http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 17 (4): 570-573, 2014
ISSN 1028-8880 / DOI: 10.3923/pjbs.2014.570.573

Correlation Between Total Lymphocyte Count, Hemoglobin, Hematocrit and CD4 Count in HIV Patients in Nigeria

[1]Charles Iheanyichi Emuchay, [2]Shemaiah Olufemi Okeniyi and [3]Joshua Olusegun Okeniyi
[1]Department of Physiology, Abia State University, Uturu, Nigeria
[2]Excellence Medical Diagnostic Laboratory Ltd., Aba, Nigeria
[3]Department of Mechanical Engineering, College of Science and Technology,
Covenant University Ota, Nigeria

Abstract: The expensive and technology limited setting of CD4 count testing is a major setback to the initiation of HAART in a resource limited country like Nigeria. Simple and inexpensive tools such as Hemoglobin (Hb) measurement and Total Lymphocyte Count (TLC) are recommended as substitute marker. In order to assess the correlations of these parameters with CD4 count, 100 "apparently healthy" male volunteers tested HIV positive aged ≥20 years but ≤40 years were recruited and from whom Hb, Hct, TLC and CD4 count were obtained. The correlation coefficients, R, the Nash-Sutcliffe Coefficient of Efficiency (CoE) and the p-values of the ANOVA model of Hb, Hct and TLC with CD4 count were assessed. The assessments show that there is no significant relationship of any of these parameters with CD4 count and the correlation coefficients are very weak. This study shows that Hb, Hct and TLC cannot be substitute for CD4 count as this might lead to certain individuals' deprivation of required treatment.

Key words: HIV, total lymphocytes count, hemoglobin, hematocrit, $CD4^+$ T cells count

INTRODUCTION

The number of people living with HIV globally continues to grow, a large number of these lives in developing and resource limited countries, like Nigeria, with millions of people in these countries requiring life sustaining highly active anti retroviral therapy (HAART) (Alavi *et al*., 2009; UNAIDS/WHO, 2005, 2006, 2007, 2009; WHO, 2004; Kumarasamy *et al*., 2002). In the resource-limited countries, the high cost of widespread and routine use of CD4+ T lymphocytes count and plasma viral load testing in the management of HIV infection has militated against its usage (Daryani *et al*., 2009; Solomon and Solomon, 2004; Kumarasamy *et al*., 2002). The World Health Organization (WHO) suggested that this limitation could be tackled using simple tools such as hemoglobin (Hb) measurement and TLC as markers to initiate HAART and as a substitute marker to monitor immune response to therapy in symptomatic HIV patients, in the resource-limited settings (WHO, 2004). There exist different arguments by studies based on this suggestion (Akinola *et al*., 2004; Spacek *et al*., 2003).

The expensive and technology limited setting attending CD4 count testing is major setback to the initiation of HAART. It is therefore resolved, in this study, to look into the capability of TLC, Hb and hematocrit (Hct) as substitute for CD4 count in HIV/AIDS patients through the correlation assessments of these parameters as alternate for CD4 count to initiate HAART in HIV/AIDS patients in Nigeria.

MATERIALS AND METHODS

Subject: One hundred "apparently healthy" male volunteers tested HIV positive, aged ≥20 years but ≤40 years, were recruited from randomly selected sites throughout South East Nigeria. These are yet to receive HAART. Exclusion criteria include known diagnosed diseases, use of chronic medication, oral temperatures>37°C, free from the use of interfering or toxic drugs. Most importantly, women are also excluded from the study because of their complication from menstrual cycle, pregnancy and lactation and other hormonal complications. All these constitute methods of subjects selection described by Oosthuizen *et al*. (2006).

Blood collection and HIV serology: Whole blood was collected with a Vacutainer system in 10 mL tubes containing EDTA. HIV status was determined with plasma samples by an enzyme immunoassay (EIA) with

Corresponding Author: Joshua Olusegun Okeniyi, Department of Mechanical Engineering, College of Science and Technology, Covenant University Ota, Nigeria

ImmunoComb® (Orgenics Isreal) and Determine test (Abbott). Blood sample for other analysis were obtained based on the instruction given in the manual of the analyzer used.

Hematological analysis: QBC™ Autoread ™ Plus Centrifugal Hematology System was used for the whole blood analysis of hematological parameters. It utilizes precision-bore glass tubes pre-coated with potassium oxalate, acridine orange fluorochrome stain and an agglutinating agent. QBC tubes made specifically for capillary blood (finger-stick samples) additionally contain a coating of anti-coagulants. During high-speed centrifugation of the blood-filled tube, the cells form in packed layers around the float, which has descended into the buffy coat. The spun tube is inserted in the QBC Autoread Plus Analyzer, where it is automatically scanned and fluorescence and absorbance readings are made to identify the expanded layers of differentiated cells. Volumes of these packed cell layers are then computed to obtain quantitative values. The obtained quantitative values include Hematocrit (Hct) (in percent), Hemoglobin (Hb) (in gram per deciliter), Platelet Count (Pl) (number of cell$\times 10^9$ L^{-1}), White Blood Cell Count (leucocytes/WBC) (expressed in number of cell$\times 10^9$ L^{-1}). Other quantitative values obtained include Granulocyte Count (Gr) (in percent and number of cell$\times 10^9$ L^{-1}) and Lymphocyte-Monocyte Count (TLC) (in percent and number of cell$\times 10^9$ L^{-1}).

CD4$^+$ T Lymphocyte analysis: Quantification of human peripheral blood CD4$^+$ T lymphocytes were carried out using Dynal® T4 Quant Kit. This utilizes immunomagnetic cell separation EDTA anti-coagulated blood samples to enable a rapid and direct quantification of CD4$^+$ T lymphocytes. The cell isolation takes place at room temperature by depletion of monocytes from blood sample using Dynabeads CD14, isolation of CD4$^+$ T cells from monocyte-depleted blood using Dynabead CD4, washing of bead bound cells to remove contaminating cells and counting of CD4$^+$ T cells.

Statistical analyses: Initial distribution of descriptive statistics for each hematological characteristic in the reference subjects was analysed using STATGRAPHICS® Centurion XVI version 16.1.11 by StatPoint Technologies, Inc. Also, linear correlation models of each of the hematological parameters and those of the combined parameters with CD4 count were analyzed and investigated using personally developed Visual Basic® program version 6.0. These were run on hp pavilion dv6-2150us (2009 model) with Intel® CORE™ i3 CPU M330 @ 2.13 GHz (Okeniyi and Okeniyi, 2012). Fittings of correlation models were studied using the correlation coefficient, R and the Nash-Sutcliffe Coefficient of Efficiency (CoE) given respectively as (Omotosho *et al*., 2011; Krause *et al*., 2005):

$$R = \frac{\sum_{i=1}^{N}(O_i - \bar{O})(P_i - \bar{P})}{\sqrt{\sum_{i=1}^{N}(O_i - \bar{O})^2} \times \sqrt{\sum_{i=1}^{N}(P_i - \bar{P})^2}} \quad (1)$$

$$CoE = 1 - \frac{\sum_{i=1}^{N}(O_i - P_i)^2}{\sum_{i=1}^{N}(O_i - \bar{O})^2} \quad (2)$$

where, O_i and P_i are respective observed and predicted CD4 count, while $\bar{O}$ and $\bar{P}$ are the respective mean of the observed and predicted CD4 count.

Ethics: Informed consent was obtained from each participant.

RESULTS AND DISCUSSION

The mean, Mean±2SD, median and 95th-percentile confidence interval for the hematological parameters and CD4$^+$ T cells of the recruited 100 subjects (between 20≤age (years) ≤40) that were tested HIV positive and are yet to receive HAART, are shown in Table 1. From the table, it could be observed that the mean values of hematological parameters obtained in this study bear similarity with those from other African studies (Akinola *et al*., 2004; Akanmu *et al*., 2001). Hct value of 33.6±5.97% from 100 male patients in this study compares well with 34.1±7% obtained from 64 male patients in Akinola *et al*., 2004), even as the WBC of 4.2±1.38 ($\times 10^9$ L^{-1}) in this study falls within the range of 4.788±2090 ($\times 10^9$ L^{-1}) obtained in that study. Also, CD4 count of 228±140.66 μL^{-1} from this study bear good comparisons with 222±189 μL^{-1} obtained in Akinola *et al*. (2004) and the 233 μL^{-1} obtained by Akanmu *et al*. (2001). However, CD4$^+$ T cells value of 279±225 μL^{-1} obtained from patients living in Iran, Asia (Alavi *et al*., 2009), is significantly higher than the results from this and other studies of patients living in Nigerian, Africa (Akinola *et al*., 2004), even though hemoglobin and hematocrit exhibited lower mean values in the former than in the latter studies. However, the lymphocyte count of 3.3±1.1($\times 10^9$ L^{-1}) obtained in this study is high compared to lymphocyte counts obtained in the other studies mentioned above.

Table 1: Measured parameter for HIV positive male adults in South Eastern Nigeria

Parameters	n	Mean±SD	Median	95% CI	p-value
Hb (g dL^{-1})	100	11.1±1.89	11.7	10.7-11.4	<0.0001
Hct (%)	100	33.6±5.97	35.4	32.4-34.7	<0.0001
TLC ($\times 10^9$ L^{-1})	100	3.3±1.1	3.18	3.0-3.5	NS
WBC ($\times 10^9$ L^{-1})	100	4.2±1.38	4.05	3.8-4.44	<0.001
$CD4^+$ T cells (μL^{-1})	100	228±140.66	200	200 - 256	<0.001

NS: Not significant

Table 2: Linear correlation of CD4 cells with other hematological parameters

Parameters	n	Cases in cutoff	Cases > cutoff with CD4 < 200	R	p-value
Hb (g dL^{-1}) < 12	100	61	0, (19[a])	0.1538	0.1265
Hct (%)< 30	100	23	19	0.1536	0.1271
TLC ($\times 10^9$ L^{-1})≤1.2	100	2	40	-0.1057	0.2954
$CD4^+$ T cells (μL)<200	100	42	42	1.000	-

[a]Cases<cutoff with CD4<200

The correlation coefficient, R and the p-value of the slope, which is also that of its ANOVA, obtained from linear relationships of each of the hematological parameters with CD4 count are shown in Table 2. Also shown in the table are cases found in each parameter's cutoff and those greater than the cutoff of CD4 count. From this, the p-values obtained from the ANOVA for each of the parametric correlations are greater than 0.05 showing that there is no statistically significant relationship between any of the parameters and CD4 at the 95.0% or higher confidence level (Table 2). Although CD4 count exhibited positive correlation with hemoglobin (R = 15.38%) and hematocrit (R = 12.71%) but negative correlation with lymphocyte count (R = -10.57%), these R-values indicate relatively weak relationship between these variables and CD4 count. This agrees with Akinola *et al.* (2004) which is also a study based on patients living in Nigerian. However, results from this study bear disagreement with that obtained in (Alavi *et al.*, 2009), save for the lack of fit encountered with the hemoglobin and the hematocrit, as well as that from (Spacek *et al.*, 2003) both of which were not based on patients from Africa. Suggestions from Lugada *et al.* (2004) identified causes for these kind of disagreements as that which could be due to environmental and genetic factors.

The combination of all these hematological parameters exhibit linear correlation with the CD4 cells which could be represented as:

$$CD4^+ = 4.1556Hb+2.3569Hct-13.8492TLC+138.9551 \quad (3)$$

For this, the correlation coefficient R = 0.1848, Nash-Sutcliffe CoE = 0.0342 and p-value = 0.3402. All these estimates of model validation parameters further reinforced that the correlation between CD4 counts and the studied hematological parameters are very poor. Specifically, the correlation coefficient showed that only 3.42% of the variability in the CD4 count could be accounted for by the fitted correlation model, while the CoE statistics showed that the observed model of CD4 count would be better than the predicted model from the fit. Also, the p-value model showed that there is not a statistically significant relationship between the hematological variables and CD4 count at the 95.0% confidence level.

This bear dire implications, especially, from the view of TLC, which has been recommended to stand as substitute marker for CD4 count for resource limited countries to initiate HAART (WHO, 2004). Out of the 100 HIV positive individuals, two (2) fell in the $\leq 1.2\times10^9$ L^{-1} cutoff of the TLC recommendation while 40 that had CD4 <200 cells μL^{-1} had TLC $>1.2\times10^9$ L^{-1}. It has been reported that with results like these, certain number of HIV positive individuals would have been deprived of needed treatment (Akinola *et al.*, 2004) for their TLC result might have been used for HAART initiation going by WHO recommendation. Results from the patients studied in this work show that neither Hemoglobin nor Hematocrit, not even TLC, can be substitute for CD4 count of the patients. Thus, further research work are required for patients in Nigeria and in other resource limited countries in Africa, investigating parameters which would bear correlation with CD4 count for adequate indication of costly CD4 in resource limited countries.

CONCLUSION

Correlation between CD4 count and hematological parameters such as total lymphocyte count, hemoglobin and hematocrit had been studied in this work. From this, conclusions that could be drawn include:

- The mean values of hematological parameters obtained in this study bear similarities with those obtained in other studies for patients living in other part of Nigeria (Africa)

- Individual correlations of total lymphocyte count, hemoglobin and hematocrit with CD4 showed that all of the parameters exhibit poor correlation with CD4 count, which were attendant with low correlation coefficients and non significant ANOVA p-values
- Combined correlation of total lymphocyte count, hemoglobin and hematocrit with CD4 showed that the resulting correlation fitting function exhibited poor correlation coefficient, R = 0.1848 and poor Nash-Sutcliffe Coefficient of Efficiency, CoE = 0.0342 and non-significant p-value = 0.3402
- These results lead to the conclusion that hematological parameters such as total lymphocyte count, hemoglobin and hematocrit are not suitable for representing CD4 even as suggestion for further study are recommended for finding suitable alternative for indicating costly CD4 count in limited resources countries

REFERENCES

Akanmu, A.S., I. Akinsete, A.O. Eshofonie, A.O. Davies and C.C. Okanny, 2001. Absolute lymphocyte count as surrogate for $CD4^+$ cell count in monitoring response to antiretroviral therapy. Niger. Postgraduate Med. J., 8: 105-111.

Akinola, N.O., O. Olasode, I.A. Adediran, O. Onayemi and A. Murainah *et al*., 2004. The search for a predictor of CD4 cell count continues: Total lymphocyte count is not a substitute for CD4 cell count in the management of HIV-infected individuals in a resource-limited setting. Clin. Infect. Dis., 39: 579-581.

Alavi, S.M., F. Ahmadi and M. Farhadi, 2009. Correlation between total lymphocyte count, hemoglobin, hematocrit and CD4 count in HIV/AIDS patients. Acta Medica Iranica, 47: 1-4.

Daryani, A., M. Sharif, M. Meigouni, F. Baba Mahmoudi and A. Rafiei *et al*., 2009. Prevalence of intestinal parasites and profile of CD_4^+ counts in HIV^+/AIDS people in North of Iran, 2007-2008. Pak. J. Biol. Sci., 12: 1277-1281.

Krause, P., D.P. Boyle and F. Base, 2005. Comparison of different efficiency criteria for hydrological model assessment. Adv. Geosci., 5: 89-97.

Kumarasamy, N., T.P. Flamigan, A.P. Mahajan, C.C. Carpenter, K.H. Mayer and S. Solomon, 2002. Monitoring HIV treatment in the Developing world. Lancet Infect. Dis., 2: 656-657.

Lugada, E.S., J. Mermin, F. Kaharuza, E. Ulvestad and W. Were *et al*., 2004. Population-based hematologic and immunologic reference values for a healthy ugandan population. Clin. Diagn. Lab. Immunol., 11: 29-34.

Okeniyi, J.O. and E.T. Okeniyi, 2012. Implementation of kolmogorov-smirnov p-value computation in visual basic®: Implication for Microsoft Excel® library function. J. Stat. Comput. Simul., 82: 1727-1741.

Omotosho, O.A., C.A. Loto, O.O. Ajayi and J.O. Okeniyi, 2011. Statistical-based performance evaluation of inhibitors on the corrosion of steel rebar in concrete. Botswana J. Technol., 19: 220-226.

Oosthuizen, W., A. van Graan, A. Kruger and H.H. Vorste, 2006. Polyunsaturated fatty acid intake is adversely related to liver function in HIV-infected subjects: The THUSA study13. Am. J. Clin. Nutr., 83: 1193-1198.

Solomon, S.S. and S. Solomon, 2004. A comparison of the delivery of antiretroviral therapy system between Brazil and India. JK Sci., 6: 168-171.

Spacek, L.A., M. Griswold, T.C. Quinn and R.D. Moore, 2003. Total lymphocyte count and hemoglobin combined in an algorithm to initiate the use of highly active antiretroviral therapy in resource-limited settings. AIDS, 17: 1311-1317.

UNAIDS/WHO, 2005. AIDS epidemic update. Joint United Nations Programme on HIV/AIDS, Geneva, Switzerland.

UNAIDS/WHO, 2006. AIDS Epidemic Update. Joint United Nations Programme on HIV/AIDS Geneva. http://data.unaids.org/pub/epireport/2006/2006_epi update_en.pdf

UNAIDS/WHO, 2007. AIDS epidemic update. Joint United Nations Programme on HIV/AIDS Geneva. http://data.unaids.org/pub/epislides/2007/2007_epi update_en.pdf

UNAIDS/WHO, 2009. AIDS epidemic update. Joint United Nations Programme on HIV/AIDS Geneva. http://data.unaids.org/pub/report/2009/jc1700_epi_ update_2009_en.pdf

WHO, 2004. Scaling up antiretroviral therapy in resource-limited settings: Treatment guidelines for a public health approach. World Health Organization, Geneva, Switzerland, pp: 1-68.