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CD4-T-Lymphocyte Reference Ranges in Uganda and Its Influencing Factors

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Abstract

Background: Given the importance of CD4-T-lymphocyte monitoring in HIV/ART management, we established CD4/CD8 reference ranges in Uganda and studied factors associated with CD4/CD8 in a normal population.

Methods: Blood samples for 206 HIV seronegative healthy volunteers from the Mbarara and Kampala districts in Uganda were analyzed using the PanLeucogating protocol.

Results: The reference ranges reported include data from 172 participants with no current or serious recent health problems. The 95% reference ranges for absolute CD4 (ACD4) count was 418-2105 cells/μL, 256-1619 for absolute CD8 count, and 0.52-4.1 for CD4/CD8 cell ratio, which is wider than the reference ranges currently used in Uganda. Recent illnesses/medications and socio-demographic factors affected the CD4-T-lymphocyte count.

Conclusion: The CD4 reference ranges in Uganda were established using a cost-effective method, recommended and available in resource-limited settings. Effect of prevalent infections and socio-demographic differences on CD4-T-lymphocyte levels would need consideration in HIV/ART clinical management.

Keywords: CD4-T lymphocytes, HIV/AIDS, hematology, Ugandans, socio-demographic factors, infectious diseases

CD4-T lymphocytes are specialized WBCs that play a central role in the body's immunity, yet the human immunodeficiency syndrome (HIV) causes their depletion while CD8 (cytotoxic cells) increase. CD4 cell monitoring remains the valid laboratory method to evaluate HIV progression, yet variations in CD4 reference values exist. Lower CD4/CD8 reference ranges have been reported for African populations and some Asian populations compared to European populations.[1-5] Intra-country and intra-regional variations in absolute CD4 (ACD4) counts reference ranges have also been documented.[4,6-8] However, in clinical practice the international standard reference ranges are used for most populations.

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Abbreviations

HIV, human immunodeficiency syndrome; ACD4, absolute CD4; SES, social economic status; VCT, voluntary counseling and testing; HCG, human chorionic gonadotrophin; WCC, white cell count; RBC, red blood cell count; GCP, Good Clinical Practice; NCST, National Council of Science and Technology; ALC, absolute lymphocyte count; ACD8, absolute CD8 cell count; ABSR, absolute CD4/CD8 cell ratio; ANC, absolute neutrophil count; LC%, lymphocyte percentage; NC%, neutrophil cell percentage; Hb, hemoglobin

Biological, geographical, social, and methodological factors like race, gender, age, altitude, physical activity, social economic status (SES), circadian rhythm, disease, and drug intake can affect the CD4-T-lymphocyte values.[5,8-14] Several studies report higher CD4 normal ranges for females.[4,8,13,15] The absolute CD4 cell count is highest during the early years of life and declines steadily to stable adult values.[7] The values then decline further in advanced age. Altitude also affects hematological values with increments in hemoglobin (Hb), hematocrit, and neutrophil numbers.[16-18] The number of lymphocytes including CD4 cells is reduced at high altitudes.[11,19] Methodological factors affect different parameters of T-lymphocytes depending on instruments used, washing techniques, and the quantity of blood used.[20] The methodological effects are more pronounced where CD4 subsets are calculated as percentages of total lymphocyte counts.[20-24] Methodological differences largely result from errors in identifying lymphocytes on flow cytometers, and the PanLeucogating method improves accuracy and precision since CD4 subsets are calculated from the total number of white cells.[25-27]

First, this study aimed at establishing hematological reference ranges in Ugandan populations using the PanLeucogating method. Secondly, we determined how infections as well as socio-demographic factors affected CD4-T lymphocytes and compared our results with previous findings.

Designs and Methods

Study Sites

Data were collected from the Mulago National Referral Hospital located in the central (Kampala) region and from the Mbarara Regional Referral Hospital in the southwestern

region of Uganda. The 2 regions differ topographically and in the socio-economics of the population. Kampala lies at approximately 1134 meters above sea level while the Rwenzori peaks modify parts of the western region to altitudes of 3477-5113 meters above sea level.[28] Kampala district is 238 square kilometers, is more industrialized, and has a population of 1,208,544, of whom 15% live below the poverty line. On the contrary, the Mbarara district is 7,346 square kilometers with a population of 1,093,388, of whom 30% live below the poverty line (district portal map). Given these differences in altitude and socio-economic status between the 2 study regions, participants from Mbarara were considered to have lived at a higher altitude and had a lower mean SES-income compared with Kampala participants.

Principles of Selection of Participants

Healthy volunteers aged 15-70 years in the central (Kampala) and western regions of Uganda were targeted between March 2007 and May 2007. The age range and sample size were based on similar previous studies.[8,20,29] Recruitment followed sensitization in the areas including medical schools. Healthy subjects presenting for purposes of the study or voluntary HIV counseling and testing were screened for the study. In the Mbarara hospital, eligible consenting participants were referred to the study team after HIV serology testing by the Mbarara HIV voluntary counseling and testing (VCT) group while in Kampala, HIV testing was done by the researcher for all consenting participants using the same kits and procedures as in the Mbarara hospital. All female participants had human chorionic gonadotrophin (HCG) tests performed on them to exclude pregnancy. All HIV seropositive volunteers and pregnant mothers were excluded from the study. Physical status of the participants was assessed using a pre-test questionnaire administered by the researcher and trained assistants. The questionnaire sought history of symptoms and diagnoses of common tropical illnesses including malaria, common colds, pneumonias, viral infection other than HIV, and any other symptom or disease reported by the subjects as current, recent, or often present. Symptoms or illnesses suffered in the previous 8 weeks (2 months) were considered as recent while those suffered before and during the previous 8 weeks were taken as often present. A period of 8 weeks was considered adequate to cover a single episode for most of the above-mentioned illnesses. Information on severity was also collected for all recent ailments, which was categorized as minor (no medication administered), moderate (only medicines administered), or severe (hospitalized). Participants with any current ailment or recent hospitalization were excluded from the study, and results from volunteers who often had disease symptoms and those with a history of recent medication were not included in the estimation of reference ranges.

Procedure for Blood Sample Collection

Two blood samples, each 5 mL, were drawn from each Kampala participant at Mulago Hospital through antecubital venous puncture using a Vacutainer (BD, Franklin Lakes, NJ) needle. One sample was collected in a 5 mL SST Gel and Clot Activator Vacutainer for HIV testing, and the second sample in a 5 mL EDTA-containing Vacutainer for determination of hematological indices. The HIV tests were performed at Mulago hospital using a commercially available enzyme immunoassay kit (Welcozyme HIV-1 and -2 kit, Murex Diagnostics, Dartford, England), and discordant samples were confirmed using the Recombigen (*env* and *gag*) HIV-1 assay kit (Cambridge Biotech, Dartford, England). Samples for hematological tests from the Kampala site were transferred on a daily basis and analyzed by Nsambya Hospital laboratory while Mbarara samples were analyzed by the Mbarara University Research Laboratory. All samples were collected between 8 AM to 11 AM and analyzed within 4 hours after collection. The hematology results produced include absolute cell counts and percentages for lymphocytes, CD4, CD8, CD4/CD8, and neutrophils. Other hematological results include total white cell count (WCC), Hb level, and red blood cell count (RBC).

Laboratory Procedure for CD4/CD8 Analyses

Analysis was done using a double platform PanLeucogating method as described previously[26,30] on an Epics XL-MCL Beckman Coulter flow cytometer (Beckman Coulter, Miami FL). Using 2 MCL Epics XL tubes (Beckman Coulter), 10 µL of the PLG CD4 monoclonal antibodies (CD4 and CD45) was added to either of the tubes, and 10 µL of the PLG CD8 monoclonal antibody to the other, as in the manufacturer's manual.[34] One hundred µL of well-mixed blood samples were pipetted into each of the tubes containing the monoclonal antibodies, gently vortexed, and incubated at 20°C-25°C in the dark for 10 minutes before 800 µL of the lyse was added. The mixture was further incubated at 20°C-25°C for 15 minutes, after which the tubes were loaded into the cytometer for analysis.

Ethical Considerations

The study followed Good Clinical Practice (GCP) guidelines and was approved by the Makerere University Institutional Review Board, the Mbarara University research committee, and the National Council of Science and Technology (NCST). All participants gave written informed consent prior to entry into the study, and volunteers who turned out to be HIV positive were referred to HIV/ART care centers for further management after their CD4/CD8 tests were completed by the research team.

Data Management and Analysis

Raw data were double checked for completeness and consistency, and the participants' clinical data were matched with their laboratory forms before data entry. Laboratory records that could not be matched with clinical data (4 patient records) were excluded. Double entries were done for 206 participants (99 Mbarara, 107 Kampala) using Microsoft Excel (Redmond, WA), and consistency was ensured prior to exporting data to Stata software (Stata 10, StataCorp, College Station, TX)[33] for analysis. Comparison of means was done for regions, gender, age groups, health status, education, social status, and previous research findings.

Since education level has been suggested as a good SES predictor for health-related factors, its effect on ACD4 count was assessed after stratifying participants according to the highest educational level reached under the 3-tier education system in Uganda regardless of whether a participant

completed a particular level. The following categories were used: 1=no formal education, 2=primary, 3=secondary, and 4=tertiary (post-secondary) education. This classification has been previously used in Uganda.[31] However, it was beneficial to consider other factors like source of income to effectively assess the effect of socio-economic status since formal adult education covers only 69% of the Ugandan population. Thus, participants were classified into the following relative socio-economic categories depending on their main income source (SES-income) as an estimation of median monthly income based on results of the 2005/2006 household survey.[35] **1=Lowest income** (23,000-27,200 Uganda Shilling/ approximately 15 USD): these listed agriculture and elementary occupation as the main source of income. **2=Second lowest** (52,200 Uganda Shilling/approximately 35 USD): these had low level service as the main source of income and included shop/market salesmen, mechanics, and saloon employees. **3=Highest income** (approximately 100 USD): these were professionals/diploma holders. Tertiary education students were included in category 3 given their lifestyle, and non-employed housewives were assigned categories based on their spouses' main source of income.

Physical condition was categorized on the basis of health histories: **Category 1**-participants with no current, recent, or frequent/chronic ailments; **Category 2**-participants with minor recent ailments where no medication was used; **Category 3**-participants with a history of recent ailments and medications; and **Category 4**-participants who often had disease symptoms.

The 95% reference ranges are given as 2.5th to 97.5th empirical percentiles. Differences between groups were assessed using t-test for continuous symmetric data and chi2-test for proportions. The observations were divided into different categories of interest in relation to the study topic and for ordered categories; the first category was chosen as a reference in the analysis. Significances are stated at a 5% level, and 95% confidence intervals are given. Distribution of data was assessed using graphical methods, and log transformation was needed for total WCC, RBC counts, absolute lymphocyte count (ALC), absolute CD4 cell count (ACD4), absolute CD8 cell count (ACD8), absolute CD4/CD8 cell ratio (ABSR), absolute neutrophil count (ANC), and CD8%, while no transformation was needed for lymphocyte percentage (LC%), CD4%, CD4/CD8%, neutrophil cell percentage (NC%), and Hb levels. All results were back transformed to the original scale.

Results

Socio-Demographic Features

Two hundred and thirty-nine participants (131 from Kampala, 108 from Mbarara) consented and were screened for the study. Eighteen participants were excluded due to acute illnesses or histories of recent serious illness/hospitalization, 10 were excluded following positive HIV tests, and 1 due to pregnancy. An additional 4 participants were excluded at the time of data entry due to incomplete results. Excluded participants did not differ from those who were included in the study with respect to their demographic features. Although data on hematological indices was collected from 206 participants (51.9% Kampala, 48.1% Mbarara), results for the final CD4/CD8 reference ranges were calculated for 172 participants. The remaining 34 participants, all with frequent or recent illnesses with drug therapy, were found to have a statistically significant low mean CD4 count (**Figure 1**) and were subsequently excluded when calculating the reference ranges.

Out of the 42 volunteers with recent illnesses, 37 reported only undiagnosed symptoms in their recent past. A proportion of 35.7% had fever as the main recent symptom, 23.8% had cough or common cold as the main recent symptoms, and 28.6% had other symptoms including headaches, body pains, skin rashes, and body swellings or wounds. Only 5 volunteers (11.9%) had specific diagnoses, which were malaria (2), diabetes mellitus (2), and abscess (1). Analgesics and other symptomatic treatments had been taken as the only medications in 53.9% of the volunteers with recent ailments. A proportion of 15.4% of volunteers with recent illnesses had used chloroquine and fansidar (sulfadoxine + pyrimethamine) either in combination or as a single drug, and 23.1% had taken antibiotics, mainly cotrimoxazole, recently. It should be noted that 8 out of these 42 volunteers with recent ailments were categorized as minor, and hence included in the reference range establishment.

Of the 172 participants' data used in the final CD4/CD8 analysis, 54.1% were males, and the mean age was 27.9 years (range 18-66). Only 39% of the participants were from the Mbarara region, and the study population from Mbarara had a lower mean SES with respect to source of income compared to Kampala participants (P=0.001*). Details of socio-demographic features of the 172 participants are presented in **Table 1**.

Hematological Results

Data are summarized as means and 95% reference ranges for the different hematological parameters in **Table 2**. The table also illustrates gender differences observed in mean hematological values of the participants. Generally, females had

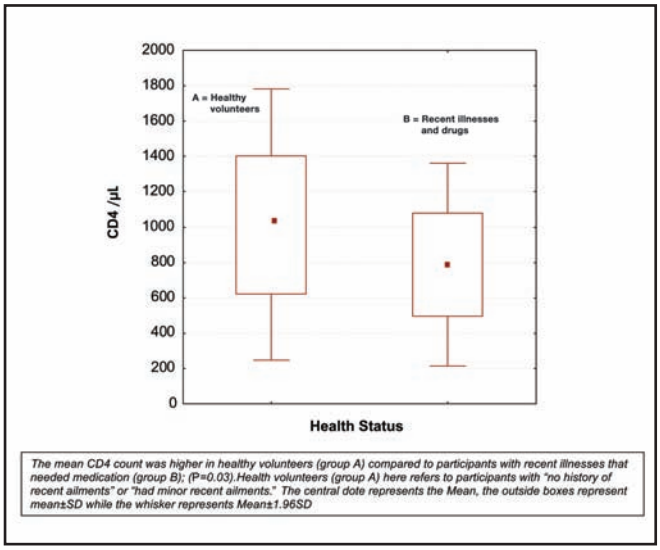

Figure 1_Graph showing mean CD4 for healthy volunteers (group A) compared with participants with a history of recent illnesses and drugs (group B).

lower means and 95% reference ranges for CD4-T lymphocytes (P=0.02*) and RBC components (P=0.01*), while male participants had a lower neutrophil percentage (P=0.037*). The details for all observed gender differences in the studied hematological indices can be found in **Table 2**.

There were 2 female participants with low ACD4 counts that warrant mentioning in this report and have been included in the reference range calculation. One was 32 years old with an absolute CD4 count of 210 cells/μL, CD4 percentage of 10.3%, but no present or recent history of illnesses. The other was 45 years old with absolute CD4 count of 194 cells/μL, CD4 percentage of 21.6%, and had a recent fever with no medication used. Mean CD4 for the HIV-positive volunteers was 352 (range 217-585 cells/μL), but these were not included in the estimation of reference ranges.

Table 1_Social Demographic Features and Health Status of Healthy Volunteers (n=172) Recruited and Forming the Basis for Reference Values in Uganda

Parameter	Subgroups	N	Percentage of the Total Study Population (%)
Gender	Female	75	43.6
	Male	93	54.1
	Missing data	4	2.3
Region	Mbarara	65	37.8
	Kampala	107	62.2
Age groups (years)	15-19=(1)	14	8.1
	20-30=(2)	112	65.1
	Above 30=(3)	46	26.7
Highest education attained (SES-Educ)	None=(1)	1	0.6
	Primary=(2)	14	8.1
	Secondary=(3)	30	17.4
	Tertiary=(4)	58	37.2
	Missing data	69	40.1
Source of income (SES-Income)	Lowest income=(1)	40	23.3
	Middle income=(2)	41	23.5
	Highest income=(3)	81	47.1
	Missing	10	5.8
Health status	No ailments=(1)	164	79.6
	Recent minor illness but no medication=(2)	8	3.9
	Recent minor + medication=(3)	10	4.9
	Chronic complaints=(4)	23	11.2
	Missing	1	0.5

Comparison of Hematological Indices Between Participants from the Kampala and Mbarara Regions

An inter-regional comparison of hematological indices showed the mean absolute CD4 was higher in Kampala participants compared to Mbarara participants (P=0.001*). Kampala participants had higher mean values for total WCC (6119 for Kampala and 5583 for Mbarara, P=0.02*) and higher lymphocyte counts (2896 for Kampala and 2279 for Mbarara, P=0.001*). Other lymphocyte subsets whose mean values were significantly higher in Kampala participants compared to Mbarara participants include CD4% (P=0.002*), ACD8 (P=0.001*) CD4/CD8 cell ratio (P=0.044*), and CD4/CD8% (P=0.001*). On the contrary, participants from Mbarara had significantly higher mean values for neutrophil and RBC component. The ANC was 2715 for Mbarara and 2436 for Kampala participants (P=0.001*), and the Hb levels were 15.8 g/dl and 14.9 g/dl for Mbarara and Kampala participants, respectively (P=0.003*).

Effect of Socio-Demographic Factors and Health Status on CD4-T Lymphocyte Counts

Although regression analysis suggested reduction of absolute CD4 after the age of 30 years, categorization of participants into different age groups revealed no significant difference in the mean CD4-T lymphocyte values. Participants in the lowest income category had a lower mean ACD4 compared to their counterparts in higher income categories (P=0.001*). Since data for the previous study by Tugume[8] was collected from blood donors in Kampala, findings from current Kampala participants were also compared with the previous study, and the 2 means were comparable and higher than that from current Mbarara participants (P=0.001*).

Analysis of the effect of illnesses and medications on CD4-T lymphocytes was done, and the results showed the mean ACD4 count for participants with chronic illnesses and those with recent ailments who needed medication (categories 3 and 4 in **Table 3**) were comparable and lower than the mean ACD4 for participants with no recent illnesses at all,[1] or those who had recent illnesses needing no medication,[2] P=0.03*. Based on this finding, which is also demonstrated in **Figure 1**, chronic or frequent infections and medications were considered to affect CD4 counts. These categories (3 and 4), also shown as group B in **Figure 1**, were not included when estimating the final hematological reference ranges. Results showing the effect of social-demographic factors and health status on CD4-T lymphocytes are shown in **Table 3** and **Figure 1**.

Discussion

This study reports variation in CD4 counts among HIV-seronegative Ugandans related to health history, socioeconomic status, and geographic locations. We have also observed a wide absolute CD4 reference range compared to those reported in the surrounding east African region.

The wide CD4 reference range observed in this study confirms a similar finding in an earlier study published in 1995.[8] Both studies demonstrate the Ugandan population has a wider CD4 reference range in comparison to surrounding east-African countries (**Figure 2**). This characteristic is apparently shared with India, a feature that may indicate genetic and/or socio-demographic diversity in populations within the 2 countries. This study, however, reports a lower mean and 2.5th and 97.5th percentile than the previous Ugandan study. The difference between the 2 Ugandan studies may largely be explained by geographical coverage and methods employed. While participants for the current study were selected from both the central and western regions, the previous study had participants recruited from the central region. The mean CD4 count for Kampala participants in this study is comparable to

Table 2_Hematological Indices of 172 Healthy Volunteers Recruited From the Kampala and Mbarara Regions, Uganda

Parameter (N)	Study Populations	Mean	95% CI	95% Reference Range	*P* Value for Gender Differences
Total WCC (cells/cm3)					
	F	5844	5427-6294	3109-10987	0.636
	M	5972	5657-6304	3541-10071	
	All	5911	5661-6172	3357-10408	
ALC (cells/cm3)					
	F	2485	2329-2650	1253-4148	0.0241★
	M	2771	2593-2962	1459-5264	
	All	2645	2526-2771	1441-4856	
LC percentage (%)					
	F	43.85	41.6-46.1	24.54-63.16	0.0105★
	M	47.79	45.8-49.8	28.62-66.97	
	All	46.14	44.7-47.6	26.71-65.57	
ACD4 (cells/μL)					
	F	861	780-949	368-2013	***0.02***★
	M	1000	923-1082	464-2154	
	All	938	882-998	418-2105	
CD4 percentage (%)	F	36.2	33.9-38.5	16.4-56.1	0.8071
	M	36.6	34.9-38.3	20.4-52.8	
	All	36.4	35.1-37.8	18.8-54.1	
ACD8 (cell/μL)	F	578	511-655	213-1572	***0.02***★
	M	692	632-756	296-1616	
	All	644	599-694	256-1619	
CD8 percentage (%)					
	F	23.05	30.0-25.3	10.7-49.5	0.3961
	M	24.3	22.5-26.3	11.6-50.8	
	All	23.8	22.5-25.3	11.3-50.1	
CD4/CD8 cell ratio					
	F	1.46	1.3-1.7	0.48-4.41	***0.947***
	M	1.47	1.3-1.6	0.54-3.96	
	All	1.46	1.4-1.6	0.52-4.1	
CD4/CD8 percentage ratio (%)					
	F	1.67	1.5-1.9	.08-3.41	0.8407
	M	1.64	1.5-1.8	.220-3.06	
	All	1.65	1.5-1.8	.098-3.2	
Absolute neutrophils (cells//cm3)	F	2671	2399-2974	1052-6779	***0.1911***
	M	2450	2269-2645	1180-5085	
	All	2538	2383-2703	1115-5776	
Neutrophil percentage (%)	F	47.1	44.6-49.5	25.5-68.6	0.0037★
	M	42.0	39.7-44.3	20.0-64.0	
	All	44.1	42.4-45.8	21.9-66.3	
Red blood cell count × 103 (cells/L)	F	4.54	4.39-4.69	3.43-5.99	***0.001***★
	M	4.97	4.86-5.08	4.05-6.09	
	All	4.77	4.68-4.86	3.70-6.14	
Hemoglobin level (g/dL)	F	14.1	13.7-14.5	10.4-17.8	***0.001***★
	Males (92)	16.1	15.8-16.4	13.0-19.2	
	All (171)	15.2	14.9-15.5	11.4-19.1	

★ *Represents a statistically significant result.*

Results are expressed as means, 95% CI and 95% reference ranges. Total number of participants was 172 except for CD8 and CD4/CD8 ratios where data was collected from 159 participants.

that of the entire previous study (**Table 3**). The other cause of variation may have risen from the fact that the PanLeucogating method was employed in the current study while previously CD4/CD8 ranges were established using Becton Dickinson FACScan flow cytometer, and the differences of these methods on CD4-T-lymphocyte identification have previously been discussed.[20-27]

The low mean absolute CD4 count and range for Mbarara participants when compared to Kampala participants (*P*=0.001*) may be attributed to differences in altitude and socio-economic status between the 2 regions. The western region lies at a relatively higher altitude,[28] and participants from this region had a poorer socio-economic status (*P*=0.001*). Studies have previously reported a reduction of lymphocyte subsets (CD4/CD3) at high altitudes and an increment of absolute CD8,[11,17] Hb, RBC count,[16-18] and neutrophils.[11,19] All of these differences were demonstrated between participants of the 2 regions. Nevertheless, the low mean socio-economic status of participants from Mbarara may have contributed to the observed difference in CD4

Table 3_The Effect of Socio-Demographic and Health Status on CD4-T Lymphocytes Among Healthy Volunteers in Uganda

Parameter	Categories (n)	Mean CD4 (95% CI)	*P* Value
Age groups (years)	15-19 (14)	951 (800-1130)	Ref. category
	20-30 (112)	1017 (946-1093)	*P*=0.55
	>30 (46)	767 (676-869)	*P*=0.08
SES-Highest education attained	No formal + primary education (15)	1015 (854-1207)	Ref. category
	Secondary (30)	963 (866-1071)	*P*=0.65
	Post-secondary (58)	1168 (1071-1273)	*P*=0.19
	Missing (69)	758 (683-839)	*P*=0.01
SES-income	Lowest (40)	773 (675-872)	Ref. category
	Middle (41)	1044 (926-1162)	*P*=0.001*
	Highest (81)	1125 (1039-1211)	*P*=0.001*
Health status	No frequent/chronic or recent ailments + recent ailments, no medication (172)	992 (957-1026)	Ref. category
	Recent ailment and medication + frequent/ chronic complaints (33)	783 (776-789)	*P*=0.003*
Gender (entire study)	Males (93)	1000 (923-1082)	Ref. category
	Females (75)	861 (780-949)	*P*=0.02*
Gender (intra-regional differences)	Males, Kampala (69)	1122 (1039-1212)	Ref. category
	Females, Kampala (34)	1006 (899-1126)	*P*=0.12
	Females, Mbarara (41)	756 (656-824)	Ref. category
	Males, Mbarara (24)	717 (616-835)	*P*=0.64
Regions	Mbarara (65)	742 (667-824)	Ref. category
	Kampala (current study) (107)	1082 (1017-1151)	*P*=0.001*
	Kampala (previous study) (182)	1256 (n/a)	n/a
Comparison with other studies	Current study, Uganda (172)	938 (882-998)	*P* value not available (n/a)
	Previous study, Uganda (182)	1256 (n/a)	
	Previous study, Tanzania (214)	843 (n/a)	

**The figures are also compared with those of other studies conducted in the region.*

count findings since the same study demonstrated that participants in the lowest socio-economic status based on income had a lower mean absolute CD4 compared to those in higher income groups (*P*=0.001*). Unfortunately, the large amount of missing data on highest education attained stands as a big limitation to findings obtained with regard to effect of socio-economic status based on education.

Participants with a history of recent illnesses and medications had lower absolute CD4-T lymphocyes compared to participants with no recent illness and those with minor recent illnesses that required no medication (*P*=0.03). This finding is in line with previous reports of reversible lymphocytopenia resulting from viral and parasitic infections among HIV-seronegative persons.[10] A study in Zambia reported a rise in absolute CD4 among HIV-seronegative malaria patients after effective malaria therapy.[10] The low CD4 counts observed with recent infections and medications in this study (**Figure 1**) could be due to the effect of viral, bacterial, or parasitic infections, and medications. Even though all participants were in good health by the time of this study, full immunological recovery may not have been reached by some participants with recent infections. There are also reports of idiopathic lymphocytopenia where HIV-seronegative persons were found to have low CD4 counts to the level of categorizing some as immune compromised. This could explain the 2 individuals reported in this study to have CD4 cell counts of 210 and 194 cells/μL. A review of studies reporting idiopathic lymphocytopenia and the influence of infectious disease suggested that such persons whose CD4 counts are significantly low may deteriorate much faster if they have HIV.[36]

Gender variations in CD4 have previously been reported with females having higher CD4 counts compared to males.[4,8,13,15] The low mean CD4 counts and reference ranges observed among female participants in this study may have been caused by socio-demographic/economic differences since more than 64.6% of the entire female population was recruited from Mbarara, a region with a lower mean CD4 count and socio-economic status. The observed gender differences in CD4 was not reflected in the intra-regional analysis, which means socio-economic and demographic differences may have contributed. Regression analysis suggested a reduction of CD4 after 30 years of age, a lower age than Jentsch-Ullrich reported (50 years) for CD4 T-lymphocytes decline.[13]

Inter- and intra-regional differences observed in the studies reviewed (**Figure 2**) could be explained by genetic differences between the populations. Differences in socio-demographics, health histories, and methods used in establishing the CD4/CD8 reference ranges may also be contributory. There is a good comparability between absolute CD4 ranges/means observed within the European studies[13,20,32] in comparison to studies from other regions (**Figure 2**). The low variability of CD4 references among European countries shown in **Figure 2** may be attributed to genetic and social homogeneity within the region.

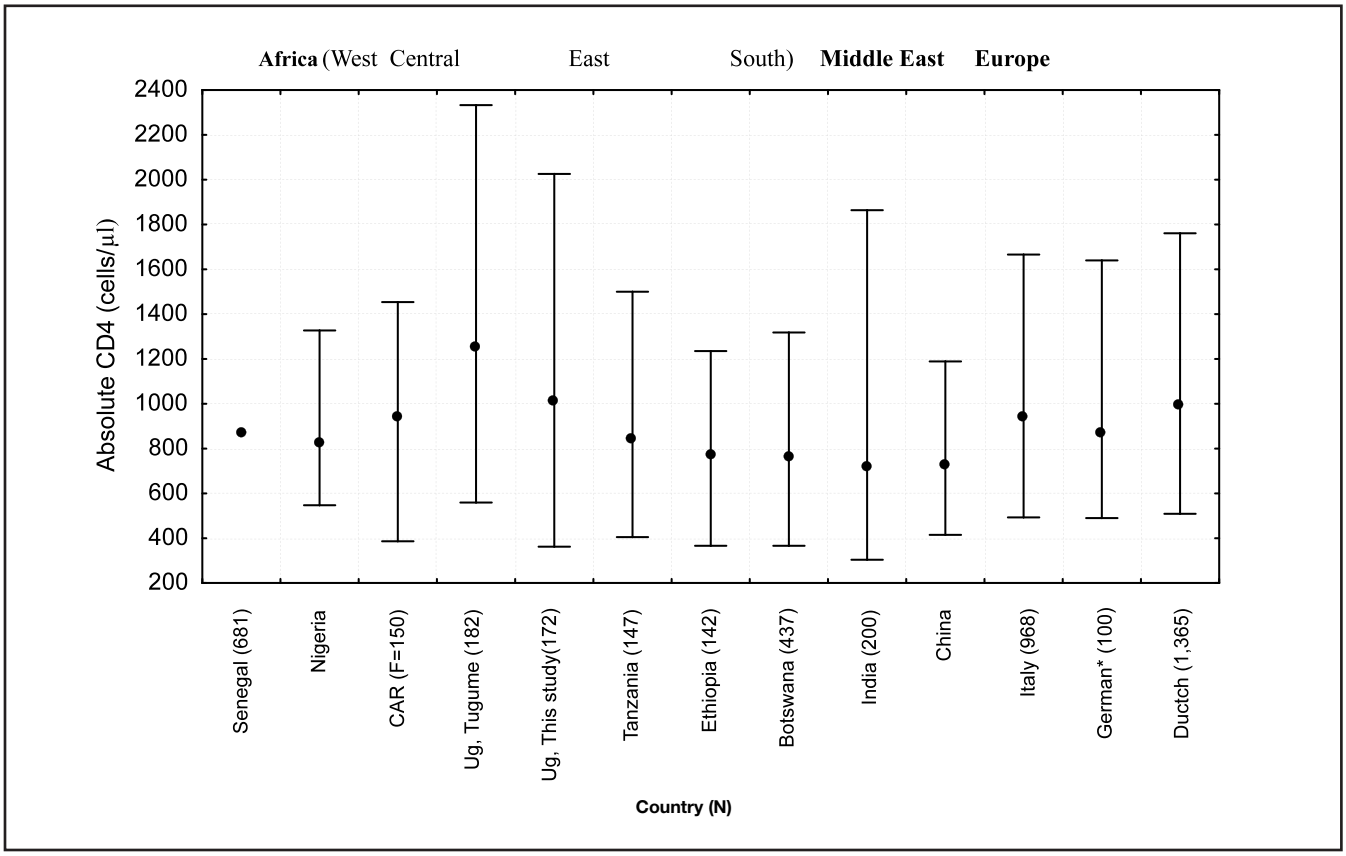

Figure 2_ACD4 means and 95% reference ranges for study populations in parts of Africa, Europe, and Asia. Data obtained from secondary analysis using Statistica software to draw the graph.
Whiskers represent 2.5th and 97.5th percentiles, while the middle point is the mean value for all countries except Germany where median was reported.

Findings from this study would encourage use of local rather than international CD4/CD8 reference ranges in managing HIV patients from regions where the above factors are prevalent. Use of general CD4 cut-off points in regions with wide CD4 reference ranges should be done with caution since some individuals with high-normal CD4 values may present with falsely high enough CD4 values in comparison to the national cut-off points for initiating therapy. The integration of the knowledge of patients' socio-economic and health histories in populations with wide CD4 reference ranges would also help in identifying those who would need treatment irrespective of national CD4 cut-off values. These unaddressed factors could be contributing to the mortality reported among treated HIV patients in developing countries.

It is acknowledged that the missing information of the highest level of education reached for many participants limits the findings related to effect of education on CD4 counts. The difference in the flow of participants screened at the 2 sites could have produced a selection bias. The limited number of participants with recent illnesses and medications also call for further evaluation of the effect of past health histories in a larger number of participants.

Conclusion

Results from this study elicit a number of factors that may be associated with lower and differing CD4 references within Africa and Asia compared to European countries. Other than the known racial and genetic differences, a higher prevalence of infections, socio-demographic differences, participant selection for reference range studies, and laboratory analysis affect CD4-T lymphocytes and may partly be responsible for differences in reference ranges reported. These factors must be considered when using CD4/CD8 results in HIV/ART management.

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1. 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;12:525-530.
2. Amatya R, Vajpayee M, Kaushik S, et al. Lymphocyte immunophenotype reference ranges in healthy Indian adults: Implications for management of HIV/AIDS in India. *Clin Immunol*. 2004;112:290-295.
3. 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;11:811-813.
4. Urassa WK, Mbena EM, Swai AB, et al. Lymphocyte subset enumeration in HIV seronegative and HIV-1 seropositive adults in Dar es Salaam, Tanzania: Determination of reference values in males and females and comparison of two flow cytometric methods. *J Immunol Methods*. 2003;277:65-74.
5. Tsegaye A, Messele T, Tilahun T, et al. Immunohematological reference ranges for adult Ethiopians. *Clin Diagn Lab Immunol*. 1999;6:410-414.
6. Breton G, Lewden C, Spire B, et al. Characteristics and response to antiretroviral therapy of HIV-1-infected patients born in Africa and living in France. *HIV Med*. 2007;8:164-170.
7. 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;11:29-34.
8. Tugume SB, Piwowar EM, Lutalo T, et al. Hematological reference ranges among healthy Ugandans. *Clin Diagn Lab Immunol*. 1995;2:233-235.
9. 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;72:27-31.
10. Van Geertruyden JP, Mulenga M, Kasongo W, et al. CD4 T-cell count and HIV-1 infection in adults with uncomplicated malaria. *J Acquir Immune Defic Syndr*. 2006;43:363-367.
11. Facco M, Zilli C, Siviero M, et al. Modulation of immune response by the acute and chronic exposure to high altitude. *Med Sci Sports Exerc*. 2005;37:768-774.
12. Krause D, Mastro AM, Handte G, et al. Immune function did not decline with aging in apparently healthy, well-nourished women. *Mech Ageing Dev*. 1999;112:43-57.
13. Jentsch-Ullrich K, Koenigsmann M, Mohren M, et al. Lymphocyte subsets' reference ranges in an age- and gender-balanced population of 100 healthy adults - A monocentric German study. *Clin Immunol*. 2005;116:192-197.
14. Winkleby MA, Jatulis DE, Frank E, et al. Socioeconomic status and health: How education, income, and occupation contribute to risk factors for cardiovascular disease. *Am J Public Health*. 1992;82:816-820.
15. Ampofo W, Torpey K, Mukadi YD, et al. Normal CD4+ T lymphocyte levels in HIV seronegative individuals in the Manya/Yilo Krobo communities in the Eastern region of Ghana. Viral Immunol. 2006;19:260-266.
16. León-Velarde F, Gamboa A, Chuquiza JA, et al. Hematological parameters in high altitude residents living at 4355, 4660, and 5500 meters above sea level. *High Alt Med Biol*. 2000;1:97-104.
17. Siqués P, Brito J, León-Velarde F, et al. Hematological and lipid profile changes in sea-level natives after exposure to 3550-m altitude for 8 months. *High Alt Med Biol*. 2007;8:286-295.
18. Tufts DA, Haas JD, Beard JL, et al. Distribution of hemoglobin and functional consequences of anemia in adult males at high altitude. *Am J Clin Nutr*. 1985;42:1-11.
19. Thake CD, Mian T, Garnham AW, et al. Leukocyte counts and neutrophil activity during 4 hours of hypocapnic hypoxia equivalent to 4000 m. *Aviat Space Environ Med*. 2004;75:811-817.
20. Santagostino A, Garbaccio G, Pistorio A, et al. An Italian national multicenter study for the definition of reference ranges for normal values of peripheral blood lymphocyte subsets in healthy adults. *Haematologica*. 1999;84:499-504.
21. Robinson G, Morgan L, Evans M, et al. Effect of type of haematology analyser on CD4 count. *Lancet*. 1992;340:485.
22. Gratama JW, Kraan J, Van den Beemd R, et al. Analysis of variation in results of flow cytometric lymphocyte immunophenotyping in a multicenter study. *Cytometry*. 1997;30:166-177.
23. Bentley SA, Johnson A, Bishop CA. A parallel evaluation of four automated hematology analyzers. *Am J Clin Pathol*. 1993;100:626-632.
24. Simson E, Groner W. Variability in absolute lymphocyte counts obtained by automated cell counters. *Cytometry*. 1995;22:26-34.
25. Pattanapanyasat K, Shain H, Noulsri E, et al. A multicenter evaluation of the PanLeucogating method and the use of generic monoclonal antibody reagents for CD4 enumeration in HIV-infected patients in Thailand. *Cytometry B Clin Cytom*. 2005;65:29-36.
26. Storie I, Sawle A, Whitby L, et al. Flow rate calibration II: A clinical evaluation study using PanLeucoGating as a single-platform protocol. *Cytometry B Clin Cytom*. 2003;55:8-13.
27. Glencross D, Scott LE, Jani IV, et al. CD45-assisted PanLeucogating for accurate, cost-effective dual-platform CD4+ T-cell enumeration. *Cytometry*. 2002;50:69-77.
28. Geography of Uganda, Encyclopedia of earth. Available at: http://www.eoearth.org/article/Uganda. Accessed July 12, 2009.
29. Reichert T, DeBruyère M, Deneys V, et al. Lymphocyte subset reference ranges in adult Caucasians. *Clin Immunol Immunopathol*. 1991;60:190-208.
30. NACO IMaFwACP. National Guidelines for the Enumeration of CD4+T-Lymphocytes with Single Platform Technology for Initation and Monitoring of ART in HIV Infected Individuals. In: India NACPN, ed. March 2007:1-47.
31. Odaga J. Health inequity in Uganda: The role of financial and non-financial barriers. *Health Policy and Development*. 2004;2:192-208.
32. Comans-Bitter WM, de Groot R, van den Beemd R, et al. Immunophenotyping of blood lymphocytes in childhood. Reference values for lymphocyte subpopulations. *J Pediatr*. 1997;130:388-393.
33. StataCorp. Copyright 1984-2008. Stata-10, 4905 Lakeway Drive, College Station, Texas 77845 USA.
34. FlowCARE PLG CD4 Reagent (CD45-FITC/CD4-PE), Beckman Coulter. Available at: http: www.beckman.com/CustomerSupport/IFU/ivdd/627807B_EN.pdf. Accessed June 30, 2009.
35. Uganda Bureau of Statistics (UBOS). Uganda National Household Survey 2005/2006. Report on the Socio-Economic Module, December 2006.
36. Laurence J. T-Cell subsets in health, infectious disease, and idiopathic CD4+ T lymphocytopenia. *Ann Intern Med*. 1993;119:55-62.