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THE EFFECTS OF HIGHLY ACTIVE ANTIRETROVIRAL THERAPY (HAART) OF STAVUDINE, LAMIVUDINE AND NEVIRAPINE ON THE CD4 LYMPHOCYTE COUNT OF HIV- INFECTED AFRICANS: THE NIGERIAN EXPERIENCE

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ABSTRACT

Objectives: The objective of this study was to investigate the short-term effect of highly active antiretroviral therapy on the CD4 lymphocyte count of HIV-infected Nigerians.

Design: A case control study of 70 HIV-infected subjects placed on highly active antiretroviral therapy. Thirty HIV-infected yet to start therapy due to unaffordability were observed as controls.
Setting: This study was carried out at the Hematology Department of the University of Port Harcourt Teaching Hospital a 500 bed tertiary hospital and one of the designated antiretroviral therapy pilot centers.

Methods: CD4 lymphocyte count was determined at baseline for subjects and controls. Subjects were placed on HAART for 12 weeks while controls that were yet to start therapy were monitored as controls. CD4 lymphocyte count was repeated after 12 weeks and the differences compared statistically.

Results: We observed that subjects and control patients did not differ significantly in their CD4 lymphocyte count at baseline ($p>0.05$), but after 12 weeks HAART in subjects and untreated control there was a mean increase in CD4 count of (39 cells/μL) in subjects, while untreated controls showed a mean decline of (12 cells/μL) $p< 0.05$. There was a statistically significant variation in the therapy dependent increases in CD4 count of HAART treated subjects based on pre-therapeutic baseline CD4 count ($\div^2 = 180.39, p<0.05$). The HAART dependent increase in CD4 counts was higher in younger subjects 19-28 years (31 cells/μL) compared to older subjects 49-58 years (21 cells/μL) ($p = 0.01$). Similarly CD4 response was found higher in females compared to males ($p = 0.01$).

Conclusion: This study indicates the importance of accessing the CD4 lymphocyte count of HIV infected patients before the initiation of HAART, its use as a prognostic maker in predicting the initial response to HAART and in determining the optimal time to initiate therapy.

Key words: HAART, CD4 lymphocyte count, Nigerians, Africans.

INTRODUCTION

Chronic HIV infection is characterized by a decline in the number of peripheral blood CD4 T-helper lymphocytes and progressive defects in the CD4 T-cell function [1,2]. Strategies such as simultaneous initiation of three antiretroviral drugs including a protease inhibitor or a non nucleoside highly active antiretroviral therapy (HAART) has been found to profoundly and durably inhibit HIV production, extend overall long term effectiveness, help in the preservation of overall- term term effect and provide a salvage treatment option should initial treatment fail, without the rapid development of drug resistance.

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These drugs have resulted in slow progression from HIV to full-blown AIDS in HIV/AIDS patients fortunate enough to have access to these drugs [3], a possibility above the reach of a vast majority of patients in Sub-Saharan Africa.HAART almost invariably reduces plasma viraernia and a rapid increase in blood CD4 lymphocyte count in a majority of patients [4,5]. Concurrent use of Zidovudine and Didanosine is associated with decreased plasma viraemia and increased CD4 count compared to monotherapy [6]. The combination of Saquinavir and lopinavir/ritonavir at a dose of 100mg of saquinavir and 100mg ritonavir daily has been shown to result in a mean CD4 increase of 94 cells/μL after 8 weeks [7]. A phase III randomized double blind trial compared

stavudine to Zidovudine in HIV infected adults with CD4 count of 50-500 cells/μL. An interim analysis of study data demonstrated that after 12 weeks of therapy, there was a mean rise in CD4 count of 20 cells/μL in subjects who received zidovudine [8].Sex related difference in immunological responses of HIV patients to HAART has been suggested and such difference is believed to be hormonally related as estrogen related effect has been described in immune function [9].CD4 T-helper lymphocyte response to HAART has been found higher in younger HIV-infected compared to older subjects [10]. One current explanation of a rapid increase in CD4 lymphocyte count is that the cells are initially redistributed from lymphoid tissue followed by a later continuous slow repopulation with newly produced naïve cells by thymopoiesis [11].Highly active antiretroviral therapy has changed the landscape of HIV and AIDS care in the developed world, but its benefits and effects are yet to be well established in Nigeria, especially with the recent sound strategy of the Federal Government to introduce HIV infected Nigerians to HARRT. This study was undertaken to determine the presence and frequency of immunological derangement in HIV-infected individuals and to investigate the effect of HAART on the CD4 lymphocyte count of symptomatic and asymptomatic HIV positive individuals initiating HAART and at different baseline CD4 counts.

SUBJECTS AND METHODS

Study Subjects

Seventy HIV- infected, previously antiretroviral naïve out-patients, recruited into the antiretroviral therapy pilot project at the Department of Hematology, University of Port Harcourt Teaching Hospital, aged 19-58 years, made up of 33 females and 37 males, with 7 asymptomatic and 63 symptomatic, between June and August 2002, constituted the subjects in this case control study. These subjects in the treatment group received HAART consisting of 40mg Stavudine, 150 mg Lamivudine and 200mg Nevirapine twice daily and were monitored for a period of 12 weeks. Thirty previously antiretroviral naïve HIV positive individuals age and sex matched with subjects in the treatment group who were yet to commence HAART due to unaffordability served as controls. CD4 lymphocyte count was measured at baseline in subjects and control patients, and also after 12 weeks in controls, and in HAART treated subjects. Eligibility criteria were age ≥18 years and CD4 count ≥100 cells/μL, Exclusion criteria were age <18 years, CD4 count < 100 cells/μL, pregnancy and previous antiretroviral therapy.

Demographic data of age, sex and written consent were obtained from all study participants.

Specimen Acquisition And Preparation

Whole blood samples were collected by venepuncture using a 10 milliliters hypodermic syringe and needle into EDTA anticoagulated tubes (5 milliliters) and non-anticoagulanted tubes (5 milliliters). Sera derived from the non-anticoagulated tubes were screened and confirmed for HIV 1&2 infection using a double ELISA confirmatory method involving the World Health Organization (WHO) approved Immunocomb HIV 1&2 kits (Orgenics, Israel)- an immunochromatographic test for the qualitative and differential detection of antibodies in HIV 1 and 2 and Genscreen HIV 1&2 ELISA kits (Bio Rad, France) -an invitro qualitative enzyme immunoassay (EIA) test for the detection of antibodies to HIV I and 2 in human serum. CD4 T-helper lymphocyte count was estimated using the Dynabeads method (Dynal ASA, Oslo, Norway)- an alternative technique to flow cytometry for quantification of human peripheral blood CD4 T-cells in resource- limited settings.

Statistical Analysis

Data was analyzed using a statistical package for personal computers (version 9; SPSS Inc. Chicago, IL). Statistical analysis of mean, standard deviation, and chi square analysis was used for discrete variables while correlation were compared by linear regression analysis. Differences were regarded as significant when $p \leq 0.05$.

RESULTS

The highest HIV infection prevalence (44%) occurred in the age group 29-38 years while the lowest prevalence occurred in the 49 - 58 years age group (11 %). HIV-1 was the predominant viral strain (96%), HIV 2 (2%) while dual HIV 1 & 2 accounted for (2%). The mean CD4 T-helper lymphocyte counts were measured at baseline. There was no statistically significant difference between the mean CD4 count of the proposed treatment group (253.57 ± 97.00 cell/μL) and controls (227.33 ± 76.34 cells/μL), ($p > 0.05$). While peripheral CD4 cell count increased significantly in the treatment group after 12 weeks therapy from 253.57 + 97.00 cells/μL to 292.86 ± 91.94 cells/μL (mean increase of 39 cells/μL), untreated controls showed a decline from 227.33±76.43 cells/μL to 215.67±79.08 cells/μL (a mean decline in CD4 count of 12 cells/μL). The difference in mean CD4 count after 12 weeks in untreated controls and HAART in subjects was statistically significant ($p<0.05$).

Table 1 shows the mean changes in CD4 lymphocyte count of subjects and control patients.Changes in CD4 counts in the HAART treated subjects and the untreated controls were assessed based on starting baseline CD4 count; <200 cells/μL, 200-350 cells/μL and >350 cells/μL. The absolute mean cell rise was 47 cells/μL, 42 cells/μL and 32 cells/μL respectively. There was a statistically significant variation in the therapy dependent increase in CD4 count based on pre-therapeutic baseline CD4 count ($\div^2 = 180.39$, $p < 0.05$). Comparatively, untreated controls showed a decline of 15 cells/μL, 9 cells/μL and 10 cells/μL respectively for those who delayed treatment with baseline CD4 counts of <200 cells/μL, 200-350 cells/μL and >350 cells/μL respectively. There was a statistically significant variation in the delayed HAART related decline in CD4 count based on pre-therapeutic baseline CD4 count ($\div^2 = 1.750$, $p = 0.05$). Table 2 shows the mean CD4 lymphocyte response to HAART based on pre-therapeutic baseline CD4 count of subjects and control patients.The mean AIDS-defining CD4 count obtained in this study was 159.53±28.4 cells/μL. The relative number of subjects with CD4 of <200 cells/μL, 200-350 cells/μL and >350 cells/μL at baseline was 26 (37.1%); 34 (48.6%) and 10 (14.36%) respectively. But after 12 weeks HAART the number became 10 (14.2%), 44 (62.9%) and 16 (22.9%) respectively. Comparatively, untreated controls with CD4 count of <200 cells/μL, 200-350 cells/μL and >350 cells/μL at Baseline was 12 (40%), 15(50%) and 3(10%) respectively but after delayed treatment for 12 weeks the number became 15 (43.3%), 16 (53.3%) and 1 (3.30%) respectively. Table 3 shows the frequency of immunological derangement in subjects on HAART and untreated controls at baseline and after 12 weeks observational period.The mean CD4 helper lymphocyte response in subjects on HAART was compared based on age groups. The CD4 lymphocyte response to HAART was higher in younger subjects 19-28 years (31 cells/μL) compared to older subjects 49-58 years (21 cells/μL). The difference in mean CD4 count between younger and older subject was statistically significant both at baseline and after 12 weeks HAART ($p < 0.05$).The CD4 helper lymphocyte response in subjects on 12 weeks HAART was compared based on gender. The therapy dependent increase in CD4 count was higher in females (43 cells/μL) than males (36 cells/μL). The difference in mean CD4 count between male and female subjects at baseline and after 12 weeks HAART was statistically significant ($p < 0.05$) and ($p < 0.01$) respectively. Severe potentially fatal Steven-Johnson's Syndrome (rash) was one of the commonest adverse clinical events observed in (4.3%) of subjects on HAART. CD4 cells restored by HAART were evaluated as a prognostic indicator for disease progression to AIDS and death; mortality was relatively higher in untreated controls (13.3%) compared to subjects on HAART (2.9%). Mortality was found clustered in subjects and control patients with CD4 count of <200 cells/μL.

Table 1: **Mean Changes In Cd4 Lymphocyte Counts of Subjects and Controls.**

CD4 Count (cells/μL)	Subjects (Mean ± 2SD)	Controls (Mean) ± 2SD)	p- Value
Baseline	253.57 ± 97.00	227.33 ±76.43	p> 0.05
Post 12 Weeks	292. 86 ± 91.94	215.67 ± 97.08	p< 0.05

Table 2: **Mean Cd4 T - Helper Lymphocyte Response to Haart Based on the Pre-Therapeutic Baseline Cd4 Count of Subjects and Controls.**

CD4 RANGES	Subjects Mean ± 2SD		χ^2	p- Value	Controls Mean ± 2SD		χ^2	p- Value
	Baseline	Post 12 weeks therapy			Baseline	Post 12 weeks		
<200 cells/μL	148 ± 31.66	195.77 ±40.42	180.39	< 0.05	154.17 ±20.21	137.17 ±22.75	1.750	< 0.05
200-350 cells/μL	288.53 ±38.23	324.12 ±33.31			258.00 ±44.75	248.00 ±45.65		
>350 cells/μL	407.00 ±39.17	439.00 ±39.29			366.67 ±11.55	356.67 ±11.55		

Table 3: **Frequency of Immunological Derangement in Subjects and Controls at Baseline and After 12 Weeks HARRT.**

CD4 Ranges	Subjects				Controls			
	Baseline		12 Weeks Post HAART		Baseline		Post 12 Weeks	
	n	%	n	%	n	%	n	%
<200 cells/μL	26	37.1	10	14.2	12	40	13	43.3
200-350 cells/μL	34	48.6	44	62.9	15	50	16	53.3
>350 cells/μL	10	14.3	16	22.9	3	10	1	3.3

DISCUSSION

In this study, we observed the highest HIV infection rate in the 29-38 years age group, a 1:1 male to female ratio and a higher HIV-1 prevalence (96%) compared to HIV-2 (2%) and dual HIV 1&2 (2%). These findings are in agreement with that of other workers in Nigeria [12,13]. The finding of a higher prevalence of HIV among adults (29-38 years) in this study may have been accounted for by the fact that adults in this age bracket are more sexually active and are more prone to high risk behaviors that makes them vulnerable to infection with HIV; maintenance of multiple sex partners and intravenous drug use. This tends to adversely affect the economy of the nation since the youths are the bedrock of the labor force.We have observed that short- term highly active antiretroviral therapy (12 weeks) of two nucleoside reverse transcriptase inhibitors (Stavudine and Lamivudine) and one non-nucleoside reverse transcriptase inhibitor (Nevirapine) induces an initial mean increase in blood CD4 T-cell number (39 cells/μL) compared to a mean decline in CD4 count of (12 cells/μL) in untreated controls. This observation is consistent with previous report by Veldkarnp *et al* [7] who observed a mean CD4 increase of 94 cells/μL after 12 weeks of Saquinavir and ritonavir therapy. The lower mean increase in CD4 count observed in this study may have been due to a shorter follow-up period, malnutrition or may in fact prove the superiority of a protease inhibitor containing regimen. The significant increase in CD4 cell count may represent redistribution of naive CD4 T cell from lymphoid tissues followed by a later continuous slow repopulation with newly produced naive CD4 cells by thymopoesis as previously suggested [21]. The observation of a decline in CD4 cell count (12 cells/μL) after 12 weeks in untreated controls in this study is consistent with previous reports [1,14] which indicated that CD4 T-helper lymphocyte decline at a rate of 25-60 cells/μL per year.The rate of decline observed among untreated controls in this study is much higher and may infarct be a reflection of malnutrition, a major problem in Sub Saharan Africa [15].The mean AIDS defining CD4 count obtained in this study was 150.53 + 28.39 cells/μL. This value is however lower than that observed in a previous study in Jos, Nigeria [15] which observed a mean AIDS defining CD4 count of 160.00± 22.00 cells/μL. The lower AIDS-defining CD4 count obtained in these studies may be a reflection of the level of immunodeficiency of patients included in these studies and malnutrition-a major problem in sub Saharan Africa.In this short-term study we have observed that there is no immunological advantage in initiating HAART at a baseline CD4 count of >350 cells/μL rather than 200-350 cells/μL or <200 cells/μL. We observed a mean HAART dependent CD4 cell increases of 47 cells/μL, 42 cells/μL and 32 cells/μL respectively for patients initiating HAART with a baseline CD4 count of <200 cells/μL, 200-350 cells/μL and >350 cells/μL, This observation is consistent with previous report by Alessandro et al [16] but at variance with current suggestion of the British HIV Association (BHIVA) of initiating HAART once CD4 count falls to 350 cells/μL.Consistent with, previous reports [10,17], our study shows a higher CD4 T-cell restoration after 12 weeks HAART among younger subjects 19-28 years (31 cells/μL) compared to older subjects 49-59 years (21 cells/μL), ($p<0.01$). This observation is possibly due to better-preserved thymic tissue and functions in younger patients compared to older patients.Consistent with previous report [9], we observed a gender-related difference in the CD4 response to HAART. We observed a higher CD4 response among females (43 cells/μL) compared to males (36 cells/μL) with a statistically significant difference ($p<0.01$). The differences in immunological response seen between

males and females in this study may be hormonally related as estrogen related effect have been described in immune functions [9].Consistent with previous report [18], which observed a 20% incidence of Steven-Johnson's Syndrome (rash) in patients who received a triple therapy of Zidovudine, Didanosine and Nevirapine compared to 9% in patients who received Zidovudine and Didanosine, we observed a 4.3% incidence of Steven- Johnson's Syndrome in our patients. The high incidence of Steven - Johnson's Syndrome observed in our study could have been due to the presence of Nevirapine in the regimen.We observed a mortality rate of 13.3% among untreated controls and 2.9% among HAART treated subject. This observation is consistent with previous reports [19,20] which observed mortality rates of 12% and 6.7% respectively in HIV /AIDS patients.Consistent with previous report [20], mortality was found in this study clustered in subjects and controls that initiated or deferred therapy at a baseline CD4 count of <200 cells/μL.In summary we have identified in this case-controlled study that CD4 measurement can be used as a prognostic marker in predicting initial response to HAART and in determining the optimal time to initiate HAART.

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