Journal of Advances in Medicine and Medical Research

23(10): 1-9, 2017; Article no.JAMMR.36033
ISSN: 2456-8899
(Past name: British Journal of Medicine and Medical Research, Past ISSN: 2231-0614, NLM ID: 101570965)

Lymphocyte Transformation and Nitro-blue Tetrazolium Reduction Rate of Neutrophils among HIV Infected Adults in Sokoto Metropolis

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Authors' contributions

This work was carried out in collaboration between both authors. Authors AY and MUK designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author AY managed the analyses of the study. Author MUK managed the literature searches. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMMR/2017/36033
Editor(s):
(1) Thomas I. Nathaniel, University of South Carolina, School of Medicine-Greenville, Greenville, USA.
Reviewers:
(1) Karpal Singh Sohal, Muhimbili University of Health and Allied Sciences, Tanzania.
(2) Neema Tiwari, Era's Luck Now Medical College and Hospital, India.
Complete Peer review History: http://www.sciencedomain.org/review-history/20902

Original Research Article

Received 9th August 2017
Accepted 29th August 2017
Published 9th September 2017

ABSTRACT

Aim: The study was aimed at evaluating the functional activity of lymphocytes and neutrophils among HIV positive adults in Sokoto, Nigeria.
Study Design: This was a cross sectional comparative study of HIV positive subjects on HAART, HAART Naïve and apparently healthy control participants.
Methodology: A total of 157 adults were recruited for the study comprised of90 HIV seropositive subjects and 67 age and sex-matched apparently healthy controls.The subjects (HIV infected participants) were further sub-grouped into four different groups based on the revised WHO standard criteria for staging of HIV/AIDS infected adults; as Clinical stage I (n=31), Clinical stage II (n=25), Clinical stage III (n=19) and Clinical stage IV (n=15).The CD_4^+ cells were evaluated using flow cytometric method, percentage transformed cells were evaluated using culture techniques and neutrophil phagocytic activity was determined using Nitro- Blue Tetrazolium reduction test (NBT).

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Data were analyzed using SPSS Version 20. A *p*-value ≤ 0.05 was considered statistically significant.

Result: The $CD4^+$ count, percentage lymphocyte transformation and Neutrophil ingestion rate of NBT were significantly lowered in HIV infected subjects compared with controls ($p<0.05$). The $CD4^+$ count, percentage transformed cells and formazan generated by neutrophil was significantly higher among female subjects compared with male subjects ($p<0.05$).

Conclusion: Our findings revealed a lowered blast formation and neutrophil ingestion rate of NBT indicating a functional derangement in the innate and adaptive immune responses.

Keywords: Lymphocyte transformation; neutrophils; NBT; CD_4^+; HIV; Sokoto.

1. INTRODUCTION

Human Immunodeficiency Virus (HIV) is a major public health problem in sub-Saharan Africa; Nigeria inclusive, it's known to cause malfunction of the immune cells thereby predisposing to profiles of morbidity and fatality [1]. AIDS was first recognized in 1981 and by 2009 had caused nearly 30 million deaths [2]. As of 2012, approximately 35.3 million people were living with HIV globally, approximately 17.2 million were men, 16.8 million were women and 3.4 million were less than 15 years old [2]. There were about 1.8 million deaths from AIDS in 2010, down from 2.2 million in 2005 [2]. Sub-Saharan Africa is the region most affected. In 2010, an estimated 68% (22.9 million) of all HIV cases and 66% of all deaths (1.2 million) occurred in this region [3]. Cell-mediated immune dysfunction in acquired immune deficiency syndrome (AIDS) is primarily characterized by a qualitative and quantitative defect of helper/inducer-T lymphocyte subset [4]. The cellular immune defects of individuals with AIDS also affects monocyte function, natural killer cell activity, proliferative responses of lymphocyte to mitogens and antigens, and the production of lymphokines [5]. HIV infection is characterized by high rate of viral replication throughout the course of the infection which results in immune mediated destruction of $CD4^+$ T cells. Consequently, the infected individual becomes susceptible to opportunistic infections, malignancies and neurological diseases [5]. Neutrophils are the major cells in the host defense against bacterial and fungal infections. The decreased number of neutrophils in individuals with HIV infection is accompanied by multiple abnormalities and/or neutrophil dysfunction, which compromise the ability to kill bacterial and fungal invaders. Other abnormalities may be impairments of chemotaxis and phagocytosis, expression of cellular adhesion molecules at the surface of the neutrophil; production of reactive oxygen species, and actin shedding. Impaired neutrophil function has been demonstrated in both symptomatic and asymptomatic individuals with HIV infection [6]. Also an accumulative evidence supporting the role played by neutrophils in the negative regulation of T cell function via production of reactive oxygen species (ROS) and arginase-1 have been reported to be impaired in HIV infection [7]. The initial spike in HIV load in the acute phase of the infection is accompanied by an initial immunologic response that tries to limit the multiplication of the viral particles within the host, Once this attempt to clear the virus fails the infected host will experience gradual drop in the population of the T lymphocytes due to exponential multiplication of the virus and this may lead to some varying degrees of impairment in host immunity. The pattern of immune deterioration or stability varies amongst individuals. Hence the need for continued assessment of HIV infected individuals in order to monitor and understand the immune responses necessary in the fight against HIV/AIDS. This present study examined the lymphocyte and neutrophil functional activities and established the role of acquired and natural immunity in HIV-infected subjects.

2. MATERIALS AND METHODS

2.1 Study Subjects

A total of 157subjects participatedin this study comprised of 90 HIV positive subjects (study group) and 67 HIV negative healthy individuals (control group). The study constituted only consenting adults that were 18 years and above. This was a cross-sectional comparative study of HIV positive patients on HAART, HAART Naïve and apparently healthy control participants. The subjects were recruited by simple random sampling technique. Using a structured interviewer administered questionnaire, socio-demographic characteristics including age, gender, marital status, tribe, occupation and

educational level of the study subjects were obtained. Newly diagnosed HIV infected subjects that had Tuberculosis, hypertension or diabetes were excluded from the study.The subjects (HIV infected participants) were further sub-grouped into four different groups based on the revised WHO standard criteria for staging of HIV/AIDS infected adults; as Clinical stage I (n=31), Clinical stage II (n=25), Clinical stage III (n=19) and Clinical stage IV (n=15). The subjects were also grouped based on their treatment regimen into six different groups as Group A (n=31): HIV infected patients that are HAART naïve. Group B (n=15): First line treatment with zidovudine and lamivudine (Combivir). Group C (n=12): First line treatment with zidovudine, lamivudine and navirapine (Combipack), Group D (n=10): First line treatment with lamivudine and tenofovir (Tenolam), Group E (n=14): Second line treatment with lopinavir and ritonavir (Kaletra), Group F (n=8): Second line treatment with atazanavir and ritonavir.

2.2 Description of the Study Area and Participating Hospitals

The study was conducted in the faculty of medical laboratory sciences Usmanu Danfodiyo University, Sokoto (UDUS), Nigeria. Samples were collected at Institute of Human Virology, Nigeria(IHVN),Usmanu Danfodiyo University Teaching Hospital (UDUTH), ART clinic of Specialist Hospital, and Murtala Muhammad Hospital Sokoto.

2.3 Ethical Approval and Informed Consent

The Ethics and Research Committee of Usmanu Danfodiyo University Teaching Hospital (UDUTH) Sokoto, and Sokoto State Ministry of Health approved the study protocol. Written informed consent was obtained from all participants before the commencement of the study.

2.4 Sample Collection and Processing

A total of eight milliliters (8 ml) of venous blood were collected; two milliliters (2 ml) into the plain vacutainer blood specimen bottle, clear unhaemolyzed serum was harvested and used to re-determine the HIV-status. Six milliliters (6 ml) of the blood were collected into a sterile EDTA vacutainer blood specimen bottle, used for enumeration of $CD4^+$Cells, Nitro Blue Tetrazolium reduction test and lymphocyte transformation test.

2.5 HIV Screening

The HIV screening was carried out using the WHO screening criteria for developing countries which entailed the use of a parallel testing algorithm for serological testing of HIV antibodies in the patient's sera using a combination of three (3) different screening methods, in a stepwise order for the detection of HIV-1 and HIV-2 in the blood. Rapid HIV Screening test kits using Determine HIV I/2, Unigold™ and Stat Pak (Tie breaker**)** were used.

2.6 $CD4^+$ Count and Neutrophils Phagocytic Function Test

The $CD4^+$ Cells were enumerated using flow Cytometric (FCM) techniques with Cyflow counter manufactured by Partec, Munster Company, Germany and Neutrophils phagocytic function test was performed using the method adopted by Onyenekwe et al. [8]. The conversion factor was derived to be 47.0.

2.6.1 Calculation

Functional activities of neutrophils were calculated as follows:

Functional activity of neutrophils (Fmol/Phag) = absorbance of a test × conversion factor.

2.7 Lymphocyte Transformation Test

Lymphocyte Transformation test was carried out using the method invented by Boyum (1964), [9]. it was carried out under three different steps

Step 1. Lymphocyte isolation using Histopaque
Step 2. Lymphocyte Viability Test using Trypan blue.
Step 3. Lymphocyte culture under mitogenic stimulation(Conc A) and the supporting media was RPMI.

2.7.1 Lymphocyte Isolation

2.7.1.1 Principle

For lymphocyte and monocyte to be isolated, defibrinated or anticoagulant-treated blood was diluted with an equal volume of Rose well Pack Memorial Institute culture media (RPMI) and layered carefully over histopaque (without

intermixing) in a centrifuge tube. After a short centrifugation at room temperature (typically at 400 g for 30 min), lymphocytes together with monocytes and platelets was harvested from the interface between the histopaque and sample layers. The harvested cells suspension was centrifuged twice in RPMI to wash the lymphocytes to remove the platelets and monocyte.

2.7.2 Lymphocyte viability test

2.7.2.1 Principle

The test is based on the principle that live cells possess intact membrane that exclude certain dyes, such as trypan blue, eosin or propidium, whereas dead cells do not. In the test, a cell suspension is mixed with dye and then is visually examined to determine whether cells take up or exclude the dye. A viable cell will have a clear cytoplasm whereas a non-viable cell will have a blue cytoplasm.

2.7.3 Lymphocyte culture

2.7.3.1 Principle

Resting lymphocytes can be induced to undergo DNA synthesis, and subsequently cell division and proliferation by a wide variety of agents, Lectins constitute the most convenient group of mitogens. Under the influence of mitogenic lectins a high proportion of T-lymphocytes differentiate into helper T-cells and functional suppressor T-cells. Some resting B-cells become activated and subsequently proliferate into plasma cells.

2.8 Data Analysis

The data obtained was analyzed using SPSS version 20. The results were expressed as mean ± SEM. Group comparisons was made using one-way analysis of variance (ANOVA), paired comparisons was carried out using the Student's t-test, and a p-value of ≤ 0.05 was considered as statistically significant.

3. RESULTS

The result in Table 1 shows the percentage distribution of HIV infection based on socio-demographic characteristics of the study subjects. The mean age of the newly diagnosed HIV- infected subjects was 33.78 years. In this study, majority of the HIV-infected subjects were married (62.2%), followed by single (24.4%), and divorced (6.7%).Likewise majority of the HIV-infected subjects were Hausa's (56.6%), followed by Ibera's (12.2%) and dakkarkari (8.8%). Occupationally, majority of the HIV- infected subjects were predominantly business people (34.4%) followed by civil servants (25.5%), and most of them are in WHO clinical stage I of HIV infection (34.4%).

Table 1 Percentage distribution of HIV-infection based on sociodemographic characteristics of the study subjects

Characteristics	Number of subjects	Percentage (%)
Marital status	90	100
Married	56	62.2
Single	22	24.4
Divorced	6	6.7
Widow	4	4.4
Widower	2	1.9
Tribe	90	100
Hausa	51	56.6
Fulani	6	6.6
Igbo	3	3.3
Yoruba	6	6.6
Igala	2	2.2
Ibera	11	12.2
Dakarkari	8	8.8
Idoma	3	3.3
Occupation	90	100
Students	16	17.8
Business	31	34.4
Civil servants	23	25.5
House wives	10	11.1
Drivers	6	6.7
Farmers	4	4.4
WHO Staging of HIV infection	90	100
Stage I	31	34.4
Stage II	25	27.7
Stage III	19	21.1
Stage IV	15	16.7

WHO= world health organization, majority of the HIV-infected patients are married (62.2%), Hausa's (56.6%), and are predominantly business people (34.4%) and most of them are in WHO stage I of HIV infection (34.4%).

The result in Table 2 shows comparison of Mean CD_4 Count, Lymphocyte transformation and Nitroblue-tetrazolium reduction among control, HAART naïve and HIV infected participants on HAART. The mean CD_4^+ of (325.21 ± 23.24 cells/µl) in HIV infected participants on HAART was significantly ($P < 0.05$) lower than corresponding value of (495.56 ± 45.96 cells/µl) in HAART naïve HIV infected participants and control participants (809.39 ± 40.93 cells/µl), ($P <$

0.01). Similarly, the mean percentage lymphocyte transformation with Con A was significantly lower in HIV infected participants on HAART (12.70 ± 0.78%), and HAART naïve (15.9 ± 1.1%) HIV infected participants compared with the corresponding value of control participants (22.7 ± 1.7%), (P < 0.01) and (P < 0.05) respectively. The mean formazan generated by neutrophils among HIV infected participants on HAART (2638 ± 248 Fmol/Phag), HAART naïve (3027 ± 429 Fmol/Phag) and control participants (5065 ± 290 Fmol/Phag), shows a statistically significance difference (P < 0.01) and (P < 0.05) respectively.

Result in Table 3 shows the comparison of $CD4^+$ count, lymphocyte transformation, and formazan generated by neutrophils based on Clinical stage of HIV-infection. The mean $CD4^+$ count between clinical stage I and other clinical stages of HIV-infection shows a statistically significant differences (*P* = 0.000). Similarly, the mean percentage transformed cells show statistical significance differences (*P* = 0.000). The formazan generated by neutrophils in HIV-infected subjects on clinical stage I and other clinical stages were significantly decrease as HIV progresses to stage IV (*P*= 0.000).

The result in Table 4 shows distribution of mean $CD4^+$count, lymphocyte transformation and nitroblue tetrazolium reduction among male and female HIV-infected subjects. The $CD4^+$ count, lymphocyte transformation and phagocytic function of neutrophils were higher among female HIV-infected subjects (397.16±31.32 cells/µl) compared with male HIV-infected subjects (291.37±31.32 cells/µl). Statistically significant (*P* = 0.024) difference was observed on mean $CD4^+$ Count. Amount of formazan generated by neutrophils in female HIV- infected subjects (3166.29 ±318.10) was significantly (P < 0.012) higher than that of male HIV-infected (2038.08 ± 26.17) and non-statistically significant differences (*P*=0.344) was observed between mean percentage transformed cells.

Result in Table 5 shows comparison of mean $CD4^+$ count, percentage lymphocyte transformation and formazan generated by neutrophil, in HIV-infected subjects that are HAART Naïve, first line, and second line treatment regimen with HAART.A statistically significance (P < 0.05 in each case) difference was observed in $CD4^+$ count, Percentage Transformedcells and formazan generated by neutrophils.

Table 2. Comparison of Mean $CD4^+$ Count, Lymphocyte transformation and Nitroblue-tetrazolium reduction among Control, HAART naïve and HIV infected participants on HAART

Groups	$CD4^+$ count (cells/µl)	Lymphocyte transformation (%)	NBT formazan (Fmol/Phag)
Group 1 (n = 67)	809.39 ± 40.93	22.66 ± 1.69	5065.48 ± 290.58
Group2 (n =31)	495.56 ± 45.96	15.88 ± 1.05	3027.38 ± 429.29
Group 3 (n = 59)	325.21 ± 23.24	12.70 ± 0.78	2638.93 ± 248.67
P-Value	0.000	0.000	0.000

Values are Mean ± SEM, n= Number of subjects, $CD4^+$= Cluster of Differentiation Type 4, % = Percentage, , NBT= Nitroblue tetrazolium reduction test, Group 1= controls, Group = HAART naïve, Group 3 = HIV infected participants on HAART.

Table 3. Comparison of Mean $CD4^+$ count, Lymphocyte transformation, and Nitroblue tetrazoliumReduction based on Clinical stagesof HIV-infection

Clinical stages of HIV infection	$CD4^+$ count (cells/µl)	Lymphocyte transformation In percentage (%)	NBT (Fmol/Phag)
Stage 1 (n= 31)	531.09 ± 33.06	16.53 ± 1.09	4015.53 ± 415.49
stage 2 (n= 25)	347.96 ± 31.26	14.68 ± 1.19	2792.79 ± 368.28
stage 3 (n= 19)	248.32 ± 34083	10.50 ± 1.34	2058.91 ± 390.98
stage 4 (n= 15)	146.33 ± 40.67	7.89 ± 1.23	853.56 ± 81.58
P-Value	0.000	0.000	0.000
F-Value	24.758	9.762	11.469

Values are Mean ± SEM, n= Number of subjects, $CD4^+$= Cluster of Differentiation Type 4, % = Percentage, Con A= Concanavallin A, NBT= Nitroblue-tetrazolium reduction test

Table 4. Comparisons of mean $CD4^+$ Count, Lymphocyte transformation and Nitroblue tetrazoliumreduction amongMale and Female HIV-infected subjects

Groups	$CD4^+$ count (cells/µl)	Lymphocyte transformation (%)	NBT (Fmol/Phag)
Male (n= 45)	291.37 ± 25.47	12.54 ± 1.04	2038.1 ± 261.17
Female (n= 45)	397.16 ± 31.32	13.81 ± 0.89	3166.29 ± 318.1
P-Value	0.024	0.344	0.012

Values are Mean ± SEM, n= Number of subjects, CD4= Cluster of Differentiation Type 4, % = Percentage, NBT= Nitroblue-tetrazolium reduction test

Table 5. Comparison of Mean $CD4^+$ Count, Lymphocyte transformation and Nitroblue tetrazoliumreduction in HIV-Infected subjects that are HAART Naïve and those that are on treatment with HAART

Groups	$CD4^+$ Count (cells/µl)	Lymphocyte transformation(%)	NBT (Fmol/phag)
Group A (n= 31)	495.56 ± 45.96	15.88 ± 1.05	3027.38 ± 429.29
Group B (n=15)	344.35 ± 42.90	45.10 ± 1.31	2700.20 ± 338.67
Group C (n=12)	443.77 ± 49.92	17.41 ± 1.47	3740.64 ± 519.29
Group D (n=10)	393.88 ± 69.75	6.38 ± 1.38	3461.50 ± 147.55
Group E (n=14)	288.80 ± 19.43	10.40 ± 2.80	1964.67 ± 444.92
Group F (n=8)	218.75 ± 61.81	975 ± 2.29	1391.50 ± 321.38
P-Value	0.000	0.000	0.021
F-value	4.366	5.277	2.498

Values are Mean ± SEM, n= Number of subjects, $CD4^+$= Cluster of Differentiation Type 4, % =Percentage, NBT= Nitroblue-tetrazolium reduction test, HAART = Highly Active Anti-Retroviral Therapy. Group A= HIV infected participants that are HAART Naïve, Group B= First line treatment with zidovudine and lamivudine (Combivir), Group C= First line treatment with zidovudine, lamivudine and navirapine (Combipack), Group D= First line treatment with lamivudine and tenofovir (Tenolam), Group E= Second line treatment with lopinavir and ritonavir (Kaletra), Group F= Second line treatment with atazanavir and ritonavir.

4. DISCUSSION

In this study, the finding revealed that 33 years is the approximate mean age of HIV-infected subjects indicating that HIV-infection is predominantly found in the young and most productive age group. This finding agreed with the earlier reports in Nigeria (FMOH, 2010), [10], that most of the HIV- infected men and women were between the age of 20 and 29 years. This may be as a result of young age involvement in economically productive ventures, couple with the quest for physiological satisfaction, which makes it easy for the spread of HIV-infection between the young age group.

In this study we observed a significantly lower $CD4^+$ countamong HIV-infected subjects. This finding is consistent with previous studies (Ezeani et al. [11]; Ukibe et al. [12] and Onyenekwe et al. [13]), who indicated a significantly lowered $CD4^+$ T cells in symptomatic and asymptomatic HIV-infected individuals. Several mechanisms were thought to be responsible for CD_4 T cell – death in HIV-infection: the programmed cell death or apoptosis, where even uninfected cells and unstimulated cells die within a particular period of time. The second mechanism is the activation associated lymphocyte death, by which cells stimulated by strong mitogenic stimuli such as phytohaemagglutinin (PHA) die after 48–72 hours due to hyperactive stimulation [14]. Onyenekwe et al. [13], also reported that antigen can induce special resting cells into activation burst, and this may be due to rapid cells proliferation and differentiation into effector cells over the period of days or weeks, this can contribute to the decline in the number of $CD4^+$ count. Evidence has also shown that, regulatory T lymphocyte (Tregs) are depleted during the course of HIV-infection [15]. This may facilitate the immune hyper activation leading to increased $CD4^+$ T cell death associated with HIV-infection.

In this study, Con A was used to stimulate lymphocyte transformation, the percentage blast formation due to Con A was significantly lowered in most of the HIV-infected subjects. Only the T cells that have not been pre-stimulated by the virus would be in a state to respond to stimulation. The observation of significantly

lowered lymphocyte function in HIV-infected subjects. This study agreed with the previous studies [16,17]. The implication is that, there is lowered blast formation (T cell function) in HIV-infection; that leads to induced cellular immune derangements. Significant differences in proliferative responses between HIV-infected on HAART, HAART naïve and control has been reported (Bocchieri et al. [18]; Shakoor, [19]. In contrast to the present study, Eggena et al. [20], found high levels of T cell activation in HIV-infected Ugandans, when compared to their corresponding seronegative counterparts. It was also observed that symptomatic HIV-subjects had lower percentage transformation than asymptomatic group. This finding may be attributed to the level of HIV disease progression. Previous studies had shown a decreased percentage lymphocyte transformation, as HIV Infection progression to acquired immunodeficiency syndrome (AIDS) [21,19].

The finding of significantly lowered neutrophil ingestion rate of NBT in HIV-infected subjects. This study is consistent with the previous study by Onyenekwe et al. [8], who reported that HIV-infection is significantly associated with lowered phagocytic activity of neutrophils. The degree of reduction was more marked in symptomatic than asymptomatic HIV-infected subjects. Like $CD4^+$ T cells, neutrophils are one of the body's effector cells performing phagocytic functions to rid the body of invading organisms including parasites. Thus, they are responsible for eliminating bacterial, fungal and protozoan parasitic organisms that are responsible for the opportunistic infections, which are common in HIV-disease. With greater HIV disease progression, the number of neutrophils gets fewer and fewer in circulation and cellular immunity continues to decline [22].

The finding of significantly higher mean $CD4^+$ counts, and formazan generated by neutrophils among female HIV-infected subjects.This study when compared with male HIV-infected subjects is consistent with the previous studies (Kumarasamy et al. [23]; Moges et al. [24]), who reported that HIV associated TB could be the contributing factor for the low $CD4^+$ count in males as the proportion of patients having TB was significantly higher in male HIV-infected subjects than females. The functional activity of neutrophil was observed to be significantly higher in HIV infected female subjects than HIV-infected male subjects. This corroborated with earlier report by Mugusi et al. [25], who reported that, the progression rates to AIDS and clinical manifestations of diseases associated with HIV infection might differ between women and men because of biological and socioeconomic factors. Males were known to seek for HIV treatment and care at a later stage when the disease had already becomes more advanced compared with females (Mugusi et al. [25]). This can be attributed, in part; due to the fact that females are having extra entry points to HIV services e.g. through PMTCT services, however this was not the case in this study, where the majority of the male subjects were tested after a long term illness. The most common reason for HIV testing in males is when they come down with AIDS related syndrome, rather than voluntary counseling and testing.

A significant positive correlation ($r = 0.044$, $P < 0.001$) was established between $CD4^+$ count and neutrophil ingestion rate of NBT. Therefore, a decrease in $CD4^+$ count is accompanied by a decrease in neutrophil ingestion rate of NBT. Hence the two parameters can substitute each other in areas where it's more feasible to determine one in preference to the other.

5. CONCLUSION

The $CD4^+$ count, percentage transformed cells and formazan generated by neutrophil was significantly higher among female subjects compared with male subjects. The lowered $CD4^+$ count suggests possible destruction of adaptive/cellular immune cells (mainly Th1 cells), while lower blast formation and neutrophil ingestion rate of NBT indicate functional derangement of the cellular immune cells and neutrophil phagocytosis respectively.

CONSENT

As per international standard or university standard, patient's written consent has been collected and preserved by the authors.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the authors.

COMPETING INTEREST

Authors have declared that no competing interests exist.

REFERENCES

1. World Health Organization. National Guidelines for HIV/ AIDS Treatment and Care in Adolescents and Adults; Federal Ministry of Health Abuja – Nigeria, January; 2010.
2. UNAIDS, United Nation programme on Acquired Immune Deficiency Syndrome. 2007;10. Retrieved 2011.
3. Centre for Disease Control and Prevention. CDC 2012 Revised Classification System for HIV Infection and Surveillance, Case Definition for AIDS among Adolescent and Adults. Morbidity and Mortality Weekly Reports, Recommendations and Report, 2011;41(RR-20):9-11.
4. Rodriguez PC, Ernstoff MS, Hernandez C, Atkins M, Zabaleta J. Arginase I-producing myeloid-derived suppressor cells in renal cell carcinoma a subpopulation of activated granulocytes. Cancer Research. 2009;69: 1553–1560.
5. Mehandru S, Poles MA, Tenner-Racz K, Horowitz A, Hurley A, Hogan C, et al. Primary HIV-1 infection is associated with preferential depletion of $CD4^+$ T cells from effector sites in the gastrointestinal tract. J of Exp Med. 2004;200(6):761–770.
6. Mantovani A, Cassatella MA, Costantini C. Jaillon S. Neutrophils in the activation and regulation of innate and adaptive immunity. Nat Rev Immun. 2011;11:519–531.
7. Muller I, Munder M, Krop FP, Hansch GM. Polymorphonuclear neutrophils and T lymphocytes: Strange bed fellows or brothers in arms? Trends Immun. 2009; 30:522–530.
8. Onyenekwe CC, Ukibe NR, Ahaneku JE, Ukibe SN, Meludu SC, Ilika A, et al. Use of absolute lymphocyte count or neutrophil ingestion rate of nitroblue tetrazolium (NBT) as alternative index to $CD4^+$ T cell count to initiate ART in the management of HIV/AIDS disease. Inter J of BioSci. 2012;6(1):99-107.
9. Boyum A. Isolation of mononuclear cells and granulocytes from human blood. Isolation of mononuclear cells by one centrifugation, and of granulocytes by combining centrifugation and sedimentation at 1 g. Scand. J of Cli Lab Inve. 1968;(Suppl. 97):77-89.
10. Federal Ministry of Health (FMOH), National HIV Sero-prevalence Sentinel Survey among Pregnant Women Attending Antenatal Clinics in Nigeria; 2010.
11. Ezeani MC, Onyemekwu CC, Wachukwu CK, Anyiam DCD, Meluclu SC, Ukube RN, et al. Detection of microbial antigenic components circulating immune complexes in hiv patients: Involvement in $CD4^+$ counts depletion. Asian Pacific J of Trop Med. 2010;10:828–832.
12. Ukibe NR, Onyenekwe CC, Ahaneku JE, Meludu SE, Ukibe SN, Ilika A, et al. $CD4^+$ T-cell count in HIV-malaria co infected adult population in nnewi, South Easthern Nigeria. Inter J of Bio Sci. 2010;4(5):1593-1601.
13. Onyenekwe CC, Ifeanyi MI, Ele PU, Ukibe NR, Meludu SC, Ezeani MC, et al. Evaluation of some cellular immune index in HIV infected participants. International Journal of Biological Scences. 2011; 5(3):1311-1313.
14. Grossman ZM, Schellersheim M, Sousa AE, Victorin RM, Paul WE. $CD4^+$ T cell depletion in HIV infection are we closer to understanding the cause. Nature Medicine. 2002;8:319–323.
15. Weiss L, Burgard M, Cahen YD. Immunological and virological features of HIV – infected patients with increasing $CD4^+$ T cell numbers despite virological failure during protease inhibitor–based therapy. International journal of AIDS. 2002;3:12–20.
16. Amulic B, Cazalet C, Hayes GL, Metzler KD, Zychlinsky A. Neutrophil function: From mechanisms to disease. Annual Rev Immuno. 2012;30:459–489.
17. Beauvillain C, Cunin P, Doni A, Scotet M, Jaillon S. CCR7 is involved in the migration of neutrophils to lymph nodes. Blood Disorders. 2011;117:1196–1204.
18. Bocchieri MH, Talle MA, Maltese LM, Ragucci IR, Hwang CC, Goldstein G. Whole blood culture for measuring mitogen induced T cell proliferation provides superior correlations with disease state and T cell phenotype in asymptomatic HIV –infected subjects. J of Immunol Methods. 1995;181:233–243.
19. Shakoor Z. Serial evaluation of percentage activated T lymphocyte in peripheral blood of human immunodeficiency virus infected individuals as a prognostic marker. Sau Med J. 2003;24(6):632–636.
20. Eggena MP, Barugahare B, Okello M, Mutyala S, Jones N, MayKityo C, et al. T-cell activation in HIV–sero-positive ugandans. Differential Association with Viral Load and $CD8^+$ T-Cell Depletion.

Journal of Infectious Disease. 2005;199: 694–701.

21. Bansal AS, Moran A, Otter M, Taycor R, Haeney MR,Mandal BK. Lymphocyte transformation responses to phytohaemagglutinin and pokeweed mitogen in patients at different stages of HIV infection are they not measuring. J of Clinil Path.1993;146:8446–8448.
22. Brooks JT, Masoba TR, Amornkul P, Mwaengo D, Valule J, et al. Neutropenia in HIV infected Kenyan women receiving ART to prevent mother- to child HIV congenital transmission. Retroviral Opportunistic Infection. 2005;22(12):817-820.
23. Kumarasamy N, Venkatesh KK, Cecelia AJ, Devaleenol B, Saghayam S, Yepthomi T. Gender-based differences in treatment and outcome among human immune virus patients in South India. J of Women Health (Larchmt). 2008;17(9):1471-1475.
24. Moges D, Monga DP, Deresse D. Immunological response among HIV/AIDS patients before and after ART therapy at Zewuditu Hospital Addis Ababa, Ethiopia. American J of Research Communication. 2013;1(1):103-115.
25. Mugusi S, Mwita J, Francis J. Effect of improved access to antiretroviral therapy on clinical characteristics of patients enrolled in the HIV care and treatment clinic, at Muhimbili National Hospital (MNH), Dares Salaam, Tanzania. BMC Public Health. 2010;291(10):1471-2458.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/20902