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IMMUNOLOGICAL RESPONSES AMONG HIV/AIDS PATIENTS BEFORE AND AFTER HAART THERAPY IN SOME SELECTED HOSPITALS IN NORTH CENTRAL, NIGERIA.

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ABSTRACT

Human Immunodeficiency Virus (HIV), is the agent that causes acquired immune deficiency syndrome (AIDS). Although, highly active antiretroviral therapy (HAART) is known to profoundly suppress viral replication, it increases CD4+ cell count and delays both disease progression and death. The aim of this prospective cohort study was to assess immunological (CD4+ recovery) responses among HIV infected individuals receiving HAART with long-term follow-up. The study was carried out in some selected ART hospitals in North Central Nigeria from June, 2013 to February, 2015. Immunological determination was carried out using Flow Cytometry (Partec- cyflow, Germany), according to manufacturer's instructions. A total of 2,322 HIV positive patients were reviewed after every three months for fifteen (15) months in this study. The mean baseline CD4+ count was 231.7 cells/µl; the mean CD4+ count at the 3rd, 6th, 9th, 12th and 15th month were 281.9, 327.4, 377.8, 416.5 and 466.8 cells/µl respectively. There was a good immune recovery at the 3rd month of therapy from the baseline mean CD4+ T cell count of 231.7 cells/µl to 281.9 cells/µl, which was statistically significant ($p<0.05$). This remarkable rise was continued in achieving the mean CD4+ count of 327.4 cells/µl at the 6th month of monitoring. In this study, although good CD4+ cells recovery in responses to ART was documented in more than 89% of follow-up cases, despite these patients were enrolled in ART program at decreased CD4+ cells count.

Therefore interventions need to be designed to promote early HIV testing and early enrollment of HIV infected individuals into ART services.

KEYWORDS: ART, CD4+ count, HIV/AIDS, HAART, Cohort.

INTRODUCTION

Human Immunodeficiency Viruses (HIV-1 and HIV-2) are the etiologic agents for Acquired Immune Deficiency Syndrome (AIDS) in humans.[5] According to Schim and Aaby,[14] HIV-1 has spread to most parts of the world, while HIV-2 has remained largely restricted to West Africa. They both cause AIDS and the routes of transmission are the same. However, HIV-2 causes AIDS much more slowly than HIV-1.[25] Gottlieb,[7] reported that AIDS was first recognized in the 1980s and is the leading cause of death in many developing countries today. AIDS is one of the most destructive epidemics in the history of mankind and it is define by the manifestation of at least 2 clinical signs associated with immune suppression in addition to a CD4+ lymphocyte count of less than 200 cells/µl.[11]

The CD4+ molecules are the major receptor of HIV, it has a high affinity for the viral envelope; thus Ellen *et al.*,[3] reported that infection can be blocked by monoclonal antibodies to CD4+ cell and by recombinant soluble CD4+ cell. The HIV co-receptor on lymphocyte is the CXCR4 chemokine receptor.[2, 12] The characteristic feature of HIV infection is the depletion of T-Helper – inducers Lymphocytes – the results of the tropism of HIV for this population of lymphocytes, which expresses the CD4+ phenotypic marker on their surface.

The consequences of CD4+Tcell malfunction caused by HIV infection are devastating because the CD4+T lymphocytes play an important role in the immune responses. It is responsible directly for induction of a wide array of lymphoid and non-lymphoid cell functions. These include activation of macrophages, induction function of cytotoxic T cells; and secretion of a variety of soluble factors that induce growth and differentiation of lymphoid cells and affect hematopoietic cells.[8, 9]

Enumeration of CD4+ T cell count has been useful to initiate and monitor therapy in HIV infected individuals taking potent ART. The CD4+ T cell count recovery shows high variability among patients.[23] The CD4 cell count response to ART varies widely, but a poor CD4+ responses is rarely an indication for modifying a virologically suppressive ARV regimen.[23] The guiding principles of good ART include: not to start ART too soon (when

CD4 cell count is close to normal) or too late (when the immune system is irreversibly damaged).[10] Antiretroviral therapy in the developed world has resulted in substantial reductions in HIV-associated morbidity and mortality, changing an HIV diagnosis from a likely death sentence into a manageable chronic infection.[26]

In Nigeria the adult prevalence of HIV was estimated to be 1.8% in 1991 to 4.5% in 1996, 5.8% in 2001, 5.0% in 2003 and 4.4% in 2005. However, the national prevalence seemed to stabilize between 2005 and 2010 as shown by the reported prevalence 4.4% (2005), 4.6% (2008) and 4.1% (2010), which ranged from 1.0% in Kebbi State to 12.7% in Benue State.[21] Based on the overall national prevalence of 4.1% obtained in 2010, it is estimated that 3.1million people in Nigeria are living with HIV/AIDS in 2010. Of these people, about 1.5 million require ARV drugs.[21] In 2014, the national prevalence was further reduced to 3.0%. Nevertheless, a prevalence of 3.0% HIV prevalence implied that over 3.4 million Nigerians are currently infected with the virus and about 2.5 million requiring ART.[22]

The goals of treatment with antiretroviral drugs are to inhibit viral replication while minimizing toxicities and side effects associated with available drugs. The inhibition of virus replication permits restoration of the immune system, suppression of HIV replication (as reflected in plasma HIV concentration) to as low as possible and for as long as possible, the preservation or enhancement of the immune function (CD4+ restoration), thereby preventing or delaying the clinical progression of HIV disease. Viral eradication from the host genome is not achievable, thus a cure for HIV is not yet possible. By using HAART, it is possible to promote growth in children and prolong the survival of all HIV infected patients, reduce their morbidity and improve their quality of life.[21]

The effects of HAART on its immunological responses rate and hematological disorders among Africans, for whom access to ART is expanding, still remain unknown.[4, 23] Highly active antiretroviral therapy confers several benefits, including reduction in viral load and longevity in HIV positive patients. However, metabolic and morphological complications have been increasingly reported among patients in the advanced industrialized countries receiving chronic HAART up to 10-20 years.[4, 23] Therefore, this prospective cohort study was aimed to assess immunological responses among HIV-infected individuals receiving highly active antiretroviral therapy (HAART) with long-term follow-up with reference to their immunological profiles.

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^{o} and 14^{o} North and Longitude 3^{o} and 15^{o} East, with total land area of $923.8x10^{3}$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.[24]

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 Clearance

Ethical clearance was obtained from Health Research and Ethics Committee (HREC) of these selected hospitals in North Central, Nigeria which include: Federal Capital Territory (FCT) Abuja, Federal Medical Centre Keffi, Federal Medical Centre Lokoja and General Hospital Suleja. Detailed explanations were given about the objectives, risks and benefits of the study to the study subjects. Strict confidentiality of responses was maintained during the study. Data were collected after obtaining informed consent obtained.

Study Design

The research was a prospective study. The recruitment of the subjects was non-randomised and they were consented to participate in the study. The laboratory monitoring (baseline) of the patients with HIV/AIDS 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.

Inclusion criteria/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 patients were recruited and were followed up after 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 hospitals, first line (HAART) regimens were administered which include: Truvada™ + Nevirapine, Truvada™ + Efavirenz, Combivir™ + Nevirapine and Combivir™ + Efavirenz.

Blood Collection and Processing

Five (5ml) millilitres of venous blood were careful drawn from the veins of each subject into well labeled Ethylene Diethyl Tetracetic Acid (EDTA) blood sample container and slightly mixed for CD4+ cell determination according to the standard protocol. The blood sample was collected at the beginning of the study for baseline determinations and subsequently for monitoring of the same parameters.

Determination of CD4+ Lymphocyte Cell Counts

The CD4+ lymphocyte counts were determined for each patient at baseline and subsequent monitoring using Flow Cytometry (Partec-cyflow, Germany), according to manufacturer's instructions. This was the adopted technique for CD4+ count estimation under the national ARV programme.[21]

Statistical Analysis of the Data

The data collected from the baseline and consecutive follow- up were entered into Excel spread sheet and analyzed. Means±SEM (standard error of mean) of the CD4+ counts and duration (months) were computed. The comparisons between means were done using one-way analysis of variance (ANOVA). All statistical analysis was performed using SPSS software (version 17.0, SPSS, Chicago, USA) and values ($p<0.05$) was statistically significance.

RESULTS AND DISCUSSION

A total of 2,322 HIV infected patients were monitored in this study; Out of these 1418 (61.1%) were female and 904 (38.9%) male patients. None of them have any other opportunistic infection during the monitoring period. The mean age of the study group was 38.0 (18 – 58) years.

The majority of ART- naive HIV patients were female. A similar finding was reported by World Health Organisation (WHO),[27] from South Africa who stated that ART-naïve patients in low- income settings were more likely to be female. This is because females are biological and socially more vulnerable to HIV infection in the developing countries. However, this does not translate that more women are infected with HIV in our population, as study in Nigeria actually found that more men were afflicted with HIV/AIDS.[13]

Most of the HIV infected patients enrolled in this study were young, aged between 18 and 40 years old who were sexually more active and thus have a higher risk of infection compared to the other age groups.[16] These findings could conform to previous reports from elsewhere in Ethiopia which reported that HIV prevalence decreases significantly to increasing level of education as well as their socio economic status.[1] Sexually active age group is a factor that predisposes people to HIV infection and high rate of co-infection is expected in such groups.
The mean baseline CD4+ count for two thousand three hundred and twenty two (2,322) HIV infected patients during the study was 231.7±1.9401 cells/µl before the initiation of the ARV drug. After the 3rd month, the mean CD4+ count was 281.9±1.8919 cells/µl, which shows an increase of 50 cells/µl. This increment was statistically significant ($p<0.05$). This first remarkable rise was continued in the achieving a mean CD4+ count of 327.4±1.9247 cells/µl at the 6th month of visit. At the end of the 9th month, the mean CD4+ count increased to 377.8±2.0872 cells/µl. At the end of the 12th and 15th months, the mean CD4+ counts were 416.5±2.0389 and 466.8±2.0285 cells/µl respectively as shown in "fig" 1. The pick recovery was noted in those patients having a baseline CD4+ of >250 cells/µl, while patients with a baseline CD4+ count <250 cells/µl showed less recovery rate. In general, there was progressive increase in CD4+ count from the 3rd month through to the 15th month and the result also indicated that the recovery was significantly in those patients who started therapy at the baseline CD4+ >250 cells/µl of the study.

The CD4+ lymphocytes are the main 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 clinics particularly in developing and poor resource settings.[19, 23]

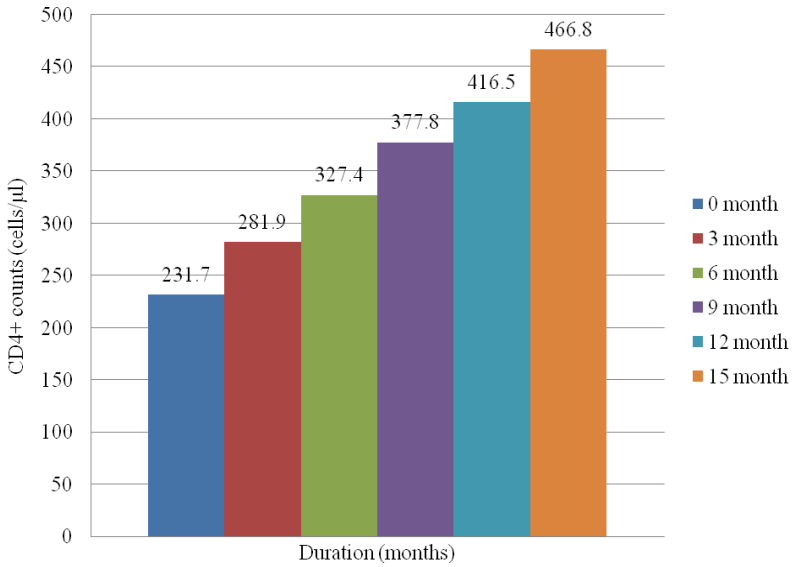

Figure 1: Mean CD4+ counts of HIV infected patients reviewed after every three Month For fifteen (15) months in North Central, Nigeria.

Normal range of CD4+ counts (365 – 1,571cells/µl).

Our data indicates that the majority of HIV patients were initiated on antiretroviral treatment with more advanced immunodeficiency status. Since the majority (74%) of 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.[18, 25] 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. Therefore, there is need for the patients to understand how to prevent HIV and advantages/disadvantages of early or late presentation to the hospital.

There was a significant improvement in the CD4+ counts (50 cells/µl) 3 months after of HAART initiation in this setting. Although, such increased in CD4+ counts was not observed after 6 months of HAART. This is an indication of viral suppression and increased in

immunological responses. Thus, the risk of infection progression to stage 3 or 4, prone a large proportion of the clients to opportunistic infections due to weakened immune systems. These invariably present symptoms such as persistent cough which may be due to *Mycobacterium tuberculosis* or *Mycobacterium avium* complex; chronic diarrhea, as a result of malabsorption of gut lining; general body weakness induced by anaemia or as a result of malnutrition and adverse effect of antiretroviral drugs. Others were oral thrush, genital herpes and recurrent vulval candidiasis.[15] This study supports the need for symptom management interventions that would increase emotional wellbeing and self-care activities for HIV infected patients.

The level of CD4+ count (> 250 cells/µl) was better and more effective for immunological recovery after ART initiation. The immune recovery was significantly higher when compared to the studies conducted in South Eastern United States and Thailand which reported a lower rate of AIDS at the initiation of ART.[6, 18, 25]

In this study, majority 859 (37%) of the patients presented at a CD4+ counts of < 250 cells/µl among treatment-naive HIV patients failed to achieved CD4+ cell count above 250 cells/µl even at the end of 15 months followed up. The lower CD4+ cell counts (< 250 cells/µl) before the initiation of ART had significantly associated with failure to achieved CD4+ cell count recovery, as the majority of the patients whose CD4+ cell count remained < 250 cells/µl at the end of 15 month were from those groups with low baseline CD4+ count. The lower baseline CD4+ cell counts therefore may correlate with poor immune responses and thus determined the degree of morbidity and mortality related to HIV/AIDS as reported by other studies.[4, 10] None of the patients showed exaggerated immunological responses which could lead to autoimmune disorder.

These findings indicate urgent need to promote early and enhanced HIV testing to enable HIV/AIDS patients to benefit from the expanding ART services; for instance, early commencement of ART at this level of CD4+ counts (> 250 cells/µl) should be encouraged in our ART Hospitals/Clinics due to its clinical importance.

CONCLUSION

In conclusion, our study, although good CD4+ cells recovery in responses to ART was documented in more than 89% of followed up cases, despite these patients were enrolled in ART program at decreased CD4+ cells counts. The ART initiation proved more effective in patients that had CD4+ counts > 250 cells/µl compared to those with lower count < 250 cells/µl. The study also revealed that at the end of the 9th month of ART, the CD4+ count and

even the clinical picture of patients tend towards normalcy and therefore interventions need to be designed to promote early HIV testing and early enrollment of HIV infected individuals into ART services.

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CONFLICT OF INTEREST

None was declared by the authors.

REFERENCES

1. Bradley H, Bedada A, Brahmbhatt H, Kidanu A, Gillespie D, Tsui, A. Educational attainment and HIV status among Ethiopian voluntary counseling and testing clients. AIDS Behav, 2007; 11(5): 736-742.
2. Daniel PS, Abba IT, Tristram GP. Retroviruses, In Medical Immunology: Prentice-Hall International Inc 9th edition, 1997; Pp334-350, 537- 538,546,751-759,794.
3. Ellen JB, Lance RP, Sydney MF. 1994. Lentiviruses; In Bailey and Scotts Diagnostic Microbiology: Mosby 9th edition, 1994; Pp 195, 266, 312, 314-315, 584-585, 644, 679-680.
4. Falster K, Petoumenos K, Chuah J, Mijch A, Mulhall B, Kelly M. Poor baseline immune function predicts an incomplete immune response to combination antiretroviral treatment despite sustained viral suppression. J Acquir Immune Defic Syndr., 2009; 50(3): 307-313.
5. Gallo RC, Montagnier L. The discovery of HIV as the cause of AIDS. N Engl J Med, 2003; 349: 2283-2285.
6. Gay C L, Napravnik S, Eron JJ. Advanced immunosuppression at entry to HIV care in the Southeastern United States and associated risk factors. AIDS. 2006; 20(5): 775-778.
7. Gottlieb MS. Past and Future. N Engl J Med. 2001; 344: 1788-1791.

8. Geo FB, Janet SB, Stephen AM. 1998. Jawtz, Melnick and Adelberg's Medical Microbiology: Apleton and Lange Stamford, Connecticut, 21st edition, 1998; Pp 427,566-582,704.
9. Hazenberg MD, Otto SA, Van Benthen, BH. (2003): Persistent immune activation in HIV-1 infection is associated with progression to AIDS: AIDS. 2003; 17(13): 1881-1888.
10. Lawn SD, Myer L, Bekker LG, Wood R. CD4+ cell count recovery among HIV- infected patients with very advanced immunodeficiency commencing antiretroviral treatment in sub-Saharan Africa. BMC Infect Dis., 2006; 6: 59-68.
11. Nassipuri I. HIV/AIDS*:* journey so far. A paper presented at the 136th NIPRD institute seminar (unpublished), 1997.
12. Norcoss MA. Chemokine receptors and HIV-1pathogenesis; a viral attraction. In Dalgliesh A, Weiss R (eds) HIV and the new viruses, second edition. Academic press, New York, 1999.
13. Ola SO, Ladipo MM, Olegbayo JA, Odaibo GN, Bamgboye E A, Nwaorgu OG, Shokunbi SW, Olaleye OD. Demographic factors in HIV infected patients seen at UCH, Ibadan, Nigeria. Afr J Med Sci. 2005; 34: 297-301.
14. Schim van der Loeff MF, Aaby P. Towards a better understanding of the epidemiology of HIV-2. AIDS, 1999; 13(Supplement A): S69-84.
15. Thejus TJ, Jeeja M, Jayakrishnan T. The functional status of patients with AIDS attending antiretroviral treatment center. Indian J Pal Care, 2009; 15: 57-60.
16. UNAIDS. 1998. HIV Epidemic a global update United Nations World AIDS day report. Health Millions, 1998.
17. Erah PO, Arute JE. Adherence of HIV/AIDS patients to antiretroviral therapy in a tertiary health facility in Benin City. Afr J Pharm Pharmacol. 2008; 2: 145-152.
18. Chasombat S, McConnell MS, Siangphoe U, Yuktanont P, Jirawattanapisal T, Fox K. National expansion of antiretroviral treatment in Thailand, 2000-2007: program scale-up and patient outcomes. J Acquir Immune Defic Syndr. 2008; 50(5): 506-512.
19. Alavi SM, Ahadi F, Farhadi M. Correlation between total lymphocyte count, hemoglobin, hematocrit and CD4+ count in HIV/AIDS patients. Actal Med Iran. 2009; 47(1): 231-234.
20. Agu KA, Ochei UM, Oparah AC, Onoh OU. Treatment outcomes in patients receiving combination ARV therapy in Central hospital, Benin City, Nigeria. Trop J Pharm Res. 2010; 9: 1-10.
21. Federal Ministry of Health. 2010. National HIV Sero-prevalence Sentinel survey, Technical report, 2010; 1-53.

22. Federal Ministry of Health. 2014 National HIV Sero-prevalence Sentinel survey, Technical report, 2015; 93-95.
23. Jong MA, Wisaksana R, Meijerink H, Indrati A, Andre J, Alisjahbana B. Total lymphocyte count is a reliable surrogate marker for CD4+ cell counts after the first year of antiretroviral therapy: data from an Indonesian cohort study. Trop Med Intl Health. 2012; 17(5): 581-583.
24. Henry, LR (Ed.) 2008. The free online Encyclopedia. (5th ed.), 2008; New York.
25. Nwokedi EE, Ochicha O, Mohammed AZ, Saddiq NM. Baseline CD4+ lymphocyte count among HIV patients in Kano, Northern Nigeria. Afr J Health Sci. 2007; 14: 4-12.
26. WHO. 2006. Antiretroviral therapy for HIV infection in adults and adolescents. Recommendations for a public health approach Geneva: 2006, World Health Organization.
27. WHO. 2004. TB/HIV a clinical Manual 2nd ed. Geneva, 2004.