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Normal CD4 Count Range among Healthy Nigerian Population in Ilorin

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Abstract

Background: For the establishment and monitoring of the immune status, CD4 count is critical. **Objectives:** To determine the CD4 count range of apparently healthy Nigerians resident in Ilorin and compare with the national value. **Methods:** An automated blood analyzer was used to determine the full blood count and CD4 count. The percentage of CD4 count was derived by using other variables. **Results:** Of the 1205 participants, the reference CD4 count (percentage of CD4) range for adult was 400 to 1288 cells/mm^3 (19%-48%) and for children was 582 to 3652 cells/mm^3 (17%-50%). CD4 count and percentage of CD4 were significantly ($P = .001$) higher in females than in males, and the CD4 count declined significantly with increasing age ($r = -.174$, $P \leq .0001$). The percentage of CD4 count shows less variation with age ($r = -.051$, $P = .076$). Adult residents of Ilorin had significantly lower absolute mean CD4 count (808 $\pm$ 260) than that of the national reference values of 847.0 $\pm$ 307.0 cells/mm^3 ($P = .001$). **Conclusion:** We therefore advocate the use of CD4 count range derived in this study is lower than that of the national reference values.

Keywords

CD4 count, range, T lymphocyte, Nigeria

Introduction

The CD4 or helper T cells are subgroups of lymphocytes and are major determinant of the integrity of human immune system. It is responsible for the way the body responds to immunogens. The estimation of CD4 counts is a critical parameter in the establishment and monitoring of the immune status of an individual both in healthy and disease states.[1-3] Determination of CD4 count is of particular importance in this era of HIV pandemic. It is used to stage the severity of HIV infection, determine the initiation of highly active antiretroviral therapy (HAART), and also monitor the response to HAART.[1-3] These qualities of CD4 count are quite relevant where viral assay is not readily available, cost effective, or just impractical as found in some resource-poor nations like Nigeria. There are several factors that affect the interpretation and the level of CD4 count in healthy and disease states; some of these are genetic inheritance, nutritional pattern, age, gender, and race.[4,5] For instance, the CD4 count in healthy children is known to be much higher than that of the adults and varies from one ethnic population to the other.[6-9] Similarly, CD4 count values in normal Africans have been reported to be lower than those of the North American and European populations.[10] Normal CD4 count in the Caucasians are said to range from 500 to 1600 cells/mm^3. Although a few studies have tried to set the reference CD4 count in Nigeria,[11,12] the result of those studies may not be comprehensive enough. This is because the studies were conducted with adults, excluding the children. Furthermore, no participant was recruited from Ilorin, Nigeria. The reference

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CD4 count of 365 to 1571 cells/mm^3 reported by the study in question for Nigerians therefore requires a validation within the Ilorin environment. The present study therefore is designed to determine the CD4 count range of apparently healthy Nigerians of all age-groups living in Ilorin, Nigeria.

Methodology

Study Design and Setting

This was a cross-sectional study carried out in Ilorin, Kwara State, from January to December 2010. The study site is in the middle belt region of Nigeria, which is about 400 km north of Lagos, lying at 8°30'N 4°33'E.

Study Population

The study population comprises apparently healthy, HIV nonreactive persons of various age-groups resident of Ilorin and its environs.

Sample Size

Using Fishers formula and 50% as the proportion of the population targeted, the sample size of 384 was arrived at as detailed subsequently: sample size (n) $= z^2p\,(1-p) = 1.962 \times 0.031 \times (1-0.031) = 384$ $d^2 = 0.052$, where z = standard error and taken as 1.96, p = proportion of the population targeted (50%), and d = desired degree of accuracy taken as 0.05. However, the number of participants recruited into the study was far in excess of the calculated sample size in order to have a good representative of the study population and increase the power of the study.

Selection of Participants

Apparently healthy, HIV nonreactive persons belonging to various age-groups were recruited into the study. The participants were selected by simple random sampling technique from schools, churches, mosques, markets, and barracks in and around Ilorin. Each consented participant was assessed clinically by an adult physician and a pediatrician for eligibility to take part in the study. The participants and their parents or guardians completed a screening questionnaire. They were assessed for signs of febrile illnesses, active infection, malnutrition, chronic medical condition, and infection. In addition, they were questioned to determine their medication history with the associated illness. Participants who gave history of systemic and local symptoms that are suggestive of diabetes mellitus, tuberculosis, cancer, and physical appearance that is typical of malnutrition were excluded outright. HIV seroreactivity of the participants was also determined, and only those who were nonreactive were recruited into the study. The consented participants were certified healthy after a thorough history taking to exclude illnesses/conditions that may cause immune suppression like HIV, diabetes, malnutrition, tuberculosis, cancers, and pregnancy. All participants with other comorbidity that can affect the outcome of this study or who cannot adequately screen them of any illness were also excluded. The following categories of people were also excluded from the study: those who received blood transfusion within the last 6 months, pregnant women, and those on steroid or other immunosuppressive therapy. This method of screening participants was used in the previous studies[7-10] and was adapted to our local setting.

Specimen Collection

Blood specimens were collected from the participants between 9.00 AM and noon each day and transported to the laboratory section of University of Ilorin Teaching Hospital (UITH), Ilorin, for immediate processing and analysis. In this study, 5 mL of venous blood was collected from the antecubital vein of all participants aseptically and dispensed in aliquots of 2.5 mL each into a plain bottle and an EDTA bottle. The specimen in the plain bottle was left on bench to retract and the serum removed for HIV sero-status determination, while the specimen in EDTA was used to determine the CD4 count and full blood count (FBC). Participants' sociodemographic data and other clinically relevant information were also obtained at the time of recruitment.

Specimen Handling

HIV sero-reactivity was determined according to the national algorithm. Parallel screening was quickly carried out using 2 rapid kits, and discordant results resolved with the third kit (tie breaker). Three kits (Start Pack, Chembio Diagnostics Systems, Inc, USA; Determine, Alere Medical C. Ltd, Japan; and Uni-gold, Trinity Biotech Plc, Ireland) were used according to manufacturer's instruction. Participants were categorized as HIV nonreactive when they do not react to HIV in at least 2 rapid kits. The CD4 count and FBC were determined within 6 hours of blood specimen collection with Cyflow SL (Partec, Münster, Germany) and automatic blood count analyzer Sysmex KX21 (Sysmex, Japan), according to the manufacturer's instruction.

Ethical Issues

Written informed consent and assent were obtained from all the participants. Ethical approval was obtained from the ethical review committee of UITH, Ilorin, Nigeria.

Pre- and Post-HIV Screening Counseling

Participants were all counseled before the HIV screening test was done and were also counseled before the results were disclosed to them. Some of the sero-reactive participants were referred to the HIV/AIDS clinic for documentation and initiation of care.

Disclosure of Results

All participants were encouraged to check personally with researchers at the UITH for the outcome of their test. Those individuals who failed to check were sent reminders through text messages to their mobile phone, especially those who provided their phone numbers at the time of recruitment. In cases where there were no responses, researchers located participants' residence based on the address given at the time of recruitment and counseled and communicated the result irrespective of their HIV status but maintained confidentiality.

Data Handling

Data generated were entered into SPSS with the results presented in tables. Comparisons of categorical data were done by chi-square analysis or Fisher exact tests as appropriate while continuous variables were described by means and compared by Student *t* test. Normality of CD4 count and its percentage were determined using 1-sample Kolmogorov-Smirnov test. The values that were normally distributed were expressed in mean, and standard deviation and nonnormally distributed variables were expressed as median and 2.5th to 97.5th percentile. P values $< .05$ were considered to be statistically significant.

Results

Sociodemographic Distribution of the Participants

A total of 1205 apparently healthy, HIV nonreactive persons resident in Ilorin and its environs were recruited in the study. The median age of the participants was 29 years, and interquartile range was 20 to 41 years. The minimum age was 1 month, while the maximum age was 65 years. In all, 595 (49.4%) were males and 610 (50.6%) were females, giving a male to female ratio of 1:1. The participants were predominantly employed (82.9%) and Muslim (63.9%), and a majority (68.6%) had tertiary education (Table 1).

Table 1. Sociodemographic Distribution of the Participants.

Parameter	Frequency (%), n = 1205
Age stratification	
Adult (>18 yrs)	935 (77)
Children (≤18 yrs)	270 (22.4)
Gender	
Male	595 (49.4)
Female	610 (50.6)
Education	
Arabic	2 (0.0)
None	58 (5.8)
Primary	72 (7.2)
Secondary	183 (18.3)
Tertiary	687 (68.6)
NA	203 (16.8)
Occupation	
Employed	830 (82.9)
Unemployed	171 (17.1)
NA	204 (16.9)
Religion	
Traditional	1 (0.08)
Islam	770 (63.9)
Christian	434 (36.02)

NA, not applicable especially in children; yrs, years.

CD4 Count of the Participants

The absolute mean CD4 count obtained for whole population of Ilorin was 1023 ± 603 cells/mm^3 (median 842 cells/mm^3, range: 408-2785 cells/mm^3). For the adult population, the mean CD4 count obtained was 808 ± 260 cells/mm^3 (median 779 cells/mm^3, range: 400-1288 cells/mm^3). The total mean absolute CD4 count obtained for the children was 1816 ± 810 cells/mm^3 (median 1792 cells/mm^3, range: 582-3652 cells/mm^3). Table 2 shows the mean CD4 count obtained for children of different age-groups.

The Percentage of CD4 Count of the Participants

The percentage of absolute mean CD4 obtained for whole population of Ilorin was 32% ± 7% (median 32%, range:18%-46%). The percentage of mean CD4 for adults was 32% ± 7% (median 32%, range: 19%-48%), and for children was 32% ± 8% (median 32.0%, range 17-50). Table 3 shows the percentage of CD4 for children and adults.

Sociodemographic Factors Associated with CD4 Count and Percentage of CD4

Females significantly had a higher absolute mean CD4 count (1077 ± 609 versus 965 ± 589) and percentage of CD4 (34% ± 6% versus 30% ± 7%) than adults; similarly, children had a significantly higher absolute mean CD4 count (1770 ± 821 versus 807 ± 260) than the adults, while there was no significant difference in their percentage of CD4 (32% ± 0% versus 32% ± 1%) in this study. Other sociodemographic variables had no significant effects on the CD4 count (Table 4). CD4 count declined significantly with increasing age ($r = -0.174$, $P \leq .0001$; Table 5). The absolute mean CD4 count of adult males and females was significantly lower in this study than in the national study (Table 6).

Discussion

This study has determined the CD4 count range of apparently healthy Nigerians of all age-groups living in Ilorin and its environs. This study presents the data on normal reference CD4 count to facilitate the assessment of degree of immune suppression, which is widely used in the stratification, treatment, and follow-up of HIV-infected individuals in clinical practice and AIDS-related research studies.

The mean CD4 count obtained in this study for adult population is comparatively lower than the national average of

Table 2. Normal CD4 Count Ranges of Nigerians Resident in Ilorin.

Population Stratification by Age	Male			Female				Total		
	Mean ± SD	Median	2.5th–97.5th Percentile	Mean ± SD	Median	2.5th–97.5th Percentile	*P* Values	Mean ± SD	Median	2.5th–97.5th Percentile
≥18 yrs	741 ± 216	715	390–1173	872 ± 281	847	411–1355	<.001	808 ± 260	779	400–1288
10–17 yrs	783 ± 217	782	528–1384	958 ± 306	918	321–1512	.023	888 ± 285	833	403–1449
2–9 yrs	1392 ± 390	1306	995–2066	1641 ± 556	1708	842–2464	.297	1527 ± 493	1474	842–2461
≤1 yr	2044 ± 693	1941	664–3187	2270 ± 725	2516	1241–3747	.043	2140 ± 712	2034	773–3347
≤1 month	1808 ± 930	1541	1048–3104	2542 ± 442	2444	2127–3155	.149	2175 ± 780	2247	1048–3166

Abbreviations: SD, standard deviation: yrs, years.

Table 3. Normal CD4 Percentage Ranges in Nigerians Residents in Ilorin.

Population Stratification by Age	Male			Female				Total		
	Mean ± SD	Median	2.5th–97.5th Percentile	Mean ± SD	Median	2.5th–97.5th Percentile	*P* Values	Mean ± SD	Median	2.5th–97.5th Percentile
≥18 yrs	30 ± 6	30	18–42	34 ± 7	34	21–48	<.001	32 ± 7	32	19–48
10–17 yrs	28 ± 8	28	5–41	33 ± 6	33	20–47	.015	31 ± 7	31	10–45
2–9 yrs	28 ± 6	27	20–39	31 ± 9	29	20–52	.331	30 ± 8	28	20–47
≤1 yr	31 ± 8	32	20–47	33 ± 8	34	16–48	.128	32 ± 8	32	16–47
≤ 1 month	32 ± 17	28	16–55	47 ± 6	50	38–52	.248	40 ± 14	43	16–55

Abbreviations: SD, standard deviation: yrs, years.

Table 4. Influence of Sociodemographic Variables on Normal CD4 Count.

Parameter	N (%)	Mean CD4 Count	*t*/*F* Statistics	*P* Value
Age				
≤18 yrs	270 (22.4)	1770 ± 821	30.9	.00
>18 yrs	935 (77.6)	807 ± 260		
Gender				
Male	595 (49.4)	965 ± 589	3.23	.00
Female	610 (50.6)	1077 ± 609		
Education				
Arabic	2 (0.2)	1003 ± 226	2.27	.06
None	58 (5.8)	876 ± 425		
Primary	72 (7.2)	879 ± 305		
Secondary	183 (18.3)	808 ± 244		
Tertiary	687 (68.6)	805 ± 256		
Occupation[a]				
Employed	830 (82.9)	815 ± 265	0.94	.35
Unemployed	171 (17.1)	795 ± 265		
Religion				
Traditional	1 (0.1)	853 ± 0.0	0.13	.88
Islam	770 (63.9)	1028 ± 601		
Christian	434 (36.0)	1013 ± 606		

Abbreviation: yrs, years.
[a] Data did not add up because of children.

Table 5. The Association between Age, Gender, and Normal CD4 Count.

Parameter	N (%)	Mean CD_4 Count	Spearman Correlation	*P* Value
Age				
≤18 yrs	270 (22.4)	1770 ± 821	−0.578	.000
>18 yrs	935 (77.6)	807 ± 260		
Gender				
Male	595 (49.4)	965 ± 589.	−0.174	.001
Female	610 (50.6)	1077 ± 609		
Education				
Arabic	2 (0.2)	1003 ± 226	−0.031	.278
None	58 (5.8)	876 ± 425		
Primary	72 (7.2)	879 ± 305		
Secondary	183 (18.3)	808 ± 244		
Tertiary	687 (68.6)	805 ± 256		

Table 6. Comparison of Mean CD4 Counts of the Present Study with Previous National Study.

Parameter	Present Study, n = 1205	Previous Study, n = 2507	*P* Value	*t* Statistics
Absolute adult mean CD4 count	808 ± 260	847.0 ± 307.0	<.001	3.815
Absolute adult male mean CD4 count	741 ± 216	782 ± 272	<.001	4.598
Absolute adult female mean CD4 count	872 ± 281	920 ± 327	<.001	4.300

847.0 + 307.0 cells/mm^3 and 828 cells/mm^3 reported in Jos Nigeria.[11,12] Similarly, when compared with other studies outside Nigeria, it is higher than the 746 cells/mm^3 in Tanzania,[13] 759 cells/mm^3 in Botswana,[14] 727 cells/mm^3 in China,[15] and 753 cells/mm^3 in Ethiopia[1] the and lower than, 830 cells/mm^3 in the United Kingdom,[16] 865 cells/mm^3 in India,[2] 910 cells/mm^3 in Thailand,[17] 1095 cells/mm^3 in Turkey,[18] and 1256 cells/mm^3 in Uganda.[19] The observed differences in CD4 reference values might be due to the heterogeneity of population as a result of ethnoracial variations, interlaboratory variability, and the differences in the laboratory methodologies of measuring CD4 count especially where the variations were not controlled.

The mean percentage of CD4 obtained in this study for adult population is similar to the Malaysian population[20] and is lower than the 42% in Romania[21] and 47% in Turkey.[18] In this study, in view of the statistical difference in both the CD4 count and the percentage of CD4 obtained for both gender in adult and childhood, it is imperative to establish a reference range separately for each of them. The reference range of CD4 count was significantly higher in females than in the males, and this result is in agreement with other studies in Africa,[8,12,14,19] India,[2,22] and Britain.[23] This reported gender difference in CD4 counts is attributed to the effect of sex hormones.[23]

In children ≤1 month of age, the mean CD4 count obtained is lower than the 3050 reported in Saudi Arabia[24] but higher than the 2000 reported in Malawi,[25] 2001 in Cameron,[26] and 1816 in Ethiopia.[27] For those between 2 months of life to 1 year, the mean CD4 count is lower than the 2200 in Malawi[25] and 2252 in Cameron,[26] but lower values were obtained in the developed countries like the Netherlands[28] and the United States.[29,30] The CD4 count observed in children of African countries was found to be lower than the values reported in children of developed countries. This difference is attributed to genetics or environmental factors or a combination of both factors. We observed that the mean CD4 count and percentage of CD4 peaked in the first year of life and then decreased during childhood. Similar trend was also observed in previous studies in Africa and elsewhere.[24,26,27]

The present study also shows that age and gender are significant determinants of CD4 count and its percentage. CD4 count and percentage of CD4 were significantly higher in females than in males and declined significantly with increasing age. From the neonatal period to adulthood, the percentage of CD4 shows less variation than that of CD4 count. The World Health Organization has stated that percentage of CD4 rather than the CD4 absolute count may be preferred as the best surrogate marker for monitoring HIV-infected children of all ages, rather than just among those aged <5 years.[31]

We also compared our findings with the previous study in Nigeria and found a statistically significant difference in the values of CD4 count obtained in the 2 studies. Considering the significant difference in parameter between the 2 studies in the same country, this result has an important implication for the use of T-lymphocyte subset measurements for the evaluation and treatment of HIV-infected patients in this locality and recruitment in clinical trials.

The strength of this study was its ability to establish a reference range in both adult and children population in Nigeria with a large sample size. However, we were limited by our inability to determine the counts of other subsets of T lymphocytes.

Conclusion

We therefore advocate the use of CD4 count range derived in this study because it is lower than the national reference values.

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Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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References

1. Tsegaye A, Messela T, Halilu E, et al. Immunohematological reference ranges for adult Ethiopians. *Clin Diag Lab Immunol*. 1999; 6(3):410-414.
2. Uppal SS, Verma S, Dhot PS. Normal values of CD4 and CD8 lymphocyte subsets in healthy Indian adults and the effects of sex, age, ethnicity and smoking. *Cytometry*. 2003;52(1):32-36.
3. Holfman RG. *Establishing quality control and normal ranges in the clinical laboratory*. Jericho, NY: Exposition Press Inc; 1971.
4. Chin SF, Cheoug SK, Lim YV, Ton SH. The distribution of immunoregulatory cells in the peripheral blood of normal Malaysia adults. *Malays J Pathol*.1993;15(1):49-52.
5. Choong ML, Yon SH, Cheong SK. Influence of race, age and sex on the lymphocyte of subjects in peripheral blood of normal Malaysian adults. *Ann Clin Biochem*. 1995;32(pt 6):532-539.
6. Shabuddin S. Quantitative differences in CD8+ lymphocytes, CD4/CD8 ratio, NK cella, and HLA-DR + activated T cells of racially different male populations. *Clin Immunol Immunopathol*. 1995;75(2):168-170.
7. Idoko JA, Njoku MO, Sirisena MD, Jelpe D. CD4+ T- lymphocyte counts in human immunodeficiency virus (HIV) infected and healthy Nigerian population. *Niger Med Pract*. 2001;39:53-56.
8. Njoku MO, Sirisena ND, Idoko JA, Jelpe D. CD4+ T-lymphocyte counts in patients with Human Immunodeficiency virus type 1 (HIV-1) and healthy population in Jos, Nigeria. *Niger Postgrad Med J*. 2003;10(3):135-139.
9. Ampofo W, Torpey K, Mukadi YD, et al. Normal CD4+ T lymphocyte levels in HIV seronegative individuals in the Manyalyila Krob0 communities in the eastern region of Ghana. *Viral Immunol*. 2006;19(2):260-262.
10. Audu RA, Idigbe EO, Akanmu AS, Mafe AG, Onyewuche J, Oparaugo CT. Values of CD4+ Tlymphocyte in apparently healthy individuals in Lagos. *Eur J Sci Res*. 2007;16:168-173.
11. Aina O, Dadik J, Charurat M, et al. Reference values of CD4 T-lymphocytes in Human Immunodeficiency virus-negative adult Nigerians. *Clin Diagn Lab Immunol*. 2005;12(4):525-530.
12. Oladepo DK, Idigbe EO, Audu RA. Establishment of CD4 lymphocyte baseline in Nigeria: Project report; 2008.
13. Ngowi BJ, Mfinanga SG, Bruun JN, Morkve O. Immunohaematological reference values in human immunodeficiency virus-negative adolescent and adults in rural northern Tanzania. *BMC Infect Dis*. 2009;9:1. doi:10.1186/1471-2334-9-1. http://www.biomedcentral.com/1471-2334/9/1. Accessed February 15, 2014.
14. Bussmann H, Wester CW, Masupu KV, et al. Low CD4+ Tlymphocyte values in human immunodeficiency virus-negative adults in Botswana. *Clin Diagn Lab Immunol*. 2004;11(5):930-935.
15. Jiang W, Kang L, Lu HZ, et al. Normal values for CD4 and CD8 lymphocyte subsets in healthy Chinese adults from Shanghai. *Clin Diagn Lab Immunol*. 2004;11(4):811-813.
16. Bofill M, Janossy G, Lee CA, et al. Laboratory control values for CD4 and CD8 T lymphocytes. Implications for HIV-1 diagnosis. *Clin Exp Immunol*. 1992;88(2):243-252.
17. Vithayasai V, Sirisanthana T, Sakonwasun C, Suvanpiyasiri C. Flow cytometric analysis of T lymphocytes subsets in adult Thais. *Asian Pac J Allergy Immunol*. 1997;15(3):141-146.
18. Yaman A, Cetiner S, Kibar F, Tasova Y, Seydaoglu G, Dundar IH. Reference ranges of lymphocyte subsets of healthy adults in Turkey. *Med PrincPract*. 2005;14(3):189-193.
19. Tugume SB, Piwowar EM, Lutalo T, et al. Hematological reference ranges among healthy Ugandans. *Clin Diagn Lab Immunol*. 1995;2(2):233-235.
20. Dhaliwal JS, Balasubramaniam T, Quek CK, Gill HK, Nasuruddin BA. Reference ranges for lymphocyte subsets in a defined Malaysian population. *Singapore Med J*. 1995;36(3):288-291.
21. Roman S, Moldovan I, Călugăru A, Regalia T, Sulică A. Lymphocyte subset reference ranges in Romanian adult Caucasians. *Rom J Intern Med*. 1995;33(1-2):27-36.
22. Thakar MR, Abraham PR, Arora S, et al. Establishment of reference CD4+ T cell values for adult Indian population. *AIDS Res Ther*. 2011;8:35. doi:10.1186/1742-6405-8-35.
23. Maini MK, Gilson RJ, Chavda N, et al. Reference ranges and sources of variability of CD4 counts in HIV seronegative women and men. *Genitourin Med*. 1996;72(1):27-31.
24. Shahabuddin S, Al-Ayed I, Gad El-Rab MO, Qureshi MI. Age-related changes in blood lymphocyte subsets of Saudi Arabian healthy children. *Clin Diagn Lab Immunol*. 1998;5(5):632-635.
25. Mandala WL, MacLennan JM, Gondwe EN, et al. Lymphocyte subsets in healthy Malawians: implications for immunologic assessment of HIV infection in Africa. *J Allergy Clin Immunol*. 2010;125(1):203-208.
26. Sagnia B, Ndongo FA, Tetang SNM, et al. Reference values of lymphocyte subsets in healthy, HIV-negative children in cameroon. *Clin Vaccine Immunol*. 2011;18(5):790-795.
27. Tsegaye A, Messele T, Tilahun T, et al. Immunophenotyping of blood lymphocytes at birth, during childhood, and during

adulthood in HIV-1-uninfected Ethiopians. *Clin Immunol*. 2003; 109(3):338-346.
28. van Gent R, van Tilburg CM, Nibbelke EE, et al. Refined characterization and reference values of the paediatrics T- and B-cell compartments. *Clin Immunol*. 2009;133(1):95-107.
29. Shearer WT, Rosenblatt HM, Gelman RS, et al. Lymphocyte subsets in healthy children from birth through 18 years of age: the Pediatric AIDS Clinical Trials Group P1009 study. *J Allergy Clin Immunol*. 2003;112(5):973-980.
30. Lugada ES, Mermin J, Kaharuza F, et al. Population-based hematologic and immunologic reference values for a healthy Ugandan population. *Clin Diagn Lab Immunol*. 2004;11(1):29-34.
31. World Health Organization. *Antiretroviral Therapy of HIV Infection in Infants and Children in Resource Limited Settings: Towards Universal Access. Recommendations for a Public Health Approach*. Geneva, Switzerland: World Health Organization; 2006. http://www.who.int/hiv/pub/guidelines/WHOpaediatric.pdf. Accessed February 15, 2014.