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Comparative Studies on the Effects of Zidovudine, Nevirapine, Lamivudine and Bioclean II on Female HIV/AIDS Cases in Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author INI designed the study and wrote the protocol. Author NII collected all data and performed the statistical analysis. Author MAO wrote the first draft of the manuscript. Author BIA did the literature search. All authors read and approved the final manuscript.

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ABSTRACT

Definite cure for HIV/AIDS is not yet documented and over 40 million people in the world are believed to be infected with the virus. The present study evaluates the effects of Zidovudine + Lamivudine + Nevirapine (HAART) and Bioclean II on female HIV/AIDS cases in Nigeria. Candidates on HAART (n=5) were recruited from Central Hospital, Stella Obasanjo Hospital and University of Benin Teaching Hospital, Benin City. The Bioclean II group (n=5) were placed on oral 10 ml twice daily Bioclean II for 8 months. Venous blood samples were collected from each individual in the two treatment groups at 0, 90 and 180 days intervals for analysis for viral load, CD_4^+T cells and sero-status. The body weights of the individuals were taken at the same time

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intervals. The mean viral load, CD_4^+T-cells count and body weights for the HAART Group were 299, 587.8 copies/ml – 199, 854.4 copies/ml; 123.2 cells/µl – 461.2 cells/µl and 44.6 kg – 48.6 kg while the Bioclean Group yielded 304161.8 copies/ml – 2785.0 cells/ml; 123.4 cells/µl – 752.6 cells/µl and 45.0 kg – 61.4 kg respectively. Though the sample size is small and therefore may not provide sufficient grounds for generalization without recourse to small sample theorem, the performance of Bioclean II in the three parameters investigated suggests that Bioclean II has a potential as an intervention tool in HIV/AIDS disease in the population investigated.

Keywords: HIV; AIDS; HAART; bioclean II; females; exposure.

1. INTRODUCTION

Thirty-one years after the emergence of HIV/AIDS pandemic, definite cure still remains elusive. The epidemic seems not to be declining but shows pockets of slowing down or stabilizing in different parts of the world [1]. In Nigeria, multi-sectional approach has been adopted in fighting the HIV/AIDS epidemic: Comprehensive antiretroviral drug treatment, prevention of mother to child transmission, voluntary counselling and testing and surveillance. These approaches have their major constraints. Take for instance, the antiretroviral drug treatment, the principal aim of the existing antiretroviral drug therapy is to suppress viral replication to as low as possible in order to delay disease progression but not to effect a cure. Consequently, the HIV/AIDS affected individuals would have to contend with a chronic, progressive infection and unpredictable outcome. The orthodox antiretroviral drugs can be categorised as follows: The reverse transcriptase inhibitors, protease inhibitors and fusion inhibitors [2,3]. Each of these groups attack one point or the other in the life cycle of HIV such as inhibition of reverse transcriptase, integrase, transactivator (tat) transcription and protease. These drugs have started showing undesirable side effects ranging from bone marrow suppression, insomnia, malaise, headaches, rashes, abdominal pains, nausea, vomiting, pancreatic disorder and kidney damage [4]. Some encourage resistance development in HIV [5]. Thus making continued search for viable alternatives or even cure very necessary.

The present study is on Bioclean II, a natural product obtained from plants in Nigeria which was aired by African Independent Television (AIT) in December 2012 in the episode titled, "Voyage of Discovery" and also reported by Ibeh et al. [6]. The efficacy of this product is to be evaluated comparatively with the standard regime of oral administration of antiretroviral in Nigeria (triple therapy) comprising 2 nucleoside reverse transcriptase inhibitor (NRTIs) plus a protease inhibitor (PI) (Zidovudine (AZT) + Lamivudine (3TC) + Nevirapine (NVP).

2. MATERIALS AND METHODS

2.1 Sample Collection

Candidates on HAART were recruited from Central hospital, Stella Obasanjo Hospital and University of Benin Teaching Hospital. The antiretroviral drug naïve came from private hospitals/private practitioners and volunteers who came on their own. Relevant documentations including informed consent forms and questioneers were administered to all of them.

2.1.1 Selection criteria

The selection criteria include, prior exposure to antiretroviral drugs (Multiple therapy), for not less than one year, female, age bracket of 25-40 years and CD_4^+ T cells of less than $200x10^6$ cells/L, sero status – HIV 1 and 2 positive for the antiretroviral drug wise group (ARDW) and strict adherence to drug regimen.

Antiretroviral drug naïve (single, double or triple therapy exposure) females, no multiple infection e.g. TB, Syphilis, Age between 25 and 40 years for the antiretroviral drug naïve group (ARDN).

2.2 Methods

The selected individuals in the antiretroviral drug naïve group were placed on 10 mls of Bioclean II twice daily for eight months. After 3 months and at the end of six months, blood samples were collected for analysis including time 0 (at the beginning of the treatment). The parameters evaluated were the viral load, CD_4^+ T cell counts, viral antibody detection, body weights and physical appearance of each candidate. Blood samples were collected from the selected individuals in the antiretroviral drug wise group and analysed for the same parameters.

2.2.1 Viral antibody detection

Briefly, 4.0 ml of venous blood were collected from each subject into a sterile vacutainer tube containing ethylene-diamine tetra acetic acid, 7.2 mg/4 ml solution (BDF Plymouth, PL67BP; UK). The tubes were arranged in racks and allowed to stand at room temperature (27±1.0℃) for 15 minutes. Using automatic pipettes, plasma was transferred from each tube into a pre-labelled plain specimen vial for the detection of antibodies to HIV1 and 2 using HIV rapid testing kits.

Each sample was screened with Determine and Unigold kits run in parallel. Every sample that reacted with both kits was regarded as positive while a non-reactive one was regarded as negative. A reaction with only one of the two kits was regarded as discordant and stat pak was used as a tie breaker. A positive result with stat pak confirmed positivity of the sample. All test kits were evaluated for potency and shelf-life before use.

2.2.2 Cd_4^+ t cells count

Briefly, the reagents – PE antibody, partec buffer and count check beads stored at 4^0C were brought out to attain room temperature (27±1.0℃). Using an automatic pipette (Eppendorf), 20 µl of PE was dispensed into a partec tube. Then 20 µl of well mixed sequestrated whole blood was added into the tube and shaken gently to mix the content thoroughly. The mixture was incubated in the dark at room temperature (27±1.0℃) for 15 minutes, mixing at 5 minutes interval. Thereafter, 800 µl of CD_4^+no lyse buffer was added to the tube and mixed gently. Then the tube was plugged to the counter (Flow cytometer; Partec Gmbh, Germany). During counting, it was ensured that the CD_4^+ bearing monocytes and noise were well separated and gated. The count check beads were run as control.

2.3 Viral Load Determination

Viral load was determined by the Polymerase Chain Reaction method. Briefly, the patient's blood and buffer were heated to a temperature of 95℃ for the hydrogen bonds of the double helix of HIV DNA to be broken, resulting in single-stranded molecules to be used as temperate. This is the denaturation stage. This was followed by lowering the temperature to 50-56℃, to allow the primers to anneal to the appropriate single-stranded DNA template, forming short segments of double-stranded DNA where the polymerase attached to begin DNA synthesis. This was followed by the extension/elongation step at 72℃ when the DNA polymerase (T_{aq}) synthesized new DNA strands which were complementary to the HIV DNA template strands. By repeating the cycle of denaturation-annealing-syntheses, copies of the viral DNA were amplified with their 5' and 3' ends both set by the primers.

2.4 Body Weight Measurement

The body weight of individuals were taken using the standing weighing balance (The Big Boss, Hana).

2.4.1 Statistics

Data generated were analysed statistically using SPSS package. The parameters determined include means, standard error, Analysis of Variance and locations of significant differences at the 95% and 99% confidence limits.

3. RESULTS

The profiles of the two groups of individuals investigated are shown in Tables 1, 1a and 1b. There was a reasonable similarity in age, sex and body weights of individuals in the two groups. The effects of treatment with Zidovudine +Lamivudine + Nevirapine and Bioclean II on body weights of the individualsinvestigated are shown on Table 2. There were increases in body weights of the individuals in the two treatment groups.

The effects of treatment with Zidovudine + Lamivudine + Nevirapine and Bioclean II on Body weights of the individuals investigated are shown on Table 2. There were increases in body weights of the individuals in the two groups at 90 days and 180 days post treatment. The Bioclean II treatment group recorded higher increases in body weight than the triple drug (Zid + Lam + Nev) group. The differences in body weight gain at the two intervals were significant ($p < 0.05$; $p < 0.001$).

The effects of treatment on the viral load of individuals investigated are shown on Table 3. Triple drug treatment and Bioclean II produced viral load reductions in the individuals investigated. The rate of viral load reduction in the Bioclean II treatment group was higher than the HAART group.

Table 4 shows the effect of treatment on the CD_4^+ T cells of the individuals investigated. There were increases in CD_4^+ T cells count at 90 and 180 days treatment intervals. The Bioclean II treatment group recorded higher increase in CD_4^+ T cells than the Zid + Lam + Nev treatment group. The differences in the rate of CD_4^+ T cells increases by the two treatments were significant ($p < 0.001$).

Table 1. Profile of HIV/AIDS cases in the HAART group

S/N	Code	Sex	Age	ARDW	Drug expt in years	CD_4^+ cells count/ml
1	A	F	36	+	$1\,1/2$	105
2	B	F	28	+	$1\,1/2$	102
3	C	F	32	+	2	110
4	D	F	31	+	$1\,1/2$	89
5	E	F	37	+	2	102

Key: ARDW = Antiretroviral drug Wise – Zidovudine + Lamivudine + Nevirapine

Table 1a. Profile of HIV/AIDS cases in the HAART group

S/N	Height (cm)	Body weight (kg)	Viral load copies/ml	Physical appearance	Sero status HIV 1 and 2
1	153	51	312,572	Gauntly with disorientation	+
2	149	43	308,314	Gauntly with disorientation	+
3	153	41	283,163	Gauntly with disorientation	+
4	152	48	340,218	Gauntly with disorientation	+
5	150	50	253,671	Gauntly with disorientation	+

Table 1b. Profile of HIV/AIDS individuals in the bioclean II treatment group

S/N	Height (cm)	Body weight (kg)	Viral load (copies/ml)	Sero status (HIV 1 and 2)	ARDW	CD4+ T cells (count/ml)	Sex	Age (years)
1	148	41	413,278	+	-	112	F	36
2	151	43	342,167	+	-	120	F	35
3	150	43	342,814	+	-	114	F	32
4	152	48	438,826	+	-	87	F	27
5	150	50	411,572	+	-	113	F	28

Table 2. Effects of Zidovudine + Nevirapine + Lamivudine and bioclean II on body weight of female HIV/AIDS cases

Item	Treatment (n=5)		Statistical T-test	Control
	Zid + Nev + Lam	Bioclean II		
Period (days)	Body weights (kg)		P value	
0	43, 41, 40, 48, 51 (mean 44.6)	43, 43, 41, 48, 50 (mean = 45.0)	P > 0.05	58.2
90	45, 44, 43, 50, 50, 53 (mean= 47.0)	60, 51, 52, 58, 61 (mean = 56.4)	P < 0.05	60.5
180	46, 48, 45, 49, 55 (mean= 48.6)	62, 58, 62, 60, 65 (mean= 61.4)	P < 0.001	60.2

Table 3. Effects of Zidovudine + Nevirapine + Lamivudine and bioclean II on viral load of HIV/AIDS cases

Item	Treatment (n=5)		Statistical T-test	Control
	Zid + Nev + Lam	Bioclean II		
Time (in days)	Viral load (copies/ml)		P value	
0	312572, 308314, 283163, 340218, 253671 (mean =299587.6)	324362, 315460, 290231, 346213, 247543 (mean=304161.8)	P > 0.05	ND
90	76, 340, 112326, 164706, 211327, 117, 471 (mean=136234)	12321, 7351, 5326, 2401, 11210 (mean=7721.8)	P < 0.001	ND
180	211370, 213052, 165725, 207682, 201443 (mean=199854.4)	ND, ND, 250, 5320, ND (mean=2785)	P < 0.001	ND

Key: ND = Not Detectable

Table 4. Effects of Zidovudine + Nevirapine + Lamivudine and bioclean II on Cd_4^+ T-cells of HIV/AIDS cases

Item	Treatment (n=s)		Statistical T-test	Control
	Zid + Nev + Lam	Bioclean II		
Time (in days)	CD_4^+ T-cells count (x/µl)		P-value	
0	112, 108, 180, 102, 114 (mean=123.2)	108, 116, 104, 182, 107 (mean =123.4)	P > 0.05	1102
90	326, 241, 372, 413, 425 (mean=355.4)	823, 706, 568, 604, 712 (mean = 682.6))	P < 0.001	1194
180	428, 392, 561, 524, 401(mean= 461.2)	892, 756, 692, 785 (mean= 752.6)	P < 0.001	1011

4. DISCUSSION

The effects of treatment with Zidovudine + Lamivudine + Nevirapine (HAART) and Bioclean II on female HIV/AIDS cases in Nigeria were investigated. Individuals in the two treatment groups were balanced in terms of sex, age, body weight and CD_4^+ T cells at the beginning of the investigation (Tables 1, 1a and 1b). HAART treatment was provided by approved Heart-to-Heart clinics in Benin City and strict compliance was a selection criterion.

Treatment with triple drugs for over one year produced improvement in the body weights of the HIV/AIDS cases investigated (Table 2). This finding is in agreement with reports of other workers [6-8]. Bioclean II produced a superior body weight gain by HIV/AIDS cases aftermonths exposure (Table 2). This finding is in agreement with our earlier report on the effect of Bioclean II on body weights of HIV cases [5]. The implication is that the two treatment methods had effect on the metabolic activities of the individuals investigated which was translated to body weight gain.

The HAART and Bioclean II treatment produced reductions in the viral load of the HIV/AIDS cases investigated (Table 3). Reduction of viral load in HIV/AIDS cases by HAART is already documented [9,10]. The findings in the present study suggest that Bioclean II has the potential to reduce the viral load of HIV/AIDS cases. That it did so at a shorter exposure time than HAART and at a higher rate is very interesting (Table 3). This result suggests that Bioclean II has an effective inhibitory effect on the replication of HIV. The mechanism by which Bioclean II interferes with the replication of HIV needs to be further investigated.

The effects of treatment with HAART and Bioclean II extended to the CD_4^+ T cells of the individuals investigated (Table 4). The two treatment methods produced increases in the CD_4^+ T cells ^{count} of the individuals investigated. Improvement in CD_4^+ T cells in HIV/AIDS cases

exposed to HAART has been documented [11,12]. The observed increases in CD_4^+ T cells of HIV/AIDS in this study is in agreement with our earlier report [5]. This finding suggests that Bioclean II is very effective in restoring depleted CD_4^+ T cells associated with HIV infection and progression to AIDS. Although the mechanism of action of Bioclean II is not yet fully understood, it is possible that there may be a direct arrest of CD_4^+ cells destruction, abolition of apoptosis of bystander cells, reduction of CD_8 cells destruction of CD_4^+ T cells infected with HIV and neutralization of oxidative stress that destroys cells [13-16].

The performance of Bioclean II in this study suggests need for further studies to understand the mechanism of action of this local herbal product with a view to ascertain its value as an intervention tool in the cycle of HIV/AIDS disease.

5. CONCLUSION

Data from this study show that HAART and Bioclean II have the potential to influence HIV replication, regulate CD_4^+ T-cells in injected individuals and improve body weight gain. The significance of these results in the management of HIV/AIDS disease needs proper evaluation.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Anderson J. Women and HIV: Motherhood and more. Curr. Opin. Infect. Dise. 2012; 25(1):58-65.
2. Coutsoudis A, Kwaan l, Thomson M. Prevention of vertical transmission of HIV-1 in resource limited settings. Exp. Rev. Anti-infect. Ther. 2010;8(10):1163-1175.
3. Coovadia H. Antiretroviral agents – how best to protect infants from HIV and save their mothers from AIDS. N. Engl. J. Med. 2004;351(3):289-292.
4. Ibeh IN, Akanu N, Mkpa AM, Isitua CC, Ogefere HO. Evaluation of Anti-human Immunodeficiency Virus (HIV) properties of DXL (Decoction X-Liquid-Bioclean II). J. Clin. Toxicol. 2013;S12:004.

   DOI: 10.4172/2161-0495. S12-004
5. Ibeh IN, Nnachi A, Mkpa AM, Okungbowa MA, Ibeh NI. Studies on the effects of Bioclean II on female HIV/AIDS cases in Nigeria (In Press); 2014.
6. Kripke C. Antiretroviral prophylaxis for occupational exposure to HIV. Amer. Faml. Phys. 2009;76(3):375-376.
7. Merril DP, Moonis M, Chou TC. Lamivudine or stavudine in two and three drug combinations against human immunodeficiency virus type 1 replication *in-vitro.* J. Infect. Dis. 1996;173:355-364.
8. Odunukwe N, Idigbe O, Kanki P, Adewole J, Onwujekwe D, Amdu R, Onyewuche J. Haematological and biochemical response to treatment of HIV-1 infection with a combination of nevirapine + stavudine + lamivudine in Lagos, Nigeria. turk. J. Haematol. 2005;22:125-131.
9. Osayande DO. Immunological assessment of HIV and AIDS patients on antiretroviral drugs. Ph.D. Thesis, University of Benin, Nigeria; 2014.
10. Raman KV, Rao R. Human immunodeficiency virus disease management in highly active antiretroviral therapy era: A comprehensive review. Ann. Trop. Med. Publ. Hlth. 2013;1(6):5-9.
11. Raman KV. Markers of HIV-1 disease progression and treatment response in highly active antiretroviral therapy (HAART) Era: A review. Am. J. Infect. Dis. Microbiol. 2013;1(5):96-100.
12. Raman KV. Effect of Highly Active Antiretroviral Therapy (HAART) on human immunodeficiency virus disease pathogenesis and progression. Am. J. Publ. Hlth. Res. 2014;2(3):68-74.
13. UNAIDS. Intensifying HIV prevention: A UNAIDS Policy Position Paper; 2005.

14. Akinbami A, Dosunmu A, Adediran A, Ajibola S, Oshinaike O, Wright K, Arogundade O. CD_4^+ count pattern and demographic distribution of treatment-naïve HIV patients in Lagos, Nigeria. AIDS Res. Treat. 2012;1:1-6.
15. UNAIDS. UNAIDS REPORT on the Global AIDS Epidemics; 2010.
16. Venkat A, Piontkowsky DM, Cooney RR, Srivastava AK, Suares GA, Heidelberger CP. Care of the HIV-positive patient in the emergency department in the era of highly active antiretroviral therapy. Ann. Emerg. Med. 2008;52(3):274-285.

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