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Possible Use of Absolute Lymphocyte Count as Surrogate for CD4⁺ Cells Count in Monitoring Response to Highly Active Antiretroviral Therapy in Sokoto.

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Abstract

Few laboratories in resource-constrained countries can afford to perform laboratory-monitoring tests required for the management of HIV therapy. The initiation of an antiretroviral therapy is based on CD4⁺ count less than 350 cells/mm³ according to the World Health Organization (WHO) and Center for Disease Control (CDC). The determination of CD4⁺ count however in resource limited localities is difficult. In this cross sectional comparative study, the relevance of absolute lymphocyte count (ALC) as a possible surrogate for CD4⁺ cell count in monitoring response to highly active antiretroviral therapy (HAART) was investigated. A total of eighty-eight (88) HIV- infected patients on highly active antiretroviral therapy (HAART) and thirty (30) HIV-negative individuals were recruited for this study. Blood samples were run for absolute lymphocyte count and CD4⁺ cell counts and the results were compared using Linear Regression analysis. A modest correlation was observed between absolute lymphocyte count and CD4⁺ cell count ($r = 0.139$, $p = >0.05$) of 88 HIV-infected persons on HAART, relevant for monitoring response to HAART. The ALC and CD4⁺ count of HIV subjects decreased significantly ($p < 0.001$) when compared with control. This study indicates a modest correlation between absolute lymphocyte count and CD4⁺ cell counts of HIV infected patients on HAART. Absolute lymphocyte count may be adopted as an inexpensive and readily available to CD4⁺ cell counts.

Key words: Absolute Lymphocyte Count, Surrogate, CD4⁺ Cells Count, Antiretroviral Therapy, Sokoto

Introduction

The Human Immunodeficiency Virus (HIV) is a lentivirus (slowly replicating retrovirus) that causes Acquired Immune Deficiency Syndrome (AIDS), a condition in human in which there is progressive failure of the immune system allowing life threatening opportunistic infections and cancers to thrive (Weiss, 1993). Infection with HIV occurs by the transfer of blood, semen, vaginal fluid, pre-ejaculate or breast milk, within these bodily fluids. HIV is present as both free virus particles and virus within infected immune cells. It infects vital cells in the human immune system such as helper T-cells (specifically CD4⁺ T cells), macrophages and dendritic cells (Cunningham *et al.*, 2010).

Human immunodeficiency virus (HIV) types, derived from primate Lentiviruses, are the etiologic agents of Acquired Immune Deficiency Syndrome (AIDS). The illness was first described in 1981 and HIV 1 was isolated by the end of 1983, since then AIDS has become a worldwide epidemic, expanding in scope and magnitude as HIV infection has affected populations and geographic locations. Millions are now affected worldwide and once infected, individuals become infected for life. Within a decade if left untreated, the vast majority of HIV-infected individuals develop fatal opportunistic infections as a result HIV-induced deficiencies in the immune system. AIDS is one of the most important public health problems worldwide at the end of the 21st century (Brooks *et al.*, 2010).

CD4⁺ cells are a type of white blood cell that fights infection; they are made in the spleen, lymph node

and thymus gland which are part of the lymph or infection fighting system. CD₄⁺ cells move throughout the body, helping to identify and destroy germs such as bacteria and viruses. HIV targets CD₄⁺ cells by binding to the surface of CD₄⁺ cells, entering the CD₄⁺ cells and becoming a part of them, as the CD₄⁺ cells multiply to fight infection, they also make more copies of HIV, continuing to replicate leading to a gradual decline of CD₄⁺ cells. HIV uses the machinery of the CD₄⁺ cells to multiply (make copies of itself). HIV infection leads to low level of CD₄⁺ cells through a number of mechanism (Garg *et al.*, 2012).

The CD₄⁺ cells count measures the number of CD₄⁺ cells in a blood samples. It helps to tell how strong the immune system is, indicate the stage of HIV disease, guides treatment and predict disease progression. Depletion of lymphocyte, primarily of the CD₄⁺ cells subset subsequent to cellular CD₄⁺ immunodeficiency has been noted as the hallmark of HIV infection (Gougeon and Montagnier, 1993).

CD₄⁺ cells count has been established as the gold standard for staging HIV/AIDS, guiding treatment decision for HIV- infected person and evaluating effectiveness of the therapy, establishing a cut-off valued for total lymphocyte count (TLC) to be used as surrogate marker for CD₄⁺ cells count in staging, monitoring and as a guide to treatment decisions in HIV infected person in resource- limited setting. The possible use of total lymphocyte count (TLC) to be used as surrogate marker for CD₄⁺ cells has been suggested particularly in areas where viral loads and CD₄⁺ counts are absent. Current World Health Organization (WHO) guidelines propose the use of Total Lymphocyte Count (TLC) in conjunction with clinical data as a criterion for initiation of antiretroviral therapy (Doka and Loha, 2008).

Antiretroviral drugs are medications used for the treatment of infection by retroviruses, primarily HIV. When several such drugs, typically 3 or 4 are taken in combination, the approach is known as highly active antiretroviral therapy (HAART). The American National Institute of health and other Organizations recommended offering antiretroviral treatment agent to all patients with AIDS (Dybul *et al.*, 2002).

Worldwide estimate of people living with Human Immunodeficiency Virus was approximately 32 million in 2007 with thousands of people getting infected every day (UNAIDS, 2007). Most people living with HIV are from developing country with less than 5% receiving antiretroviral therapy. In

2009, an estimate of 2.6 million people became infected in sub-Saharan Africa (UNAIDS, 2010).

The initiation of antiretroviral therapy is based on CD₄⁺ count less than 350 cells /mm³ according to the World Health Organization (WHO) and Center for Disease Control (CDC). The determination of CD₄⁺ count however in resource- limited localities is difficult. A total lymphocyte count of <1200 cell/mm³ has been recommended in addition to WHO staging (stage 2) of the disease for the initiation of antiretroviral therapy in such localities. The use of absolute lymphocyte count as a marker for HIV progression has been argued in many quarters. Over the years, studies have suggested that when the absolute lymphocyte count is used in conjunction with blood hemoglobin it gives a more sensitive marker for HIV progression (Crowe *et al.*, 2003; Erhabor *et al.*, 2006). In April 2002, the WHO recommended the use of absolute lymphocyte count as an alternative marker when a CD₄⁺ cell count is not available or is not affordable. A total lymphocyte count of less than 1,000- 1,200 lymphocytes/ mm³ could be used as a threshold value to initiate antiretroviral therapy (Akintola *et al.*, 2004).

WHO has suggested that total lymphocyte count (TLC) could work as a potential marker for immunosuppression whenever CD₄⁺ cell count are unavailable, because TLC is easily obtained from routine complete blood cell counts by multiplying the percentage of lymphocytes by the white blood cell count. Several studies have demonstrated a good correlation between CD₄⁺ T cell count and the total lymphocyte count (TLC) in HIV-I infected patients however, others have showed no correlation between TLC and CD₄⁺ cell count (Kumarasamy *et al.*, 2002). This disagreement in various study setting necessitated the need for this study to be carried out.

Findings from this study can potentially be used to justify the use of absolute lymphocyte count which does not require complex laboratory equipment and inexpensive compared to CD₄⁺ cell count as guideline for clinical management of HIV- infected person on HAART and to justify the determination of the optimal time to initiate HAART and monitor disease progression. The aim of this study is to ascertain if absolute lymphocyte count could be used as a surrogate marker for CD₄⁺ cells count in the monitoring of response to highly active antiretroviral therapy (HAART) in Sokoto.

Material and Methods

Study Area

The study was carried out in Usmanu Danfodiyo University Teaching Hospital (UDUTH) Sokoto, Sokoto State. Sokoto State is located in the extreme North-West of Nigeria, near to the confluence of Sokoto River and Rima river, with an annual average temperature of 28.3^oc (82.9^oF). Sokoto is on the whole a very hot area. However, maximum daytime temperature is for most of the year generally under 40^oc (104.0^o F). The warmest months are February-April when daytime temperature can exceed 45^oc (113.0^o F) the rainy season is from June to October during which showers are a daily occurrence. There are two major seasons wet and dry which are distinct report from the 2007 population commission indicated that the State head a population of 3.6 million with projected population of 4,695,188.8 million in 2015 (UNFPA, 2013). The State has a landed area of 25,973 km² and is located between longitude 11.300 to 13.500 east and latitude 40 to 60 north. It is bounded to the North by Niger Republic, Kebbi State to the South-West and to the East by Zamfara State. The State composed of 23 local governments (Collins, Maps, 2013).

Study Subjects

A total of 118 subjects participated in this study comprising of 88 HIV- positive patients on HAART (study group) and 30 HIV - negative healthy individuals (controls group).

Study Design

This was a cross sectional comparative study of HIV- positive patient on HAART attending MSH clinic Usmanu Danfodiyo University Teaching Hospital, Sokoto and simple random sampling method was used.

Inclusion Criteria

All adult (≥ 18 years) consenting HIV- infected individuals undergoing HAART constituted the subjects for this study. HIV- negative consenting individuals used as control.

Exclusion Criteria

Non adult (<18 years) and Non- consenting HIV- positive individuals on HAART and non-HIV infected individuals were excluded from participating as subjects in this study.

Study Site and Participating Hospital

This study was conducted in the Faculty of Medical Laboratory Science of Usmanu Danfodiyo University Sokoto (UDUTH) in collaboration with

the Management Science for Health (MSH) in UDUTH Sokoto.

Ethical Consideration

Approval for the entire study was obtained from the ethical committee of Usmanu Danfodiyo University Teaching Hospital Sokoto while verbal informed consent was obtained from each of the participants.

Sample Collection

Five milliliters (5ml) of blood sample was drawn with minimum stasis into an EDTA vacutainer blood collection tube via the antecubital vein. Each sample was then mixed gently and thoroughly to ensure anticoagulation. The sample was then analyzed for CD4⁺ cells count, differential white cell count and total leucocyte count.

Estimation of CD4⁺ cell count

The CD4⁺ cells was enumerated using flow cytometry (FCM) method described by Cassens *et al.*, (2004), using Cyflow Counter manufactured by Partec, Munster, Germany.

Differential White Cell Count

Differential white cell count provides information on the different white cells present in the circulating blood. Provided the total WBC count is known the absolute number of each white cell type can be calculated (Cheesbrough, 2006).

Calculation (Cheesbrough, 2006).

Absolute Lymphocyte Count (ALC) = % of Lymphocyte Count X Total WBC count

Total Leucocyte Count (TLC).

Total leucocyte count is also known as white blood cell count. It is used to investigate HIV/AIDS and monitor treatment which can cause leucopenia (Cheesbrough, 2006).

Instrument and Method of Data Collection

The socio-demographic data and other relevant information of each participant was obtained using self-administered questionnaire.

Statistical Analysis

Statistical analysis was performed using Graph Pad Instat3 version 3.02 (Graph Pad Corp., San Diego, USA). Data are presented as mean \pm SEM. Statistical comparison between groups were made using unpaired t test. Pearson r correlation of HIV infected individuals on HAART was studied. P value ≤ 0.05 was taken as statistically significant.

Results

Eighty eight (88) HIV-infected patients on HAART constituted the subjects for this case-control study and thirty HIV-negative persons were monitored as controls. The results indicated that ALC and

CD4⁺cell count of HIV subjects decreased significantly ($p < 0.001$) when compared with control as shown in Table 1 and that ALC correlated positively with CD4⁺ cell count of HIV subjects on HAART as shown in Table 2.

Table 1: Comparison between ALC and CD4⁺ cell count of controls and HAART patients

Parameters	Study subjects	
	Control	HIV positive
Absolute Lymphocyte Count (mm ³)	3918.90±330.28	2503.56±130.24***
CD4 cell count (cell/μL)	1042.80±70.48	354.56±21.03***

Values are presented as mean± SEM. ***statistically significant ($p < 0.001$) as compared with control.

Table 2: Relationship between Absolute lymphocyte count and CD4⁺ cell count of HIV patients on HAART

Correlation between parameters	HAART patients		r-value	p-value
ALC and CD4	ALC	CD4	0.139	> 0.05
	3918.90±130.24	354.56±21.03		

ALC=absolute lymphocyte count (mm³), CD4=(cell/μL).

Discussion

The availability of highly active antiretroviral therapy (HAART) and the presence of effective facilities for the management of HIV infection have dramatically changed the landscape of human immunodeficiency virus (HIV). This possibility however is beyond the reach of a vast majority of people living with HIV in sub-Saharan African where paradoxically more than 23 million people are living with the virus but with less 8% having access. This is an ethical and public health issue that should be of great concern to the developed world (WHO, 2002). In this cross-sectional comparative study, the relevance of absolute lymphocyte count as a surrogate for CD4⁺ cell count was evaluated for monitoring of response among HIV-infected patients on HAART in Sokoto, Nigeria.

We observed a modest overall correlation coefficient ($r = 0.139$) between CD4⁺ cell count and absolute lymphocyte count of 88 HIV-infected patients on HAART. This value is lower than a value of ($r = 0.25$) obtained in a similar study involving 32 HIV-infected Nigerians (Akanmu *et al.*, 2001). Our observed correlation coefficient is also lower than that obtained in a study involving 2774 HIV positive persons in ($r = 0.70$) in South Africa (Van der Ryst *et al.*, 1998). The differences between our result and those of other authors could be due to difference in

ethnicity, racial, epidemiology and socioeconomic factors.

Our findings is also consistent with previous study (Blatt *et al.*, 1996), which indicated that absolute lymphocyte count was a better predictor of CD4 for symptomatic patients than for patients that are asymptomatic and have higher CD4 cell counts. A similar study conducted in Rivers by Erhabor and colleagues, observed a correlation coefficient of $r = 0.51$ between ALC and CD4 count. They observed a statistically significant positively correlation between absolute lymphocyte count and CD4 lymphocyte particularly at threshold relevant for the clinical management of HIV-infected Nigerians. Our finding is in agreement with the World Health Organization recommendation that in resource-limited settings where CD4 monitoring facilities are unavailable, absolute lymphocyte count could be used as a readily available minimal alternative for CD4 lymphocyte count.

Conclusion

In conclusion, this study revealed that there is a positive correlation between Absolute Lymphocyte Count (ALC) and CD4⁺cell count among HIV-infected person on Highly Active Antiretroviral Therapy (HAART).

Recommendation

From this study, it is recommended that absolute lymphocyte count (TLC) which is less expensive and easily obtained compared to CD4⁺ cell count should be used as a surrogate for CD4⁺ count whenever CD4⁺ cell count are unavailable for monitoring of response to HAART in Sokoto Nigeria.

Conflict of Interest

The authors disclose no conflict of interest

References

- Akanmu, A.S., Akinsete, I., Eshomonie, A.O., Davies, A.O and Okanny, C.C. (2001). Absolute lymphocyte count in monitoring response to antiretroviral therapy. *Nigerian Postgraduate Medical Journal*; 8:105-111.
- Akinola, O.O., Adeniran, I.A. and Onayemi, O. (2004). The search for a predictor of CD4 cell count continues: total lymphocyte count is not a substitute for the CD4 cell count in the management of HIV Infected individual in a resource –limited setting. *Clinical Infectious Diseases*; 39:579-89.
- Blatt, S.P., Lucey, C.R., Butzin, C.A., Hendrix, C.W. and Lucey, D.R. (1993). Total lymphocyte count as a predictor of absolute CD4 count and CD4 percentage in HIV infected person. *Journal of American Medical Association*; 269:622-628.
- Brooks, G.F., Carnoll, K.C., Janet, S., Butel, Morse, S.A. and Mietne, T.A. (2010) “Jawetz, Melnick S Adel Bergs Medical Microbiology”. 25th Edition. McGraw-Hill Companies North American: 406-407.
- Cheesbrough, M. (2006): Haematological tests in: District Laboratory Practice in Tropical Countries. Cambridge University Press: 321- 325.
- Collins Maps (2013). Geographical Information for Sokoto. www.collinsmaps.com (last accessed, 14/7/2013).
- Crowe, S., Turnbull, S., Oelrichs, R. and Dunne, A. (2003). Monitoring of human immune deficiency virus infection in resource constrained countries. *Clinical Infectious Diseases*, 37(1):25-35.
- Cunningham, A.L., Donaghy, H., Harman, A.N., Kim, M. and Turville, S.G. (2010). Manipulation of dendritic cell functions by viruses. *Current opinion in microbiology*; 13(4): 524-529.
- Doka, D. and Loha, E. (2008). Relationship between total lymphocyte count (TLC) and CD4 count among people with HIV, Southern Ethiopia: a retrospective evaluation. *AIDS Research and Therapy*; 5:26.
- Dybul, M., Fauci, A.S., Bartlett, J.G., Kaplan, J.E. and Pau, A.K. (2002). Panel on Clinical Practices for Treatment of HIV “*Annals of Internal Medicine*”; 137(5 pt2): 381-433.
- Erhabor, O., Uko, E.K., Adias, T. (2006). Absolute lymphocyte count as a marker for CD4 T-lymphocyte count: criterion for initiating antiretroviral therapy in HIV infected Nigerians. *Nigerian Journal of Medicine*; 15(1):56-59.
- Garg, H., MohI, J. and Joshi, A. (2012). HIV-I induced bystrander apoptosis viruses; 4(11): 3020-3043.
- Gougeon, M.L. and Montagnier, L. (1993). Apoptosis in AIDS. *Science*; 260 (5112): 1269-1270.
- Kumarasamy, N., Mahajan, A.P., Flanigan, T.P., Hemalatha, R., Mayer, K.H., carpenter, C.C., Thyagarayan, S.P. and Solomon, S. (2002). Total lymphocyte count (TLC) is a useful tool for the timing of opportunistic infection prophylaxis in India and other resource –constrained countries. *Journal of Acquire immune deficiency Syndrome*; 31(4): 378-383.
- UNAIDS (2010): Report on the Global AIDS Epidemics..
- UNAIDS/WHO (2007): Joint United Nations Programme on HIV/AIDS, AIDS epidemic update. Geneva, Switzerland: UNAIDS/WHO.
- United Nations fund For Population Activities (UNFPA) (2013): Population Projection Health Care Services in Sokoto State, Nigeria.
- Weiss, R.A. (1993). How does HIV cause AIDS? *Science*; 260 (5112): 1273-1279.
- WHO (2002). Scaling up antiretroviral therapy in resource limited settings. Guidelines for a public health approach.