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HAEMATOLOGICAL PARAMETERS IN HUMAN IMMUNODEFICIENCY VIRUS POSITIVE INDIVIDUALS ON DIFFERENT HAART REGIMEN.

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ABSTRACT

Highly active antiretroviral viral therapy (HAART), a combination of three antiretrovirals from at least two drug classes for optimization of hindrance to HIV replication has greatly increased life expectance. There however, exist numerous of these combinations and thus the questions of which is the best HAART combination with respect to the individual's haematological status. To investigate this, blood samples were collected from 231 retropositive subjects on six different HAART combinations at least six months after HAART commencement, assayed for CD4 and some haematological parameters using cyflow and sysmex(KX-21) autoanalysier. Baseline data was accessed from the LAMIS data base. The difference between baseline and values after HAART was taken and statistically compared. HAART evaluated includes, Combivir(NVP), Combivir(EFV), Truvada(NVP), Truvada(EFV), Lanten(NVP) and Lanten(EFV). The difference in the parameters assayed are: CD4(cellmm^{-3}): 154, 205, 172, 206, 262, and 230(P=0.478). Haemoglobin(gdl-1): -0.78, -0.73, 2.35, 1.48, 1.11 and 1.27(P=0.010). PCV(%): -2.34, -2.19, 7.65, 7.02, 3.36 and 3.12(P=0.0001). WBC($10^3\mu l^{-1}$): -1.19, -1.02, -0.37, 0.06, 1.14 and -0.63(P=0.001). Neutrophil(%): -1.51, -1.83, 3.87, 2.07, 2.71, 2.71 and 1.97(P=0.868). Lymphocyte(%): -4.26, -2.87, -1.45, -0.19, 2.36 and 3.40(P=0.790). Eosinophil(%): -0.41, 0.14, -2.65, 0.14, -0.46 and 0.12(P=0.094). Platelet($10^3\mu l^{-1}$): -49, -39, -

53, -53, -47 and -35(P=0.931). The Zidovudine based combinations showed anaemic tendencies; Nevirapen based combinations showed Eosinopenic tendencies. All HAART used induced good immunologic responses along with thrombocytopenic tendencies. The data generated was however insufficient to discriminate one combination as being better than the other, rather it was observed that the haematological profile of clients must be well considered when selecting HAART.

KEYWORDS: HIV, anaemia, thrombocytopenia, HAART, haematotoxicity, CD4 cells.

1. INTRODUCTION

Highly active retroviral therapy (HAART) is a cocktail consisting of at least three drugs belonging to at least two types or classes of antiretroviral agents. Typically, these classes are two nucleoside analogue reverse transcriptase inhibitors (NRTIs) plus either a protease inhibitor (PIs) or a non-nucleoside reverses transcriptase inhibitor (NNRTI).[1, 2] Other classes of drugs such as entry inhibitors provide treatment options for patients who are infected with viruses already resistant to common therapies, although they are not widely available and not typically accessible in resource limited settings.[1]

HIV infection is characterized by a progressive damage of the body's immune systemwhich in turn results in a number of opportunistic infection, immunological and haematological complications.[3] The goal of HAART is however, firstly, to suppress HIV viral load in plasma to a level below detection and secondly to restore immune function as demonstrated by an increased number of CD4+ T-cells. This has greatly increased chances of long term survival as it leaves more drugs available to the patient for longer period of time.[4]

Haematological complications have been documented to be the second most common cause of morbidity and mortality in HIV patients[5, 6] and are generally marked with cytopoenias such as anaemia, neutropenia, lymphopenia and thrombocytopenia.[7] The incidence and severity of the cytopenia generally correlate to the stage of the disease with anaemia being the most commonly encountered haematologic abnormality and a significant predictor of progression to AIDS or death.[7, 8]

The role of HAART in improving bone marrow function in HIV-infected individuals remains an area of intense investigation. Prior work has indicated that the primitive hematopoietic progenitor, CFU-GEMM, is not infected by HIV per se,[9] although the function of this cell in

terms of subsequent growth of committed progenitor cells is clearly abnormal.[10] As a result of cell surface interactions with the HIV envelope protein, gp120/160, decreased colony growth of BFU-E, CFU-GM, CFU-G, and CFU-M has all been reported.[10,11] Exposure of CD34+ progenitor cells to HIV may also promote apoptosis in these cells, with marked decrease in colony growth. In addition, the microenvironment of the marrow, which is necessary for normal blood cell growth and development, is also abnormal.

Anemia is very common among HIV-infected individuals, occurring in approximately 30% of patients during the initial asymptomatic period of infection and in over 80% during the course of disease. The prevalence and correlates of anaemia have been studied in several large cohort studies.[12] USDHHS[8] and Gea-Banacloche[13] separately reported improvements in haematocrit and haemoglobin values resulting in reduction in morbidity and mortality of HIV patients and [14] reported no improvement in haematocrit values of HAART treated HIV patients compared with HAART-naïve patients. HAART though effective in improving on haematocrit and decreasing the prevalence of anaemia could lead to a decrease in platelet counts.

In addition to anaemia, patients with HIV infection may also have neutropenia, granulocytopenia and monocytopenia. Thus, abnormal Fc processing by macrophages has been described, while decreased opsonization and intracellular killing of bacterial or fungal organisms by granulocytes has also been reported.[15]

Cytopenia involving more than one haematopoietic linage impairment are common complications of HIV.Bone marrow dysfunction has been suggested as a possible mechanism of thios complication.[16] Some antiretroviral drugs have also been documented to have cytopenic effect especially when use as monotherapy.[17] Some of these complications as attributed to Nevirapine are eosinophilia and granulopenia. Those attributed to Zidovudine and Stavudine include neutropenia, anaemia and thrombocytopenia.

Although haematological abnormalities are common manifestations of HIV infection and AIDS, and may have considerable impact on patients' well-being, treatment and care, few studies on haematological parameters in HIV-infected persons comparing HAART have been undertaken in Nigeria. Such information for HIV-infected adults on HAART may help to inform treatment of HIV-infected individuals in this region.

HIV infected population are prone to liver disease.[18] Initiation of HAART further worsens this due to the toxic effects of antiretroviral therapy. Some of these complications of HAART include hepatotoxicity, hyperlipidemia, hyperglycemia, hypolactatemia, lactic acidosis, lipodystrophy, hepatic steatosis.[19,20] Other complications include neurological complications and organ related malignancies, bone and connective tissue disorders, cardiovascular, renal and hepatic diseases are some of the serious non-AIDS events which are responsible for morbidity and mortality in HAART era.[21,22,23]

Several HAART combinations are now being used in Nigeria hence it becomes imperative to study their effects on haematological parameters and compare the performance of each combination with regards to haematological parameters.

2. METHOD

Study design

This study is a cross sectional study, participants were confirmed cases retro positive clients attending Heart to Heart Center, General Hospital Calabar. HIV status was confirmed using the HIV serial testing algorithm.[24] All subjects satisfied the conditions of being non-pregnant, with records of CD4 cell count and Haematological parameter assessment pre-HAART commencement, commencement of HAART at least six months and maintaining a single HAART regimen which must also be the first ever used. In addition, candidates were not on any anti-tuberculosis therapy and must be on one of the ART combination of interest. The pre-HAART CD4 count, haematological parameter assay result and HAART regimen details were collected from the LAMIS data base of the facility. Two hundred and thirty one (231) blood samples were collected from Retro positive clients that have been on the following HAART regimen for at least six months. Ethical approval to access baseline data of freely consenting client's as they come for their periodic checkup was sort from the facility.

Antiretroviral therapy combinations of used in this study

	NAME OF HAART	Components		
1	**COMBIVIR(NVP)**	Zidovudine	Lamivudine	Nevirapine
2	**COMBIVIR(EFV)**	Zidovudine	Lamivudine	Efavirenz
3	**TRUVADA(NVP)**	Tenofovir	Emtricitabine	Nevirapine
4	**TRUVADA(EFV)**	Tenofovir	Emtricitabine	Efavirenz
5	**LANTEN(NVP)**	Tenofovir	Lamivudine	Nevirapine
6	**LANTEN(EFV)**	Tenofovir	Lamivudine	Efavirenz

Sample Collection

2ml of venous blood was collected from each patient into a vacutainer containing EDTA, following standard phlebotomy procedure six month after HAART initiation.

Data Collection

The pre-HAART (baseline) of CD4 count, haematological parameter assay result and HAART regimen details were collected from the LAMIS data base of the Heart to Heart center.

Sample analysis

CD4 count was carried out by Flow Cytometric method using the automated PARTEC Cyflow machine (version 2.4), haematological parameters were analyzed using the SYSMEX KX-21 automated haemoanalyser machine.[25]

Data obtained were collected was analyzed with PASW (version 18) statistical package.

Statistical analysis: One way ANOVA was carried out on data collected.

3. RESULTS

Amongst the clients sampled 68.8%(157) were placed on Combivir(NVP), 15.7%(37) on Combivir(EFV), 5.1%(12) on Truvada(NVP), 5.5%(13) on Truvada(EFV), 3.4%(8) on Lanten(NVP) and 3.0%(7) on Lanten(EFV).

The mean values and standard errors of mean plot for CD4 count and other haematological parameters as presented in figure 1.

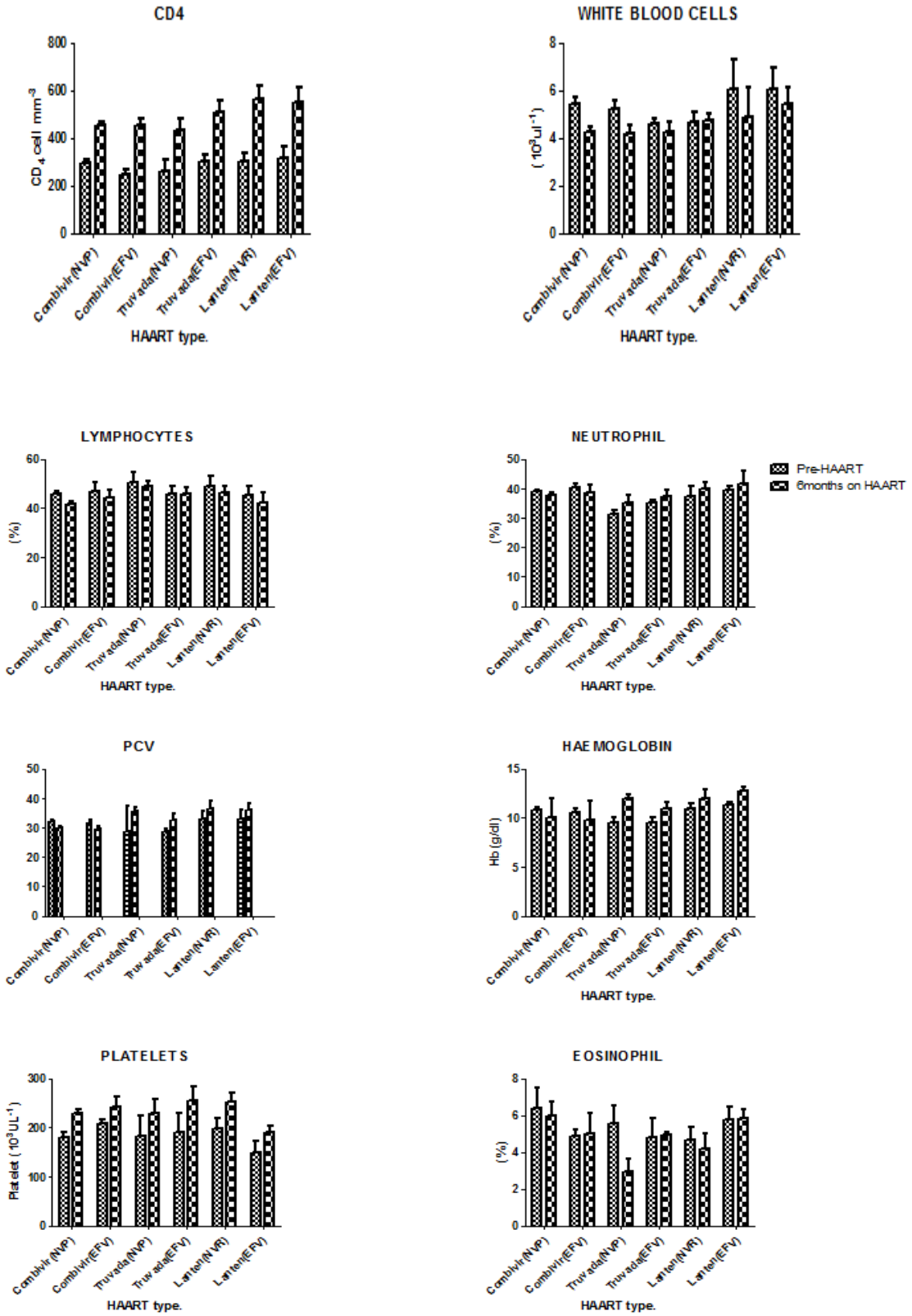

Figure 1. Shows CD4 count, WBC, Lymphocytes, Neutrophils, PCV, HGB, Platelet count, Eosinophils pre and 6months post commencement of HAARTS.

Table 1: Difference in mean values of Pre-HAART (baseline), six months Post-HAART treatment and P-value at 95% confidence limit.

	COMBIVIR (NVP)	COMBIVIR (EFV)	TRUVADA (NVP)	TRUVADA (EFV)	LANTE N (NVP)	LANTEN (EFV)	P-VALVE (95%confidence limit)
CD4 (cell mm-3)	154	205	172	206	262	230	0.478
HAEMOGLOBIN (g/dl)	-0.78	-0.73	2.35	1.48	1.11	1.27	0.01*
PCV (%)	-2.34	-2.19	7.05	7.02	3.36	3.12	0.001*
WBC ($10^3\mu L^{-1}$)	-1.19	-1.02	-0.37	-0.06	-1.14	-0.63	0.01*
NEUTROPHIL (%)	-1.51	-1.83	3.87	2.07	2.71	1.97	0.868
LYMPHOCYTE (%)	-4.26	-2.87	-1.45	-0.19	2.36	3.4	0.79
PLATELET ($10^3\mu L^{-1}$)	-49	-39	-53	-53	-47	-35	0.931
EOSINOPHIL (%)	-0.41	0.14	-2.65	0.14	-0.46	0.12	0.094

- PVC=Packed Cell Volume.
- WBC= White Blood Cell.
- * = statistically significant

4. DISCUSSION

In this study, increases in CD4 cell count ranging from 154cellmm^{-3} to 262 cellmm^{-3} were recorded, indicating a promising life expectancy as the immune cells which are the major target of the virus appreciated in number. The variations in increases of CD4 counts between the groups was not statistically significant ($p>0.05$). This observation is supported by the report of Nacoulma *et al.*,[26] who reported an immunological efficacy of 119 cellmm^{-3} at the end of the firstsix months of HAART.

The mean values of packed cell volume and haemoglobin estimation decreased in clients on Combivir(NVP) and Combivir(EFV). Incidentally both drugs are zidovudine based drugs when compared with their various baseline values. Those on drugs devoid of zidovudine showed an appreciation ($P<0.05$) in the mean haemoglobin and packed cell volume values. This findings well agrees with the report of Mildvan *et al.*,[27] who reported an association of anaemia, neutropenia and leucopenia with zidovudine based HAART. This is as a result of haematological toxicity caused by inhibition of haematological progenitor cells, resulting in myelosuppression.[28]

Ayer *et al.*,[29] in their report compared HAART naïve clients and those basically on zidovudine based HAART regimen and proposed that the HAART naïve clients have a five times tendency of developing anaemia than those on zidovudine based HAART. Ippoliton and Puro[30] demonstrated the reversibility of zidovudine induced haematological toxicity in experimental animals. A similar study in uninfected health workers by Owiredu *et al.*,[31] showed that the anaemia is reversible.

The mean neutrophil count reduced in the groups treated with Combivir(NVP) and Combivir(EFV) i.e. zidovudine based drugs. In contrast, there was increased neutrophil count in the group given Truvada(NVP), Truvada(EFV), Lanten(NVP) and Lanten(EFV). The latter four are tenofovir based and the observed increases is supported by the findings of Owiredu *et al.*,[31] who reported an increase in haemoglobin and Neutrophil count after a switch from HAART regimen containing zidovudine/lamivudine (combivir) to those containing tenofovir. There was however, no statistically significant difference ($P>0.05$) in the Neutrophil count obtained from various HAART combinations.

A general trend of reduction ($P>0.05$) in platelet count throughout the various HAART combination groups was noted. This is indicative of the tendency of HAART to potentiate thrombocytopenia. Yakubu *et al.*,[19] reported a little increase in platelet count in clients on zidovudine based HAART which include Combivir(NVP) and Combivir(EFV). Racial differences in the study population may explain the differences in the observations. There could also be a counter balancing struggle between the haemopoiesis depressing effect HIV of on the platelets and attempt of HAART to ameliorate those effects.

USDHHS[8] and Odunukwe *et al.*,[19] in separate study reported an association of eosinopenia with Nevirapin based HAART, this is in total agreement with our findings as it was observed that there were decreases ($P>0.05$) in the eosinophil count of subjects on Combivir(NVP), Truvada(NVP) and Lanten(NVP) which are all Nevirapin based.

It was observed that zidovudine based HAART has many side effects on the haematological parameters but promotes life expectancy as evidenced by the increase in CD4+ counts. This underlines the importance of haematological monitoring of individuals placed on a zidovudine based HAART regimen.

In studying effects of HAART on haematological parameters, if the difference in handling HAART in non-infected experimental subject and infected subject will most likely present different scenario because HIV in itself has a great damaging effect on haematological parameters and HAART would have a lot of work to do to ameliorate these effects as against non-infected subjects which may have every condition been normal.

5. CONCLUSION

To a large extent, it was observed that zidovudine based HAART has many side effects on the haematological parameters. It however has a lot of advantages also in its promotion of life expectancy. It thus makes it very difficult to discard these drugs as it is also a fact that there is no one drug without side effects. Other drugs indeed may not have showed too many side effects on the haematological parameters as zidovudine as done but there are many physiological and chemical side effects some of which may be irreversible reported by other literatures. It however becomes very critical that proper haematological investigations and considerations be made before and during zidovudine based HAART therapy.

Many recent studies have been done and are still on going on the use of some haematological investigations and parameters as a surrogate for some more complex and expensive investigations required for the monitoring of response of clients on HAART in rural areas. It however becomes pertinent to give some more considerations to likely variations that are likely to occur due to various HAART combinations.

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