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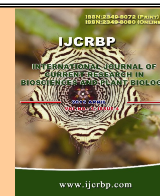

Original Research Article

Comparative Study of CD8+ T-Cell Count in Human Immunodeficiency Virus Infection in Umuahia, Nigeria

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A b s t r a c t

In this study, a total number of ninety (90) subjects within the age of 18-60 years were used for the study. The subjects were divided into three (3) groups of thirty (30) subjects in each group. Group I comprised of control subjects. Group II were HIV- subjects not on therapy, and group III were AIDS-subjects not on therapy. Blood samples were collected into commercially prepared dipotassium EDTA vacutainer for both test and control subjects after informed consent. These were used in determining their CD8+ cells counts. The results showed that the CD8+cells count of group II was significantly higher than that of group I while the CD8+ cells count of group III CD8+ showed a statistically higher level when compared with those of groups I and II at $p<0.05$. The results therefore showed that following HIV infection there is expansion of CD8+ cells.

K e y w o r d s

CD8 T-cell counts

Human Immunodeficiency Virus

Immune system

Introduction

The immune system plays a central role not only in health maintenance but also in pathogenesis. It is a system of biological structures and processes within an organism that protects against disease. For it to perform its functions, it must ensure continuous reception and processing of information about the antigenic state of the organism. This perception must allow an evaluation of how serious is any divergence from the norm whose notion varies during the development of the organism and between different organisms (Igor et al., 2010). Surveillance of the human body is accomplished in part by means of patrolling white blood cells or leucocytes (Darius et al., 2003). There are two major arms of the immune system, one which works through antibodies, which are produced by B-cells and plasma cells, and the other that works through direct cellular action and which relies heavily on CD4+ T-cells. The first is called

antibody-mediated or humoral immunity, and the second is called cell-mediated immunity. It is the cell-mediated arm of the immune system that is found to be profoundly suppressed in people diagnosed with AIDS. The antibody-mediated arm of the immune system, however, is usually hyper stimulated in the early stages, with "increasing levels of humoral antibodies and plasma cells". The fact that antibody levels are increased is what allows the HIV antibody screening tests to use serum that has been diluted 400 times, unlike other antibody tests that usually use straight, undiluted serum. In these early stages the lymph nodes may grow in size and be chronically enlarged. In late stages, however, both the cell-mediated and antibody-mediated arms of the immune system begin to fail and lymph node atrophy results (Irwin, 2001).

The CD4/CD8 ratio is considered a marker of disease progression in HIV and AIDS, and is often found to be inverted meaning that there are less CD4 cells than CD8 cells, resulting in a ratio of less than 1. CD8 cells are often increased, especially in less advanced stages of AIDS, and this combination of lowered CD4 counts and increased CD8 counts are commonly thought to occur only in people diagnosed HIV-positive. Another finding that is common in people diagnosed HIV-positive is reduced lymphocyte activity and function, as measured by their responsiveness to foreign antigens. This can result in a state of "anergy", where people's skin fails to respond when antigens are injected under it (Irwin, 2001).

CD8 T cells are powerful components of the adaptive immune system, yet were not formally recognized until long after the discovery of antibody. CD8 T cells contribute to the eradication of intracellular infections and to the control of many chronic infections (Masopust et al., 2007). The CD8 (cluster of differentiation 8) which is a transmembrane glycoprotein that serves as co-receptor for the T-cell receptor binds specifically to class 1 major histocompatibility complex (MHC) molecule. Its responses in HIV infection can be divided into (1) the lytic response (Cytotoxic T Lymphocytes, CTLs) which make use of proteins in their cytoplasm such as peforin and granzymes for cell lysis (direct killing response) and (2) Non-lytic responses (chemokines) which are soluble substances secreted by CD8 cells e.g. cytokines that work by either inhibiting HIV replication or inhibiting viral entry into target cells changing the viral set point. During many infections, all T lymphocytes regardless of specificity may undergo cytokine- driven phenotypic changes –so called bystander activation , but only those T-cells that recognize pathogen- encoded antigen go through multiple rounds of replication to generate enormous numbers of CTL (Cytotoxic T Lymphocytes) effector progeny that are the foot soldiers of the adaptive immune response (Nu and Michael, 2011). The present study has been aimed to comparatively study CD8+ cell counts in HIV /AIDS subjects and apparently healthy subjects in Umuahia, Nigeria.

Materials and methods

The study was conducted at the HIV/AIDS clinic of Federal Medical Centre (F.M.C.), Umuahia which is located at the heart of Abia State capital territory. Umuahia covers a land mass of 245km^2, with Latitude of 5.5267 (decimal degree) North and Longitude 7.48959 (decimal degree) East with a population of 264,662 (Mongobay, 2012).

Ethical considerations

This study was done through oral consent of the subjects prior to sample collection.

Study population and enrolment

A total number of Ninety (90) subjects were used which was calculated based on the prevalence rate of HIV infected subjects. These subjects with the age of 18-60 years were grouped into three (3) of thirty (30) each. Group I was made up of control subjects while groups II and III were made up of HIV subjects and AIDS subjects respectively. The HIV subjects were selected from donors that came to donate blood at the hospital and those that came for premarital counselling and testing after testing only positive to HIV screening. The AIDS patients were selected from ART clinic at F.M.C. Umuahia. The control subjects were selected from donors who were fit and from voluntary workers at the laboratory department F.M.C Umuahia.

Selection criteria

Oral consents were obtained from the subject who also accented to the sample collection. The group I (controls) were selected on the basis that they were

apparently healthy and showed no signs and symptoms of any viral (hepatitis B and C), systemic or cardiovascular diseases from the pre donating screening done on them such as clerking, HIV test, Hepatitis test, etc.

The group II (HIV-patients) were selected having being confirmed of having HIV infection by the standard technique and not reactive to any other viral infections (hepatitis B and C), no other complications associated with the HIV infection and that are not sicklers or immunocompromised from their CD4 cell count. Group III (AIDS- Patients) were picked on the basis that they were confirmed of having AIDS and showed all AIDS indicator conditions (but no history of tuberculosis or reactive to any other viral infection.

Exclusion criteria

The subjects showing any underlying signs and symptoms of diseases other than HIV and AIDS for the test subjects were excluded from the study. While the control groups reactive to any viral infections including HIV/AIDS or that are immunocompromised were excluded.

Sample collection

About 6ml of venous blood was aseptically collected from the patients using a standard venipuncture technique. About 3ml was dispensed into a commercially prepared dipotassium EDTA Vacutainer (Beckon, Dickson and Company) while the remaining 3ml was dispensed into a dry plain plastic tube and allowed to clot. The samples were centrifuged at 3000 rpm for 10minutes to separate the plasma and the serum respectively. Whole blood was used for analysis of CD8 while the serum was used for confirmation of HIV and other viral infections. All the reagents and kits were bought commercially from a reputable company as the standard operating procedures were strictly adhered to.

CD8 count

The CD8 count was done using flow cytometer (partec flow cytometer).While the cells are passing through a flow cuvette one-by-one, the cells are individually illuminated by the light spot of the laser. Due to the excitation, the dye molecules emit fluorescence of characteristic colour (emission wavelength spectrum). The fluorescence light is separated into colour ranges by means of optical filters. The intensity of each colour is analyzed for each single cell (Fryland et al., 2006). About 20ul of sample (whole blood) was added with 20ul of $CD8^{+}$ fluorochrome conjugated monoclonal antibodies into a test tube, mixed and incubated at room temperature in the dark for 10 minutes. After the incubation period, 800ul of the buffer was added. The sample tube was then inserted unto the sample port on the flow cytometer until a click sound was heard, sample aspirated and measurement starts automatically.

HIV testing

The HIV status of the patients was done using both screening kits (Stat pak and Determine test kit) and confirmatory kit (Orenic Immunocomb II HIV 1&2 comfirm).

HIV screening test

Chembio HIV 1/2 STAT-PAK (USA): The Chembio HIV 1/2 STAT-PAK assay is a single use, immunochromatographic test used to detect antibodies to Human Immunodeficiency Virus Type 1 (HIV-1) and Type 2 (HIV-2) in finger stick, whole blood, venous whole blood, serum and plasma specimens. This employs a unique combination of a specific antibody binding proteins, which is conjugated to colloidal gold dye particles, and HIV- 1/2 antigens, which are bound to the membrane solid phase. The sample is applied to the sample(s) well followed by the addition of running buffer. This facilitates the lateral flow of the released products and promotes the binding of antibodies to the antigens. If present, the antibodies bind to the gold conjugated antibody binding protein.

In a reactive 9sample, the dye conjugated- immune complex migrates on the nitrocellulose membrane and is captured by the antigens immobilized in the test (t) area, producing a pink /purple line while in non-reactive sample, the pink/purple colour is absent but there will always be a pink/purple colour in the control region containing immunoglobulin G antigens indicating a proper performance. With the sample loop provided, 5ul of the sample was taken and applied on the sample pad of the device. Then three drops (105ul) of the running buffer were added on the sample well also. The result was then read after 10 min.

Interpretation of test performance: When the test was completed, a pink/purple line appeared in the control (C) area of the test device on nonreactive as well as reactive samples. This control line served as an internal control and gave confirmation of sample addition and proper test performance. Pink/purple lines in both the TEST (T) and CONTROL (C) areas indicated a reactive sample. Specificity= 100%, and sensitivity= 100%.

Alere Determine HIV- 1/2 Kit (JAPAN)

Alere Determine HIV- 1/2 Kit is an in vitro, visually read qualitative immunoassay for the detection of antibodies to HIV- 1 and HIV- 2 in human serum, plasma or whole blood. The test is intended as an aid to detect antibodies to hiv-1/ hiv-2 from infected individuals. Alere Determine hiv-1/2 is an immunochromatorapic test for the qualitative detection of antibodies to hiv-1/hiv-2. As sample is added to the sample pad and allowed to migrate through the conjugate pad, it reconstitutes and mixes with the selenium- colloid-antigen conjugate.

The mixture continues to migrate through the solid phase to the immobilized recombinant antigens and synthetic peptide at the patient’s window site. If antibodies to HIV-1 and/or HIV -2 are present in the sample, they bind to the antigen- selenium colloid and to the antigen at the patient window forming a red line at the patient’s window site, but if the antibodies to HIV-1 and HIV -2 are absent, the antigen-selenium colloid flows past the patient window, and no red line is formed at the patient window site.

The desired number of test units from the 10- test card was removed by bending and tearing at the perforation. The protective cover from each test was removed. 50ul of sample (serum) was added to the sample pad and allowed to flow through the solid phase. The result was read within a minimum of 15 min.

Interpretation of results

Positive result (two bars): Red bars appeared in both the control window (labelled " control") and the patient window (labelled "patient") of the strip. Visible red colour in the patient window was interpreted as positive.

Negative result (one bar): One red bar appeared in the control window of the strip (labelled " control") and no red bar appeared in the strip (labelled "patient").

Invalid (No bar or one bar only in patient window): If there is no red bar in the control window of the strip and even if a red bar appears in the patient window of the strip, the result is invalid and should be repeated. Specificity=99.75% and Sensitivity=100.00%

HIV confirmatory test

The HIV confirmatory testing was done using an indirect solid phase Enzyme Immunoassay (EIA) kit (Orgenic Immunocomb II HIV 1&2 combfirm kit). The ImmunoComb® II HIV 1 & 2 CombFirm test is an indirect solid-phase enzyme immunoassay (EIA). The solid phase is a card with 12 projections ("teeth"). Each card has 6 pairs of teeth, with six antigen spots per pair (3 spots on each tooth). The left tooth of each pair carries an upper spot sensitized with human immunoglobulin (Internal Control), and the two protein markers p24 (*gag)* and p31 (*pol*). The right tooth has three *env*-derived protein spots gp41, gp120 and gp36. The Developing Plate has 6 rows (A-F), each row containing a reagent solution ready for use at a different step in the assay. The test is performed stepwise, by moving the Card from row to row, with incubation.

To start the test, serum or plasma specimens are added to the diluents in the wells of row A of the Developing Plate. The Card is then inserted in the wells of row A. Anti-HIV antibodies, if present in the specimens, will specifically bind to the HIV antigens on the teeth of the Card. Unbound components are washed away in row B. In row C, the anti-HIV IgG captured on the teeth, and the human immunoglobulin on the upper spots (Internal Control), will react with anti-human IgG antibodies labelled with alkaline phosphatase (AP). In the next two rows, unbound components are removed by washing. In row F, the bound alkaline phosphatase will react with chromogenic components. The results are visible as gray-blue spots on the surface of the teeth of the Card. The kit contains 3 plastic Cards. Each Card has 6 pairs of teeth, one pair for each test. Both teeth of a pair carry identical numbers. Each pair of teeth is sensitized with six reactive areas.

Developing plates: The kit contains 3 Developing Plates, covered by aluminium foil. Each developing Plate contains all reagents needed for the test. The developing Plate consists of 6 rows (A–F). Row A is divided into 6 wells, whereas the other rows are divided into 12 wells each. The contents of each row are as follows:

Row A: specimen diluent

Row B: washing solution

Row C: alkaline phosphatase-labeled goat anti-human IgG antibodies

Row D: washing solution

Row E: washing solution

Row F: chromogenic substrate solution containing 5-bromo-4-chloro-3-indolyl phosphate (BCIP) and nitro blue tetrazolium (NBT).

Statistical analysis

The results were expressed as mean and standard deviation (x±SD) the analysis was done using student's t-test and Pearson correlation analysis with the statistical package for social science (SPSS) version 13. The level of significance was at $p<0.05$.

Results and discussion

The results of the ninety subjects (90) used for the study was as tabulated below and the results obtained have been summarized below in Figs. 1, 2 and 3. From the Figures, it was observed that the CD8 of group II was statistically higher when compared with that of the control group I while that of the group II showed statistical difference when compared with both the groups I and II at $p<0.05$ (Fig. 1).

Fig. 1: The effect of CD4 counts on coagulation parameters among the study groups.

Fig. 1: CD8$^+$ cells/ml of the studied groups (I, II and III). I = Control; II = HIV-infected patients not on therapy; III= AID-patients not on therapy; CD8 = Cluster of differentiation 8; Trend line indicates the CD8+ cells variation trend among study groups.

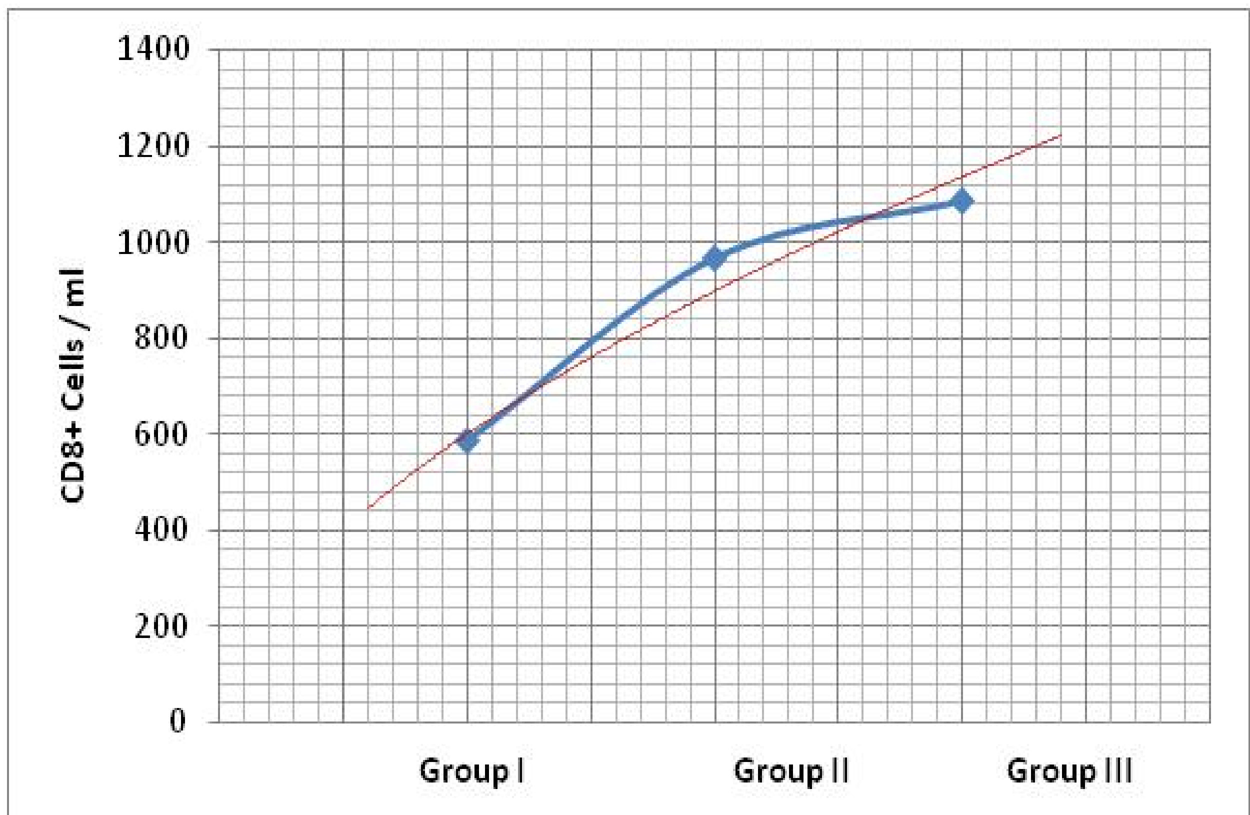


Fig. 2: Gender-wise distribution of studied groups (I, II and III). I = Control; II = HIV-infected patients not on therapy; III= AID-patients not on therapy.

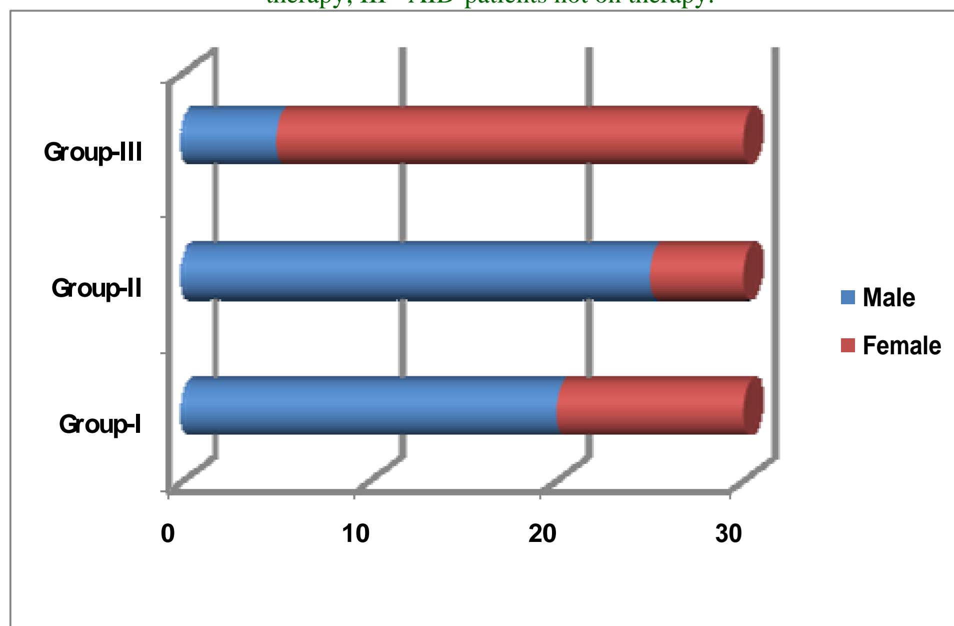
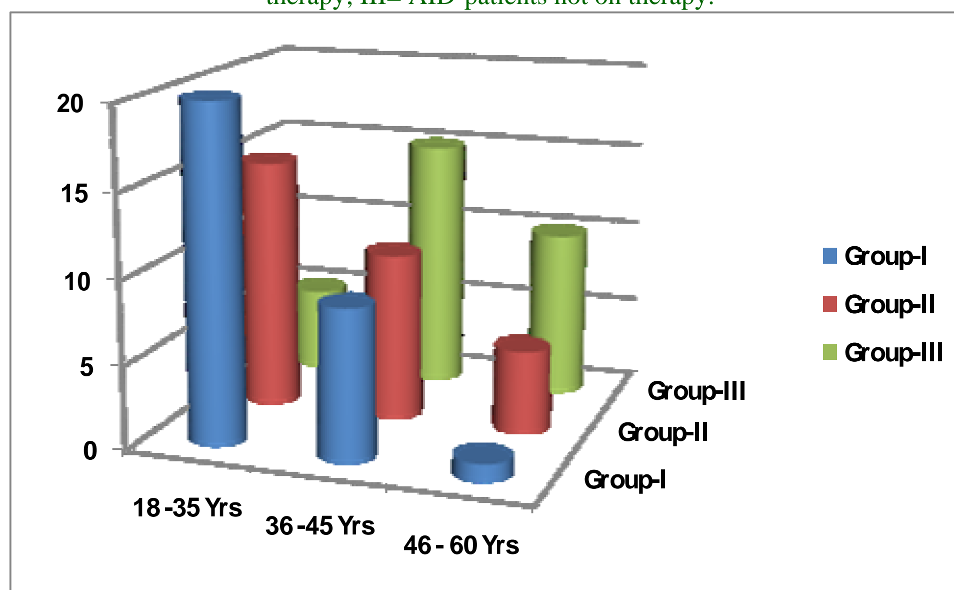


Fig. 3: Age-wise distribution of studied groups (I, II and III). I = Control; II = HIV-infected patients not on therapy; III= AID-patients not on therapy.



In this study it was observed that the CD8 cells of the AIDS patients are significantly higher when compared with those of the HIV patients and the control groups (III > II > I). This is in line with the finding that during HIV infection, HIV replication leads to activation of the innate and adaptive immune system ,generating an inflammatory environment associated with the induction of type 1 IFN (Tilling et al., 2002),whose chronic exposure is detrimental to T cell homeostasis and survival (Boasso et al., 2008). This stands to reason that as the HIV infection advances, there is a compromise in leucopoiesis which could arise from defect in the production of leukopoietin that regulates leucopoiesis. The highest mean value of CD8 cells was observed in AIDS patients (group III) indicating increased activation of immune cells as the disease progresses (Krishna et al., 2006). According to Michael (2011), overstimulation of CD8 response and its elevated count has been associated with accelerated HIV disease progression.

In HIV infection, there is reduction in numerical and functional strength of CD4 cells with the expansion of CD8 cells which are cytotoxic cells, with consequent decline in immunocompetence as the disease progresses (Martha et al., 2011). In an average healthy adult between 50 to 70 billion cells die per day due to apoptosis (Karam, 2009) but this may be increased due also to increased apoptosis occasion by increased production of inflammatory cytokines secondary to HIV infection. This study showed increase in proliferation of T lymphocytes measured by CD8 counts in HIV infection; therefore it would be of immense health benefit to include CD8 count to serve as a prognostic guide in virologic treatment and management of HIV/AIDS patients.

Conclusion

The CD8+cell count of HIV positive patients showed statistical difference when compared with apparently healthy controls while CD8+cell count of AIDS patients showed a remarkable difference when compared with both HIV patients and apparent healthy individuals.

References

Boasso, A., Shearer, G. M., 2008. Chronic innate immune activation as a cause of HIV immunopathogenesis. Clin. Immunol. 126, 235–245.

Darius, M., Mark, S. F., 2003. Immune System Overview. Encyclopedia of the Neurological Sciences. pp.633 -639.

Fryland, M., Chaillet, P., Zachariah, R., Barnaba, A., Bonte, L., Andereassen, R., Charrondière, S., Teck, R., 2006 .The Partec CyFlow Counter could provide an option for CD4+ T-cell and CD8+ T-cell monitoring in the context of scaling-up antiretroviral treatment at the district level in Malawi. Transac. Royal Soc. Trop. Med. Hygiene 100(10), 980-985.

Igor, M. D., Dresser, D., 2010. Immune system as a sensory system. Int. J. Biomed. Sci. 6(3), 167–175.

Irwin, M., 2001. Low CD4 T Lymphocyte Counts: A Variety of Causes and their Implications to a multifactorial Model of Aids. Virus Myth Homepage. 1p.

Karam, J. A., 2009. Apoptosis in Carcinogenesis and Chemotherapy. 2nd Edn. Springr Netherlands. 45p.

Krishna, R., Gupta, S. M., Manju, B., Sumathi, M., Joginder, K., 2006. CD4: CD8 lymphocyte count in healthy, HIV positive individuals and AIDS patients. Indian J. Med. 124(1), 319–330.

Masopust, D., Vezys, V., John Whenry, E., Ahmed, R., 2007. A brief history of CD8 T cells. Eur. J. Immunol. 37(1), 103-110.

Michael, C., 2011. Elevated CD8 counts during HAART are associated with virologic treatment failure. J. Acquired Imm. Deficiency Synd. 10, 15–20.

Milstein, O., Hagin, D., Lask, A., Reich–Zeliger, S., Shezan, E., Ophir, E., Eidelshtein, Y., Afik, R., Antebi, Y. E., Dustin, M. L., Reisner, Y., 2011. CTLs respond with activation and granule secretion when serving target for T cell recognition. Blood 117, 1042–1052.

Mongobay, 2012. Population of Umuahia, Nigeria Population . mongobay.com/../umuahia.

Nu, Z., Michael, J. B., 2011. CD8 T cells: foot soldiers of the immune system. Immun. 35(2), 161–168.

Tilling, R., Kinloch, S., Goh, L. E., 2002. Parallel decline of $CD8^{+}/CD38^{++}$ T cells and viraemia in response to quadruple highly active antiretroviral therapy in primary HIV infection. AIDS 16, 589-596.