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ASSESSMENT OF SOME HAEMATOLOGICAL AND IMMUNOLOGICAL PARAMETERS IN HIV-INFECTED PATIENTS ON HAART AND HAART naïve IN EKITI STATE

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ABSTRACT

The aim of this study is to evaluate the haematological and immunological outcomes in HIV infected patients on HAART and HAART naïve in Ekiti State. The Human Immunodeficiency Virus (HIV) infection causes the Acquired Immunodeficiency Syndrome (AIDS), which is a systemic disorder characterized by severe impairment and progressive damage of both cellular and humoral immune responses. Haematological abnormalities are the strong independent predictors of morbidity and mortality in HIV-infected individuals. About 400 HIV infected participants were recruited for this study, 200 were HIV infected patients on antiretroviral therapy while another 200 HIV infected patients were not on antiretroviral therapy. 4ml of blood samples were collected into K_2 EDTA bottles for the analysis of haematological and immunological parameters. Ethical clearance and Permission for the conduct of the study was obtained from the Ethical Committee, Federal Teaching Hospital, Ido-Ekiti. Mean$\pm$ SD of CD4, WBC, PCV, PLT, and LYM for patients on antiretroviral therapy (ART) were significantly ($p<0.05$) higher compared to patients that were not on antiretroviral therapy. This study provides information about the effectiveness of antiretroviral drugs therapy among HIV positive patients in this part of world despite the reported side effects.

Keywords: Antiretroviral therapy, HIV, Haematological and Immunological

Abbreviations: PCV= Packed cell volume, PLT= Platelets, WBC= White cell count, LYM= Lymphocytes, NEU= Neutrophlis, MXD= Mixed, SD= Standard deviation, HAART= Highly active antiretroviral therapy

1. INTRODUCTION

The introduction of highly active antiretroviral therapy (HAART) in developed countries in the late 90s has been associated with a remarkable decrease in AIDS-related mortality. This decrease in mortality has changed the perspective of HIV infection from that of a rapidly fatal to a chronic manageable infection [1] Clinical benefits of HAART are due to its effectiveness in decreasing disease progression in HIV infected patients by sustained suppression of viral replication [2].

The Human Immunodeficiency Virus (HIV) infection causes the Acquired Immunodeficiency Syndrome (AIDS), Which is a systemic disorder characterized by severe impairment and progressive damage of both cellular and humoral immune responses [1,2]. Besides immunological and infectious complications of HIV disease, blood cell abnormalities have been reported in HIV infection, of which anaemia and neutropenia are reportedly the most common [3,4,7]. These haematological abnormalities are the strong independent predictors of morbidity and mortality in HIV-infected individuals although it is not part of the criteria for initiating therapy nor used by the World Health Organization (WHO) for staging HIV, peripheral blood cell abnormalities in an abnormal hemogram are important prognostic tools for morbidity in HIV infection and AIDS [1,3,5,6,7].

Anaemia and neutropenia are generally caused by inadequate blood cell production because of bone marrow suppression by HIV infection mediated by abnormal cytokine expression and alteration of the bone marrow microenvironment [5,8-11]. HIV replicates not only in CD4 lymphocyte cells, but also in macrophages and dendritic cells. Such replication is followed by immune system depression, which can lead to life threatening opportunistic infections [1,2,10]. Mild-to-severe anaemia in HIV-infected patients is associated with CD4 cell depletion and progression to AIDS and is one of the strongest predictors of poor responses to antiretroviral therapy (ART) and HIV-related mortality [6,8,9,10,11].

Neutropenia is frequently observed in advanced stages of HIV infection after development of AIDS, and has been associated with certain types of antiretroviral medications used to treat HIV infection [1,6,8,12,]. Thrombocytopenia is characterized by low platelet counts below 125×103/mm3, and also frequently occurs in HIV-infected patients. Thrombocytopenia is usually caused by immune-mediated destruction of platelets, in addition to inadequate production [13-15]. Other causes of cytopenias in HIV infected patients include treatment-related adverse events, opportunistic infections, malignancies, drugs (zidovudine and cotrimoxazole) and other pre-existing or co-existing medical problems [3,4].

Haematological parameters mainly anaemia and leukopaenia in HIV-infected ART-naïve patients result in poor ART-treatment outcome and otherwise strongly predict mortality, although haematological abnormalities are common manifestations of HIV infection and AIDS, and may have considerable impact on patients' well-being, treatment and care [1]. Antiretroviral drugs are medication for the treatment of infection by retro viruses primarily HIV which act at different stages of HIV cycle, combination of antiretroviral drugs create multiple obstacles to HIV replication to keep the number of offspring low and reduce the possibility of a superior mutation arising which convey resistance to one of the drug being taken, while other drug continue to suppress reproduction of that mutation. Antiretroviral therapy has benefitted greatly in marked reductions in morbidity and mortality in HIV infected patients [7,11,13].

This contributes to knowledge on the treatment of HIV-infected individuals because antiretroviral regimens containing zidovudine should not be used by patients having severe anaemia or pancytopenia. Therefore, the aim of this study is to evaluate the haematological

and immunological outcomes in HIV infected patients on HAART and HAART naïve in Ekiti State.

2. MATERIALS AND METHODS

2.1 Study design

About 400 HIV infected participants were recruited for the study, 200 were on HAART and 200 were on HAART naïve. 4ml of blood were collected into di-potassium ethylenediaminetetracetic acid (K_2 EDTA) vaccutainer bottles for the analysis of haematological and immunological (CD4) parameters. The study is a cross-sectional study, Consenting HIV infected individuals who are on HAART and HAART naïve were recruited into the study for those who attended HIV clinic at Federal Teaching Hospital, Ido-Ekiti, Nigeria (FTH) between August 2013 and February 2014 were enrolled for the study.

2.2 Ethical considerations

Ethical clearance and Permission for the conduct of the study was obtained from the Ethical Committee, Federal Teaching Hospital, Ido-Ekiti. After informing study participants of the objectives of the study and assuring them of confidentiality of their data, written informed consent was taken from all the participants.

2.3 Haematological Parameters Analysised Using Haematology Analyser (Sysmex Automated Haematology Analyser Model KX-21N, Maunfactured By Sysmex Co-Operation Kobe, Japan)

Three parts differential haematology analyser was used which consist of lymphocyte count (LYM), neutrophil count (NEU), sum of eosinophil, basophil and monocyte as mixed (MXD).

a) Principle

The aspirated blood sample is measured to a predetermined volume diluted at the specified ratio and then fed into each transducer chamber, which has a minute hole aperture and also contains electrodes through which direct current flows. Blood cells suspended in the diluents sample, pass through the aperture, causing direct current resistance to change between the electrodes, blood cell size is detected by electric pulses. Blood cell count is calculated by counting the pulses and the histogram determined by the pulse sizes [16].

b) Procedure

Sysmex machine was inspected (for instrument, reagents, waste bin and printer paper) before switch on the machine from power source, machine was calibrated before used and control sample was run along each batches of sample analysis. Well mixed EDTA blood sample was used for the analysis of complete blood count, blood sample was aspirated through the sample probe one after another by pressing start switch, sample was analyzed, rinsed and display the result on the LCD screen of the machine also printed the results out. After the analysis, machine was shut down by aspirating cell clean which washed and rinsed the machine before finally shutdown and switch off from the power source [16].

CD4 Count was analyzed using flow cytometry (Cyflow counter)
Calibration of Cyflow counter

Research samples for CD4 count were prepared and run on the Partec cyflow counter (Partec flow cytometer, GMBH, Munster, Germany) according to the manufacturer's instructions. Partec flow cytometer (Cyflow counter) was first calibrated to ascertain optimal equipment performance by using count check beads of already known concentration following daily cleaning procedure. Samples from normal subjects were tested along with research samples to ensure reagent control and quality of results. A well calibrated cyflow counter must give count check beads reagent control within ±10% of reagent concentration. CD4 monoclonal antibodies were used within the expiry dates. Values within ±10% of known results validated the potency of the CD4 monoclonal antibodies used for our research procedure [17].

(i) Cyflow counter count check beads calculation

A specific count check bead used during analysis of our research samples had known concentration of 23,470 cells/ml. The equipment displayed absolute CD4 count value of the count check bead as 966 cells/µl, and the pre-set dilution factor is 42, then calculated concentration of the count check beads in cells/ml from the flow cytometer. Since the calculated value of count check beads concentration fell within -10% of known value, the equipment was successfully calibrated [17].

(ii) Principle and procedure of flow cytometry for CD4 count

The cyflow counter operation is based on the simultaneous measurement of multiple physical characteristics of CD4 count in a single file as it flows through the cyflow counter. The counter separated the CD4+ T cell from the monocytes- CD4 bearing cells and noise using a gating system. We prepared the samples and analysed them for CD4 count according to the manufacturer instructions. 20 µl of well-mixed whole blood sample was added to 20 µl of CD4 MAB (monoclonal antibody) in a Rhören tube. This was incubated for 15 minutes in the dark. 800µl of CD4 no-lyse buffer was added (carefully without introducing bubbles) and the mixture was analyzed on Partec cyflow counter and results recorded in cells/µl [17].

(c) Statistical Analysis

Results obtained were analyzed using student t-test to compare the means. Analysis was performed using computer database software from the statistical package for social sciences (version 16.0 SPSS). A P-value of < 0.05 was considered statistically significant in all clinical comparisons at 95% confidence interval.

3. RESULTS

Mean± SD of CD4, WBC, PCV, PLT, and LYM for patients on antiretroviral therapy (ART) were significantly ($p<0.05$) higher compared to patients that were not on antiretroviral therapy, however Mean±SD of NEU and MXD were significantly ($p<0.05$) lower in patient on antiretroviral therapy compared to patients that were not on antiretroviral therapy. Hence, t – value obtained also confirmed the significant difference in the parameters as showed in table 1 and 2.

TABLE: 1 Mean± SD OF SOME HAEMATOLOGICAL AND IMMUNOLOGICAL PARAMETERS FOR PATIENTS ON HAART naïve

Parameters	Mean+ SD	t-value	p-value
CD4(cells/µL)	152.72+177.22	12.19	0.00
WBC(109/L)	4.80+2.63	25.86	0.00
PCV(%)	32.24+6.29	27.47	0.00
PLT(109/L)	164.29 +150.38	15.45	0.00
LYM(%)	37.06 +14.57	35.98	0.00
NEU(%)	51.52 + 15.49	46.84	0.00
MXD(%)	11.18 + 7.37	21.06	0.00

N.B: One sample t-test: P<0.05 significance, P>0.05 no Significant

TABLE: 2 Mean± SD OF SOME HAEMATOLOGICAL AND IMMUNOLOGICAL PARAMETERS FOR PATIENTS HAART

Parameters	Mean+ SD	t-value	p-value
CD4(cells/µL)	543.73+295.79	25.99	0.00
WBC(109/L)	5.08+1.85	38.76	0.00
PCV(%)	35.37+5.35	93.54	0.00
PLT(109/L)	251.91 +73.87	48.23	0.00
LYM(%)	46.73 +12.25	53.97	0.00
NEU(%)	41.53 + 12.87	45.65	0.00
MXD(%)	11.85 + 8.48	19.25	0.00

4. DISCUSSION

Patients with HIV /AIDS are reported to experience a wide range of haematological and immunological complications which have been found to be the most common cause of mortality/ morbidity in HIV infected patients. However, antiretroviral therapy has been acknowledged as a good remedy to the threat of HIV/AIDS infection in the society. Based on this present study, it was observed that antiretroviral therapy aid the healthy living of patients living with HIV/AIDS. Similar to this study, Bamlaku et al [18] reported that mean ± SD WBC, PCV, PLT and CD4 were 5.2 ± 1.9 ×103/µl, 41.4 ± 4.4 , 258.6 ± 82.9 and 415.4 ± 218.8 cells respectively in patients on HAART and 6.3 ± 2.3 × 103/µl, 40.4 ± 6.4, 253.1 ± 95.2 and 361.1 ± 224.4 cells respectively for HAART naïve patients.

This present study shows that prevalence of anemia is higher in treatment naïve patients compared to patients on treatment. This is consistent with previous study done by Owiredu [19] and Mildvan [20]. The findings of this study affirm that hematological disorders are corrected by combination antiretroviral therapy which also decreases the viral load. Thus HIV patients who were on HAART had greater numbers of blood cells within six months of beginning treatment and hematological disorders were corrected [21] This present study shows that Patients on HAART were statistically significant increase in leucopenia (WBC) and neutropenia compared to their HAART-naïve counterparts. Similarly when patient's CD4 count decreases prevalence of leucopenia and lymphopenia increases as we observed in this study. This may be due to suppression of bone marrow and direct infection of T cells. Having CD4 count less than 200cell/µl was higher in HAART naïve patients than those on HAART.

This condition reduces the body's resistance to many opportunistic infections and the patient becomes more susceptible to bacterial infections and needs medical attention, the condition may become life-threatening due to low immunity. This may be caused by direct and indirect effect of HIV infection (viral load), opportunistic infections, and toxicity of the

drugs [22,23]. This study shows that, CD4 counts of patients on HAART were significantly higher compared to HAART-naïve, this study support the fact that HAART resulted in the significant increase in CD4 T cell counts in majority of patients. This possibly was due to suppression of plasma HIV RNA [24]. Degree of thrombocytopenia in this study was higher in HAART naïve patients than those on HAART. It was reported that highly active antiretroviral therapy results in a sustained increase in the platelet count in HIV-infected patients with thrombocytopenia.

Thrombocytopenia increases as CD4 decreases, this possible cause of thrombocytopenia may be due to immune destruction of platelets. It is known that many chronic human diseases may have an underlying autoimmune mechanism. However, thrombocytopenia probably increases as immunological incompetence worsens thus leading to increased risk of excessive bleeding [25,26].

5. CONCLUSION AND RECOMMENDATIONS

This study provides information about the effectiveness of antiretroviral drugs therapy among HIV positive patients in this part of world despite the reported side effects. This study also serve as supporting information for health workers to emphasize on the use of Antiretroviral drugs (ARVDs) in their campaign, for HIV management and encourage the HIV patients in making themselves available to be treated especially at the early stage and to ignore the experienced side effects.

We recommend that drugs like zidovudine and cotrimoxazole which are myelotoxic should not be administered to patients with severe cytopenias, thus all HIV patients, especially those with advanced disease, should systematically do a full blood count before initiation of antiretroviral therapy.

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