±3.945 to 329.9±5.3998cells/µl and viral load decreased from 22821.62±4098.53 to 10246.82±2169.98copies/ml. The differences in the values were statistically significant (p<0.05). In conclusion, the reports revealed that at the end of the 9th month of ART, the CD4+ counts and suppression of the viral particles and even the clinical picture of patients tend towards normalcy in HIV mono-infected but these analytes were not adequate even at the 15th month in detecting the success of ART in co-infected patients, when their CD4+ counts were compared with the normal adult range (365–1,571cells/µl). Therefore, clinicians should give more attention and care to those patients co-infected in order to offer quality care.

KEYWORDS: HIV/AIDS; ART; HCV; ARV, HIV/HCV, PCR.

INTRODUCTION

The Human Immunodeficiency Virus (HIV) and Hepatitis C Virus (HCV) are two viruses with similar characteristics having in common diversities in numerous subtypes and capacities for mutation. Huge resources and technical inputs for preventive vaccines have remained largely futile in spite of decades of research in this area.[41] The two viruses are blood-borne infections. A significant number of people are chronically infected with both HIV and HCV, a condition known as co-infection. Co-infection is the simultaneous infection with two or more different disease causing organisms. The widespread use of increasingly effective antiviral therapies for HIV has greatly improved long-term survival among HIV infected persons. As HIV/AIDS survival has improved, HCV-related illness and deaths have increased among co-infected persons.[42, 43]

Co-infections are common public health problems and recognized worldwide.[1] Co-infection with hepatitis viruses (hepatotropic viruses) are known to influence progression, management as well as outcome of HIV infection.[1, 2] The viruses are blood borne pathogens and share similar routes of transmission.[1, 2] It is a growing problem and is associated with increased risk of antiretroviral related hepatotoxicity and increased risk of progression to liver diseases which is a major cause of morbidity and mortality in HIV infected patients.[3, 4]

Other infections are seen in HIV positive individuals, which include Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), dual HBV and HCV.[5] Worldwide, prevalence of co-infection among humans is unknown.[6] The prevalence is high worldwide, high prevalence of hepatotropic viruses in HIV positive individuals may be as a result of shared modes of transmission between HIV

and hepatotropic virus infections, secondly, it may be due to the phenomenon of reactivation of hepatotropic viruses in the setting of HIV immunodeficiency and lastly, socio-demographic factors: the unawareness of the population at large of the mode and route of transmission.[7]

Co-infections and opportunistic infections are the major causes of deaths among HIV positive individuals due to risk factors, type of exposure and geographic region.[8] Co-infections are major burdens in the health care system Africa. Infections with hepatotropic viruses are characterized by a higher prevalence of injection drug use, poverty and psychiatric disorder.[9]

Co-infection affects disease progression related to HIV and hepatitis viruses and complicates treatment.[10, 11] The rate of liver cirrhosis is up to six times higher in HIV co-infected persons than hepatitis viruses mono-infected.[12,13] Infection of HIV with other viruses have been recognized worldwide in individuals exposed to blood borne diseases, but limited data are available on the extent of co-infection, effect of these viruses on the immune system and liver in developing countries.[7]

Nigeria is having high burden of viral hepatitis. Few studies have been done on the prevalence of HIV/Hepatitis virus co-infection in Nigeria but the knowledge about the interrelationship between these viruses and their effect on the immune system still remains unclear.[7] Clinical management and treatment of HIV co-infected patients is controversial, challenging and complex.[11, 14] Abnormal hepatic function is one of the most common complications occurring among HIV infected individuals receiving antiretroviral therapy.[11, 15] It is well documented that all HIV co-infected individuals should be screened for HCV, HBV and those with co-infection should be considered for anti HCV treatment.[11, 15, 17] However, treating HIV first is clearly indicated when CD4+ lymphocyte count is very low (<200cells/μl).[14]

Therefore, this study was aimed to determine the trend of the changes in CD4+ counts and HIV-RNA load in responses to ART of HIV/AIDS and HIV/HCV co-infected patients and to ascertain the socio-demographic information of those co-infected in North Central, Nigeria.

MATERIALS AND METHODS
Study Areas
Nigeria is a federal constitutional republic comprising thirty-six states and Federal Capital Territory. The country is located in West Africa between latitudes 4^0 and 14^0 North and Longitude 3^0 and 15^0 East, with total land area of 923.8×10^3 square kilometres bordered with the Republic of Benin in the west, Chad and Cameroon in the east, and Niger in the north. Its coast lies on the Gulf of Guinea, a part of the Atlantic Ocean, in the south. The capital city is Abuja. The country Nigeria

comprises of six geo-political zones, which includes South West, South South, South East, North West, North East and North Central or Central Nigeria known as the middle belt of Nigeria.[30]

Three states from the North Central geo- political region and FCT of Nigeria were selected for the study. The states were Kogi, Nasarawa and Niger. The three states were selected using systematic random sampling technique. This method of selection guarantees that all states are equally likely to be drawn.

Ethical Considerations
Ethical considerations and approval for the study was sought from the Health Research and Ethics Committee of the studying hospitals in North Central, Nigeria to include: Federal Capital Territory (FCT) Abuja, FMC Lokoja, FMC Keffi and General Hospital Suleja. The patients were enrolled after they were sufficiently counseled, their written informed consents was obtained. Relevant confidentiality was maintained throughout and after the study period.

Questionnaires
In order to ascertain the socio-demographic information, a questionnaire was used in studying the possible risk factor for transmission. These include age, sex, present place of abode, HIV status (if known), occupation, history of previous blood transfusion, phone number.

Study Design
The research was a prospective study. The recruitment of the subjects was non-randomised and questionnaires were administered on those that consented to participate in the study. The laboratory monitoring (baseline) of both HIV/AIDS and HIV/HCV co-infected patients on ART were reviewed after every three months for fifteen (15) months after pre-ART counseling/registration process at the ART clinics of the various hospitals. They were enrolled into the study when found eligible for ART as per the national guidelines on ART and fulfillment of the following inclusion/exclusion criteria. HIV infected adults, above 18 years of age, from both sexes and not on ART prior to the study was included while patients that are critically ill and on ART prior to the study were excluded.

Study Population
A total of two thousand three hundred and twenty two (2,322) HIV/AIDS and 109 HIV/HCV co-infected patients were recruited and were followed up every three months for fifteen (15) months in this study. The age range of the patients was 18- 58 years with mean of 38.0 years. In these clinics, first line (HAART) regimens were administered to include: Truvada™, Nevirapine, Combivir™ and Efavirenz.

Blood Collection, Storage and Processing
Five (5ml) millilitres of venous blood were carefully drawn from the veins of each subject into well labeled

Ethylene Dietheyl Tetracetic Acid (EDTA) blood sample container for CD4+ cell counts and viral load according to the standard protocol. The blood sample was collected for baseline analysis and subsequent monitoring. After, the analysis of CD4+ count parameter, samples was centrifuged at 4,000 × g for 10 minutes. The plasma samples were aliquoted into cryovials and stored at -80°C freezer for further assay.

Determination of CD4+ Lymphocyte Cell Count
The CD4+ lymphocyte counts were determined for each patient at baseline and subsequently during follow up using Flow Cytometry (Partec-cyflow, Germany), according to manufacturer's instructions. This was the adopted technique for CD4+ counts estimation under the national ARV programme.[34] The CD4+ lymphocyte counts were expressed as cells/µl of blood.

Viral load estimation (HIV-1 RNA)
The viral load of each patient were quantified on plasma samples using the PCR based Amplicor HIV-1 monitor version 1.5 (Roche Diagnostic Systems, Branchburg, NJ, USA), according to manufacturer's instructions. The viral load was expressed as viral copies/ml of blood sample.

STATISTICAL ANALYSIS OF THE DATA
Data collected through a standardized questionnaire through out the study were entered into Excel spread sheet and analyzed. Means±SEM (standard error of mean) of the analytes (CD4+ counts and viral load) and duration (months) were computed. The comparisons between means were done using one-way analysis of variance (ANOVA). The level of significance was set at 0.05. All statistical analysis was performed using statistical product and service solutions (SPSS software) version 16.0, SPSS, Chicago, USA.

RESULTS
The outcomes of the 2,322 HIV infected patients monitored in this study; Out of these 1418 (38.9%) were female and 904 (38.9%) male patients. The HIV/HCV co-infected persons were 109 (4.7%); of these 74 (3.2%) were females while 35 (1.5%) are males. None of them have any other opportunistic infection during the time of follow up. The mean age of the study group was 38.0 (18 – 58) years.

A total of two thousand three hundred and twenty two (2,322) HIV infected patients monitored for the CD4+ Counts and Viral load estimations are shown in figure 1 and 2 respectively. The mean baseline CD4+ count was 231.7±1.9401cells/µl before the initiation of the ARV drug. After three month, the mean CD4+ count was increased to 281.9±1.8919cells/µl. At the end of the 6th month, the mean CD4+ count increased further to 327.4±1.9247cells/µl. At the end of the 9th month, the mean CD4+ count went up to 377.8±2.0872cells/µl. At the end of the 12th and 15th months, the mean CD4+ counts were 416.5±2.0389 and 466.8±2.0285cells/µl

respectively. In general, there was progressive increased in CD4+ count from the 3rd month through the 15th month of the study. This increment was statistically significant ($p<0.05$). The mean baseline viral load before commencement of the ARV drug was 17786.59±3316.36 copies/ml. At the end of the 3rd month, the mean viral load reduced to 12071.4±884.09 copies/ml. At the end of the 6th month, the mean viral load was 9974.98±736.68 copies/ml. At the end of the 9th month, the mean viral load reduced to 6977.08±510.73 copies/ml. At the end of the 12th month, the mean viral load was 1810.67±144.18 copies/ml. At the end of the study in the 15th month, the mean viral load was 1371.86±131.04 copies/ml.

Of the one hundred and nine (109) HIV/HCV co-infected patients monitored for the CD4+ Counts and Viral load estimations are shown in figure 3 and 4 respectively. The mean baseline CD4+ Count was 157.4±3.9457cells/µl. After the 3rd month, the mean CD4+ Count was 188.2±4.3657cells/µl. At the end of the 6th month the CD4+ counts increased to 222.5±4.5088cells/µl. At the end of the 9th month, the mean CD4+ count was 251.5±5.3998cells/µl. At the end of the 12th month, the mean CD4+ count was 291.4±5.3998 cells/µl, and at the end of the 15th month, the mean CD4+ count was 329.9±5.8086 cells/µl. The increase in CD4+ counts was statistically significant ($p<0.05$). The mean baseline viral load quantification was 22821.62±4098.53 copies/ml. After the 3rd month, the mean viral load reduced to 18981.48±3176.08 copies/ml. At the end of the 6th month, the mean viral load was 16242.32±2447.44 copies/ml. At the end of 9th month, the mean viral load quantification was 13892.37±1908.21 copies/ml. At the end of the 12th and 15th months, the mean viral load quantifications were 9446.0±1286.75 and 10246.82±2169.98 copies/ml respectively. The decreased in the viral load was statistically significant ($p<0.05$).
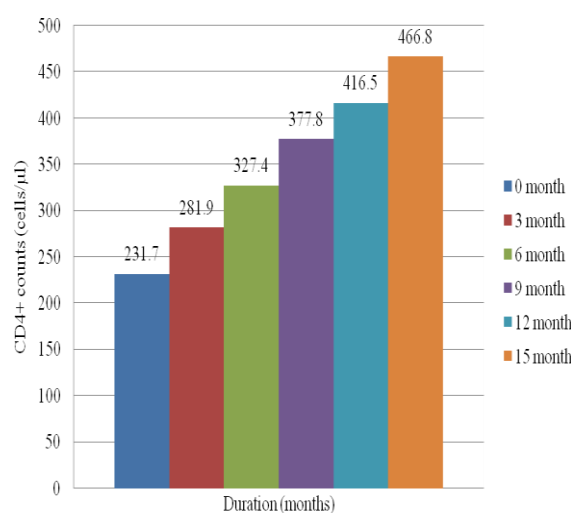

Figure 1: Mean CD4+ counts of HIV infected patients in North Central, Nigeria.
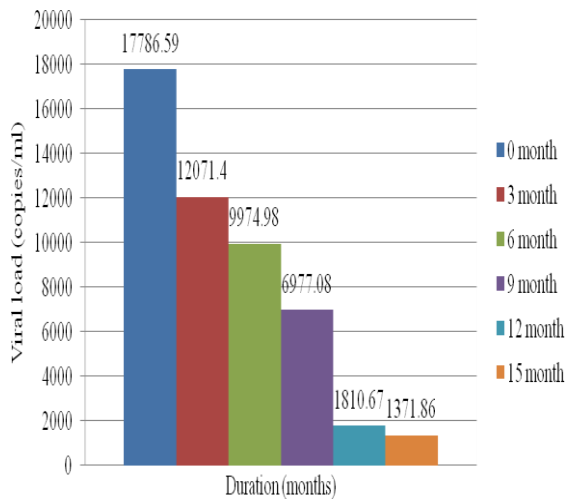Normal range of CD4+ counts (365 – 1571 cells/µl).

Figure 2: Mean viral load of HIV infected patients in North Central, Nigeria.
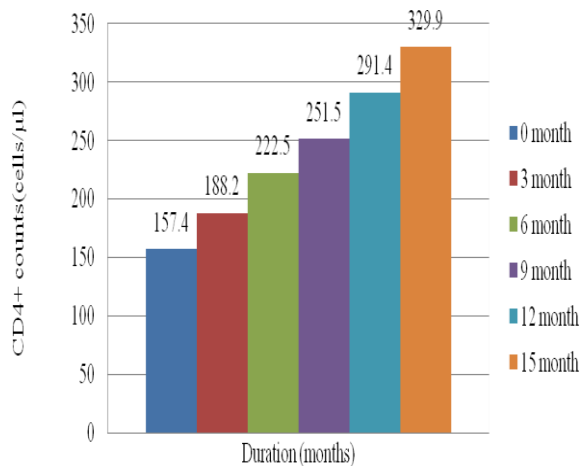

Figure 3: Mean CD4+ count of HIV/HCV co-infected patients in North Central, Nigeria.
Normal range of CD4+ counts (365 – 1,571 cells/µl).
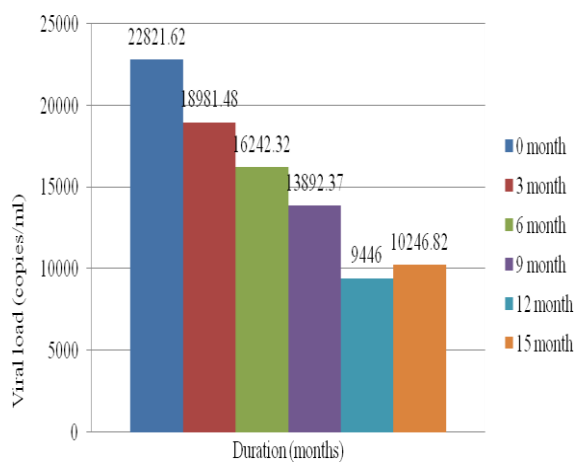

Figure 4: Mean viral load of HIV/HCV co-infected patients in North Central, Nigeria.

DISCUSSION
The CD4+ lymphocytes are the principal target cells for HIV virus infection. The number of CD4+ T cell count remains a useful marker of disease progression and widely used as indicators for starting ART, monitoring of treatment or primary prophylaxis for opportunistic infections. However, accurate determination of CD4+ cell count needs to be established by the use of Flow Cytometry, an inexpensive technique that is not available in the majority of ART centres laboratories particularly in developing and poor resource settings.[27,38] HAART has transformed patients with HIV/AIDS from a uniformly fatal illness into manageable chronic infection and has been shown to be able to restore CD4+ cells in HIV infected patients.[18] The gain of HAART could be compromised by co-infection with hepatitis viruses as they are known to have adverse effects on the prognosis of HIV infection.[33] Consequently, increased attention has to be paid on co-infection of hepatitis viruses and HIV especially in the developing countries like Nigeria where these groups of viruses are endemic.

In this study, 109 (4.7%) HIV infected individuals that were co-infected with HCV were monitored for fifteen (15) months for their immune responses to HAART. The mean CD4+ count values for this group was 157.4 cells/µl which is lower than that of HIV mono-infected persons (mean = 231.7 cells/µl) for the initiation of ARVDS, as evidenced by the higher CD4+ counts in HIV mono-infected than HIV-coinfected with HCV. Contrary to observations made by Ortegbayo *et al* in South-western and Idoko *et al* in North-Central part of Nigeria.[22, 29]

The lower count observed in this study was also in agreement with finding by other researchers.[18, 32, 38] The natural history of HCV is known to be complicated by HIV co-infection but the effect of HCV on the outcome of patients infected with HIV-1 is controversial.[19] We have demonstrated that co-infection of HIV and HCV appears to decreased the CD4+ counts of patients at end of the study, HIV/HCV co-infection and mono-HIV-infected were 329.9 cells/µl and 466.8 cells/µl respectively.

Generally, we showed in our study that the overall mean CD4+ counts increased with continued use of ARVDs during the fifteen (15) months of follow-up, which is consistent with many previous findings.[20, 24, 31] The three months reviewed was statistically significant (p<0.05).

Our data indicates that the majority of HIV patients started antiretroviral treatment with more advanced immunodeficiency status. Since the majority (74%) of the HIV patients had AIDS as defined by their CD4+ cell counts of < 200 cells/µl. This was significantly higher when compare to the other studies conducted in Nigeria, South eastern United States and Thailand which reported a lower rate of AIDS at the initiation of ART.[23, 37] This

was in agreement with the study of Agu _et al.,_[39] that large proportion of the patients are presented at very late stage for treatment with very poor baseline CD4+ counts and WHO clinical stage 3 or 4. However, Erah and Arute,[35] reported that the CD4+ counts for 44.1% of the patients included in their study were higher than 350 cells/μl, while less than 200 cells/μl were the minority. The reasons for the late presentation to the hospital may be due to denial and stigmatization which prevent acknowledgment of the problem and care seeking. Also, tradition beliefs and practices affect understanding of health, the disease and acceptance of conventional medical treatment. Furthermore, lack of information needed to understand and prevent HIV is contributing to late presentation of the disease at the hospital. Therefore, there is dire need for a rapid scale up of counseling and testing for early detection of asymptomatic cases in developing countries like Nigeria.

There was a significant improvement in the CD4+ counts (50cells/μl) 3 months after commencement of HAART in this setting. Though, such escalation of CD4+ counts was not observed after 6 months of HAART. This is an indication of viral load suppression and enhancement in immunological responses. The risk of disease progression to stage 3 or 4, prone a large proportion of the patients to opportunistic infections due to weakened immune systems.

We have also demonstrated that co-infection of HIV and HCV is on increase in Nigeria and appears to decrease the CD4+ counts of patients who are co-infected. The result of the study is comparable with the finding observed of a previous work in Jos, Nigeria.[26] Although the reasons for the CD4+ count declined is not clear but it is known that there is an in-balance in peripheral blood T-lymphocytes subsets and turbulence in cellular immunity in the individuals with chronic hepatitis infections.[28] Treatment of either hepatitis virus is complex because of pharmacokinetic interactions with components of HAART regimens. Thus, the phenomenon of HIV and HCV co-infection is a cause for concern. The medical community in Nigeria therefore needs to be alerted to this phenomenon as smart treatment options would need to be instituted in such individuals if treatment is to be meaningful.

This study also yielded several major findings in relation to viral load. HIV load is the strongest predictor for disease progression. In the cross sectional analysis, early levels of HIV load were inversely associated with CD4+ cell count, which has been used as an intermediate or surrogate measure of HIV progression. When compare to HIV mono-infected individuals, the viral load of those co-infected with HCV was higher both at baseline and even at the end of 15th months of ART in this study. These were similar to observations made by Thomas _et al.,_[21] and Di Martino _et al_.,[36] that HIV-HCV co-infection is generally associated with higher mean serum HCV-RNA levels than in patients with HCV infection

alone and the increase of HCV-RNA values is more marked in subjects with lower CD4+ cell counts.

The more elevated titres of HCV viral load in co-infected individuals probably reflect the unimpaired control of HCV replication caused by HIV-induced immunodeficiency, but the real impact of increased HCV viremia on the progression of chronic hepatitis C in HIV-positive subjects is still uncertain.[21] Although, there was continued increased in CD4+ count while the viral load was appreciably suppressed in the two groups of patients monitored and this compare to similar study by Marscher _et al.,_[25] who concluded that CD4+ counts continued to increase even when the concurrent viral load is detectable. It is plausible that HIV preferentially infects CD4+lymphocytes which are activated against HCV. This infection might remarkably reduce the cell-mediated immunologic responses to HCV during acute hepatitis C and contribute to viral persistence.[40]

CONCLUSION
In conclusion, the study revealed that laboratory investigation of patients for immunological responses and viral load estimation were adequate to detect success of the ART at the 9th month of treatment, because suppression of the viral particles and even the clinical picture of patients tend towards normalcy in HIV mono-infected patients when compared to their normal adult values. The study also proved that the investigation into immunological responses and viral load quantifications was not adequate even at the 15th month in detecting the success of ART in HIV/HCV co-infected patients when CD4+ counts were compared with the normal adult range (365 – 1,571 cells/μl) and thus, clinicians should give more attention and care to those patients co-infected with HIV/HCV.

CONFLICT OF INTEREST
The authors declare that they have no conflict of interest.

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