Research Article

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Comparison of Viral Load and CD4+ Values of ARV Naïve and ARV Challenged HIV Patients in Taraba State Nigeria

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ABSTRACT

Taraba State has the second highest prevalence HIV infection in Nigeria, of 10.5% (NACA, 2015). Increased HIV viral load indicates increase in infectivity and weaker immune system by decrease in CD4 counts. Antiretroviral therapy (ART) helps to reduce the risk of transmission and infectivity of the virus and also helps to improve the health and life expectancy of the patient. This study evaluated the baseline viral load and CD4 values of newly enrolled HIV positive patients; also at three and six months for both those that commenced ART (ARV challenged patients) and those who did not commence ART(ARV naïve patients) and compared these values at three and six months after commencement of ART or not. A prospective longitudinal observational cohort study design was adopted at the Federal Medical Centre, Jalingo, Taraba State, Nigeria. Newly enrolled HIV positive adult patients 18years and above were recruited. Data was obtained from each patient by administration of questionnaires. Intravenous blood samples were collected during clinical visits; processed and analyzed for current HIV Viral load and CD4 counts using the COBAS Ampliprep/TaqmanMechine (Polymerase Chine Reaction) and the Cyflow Counter respectively. Data was analyzed using SPSS statistical software version 18.0. A total of 171 HIV drug naïve adult patients were recruited into this study. The ratio of female (70.2%) to male patients (29.8%) was 2.3:1. Baseline assessment of the 171 enrollees showed 74 patients with CD4 count ≥ 500cells/µl and 97 patients with CD4 count <500cells/µl; females having higher CD4 count than males; p= 0.008. CD4 count and viral load of the 171 ARV naïve patients showed significance at baseline assessment (p<0.0001); then showed no significance at three months assessment for both ARV challenged and ARV naïve groups. There was significance for CD4 and viral load at six months, for both ARV challenged, p = 0. 019 and ARV naïve, p = 0.007groups respectively. Nineteen (19.6%) ARV challenged patients had an improved CD4 count from<500cells/µl at baseline assessment to ≥ 500cells/µl six months on therapy and 40 (41.2%) patients achieved viral suppression of<1000 copies /ml over the period, due to effective use of HAART. Also, 30 (30.9%) ARV challenged patients had CD4 count ≤ 200 cells/µl and 57 (58.7%) had viral load >1000 copies/ml after being on HARRT therapy for six months. This study confirms the importance of CD4 and viral load in monitoring disease progression or regression in HIV infected patients.

Keywords

CD4, Viral load, Antiretroviral naive, Antiretroviral challenged, HIV.

Introduction

Human Immunodeficiency Virus (HIV) disease is a global epidemic, with sub-Saharan Africa being the most affected region, having about 25.8 million people living with HIV as at 2014 [1]. Sub-Saharan Africa accounts for almost 70 percent of the global total of new HIV infections. Approximately 36.7 million people worldwide are living with HIV as at the end of 2016 [2]. Of these, 2.1 million are children (<15 years old). Almost 76.1 million

people have been infected with the HIV virus and about 35 million people have died of HIV since the beginning of the epidemic [2].

Human Immunodeficiency Virus is a member of the genus Lentivirus, part of the family Retroviridae (International Committee on Taxonomy of Viruses) [3]. They are single-stranded, positive-sense, enveloped RNA viruses. If allowed to undergo its natural cause of infection would lead to a syndrome, known as the "Acquired Immune Deficiency Syndrome (AIDS)" [4]. The virus was first isolated in 1983 by Dr. Montagnier of France, but later in 1984 by Gallo and Lavy of the United States of America. The name "Human Immunodeficiency Virus" was given at the international conference in 1986 [5]. The primary method of spread of HIV infection worldwide is through sexual exposure. In the areas of highest HIV prevalence globally, heterosexual intercourse is the primary mode of transmission, accounting for approximately 70% of the overall sexual transmission. There are many risk factors that contribute to the spread of HIV, including prostitution, high-risk practices among itinerant workers, high prevalence of sexually transmitted infections (STI), high-risk heterosexual and homosexual practices, international trafficking of women, and irregular blood screening [6].

The HIV epidemic in Nigeria is complex and varies widely by region. In some states, the epidemic is more concentrated and driven by high-risk behaviors, while other states have more generalized epidemics that are sustained primarily by multiple sexual partnerships. In more recent times, internally displaced persons (IDPs'), due to terrorism and crisis. Youth and young adults in Nigeria are particularly vulnerable to HIV, with young women at higher risk than young men [7,8]. The virus has been isolated from blood, seminal fluid, pre-ejaculate, vaginal secretions, cerebrospinal fluid, saliva, tears, breast milk of infected individuals, and genital fluids; though no case of HIV infection have been documented to arise from contact with non-bloody saliva or tears [9]. Non-sexual HIV transmission can occur through transfusion with contaminated blood products, injection drug use, occupational exposure, or accidental needle sticks. Breast-feeding is also a risk factor for HIV transmission. Approximately one-third of cases of mother-to-child transmission result from breast-feeding, and the risk increases with the duration of breast-feeding [10]. Viral load refers to the amount of HIV genetic materials or viral particles known as 'copies' in a milliliter sample of plasma. These copies may be very large, hence quantified sometimes using the powers of ten, or 'log scale'.

Increase in HIV viral load indicates an increase in infectivity and a weaker immune system by decrease in CD4+ levels. HIV viral load test provide information on the patients' health status and antiretroviral therapy (ART) management. ART helps to reduce the risk of transmission and infectivity of the virus and also helps to improve the health and life expectancy of the patient. The goal of ART is to decrease HIV viral load to an "undetectable" level of less than twenty (20) copies/ml of plasma (depending on the laboratory analyzing the test), though viral load below this "undetectable" level indicates the inability of the assay to detect HIV in the plasma, but does not indicate absence or clearance of the virus from the body. Human Immunodeficiency Virus can still exist in semen, vaginal and rectal fluids, breast milk, and other parts of the body. The use of ART greatly reduces the risk of transmission of the virus and also the development of drug resistance. Human Immunodeficiency Virus infects vital cells in the human immune system such as helper T-cells (especially CD4+ T-cells), macrophages, and dentritic cells [11].

Human immunodeficiency virus (HIV) causes acquired immunodeficiency syndrome (AIDS) by destroying CD4+ T-cells [12]. Infection with the virus can result in low levels of CD4+ T cells through various mechanisms such as apoptosis of uninfected cells around infected cells [13], direct viral killing of infected cells, and killing of infected CD 4+ T cells by CD8 T cells cytotoxic lymphocytes that recognize infected cells [14]. CD4+count are used to measure the degree of immunosuppression in HIV-positive patients. There is an inverse relation between CD4+ count and degree of viral load [8]. CD4+ count is used in monitoring disease progression, deciding when to commence therapy, staging the disease, determining treatment failure, and defining the risk for mother-to-child transmission. Viral load is very important in the measurement of viral replication. Viral load estimation can be used in discriminating between treatment failure, non-adherence, and can serve as a proxy for the risk of transmission of the HIV virus at the population level [15], though the test is very expensive when compared to CD4+ count test. Morbidity and mortality risk are associated with CD4+ count; while transmission risk depends on plasma viral load (VL) values, which is the gold standard. In addition, measuring viraemia loads provide a measure of infectivity. When infected patients have low or suppressed viral loads, they have been shown to significantly lower transmission rates [16]. Thus, the laboratory markers used in monitoring management in HIV-positive patients are viral load and CD4+ count. CD4+ count together with viral load can be used in deciding when to initiate ART in HIV-positive patients [17].

The use of HAART as a regimen for the treatment of HIV positives has resulted in a dramatic decrease in AIDS-related morbidity and mortality and a great improvement in CD4+ count in patients [18]. However, some asymptomatic HIV-positive patients do not require antiretroviral (ART) drugs on enrollment because of their high CD4+ count at registration(naïve patients), hence their inability to meet criteria for initiation of therapy laid down by various organizations like World Health Organization (WHO), Centre for Disease Control, (CDC) Atlanta, and the Presidential Emergency Program for AIDS relief (PEPFER).

In Nigeria, the first case of HIV/AIDS was reported in 1986. Worldwide, Nigeria has the second highest number of new HIV/AIDS infections reported each year [19]. Nigeria is second in Africa with 3 million people infected. In 2009 an estimated 3.6% of 150 million Nigerians are living with HIV and AIDS [20]. Approximately 215,000 people died of HIV/AIDS in Nigeria in 2010 [19]. A report from the National Agency for the Control of AIDS (NACA) in 2015, places Taraba State as the second highest

prevalence state for HIV infection in Nigeria, with a prevalence of 10.5% [20].

This study is a comparative study to assess the impact of viral load and CD4 cell count on HIV disease regression and progression and its dynamics on the Taraba state populace. This study provides information on viral load and CD4 count of HIV infected naïve patients who commenced ARV and compared these to those who did not commence ARVs at enrollment into the HIV clinic in Jalingo. Estimation of Viral Load and CD4 count is vital in the treatment and management of HIV positive patients. In ARV compliant patients the Viral Load is expected to reduce inversely with increase in CD4 count. The comparison of the Viral Load and CD4 counts of HIV positive patients in Jalingo provided suggestive insights to the patients' ARV compliance profile and further information for patients´ management.

Methodology

Study Area

The study was a facility based study carried out at the Federal Medical Centre, Jalingo, Taraba State, Nigeria. It is a federal tertiary health institution that provides specialized health services to the state populace and serves as a referral centre to other neighboring states in the area of HIV diagnosis. The facility hosts the only EID/PCR laboratory situated in the state where viral load and CD4+ estimations are carried out. Jalingo is the capital of Taraba State, and has an estimated population of 118,000 [21].

Study Design

This study consisted of 171 newly recruited adult HIV patients at baseline before initiation of highly active antiretroviral therapy (HAART) to those who had CD4+ count <500cell/µL. This study utilized a prospective longitudinal observational cohort study design carried out over a period of seven (7) months at the Federal Medical Centre, Jalingo, Taraba State, Nigeria. Data analysis was done using Microsoft excel, Kruskal-Wallis test, linear regression, and SPSS software version 18.0.

Sampling Technique

5ml intravenous blood samples were collected from each patient into EDTA bottles. 20µl of each blood sample was analyzed for CD4+ count and 1100µl of plasma was extracted from each sample and analyzed for viral load. Current HIV Viral load and CD4+ count values were estimated using the COBAS Ampliprep/ Taqman PCR machine and the Cyflow Counter (Sysmex-Partec GmbH, Munster, Germany) respectively.

Study Population

The study population included all HIV positive adult patients from 18years and above who had recently enrolled into the ART clinic, Federal Medical Centre, Jalingo. Patients that have been tested and found to be HIV positive are regularly referred from various primary and secondary hospitals throughout the state to the Federal Medical Centre ART clinic for management. Samples were collected during clinical visits; processed and analyzed for current HIV Viral load and CD4+ counts using the COBAS Ampliprep/ Taqman Mechine and the Cyflow Counter respectively.

Principle 1

For CD4+ Analysis Using The Cyflow Automated Counter: The Cyflow Automated Counter works on the principle of light scatter (based on different size or granularity of the cell) combined with fluorescence of cells after staining with monoclonal antibodies to cell surface markers tagged to fluorescent dyes. The counter separated the CD4+ T cell from the monocytes-CD4+ bearing cells and noise using a gating system. Bring all reagents to ambient temperature and fill the sheath fluid bottle to the 800ml mark and tighten the cork. Then switch on the counter. 20ul of CD4+ PE antibody is added into a partec tube. 20µl of well mixed whole blood (EDTA) is then added to the test tube. This is mixed gently by tapping and incubated in the dark for fifteen (15) minutes at room temperature. Mixing is repeated every five (5) minutes during incubation. 800ul of CD4+ non- lyse buffer is then added to the test sample tube, mixed gently and plugged onto the counter for counting while ensuring that the CD4+ cells, monocytes and noise are well separated and gated. The result is then printed and saved.

Principle 2

For Viral load estimation: The COBAS AmpliPrep/COBAS TaqMan HIV-1 Test, v2.0 is a nucleic acid amplification test for the quantitation of Human Immunodeficiency Virus Type 1 (HIV-1) RNA in human plasma. The COBAS AmpliPrep/COBAS TaqMan HIV-1 Test, v2.0 is based on three major processes: specimen preparation to isolate HIV-1 RNA, reverse transcription of the target RNA to generate complementary DNA (cDNA), and simultaneous PCR amplification of target cDNA and detection of cleaved dual-labeled oligonucleotide detection probe specific to the target.

Quantitation of Viral Load Using the Cobas Ampliprep/ Taqman HIV-1 Version 2.0 RNA Instrument

The quantitation of viral load is carried out using a Polymerase Chain Reaction (PCR) system from Roche Molecular Diagnostics. The Cobas AmpliPrep/ Taqman instrument is such that uses automation in the preparation of samples. This instrument uses an Amplilink software. The AmpliPrep instrument processes the sample to extract the DNA, while the Taqman thermocycler analyzer, amplifies, detects and quantifies the viral particles. This test is used for the quantification of the Human Immunodeficiency Virus Type 1 (HIV-1) RNA in human plasma. The test can quantitate HIV-1 RNA over the range of 20-10,000,000 copies/ mL. Sample processing lasts for about 51/2 hours.

Results

A total of 171 HIV patients were recruited into this study from June to July, 2016 and were followed up for six months. This comprised of; 120 (70.2%) female and 51 (29.8%) male with female to male ratio of 2.3:1 (Figure 1). The mean age of study participants was 33.3 ± 9.3 years. Male participants were significantly older than female participants, $p<0.0001$ with a mean age of 38.3 ± 10.8 years in contrast to 31.2 ± 8.2 years of female study participants.

Sex and Age		Median Log VL (IQR)							
Charateristics		N (%)	Baseline	3 Months	6 months	Baseline	3 Months	6 months	P Values
Sex	Male	51 (29.8)	297 (155-537)	274 (186-559)	328 (191-495)	4.3 (2.8-5.6)	3.7 (2.9-5.3)	3.5 (1.9-4.2)	< 0.0001
	Female	120 (70.2)	524 (276- 764)	497 (303- 681)	477 (320- 685)	3.8 (2.0- 5.0)	3.5 (2.1- 4.9)	3.1 (2.0- 4.7)	
Mean Age (Years)	33.3 ± 9.3								< 0.0001
Male Mean Age		38.3 ± 10.8							
Female Mean Age		31.2 ± 8.2							
Age Group (Years)	18-30	79 (46.2)	478 (279-682)	462 (274-641)	462 (305-574)	4.3 (2.6-5.4)	4.0 (3.0-5.3)	3.6 (2.5-4.8)	0.396
	31-40	57 (33.3)	379 (193-668)	418 (213-605)	394 (259-641)	3.6 (1.9-4.9)	3.3 (2.0-4.9)	3.1 (1.9-3.9)	
	41-50	30 (17.6)	319 (202-605)	302 (227-584)	374 (195-471)	3.3 (2.0-5.0)	3.1 (2.0-4.7)	2.8 (1.9-4.1)	
	>50	5 (2.9)	580 (302-835)	537 (330-752)	500 (354-608)	4.6 (3.8-4.7)	3.9 (3.3-4.8)	3.5 (3.2-4.9)	

Table 1: Sex and Age demographic distribution of study population (N=171). Where P value ≤ 0.05 is significant and P value > 0.05 is not significant.

Socio-demographic characteristics	N(%)	Median CD4 (IQR) Baseline	3 Months	6 months	Median Log VL(IQR) Baseline	3 Months	6months	*P* Values
Marital Status								0.021
Single	44(25.7)	393(203-596)	391(258-539)	404(305-519)	4.7(3.2-5.8)	4.5(3.3-5.8)	3.8(2.7-5.2)	
Widowed	26(15.2)	558(288-827)	512(311-726)	422(327-598)	4.0(1.7-5.0)	3.8(2.3-4.9)	3.5(1.9-5.0)	
Divorced	22(12.9)	490(235-697)	519(263-750)	505(264-760)	3.9(1.9-4.9)	3.6(2.0-4.9)	3.0(2.4-4.3)	
Married	74(43.3)	365(177-647)	418(213-652)	410(241-621	3.4(2.2-4.9)	3.1(2.1-4.6)	3.0(1.9-3.9)	
Missing Value†	5(2.9)							
Occupation								0.732
Farming	37(21.6)	321(208-599)	391(260-608)	406(222-606)	4.0(2.6-5.7)	3.7(2.8-4.6)	3.4(1.9-4.6)	
Business	64(37.4)	542(204-754)	453(225-648)	401(244-646)	3.2(1.9-5.1)	3.3(2.0-5.0)	3.0(1.9-4.5)	
Civil Servant	17(9.9)	350(190-797)	386(171-736)	419(210-612)	3.6(1.9-5.2)	3.4(2.1-5.0)	3.6(1.9-5.1)	
House Wife	21(12.3)	483(241-785)	492(266-680)	477(359-703)	4.4(2.5-5.1)	3.8(2.3-5.1)	2.9(1.9-5.4)	
Student	12(7.0)	405(205-642)	405(219-552)	384(244-556)	4.4(2.7-5.8)	4.2(3.2-5.4)	3.6(2.9-5.3)	
Others	17(9.9)	385(263-563)	462(300-612)	502(411-567)	4.6(3.7-5.5)	4.3(3.1-4.9)	3.3(2.8-4.5)	
Missing Value†	3(1.8)							
Educational Status								0.933
No formal education	16(9.4)	425(291-809)	513(312-804)	535(417-735)	4.1(1.9-4.9)	3.8(2.0-4.6)	2.9(1.9-4.1)	
Primary education	25(14.6)	349(196-606)	290(205-580)	344(299-631)	4.1(2.8-4.9)	3.5(2.3-4.9)	3.1(1.9-4.7)	
Secondary education	62(36.3)	416(248-676)	400(257-652)	440(243-565)	3.9(2.5-5.3)	3.6(2.7-5.3)	3.3(2.1-4.7)	
Tertiary education	38(22.2)	517(199-744)	427(232-594)	384(215-579)	3.7(2.1-5.5)	3.4(2.7-4.9)	3.6(1.9-5.2)	
Arabic education	27(15.8)	461(173-664)	525(225-620)	483(312-706)	4.2(2.2-5.3)	3.9(1.9-5.0)	3.1(1.9-4.2)	
Missing Value†	3(1.8)							
Family Awareness								0.404
Yes	125(73.1)	429(206-647)	423(244-595)	405(243-617)	3.8(2.1-5.2)	3.7(2.3-4.9)	3.2(1.9-4.7)	
No	44(25.7)	414(291-773)	453(283-683)	467(326-578)	4.2(2.6-5.2)	3.6(2.8-4.9)	3.5(2.0-4.4)	
Missing Value†	2(1.2)							
Family Support								0.74
Yes	124(72.5)	430(213-619)	423(245-592)	411(246-612)	3.9(2.2-5.1)	3.7(2.4-4.9)	3.2(1.9-4.7)	
No	31(18.1)	414(280-739)	442(280-621)	501(365-622)	3.9(2.0-5.2)	3.5(2.0-4.7)	3.0(1.9-4.4)	
Missing Value†	16(9.4)							

Table 2: Other Socio-demographic characteristics of the study population. † Participant did not provide any response. Where P value ≤ 0.05 is significant and P value > 0.05 is not significant.

CD4 (cells/µl)	Total	Male	Female	VL (copies/ml)			
				<1000		>1000	
				Male	Female	Male	Female
≤ 200	41 (24.0)	19 (11.1)	22 (12.9)	3 (1.8)	5 (2.9)	16 (9.4)	17 (9.9)
201-499	56 (32.7)	18 (10.5)	38 (22.2)	4 (2.3)	6 (3.5)	14 (8.2)	32 (18.7)
≥ 500	74 (43.3)	14 (8.2)	60 (35.1)	7 (4.1)	34 (19.9)	7 (4.1)	26 (15.2)
Total	171 (100.0)	51 (29.8)	120 (70.2)	14 (8.2)	45 (26.3)	37 (21.7)	75 (43.8)

Table 3: Baseline values of CD4 and Viral Load of 171 patients reporting for care at the HIV clinic in Taraba State. N (%).
CD4 and VL; $p < 0.0001$, VL and Sex; $p = 0.206$; $\chi 2 = 1.599$; $df = 1$, CD4 and Sex; $p = 0.008$; $\chi 2 = 9.693$; $df = 2$.

CD4⁺ (cells/µl)	Total	Male	Female	VL (copies/ml)			
				<1000		>1000	
				Male	Female	Male	Female
≤ 200	36 (37.1)	19 (19.6)	17 (17.5)	4 (4.1)	5 (5.2)	15 (15.4)	12 (12.4)
201-499	53 (54.6)	16 (16.5)	37 (38.1)	2 (2.1)	7 (7.2)	14 (14.4)	30 (30.9)
≥ 500	8 (8.2)	2 (2.1)	6 (6.2)	2 (2.1)	1 (1.0)	0 (0.0)	5 (5.2)
Total	97 (100.0)	37 (38.1)	60 (61.9)	8 (8.3)	13 (13.4)	29 (29.8)	47 (48.5)

Table 4: Three months post baseline assessment of CD4⁺ and Viral Load of ARV challenged. N (%).
CD4⁺ and VL; P = 0.349; VL and Sex; P=0.996; CD4⁺ and Sex; p=0.072.

CD4⁺ (cells/µl)	Total	Male	Female	VL (copies/ml)			
				<1000		>1000	
				Male	Female	Male	Female
≤ 200	1 (1.4)	0 (0)	1 (1.4)	0 (0)	0 (0)	0 (0)	1 (1.4)
201-499	11 (14.9)	3 (4.1)	8 (10.8)	2 (2.7)	4 (5.4)	1 (1.4)	4 (5.4)
≥ 500	62 (83.8)	11 (14.9)	51 (68.9)	4 (5.4)	23 (31.1)	7 (9.4)	28 (37.8)
Total	74 (100.0)	14 (18.9)	60 (81.1)	6 (8.1)	27 (36.5)	8 (10.8)	33 (44.6)

Table 5: Three months post baseline assessment of CD4⁺ and viral load of ARV naïve patients. N (%).
CD4⁺ and VL; p= 0.529; VL and Sex; p= 0.885; CD4⁺ and Sex; p= 0.674.

CD4⁺ (cells/µl)	Total	Male	Female	VL (copies/ml)			
				<1000		>1000	
				Male	Female	Male	Female
≤ 200	30 (30.9)	14 (14.4)	16 (16.5)	6 (6.2)	11 (11.3)	8 (8.2)	5 (5.2)
201-499	48 (49.5)	19 (19.6)	29 (29.9)	5 (5.2)	8 (8.3)	14 (14.4)	21 (21.6)
≥ 500	19 (19.6)	4 (4.1)	15 (15.5)	1 (1.0)	9 (9.3)	3 (3.1)	6 (6.2)
Total	97 (100.0)	37 (38.1)	60 (61.9)	12 (12.4)	28 (28.9)	25 (25.7)	32 (33.0)

Table 6: CD4⁺ and Viral load of ARV challenged patients six months post baseline assessments. N (%).
CD4⁺ and VL; p=0.019; VL and Sex; p =0.167; CD4⁺ and Sex; p=0.190.

CD4⁺ (cells/µl)	Total	Male	Female	VL (copies/ml)			
				<1000		>1000	
				Male	Female	Male	Female
≤ 200	6 (9.7)	2 (3.2)	4 (6.5)	1 (1.6)	2 (3.2)	0 (0)	3 (4.8)
201-499	20 (32.3)	3 (4.8)	17 (27.4)	0 (0)	3 (4.8)	4 (6.5)	13 (21.0)
≥ 500	36 (58.1)	6 (9.7)	30 (48.4)	3 (4.8)	18 (29.0)	3 (4.9)	12 (19.4)
Total	62 (100.0)	11 (17.7)	51 (82.3)	4 (6.4)	23 (37.0)	7 (11.4)	28 (45.2)

Table 7a: CD4⁺ and viral load of ARV naive patients 6 months post baseline assessment. N= 62 (%).
CD4⁺ and VL; p=0.007; VL and Sex; p=0.596; CD4⁺ and. Sex; p=0.950.

CD4⁺ (cells/µl)	Total	Male	Female	VL (copies/ml)			
				<1000		>1000	
				Male	Female	Male	Female
≤ 200	2 (16.7)	2 (16.7)	0 (0.0)	0 (0.0)	0 (0.0)	2 (16.7)	0 (0.0)
201-499	6 (50.0)	0 (0.0)	6 (50.0)	0 (0.0)	3 (25.0)	0 (0.0)	3 (25.0)
≥ 500	4 (33.3)	1 (8.3)	3 (25.0)	1 (8.3)	2 (16.7)	0 (0.0)	1 (8.3)
Total	12 (100.0)	3 (25.0)	9 (75.0)	1 (8.3)	5 (41.7)	2 (16.7)	4 (33.3)

Table 7b: Six months assessment of ARV naïve patients who became drug challenged post three months assessment due to reduced CD4+ values. N= 12 (%). CD4⁺and VL; p=0.368; VL and Sex; p=0.505; CD4⁺ and Sex; p=0.018

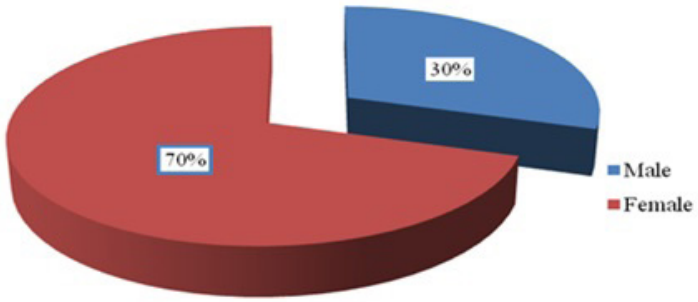
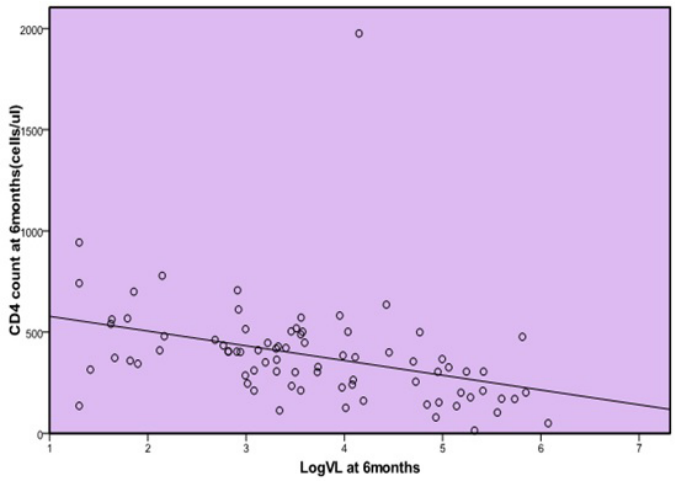


Figure 1: Sex distribution of study population at baseline.

Out of all the age groups, group's 18-30years and 31-40 year had the most recruits (Table 1). From the study population, 74 (43.3%) patients were married, 44 (25.7%) were single, 26 (15.2%) were widowed, and 22 (12.9%) were divorced. Although there was no statistically significant difference between the median CD4 of the different marital status groups, the mean rank of the median viral load for patients who were single was significantly higher than the other marital status groups, P=0.021 (Figure 2). Other socio demographic characteristics such as occupation, educational status, family awareness, family support, had no significant impact on the CD4 and viral load of the population (Table 2).



Discussion of the Findings

This study adopted the Integrated National Guideline for HIV Prevention- Treatment-and-Care, Nigeria (2014) criteria for initiating antiretroviral therapy (ART) in adults and adolescents, which stipulates that all HIV positive individuals with a CD4+ cell count less than 500cells/mm3 are eligible for ART and should receive ART irrespective of clinical symptoms. This guideline was still being in use at the Federal Medical Centre, Jalingo, as at the time of this study. Also, the WHO's strategy for the surveillance and monitoring of HIV drug resistance in low- and middle- income countries (LMICs) was adopted. It states that a viral load of <1000 RNA copies/ml should be taken as evidence of viral suppression [22,23].

In likewise, using the definition of virological failure as plasma viral load above 1000 copies/ ml based on two consecutive viral load measurements after 3 months, with adherence support or viral load > 1000 copies/ml after at least six months on ART. Thus, early intervention in asymptomatic patients would involve the commencement of antiretroviral therapy once the CD4+ count of a HIV patient was less than 500 cells/µl. A total of 171 HIV drug naïve adult patients who were newly enrolled into the HIV clinic at the Federal Medical Centre, Jalingo; were recruited into this study. Baseline assessment of these patients showed 74 patients had CD4+ count ≥ 500 cells/µl and 97 patients had CD4+ count <500 cells/µl. Patients with baseline CD4+<500 cells/µl, were immediately commenced on Highly Active Antiretroviral Therapy (ARV challenged patients) while those with CD4+ ≥ 500 cells/µl were not commenced on therapy (ARV naïve patients).

This study observed a significant CD4+ count increase of 49.1% at a rate of 39.9 cells/µl per month and viral load decrease of 1.4 log10 copies/ml in ARV challenged patients six months post baseline assessment. Nineteen (19.6%) patients had an improved CD4+count from <500 cells/µl at baseline assessment to ≥ 500cells/µl six months on therapy and 40 (41.2%) patients achieved viral suppression of<1000 copies /ml over the period, due to effective use of HAART. There was also a significant CD4+ decline of 23.3% at a rate of 54.5 cells/µl per month and viral load increase of 0.5 log10 copies/ml in ARV naive patients six months post baseline assessment. Thirty-eight (52.7%) patients with CD4+ count ≥ 500 cells/µl at baseline assessment had CD4+ decline of<500 cells/µl at six months post baseline assessment and 41 (56.9%) patients had high viral load > 1000 copies/ml, showing increased viral replication and depressed immunity in the absence of treatment. Hence, the need to recruit these patients for ARV treatment and management.

Demographics of Study Population

The ratio of female patients (70.2%) to male patients (29.8%) recruited in this study at the Federal Medical Centre, Jalingo was 2.3:1. This is similar to the findings of Nwozor and Nwankwo [7] which was 2.2:1, but slightly higher than 1.8:1 of Omoti et al. [24] and 1.6:1 of Akinbami et al. [8]. This may be linked to the fact that females make up more than 58% of persons living with HIV in Nigeria and each year, 55% of AIDS death occurs among women and girls [25]. This finding is also supported by a research carried out by the Centre for Disease and Control (CDC) in seven countries (Botswana, Côte d'Ivoire, Haiti, Nigeria, Mozambique, Swaziland, and Zambia), where point estimates of the ratio of female-to-male new ART enrollees increased more sharply among persons with HIV [26]. Females are more likely to be higher in number than their male counterparts because they tend to seek health care earlier than the men and are more readily available for testing. High number of female visits to clinics for antenatal care during pregnancy, where HIV screening and counseling are routinely carried out as part of antenatal care to recruit HIV positive mothers for ART to prevent mother-to-child transmission (PMTCT) of HIV could be also be a reason [27].

Furthermore, females are more predisposed to contracting HIV because of early marriage, polygamous relationships and pelvic

inflammatory disease which predisposes to microulceration of the genital tract thus increasing the risk of HIV infection [28-30]. Sexual intercourse during menstruation and presence of genital ulcers and the use of oral contraceptive, which could induce cervical ectopia due to replacement of squamous by columnar epithelium, also increases the risk of HIV infection for women 5-fold [8]. The study showed a mean age of 33.3 ± 9.3 years for participants. Reason could be attributed to the fact that these age groups are in their youth, hence are more sexually active and prone to unprotected sexual intercourse, drug abuse and acts associated with youthful exuberance. This finding is consistent with that of Nwauche et al. [31] who had a mean age of 35.04 years in Port Harcourt and similar to that of Omoti et al. [24]; Nwanzor and Nwankwo [7]; and Martinson et al. [32].

Male participants were significantly older than female participants, ($p<0.0001$) with a mean age of 38.3 ± 10.8 years for males in contrast to 31.2 ± 8.2 years for female participants. This could be attributed to the fact that women marry younger than men, and marriage has been found to be a risk factor for HIV transmission. Also, those women often have older partners while men rarely have partners much older than themselves [33]. This finding is similar to the work of Omoti et al., [24]; and Akinbami et al. [8]. The largest proportion of participants 74 (43.3%) were married and this has been identified as a risk factor for HIV transmission [34]. Thejus et al. [35] in a study in India showed that 80% of HIV/AIDS patients were married. This study can be compared to that of Nwauche et al., [31]; who reported a proportion of 41.1% married Individuals. Although there was no statistical significant difference between the median CD4+ of the different marital status groups, the mean rank of the median viral load for single patients was significantly higher than those who were married, divorced and widowed; ($P= 0. 021$). This may be due to the fact that singles which are a large percentage of the youths are more likely to engage in risky behaviours such as having multiple sex partners which exposes them to acquiring multiple stain of the Human Immunodeficiency Virus. Also, it may be possible that these youths were being diagnosed promptly [36]. Seventy- four (43.3%) patients presented to clinic at baseline assessment with CD4+ >500 cells/µl. This provides feedback regarding outcome of recent HIV campaigns on 'Know your HIV statuses and stronger polices on the reduction of discrimination and stigmatization against HIV patients in the country. Thus, many patients are promptly diagnosed because of early presentation to clinics. This study is contrary to Agu et al., [37]; Bello [34]; where a larger proportion of the patients presented to clinic very late with CD4+ count <200 cells/µl.

CD4+ Counts and Viral Load of Study Population

At baseline assessment of the 171 patients enrolled for this study, 74 patients had CD4+ count greater than 500cells/µl and 97 patients had CD4+ count less than 500 cells/µl, with females having higher CD4+ count when compared to males; ($p= 0.008$). This could be due to the fact that females readily assess health care and treatment when they are ill than their male counterparts and hence have a better immunological response than the men. Though viral load showed no significance between both sexes. There was significant difference in the CD4+ cell count and viral load of the 171 naïve patients at baseline assessment ($p<0.0001$); then showed no significance at three months assessment for both ARV challenged (i.e. those patients that commenced HAART post baseline assessment), ($p = 0.349$) and ARV naïve group (i.e. those patients that remained without therapy because they had high CD4+ at baseline assessment), ($p = 0.529$).

But at six months assessment, CD4+ and viral load showed a significant difference again when compared for both ARV challenged, ($p = 0. 019$) and ARV naïve, ($p = 0.007$) groups respectively. This may be because at baseline assessment, patients with higher CD4+ count values had high tendency of having much lower viral load values, while patients with lower CD4+ count had high tendency of having higher viral load values in the absence of treatment and management. Three months post baseline assessment showed that of the 97 (57%) ARV challenged patients that had baseline CD4+ count <500 cell/µl, and hence commenced treatment; 8 (8.2%) of them now had CD4+ ≥ 500 cell/µl. This was not significant, and may be due to slow recovery of the immune system, because it can take approximately two years to be able to ascertain the full extent of immune system recovery or this may be due to poor adherence to treatment in the part of the patients. This finding is contrary to that of Bello [34] where there was an escalated increase of CD4+ at three months on HARRT.

The 74 (43%) ARV naïve patients also experienced a decline in their CD4+, with 12 (16.2%) patients who had a baseline CD4+ count ≥ 500 cell/µl, now having CD4+<500 cell/µl at three months assessment. Though this count was not significant, it shows that there is a reduction in immunity and this may be due to viral replication and depletion of CD4+ cells; thus, a gradual progression to disease state. At six months assessment, ARV challenged patients had a significant CD4+ count increase of 49.1% at a rate of 39.9 cells/µl per month and viral load decrease of 1.4 log10 copies/ml with 19 (19.6%) patients who experienced an improved CD4+ count from <500cells/µl at baseline assessment to ≥ 500 cells/µl six months on therapy and 40 (41.2%) patients achieved viral suppression of<1000 copies /ml over the period, due to effective use of HAART. This is so because better immunological response would lead to lower viral replication and vireamia. The ARV drug naïve patients also had a significant CD4+ count decline of 23.3% at a rate of 54.5 cells/µl per month and viral load increase of 0.5 log10 copies/ml with 38 (52.7%) patients who had initial CD4+ count ≥ 500 cells/µl at baseline assessment, now experienced CD4+ decline of <500 cells/µl and 41 (56.9%) patients with high viral load >1000 copies/ml, showing increased viral replication and depressed immunity in the absence of treatment. Thus using CD4+ count alone as criteria for recruitment into HAART for relatively healthy HIV patients may not be reliable enough since high plasma HIV-1 RNA would lead to rapid depletion of CD4+ cells, and also increases the risk for HIV transmission.

This finding is supported by Okeke [15]; Hughes et al. [16] and Govender et al. [38]. Effective HAART can reduce viremia and transmission of HIV to sexual partners by more than 96% [39].

This study shows that there is an inverse relationship between CD4+ and viral load as evidenced in the linear regression model between viral load and CD4+ count for ARV challenged patients. Reinstating the fact that effective use of Highly Active Antiretroviral Therapy (HARRT) enhances viral suppression and thus enables the immune system to recuperate. This finding is supported by Akinbami et al., [8]. Six months post HARRT therapy on ARV challenged patients showed that 30 (30.9%) patients still had CD4+ count ≤ 200 cells/µl. This may be due to immunological failure, lack of drug adherence, resistant strains of HIV which could be acquired though risky behaviors, poor nutrition, malnutrition (due to wasting syndrome), etc. It is also clear that late starters of highly active antiretroviral therapy with CD4+ count <200 cells/µl have significantly poor response to therapy compared to those who start therapy with a higher CD4+ T cell count. Fifty-seven (58.7%) ARV challenged patients also had viral load >1000 copies/ml after being on treatment for six month, which means that these patients have not been able to attain viral suppression. This may be due to non-adherence to prescribe antiretroviral or these patients may be harboring viral subtypes (acquired through risky behaviors such as having unprotected sex, multiple sex partners); which are resistant to prescribed drug combinations. Possible drug- drug interaction when patient use other medications apart from prescribed regimen or virological failure suggesting switch of antiretroviral combination may be possible reasons. Despite attaining viral suppression of <1000 copies/ml while on therapy, 17 (17.5%) ARV challenged patients continued to have low CD4+ count of ≤ 200 cells/µl. This may be due to lack of compliance to drug in-take, nutrition, recent vaccination, opportunistic infections, or certain diseases such as hepatitis, tuberculosis, non HIV related cancers (which were not taken into consideration in the cause of this study) maybe possible factors for incomplete CD4+ count recovery, coupled with the fact that it may take about two years to fully ascertain the extent of immunologic recovery; which is a time frame that exceed the scope of this study.

Persistently low CD4+ cell counts despite ART-mediated viral suppression are associated with increased risk of morbidity and mortality. Thus, early HIV diagnosis and prompt initiation of therapy is paramount to survival. Hence the need to implement the new "test and treat" strategy by the National Agency for Control of AIDS (NACA), Nigeria as recommended by WHO, 2015 across all states of the Federation. This study identified a small proportion of ARV treatment naive patients that maintain undetectable viral load levels of <20 copies/ml with high CD4+ counts throughout the period of this study. Baseline assessment detected 14 patients who had undetectable viral load with a median CD4+ count of 710 cells/µl. At six months post baseline assessment 5 of these ARV drug naïve patient still had undetectable viral load with median CD4+ count of 943 cells/ µl. This is a 2.9% prevalence rate in the study population.

This may be because these patients presented to clinic at very early stages of HIV infection; may be on good nutrition and having positive behavioral change; or they could be "long term non progressors (LTNP) or possibly "aviremic or elite controllers" [40,41] because their system can rapidly control replication of viruses despite not receiving antiretroviral drugs. It may be important to follow up these patients to ascertain if they are truly long term non progressors or elite controllers. This study is similar to that of Martinson, et al, 2015 in Soweto, who observed a 2.6% long term non progressors in their study, but contrary to the finding of Odaibo et al. [42] in UCH, Ibadan, who had 10% prevalence. This higher prevalence may be due to the less sensitive technique with Roche Amplicor HIV version 1.5 used by Odaibo et al. [42] which could not detect viral load values less than 400 copies/ml.

Summary of Findings

This study observed a significant CD4+ count increase of 49.1% and viral load decrease of 1.4 log10 copies/ml in ARV challenged patients over the six months study period, due to effective use of Highly Active Antiretroviral Therapy (HAART). While there was a significant CD4+ decline of 23.3% and viral load increase of 0.5 log10 copies/ml in ARV naive patients during the six months study period, due to increased viral replication and depressed immunity in the absence of treatment. Hence, the need to immediately recruit all HIV positive patients for ARV treatment and management is crucial to these patients living a relatively healthy life and thus, reduce the risk of transmissibility of the disease. Six months post HARRT therapy showed that 57 (58.7%) patients out of the 97 (100.0%) ARV challenged patients still had viral load >1000 copies/ml and thus have not been able to attain viral suppression; while 30(30.9%) patients had CD4+ count ≤ 200 cells/µl despite being on therapy for six months. This findings point mainly to low compliance and lack of adherence among the study population. This study identified a 2.9% prevalence rate in the study population of ARV treatment naive patients that maintained undetectable viral load levels of <20 copies/ml with high CD4+ counts throughout the period of this study which could be elite controllers or Long Term Non- Progressors.

Conclusion

This study confirms the importance of CD4+ and viral load enumeration in monitoring HIV disease progression or regression in infected patients. This is shown by the inverse relationship between CD4+ and viral load; in the sense that as CD4+ increased, viral load decreased and vice versa. However, CD4+ may sometimes be used to monitor patients' treatment progress in the absence of viral load especially in resource limited settings such as ours. Without antiretroviral therapy (ART), most HIV-infected individuals will eventually develop progressive immunodeficiency marked by CD4+ T lymphocyte (CD4+) cell depletion, leading to AIDS-defining illnesses and death. Thus, the need for durable viral suppression to improve immune function and overall quality of life of patients, lower the risk of both AIDS-defining and non-AIDS-defining complications, and prolong life.

Recommendation

- CD4 count alone should not be used as criteria for admission into ART for relatively healthy HIV patients; rather the new policy on "test and treat" ART eligibility guideline – which states that regular testing of adults, and offering treatment to all

infected persons with ART, regardless of CD4 cell test results -; be implemented in all health centres across the federation. Hence, the need for government, and non -governmental organizations to support and strengthen more centres by the provision of adequate trained man power, machinery, drug supply and establishment of newer centres.

- There should be increased counseling and adherence support to patients on ART.
- Further research be carried out to follow- up and possibly identify long term non progressors or elite controllers; as this could be the key to cure for HIV/AIDS.
- There should be increased ART coverage especially among men with HIV to reduce morbidity and mortality in this group and contribute to reducing HIV incidence among their sexual partners.
- More enlightenment programs on HIV/AIDS should be carried out in the state to create more awareness and reduce stigmatization.

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