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Some haematological profile of HIV/AIDS patients on Highly Active Antiretroviral Therapy (HAART) in Usmanu Danfodiyo University Teaching Hospital Sokoto, North Western Nigeria

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Abstract
Treatment of HIV/AIDS patients, with HAART is the common practice in most of our health institutions, and the effect of this drug on anemia, leucopenia and thrombocytopenia in this group of patients has not been fully studied in our locality. With the wide spread side effect associated with HAART drugs, there is need to evaluate the role of HAART as it affect hematological indices. Using haematocrit centrifuge method, Burker and haemocytometer method, Packed cell volume (PCV), Total leucocyte count (TLC) and platelet count (PLC) were evaluated and the values obtained in HIV/AIDS patients not on HAART and HIV/AIDS patients on HAART were compared with apparently healthy controls. The result indicated that the mean PCV values of 38.300±4.547%, 32.467±7.133% and 34.263±7.668%, TLC, 6.360±1.567×109/L, 3.423±1.657×109/L and 3.501±1.132 ×109/L, PLC, 253.33±65.090 ×109/L, 207.37±74.65×109/L and 216.95±73.92×109/L in controls, HIV/AIDS patients not on HAART and in HIV patients on HAART treatment respectively. There were statistically significant difference ($P< 0.05$), between HIV patients on HAART and the controls. However there were no statistically significant difference ($P>0.05$) between the HIV positive on HAART and HIV positive not on HAART. However, the report from the laboratory analysis of the blood sample showed that HAART therapy increased PCV, TLC, and PLC cell count of the subjects. Therefore, we concluded that, HAART treatment from these studies has the capability of reducing the incidence of anemia, lymphopoenia and thrombocytopenia which are associated with HIV/AIDS disease progression and death in infected patients. Total lymphocyte count, Packed Cell Volume and platelet count could also serve as useful predictive tools in the management and monitoring of HIV/AIDS infected patients in resource limited settings.

1. Introduction

Acquired immune deficiency syndrome (AIDS) is caused by the human

immunodeficiency virus (HIV) and It can be contracted through sexual contact, exposure to blood/product, sharing of contaminated needle and syringe and by certain blood product of body fluid. Clinical presentation include pneumonia, fever, pyrexia, loss of vision, chronic diarrhea, weight loss, lymphadenopathy, cough itch maculopapular, generalized skin rash, blue discoloration, anemia and hairy leukoplakia [1,2,3] and is characterized by progressive damage to the body's immune system which results in a number of opportunistic infections, immunological and haematological complications [4].

Infection with HIV has been associated with a broad range of clinical outcomes involving the hematopoietic system. 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, neutropoenia, lymphopoenia and thrombocytopenia [7].

The incidence and severity of the cytopoenia 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 [8, 9].

Antiretroviral drugs Highly Active Antiretroviral Therapy drugs (HAART) are medication for the treatment of infection by retroviruses primarily HIV. Different classes of HAART act at different stages of HIV cycle [10].

The introduction of highly active antiretroviral therapy (HAART) for treatment of HIV infection has generally been accepted as the gold standard in the management of HIV/AIDS patients [9, 11], with reported improvements in haematocrit and haemoglobin values resulting in reduction in morbidity and mortality of HIV patients. However, Omoregie and coworkers reported no improvement in haematocrit values of HIV/AIDS patients treated with HAART when compared with HAART-naïve patients in his study [12]. They further noted that, the use of three or four combination different types of antiretroviral therapy drugs are known as Highly Active Anti Retroviral Therapy (HAART) as supported by other researchers may create multiple obstacles to HIV replication and keep the number of offspring low and reduce the possibility of a superior mutation which convey resistance to one of the drugs being taken [13]. This allows other drug in the combination to continue to suppress and prevent mutation of the virus with rare exceptional cases; no individual on HAART has been demonstrated to suppress HIV infection for long. Thus HAART drugs must be taken in combination in order to have an effective and lasting effect [14].

Limitation of Anti-Retroviral Therapy (ART) including HAART to HIV infection is evident and restricts patient with the disease to limited option. A large combination of HAART known as mega-HAART or salvage therapy often increase the drug side effect and treatment list [15].

However, a Week after administration of highly active anti-retroviral therapy there were steep decline in plasma HIV RNA with coincidental abrupt rise in CD4+ T-cell circulating in the blood indicative of the suppressive effect of the drug on virus replication and boosting of blood immune response [16]. This creates therapeutic perturbation of a steady-state relationship and dynamic equilibrium between viruses-medicated cell killing and endogenous production of uninfected CD4+ T lymphocytes [16].

Some workers concluded that, decreased viral killing quickly top the balance in favor of lymphocytes production which allow at least a partial immune reconstitution to occur [17].

Thrombocytopenia is a common hematologic disorder in persons infected with the human immunodeficiency virus (HIV) [18]. Although often asymptomatic, the thrombocytopenia in these patients may be associated with a variety of bleeding abnormalities. Treatment with corticosteroids, intravenous immune globulin, and interferon can improve HIV-associated thrombocytopenia, though with attending relapse after the cessation of treatment [19]. Several studies have indicated an increase in platelet counts in HIV-infected patients with thrombocytopenia treated with zidovudine [18, 19, 20]. In contrast however, many other workers reported that the adult immune system might not have capacity for the rapid CD4+T cell regeneration proposed, and that evidence are lack for heightened CD4+T cell turnover in HIV-infected individual. The observed increase are in lymphoid subset is in blood rather than a specific increase in CD4+ cell that are the target of HIV infection [21, 22, 23].

However, it is against these background of such conflicting reports which cast doubts on the efficacy and the need for HAART in this HAART era in our locality. Therefore, this study was conducted to assess the impact of HAART in resolving haematological complications in HIV patients by comparatively analyzing the results (haematologically) of HIV/AIDS patient on HAART and those not on HAART so as to establish the continual need of HAART in the management and treatment of HIV/AIDS patients in our community.

The present study was also conducted to investigate the observed trend in the Hematological parameters in UDUTH, Sokoto, Nigeria, since available information on antiretroviral drugs for HIV/AIDS patient has not been established in this region. The few available data focus on the side effects of HAART in these regions. This study however will further provide information about the effectiveness of antiretroviral drugs therapy among HIV/AIDS positive patient in this part of country, Nigeria despite the reported side effects [24]. This will also serve as supporting information for health workers to emphasize the use of HAART in their campaign, for HIV/AIDS management and encourage the HIV patients in making themselves available for treatment especially at the early stage and to ignore the experienced side effects.

From the above studies, it becomes evident that, data coming from various laboratories on the side effect of antiretroviral therapy on haematological indices varied [25], suggestive of the need for further research in this area of

antiretroviral treatment in every locality. The current study was therefore designed to evaluate the side effects of HAART treatment on PCV, TLC and PLC on HIV/AIDS patient attending UDUTH, North Western of Nigeria.

2. Materials and Methods

2.1. Study Population

The study subjects consisted of the HIV/AIDS patients attending HIV/AIDS Clinic of Usmanu Danfodiyo University Teaching, Hospital, Sokoto North Western Nigeria. A cross-sectional study was conducted at the ART Clinic of UDUTH, from February to May, 2012 (six months). A total of 170 subjects were recruited in this study. These consisted of 100 HIV/AIDS patients on HAART, 30 are HIV/AIDS patients not on HAART and 40 age- and sex-matched apparently healthy individuals as controls. Ethical clearance for the study was obtained from the Ethics and Research Committee of Usmanu Danfodiyo University Teaching, Hospital, Sokoto. Informed consent for the study was sought from each subject before commencement of the study. Agreed inclusion/ exclusion criteria were strictly followed by the research team.

2.2. Sample Collection

Data on the socio-demographic and clinical characteristics of the study participants were collected using a pre-tested structured questionnaire by interview and review of medical history. Five milliliter syringe was used to draw blood from the anti-cubital vein of each subject. The blood was dispensed into a tube containing ethylenediamine tetra-acetic acid (EDTA) and was used immediately for haematological profile.

2.3. Haematological Study

Haematological parameters were measured using manual standard operative procedures to obtain the packed cell volume (PCV), total white blood cell count and platelet count. HIV status and of all HIV/AIDS positive patients were confirmed using STAT- PAK HIV 1&2 assay test kit. CHEMBIO method.

2.4. Determination of Packed Cell Volume Using WHO recommended method 2000

Packed cell volume was determined using a method recommended by (WHO, 2000) [41]. In this method, blood sample collected was gently mixed by simple inversion drawn into capillary tube sealed and placed in a haematocrit centrifuge. Centrifugation was done at approximately 12000g for 5 minutes. PCV value was determined using haematocrit-reader, using the following formulae:

$$\text{PCV}(\%) = \frac{\text{Height of red cell column}}{\text{Height of total blood column}} \times 100$$

2.5. Total Leucocytes Count

Total leucocytes were enumerated using the Burker method:

Bürker double ruling, with or without spring clips The ruling shows 9 large squares of 1 mm2 each. These are used for counting leucocytes. Each large square is subdivided by double lines (0.05 mm apart) into 16 group squares with 0.2 mm sides. The group squares correspond in size to the Neubauer counting chamber, but have no further subdivisions. The double lines form mini squares with an area of 0.0025 mm2. [42]

2.6. Platelet Count

This was done using Haemocytometer method:

Platelets were enumerated using Haemocytometer method, recommended by Becton-Dickinson. Unopette WBC/Platelet determination for manual methods. [43]

2.7. HIV Screening

The HIV screening was carried out using the (WHO, 2010) screening criteria for developing countries. Initial HIV screening was carried out using Determine HIV 1 & 2 test kit (100% sensitivity) manufactured by Abbort Laboratories, Japan. All the positive cases from the initial screening were confirmed using STAT- PAK HIV 1&2 assay test kit(98% specificity) manufactured by Chembio Diagnostic System Inc, USA.

2.8. Statistical Analysis

All statistical analyses were carried out using GraphPad Prism version 5.0 for windows (GraphPad software, San Diego California USA). Results are presented as Means ± SEM. Unpaired t-test was used to compare the means of all continuous variables. Categorical data were analyzed using Fisher's exact test or x2 for trend. Linear regression was used to test for the degree of association between test parameters. A P-value of less than or equal to 0.05 ($P<0.05$) was considered to be statistically significant.

3. Result

3.1. Baseline Characteristics of the Study Population

A total of 170 subjects were included in the study and were categorized in to three groups: group I (n=100): HIV/AIDS patients on HAART; group II (n=30): HIV/AIDS patients not on HAART and group III (n=40): HIV negative controls. Out of the 150 HIV/AIDS patients studied, 80 (47.0%) were females (50 on HAART and 30 not on HAART) and 50 (29.6%) were males (30 on HAART and 20 not on HAART). The overall mean age was 34 ± 9.2 years (age range: 16 – 65 years). Majority of the patients, 99 (58.2%), were within 26 – 56 years of age (Table 1).

Table 1. *Baseline Characteristics in HIV/AIDS patients at UDUTHS (from February – May 2012)*

Variables	Group I (HIV/AIDS+HAART) (n = 80)	Group II (HIV/AIDS-HAART) (n = 50)	Group III (Controls) (n = 40)	Total Subjects (n = 170)
Age (years)				
>26	18(22.5%)	11(22.0%)	9(22.5%)	29(17.0%)
26-35 years	42(52.5%)	11(22.0%)	13(32.5%)	53(31.1%)
36-45 years	25(31.2%)	7(14.0%)	13(32.5%)	32(18.8%)
46-55 years	13(16.3%)	1(2.0%)	4(10.0%)	14(8.2%)
<56	2(2.5%)	0(0.0%)	1(2.5%)	2(1.7%)
Gender				
Male	30(37.5%)	20(40.0)%	15(37.5%)	50 (29.6%)
Female	50(62.5%)	30(60.0)%	25(62.5%)	80 (47.0%)

3.2. Haematological Parameters of HIV/AIDS Patients on HAART

The mean TLC, PCV and PLT were 3.501±1.132 (cells×109)/L, 34.263±7.668% and 216.95±73.92 (cells×109)/L respectively in HIV/AIDS patients on HAART; 3.423±1.657 (cells×109)/L, 32.467±7.133% and 207.37±74.65 (cells×109)/L cells respectively for HIV/AIDS patients not on HAART; 6.360±1.567 (cells×109)/L, 38.300±4.547% and 253.33±65.090 (cells×109)/L respectively for HIV-negative controls. There were statistically significant differences between the controls subjects and HIV/AIDS patients on HAART (P<0.05) and there were no statistically significant differences between HIV/AIDS patients on HAART and HIV/AIDS positive not on HAART (P>0.05) (Table 2). There were no statistically significant differences between HIV/AIDS patients on HAART and HIV/AIDS patients not on HAART (P>0.05) as shown in Table 3.

Table 2. *Haematological Parameters in HIV/AIDS Patients on HAART and Controls at UDUTH (from Feb 2012 – Jun, 2012)*

Parameter	Total Subjects (n = 170) (Mean ± SD)	HIV/AIDS +HAART (n = 80) (Mean ± SD)	Control Subjects (n=40) (Mean ± SD)	P-value
PCV %	39.45 ± 1.34	34.26 ± 7.67	38.30 ± 4.55	
TLC (cells×10^9)/L	4.134±1.245	3.501±1.132	6.360±1.567	(P<0.05)
PLT Count (cells×10^9)/L	275.34±2.50	216.95±73.92	253.33±65.090	

PCV=Pack cell volume; TLC= Total leucocytes count; PLT=Platelet

Table 3. *Haematological Parameters in HIV/AIDS Patients Based on Therapy Status*

Parameter	Total Subjects (n = 170) (Mean ± SD)	HIV/AIDS +HAART (n = 80) (Mean ± SD)	HIV/AIDS- HAART (n = 50) (Mean ± SD)	P-value
PCV %	39.450±1.34	34.263±7.668	32.467±7.133	
TLC (cells×10^9)/L	4.134±1.245	3.501±1.132	3.423±1.657	(P>0.05)
PLT(cells×10^9)/L	275.34±2.50	216.95±73.92	207.37±74.65	

PCV=Pack cell volume; TLC= Total leucocytes count; PLT=Platelet

4. Discussion

Patients with HIV /AIDS are reported to experience a wide range of haematological complications and have been found to be the most common cause of mortality/ morbidity in HIV infected patients. Pancytopenias are the most frequent during the advanced stage of disease [26]. Some Pancytopenias are associated with the type of treatment taken and adequate information in these regards may guide clinician while taking medical decision on HIV/AIDS patient before commencement of antiretroviral therapy including HAART.

In this study we have evaluated some haematological parameters in HIV/AIDS positive patients who are on treatment with HAART, and those not yet on HAART on their first visit to HAART ART Clinic of Usmanu Danfodiyo University Teaching Sokoto, and the results compared with age- and sex-matched apparently healthy controls. Derangements in both haematological parameters including anaemia, leucopenia and thrombocytopenia and immunosuppression were the most commonly observed.

The PCV value of HIV/AIDS positive patients on HAART therapy and those not on HAART when compared with control indicated a mild anemia, respectively. This is in agreement with the findings of previous researchers [27, 28, 29]. The improvement in haemoglobin levels could be due to increase in the proportion of erythrocytes in relation to total blood volume as a result of elevated PCV see (Table 2). The reduced incidence of anaemia, the reduced risk of developing moderate to severe anaemia and the overall improvement in PCV and haemoglobin concentration when on HAART have been confirmed in this study. The effectiveness of HAART in improving the quality of life of HIV patients has also been confirmed. Previous studies have reported improved haematocrit values, increased haemoglobin concentration and decreased prevalence of anaemia in HIV infected population

[30, 9]. Our result is also in agreement with Abrams and co workers who reported small increases in haemoglobin level (up to 2 g dL−1) and were associated with a beneficial effect on total quality of life [31].

Our study had further confirmed the previously reported generalized effects of HIV on haematopoietic system and blood cells [32]. However with HAART therapy, PCV values in HIV/AIDS on HAART increases over those who were not on HAART therapy (Table 3). This suggests that, HAART therapy has ability to promote blood cells production.

The reported reduction in Platelet count in this study was consistent with the established diagnosis of HIV/AID [18]. However the observed reduction in platelet was not less than 46×109/platelet. But during the HAART therapy there was an increase in the Platelet count, which was consistent with the work of pervious researchers [20, 33].

The result in this study is also in agreement with the studies of Attilli et al., [34] who reported a thrombocytopenic incidence of 4.8% in HIV patients. The platelet counts in these patients also increased after HAART therapy [34]. An incidence of about 50% was calculated in both HAART-naïve and patients on HAART which almost agrees with the findings of the study conducted by Pechere and co workers who reported a thrombocytopenic incidence of 40% [35].

Depletion of lymphocytes, primarily of the CD4 cell subset subsequent to cellular CD4 immunodeficiency has been noted as the hallmark of HIV infection [36], with leucopoenia and lymphopoenia being documented in different proportions in HIV-positive patients [37]. The slight increase in the total leukocyte count observed in this study was an indication of the ability HAART to boost the immune system and reduce the risk of an opportunistic infection [38]. Although, the study of other researchers suggested that adult immune system might not have the capacity for rapid CD4+ T cell regeneration but there were increases in the lymphoid subset in the blood [21, 23, 39].

A further observation of significant increments in the mean total leucocyte count in HIV-positive patients on HAART gives proof that the observed leucopenia may have been corrected and improved upon by an intervention with HAART usage [40].

5. Conclusion

In conclusion, our study supported the earlier findings of other workers that, the use of HAART therapy increase PCV %, TLC and Platelet count and thus has the ability to boost the immune system of the body amidst the reported side effects [32,19,38]. The ability of the drugs to boost the immune system seems to override the reported side effects and the use of HAART should therefore be encouraged in the management of HIV/AIDs patients despite the side effects. The effectiveness of the HAART drugs will become significant if proper management start at the earlier stage of HIV infection. This will also serve as supporting information for health workers to emphasize the use of HAART in their campaign, for HIV management and encourage the HIV patients in making themselves available for treatment especially at the early stage of the HIV disease and try to adhere to the initial HAART-related side effects that accompanied antiretroviral therapy.

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