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Anaemia and immunological markers in HIV patients on antiretroviral drugs (HAART)

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Abstract

Among those with HIV, anaemia is a strong risk factor for disease progression and death independent of CD4 count. This study is aimed at evaluating the effect of ARV drugs on anaemia and immunological markers in HIV patients. A total of forty four patients placed on HAART (Niverapine + Stavudine/Zidovudine + Lamivudine) were enrolled for the study. CD4 cell count was done using Becton Dickinson (BD) FACSCount analyser, while platelets, lymphocytes%, neutrophils%, and Haemoglobin (Hb) were determined using QBC Autoread analyser. All the parameters were repeated at 12^{th} week and 24^{th} week after placement on drug regimen. The mean CD4 cells count was 198.26 cells/µl, 240.37 cells/µl and 360.45 cells/µl for baseline, 12^{th} and 24^{th} week respectively. Total WBC count was $7.62x10^9$ /L, $7.3x10^9$ /L and 7.39 $x10^9$ /L, while lymphocytes% count was 39.2%, 43.2% and 43.2%. The neutrophils% count was 58.8%, 39.7% and 59.7%. The platelets count was 29.5 $x10^9$ /L, 303.6 $x10^9$ /L and 286 $x10^9$ /L (Values represent baseline 12^{th} week and 24^{th} week respectively). Results between baseline and 12^{th} week, CD4, Hb, and WBC count showed significant increase ($P<0.05$) as well as 12^{th} and 24^{th} week CD4, Hb and ESR, showed significant increase. It was concluded that HAART results in improved in immunological response and reduces incidence of anemia in HIV/AIDS patients.

Keywords

HAART, CD4, Baseline, BD FACSCount, Regimen

1. Introduction

HIV infection has a variety of effects on haematopoiesis and pancytopaenia (Ballah *et al.,* 2013). Cytopaenia is a common complication of infection with human immunodeficiency virus type 1(HIV-1) and, in the course of the disease, more than 70% of the patients develop anemia, frequently requiring transfusion (Bernstein, *et al.,* 1989). Neutropaenia, lymphopaenia and thrombocytopenia may occur indicating that more than one haemopoietic lineage may be impaired. Dysfunction of the bone marrow has been suggested as a possible mechanism; the degree of cytopaenia often reflects the severity of the disease (Koka *et al.,* 1999). HIV-1 infection of marrow stromal cells is sufficient to result in anemia and other cytopaenias (Bahner *et al.,* 1997). Cytopenia is a common complication of infection with human immunodeficiency virus (HIV), and, in the course of the disease, more than 70% of the patients develop anemia, frequently requiring transfusion (Jacobson, *et al.,* 1990). HIV severely compromises the cellular immune system; immunomodulation offers one promising therapeutic strategy. The main immunological complication and hallmark of HIV infection is cellular depletion for various mechanisms: HIV induced cytolysis; dysregulation of cytokines; cytotoxic T – lymphocytes response and HIV auto immune reactions (Okolie, *et al.,* 2003). It has been clearly demonstrated that HIV infection results in a significant loss of total body $CD4^+$ T cells. In this regard, it is estimated that HIV-infected individuals with <200 $CD4^+$ T cells/µL have a total body count of approximately 1x 10^{11} mature $CD4^+$ T cells; this is half the expected count for an uninfected male under age 30 years (McCune, 2001). However, there is a considerable controversy regarding the relative contribution of various mechanisms for the depletion of $CD4^+$ T cells during the course of HIV infection. As with many other areas of HIV

immune pathogenesis, evaluation of patients who initiate, and subsequently withdraw, effective antiretroviral therapy have provided fundamental insight into the understanding of the potential contributions of increased destruction, decreased production and redistribution as mechanisms for $CD4^+$ T- cell depletion in HIV-infected individuals (Williams, 2003).

The immunodeficiency induced by HIV must be considered in the context of microenvironment in which immune response are generated (Janet *et al.,* 1993). Because $CD4^+$ T – cells play a critical role in the orchestration of normal immune responses, it is not surprising many of the immune defects observed during HIV disease are secondary to the progressive decline in the number and function of CD4+ T – cells (Williams, 2003).

Recent progress in understanding the pathogenesis of HIV disease combined with the development of potent antiretroviral agents have resulted in an abundance of treatment options for HIV-infected individuals. Combination therapy with at least three different agents, known as highly active antiretroviral therapy (HAART) has resulted in the suppression in plasma HIV RNA and significant increase in $CD4^+$ T – cell counts in a majority of patients (Hammer *et al.,* 1997). HAART has the capability of reducing the incidence of anemia and lymphopaenia which are associated with the disease progression and death in HIV infected patients (Owiredu, 2011) and may ameliorate many of these effects in an indirect manner simply by decreasing the HIV viral burden (Semba *et al.,* 2001) .

Failure of normal haematopoiesis is an obvious candidate mechanism to account for depletion of CD4+ T – cells during HIV infection (Williams, 2003). There is improvement in the haematocrit and Hb values resulting in reduction in morbidity and mortality of HIV patients. The incidence and severity of the cytopaenia generally correlate to the stage of the disease with the anemia being the most commonly encountered hematologic abnormality and a significant predictor of progression to AIDS or death (Volberding, 2002).

2. Materials and Method

2.1. Sample Size

The study was carried out at the antiretroviral therapy (ART) Comprehensive Laboratory of the Hasiya Bayero Pediatric Hospital, Kano. Fourty four HIV/AIDS patients who met the inclusion criteria as outlined by the National Guideline for HIV/AIDS treatment and enrolled for ART in the centre were studied.

2.2. Inclusion Criteria

The inclusion criteria for enrollment of patients for the study specified that subjects must be males or females aged 15 years and above, must have laboratory evidence of HIV infection; have history of no previous antiretroviral therapy and have CD4 cell counts between 100 and 350 cells/μL.

2.3. Ethical Considerations

The major ethical consideration for the study was put in place before and during the treatment.

2.4. Clinical Management

Each of the 44 patients were placed on Zidovudine 300mg daily/Stavudine 30mg twice daily + Lamivudine 150mg twice daily + Nevirapine 200mg daily. The regimen was continued for two weeks after which the dosage of Nevirapine was increased to twice daily if there were no side effects especially skin rashes.

Before the commencement of ART, hematological and CD4 monitoring assays were done for the patient and at the 12^{th} week and 24^{th} week of commencement of the ART to ascertain the response of the patients to the drugs.

2.5. Confirmation of HIV Serostatus

Prior to initiation of treatment, the HIV serostatus of each patient was reconfirmed, serum samples from each of the patient was used for the test following national algorithm for HIV testing that is unigold then determine followed by stat pack as tie breaker.

3. Sample Collection

Four milliliters of blood was collected into EDTA bottle form each subject using vacutainer needle by vein puncture. The blood was mixed using multifunctional mixer.

3.1. Hematological Analysis

QBC machine was used for the Hematological analysis.

Principles; QBC hematology tests utilize capillary tubes pre-coated with potassium oxalate, acridine orange and coating of an anticoagulant. During high speed centrifugation of the blood filled tube, the cell form in packed layers around the coat which has descended into buffy coat. QBC scanned and read fluorescence and absorbance to identify the layers of differentiated cells:

3.1.1. Procedure for Hematological Analysis

Tubes were filled with thoroughly mixed blood and sealed. The float was then inserted and centrifuged for 5minutes. It was then placed into QBC reader after which Hb, platelets, WBC count, lymphocytes %, and neutrophils % were read (QBC AUTOREAD plus User's guide, 2008)

3.1.2. Controls

Calibration rod provided with the QBC auto reader from the manufacturer was used as control throughout tests.

3.2. CD4 Analysis

BD FACSCount machine was used.

Principle of test: When whole blood is added to the reagents, flourochrome–labeled antibodies in the reagents bind specifically to lymphocyte surface antigen. After a fixative solution is added to the reagent tubes, the sample is

run on the instrument. Here the cells come in contact with the laser light, which causes the flourochrome–labeled cells to fluoresce. This fluorescent light provides the information necessary for the instrument to count the CD4 cells.

3.3. Procedure for CD4 Analysis

The reagents were vortexed upside down and upright for five seconds each. It was then opened with the coring station. A 50µL of whole blood was dispensed into the reagent and vortexed upright for 5 seconds. The reagent was incubated in the work station for 30minutes. Then 50 µL of fixative was dispensed and vortexed for 5 seconds. The reagent was then put in the BDFACS machine to read the CD4 cells (BD FACSCount User's guide, 2008)

3.4. Erythrocyte Sedimentation Rate (ESR)

Principle: When inflammatory response to tissue injury alters serum protein concentration, increase in fibrinogen and C-reactive protein and decrease in albumin tends to increase sedimentation rate of citrated blood.

3.5. Procedure for ESR

In a small container 0.4 ml of sodium citrate was placed and 6ml of venous blood (thoroughly mixed in an EDTA bottle) was added and mixed well. The blood was drawn using safe suction to the 0 mark of the westegren pipette avoiding air bubbles, and was allowed to stand on westegren rack undisturbed for 1 hour and it was then read (Cheesbrough, 2006).

3.6. Data Analysis

Data were generated for each patient at baseline and at weeks 12^{th} and 24^{th} weeks during treatment Lab. data include CD4, Hb, ESR, lymphocyte (%), neutrophils (%) and platelets count All the data were collected and analysed statistically using the open source epidemiologic statistics for public health version 2.2.1

4. Results

Table 1. *Age and Sex distribution of HIV patients on HAART drugsn=44*

Age Distribution	Males (%)	Females (%)	Total prevalence (%)
0 – 20	0 (0)	2 (4.5)	2 (4.5)
21 – 40	11(25)	18 (41)	29 (66)
41 – 60	4(9.1)	6 (13.6)	10 (22.7)
61 – 80	2(4.5)	1(2.3)	3(6.8)
Total	17(38.6%)	27(61.4%)	44(100%)

Table 2. *Mean of measured parameters of the subjects at baseline and 12^{th} week*

Parameters	12^{th}			24^{th}			
	Range	Mean	SD	Range	Mean	SD	P–Value
CD_4 (cells/µl)	110 – 341	198.26	83.15	130 – 362	240.27	84.90	0.020
Haemoglobin (g/dL)	5.7 – 16.3	10.6	3..05	5.6 – 16.0	11.7	2.99	0.030
ESR (mm/hr)	1– 140	40.35	41.73	1– 128	33.60	36.11	0.065
Total WBC ($xl0^9$/L)	3.1 – 14.6	6.20	3.28	32 –13.2	7.30	2.86	0.040
Lymphocyte (%)	25 – 57	39.2	7.3	22 –70	43.2	6.40	0.380
Neutrophil (%)	33 – 75	58.8	8.7	30 –78	59.7	8.30	0.759
Platelets ($xl0^9$/L)	220 – 350	290.5	30.6	230 – 400	305.6	33.70	0.226

Table 3. *Mean of measured parameters of the subjects at 12^{th} and 24^{th} week*

Parameters	12^{th}			24^{th}			
	Range	Mean	SD	Range	Mean	SD	P–Value
CD_4 (cells/µl)	130 – 362	240.27	84.90	145 – 465	360.5	86.72	0.010
Haemoglobin (g/dL)	5.6 – 16.0	11.7	2.99	6.6 – 16.6	13.4	2.82	0.010
ESR (mm/hr)	1– 128	33.60	36.11	2 – 65	26.70	20.89	0.030
Total WBC ($xl0^9$/L)	32 –13.2	7.30	2.86	4.1 – 13.4	7.39	2.59	0.420
Lymphocyte (%)	22 –70	43.2	6.40	24 –57	35.70	8.8	0.244
Neutrophil (%)	30 –78	59.7	8.30	33 –76	58.30	6.9	0.132
Platelets ($xl0^9$/L)	230 – 400	305.6	33.70	2-0 –320	286.70	33.8	0.413

A total sample of 44 HIV positive patients on HAART was examined. This comprises of 27(61.4%) females and 17(38.6%) males. The least prevalence of 2(4.5%) appeared within the age range of 0 – 20 years whose number of females was 2(4.5%) with no male. The age range 21 – 40 years reflected the largest number of 29 with the overall prevalence of 66% comprising of 11(25%) and 18(41%) males and females respectively. This is followed by the age range of 41 – 60 years with a total prevalence of 10(22.7%) of which 4(9.1%) were males, and 6(13.6%) females. The range of 61 – 80 years had prevalence of 3(6.8%) consisting of 2(4.5%) males and 1(2.3%) female (Table 1). Table 2 showed the mean and standard distribution of measured parameters of HIV positive patients HAART at the baseline and 12^{th} week. Some of the parameters (CD4, Hb, and Total WBC Count) showed significant differences between baseline and 12^{th} week of HAART initiation ($P<0.05$), while ESR, lymphocyte%, neutrophils% and platelets showed no significant difference, although, all appeared within the normal range with the exception of ESR ($P>0.05$). Table 3 showed the mean and standard distribution

of measured parameters of HIV positive patients on HAART at the 12^{th} and 24^{th} week. Also CD4, Hb, and ESR showed significant differences between 12^{th} and 24^{th} week of HAART initiation ($P<0.05$), while Total WBC, lymphocyte%, neutrophils% and platelets showed no significant difference, although, all appeared within the normal range with the exception of lymphocyte that showed slight abnormality at 24^{th} week ($P>0.05$).

5. Discussion

Antiretroviral therapy has been acknowledged as a good remedy to the threat of HIV/AIDS infection in the society. Haemalogical abnormalities are among the most common complications of HIV which involves all lineages of blood cells (Kirchhoff and Silvestri, 2008).

In this study, the prevalence of female HIV patients on HAART is higher than males, this agrees with findings of Daniel and Evelyn, 2011. This also agrees with the findings of World Health Organization (WHO) which reported that HIV/AIDS affects most severely in Sub Saharan Africa and women in the world make up 57% of adults living with HIV accounting for up to 80 % of HIV infected women in the world (WHO, 2004). This may likely be due to their exposure to unprotected heterosexual intercourse than men and in many countries, women are less likely to be able to negotiate condom use and are more likely to be subject to non – consensual sex (UNAIDS, 2010). And in many societies women are expected to be innocent and submissive when it comes to sex, preventing them from accessing sexual information and services (International Planned Parenthood Foundation, 2009).

According to this finding, the age bracket 21 – 40 contained about 66% of the study population which is known to be sexually active group. This is in agreement with the findings of Amorncul *et al.,* (2009) and it also buttressed the findings by the Ghana HIV/AIDS Commission report, 2001.

According to this finding, CD4 counts increased significantly from baseline up to 12^{th} and 24^{th} week after initiation of HAART which normally starts when the CD4 counts were <350 cells/mm^3. This in conformity with the findings of Hammer *et al.,* (1997) which indicated 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.

Using a total white blood cell count (<$2.5x10^{9}/L$), a lymphocyte count (<40%) and neutrophil count (<60%) as indicators of leucopenia, lymphopaenia and neutropaenia respectively in accordance with Owiredu *et al.,* (2011), there was no indication of leucopenia in baseline, 12^{th} and 24^{th} week data while lymphopaenia and neutropaenia are evidenced in baseline and 24^{th} week. This could be as a result of possible HAART default after 12^{th} week of initiation.

The definition of anemia lies on hemoglobin level < 10 g/dL or a physician's diagnosis of anemia, Anemia is prevalent among HIV/AIDS patients, it is said to be present in 10 – 20% patients and in over 70% over the course of the disease (Olayemi *et al.,* 2008). This study did not indicate severe anemia at baseline, this possibly due to nutritional proactiveness which is normally encouraged during HIV counseling and testing (HCT) or absence of opportunistic infection as a result of robust innate immunity.

The ESR was continually not within the normal range from baseline up to the 12^{th} and 24^{th} week in spite of the linear decrease noticed. This agrees with findings of Ramana, 2013, that revealed significantly positive correlation of ESR before and after retroviral therapy. ESR usually increased in febrile conditions peculiar with HIV infection, therefore on initiation of treatment with HAART regimen, there was improved immune status and that may result in the decrease of opportunistic infections.

In this study there was no significant variation in platelet count at baseline, 12^{th} week and 24^{th} week. In contrast to the findings of Jose' *et al.,* (1999) whose data indicated that highly active antiretroviral therapy results in a sustained increase in the platelet count in HIV-infected patients with thrombocytopenia and that this increase is independent of the increase in CD4 T cells.

6. Conclusion

HIV infected patients are affected by immunological and haematological changes affecting $CD4^{+}$ T-cell count, lymphocyte, neutrophil counts and ESR. It was concluded that Stavudine/Zidovudine + Nevirapine + Lamivudine (HAART) combination results in improved aforementioned values among HIV patients.

Recommendations

Although the treatment outcome of patients with HAART indicates good response, nevertheless, there is need for more studies on a larger population including patients with severe anemia to determine the actual reason for the response obtained. This also calls for further study on a larger sample size for a longer period of time.

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