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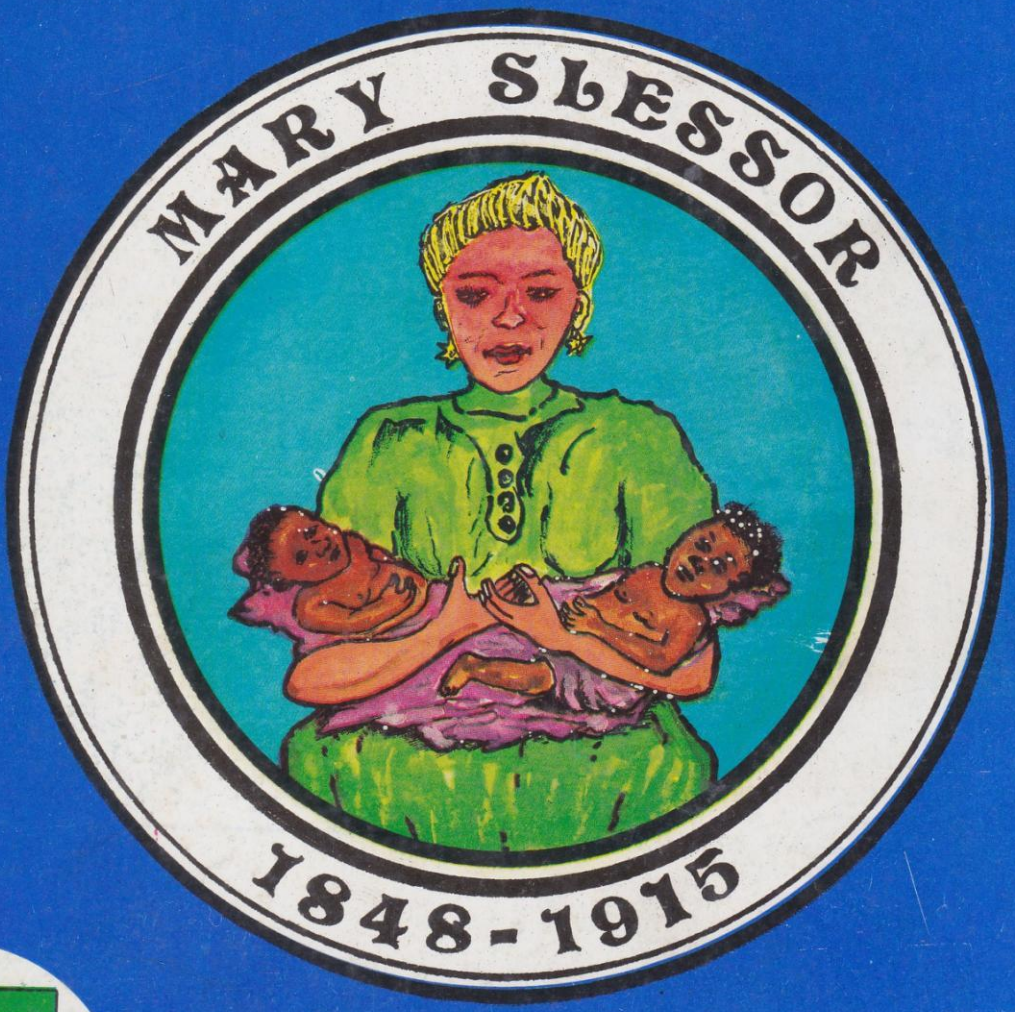

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Assessment of Some Haematological Parameters in HIV Infected Patients on Anti-Retroviral Herbal Preparation ("Winnie Cure") in Nigeria

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Abstract: The study was to assess the haematological parameters [Haemoglobin (Hb), packed cell volume (PCV), total white blood cells (TWBC), differential White Blood Cells (WBC), platelet count and erythrocyte sedimentation rate (ESR)] of confirmed thirty-one (31) HIV patients before and after being treated with a herbal preparation called Winnie Cure®, a supposed anti-retroviral agent. The preparation was prescribed at a dose of 5ml (one tea spoonful) containing 250mg Winnie Cure® extract, taken three times daily for five days and then two times daily for the period of this study (nine weeks). Samples were collected from the 31 confirmed HIV infected patients on their visit (before they started the treatment) and at three weeks interval from the time they started treatment till the 9th week (second, third and fourth visits). Blood collected was by standard veno-puncture method while ESR was estimated by Westergrene method. Other haematological parameters were evaluated using Coulter ACT diff Analyzer. At the first, second, third and forth visits the ESR (mm/h) values obtained were decreasing thus indicating a positive patient response to the drug. It was observed that all the parameters showed significant variation at the 3rd visit, with platelets and PCV values being significantly raised at the 3rd visit (six weeks of treatment). We observed that this herbal preparation (Winnie Cure®) seems to attain peak activity at 3rd and 4th visits (that is the sixth and ninth weeks of treatment), as indicated by appreciative changes of some haematological parameters. Clinical and pharmaceutical evaluation of this preparation for possible use in the treatment of HIV patients is suggested.

Key words: Anti-Retroviral, Herbal Preparation, Haematological Parameters, Nigeria

Introduction

Human immunodeficiency virus (HIV) is one of the most important emerging infectious pathogens of this century. This lymphotrophic virus was first isolated in 1983 from a patient with acquired immunodeficiency syndrome (AIDS) and AIDS related complex[1]; it is probably the only disease with multiple impacts on persons, families, communities and the entire society. HIV/AIDS is today the fourth-biggest killer world wide[2]. It ranks as the leading cause of death in sub-Sahara Africa where death from HIV infection in adults is said to have surpassed that of malaria[3]. Nigeria has recorded a steady increase in the prevalence since HIV/AIDS was first reported. Prevalence rates have increased from 1.8% in 2001 to 5.4% in 2004 with some states in the country having prevalence of over 10%[4]. With HIV/AIDS epidemic only 15 years old in Nigeria, it had

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already threatened to ravage the country with multiple epidemics[5].

Globally, it was estimated that 5.6 million new HIV infection and 2.6million AIDS death occurred in 1999 and almost 3.4 million adults and children are currently living with HIV/AIDS, the great majority are in developing countries[6]. Reports in Nigeria indicate that adolescents, youths, and children are in a precarious situation as far as HIV/AIDS problem is concerned[7]. Out of 2.7 million children estimated to be harbouring HIV in the world, Africa account for 2.4 million and Nigeria account for 10% of Africa borden[8]. In year 2000 alone there were 600,000 infected children and of this 90% of the cases were from Africa[9].

The emergence of drug development programme by government and industry have led to many antiretroviral drugs (ARVs). The ARVs are classified according to their mode of action with each of them acting on the HIV by interfering with its reproductive cycle. These ARVs include nucleoside (and nucleotide) reverse transcriptase inhibitors (NRTI), non-nucleoside reverse transcriptase inhibitors (NNRTI), protease inhibitors (PI) and fusion inhibitors (FI). These drugs aim at reducing HIV-related morbidity and mortality, reducing the viral load (to undetectable levels) for as long as possible in order to halt disease progression and prevent or reduce resistant variants. They also achieve immune reconstitution that is quantitative (CD4 count in normal range) and qualitative (fewer infections and illnesses). In addition they provide an antiretroviral regimen which has a high likelihood of success, preserve future therapeutic options, has relatively few side effects, and is tailored to individual needs for adherence.

These drugs can have serious side effects such as diarrhoea, nausea or abnormal distribution of body fats[10]. Some common side effects on haematological parameters have been reported, most especially from the use of Zidovudine (AZT) [11]. These side effects include among others anaemia and neutropenia[12]. The Centre for Disease Control (CDC) [13] advocates that before the use of any anti-retroviral therapy the safety and efficacy of the drug should be tested.

In view of the various questions raised on the side effect of anti-retroviral therapy it became important for us to study the effect of this herbal preparation, used as ARV agent, on some haematological parameters. The anti-retroviral herbal preparation, produced in Nigeria by Winners Medical Diagnostic and Research Institute, Abuja is called Winnie Cure® and is in use in many health care settings.

This research work sets out to evaluate some haematological values in HIV infected patients and assess influence of an herbal preparation treatment (Winnie Cure®) on these haematological parameters (Hb, PCV, TWBC, differential WBC, ESR and platelet count).

Materials and Methods

Participation in the study was voluntary. A total of 31 confirmed HIV positive patients, aged between 28 and 47 years who volunteered to take Winnie Cure® at a prescribed dose of 5ml (one teaspoonful) containing 250mg Winnie Cure® extract, taken three times daily for five days and then two times daily for the period of this study (9 weeks) were recruited. Samples were collected at intervals of three weeks for the period of this study.

Blood was collected into EDTA bottle to give blood anti-coagulant concentration of 2mg per one ml of blood. Blood collection was by standard venepuncture method described by Dacie and Lewis[14].

Hb, PCV, TWBC, differential WBC and platelet count were analysed using haematology analyzer (Coulter ACT diff Analyzer). The working principle of the machine is that when a suspension of blood cells is passed through a small orifice with an electric current, it introduces an impedance change in the orifice determined by the size of the cell. The system counts the individual cells and provides cell size distribution.
Erythrocyte sedimentation rate (ESR) measurement was carried out using Westergreen method described by Dacie & Lewis[14]. Two ml of blood was diluted in 0.5ml of trisodium citrate solution. The Westergreen pipette was filled to a zero mark

and mounted on the Westergreen stand for one hour for the red cells to sediment with the aid of gravitational force. Then the column of the sedimented red cells was read at exactly 1 hour and results were recorded in mm/hr.
First time call or visit of patients without any previous HIV treatment were used as controls over the parameters on commencement of treatment.

Specifically, the changes in some haematological parameters of HIV patients on this anti-retroviral herbal preparation were assessed and haematological values before and after treatment compared. A comparative statistical analysis was made between the 1st visit (before treatment) and subsequent visits (during treatment) using student t-test at a 5% level of significance.

Results

18 (58%) out of the 31 HIV positive patients were male while 13 (42) were female. Table I summarises the mean standard deviation of the haematological parameters, (Hb, PCV, TWBC, differential WBC and platelet count), of the HIV patients before treatment (1st visit) with the anti-HIV herbal preparation (Winnie Cure®), and subsequent appointments (check-up) at three weeks interval, otherwise referred to as visits. It was observed that there was a consistent reduction of ESR value among the patients from the 2nd visit (during treatment) till the 4th visit (still on treatment), (table 1), but there was no significant difference, (table 2, ($P>0.05$)).

The total white blood cells (TWBC) count showed a slight reduction at second visit ($6.03 \pm 1.48 \times 10^9$/L) compared to $6.11 \pm 1.68 \times 10^9$/L at first visit. There was a marked reduction at the 3rd and 4th visits $4.80 \pm 0.80 \times 10^9$/L and $4.85 \pm 1.63 \times 10^9$/L respectively, (table 1). The difference was statistically significant ($p<0.05$) (table 2). There was a significant reduction observed in absolute lymphocyte count at 3rd and 4th visit, (2.18 ± 0.66 and 2.90 ± 0.71 respectively), compared to the initial and 2nd visits, (3.01 ± 1.32 and 3.67 ± 1.47 respectively) , Table 1. The difference was statistically significant ($p<0.05$) Table 2. Platelet number was observed to be increased at the 3rd and 4th visits ($233.9 \pm 20.25 \times 10^9$/L and $248.74 \pm 53.54 \times 10^9$/L respectively). The difference at 3rd and 4th visits compared with the first visit was significant, (table 1), ($p<0.05$). Absolute granulocytes showed significant reduction in subsequent visits (table 1), while increased values were observed at the 3rd visit in differential granulocytes. ESR and Hb values progressively improved as treatment progressed with a consistent reduction in the ESR value and increase in the Hb concentration. An increase ($29.87 \pm 5.14\%$) in PCV was observed at the 3rd visit, compared to 24.46 ± 6.58 at 1st visit, (table 1). The difference was statistically significant ($p<0.05$).

Discussion

HIV infection has been reported to cause diverse degree of immuno-depression in man[15] and this has enormous haematologic consequences.

The haematological consequences of HIV infection are dominated by peripheral blood cytopaenia, which have become more common with the advent of anti retroviral therapy and treatments for HIV related infections and malignancies[4]

Winnie Cure® is reported to inhibit viral replication and could restore human immunity by activating and mobilising white blood cells (WBC) especially the CD4+ cells and other phagocytes for phagocytosis[16]. As a supposed anti-retroviral agent, it may have haematological consequence as reported for some other anti-retroviral agents[17]. The haematological parameter of HIV patients on treatment with Winnie Cure® compared with their values before commencement of the treatment showed significant variation. These included lymphocyte and granulocytes counts. The major clinical significance of the HIV induced neutropenia is that it often precludes therapy with ZDV and some drugs used for the treatment of opportunistic diseases in HIV patients[18].

The reduction in the lymphocyte count at the 3rd visit may be, as reported by Sten et al[19], that transient lymphopenia is common and a third of the patients may have atypical lymphocytes in the peripheral blood smear. More so the gradual rise in the lymphocyte at

Table 1: Summary of the mean ± standard deviation of the haematological parameters of the HIV Patients on treatment with Winnie Cure®.

Parameters	1st Visit	2nd Visit	3rd Visit	4th Visit
ESR (mm in 1 hr.)	49.56±51.04	49.29±52.48	48.23±45.46	37.13±35.59
Total WBC ($x10^9$/l)	6.11±1.66	6.03±1.48	4.80±0.80	4.85±1.63
Absolute Lymphocytes ($x10^9$/l)	3.01±1.32	3.67±1.47	2.18±0.66	2.90±0.71
Absolute Granulocytes($x10^9$/l)	3.10±1.42	2.46±1.19	2.60±0.73	2.95±0.92
Differential Lymphocytes (%)	49.26±16.58	58.47±16.87	45.50±11.90	61.00±5.66
Differential Granulocytes (%)	50.74±16.58	41.53±16.87	54.50±11.90	39.00±5.66
Haemoglobin (g/dl)	8.15±2.15	8.64±2.27	8.14±1.56	11.24±0.81
Packed Cell Volume (%)	24.46±6.58	25.92±6.40	29.87±5.14	34.75±1.53
Platelet ($x10^3/\mu l$)	187±78.11	220.60±86.42	233.9±70.25	248.74±53.54

Table 2: Comparative statistical analysis of ESR, total white cells and lymphocyte counts values of the HIV patients between 1st visit (before treatment) and subsequent visits (during treatment).

Parameters	Visit	t- Test	P- value
ESR	1st / 2nd	0.02	p>.05
	1st/3rd	0.11	p>0.05
	1st/4th	0.12	P>0.05
	2nd /3rd	0.09	P>0.05
	2nd/4th	1.07	p>0.05
	3rd /4th	1.07	p>0.05
Total White Cells	1st / 2nd	0.50	p>.05
	1st/3rd	3.97	p>0.05
	1st/4th	3.0	P>0.05
	2nd /3rd	4.10	P>0.05
	2nd/4th	2.95	p>0.05
	3rd /4th	0.15	p>0.05
Absolute Lymphocyte Counts	1st / 2nd	2.17	P<0.05
	1st/3rd	1.03	p>0.05
	1st/4th	3.73	P<0.05
	2nd /3rd	3.50	P<0.05
	2nd/4th	0.79	p>0.05
	3rd /4th	6.54	P<0.05

Table 3: Comparative statistical analysis of granulocyte and Hb Concentration values of the HIV patients between 1st visit and subsequent visits.

Parameter	Visit	t- Test	P- value
Granulocytes	1st / 2nd	2.17	P<0.05
	1st/3rd	1.03	p>0.05
	1st/4th	3.73	P<0.05
	2nd /3rd	3.50	P<0.05
	2nd/4th	0.79	p>0.05
	3rd /4th	6.54	P<0.05
Hb Concentration	1st / 2nd	0.23	p>0.05
	1st/3rd	4.02	P<0.05
	1st/4th	2.77	P<0.05
	2nd /3rd	4.12	P<0.05
	2nd/4th	0.77	p>.05
	3rd /4th	7.50	P<0.05

the 4th visit may contribute to the fall in granulocyte count. The count at the 3rd and 4th visits may indicate the effect of the drug at its peak of assimilation and absorption being at 6 to 9 weeks.

The TWBC count shows a consistent reduction at all the visits thus indicating a direct drug effect on TWBC, with no significant difference (p>0.05). There was a reduction in Hb concentration at the 3rd visit. The drop of Hb concentration at the 3rd visit also indicates that the 6th week of treatment is when the peak drug effect at third dosage is achieved. Tierny et al[13], reported anaemia and neutropenia as some common side effects of Zidovudine (ZDV) on haematological parameters. However at the 4th visit there was a significant increase in the value of the Hb when compared with the values at 1st and 3rd visit (P<0.05). This rise of Hb concentration up to the 4th visit may be an indication of the patient's recovery. A rise of PCV at 3rd visit observed may also indicate a drastic influence of the herbal drug on some haematological parameters at 6 weeks of treatment. This rise in PCV of the 3rd visit could be due to either direct drug effect on red cell metabolism or a dehydration process in the metabolism of the drug.

Also a significant increase of platelet count was observed at the 3rd visit compared to the counts in other visits. This may equally indicate that the drug effect on some haematological indices are active at about 6 weeks of treatment. Within this period of treatment the drug may initiate a partial or minor gastro-intestinal bleeding which may be the cause of the sudden increase in platelet count at the 3rd visit.

Actually the test which promotes the best example is the ESR because it is, like HIV

antibody test, associated with elevation of antibodies and acute phase proteins. Indeed there is evidence that an elevated ESR is a superior predictive marker for the development of clinical AIDS than is the decrease in the CD4 cell count[20]. The ESR in this study was observed to start reducing from the 2nd visit to the 4th visit, although the reduction was not significant (P>0.05). The apparent reduction of ESR from 2nd to 4th visit may indicate the drug positive activity. Therefore this reduction could possibly be related to the activity of the herbal preparation (Winnie Cure®). At this phase the drug reaction may be taking over and mobilizing CD4 lymphocytes and phagocytosis and bring down the viral load.

The preparation's main effect on some of these haematological parameters is at 3rd visit (6th week of treatment). From the results obtained in this study we conclude that this herbal preparation Winnie Cure®) starts its action at about 6th to 9th weeks of treatment indicated by appreciative changes of some haematological indices. A longer period of trial is recommended and CD4 count also included in the analysis to find out if significant and beneficial changes could be observed.

We suggest pharmaceutical/clinical evaluation of this herbal preparation to ascertain the drug interaction and activity on HIV in man for the possibility of using it for treatment or management of HIV patients.

References

1. Centre for Disease Control. Natural HIV sero-surveillance summary results through 1992. Atlanta, US Department of Health and Human Services. 1994; 3:1 – 18.
2. World Health Organization. AIDS; image of epidemics. WHO Geneva. 1994; 24:1 –6.
3. Scheibar GB, Bush MP, Klanmian SH, Korelitz, JJ.The risk of transfusion transmitted viral infections. N. Engl J Med 1996; 34: 85 – 90.
4. Nwosu, T. Basic facts on HIV/AIDS. Algke Jeepac, Lagos, 1st ed.1998; 3 –9.
5. Nwosu ABC.Technical Report on HIV/ Syphillis Sero Prevalence and STD Syndromes Sentinel survey Among PTB and STD Patients in Nigeria. FMOH, ABUJA. 2001; Vol.1:3.
6. McFarland W; Mvere D; Shandera W; Reingold A. The Epidemiology and Prevention of Transfusion-Associated HIV infection in sub-Sahara Africa. Vox Sang 1997; 72: 85 – 92.
7. The Joint United Nations Programme on HIV/AIDS Epidemic update. December 1999.1 – 20.
8. UNAIDS/WHO. Global survey of the HIV/AIDS Epidemics as at December 2003. http://www.unaids/wad 2003/epiupdate 2003 enlarglobal htm.
9. Dennis B. Mother to child transmission of HIV/AIDS. JAMA 2001; 2:1 – 3.
10. Gary DV. Combination of Anti-HIV drugs. http://www.cdc/gov/nchstp/hiv-aids media/pepfact3.htm, 2003.
11. Scandem D.T zon L.I Groopman JE. Pathophysiology and Management of HIV-associated haematologic disorders. Blood 1989; 74:55 – 63.
12. Tierney LM; Mcphee SJ Paadakis MA. Current Medical Diagnosis and Treatment. Lange Medical Books/McGraw-Hill, London 40th ed. 2001;1289 – 1299.
13. Hammer SM, Gillis, JM Pinkston, P Rose RM. Effect of Zidovudine and granulocyte-macrophage colony-stimulating factor on human immunodeficiency virus replication in alveolar macrophages. Blood 1990; 75: 1215-1219.
14. Dacie JV, Lewis SM. Practical Haematology, 9th ed. Churchill Livingstone, London, 2001; 19-56.
15. Wsykind BA, Dom HH Kelly, WB, Specific tropism of HIV-1 for microglical cells in primary human brain cultures. Science 1990; 249: 549-553.
16. Winners Medical Diagnostic/Research Institute (unpublished information), 2004.
17. Pluda JM, Mitsuya H, Yarchaan R. Haematologic effects of AIDS therapies. Hematol Oncol Clin North Am S: 1991; 229-248.
18. Zon LI, Arkin C Groopman JE. Haematologic manifestations of the HIV. Br J, Haematol 1987; 66: 251-256.
19. Stein DS, Korvick JA Vermund SH. CD+ lymphocyte cell enumeration for prediction of clinical course of human immunodeficiency virus disease. A review. J. Infect. Dis. 1992; 165: 352-363.
20. Lefrere JJ, Gozin D Leratte J. Circulating anti-coagulatant in asymptomatic persons sero-positive for HIV. Ann Intern Med. 1998; 108: 770-771.

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