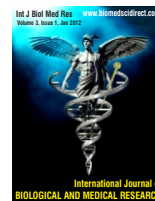


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Original Article

Effect of HAART on some haematological parameters, correlations between total lymphocyte and CD4 counts of HIV clients attending ssh, ikole-ekiti

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ABSTRACT

Several authors have reported anaemia, neutropenia, thrombocytopenia, eosinophilia etc. as the adverse effects of ARD especially when used as mono-therapy. In this study, the effect of HAART on some haematological parameters and correlation between total lymphocyte count and CD4 cells count was carried out on eighty patients accessing care in State Specialist Hospital, Ikole Ekiti, Ekiti State. The results obtained in this study show a positive relationship between haematocrit (HCT), haemoglobin (HGB) and Total Lymphocyte Count (TLC) with CD4 count in the course of receiving HAART. Statistically significant difference was observed in CD4 count, 137.72 18.66, lymphocytes 32.22 2.03, Neutrophil 60.19 2.25, HCT 30.38 0.74 and TLC 1466.41 107.97 at baseline when compared to the values at 3 months; CD4 count 313.53 35.16, lymphocyte 44.31 2.17, Neutrophil 48.91 2.21, HCT 32.5 0.70 and TLC 1999.31 120.69 ($P < 0.05$). When values at 3 and 6 months were compared, the difference was not statistically significant ($P > 0.05$). Also, statistically significant difference (increase) was seen in CD4 count 343.94 36.81, lymphocytes 44.28 1.74, HCT 32.94 0.60 and TLC 1993.12 127.52 at 6 months when compared with the baseline; while statistically significant difference (reduction) was observed in Neutrophil 49.69 1.65 and monocytes 3.97 0.26, when compared with the Neutrophil 60.19 2.25 and monocytes 4.84 0.34 at baseline ($P < 0.05$). No statistically significant difference was observed in the other parameters ($P > 0.05$). Our findings indicate that HAART results in improved haematologic values of HIV patients. In addition to the use of TLC for initiating HAART, as proposed by WHO guidelines, it can also be used as an inexpensive surrogate for monitoring the immunological response to HAART in resource-limited settings as significant correlation was observed in the TLC and CD4 counts at all points of the investigations. Significant correlation ($r, 0.632, P < 0.01$) was observed at the baseline, ($r, 0.730, P < 0.01$) at 3 months, ($r, 0.697, P < 0.01$) at 6 months and, ($r, 0.729, P < 0.01$) for all test groups.

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1. Introduction

Since its identification in 1981, Human Immunodeficiency Virus (HIV) and Acquired Immune Deficiency Syndrome (AIDS) remains the most challenging significant emerging infectious agent in the last century, and it continues to create health and socio-economic challenges in Nigeria and the world at large.

Every day, over 6,800 persons become infected with HIV and over 5,700 persons die from AIDS, mostly because of inadequate access to HIV prevention and treatment services. The HIV pandemic remains the most serious of infectious disease challenges to public health. Nonetheless, the current epidemiologic assessment has encouraging elements since it suggests: the global prevalence of HIV infection (percentage of persons infected with HIV) is remaining at the same level, although the global number of persons living with HIV is increasing because of ongoing accumulation of new infections with longer survival times, measured over a

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continuously growing general population; there are localized reductions in prevalence in specific countries; a reduction in HIV associated deaths, partly attributable to the recent scaling up of treatment access; and a reduction in the number of annual new HIV infections globally.

Examination of global and regional trends suggests the pandemic has formed two broad patterns: generalized epidemics sustained in the general populations of many sub-Saharan African countries, especially in the southern part of the continent; and epidemics in the rest of the world that are primarily concentrated among populations most at risk, such as men who have sex with men, injecting drug users, sex workers and their sexual partners²⁰.

Sub-Saharan Africa remains the most seriously affected region, with AIDS remaining the leading cause of death. Although percentage prevalence has stabilized, continuing new infections (even at a reduced rate) contribute to the estimated number of persons living with HIV, 33.2 million [30.6–36.1 million] in 2007 and 33.4 [31.1- 35.8 million] in 2008. HIV prevalence tends to reduce slowly over time as new infections decline and through the death of HIV-infected persons; it can increase through continuing HIV incidence and through reduced mortality of HIV-infected persons on antiretroviral treatment. Global HIV prevalence—the percentage of the world's adult population living with HIV—has been estimated to be relatively stable since 2001. Downward trends in HIV prevalence are occurring in a number of countries, where prevention efforts aimed at reducing new HIV infections since 2000 and 2001 are showing results. In most of sub-Saharan Africa, national HIV prevalence has either stabilized or is showing signs of a decline²¹.

The estimated number of deaths due to AIDS in 2007 was 2.1 million [1.9–2.4 million] worldwide, of which 76% occurred in sub-Saharan Africa; and 2.0 million [1.7–2.4 million] in 2008. Declines in the past two years are partly attributable to the scaling up of antiretroviral treatment services. AIDS remains a leading cause of mortality worldwide and the primary cause of death in sub-Saharan Africa, illustrating the tremendous, long-term challenge that lies ahead for provision of treatment services, with the hugely disproportionate impact on Sub-Saharan Africa ever clearer. The annual number of new infections declined from 3.0 million in 2001 to 2.7 million in 2008²¹.

In Nigeria, the annual HIV positive birth is estimated at 56,681 while the annual AIDS death in 2008 was 280,000. The total number of People Living with HIV/AIDS (PLWHA) requiring ART is 833,000 while the total number of AIDS orphan stands at 2.23 million¹⁴.

Ekiti State, though reported to have the lowest HIV prevalence of 1% in 2008, the available data from the State Ministry of Health from the Counseling and Testing points in the 16 Local Government Areas as at December 2008 revealed annual HIV prevalence exceeding 5%⁶. Though no curative measure has been discovered so far for HIV infection, successful treatment of the infection

has been achieved through successful implementation of highly active antiretroviral therapy, frequently referred to as HAART. Right from the discovery of the Antiretroviral Drugs (ARD), the death profile through HIV/AIDS has been drastically reduced. Several reports have confirmed the efficacy of antiretroviral drugs in the clinical management of HIV patient⁷.

Treatment with a combination of two nucleoside reverse transcriptase inhibitors (NRTI's) and a potent protease Inhibitors (PI) or non nucleoside reverse transcriptase inhibitors constitute the Highly Active Antiretroviral Therapy (HAART) regimen, which has been generally taken as a gold standard for management of HIV patient¹⁵. It has been reported that morbidity and mortality associated with HIV infection reduces remarkably with introduction of HAART. Recent report indicated that, as HIV disease progresses, the prevalence and severity of anaemia increase. Anaemia has been shown to be a statistically significant predictor of progression to the acquired immunodeficiency syndrome and is independently associated with an increased risk of death in patients with HIV⁴.

Some antiretroviral (ARV) drugs have been documented to have cytopenic effect, especially when used as a mono-therapy¹⁶. Adverse effects of Lamivudine in combination with Zidovudine include Neutropenia, anaemia, thrombocytopenia and transient rise in liver enzymes¹, while that of Nevirapine as been reported as eosinophilia, granulopenia, and jaundice. Anaemia, neutropenia, thrombocytopenia have also been reported as adverse effects of Stavudine¹¹.

Although the depletion of CD4 T cells remains the most reliable marker for estimating the degree of immuno suppression in HIV-1-infected individuals, several authors have investigated the possible prognostic and predictive role offered by total lymphocyte count (TLC) as a surrogate of the CD4 cell count in resource-limited settings (RLS). TLC offers the advantages of being less expensive and less complicated than the CD4 cell count. In addition, the equipment needed is already available in most RLS. Despite these potential advantages, papers recently published on this issue do not demonstrate that TLC fulfils the rigorous requirements as to sensitivity and specificity, particularly needed when methods already proved to be accurate detectors of immuno suppression (such as CD4 cell count) are available⁹.

As a result of inconsistency observed in the reports on the use of Total Lymphocyte Count (TLC) as a surrogate of CD4 cell count for monitoring HIV-infected patients receiving HAART in resource-limited settings, there is need for more research to ascertain the correlation between the two²³.

This study attempt to evaluate the effect of HAART on some haematological parameters. Also, to study the correlations between the Total Lymphocyte Count (TLC) and the CD4 Cells count, and to see if the TLC with other haematological parameter could be used as an alternative for initiating ARD and monitoring patient on treatment in situation where facilities for CD4 Cells count are not readily available or resources are limited.

2. Materials and Methods

Study area: This study was carried out in the Anti-Retroviral Clinic of State Specialist Hospital, Ikole Ekiti, Ekiti State. Ikole is a local Government Area of Ekiti State, Nigeria. Its headquarters are in the town of Ikole. It has an area of 321 km² and a population of 168,436 at the 2006 census. The hospital is centrally located and serves as a referrer center for many local governments in the State; such as Oye, Ggonyin, Ekiti East, Ilejemeje, Moba, Ekiti West, Ido-Osi, and Ikole Local Governments. It is one of the three hospitals in the State, where facilities for the treatment of HIV patients are available. It is strategically located along Abuja road.

Study population: Eighty clients were recruited into this study (male and female of fifteen years and above). Twenty of the subjects were sero-negative clients, serving as Negative Control. The remaining sixty subjects were with laboratory evidence of HIV-infection and have history of no previous antiretroviral therapy. Eight of these clients were dropped before the end of the study. The consent of the clients was sought for before the recruitment. Permission was taken from the management of the Hospital where the study was carried out.

Haematological Analysis

Five milliliters (5ml) of venous blood was collected from each subject with minimum stasis using vacutainer EDTA bottle and needle. The blood was gently mixed. All blood specimens were collected between 9:00am and 12:00noon and the analysis done within 2 hours. The parameters analyzed include: Haematocrit, Haemoglobin (HGB), White Blood Cells (WBC) count (Differential and Total), Platelets and Film reading (for possible correction).

The samples were analyzed using Sysmex KX-21N Automated Haematology Analyzer (Sysmex Corporation, Kobe, Japan) 19. Blood films were made and stained with Leishman's stain and examined under oil immersion ($\times 100$) microscope lens, for general film reading and for confirmation of the WBC differential count⁵.

Total Lymphocyte Count (TLC) is easily obtained from routine complete blood cell counts by multiplying the percentage of lymphocytes by the WBC count².

$TLC \text{ (cells}/\mu\text{l)} = \text{Total WBC (cells}/\mu\text{l)} \times \% \text{ Lymphocytes}$
CD4 Cell Count

The CD4 Cell Count was obtained using BD FACS Count System²².

The CD4 and the haematological parameters were done as baseline and monitored through a three-monthly evaluation (i.e. at 12th week and 24th week)

3. Results

The results are shown in Table 1 and II. The data were entered in Mean \pm Std. Error of Mean. T-test was used to compare the mean of the study groups and Levene's test for equality of variances.

Table 1 revealed the general pattern of the haematological parameters and CD4 results in HIV sero-negative individuals (HIV Negative Control), HIV positive Clients not on HAART (HIV Positive Control), and HIV positive Clients on HAART (Test Group).

Comparison of means of the test group at: baseline and 3 months, 3 months and 6 months, and, baseline and 6 months are shown in Table II. Positive statistically significant difference were observed in CD4 count, 137.72 18.66, lymphocytes 32.22 2.03, haematocrit 30.38 0.74 and total lymphocyte count 1466.41 107.97 at baseline when compared to the values at 3 months; CD4 count 313.53 35.16, lymphocyte 44.31 2.17, haematocrit 32.5 0.70 and TLC 1999.31 120.69 ($P < 0.05$) whereas negative statistically difference was observed in Neutrophil i.e. Neutrophil 60.19 2.25 (baseline) and Neutrophil 48.91 2.21 (3months). When values gotten at 3 and 6 months were compared, the difference was not statistically significant ($P > 0.05$).

Also, statistically significant difference (increase) were observed in CD4 count 343.94 36.81, lymphocytes 44.28 1.74, haematocrit 32.94 0.60 and TLC 1993.12 127.52 at 6 months when compared with the baseline; CD4 count, 137.72 18.66, lymphocytes 32.22 2.03, haematocrit 30.38 0.74 and total lymphocyte count 1466.41 107.97 ($P < 0.05$), while statistically significant difference (reduction) were observed in Neutrophil 49.69 1.65 and monocytes 3.97 0.26, when compared with the Neutrophil 60.19 2.25 and monocytes 4.84 0.34 at baseline ($P < 0.05$). No statistically significant difference was observed in the other parameters ($P > 0.05$) at this point. There was correlations between the CD4 count and the Total Lymphocyte count at every point of assessment i.e. baseline ($r, 0.632$ and $P < 0.01$), 3 months ($r, 0.730$ and $P < 0.01$), 6 months ($r, 0.697$ and $P < 0.01$) and in the entire test group together ($r, 0.729$ and $P < 0.01$).

Table 1: Showing the general pattern of the haematological parameters and CD4 results (Mean ± SEM) in HIV sero-negative individuals (HIV Negative Control), HIV positive Clients not on HAART (HIV Positive Control), and HIV positive Clients on HAART (Test Group).

		CD4 Cells/ μ l	Platelet Cells/ μ l	TWBC Cells/ μ l	Lymphocyte %	Neutrophil %	Monocyte %	Eosinophil %	Basophil %	Haematocrit %	Haemoglobin g/dl	TLC %cells/ μ l
HIV Sero negative Control Group (Negative Control)	Baseline N=20	942.16 \pm 21.32	1.79(105) \pm 1.3(10 ⁴)	5200 \pm 293.16	46.24 \pm 2.41	49.42 \pm 1.62	2.30 \pm 0.22	2.04 \pm 0.44	0.00 \pm 0.00	39.62 \pm 0.84	11.53 \pm 0.24	2206.24 \pm 162.29
	3 Months N=20	961.04 \pm 17.34	1.92(105) \pm 1.5(10 ⁴)	5472 \pm 304.21	44.16 \pm 3.24	50.26 \pm 2.04	3.87 \pm 0.38	1.71 \pm 0.24	0.00 \pm 0.00	40.21 \pm 0.62	11.81 \pm 0.32	2412.56 \pm 120.44
	6 Month N=20	902.00 \pm 31.04	2.02(105) \pm 1.6(10 ⁴)	5432 \pm 341.05	45.72 \pm 2.46	52.64 \pm 2.41	1.64 \pm 0.21	0.00 \pm 0.00	0.00 \pm 0.00	39.84 \pm 0.72	11.74 \pm 0.42	2476.00 \pm 148.51
HIV Positive Not on HAART (Positive Control)	Baseline N=20	448.44 \pm 91.78	2.30(105) \pm 1.5(10 ⁴)	5294 \pm 362.41	43.44 \pm 3.20	47.56 \pm 2.81	5.39 \pm 0.46	3.72 \pm 0.52	0.00 \pm 0.00	35.39 \pm 0.70	11.54 \pm 0.25	2221.33 \pm 186.27
	3 Months N=20	410.94 \pm 77.05	2.35(105) \pm 1.6(10 ⁴)	5328 \pm 413.72	39.44 \pm 2.61	52.89 \pm 2.52	4.94 \pm 0.34	2.72 \pm 0.39	0.00 \pm 0.00	35.67 \pm 0.82	11.71 \pm 0.29	2057.94 \pm 186.08
	6 Month N=20	400.50 \pm 71.53	2.70(105) \pm 2.1(10 ⁴)	5250 \pm 408.91	37.22 \pm 2.70	53.44 \pm 2.89	5.50 \pm 0.47	3.78 \pm 0.48	0.06 \pm 0.56	35.39 \pm 0.92	11.49 \pm 0.30	1804.59 \pm 202.78
HIV Positive on HAART (Test Group)	Baseline N=32	137.72 \pm 18.66	2.5(105) \pm 2.09(10 ⁴)	4806 \pm 354.40	32.22 \pm 2.03	60.19 \pm 2.25	4.84 \pm 0.34	2.69 \pm 0.30	0.03 \pm 0.31	30.38 \pm 0.74	10.11 \pm 0.36	1466.41 \pm 107.97
	3 Months N=32	313.53 \pm 35.16	2.33(105) \pm 1.85(10 ⁴)	4691 \pm 289.86	44.31 \pm 2.17	48.91 \pm 2.21	4.34 \pm 0.30	2.34 \pm 0.26	0.09 \pm 0.52	32.50 \pm 0.70	10.53 \pm 0.25	1999.31 \pm 120.69
	6 Month N=32	343.94 \pm 36.81	2.34(105) \pm 1.35(10 ⁴)	4538 \pm 257.77	44.28 \pm 1.74	49.69 \pm 1.65	3.97 \pm 0.26	2.03 \pm 0.23	0.03 \pm 0.03	32.94 \pm 0.60	10.77 \pm 0.25	1993.12 \pm 127.52

*95% Confidence Interval of the Difference

Table II: Showing the comparison of Mean ± SEM of the haematological parameters and CD4 results of the HIV positive Clients on HAART (Test Group).

TEST GROUP		CD4 Cells/ μ l	Platelet Cells/ μ l	TWBC Cells/ μ l	Lymphocyte %	Neutrophil %	Monocyte %	Eosinophil %	Basophil %	Haematocrit %	Haemoglobin g/dl	TLC %cells/ μ l
Baseline and 3rd Month	Baseline	137.72 \pm 18.66	2.5(105) \pm 2.09(10 ⁴)	4806 \pm 354.40	32.22 \pm 2.03	60.19 \pm 2.25	4.84 \pm 0.34	2.69 \pm 0.30	0.03 \pm 0.31	30.38 \pm 0.74	10.11 \pm 0.36	1466.41 \pm 107.97
	3 Months	313.53 \pm 35.16	2.33(105) \pm 1.85(10 ⁴)	4691 \pm 289.86	44.31 \pm 2.17	48.91 \pm 2.21	4.34 \pm 0.30	2.34 \pm 0.26	0.09 \pm 0.52	32.50 \pm 0.70	10.53 \pm 0.25	1999.31 \pm 120.69
	t-Values	-4.417	0.613	0.253	-4.069	3.577	1.099	0.861	-1.025	-2.090	-0.978	-3.291
	p-Value	p<0.05	p>0.05	p>0.05	p<0.05	p<0.05	p>0.05	p>0.05	p>0.05	p<0.05	p>0.05	p<0.05
3rd Month and 6th Month	3 Months	313.53 \pm 35.16	2.33(10 ⁵) \pm 1.85(10 ⁴)	4691 \pm 289.86	44.31 \pm 2.17	48.91 \pm 2.21	4.34 \pm 0.30	2.34 \pm 0.26	0.09 \pm 0.52	32.50 \pm 0.70	10.53 \pm 0.25	1999.31 \pm 120.69
	6 Months	343.94 \pm 36.81	2.34(10 ⁵) \pm 1.35(10 ⁴)	4538 \pm 257.77	44.28 \pm 1.74	49.69 \pm 1.65	3.97 \pm 0.26	2.03 \pm 0.23	0.03 \pm 0.03	32.94 \pm 0.60	10.77 \pm 0.25	1993.12 \pm 127.52
	t-Values	-0.597	-0.052	0.395	0.011	-0.283	0.939	0.905	1.025	-0.477	-0.678	0.035
	p-Value	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05
Baseline and 6th Month	Baseline	137.72 \pm 18.66	2.5(10 ⁵) \pm 2.09(10 ⁴)	4806 \pm 354.40	32.22 \pm 2.03	60.19 \pm 2.25	4.84 \pm 0.34	2.69 \pm 0.30	0.03 \pm 0.31	30.38 \pm 0.74	10.11 \pm 0.36	1466.41 \pm 107.97
	6 Months	343.94 \pm 36.81	2.34(10 ⁵) \pm 1.35(10 ⁴)	4538 \pm 257.77	44.28 \pm 1.74	49.69 \pm 1.65	3.97 \pm 0.26	2.03 \pm 0.23	0.03 \pm 0.03	32.94 \pm 0.60	10.77 \pm 0.25	1993.12 \pm 127.52
	t-Values	-4.997	0.639	0.613	-4.520	3.761	2.047	1.735	0.000	-2.700	-1.534	-3.152
	p-Value	p<0.05	p>0.05	p>0.05	p<0.05	p<0.05	p<0.05	p>0.05	p>0.05	p<0.05	p>0.05	p<0.05

*95% Confidence Interval of the Difference

DISCUSSION

In this present study, the average CD4 counts of the test group i. e. HIV positive clients receiving Highly Active Antiretroviral Therapy (HAART) at baseline was lower than that of the control group i. e. HIV positive clients not yet receiving antiretroviral drug. This sharp significant difference observed in the CD4 counts between the control groups and the test group baseline was due to the fact that most of the clients that are initiated on ART have CD4 \leq 250 cells/ μ l while most of the clients in the positive control group still have relatively high CD4 cells count which can still protect them against opportunistic infections. On comparison of the CD4 counts of the two groups at the third month, there was no significant difference. This could be as a result of the positive effect of HAART on the recovery of CD4 cells (Table II).

The significantly low haematocrit and haemoglobin seen in the test group at baseline, 3 months and 6 months when compared to the control group was due to disease progression. This supports the previous reports of anaemia as the most commonly encountered haematological abnormality in HIV positive patients, occurring with increasing frequency as the disease progresses²⁴. Sullivan reported that anaemia is related to disease progression and survival in patients with HIV infection. Also, recovering from anaemia has been linked to improved survival outcomes¹⁸. The low haematocrit and haemoglobin could be as a result of the direct attack of the reticuloendothelial cells by the virus, as reported by some workers¹⁵.

However, on comparison of the results at the baseline with that of after 3 months of receiving HAART, a significant increase was seen in CD4 Counts, Differential and Total Lymphocyte count, and haematocrit with significant reduction in Differential Neutrophil counts. The same pattern of increase was seen between the baseline and at 6 months. Though, no significant increase was observed in these parameters when compared at 3 months with the results at 6 months of HAART, mild increase was observed. These results were contrary to the previous reports of anaemia as adverse effects of Zidovudine and Lamivudine. This might be an improvement over mono-therapy. This findings supports the recent report by Odunkwe et al., that the use of HAART is associated with significant increase in haemoglobin concentration and a decrease in the prevalence of anaemia¹⁵.

This study is not in line with the Mildvan reports that many HIV positive patients receiving HAART still develop mild to moderate anaemia and associated quality of life impairment¹³. A significant correlation was observed in the Total Lymphocyte count and CD4 counts at all points of the investigations. Significant correlation (r , 0.632, $P < 0.01$) was observed at the baseline, (r , 0.730, $P < 0.01$) at 3 months, (r , 0.697, $P < 0.01$) at 6 months and (r , 0.729, $P < 0.01$) for all test groups. This presents TLC as a good CD4 marker and a surrogate marker of the CD4 cell count in HIV infected person being considered for HAART. This result is in line with the report by Kanya et al., in Kampala and, Badri and Wood of South Africa but contrary to that of Giuseppe and his colleagues^{2,9,10}.

In addition to the proposed use of TLC for initiating ART in resource-limited settings in the recent WHO guidelines²⁵, TLC may have a role in inexpensive monitoring of the immunologic response to HAART. In a study from the UK, Beck et al., observed a high correlation (r , 0.76) between TLC and CD4 cell count. They found a consistent significant correlation in: asymptomatic patients (r , 0.64), symptomatic non-AIDS HIV-infected patients (r , 0.72) and AIDS patients (r , 0.73)³. Study by Lai¹², Fournier et al.⁸, and Pulido et al.¹⁷ have also demonstrated significant correlation between TLC and CD4 cell count particularly in patients with CD4 cell count $< 200 \times 10^6/l$.

CONCLUSION

In this study, our findings indicate that HAART results in improved haematologic values of HIV patients. And, in addition to the use of TLC for initiating HAART, as proposed by WHO guidelines, it can also be used as an inexpensive surrogate for monitoring the immunological response to HAART in resource-limited settings. This work is particularly relevance to sub-Saharan African where laboratory infrastructure to perform CD4 cell and viral load measurement is frequently not available and current international initiatives for facilitating access to ART is increasing the number of patients accessing the care. Where equipments are available to carry out CD4 Count and Viral load, reagents are not always available due to poor logistics.

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