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Viral Load Monitoring and Haematological Parameters in Human Immunodeficiency Virus patients on Antiretroviral Therapy.

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Abstract

A study on Viral Load Monitoring and Haematological Parameters in Human Immunodeficiency Virus patients on Antiretroviral Therapy (ART) was carried out to monitor the effect of therapy on these parameters. A total of forty (40) naive anti-retroviral therapy HIV patients, of which 14 and 26 were male and female; age range of 20-45 years attending Rivers State University Teaching Hospital, Port Harcourt, were monitored for their viral load and haematological parameters, with a follow up period of 6 months which included baseline, three months and six months respectively from October 2017- March 2018. Viral load was determined using real time PCR and Haematological parameters were determined using automation. All data management and statistical analysis were performed using S 9.4 Statistical analysis software (SAS) (SAS Institute, Cary, NC, USA). P-Value of <0.05 was significant in the following parameters during the study periods: viral load, Haematocrit (HCT) and haemoglobin (HB). Viral load, Haematocrit (HCT) and haemoglobin (HB). Viral load decreased sharply from baseline value of 21476 ± 56914 cp/ml by 100% following 3 months of follow-up to 706 ± 108 cp/ml and later by 96% from 4-6 months of follow-up to 30 ± 3 cp/ml for a total of 100% overall decrease from baseline at the end of 6 months of follow-up. HB increased by 17% from 9.63 ± 0.35 g/dl following 3 months follow-up to 11.30 ± 0.18 g/dl, while 8% increase in HB occurred between 4-6 (12.20 ± 0.09) g/dl months for a total of 23% after 6 months of follow-up. Platelets at baseline was 269.03 ± 7.26 x $10^{9/}$L, this value reduced significantly to 239.00 ± 6.54 x $10^{9/}$L at the end of 6 months (p=0.01). The white blood cell count reduced as well from 5.02 ± 0.17 x 10^{9}/L at baseline to 4.54 ± 0.16 x 10^{9}/L after 3 months and further reduced to 4.14 ± 0.11 x 10^{9}/L by the end of 6 months. The difference was significant (p=0.0001). Neutrophils percentage reduced significantly from 47.24 ± 1.95 % to 39.49 ± 1.18% at 3 months and 33.93 ± 0.84% by 6 months (p<0.0001). Findings from the study show that the quality of life of patients enrolled in the study was improved due to administration of antiretroviral therapy and enhanced adherence counselling.

Keywords: Viral Load, Haematocrit, white Cell count, platelet count, Human Immunodeficiency Virus and Antiretroviral Therapy.

Introduction

HIV has been a major global public health concern since it was first identified in 1984. It is a retrovirus and has a predilection to infecting the T-helper lymphocytes usually referred to as CD4 cells, thus progressively suppressing the immune system. If the infection is not identified and treated promptly, it may progress to Acquired Immunodeficiency Syndrome (AIDS). Multiple organ systems are affected and one of such is the haemopoietic system which may lead to anaemia, leucopenia, thrombocytopenia and some AIDS associated blood cancers. This study sought to evaluate the changes that occur in some haematological parameters and the viral load of newly diagnosed HIV patients commencing Antiretroviral therapy.

The viral load (VL) is a measure of viral copies present in a milliter of blood. An elevated viral load speaks of the severity of the viral infection. HIV viral load (VL) monitoring is the fundamental means of appraising the efficacy of ART and risk of viral transmission. The World Health Organization (WHO) is making frantic efforts to increase the assess to VL measurement. (WHO, 2016 & Carmona *et al*., 2017).

Materials and Methods

Study Area and subjects

The study was carried out in Rivers State University Teaching Hospital, Port Harcourt, Rivers State, Nigeria. The geographical location of Rivers State is Latitude 4°31′ - 5°31′ and longititude 6°30′- 7°21′. Rivers State University Teaching Hospital, Port Harcourt is a 346 bed specialist hospital owned by Rivers State Government.

Study population

The study spanned for a period of six months during which a total of forty (40) naive anti-retroviral therapy HIV patients, of which 14 and 26 were male and female; age range of 20-45 years were recruited, attending Rivers State University Teaching Hospital, Port Harcourt, and were monitored for their viral load and haematological parameters, with a follow up period of 6 months which included baseline, three months and six months respectively from October 2017 and March 2018. The inclusion criteria were only patients diagnosed and confirmed HIV positive, only ART naive patients who have just been diagnosed and confirmed HIV positive, only subjects within the age bracket of 20-45 and Patients enrolled into Art clinic after confirmation of HIV status. Exclusion criteria were Patients who have not been confirmed HIV positive, HIV patients that are ART naive not willing to enrol into ART clinic after confirmation, Patients less than 20 years, HIV patients more than 45 years old and Patients already on ART. All patients enrolled into the study were given questionnaire to obtain some demographic characteristics and informed consent was obtained from them before a sample of blood was taken.

Ethical consideration

Ethical clearance to conduct academic research was obtained from the ethical committee of the Rivers State Ministry of Health

Blood Sample Collection and Processing.

The total amount of blood collected from each patient was 6ml through venous puncture, placing 4.0 mL into EDTA anticoagulant bottle for viral load testing and 2.0 mL of blood into another EDTA bottle for haematological parameters.

Determination of Viral Load values using the cobas® Ampliprep/cobas® taqman® 96 (Real time PCR)

Procedure

Start up procedures were Performed and reagents were Loaded onto the COBAS® AmpliPrep Instrument. Samples were removed from storage. Consumables were Loaded on the COBAS® AmpliPrep Instrument Orders were created and the sample rack was loaded onto the COBAS® AmpliPrep Instrument. The start button of the COBAS® AmpliPrep Instrument was activated. Review and acceptance of results using AMPLILINK software was made.

Determination of haematological parameters by haematology auto-analyzer

The haematological parameters investigated include Haematocrit, Haemoglobin, Red Blood Cells, Platelets, White blood cell, Netrophils, MXD (The MXD comprise of Basophils, Eosinophils and Monocytes generated by a three- part automated haematology analyzer). Sysmex Xp-300 Haematology Auto- Analyser, Model NO: XP-300 KOBE Japan.

Procedure

Samples were allowed to mix for 10 minutes in the mixer. The power switch was turned on. Self check, auto-rinsed and background check were automatically performed. Control samples were introduced into the instrument through the probe. Introduction of sample was made through the probe with a gentle tap on the start button for easy aspiration of sample. A buzzer sound was heard (beep, beep) two times, with a subsequent display of analysing then the sample tube was removed. The result of the test was displayed on the LCD screen.

Data Analysis

All data management and statistical analysis were performed using S 9.4 Statistical analysis software (SAS Institute, Cary, NC, USA). Analysis of variance and t-test were used to determine if significant differences existed by the following independent factors considered in the study, viz: sex, age group and follow-up period. Where significant differences existed mean values for a given parameter was tested using student's t or Turkey's HSD as applicable depending on the number of measured parameter levels.

Results

A total of 40.newly diagnosed HIV patients were enrolled into this study and followed up for 6 months. There were 14 males and 26 females. Table 1 shows the mean values of the viral load, haematocrit, haemoglobin and red blood cell count according to their demographic characteristics and follow-up periods of treatment, viral load decreased drastically and significantly from 21476±56914 copies /ml at baseline to 706±108 copies/ml at 3 months and by 6 months, the viral load had fallen to as low as 30±3.0 copies/ml ($P<0.0001$). The mean ± SEM of viral load was not affected by gender and age groups ($P<0.05$). For haematocrit, haemoglobin and red blood cell count, a steady significant increase was observed from baseline throughout the period of 6 months. Haematocrit increased from 31.65±1.15% at baseline to 36.44±0.68% at 3 months and 38.96±0.43% at 6 months ($p<0.0001$). Similar pattern was observed for haemoglobin where the value was from 9.63±0.35 g/dl at baseline to 11.30±0.18 g/dl at end of 3 months interval and 12.20±0.09 g/dl at the end of 6 months ($p<0.0001$). Similar pattern was observed for red blood cell count.

The pattern of decrease and increase in these parameters is shown as a trend graph using box plots (Fig 1 - Fig 4).

Table 1: Comparisons of Mean ± SEM Haematologic Parameters by Sex, Age group and Follow-up Period

Characteristic	N	Viral Load (cp/ml)		HCT (%)		HBg/dl		RBC($10^{12}/^{L}$)	
		Mean ± SEM	*P-Value*	Mean ± SEM	*P-Value*	Mean ± SEM	*P-Value*	Mean ± SEM	*P-Value*
Sex									
Female	14	79565±30684	0.633^{ns}	35.77±0.60	0.834 ^{ns}	11.13±0.19	0.485 ^{ns}	4.40±0.09 ^{a}	0.047*
Male	26	58431±19194		35.53±1.09		10.88±0.32		4.75±0.17 ^{b}	
Age Group (years)									
20-24	4	77875±44342	0.748 ^{ns}	31.01±1.87 ^{a}	0.038*	9.45±0.73 ^{a}	0.019*	3.90±0.21	0.060 ^{ns}
25-30	4	10858±7584		38.30±1.23 ^{b}		11.66±0.22 ^{b}		4.89±0.21	
31-40	26	69365±29095		35.92±0.64 ^{c}		11.22±0.20 ^{bc}		4.50±0.10	
41-50	6	107380±45795		35.76±1.47 ^{c}		10.85±0.42 ^{c}		4.71±0.23	
Follow-up Period									
Baseline	40	21476±56914^{a}	<0.0001****	31.65±1.15^{a}	<0.0001****	9.63±0.35^{a}	<0.0001****	4.07±0.17^{a}	<0.0001****
3 Months	40	706±108 ^{b}		36.44±0.68 ^{b}		11.30±0.18 ^{b}		4.55±0.13 ^{b}	
6 Months	40	30±3 ^{c}		38.96±0.43 ^{c}		12.20±0.09 ^{c}		4.95±0.10 ^{c}	

SEM: Standard Error of Mean.
Within parameter, mean ± SEM with different superscript are significantly different at p<0.05.
*Significance Level: *=p<0.05; **=p<0.01; ***=p<0.001; ****=p<0.0001; ns= not significant (p>0.05)*

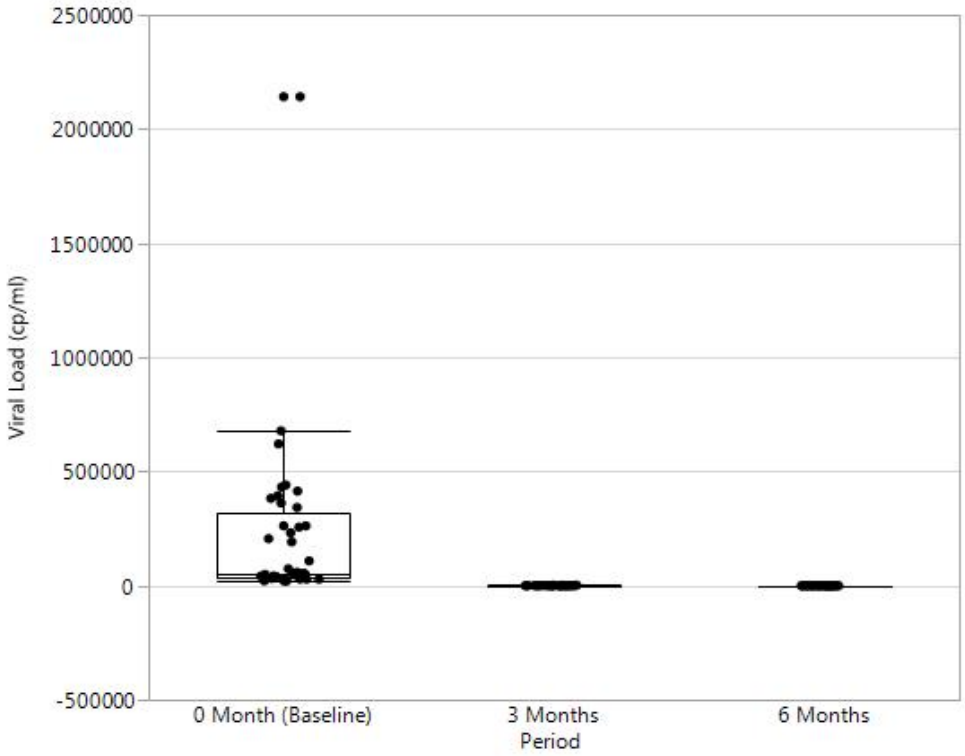

Figure 1: Box Plot of Viral Load by Follow-up Periods

Box plot describes median (line inside of box), lower and upper quartiles (bottom and top of box), minimum (horizontal line) below the outliers: (open circles) maximum (horizontal line) above and outliers (open circles) Outliers are data points above or below the upper or lower adjacent value.

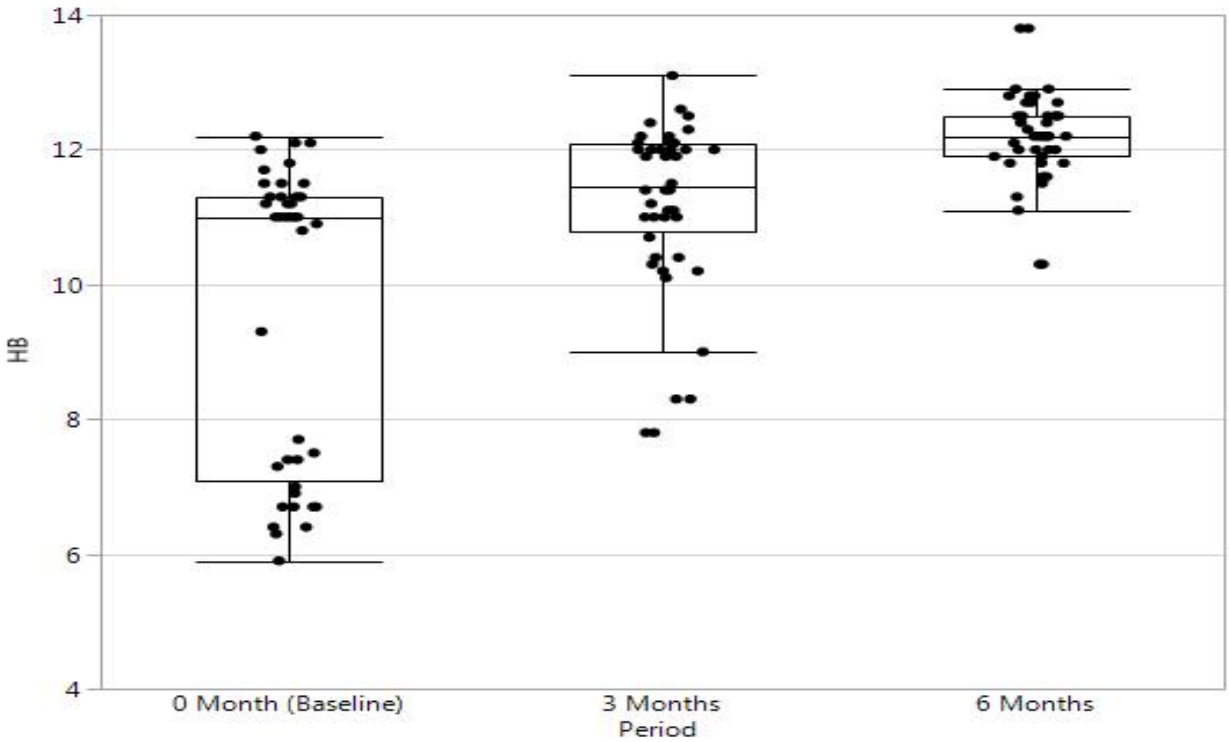

Figure 2: Box Plot of HB (g/dl) by Follow-up Periods

Box plot describes median (line inside of box), lower and upper quartiles (bottom and top of box), minimum (horizontal line) below the outliers: (open circles) maximum (horizontal line) above and outliers (open circles) Outliers are data points above or below the upper or lower adjacent value.

Table 2 shows the mean ± SEM values of platelets, total white blood cell count and neutrophils. The mean ± SEM of platelets at baseline was 269.03 ± 7.26 x $10^{9/}$L, this value reduced significantly to 239.00 ± 6.54 at the end of 6 months (p=0.01). The white blood cell count equally reduced from 5.02 ± 0.17 at baseline to 4.54 ± 0.16 after 3 months and further reduced to 4.14 ± 0.11 by the end of 6 months.

The difference was very significant (p=0.0001). Neutrophils percentage reduced significantly from 47.24 ± 1.95 % to 39.49 ± 1.18% at 3 months and 33.93 ± 0.84% by 6 months (p<0.0001). The total white blood cell counts and neutrophils were highest among subjects below the age of 25 years and lowest among 25-30 years age group. (P=0.009 and P=0.013) for total white blood cell and neutrophils respectively. The mean ± SEM values of the total white blood cell count in females was significantly lower (4.41 ± 0.11x$10^{9/}$L than their male counterparts (4.85 ± 0.16x$10^{9/}$L) (P=0.02). Figures 5 to 7 show the box plots of the parameters described above and revealed the trend and pattern of increase or decrease in the parameters.

Table 2: Comparison of Mean ±SEM Haematologic Parameters by Sex, Age group and Follow-up Period (Cont'd)

Characteristic	n	PLT x 10^9/L		WBC x 10^9/L		N (%)	
		Mean ± SEM	*P-Value*	Mean ± SEM	*P-Value*	Mean ± SEM	*P-Value*
Sex							
Female	26	258.47±5.36	0.146 ns	4.41±0.11 a	0.020*	39.92±1.09	0.728 ns
Male	14	246.07±5.85		4.85±0.16 b		40.60±1.79	
Age Group (years)							
20-25	4	252.80±10.10	0.870 ns	5.57±0.35 a	0.009**	49.10±4.69 a	0.013**
25-30	4	243.10±13.89		4.34±0.26 b		34.85±1.00 b	
31-40	26	255.74±5.45		4.50±0.11 b		40.01±1.04 c	
41-50	6	254.05±7.94		4.43±0.18 b		39.03±2.50 c	
Period							
Baseline	40	269.03±7.26 a	0.010**	5.02±0.17 a	0.000***	47.24±1.95 a	<0.0001****
3 Months	40	254.38±6.64 b		4.54±0.16 b		39.49±1.18 b	
6 Months	40	239.00±6.54 c		4.14±0.11 c		33.93±0.84 c	

SEM: Standard Error of Mean.

Within parameter, mean ±SEM with different superscript are significantly different at p<0.05.
*Significance Level: *=p<0.05; **=p<0.01; ***=p<0.001; ****=p<0.0001; ns= not significant (p>0.050)*

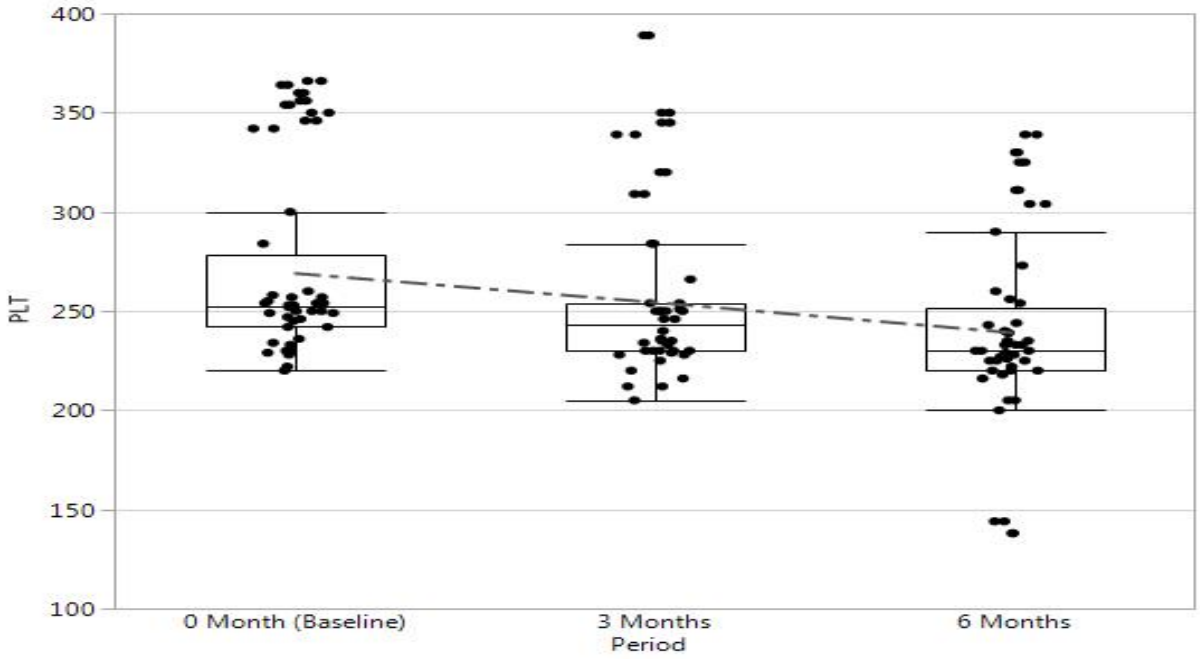

Figure 3: Box Plot of PLT (x10^9/L) by Follow-up Periods

Box plot describes median (line inside of box), lower and upper quartiles (bottom and top of box), minimum (horizontal line) below the outliers: (open circles) maximum (horizontal line) above and outliers (open circles) Outliers are data points above or below the upper or lower adjacent value.

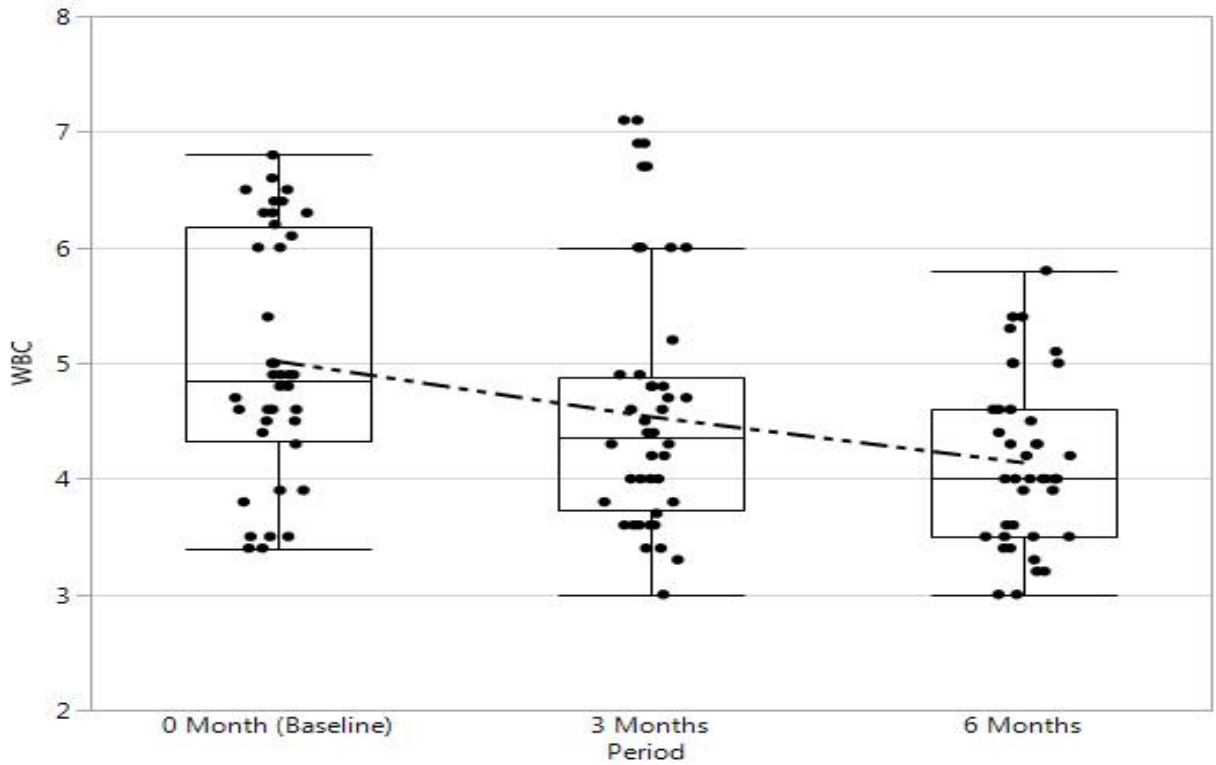

Figure 4: Box Plot of WBC (x 10^9/L) by Follow-up Periods

Box plot describes median (line inside of box), lower and upper quartiles (bottom and top of box), minimum (horizontal line) below the outliers: (open circles) maximum (horizontal line) above and outliers (open circles) Outliers are data points above or below the upper or lower adjacent value.

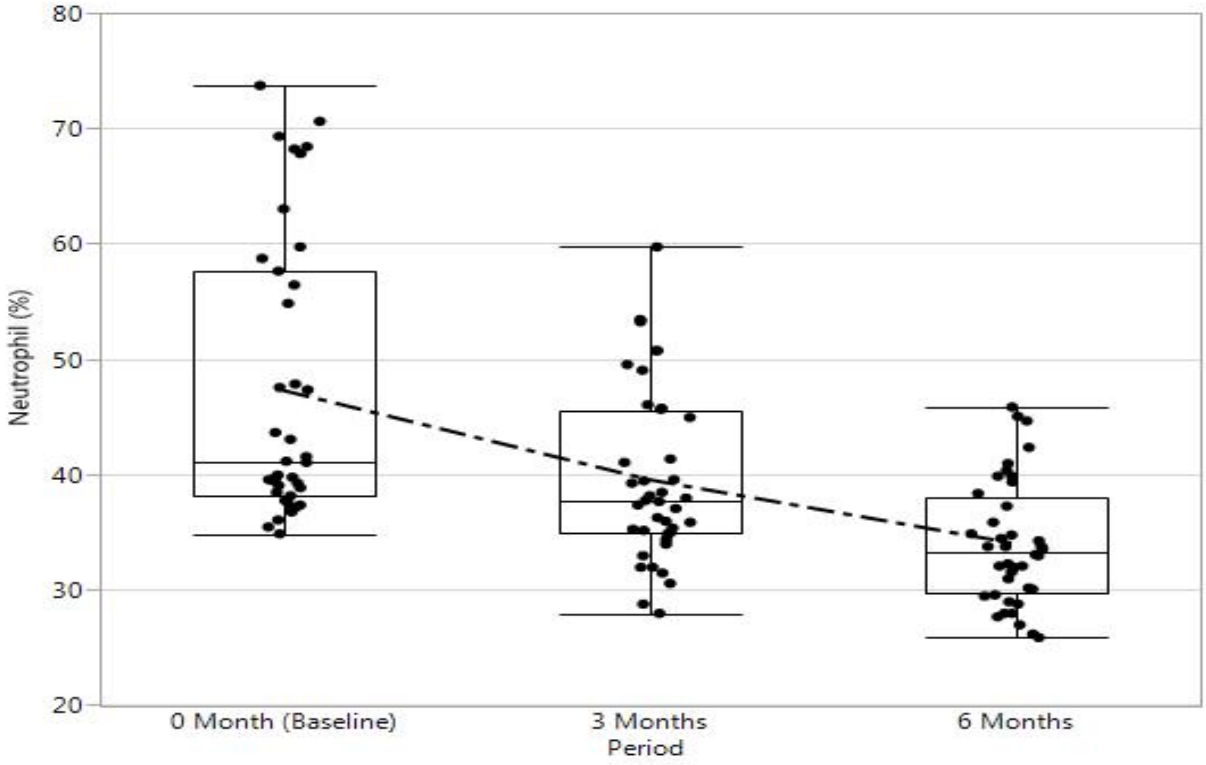

Figure 5: Box Plot of Neutrophil (%) by Follow-up Periods

Box plot describes median (line inside of box), lower and upper quartiles (bottom and top of box), minimum (horizontal line) below the outliers: (open circles) maximum (horizontal line) above and outliers (open circles) Outliers are data points above or below the upper or lower adjacent value.

The comparison of mean ± SEM of the remaining haematological parameter (MXD) is shown in Table 3. The MXD comprise of Basophils, Eosinophils and Monocytes generated by a three-part automated haematology analyzer. The values increased from baseline value of 13.49±0.33 to 14.59±0.16 and then to 15.57±0.12 at the end of 6 months. The difference was statistically significant (P<0.0001). Figure 8 shows the trend of the increase in the MXD portion of white blood cells.

Table 3: Comparisons of Mean ±SEM Haematologic Parameters by Sex, Age group and Follow-up Period (Cont'd)

Characteristic	n	MXD (%)	
		Mean ± SEM	*P-Value*
Sex			
Female	26	14.59±0.14	0.705 [ns]
Male	14	14.47±0.35	
Age Group (years)			
20-25	4	14.12±0.49	
25-30	4	15.13±0.24	
31-40	26	14.47±0.21	0.473 [ns]
41-50	6	14.77±0.26	
Period			
Baseline	40	13.49±0.33[a]	
3 Months	40	14.59±0.16 [b]	<0.0001****
6 Months	40	15.57±0.12[c]	

SEM: Standard Error of Mean.
Within parameter, mean ± SEM with different superscript are significantly different at p<0.05.
*Significance Level: *=p<0.05; **=p<0.01; ***=p<0.001; ****=p<0.0001; ns= not significant (p>0.05).*

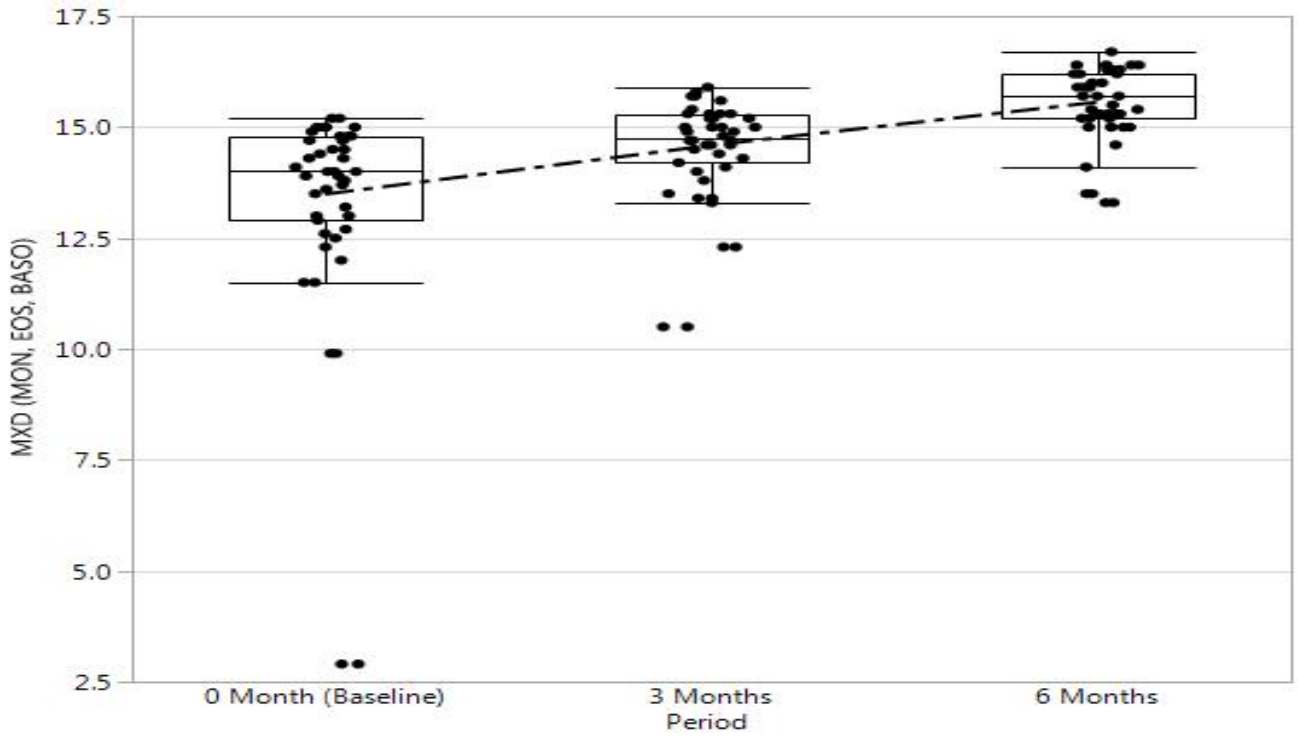

Figure 6: Box Plot of MXD (%) by Follow-up Periods

Box plot describes median (line inside of box), lower and upper quartiles (bottom and top of box), minimum (horizontal line) below the outliers: (open circles) maximum (horizontal line) above and outliers (open circles) Outliers are data points above or below the upper or lower adjacent value.

Discussion

In 2014, the Joint United Nations Programme on HIV/AIDS (UNAIDS) and partners launched the 90–90–90 targets; the intention was to establish 90% of all HIV-positive people, make available antiretroviral therapy (ART) for 90% of those established, and attain viral suppression for 90% of those assessing therapy by 2020. The reason was to achieve 73% viral suppression for those with HIV which is key if the AIDS epidemic must be ended by 2030 (UNAIDS, 2016). The aim of this vision is to reduce morbidity and mortality due to HIV and also remarkably reduce the risk of transmission of the virus.

In this study, the mean values for viral load decreased significantly from 21476±56914 copies /ml at baseline to 706±108 copies/ml at 3 months and by 6 months, the viral load had fallen to as low as 30±3.0 copies/ml ($P<0.0001$), this exceeded the UNAIDS vision goal.

In comparison with our work, Matthew *et al*., (2016), reported that their study subjects' attained viral remission roughly one year after start of therapy. This, however, is in sharp contrast with our finding as our study subjects attained 100% remission after therapy commencement at three months. This variation may be due largely to poor health behaviours on the part of their subjects, while our study subjects strictly adhered to their therapy after enhanced adherence counselling. The finding is in line with previous study done in Ethiopia and Benin City Nigeria (Eneyew *et al* ., 2016; Shakirat *et al*., 2014).

ART radically suppresses viral load and the aim of treatment is to suppress viral load to untraceable levels and sustain viral remission. There are proofs to show that these antiretrovirals repress duplication but do not get rid of HIV from all parts of the body (Chen *et al*., 2007).

Anaemia has been shown to be a an independent predictor of disease progression and death in HIV (Moore *et al*. , 2002), In one of the earlier works done on the use of antiretroviral drugs (ARVDs) it was reported that therapy improved haemoglobin level, and therefore has the ability to enhance the quality of life despite the side effects (Chukwurah *et al*., 2007 & Wei *et al*., 1995). Contrary to their opinion is Blockman's whose work states that both HIV positive subjects on ARVDS therapy and those not on ARVDS therapy had their heamatocrit value reduced such that they developed mild anaemia (Blockman, 1991).

However, subjects on ARVDS therapy had their hematocrit value higher than those not on ARVDs therapy. Hence they held that ARVDs therapy is effective to correct anaemia in HIV infection.

Although, from the analysis of Oyeka *et al*., (2014) it was observed that the haematocrit values off females was statistically lower than that of their male counterpart when compared for age and stage of infection, the haematocrit however reduced as the infection persisited.

Some reports have shown that increasing haemoglobin levels is a sign of treatment success (Paton *et al*., 2006).

According to Kettner *et al*., (2004), a lot of factors affect alteration in Haematological indices. Such factors are direct bone marrow infiltratiion by HIV, influx of opportunistic infection, alteration in regulatory mechanism and treatment therapies (Kettner *et al*., 2004). Alteration in Hematological parameters can affect the prognosis of people living with HIV infection. This is the finding of Sabin *et al*., (2002).

The correction of haematological disorders can be done by antiretroviral therapy which also brings down the number of viral particles. Hence patient placed on ART had superior values of blood cells during six months of starting therapy with visible correction in haematological markers (Servais *et al*., 2001). This findings strongly corresponds with our work in which a steady significant increase was observed from baseline throughout the period of 6 months (Haematocrit, haemoglobin and red blood cell count), with a resultant fall in viral load values.

Conclusion

Early commencement of ART is useful for reduction of viral load and correction of abnormalities in haematological parameters such as haemoglobin, this is usually associated with improved quality of life.

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