Haematological and biochemical response to treatment of HIV-1 infection with a combination of nevirapine + stavudine + lamivudine in Lagos Nigeria

Nkiru ODUNUKWE[1], Oni IDIGBE[1], Phyllis KANKI[2], Taiwo ADEWOLE[1],
Daniel ONWUJEKWE[1], Rosemary AUDU[1], Joseph ONYEWUCHE[1]

[1] Nigerian Institute of Medical Research, Clinical Sciences, Lagos, Nigeria
[2] Harvard School of Public Health, Immunology and Infectious Disease, Boston, USA

ABSTRACT

To evaluate the effect of a combination of nevirapine + stavudine + lamivudine on Haematological and Biochemical values of HIV-1 positive patients in Lagos. Fifty patients who met the enrollment criteria for accelerated clinical trial were studied. Ten millimeters of blood was taken from each patient at first visit for basic haematological and biochemical values. Viral load and CD4 cell counts were also analyzed. All the values were repeated at 12 weeks, and 24 weeks, after patients were placed on drug treatment regimen. All the data were analyzed using Epi-info version 6.4D. The mean erythrocyte sedimentation rate (ESR) results were 53.3 ± 41.8 mm/1 hr, 48.2 ± 40.6 mm/1 hr and 28.6 ± 20.7 mm/1 hr. Haemoglobin (Hb) 123 ± 15 g/L, 124 ± 21 g/L and 132 ± 14 g/L. Packed cell volume 36.8 ± 4.5%, 37.6 ± 4.8%, and 40.3 ± 3.3%. Total white blood cell (WBC) 4.2 ± 1.0, 5.0 ± 1.5 and 4.6 ± 1.0 (baseline, 12 weeks and 24 weeks respectively). Creatinine, 1.2 ± 0.68 g/L, 1.2 ± 0.7 g/L and 1.04 ± 0.3 g/L at (baseline, 12 weeks and 24 weeks respectively). Serum amylase 37.9 ± 15.1 IU/L, 38 ± 23.9 IU/L and 24.3 ± 11.6 IU/L. Triglyceride 95.2 ± 48.3 IU/L, 92.38 ± 54.3 IU/L, and 78.0 ± 35.6 IU/L. Serum bilirubin 0.18 ± 0.09 μmol/L, 0.29 ± 0.28 μmol/L and 0.33 ± 0.24 μmol/L. Alanine transaminase (ALT) 9.9 ± 3.3 IU/L, 15.1 ± 9.0 IU/L and 14.1 ± 9.3 IU/L. Serum aspartate transaminase (AST) 8.2 ± 6.2 IU/L, 9.4 ± 5.2 IU/L and 9.1 ± 6.0 IU/L. On comparison of the results between baseline and 12th week, all parameter were similar except PCV, Hb, serum bilirubin, serum ALT, and total WBC, which were significantly high at 12th week. (p≤ 0.05). On comparison of results between 12th week and 24th week all parameters were similar except Hb and PCV (which were significantly higher at 24th week) while ESR, was significantly lower at 24th week (p≤ 0.05). It was concluded that nevirapine + stavudine + lamivudine combination results in improved haematological values of HIV/AIDS patients. The effect of the drug combination on biochemical parameter in a short period of 24 weeks may not be much. Clinical response and haematological response alone may be used for patient monitoring in a resource poor setting where CD4 count and viral load analysis is impossible.

Key Words: HIV, Haematological values, ART response, Nigeria.

Odunukwe N, Idigbe O, Kanki P, Adewole T,
Onwujekwe D, Audu R, Onyewuche J.

Haematological and biochemical response to treatment of
HIV-1 infection with a combination of nevirapine +
stavudine + lamivudine in Lagos Nigeria

INTRODUCTION

Cytopenia is a common complication of infection with human immunodeficiency virus (HIV) type 1 (HIV-1), and in the course of the disease more than 70% of the patients develop anaemia, frequently requiring transfusion[1]. Neutropenia, lymphopenia and thrombocytopenia are regularly seen indicating that more than one haematopoietic linage may be impaired. Dysfunction of the bone marrow has been suggested as possible mechanism. Degree of cytopenia often reflects severity of the disease[2].

Some antiretroviral (ARV) drugs have been documented to have cytopenic effect, especially when used as monotherapy[3].

Adverse effects of lamivudine in combination with zidovudine included neutropenia, anaemia, thrombocytopenia, transient rise in liver enzymes and rise in serum amlyase[4-6].

Adverse effect attributable to nevirapine has been reported as eosinophilia, granulopenia, jaundice, increase alanine transaminase (ALT) and aspartate transaminase (AST). Stavudine has also been reported to cause elevation of AST, ALT, serum bilirubin and serum amylase. Anaemia, neutropenia, thrombocytopenia have also been reported as adverse effect of stavudine[7].

Several reports have confirmed the efficacy of antiretroviral drugs in the clinical management of HIV patients[8-10].

Treatment with a combination of two nucleoside reverse transcriptase inhibitors (NRTI's) and a potent protease (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 patient[11,12].

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 anemia 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 HIV[13].

However it is essential to evaluate the haematological and biochemical response to HAART in Nigeria. This response may also be compared with the effect of HAART on CD4 count in other to consider using the response as a monitoring tool for patients on treatment.

MATERIALS and METHODS

The study was carried out at the Clinical Research Centre of the Nigerian Institute of Medical Research (NIMR), Lagos. Fifty (50) HIV/AIDS patients who met the inclusion criteria as outlined in the National Protocol and enrolled for ARV treatment (antiretroviral therapy) in the centre were studied.

A structured questionnaire to obtain age, sex, weight and clinical history was completed for each patient. Ten millimeters of venous blood was aseptically obtained from each patient. The blood samples were analyzed to establish baseline values of haematological, biochemical parameters, CD4 cell counts, and viral load levels. The patients were then placed on a combination of nevirapine + stavudine + lamivudine regimen adopted for the national programme. The profile of opportunistic infections was also recorded for each patient prior to initiation of therapy.

The patients were subsequently monitored for their response to the antiretroviral therapy through a 3-montly evaluation of all the parameters estimated at baseline as treatment progressed.

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.

Haematological and biochemical response to treatment of
HIV-1 infection with a combination of nevirapine +
stavudine + lamivudine in Lagos Nigeria

Odunukwe N, Idigbe O, Kanki P, Adewole T,
Onwujekwe D, Audu R, Onyewuche J.

Ethical Considerations

The major ethical considerations for the study were put in place before and during the treatment. The patients were enrolled only after they were sufficiently counseled and their written informed consents were obtained. Relevant confidentiality was maintained throughout the study period.

Clinical Management

Each of the 50 patients was placed on oral nevirapine (Nevimal®, Cipla) 200 mg daily, lamivudine (Lamivir®, Cipla) 150 mg twice daily and stavudine (Stavir®, Cipla) 40 mg twice daily. This 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.

After the commencement of ARV therapy, patients were placed on weekly appointment for the first four (4) weeks, then fortnightly for the next eight (8) weeks and thereafter monthly. At every visit, clinical information was sought to ascertain drug-compliance/tolerance, side effects and the presence of opportunistic infection.

Confirmation of HIV Serostatus

Prior to initiation of treatment, the HIV serostatus of each patient was reconfirmed. Plasma samples from each of the patients were tested using double rapid tests (Cappillus and Unigold: Trinity BioTech, Dublin Ireland) a rapid (Cappillus) and an ELISA (Genie II HIV-1 & 2 kit (Biorad). It was ensured that the two tests used for reconfirmation of the serostatus of each patient have different antigenic compositions.

HAEMATOLOGICAL ANALYSIS

Five milliliters of blood (5 mLs in EDTA bottle) collected from each subject was analyzed manually for haematocrit (PCV), hemoglobin concentration (Hb), white blood cell count (WBC) and platelet count. Blood films were stained with Leishman's stain and examined for differential WBC count by a single microscopist using x 100-oil immersion lens x 7 eyepieces. Erythrocyte sedimentation rate (ESR) was determined by westergren methods[14].

BIOCHEMICAL ANALYSIS

For each patient 5 mL of whole blood was obtained. The blood was left to stand in plain bottle at room temperature. Retraction and centrifugation followed to obtain serum.

Commercially available kits were used to assay for he following: serum amylase, serum and urine creatinine, triglyceride (BIOLABO), serum ALT, serum AST, serum bilirubin (RANDOX) and alkaline phosphatase (TECO).

The procedures for each test were followed according to manufacturer's instructions. Absorbance was measured with the spectrophotometer and the results were calculated. Normal ranges were reported based on the test kits.

CD4 Lymphocyte Cell Count

The CD4 lymphocyte counts were determined for each patient at baseline and subsequently during monitoring using the Dynabead Technique (Dynal A.S. Oslo Norway). This was the adopted technique for CD4 estimation under the national ARV programme. The CD4 lymphocyte counts were expressed as cells/µL of blood.

Viral Load Estimation (HIV-1 RNA)

The viral load of each patient was quantified on plasma samples using the PCR based Amplicor HIV-1 monitor version 1.5 (Roche Diagnostic Systems, Branchburg, NJ, USA). The viral load was expressed as viral copies/mL of blood sample. Prior to assay, the samples were not subjected to ultracentrifuge, thus the cut-off point of non-detectable limits of viral load in this study was $\leq$ 400 copies/mL.

Analysis of Data

Data were generated for each patient at baseline and at weeks 12 and 24 during treatment. Clinical and laboratory data recorded included the body weights, the frequency and severity of opportunistic infections, drug

Odunukwe N, Idigbe O, Kanki P, Adewole T,
Onwujekwe D, Audu R, Onyewuche J.

Haematological and biochemical response to treatment of
HIV-1 infection with a combination of nevirapine +
stavudine + lamivudine in Lagos Nigeria

side effects, drop-out to follow up, number of deaths, CD4 cell counts and viral load estimations. All the data were collated and analyzed statistically using the Epi-Info version 6.4D software.

RESULTS

All the patients were confirmed HIV-1 positive. Demographic result and age distributions are shown in figures 1 and 2.

Tables 1 and 2 show the mean results of ESR, haemoglobin (Hb), packed cell volume (PCV), and total WBC (baseline, 12 weeks and 24 weeks respectively).
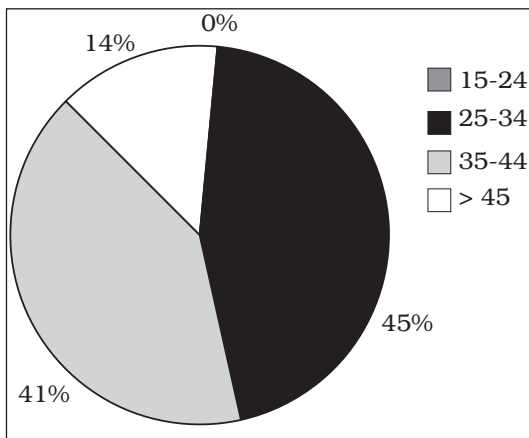

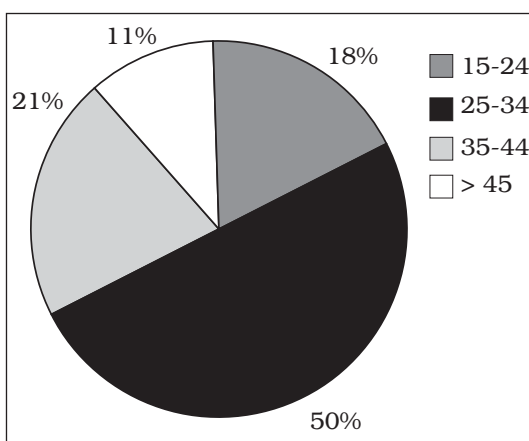**Figure 1.** Age distribution of the male study population.

Figure 2. Age distribution of the female study population.

On comparison of the results between baseline and 12th week, all parameter were similar except PCV, Hb serum bilirubin, serum ALT, and total WBC, which were significantly high at 12th week (p≤ 0.5). On comparison of results between 12th week and 24th week all parameters were similar except Hb and PCV (which were significantly higher at 24th week) while ESR, was significantly lower at 24th week (p≤ 0.5). Mean creatinine results serum amylase, triglyceride, serum bilirubin, ALT and serum AST (baseline, 12 weeks and 24 weeks respectively) are shown in Tables 1 and 2.

At baseline 38% of the population presented with various opportunistic infections. There was a fall to 23.9% and 19.7% at week 12 and week 24 respectively. There was a significant difference between the opportunistic infections at baseline and at week 24 (p< 0.05). The CD4 cells were significantly increased at week 24 (p< 0.05), and viral load was also significantly reduced (p< 0.05).

DISCUSSION

In this study the hematological result at the baseline did not indicate severe anaemia. However there was evidence of mild to moderate anaemia in most of the patients. Absence of severe anaemia in this group of patients can be explained by the fact that the exclusion criteria in this study, excluded the patients with CD4 below 100 cells/μL. Patients with CD4 below 100/μL are more likely to be progressing to AIDS and are more likely to have severe anaemia[2].

Anaemia has been reported severally in HIV/AIDS infection[1,15,16]. Sullivan reported that anemia is related to disease progression and survival in patients with HIV infection. Also that recovery from anaemia has been linked to improved survival outcomes[15]. Volberding reported that anaemia is the most commonly encountered haematologic abnormality in HIV-positive patients, occurring with increasing frequency as the disease progresses[16]. Some workers reported that the anaemia observed in HIV/AIDS patients

Haematological and biochemical response to treatment of
HIV-1 infection with a combination of nevirapine +
stavudine + lamivudine in Lagos Nigeria

Odunukwe N, Idigbe O, Kanki P, Adewole T,
Onwujekwe D, Audu R, Onyewuche J.

Table 1. Comparison between baseline values and 12 weeks values

| | Baseline | | 12 weeks | | |
Parameters	Range	Mean ± SD	Range	Mean ± SD	p value
ESR (mm/1hr)	4-135	53 ± 41	1-157	48 ± 40	0.086
Haemoglobin (g/L)	87-153	123 ± 12	133-150	124 ± 20	**0.038**
Neutrophile (%)	40-74	59.7 ± 7.6	36-79	58.9 ± 8	0.438
Lymphocyte (%)	26-56	38.8 ± 6.1	21-64	40.8 ± 8	0.112
Haematocrit (%)	26-46	36.8 ± 5	20-45	38.6 ± 5	**0.010**
Total WBC (x 10^6/L)	2.6 -7.6	4.2 ± 1.0	2.4-9.4	5 ± 1.5	**0.020**
Bilirubin (μmol/L)	0.04-0.58	0.18 ± 0.09	0.01-1.3	0.29 ± 0.28	**0.02**
Triglyceride (IU/L)	41.2-200	95.2 ± 48.3	21.0-230.0	92.4 ± 54.3	0.739
AST (IU/L)	3.0-34.0	8.2 ± 6.2	0.8-29.0	9.4 ± 5.2	0.9
ALT (IU/L)	3.9-18.9	9.9 ± 3.3	1-44.7	15.1 ± 9.1	**0.001**
Amylase (IU/L)	11-78	37 ± 15.1	11-100	38.2 ± 23.9	0.75
Creatinine (mg/dL)	0.6-4.8	1.2 ± 0.7	0.7-5.6	1.2 ± 0.7	0.77

Table 2. Comparison between 12th week data with 24th week data

| | 12th weeks | | 24th weeks | | |
Parameters	Range	Mean ± SD	Range	Mean ± SD	p value
ESR (mm/1hr)	1-157	46 ± 40	2-70	28.6 ± 20.7	**0.02**
Haemoglobin (g/L)	83-150	124 ± 20	90-160	132 ± 14	**0.01**
Nutrophile (%)	36-79	58.9 ± 8	45-75	58.1 ± 6.7	0.57
Lymphphocyte (%)	21-64	40.8 ± 8	24-54	40.9 ± 6.4	0.76
Haematocrit (%)	20-45	38 ± 5	35-50	40.3 ± 33	**0.01**
WBC (x 10^6/L)	2.4-9.4	5 ± 1.5	1.4-6.4	4.6 ± 1.0	0.44
Bilirubin (μmol/L)	0.01-1.3	0.29 ± 0.28	0.07-1.77	0.3 ± 0.28	0.41
Triglyceride (IU/L)	21-230	92.4 ± 54.3	32-184	78 ± 35.6	0.10
AST (IU/L)	0.8-29.0	9.4 ± 5.2	4.6-48	14.2 ± 9.3	0.30
ALT (IU/L)	1-44.7	15.1 ± 9.1	1.0-27.5	9.1 ± 6.0	0.2
Amylase (IU/L)	11-100	38.2 ± 23.9	11.0-48.0	24.3 ± 11.6	0.79
Creatinine (mg/dL)	0.7-5.6	1.2 ± 0.7	0.6-1.8	1.04 ± 0.3	0.42

may be as a result of the direct attack of the reticuloendothelial cells by the virus. This hypothesis was buttress by the cytopenia that has been reported involving all the cell lines in the marrow[1]. That could also explain the increase in the haematocrit as the viral load decreases.

Several factors play a role in the development of anemia in patients with HIV, including chronic disease, opportunistic infections, and certain nutritional deficiencies. In this study, there was significant increase in the haematocrit and haemaglobin level after 12 weeks of treatment. This increase was

Odunukwe N, Idigbe O, Kanki P, Adewole T,
Onwujekwe D, Audu R, Onyewuche J.

Haematological and biochemical response to treatment of
HIV-1 infection with a combination of nevirapine +
stavudine + lamivudine in Lagos Nigeria

sustained till the 24th week. The increase in these two values correlates with progressive increase in the CD4 and progressive decrease in the presence of opportunistic infection and viral load. This average increase in the haemoglobin and PCV did not support the earlier reports of anaemia as adverse effects of lamivudine and stavudine[4-7]. It could be either that these drugs when used together with nevirapine has a different effect on the bone marrow or that some individuals have some unique factors that result in improved haematocrit on treatment with HAART. Very recently, the use of HAART has also been associated with a significant increase in haemoglobin concentrations and a decrease in the prevalence of anaemia[13].

However, Mildvan reported that many HIV-positive patients receiving HAART still develop mild to moderate anaemia and associated quality of life (QOL) impairment. He also reported that many HIV-positive patients are co-infected with HCV and standard HCV therapy (interferon alfa/ribavirin) can cause anaemia[20]. Our patients were not screened for HCV and were not on any other therapy apart from HAART.

Decrease ESR reported at the 24th week further confirmed the clinical progress recorded in the patients. Frequency of the opportunistic infections decreased and the patients recorded higher periods of general well being.

HIV infected patients are likely to be having more frequent attacks of malaria and opportunistic infections as a result of their low immune status in malaria endemic areas. These infections may be the cause of the frequent fever usually experienced by these patients. ESR is usually increased in febrile conditions therefore on treatment with ARV drugs, there was improved immune status and that may result in decrease opportunistic infections including malaria attacks with subsequent decrease in the ESR which was significantly reduced at the 24th week. Kate Grimwade et al reported that more HIV-infec-

ted patients had severe or complicated malaria than HIV-uninfected patients (47% versus 30%) and significantly more HIV-infected patients died (20% versus 3.8%)[19].

The range of total WBC count in this study group was not different from what is recorded for the normal population. This could be as a result of leucocytosis that may follow malaria infection or any opportunistic infection, which may have masked the leucopenia that was supposed to have been recorded at baseline. During the course of the treatment while the immunity was improving, the haemopoietic cells were also recovering with resultant unchanging count, hence there was no significant difference between leucocyte count at baseline and at 24th week. It could also be as a result of simultaneous occurrence of eosinophilia and leucopenia at the same time.

However there was a slight increase of the leucocyte count by the 12th week.

On comparison of the results between baseline and 12th week, bilirubin, and serum ALT, were significantly higher at 12th week (p≤ 0.5), but when compared with 24th week result there was no significant change. This increase is in support of earlier reports[3-5].

However this appeared to be transient since there was no change at level at 24th week.

Nevertheless there is need for more studies on a bigger population including patients with severe anaemia to determine the actual reason for the response obtained in this group of patients in Nigeria. This also calls for another study for on a bigger sample size for a longer period of time.

It was concluded that nevirapine + stavudine + lamivudine combination results in improved haematologic values of HIV-1 patients. The effect of the drug combination on biochemical parameter in a short period of 24 weeks may not be much. Clinical response and haematological response alone may be used for patient monitoring in a setting where CD4 count is impossible.

130

Haematological and biochemical response to treatment of
HIV-1 infection with a combination of nevirapine +
stavudine + lamivudine in Lagos Nigeria

Odunukwe N, Idigbe O, Kanki P, Adewole T,
Onwujekwe D, Audu R, Onyewuche J.

REFERENCES

1. Jacobson MA, Periperl L, Volberding PA, Porteous D, Toy PT, Feigal D. Red cell transfusion therapy for anaemia in patients with AIDS and ARC: incidence associated factors, and outcome. Transfusion 1990; 30:133.

2. Scadden DT, Zeira M, Woon A Wang 2, Schieve L, Ikeuchi K, Lim B, Groopman JE. Human immunodeficiency virus infection of human bone marrow fibroblast. Blood 1990;76:317.

3. Physicians Desk Reference 2001;55:2838-42, 1374-7, 1072-5.

4. ABPI compendium of data sheets and summaries of product characteristics 1999-2002:20-1, 486-9, 288.

5. Cooper DA, Gold J, Maclean P. Acute AIDS retrovirus infection. Definition of a clinic illness associated with sero-conversion. Lancet 1985:1537-40.

6. Anderson RM, Boily MC, et al. Contact and the predicted demographic impacts of AIDS. Nature 1991;352:581-92.

7. Kofu K, Gresham-Dame GM, Peters M, et al. Rapid Spread of HIV infections in Abidjan, Ivory Coast. Europe. J Clin Microbiol Infect Dis 1992;11:27 1-3.

8. Ekweozor CC, Olaleye OD, Tomori O, Saliu I, Essien E. IV sentinel surveillance in Ibadan/Ogbomosho zone of Oyo State: a pilot study. Niger Med J 1993; 24:1-4.

9. Nigeria Bulletin of Epidemiology. No: 2, 1992;2.

10. Blattner WA. HIV epidemiology: past present and future. FASEB 1991;5:2340-9.

11. De Cock KM, Barrere BB, Diaby L, La Fontane MF, et al. AIDS the leading cause of adult death in West African city of Abidjan. Ivory Coast Science 1990;249:793-6.

12. Ryder RW, Rayfield M, Quinn T, et al. Transplacental HIV transmission in African newborns. Presented at the Fourth International Conference on AIDS. Stockholm 1988:12-6.

13. Belperio PS, Rhew DC. Prevalence and outcomes of anemia in individuals with human immunodeficiency virus: a systematic review of the literature. Am J Med 2004;116(Suppl 7A):27-43.

14. Dacie JV, Lewis SM. Estimation of the erythrocyte sedimentation rate (ERS). Practical Haematology. 6th ed. Churchill Livingstone, 420.

15. Sullivan P. Associations of anaemia, treatments for anaemia, and survival in patients with human immunodeficiency virus infection. J Infect Dis 2002;185 (Suppl 2):138-42.

16. Volberding P. The impact of anemia on quality of life in human immunodeficiency virus-infected patients. J Infect Dis 2002;185(Suppl 2):110-4.

17. Moore RD. Anemia and human immunodeficiency virus disease in the era of highly active antiretroviral therapy. Semin Hematol 2000;37(4 Suppl 6):18-23.

18. Antoniou T, Gough K, Yoong D, Arbess G. Severe anaemia secondary to a probable drug interaction between zidovudine and valproic acid. Clin Infect Dis 2004;38:38-40.

19. Kate Grimwade, et al. HIV infection boosts severe malaria risk. AIDS 2004;18:547-54.

20. Mildvan D. Implications of anemia in human immunodeficiency virus, cancer, and hepatitis C virus. Clin Infect Dis 2003;37(Suppl 4):293-6.

Address for Correspondence:

Nkiru ODUNUKWE, MD

Nigerian Institute of Medical Research

Clinical Sciences

Lagos, NIGERIA

e-mail: *nodunukwe@nimr-ng.org*