CD4$^+$ count and Nitro-Blue Tetrazolium reduction rate of neutrophil in newly diagnosed HIV-infected adults in Sokoto Metropolis

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ABSTRACT

Human immunodeficiency Virus (HIV) is a major public health problem in sub-Saharan Africa. It is known to cause malfunction of the immune cells thereby predisposing to profiles of morbidity and fatality. The current study evaluated the functional activity of neutrophils and lymphocyte sub set (CD$_4^+$ count) in newly diagnosed HIV – infected adults in Sokoto, Nigeria. A total of 126 male and female adults were recruited for the study, comprising of 64 newly diagnosed HIV– infected subjects and 62 age-and sex-matched apparently healthy individuals as controls. CD$_4^+$ cells were enumerated using flow Cytometric method and neutrophil phagocytic activity was determined using Nitro- Blue Tetrazolium (NBT) Reduction test. Data was analyzed using Microsoft Excel and independent sample t-test for comparison of means. P-value less than or equal to 0.05 (P $\leq$ 0.05) was regarded as statistically significant. The CD4$^+$ count and neutrophil ingestion rate of NBT were significantly (P = 0.000) lower in newly diagnosed HIV- infected subjects compared with the values in control. The CD4$^+$ count and formazan generated by neutrophil were significantly (P = 0.024 and 0.012 respectively) higher in newly diagnosed HIV– infected female compared with male subjects. The decreased CD$_4^+$ count and neutrophil phagocytic activity in ART-naïve HIV- infected subjects is an indication that HIV suppresses both innate and adaptive arms of immune response. However, this effect was more pronounced in male subjects. The possible mechanism for this gender differences are discussed.
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Keywords: Neutrophil phagocytosis, CD$_4^+$, NBT, HIV, Sokoto.

INTRODUCTION

Human immunodeficiency virus (HIV) is a member of lentivirus (a subgroup of retrovirus) that causes progressive degeneration of the immune system, leading to the development of acquired immune deficiency syndrome (AIDS) (Alavi, 2013; Tudela, 2014). HIV has infected more than 60 million and killed 30 million people around the world. HIV infects vital cells of the human immune system such as helper T cells (specifically $CD4^+$ T cells), macrophages, and dendritic cells (Cunningham et al., 2010). HIV infection may lead to low levels of $CD4^+$ T cells through a number of mechanisms, including apoptosis of uninfected bystander cells (Garg et al., 2012), Direct viral killing of infected cells, and killing of infected $CD4^+$ T cells by $CD8^+$ cytotoxic lymphocytes that recognize infected cells (Kumar, 2012). When $CD4^+$ T cell decline below a critical level, cell-mediated immunity is lost, and the body becomes progressively more susceptible to opportunistic infections.

Neutrophils are the most abundant leukocyte population in humans and are essential effector cells of the innate immune system in the host defense against invading pathogens. They are usually the first cell to reach the site of infection where they commence phagocytosis (Muller et al., 2009; Amulic et al., 2012). Neutrophils co-localize and actively communicate with T cells at sites of infection and migrate to the draining lymph nodes where they are involved in the induction and regulation of cellular and humoral immune responses by exerting pro-inflammatory or anti-inflammatory function (Beauvillain et al., 2011). Therefore, the present study examined the status of lymphocyte sub set and functional activity of neutrophil, in order to establish the role of both immune cells play in HIV infected individuals and also considered if gender played any significant roles in performance of these immune cells.

MATERIALS AND METHODS

Study subjects

A total of 126 subjects participated in this study. These comprised of 64 newly diagnosed HIV – infected subjects that are in stage 1 of HIV infection and 62 age- and sex-matched apparently healthy individuals as control. This was a cross sectional case control study of newly diagnosed HIV-infected subjects attending ART Clinic of Usmanu Danfodiyo University Teaching Hospital, Sokoto and Murtala Muhammad Specialist Hospital, Sokoto. 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. The subjects included in the study were HIV – infected ART-naïve adult males and females, with age range of 18 years and above. Subjects were ineligible if they were known to have tuberculosis, hypertension or diabetes.

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.

Blood sample collection and processing

A total of six millilitres (6.0 ml) of venous blood was collected; two millilitres (2.0 ml) into the plain vacutainer blood specimen bottle. The blood was allowed to clot and centrifuged at 3000 rpm for 5 min and clear unhaemolyzed serum was harvested and used to re-determine the HIV-status. Four millilitres (4.0 ml) of the blood was collected into a sterile EDTA vacutainer blood specimen bottle, used for enumeration of $CD4^+$ T cell, and Nitro Blue Tetrazolium reduction test.

HIV screening

The HIV screening was carried out using the WHO screening criteria for developing countries which entails 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.

CD4+ count and neutrophils phagocytic function test

The CD4+ cells were enumerated by flow Cytometric (FCM) technique using Cyflow counter manufactured by Partec, Munster Company, Germany. Neutrophil phagocytic function was performed using the method adopted by Onyenekwe et al. (2012). The conversion factor was derived to be 47.0.

Calculation

Functional activities of neutrophils were calculated as follows:
Functional activity of neutrophils (Fmol/Phag) = absorbance of a test × conversion factor.

Data analysis

The data obtained was analyzed using SPSS version 20. The results were expressed as mean ± SEM. Paired comparisons was carried out using the Student's t-test, and p-value of equal to or less than 0.05 ($P \leq 0.05$) was considered as significant.

RESULTS

The result in Table 1 shows the percentage distribution of HIV infection based on marital status of the study subjects. Majority of the HIV-infected subjects were married (46.8%), followed by single (29.6%), and divorced (10.9%). Table 2 shows percentage distribution of HIV infection based on tribe. Majority of the HIV infected subjects were Hausa (53.1%), followed by Ibera (15.6%) and dakarkari (10.9%). The result in Table 3 shows the percentage distribution of HIV infection based on occupation of the study subjects. Majority of the HIV infected subjects were predominantly business people (32.8%) followed by civil servants (24.4%). The mean age of the newly diagnosed HIV-infected individuals is 33.78 years.

The result in Table 4 shows comparison of mean CD4+ count and Nitroblue tetrazolium reduction among control and newly diagnosed HIV-infected subjects. The result indicated that mean CD4+ count was significantly ($P < 0.05$) lower in newly diagnosed HIV infected subjects (495.56 ± 45.96 cells/µl) compared with controls (809.39 ± 40.93 cells/µl). The mean formazan generated by neutrophils was significantly ($P < 0.05$) lower in newly diagnosed HIV- infected subjects (3027 ± 429 Fmol/Phag) compared with controls (5065 ± 290 Fmol/Phag).

The comparisons of mean CD4+ count and nitroblue tetrazolium reduction in male and female HIV- infected subject is presented in Table 5. The result indicated that mean CD4+ count and formazan generated by neutrophils were significantly ($P = 0.024$, $P = 0.012$ respectively) higher in female HIV-infected subjects (397.16 ± 31.32 cells/µl and 3166.29 ±318.10 Fmol/Phag respectively) compared with male HIV- infected subjects (291.37 ± 31.32 cells/µl and 2038.08 ± 26.17 Fmol/Phag respectively). A significant positive correlation was observed between CD4+ count and neutrophil ingestion rate of NBT ($r = 0.044$, $P < 0.001$).

Table 1: Percentage distribution of HIV infection based on marital status of the study subjects.

Characteristics	Number of subjects	Percentage (%)
Marital status	64	100
Married	30	46.8
Single	19	29.6
Divorced	7	10.9
Widow	5	7.8
Widower	3	4.6

Table 2: Percentage distribution of HIV infection based on tribe of the study subjects.

Characteristics	Number of subjects	Percentage (%)
Tribe	64	100
Hausa	34	53.1
Fulani	4	6.2
Igbo	2	3.1
Yoruba	4	6.2
Igala	1	1.5
Ibera	10	15.6
Dakarkari	7	10.9
Idoma	2	3.1

Table 3: Percentage distribution of HIV infection based on occupation of the study subjects.

Characteristics	Number of subjects	Percentage (%)
Occupation	64	100
Students	12	18.7
Business	21	32.8
Civil servants	17	26.5
House wives	8	12.5
Drivers	4	6.2
Farmers	2	3.1

Table 4: Comparison of mean $CD4^+$ T cell count and Nitro-blue Tetrazolium reduction of neutrophil in control and newly diagnosed HIV- infected subjects.

Parameter	Controls (n=62)	Newly Diagnosed HIV- Infected (n=64)	P-Value
$CD4^+$ T count (cells/µl)	809.39 ± 40.93	495.56 ± 45.96	0.000
NBT formazan, (Fmol/Phag)	5065.48 ± 290.58	3027.38 ± 429.29	0.000

Values are Mean ± SEM, n= Number of subjects, $CD4^+$= Cluster of Differentiation Type 4, NBT= Nitroblue Tetrazolium, P value < 0.05 is considered as statistically significant.

Table 5: Comparisons of mean $CD4^+$ T cell count and Nitroblue Tetrazolium reduction in male and female HIV- infected subjects.

Parameter	Male (n = 32)	Female (n = 32)	P-Value
$CD4^+$ count (cells/µl)	291.37 ± 25.47	397.16 ± 31.32	0.024
NBT formazan (Fmol/Phag)	2038.1 ± 261.17	3166.29 ± 318.1	0.012

Values are Mean ± SEM, n= Number of subjects, CD4= Cluster of Differentiation Type 4, NBT= Nitroblue Tetrazolium.

DISCUSSION

The current study revealed that 33 years is the approximate mean age of HIV-infected subjects, indicating that HIV infection is predominantly found in the middle age group. This finding is in agreement with the earlier reports in Nigeria (FMOH, 2008, 2010), that most of the HIV infected men and women were between the ages of 20 and 29 years. This may be as a result of middle age involvement in economically productive ventures, couple with the quest for physiological satisfaction, which makes it easy for the spread of HIV infection among the middle age group.

In this study we observed a significantly lower $CD4^+$ count in newly diagnosed HIV- infected subjects. This finding is consistent with previous studies (Ezeani et al., 2010; Ukibe et al., 2010; Onyenekwe et al., 2011), who indicated a significantly lowered $CD4^+$ count 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 (Grossman et al., 2002). Onyenekwe et al. (2011) 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 $CD4^+$ T cell count. Evidence has also shown that regulatory T lymphocyte (Tregs) are depleted during the course of HIV infection (Weiss et al., 2002). This may facilitate the immune hyper activation leading

to increase $CD4^+$ T cell death associated with HIV infection.

The finding in this study of significantly lower neutrophil ingestion rate of NBT in newly diagnosed HIV infected subjects compared with control subjects is consistent with the previous study of Onyenekwe et al. (2012), who reported that HIV infection is significantly associated with lower 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 neutrophil get fewer and fewer in circulation and cellular immunity continues to decline (Brooks et al., 2005).

The significantly higher mean $CD4^+$ count, and formazan generated by neutrophils among female HIV- infected subjects when compared with the corresponding values of male HIV-infected subjects is consistent with the previous researchers (Kumarasamy et al., 2008; Moges et al., 2013), who reported that HIV associated TB could be the contributing factor for the low $CD4^+$ count in males as 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 among newly diagnosed HIV-infected females compared with corresponding HIV-infected males. This corroborated with earlier report by Mugusi et al. (2010) who found 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., 2010). 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.

COMPETING INTERESTS

Authors have declared that no competing interest exists.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between all the authors. UKM and CCO designed the study. IA, NMB, UKM and MB managed and analyzed samples. AY, BRA, MHY and KMH performed the statistical analysis, and wrote the first draft of the manuscript. UKM, NMB and ABI managed the literature searches. All the authors read and approved the final manuscript.

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REFERENCES

Alavi SM, Moradzadegan H, Khoshkhoy MM. 2013. Seroprevalence of HIV in newly detected pulmonary tuberculosis patient in Khuzestan region. *Jundish J. Micro.*, **6**(2): 193-196. DOI: 10.5812/jjm.5222.

Amulic B, Cazalet C, Hayes GL, Metzler KD, Zychlinsky A. 2012. Neutrophil Function: from Mechanisms to Disease. *Ann. Rev. Immu.,* **30**: 459–489. DOI: 10.1146/annurev-immunol-020711-074942.

Beauvillain C, Cunin P, Doni A, Scotet M, Jaillon S. 2011. CCR7 is involved in the Migration of Neutrophils to Lymph nodes. Blood Disorders 117: 1196–1204. DOI: 10.1182/blood-2009-11-254490.

Brooks JT, Masoba TR, Amornkul P, Mwaengo D, Valule J, Fowler M, Slutsker L, Decock K. 2005. Neutropenia in HIV infected Kenyan Women Receiving ART to prevent mother- to child HIV Congenital transmission, *Retro. Opport. Infect.,* **22**(12): 817-820. DOI: 10.1097/QCO.0b013e3282f47041.

Centre for Disease Control and Prevention 2011. 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, **41**(RR-20): 9-11.

Cohen MS, Hellmann N, Levy JA, DeCock K, Lange J. 2008. "The Spread, Treatment, and Prevention of HIV-1: Evolution of a Global Pandemic". *J. Clin. Invest.* **118**(4): 1244–1254. DOI: 10.1172/JCI34706.

Cunningham AL, Donaghy H, Harman AN, Kim M, Turville SG. 2010. Manipulation of Dendritic Cell Function by Viruses. *Curr. Opini. Micro.*, **13**(4): 524–529. DOI: 10.1016/j.mib.2010.06.002.

Ezeani MC, Onyenekwe CC, Wachukwu CK, Anyiam DCD, Meluclu SC, Ukebi RN, Ifeanyichukwu M, Onochie A, Anahalu I, Okafor UU. 2010. Detection of Microbial Antigenic Components Circulating Immune Complexes in HIV Patients: Involvement in CD4$^+$ Counts Depletion. *Asian Pac. J. Tro. Med.,* **10**: 828 – 832.

FMOH (Federal Ministry of Health). 2010. National HIV Sero-prevalence Sentinel Survey among Pregnant Women Attending Antenatal Clinics in Nigeria. Federal Ministry of Health Abuja, Nigeria.

FMOH (Federal Ministry of Health). 2008. National HIV/AIDS and Reproductive Health Survey 2007 (NARHS plus). Federal Ministry of Health Abuja, Nigeria.

Garg H, Mohl J, Joshi A. 2012. *HIV-1 Induced Bystander Apoptosis.* Viruses. Center of Excellence for Infectious Disease, Department of Biomedical Science, Texas Tech University Health Sciences Center: Texas, USA; 3020–3043. DOI:10.3390/v4113020.

Grossman ZM, Schellersheim M, Sousa AE, Victorin RM, Paul WE. 2002. CD4$^+$ T cell Depletion in HIV infection are we closer to Understanding the Cause. *Nat. Med.,* **8**: 319 – 323. DOI: 10.1038/nm0402-319.

Kumarasamy N, Venkatesh KK, Cecelia AJ, Devaleenol B, Saghayam S, Yepthomi T. 2008. Gender-based Differences in Treatment and Outcome among Human Immune Virus Patients in South India. *J. Wom. Hea.*, (Larchmt): **17**(9): 1471-1475. DOI: 10.1089/jwh.2007.0670.

Kumar V. 2012. *Robbins Basic Pathology* (9th Edn). ELSEVIER; 147-148.

Mehandru S, Poles MA, Tenner-Racz, K, Horowitz A, Hurley A, Hogan C, Boden, D, Racz P, Markowitz M. 2004. Primary HIV-1 Infection is Associated with Preferential Depletion of CD4$^+$ T cells from Effector Sites in the Gastrointestinal Tract. *J Exp Med.*, **200**(6): 761–770. PMID: 15365095.

Moges D, Monga DP, Deresse D. 2013. Immunological Response among

HIV/AIDS Patients Before and After ART Therapy at Zewuditu Hospital Addis Ababa, Ethiopia. *Ameri. J. Resea. Commu.,* **1**(1): 103-115.

Mugusi S, Mwita J, Francis J. 2010. 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. *B.M.C. Public Health*, **291**(10): 1471-2458. DOI**:** 10.1186/1471-2458-10-291.

Muller I, Munder M, Krop FP, Hansch GM. 2009. Polymorphonuclear Neutrophils and T lymphocytes: Strange Bed Fellows or Brothers in Arms? *Tren. Immu.,* **30**: 522–530.

Onyenekwe CC, Ifeanyi MI, Ele PU, Ukibe NR, Meludu SC, Ezeani MC, Ezeachukwu CC, Amilo GI, Umeanaeto PU. 2011. Evaluation of Some Cellular Immune Index in HIV Infected Participants. *Int. J. Biol. Chem. Sci.*, **5**(3) 1311-1313.

Onyenekwe CC, Ukibe NR, Ahaneku JE, Ukibe SN, Meludu SC, Ilika A, Ifeanyichukwu M, Ezeani M, Igwegbe AO. 2012. 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, *Int. J. Biol. Chem. Sci.*, **6**(1): 99-107. DOI: http://dx.doi.org/10.4314/ijbcs.v6i1.9

Tudela EV, Singh MK, Lagman M, Ly J, Venketaraman V. 2014. Cytokine Level in plasma samples of individuals with HIV Infection, *Aust. J. Clin. Immu.,* **6**(1) 193-196.

World Health Organization. 2010. *National Guidelines for HIV/AIDS Treatment and Care in Adolescents and Adults*. Federal Ministry of Health: Abuja, Nigeria.

Ukibe NR, Onyenekwe CC, Ahaneku JE, Meludu SE, Ukibe SN, Ilika A, Ifeanyichukwu MO, Ezeani MC, Igwegwe AO, Ofiaeli N, Onochie A, Abor N. 2010. CD4$^+$ T-cell count in HIV-Malaria Co-infected adult Population in Nnewi, South Easthern Nigeria. *Int. J. Biol. Chem. Sci.*, **4**(5): 1593-1601. DOI: http://dx.doi.org/10.4314/ijbcs.v4i5.65533.

Weiss L, Burgard M, Cahen YD. 2002. Immunological and Virological Features of HIV – Infected Patients with Increasing CD4$^+$ T cell Numbers Despite Virological Failure During Protease Inhibitor–Based Therapy. *Inter. J. AIDS,* **3**: 12 – 20.

Weiss RA. 1993. How does Human Immune Virus Cause Acquired Immune Deficiency Syndrome. *Science*, **260**(5112): 1273–1279.

World Health Organization. 2007. HIV/AIDS Care and Treatment. A clinical Course for People Caring for Persons Living with HIV/AIDS. Part A, Module A Session 1.