Journal of Advances in Biology & Biotechnology

15(2): 1-9, 2017; Article no.JABB.35824
ISSN: 2394-1081

Predicting CD4+ T Lymphocyte Count Using IgG and Total Lymphocyte Count in Newly Diagnosed HIV Infected Patients in Specialist Hospital Sokoto North West Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author IA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MNA and MAM managed the analyses of the study. Author MAM managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JABB/2017/35824
Editor(s):
(1) Joana Chiang, Department of medical laboratory Science and Biotechnology, China Medical University, Taiwan.
Reviewers:
(1) Nélida Virginia Gómez, Buenos Aires University, Argentina.
(2) Lívia Garcia Bertolacci-Rocha, Universidade Federal de Goiás, Brasil.
Complete Peer review History: http://www.sciencedomain.org/review-history/21283

Original Research Article

Received 30th July 2017
Accepted 15th August 2017
Published 7th October 2017

ABSTRACT

CD4+ T cell count and viral load monitoring are expensive and unavailable to most human immunodeficiency virus (HIV) infected people in Africa. There is a need to have another marker of HIV infection that is less resource demanding. Studies in human immunodeficiency virus infected adults have demonstrated association of total lymphocyte count (TLC) <1200/ÂµI and subsequent disease progression or mortality. This study was carried out to determine the relationship of CD4+

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T lymphocyte counts with total lymphocyte count (TLC) and immunoglobulin G (IgG) in HIV-positive highly active antiretroviral therapy (HAART) naÃ¯ve patients attending Specialist Hospital Sokoto northern region, Nigeria. The study population comprised of 100 adult HIV-positive HAART naÃ¯ve patients aged 19-65 years. The CD4+ T cell counts and these alternate biomarkers of study participants were measured. Spearman's rank order correlation was used for statistical analysis. CD4+ T cell count was positively correlated to TLC in group 1 and 3 (r = 0.558 and r = 0.530 respectively), and inversely correlated to IgG in group 1, 2 and 3 (r = -0.016, r = -0.002 and r = -0.276 respectively). Among the tested biomarkers, it was seen that TLC Ë,135 cells/ÂµI and IgG Ëƒ20 g/l could be helpful in predicting CD4 cell counts of Ë,200 cells/ÂµI.

Keywords: Immunoglobulin G; total lymphocyte count; CD4+ T cell count; HIV infected patients.

1. INTRODUCTION

Human immunodeficiency virus (HIV) is the causative agent of Acquired immunodeficiency syndrome (AIDS), which is characterized by progressive damage to the body's immune system that results in a number of opportunistic infections, immunological and haematological complications. HIV induced cytolysis; dysregulation of cytokines; cytotoxic T-lymphocyte responses and HIV induced autoimmune reactions have been reported [1]. AIDS is a severe disease representing the late clinical stage of HIV infection [1]. $CD4^+$ T-helper lymphocyte, which are depleted in AIDS patients, are the primary targets of HIV, because of the affinity of the gp120 (a glycoprotein component of the viral envelope) for the CD4 molecules found on the cell surface of these cells. The majority of infected individuals (70-80%) experience intermediate disease progression in which they show HIV RNA rise, $CD4^+$ T- cell decline and later development of AIDS related illness in about 6-10 years. The World Health Organization (WHO) currently recommends initiation of antiretroviral therapy (ART) in people living with HIV/AIDS with CD4 T lymphocyte counts <350 cells/µl irrespective of the WHO clinical staging (WHO, 2010). According to the WHO; patients are categorized according to CD4 count into three groups or categories (>500 cell/µl, 200-500 cell/µl, and <200 cell/µl), or according to the total lymphocyte count (TLC) into (>2000 cell/µl, 1000-2000 cell/µl, and <1000 cell/µl), this system was found prognostically valuable [2].

In the absence of viral loads and CD4 counts for monitoring HIV disease, the value of total lymphocyte count (TLC) as a surrogate for CD4 has been suggested [3]. Viral loads and CD4 counts demand highly skilled laboratory personnel and costly maintenance of sophisticated equipment. The focus of research regarding TLC has been on its use in determining when to start therapy [3]. Current World Health Organization (WHO) guidelines only commit to using TLC in conjunction with clinical data as a criteria to initiate highly active antiretroviral therapy (HAART) in resource poor settings [4]. TLC is relatively inexpensive and simple to perform and has been shown to be reliable in the HAART-naïve population, but its value may not only be in its ability to identify the need to start HAART but to monitor patients' progress and immune status during HAART as well [5]. As expected, the production of antibodies is higher in HIV-infected patients than in healthy individuals [6]. Patients with human immunodeficiency virus (HIV) infection exhibit a generalized, non-HIV specific polyclonal B-cell activation resulting in hypergammaglobulinemia of all immunoglobulin (Ig) isotypes, as well as increased production of HIV-specific immunoglobulin G (IgG) and immunoglobulin M (IgM) [7]. Whilst infection with HIV type 1 (HIV-1) is associated with profound immunologic abnormalities amongst T and B lymphocytes, the specific character and magnitude of this HIV-induced humoral immune response is poorly understood [8]. HIV neutralizing activities have been attributed to IgG and IgA isotypes, whilst IgG subclasses have been associated with virus-specific antibody-dependent cellular cytotoxicity [9]. Serum immunoglobulin concentrations increase with progression from asymptomatic to symptomatic HIV infection [10]. Furthermore, the few studies conducted in Africa show higher immunoglobulin (Ig) concentrations for both HIV-positive and negative persons compared with those in industrialized countries, suggesting a genetic and environmental influence [11]. Although access to ARV drugs in Africa has improved through free medication price reductions, standard monitoring of disease progression using $CD4^+$ T cell count and viral load measurement remain expensive. $CD4^+$ T cell count and viral load measurement are only

available in a few urban health and research facilities, owing to their technical complexity and the requirement for expensive equipment. Immunoglobulin measurement is relatively inexpensive and may potentially serve as simple surrogate markers to evaluate response to ARVs in HIV patients. To evaluate the feasibility of immunoglobulin G (IgG) concentrations as alternative surrogate marker for HIV/AIDS disease monitoring, we measured and compared concentrations of immunoglobulin G (IgG) in adults with and without HIV in Sokoto. At $CD4^+$ count below 200 cells/µl most of the opportunistic infections begin to manifest.

2. MATERIALS AND METHODS

2.1 Study Area

The study was carried out in the Department of Immmunology, Faculty of Medical Laboratory Sciences, Usmanu Danfodiyo University Sokoto and Antiretroviral Therapy (ART) Clinic of Specialist Hospital Sokoto, a Tertiary Health Facility located in Sokoto, the Sokoto State Capital.

Sokoto State is located at the extreme part of North-Western Nigeria between longitude 3° and 7° east and between latitude 10° and 14° north of the equator. It shares borders with Niger-Republic to the North, Kebbi State to the South-West and Zamfara State to the East [12]. The total population and annual growth rate stood at 3.69 million and 3.0% respectively in the 2006 National Population Census [13]. Sokoto State has semi-arid climate and vegetation is largely Sudan Savannah with an annual rainfall between 500 – 1300 mm and temperature ranges between 15°C and over 40°C during warm days [12].

2.2 Study Subjects

A total of 150 participants were consecutively selected for the study. The target populations were male and female HIV-infected adult patients aged 18 to 65 years who were attending Antiretroviral Therapy (ART) Clinic at Specialist Hospital Sokoto, Nigeria. These consisted of 100 newly diagnosed HIV-positive that are not yet on treatment with Highly Active Antiretroviral Therapy (HAART-naive) from April to August, 2015. The HIV statuses of the subjects were confirmed using the WHO screening criteria for developing countries. The control samples comprised 50 adult persons, sex-and age-matched HIV-negative (apparently healthy) individuals were selected as blood donors at the blood bank of the Specialist Hospital, Sokoto and volunteer staff. In all the patients and controls, informed consent was obtained from each prior to the commencement of the study.

2.2.1 Sampling technique

Randomized sampling technique was used to select consenting patients and a detailed questionnaire was developed to capture bio-data, medical history, socio-economic status and other relevant information.

2.2.2 Ethical approval

The ethical approval for this research was obtained from the Ethics and Research committee of Specialist Hospital Sokoto.

2.3 Study Design

The study was a cross sectional pilot study, where eligible adult HIV-positive HAART naive patients admitted to, or attending outpatient departments and blood donors donating blood to Specialist Hospital Sokoto, between April and August, 2015. The study groups, at the time of their enrollment, were not on antiretroviral therapy and those who were presently on HAART were excluded from the study. At enrolment a structured interview-administered questionnaire was administered to each patient and information on patient's demographic and socioeconomic characteristics including sex, age, marital status, occupation and education were obtained. The study participant data on the two immune parameters (surrogate markers) Human Immunoglobulin G (IgG) and total lymphocyte count (TLC) were divided into 3 categories based on the CDC Classification System for HIV-Infected Adults and Adolescents, namely, Category 1: CD4 cells> 500 cells/µl; Category 2: CD4 cells 200-499 cells/µl; Category 3: CD4< 200 cells/µl [14].

2.4 Sample Collection and Preparation

From each selected subject, a total of ten milliliters (10.0 ml) of whole venous blood samples was collected using a sterile vacutainer blood specimen bottle, holder and needle. Five milliliters (5.0 ml) of blood specimen were collected into a sterile EDTA vacutainer blood

specimen bottle for $CD4^+$ cell count and full blood count (FBC) analysis respectively within 3 hours of the blood sample collection. Five milliliters (5.0 ml) of blood specimen was collected into a sterile plain vacutainer blood specimen bottle, and allowed to clot at room temperature and later centrifuged at 3000 rpm for 5 minutes to obtain a clear unhaemolyzed serum. The sera were harvested into sterile serum-separation tubes and rapidly stored at -20°C until assayed in batches; for Human immunoglobulin G (IgG) using Enzyme Linked Immunosorbent Assay (ELISA) technique.

2.5 Analytical Methods

2.5.1 $CD4^+$ cell count

The CD4+ T-cells were enumerated using flow cytometry method, using Cyflow Counter manufactured by Partec, Munster, Germany.

2.5.1.1 Principle

In flow cytometric analysis, the fluorescent monoclonal antibody (CD4 monoclonal antibody) bind to the CD4-antigen on the mononuclear cells and in a buffer suspension; the complex is passed through the flow cuvettes in a single stream of flow. The complex is excited by the solid state laser at a wavelength spectrum which is scattered by the cells and captured by a photomultiplier tube and transmitted into digital readout as counts.

2.5.2 Determination of human IgG

Human IgG concentration was determined using IgG Human ELISA (enzyme-linked immunosorbent assay) kit manufactured by Abcam, UK.

2.5.2.1 Principle

This assay employs an antibody specific for Human IgG coated on a 96-well plate. Standards and samples are pipette into the wells and IgG present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinyl anti-Human IgG antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipette into the wells. The wells are washed, a TMB substrate solution was added to the wells and color develops in proportion to the amount of IgG bound. The stop solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

2.5.3 Determination of full blood count (automated haematology analyzer) and total lymphocyte count

This was based on the method of Automated Full blood count using Sysmex KX-21N Haematology Analyzer (Sysmex corporation Kobe, Japan).

2.5.3.1 Principle

Automated Haematology Analyzer (Sysmex KX-21N) is used to assess full blood count using whole blood. With the use of CELLPACK, blood sample is diluted beforehand to 1:26. This sample is aspirated from the sample probe into the sample rotor valve; 78µl of blood measured by the sample rotor valve is transferred to the WBC transducer chamber along with 1.922 ml of diluents. At this time, 1.0 ml of WBC/HGB lyses reagent is added to prepare 1:1000 dilution samples. When the solution is made to react in this status for 10 seconds, RBC is haemolysed and platelets shrink with WBC membrane held as they are. At the same time haemoglobin is converted into red colored methaemoglobin. Of the diluted/haemolysed sample in the WBC transducer chamber, approximately 1.0ml is transferred to the HGB flow cell. 500µl of sample in the WBC transducer is aspirated through the aperture. The pulses of the blood cells when passing through the aperture are counted by the DC detection method. In the HGB flow cell, 555nm wavelength beam irradiated from the light emitting diode (LED) is applied to the sample in the H GB flow cell. Concentration of this sample is measured as absorbance. This absorbance is compared with that of the diluents alone that was measured before addition of the sample, thereby calculating HGB (hemoglobin) value.

3. RESULTS

A total of one hundred and fifty (150) subjects were enrolled into the study. These comprised of one hundred (100) HIV-positive not on HAART patients and fifty (50) HIV-negative people who served as control. HIV-positive patients were stratified into three groups based on their $CD4^+$ T cell count: Group 1 (n=36): HIV positive with $CD4^+$ T cell count <200 cells/µl. These were made up of 13 males and 23 females, aged 26 to 41 years (mean age 33.06±1.04 years); Group 2 (n=38): HIV positive with $CD4^+$ T cell count 200-499 cells/µl. These were made up of 13 males

and 25 females, aged 19 to 64 years (mean age 31.55±1.59 years); Group 3 (n=26): HIV positive with $CD4^+$ T cell count ≥500 cells/µl. These were made up of 7 males and 19 females; aged 19 to 52 years (mean age 30.15±7.42 years).

The mean age of the HIV-positive patients and HIV-negative subjects were 31.73 ± 9.84 years ranging from 19-65 years and 32.16 ± 7.82 years ranging from 16-60 years respectively. A large number of HIV-positive patients, 53 (53.0%) were aged 21-30 years followed by 31-40 years 24 (24.0%) and the least 2 (2.0%) aged 61-70 years. The mean values of $CD4^+$ T cell count in HIV-negative controls (1190.12 ± 141.39 cells/µl) and HIV-positive HAART naive patients (group 1, 2 and 3) (110.35 ± 45.65, 319.66 ± 76.98 and 727.88 ± 222.13 cells/µl respectively). Patients with HIV had a significantly lower concentration of $CD4^+$ T cell count compared with HIV-negative subjects.

Table 1 showed the mean, standard deviation of IgG, TLC and $CD4^+$ T cell count among each category of HIV-positive HAART naïve patients. The present study used a CD4 cell cutoff of <200 cells/µl and compared the usefulness of alternate biomarkers. TLC<1354 cells/µl predicts CD4 cell count<200 cells/µl and IgG>13 g/l predicts CD4 cell count<200 cells/µl.

Table 2 showed the spearman rank correlation of $CD4^+$ T cell count with markers among HIV positive HAART naïve patients. In addition to the calculation of correlation value, the obtained data were used to draw Scatter grams to show the correlation between changes in $CD4^+$ T cell count with changes in TLC and IgG level. Figure 1 show a positive correlation between changes in $CD4^+$ cell count and TLC among HIV positive HAART naïve patients groups when the whole data were used. Figure 2 shows a negative correlation between changes in $CD4^+$ T cell count and IgG level among HIV positive HAART naïve patients group when the whole data were used.

4. DISCUSSION

HIV patients live relatively normal lives as non-infected subjects when diagnosed early and aggressive interventions are adopted. However, in many resource-poor countries, most facilities do not have cyflow counter for the performance of $CD4^+$ count analysis required for the initiation of antiretroviral therapy. Amazingly, in Sub-Saharan Africa where nearly 24 million people are living with HIV, less than 8% have access to highly active antiretroviral therapy (HAART) partly due to lack of access to $CD4^+$ count test [15]. This study have evaluated the relevance of total lymphocyte count (TLC), and immunoglobulin G (IgG) as a readily accessible and alternative immunological marker for $CD4^+$ count in determining the optimal time to initiate highly active antiretroviral therapy in HIV-infected Nigerians especially those presenting late to testing and treatment centres. The World Health Organization recommended that in treatment centres where there is no facility for performing absolute $CD4^+$ count test, clinicians need not wait until $CD4^+$ counters are available but total lymphocyte counts of <1200 and <1,500 cells/µl corresponding to $CD4^+$ count of <200 and <350 cells/µl respectively, should be used as a surrogate markers [16,17].

Table 1. Immunoglobulin G, total lymphocyte count and CD4 cell count in different categories of HIV patients based on CD4 cell count

Subjects CD4 (cells/µl)	n	IgG (g/l)	TLC (cells/µl)
HIV negative 1190.12±141.38	50	13.14± 61	2966±698
HIV positive CD4 <200 319.66±76.98[a]	36	20.34 ±3.12[a]	1354.66±728.79[a]
CD4 200-499 110.35±45.65[b]	38	18.84 ±3.00[b]	2099.12±1678.81[b]
CD4 ≥50 727.20±141.38[c]	26	16.34±2.64[c]	2828.69±1257.36[c]
t-test	p=<0.001	p=<0.001	p=<0.001

Values are mean ± standard deviation, n = number of subjects, IgG = Immunoglobulin G, TLC = total lymphocyte count, CD4 = cluster of differentiation 4. Data with different superscript [a, b and c] indicates statistical significance at p<0.05

Table 2. Correlation of CD4 cell count with TLC and IgG in HIV patients

Marker	CD4 cell count (cells/µl)	
	r	p-value
IgG (g/l)	0.434	<0.001
TLC (cells/µl)	0.431	<0.001

HIV = Human immunodeficiency virus, CD4 = Cluster of differentiation 4, TLC = Total lymphocyte count, IgG = Immunoglobulin G, r = Correlation coefficient

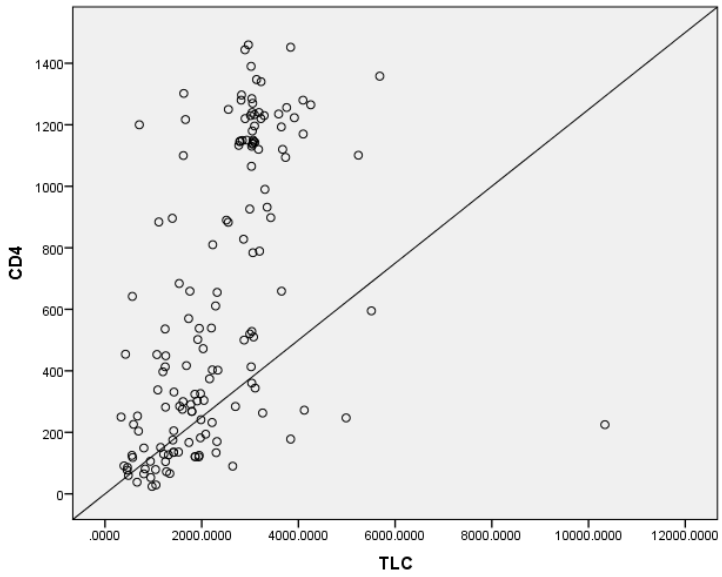

Figure 1. Scatter diagram showing the correlation of the $CD4^+$ T lymphocytes with total Counts (TLC) among HIV positive patients. r = 0.431, p < 0.001

Key: O = CD4 count; — = Linear (CD4 count)

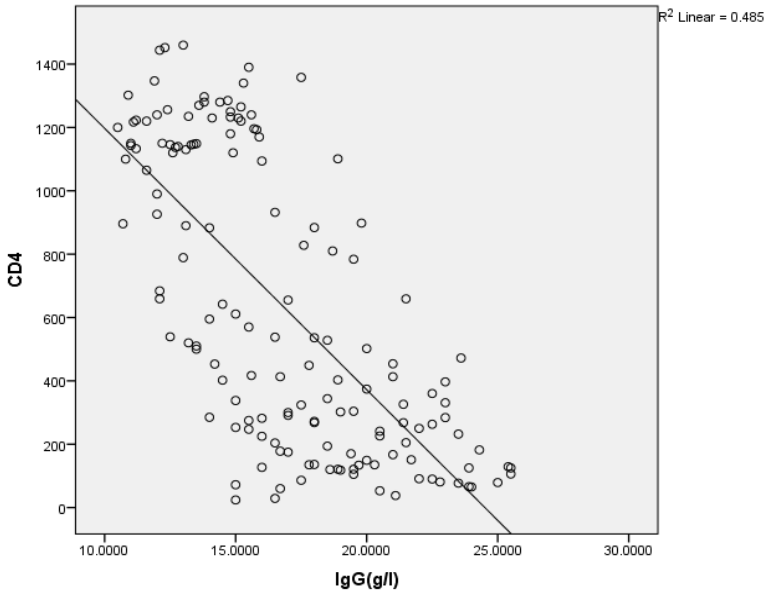

Figure 2. Scatter diagram showing the correlation of the $CD4^+$ T lymphocytes with immunoglobulin G (IgG) among HIV positive patients. r = 0.434, p < 0.001

Key: O = CD4 count; — = Linear (CD4 count)

The aim of this researches was to find the best (relatively cheap) predicting markers of disease progression in HIV positive HAART naïve patients so as to determine when to start or to stop antiretroviral therapy in order to avoid the complete dysfunction of the immune system and to avoid opportunistic infections [18]. The present study used CD4 cell cutoffs of < 200 cells/µl and it compared the usefulness of alternate biomarkers. Among the tested alternative biological markers, a TLC of <1354 cells/µl predicted CD4 counts of < 200 cells/µl. According to our results the TLC was found to have a positive correlation to the changes in CD4 count (r = 0.558). This agrees with Akanmu and his colleagues (2001) obtained from a study involving 100 HIV-infected Nigerians. In a similar study, Badri et al. [19] followed 266 patients at a hospital in South Africa to determine the usefulness of TLC versus CD4 count and viral load measurement for monitoring patients on ART and found significant correlation between changes in TLC and changes in CD4 count. While it is slightly lower than that obtained from the United Kingdom study in a larger population (n=1535) of HIV-infected patients [20]. It is higher than that obtained from similar studies involving 32 HIV- infected Nigerians [21]. This may be due to the improved clinical setting (multiple testing sites) approach, trained counselors and testers, more aggressive 'Know your HIV status' campaign and policy formulations on reduction of discrimination and stigmatization. Based on such clinical studies of CD4 count and TLC, the WHO recommended in its 2003 guideline and 2006 news letter that health facilities without the ability to perform CD4 measurement should use TLC to guide decision on when to start ART in patients who are symptomatic [22].

Among HIV positive HAART naïve patients, immunoglobulin G (IgG) concentration was negatively correlated with $CD4^+$ T cell count (r= -0.016). A similar observation was made in a study conducted in the USA, in which the number of IgG secreting B-cells was inversely correlated with $CD4^+$ cell counts, suggesting an association between polyclonal B-cell activation and defective $CD4^+$ cell function [23]. This finding was not consistent in a study in Tanzania [24] which reported insignificant correlation between high serum immunoglobulin G levels and $CD4^+$ count. Evidence from some laboratories indicates that the viral envelope proteins especially gp41 induce this polyclonal B-cell response in the presence of HIV infection has been source of debate for many years [25].

5. CONCLUSIONS

1. A significant positive correlation was established between $CD4^+$ T lymphocyte count and TLC in HIV infected adult patients.
2. A significant negative correlation was established between $CD4^+$ T lymphocyte count and IgG among HIV infected adult patients.

6. RECOMMENDATIONS

1. In developing countries like ours where facilities for $CD4^+$ cell count and viral load are not widely available, routine screening of TLC and IgG could be useful in predicting CD4+ T cell counts and HIV disease progression in resource poor settings.
2. The blood levels of TLC and serum IgG to be used in predicting $CD4^+$ cell counts needs to be validated in large scale, multi-centric studies in Nigeria so as to investigate the utility of such cheaper laboratory test modalities in predicting HIV disease progression and initiating antiretroviral therapy in resource limited settings.
3. Further studies should include the evaluation of serum levels of IgA, IgM, interleukin 2 (IL-2), interleukin 10 (IL-10), interleukin 16 (IL-6) and interferon (IFN) as surrogate markers for HIV disease progression is hereby suggested, for making treatment decision in resource poor settings when $CD4^+$ count is not available.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/21283