Original article

Immunological and hematological reference values for apparently healthy HIV-negative adults in Bahir Dar Town, Ethiopia

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Abstract

Background: Immunological and hematological reference values differ among different human beings with respect to sex, ethnicity, nutrition, altitude and health conditions. These could not be exceptional in the Ethiopian heterogeneous population. However, there are no nationally established reference values.

Objective: The aim of the study was to determine reference values of immunological and hematological parameters for apparently healthy HIV-negative adults in Bahir Dar Town.

Methods: A cross-sectional study was conducted from May to June, 2010 in Bahir Dar Town. Adults of both sexes above 18 years of age were recruited from the voluntary HIV counselling and testing centre in Felege Hiwot referral hospital. $CD4^+$ and $CD8^+$ T cells were enumerated using FACS count (Becton Dickinson) and hematological analyses were performed using Cell-DYN 1800 (Abbott Lab. USA).

Results: A total of 405 adults consisting of 238 (58.7%) males and 167 (41.3%) females with the median age of 24(range 18 to 60) years were recruited. The median, mean (± SD) and 95% percentile ranges of immunological and hematological values were determined. The mean (±SD) values were: $CD4^+$ T cells, 799 ± 218 (females) and 676 ± 235.6 (males); $CD8^+$ T cells, 582 ±247 (females) and 659.5 ± 343 (males); CD4/CD8, 1.53±0.59 (females) and 1.19 ± 0.49 (males); erythrocyte counts (10^{12}/liter), 4.9±0.4 (female) and (5.4±0.5 male); hemoglobin (g/dl), 14.7±2 (females) and 16.5±1.8 (males); haematocrit (%), 44±4 (females) and 49±4.5 (males); platelets (10^9/litre), 277 ± 20 (both sex); absolute leukocyte (WBC) counts 6.6±3.6 x10^9/liter (both sexes); lymphocyte, 2.15±.59 x10^9/liter (both sexes); granulocytes (neutrophils), 3.7±1.6 x10^9/liter (both sexes).

Conclusions: Absolute $CD4^+$ T cell counts were lower than the reference value, which Ethiopia has adopted for HIV/AIDS therapy. Females had higher $CD4^+$ T cell counts than males. Thus, considering these differences may be important in the process of using the national ART laboratory guideline for HIV/AIDS therapy. Establishing local reference values could have paramount importance for quality of health care in the clinical management of patients. [*Ethiop. J. Health Dev.* 2012;26(3):152-159]

Introduction

Reference values are ranges of upper and lower limits based on which values are interpreted as normal and abnormal. Studies have shown that the reference values of immunological and hematological values differ with respect to sex, age, ethnicity, nutrition, altitude and infections (1-3). Several studies have, for example, shown differences in reference values of lymphocytes among different populations (4-6). Moreover, CD4 counts normally differ from one locality to another and vary among different ethnic, age and gender groups (7).

In Ethiopia, where heterogeneous population and different geographic areas are found, there are no nationally established reference values for immunological and hematological parameters except those obtained in some studies done in Addis Ababa (2, 8). $CD4^+$ T cell counts and hematologic parameters have been used in Ethiopia for initiation of anti-retroviral therapy (ART) and for monitoring HIV/AIDS patients and other hematological disorders (9). It is therefore necessary to establish reference values for different population groups and different regions to provide quality health care (10). The present study was undertaken to determine normal reference value of immuno-hematology tests in apparently healthy HIV-negative adults in Bahir Dar Town.

Methods

Study Design:

This cross-sectional study was undertaken in Voluntary Counseling and Testing (VCT) centers at Bahir Dar Town from May-June, 2010.

Study Area and Population:

The study was conducted in the VCT center at Felege Hiwot Referral Hospital in Bahir Dar, the capital of the Amhara National Regional State. The town is located at an altitude of 1,830 meters above sea level and with a total population of 220,344 (11).

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Adults 18, years and above who were seeking VCT services were recruited. A total of 405 adults of both sexes, who fulfilled the inclusion criteria were recruited by convenience sampling for the study.

Inclusion Criteria:
The inclusion criteria were: negative for anti-HIV antibodies, non-pregnant women, non smokers, Body Mass Index (BMI) $\geq$18.5 kg/m^2, no history of current or recent morbid conditions such as gastrointestinal tract infections, active tuberculosis, no major surgery, no medication or allergy to drugs, no autoimmune diseases, and no history of blood transfusion.

Data Collection:
Socio-demographic data such as age, sex and ethnic background were obtained using a structured questionnaire. To determine BMI, weight and height of participants were measured. BMI was calculated by dividing body weight in kilograms by the square of height in meters.

Laboratory Investigation:
Four ml venous blood was collected from each participant using K3 EDTA vacuationer tube from 9:00-12.00 AM in the morning. Participants were screened for HIV infection using a standard national HIV testing algorithm. Fluorescence-activated cell sorter (FACS Count) (Becton Dickinson, USA) is a single platform (SP) system that provides count of cells based on the ratio of the number of events counted versus the number of reference beads counted by the machine. FACS Count system uses paired-reagent tubes for enumeration of absolute $CD4^+$ and $CD8^+$ T cells count in whole blood using a no-lyse and no-wash method. The first tube determines absolute number of $CD4^+$ T-cells using anti-human CD4 conjugated to phycoerythrin. The second tube determines the absolute number $CD8^+$ T-cells using anti-human CD8 antibody conjugated to phycoerythrin. Both tubes, in addition to the monoclonal antibodies, contain a known number of fluorochrome-labeled reference beads.

According to the manufacturer's instructions, 50 µl of whole blood from K_3EDTA collecting tube was added using an automated electronic pipette to each tube provided. The tubes were vortexed upside down then up right for 5 seconds and incubated for 60-120 minutes in the dark at room temperature. Then 50 µl of the fixative solution provided in the reagent kit was added into each tube. After the tubes were vortexed, stained samples were analyzed by the FACS Count double tube software.

Hematological Analyses:
An automated hematological analysis was performed using automated Cell-DYN 1800 (Abbott Lab., USA). As per the manufacturer's instruction, the machine aspirated 130 µl of blood into a chamber and diluted with a balanced isotonic saline solution. The diluted blood sample was split into two parts: one went for RBC and platelet counting and the other for total WBC and differential counting. The total leucocyte and lymphocyte counts were reported in micro-liter.

Quality Control:
For $CD4^+$ T cells, BD FACS Count™ controls for zero, low, medium and high controls were used during enumeration. In addition, for hematologic analysis, Cell-Dyn 1800® calibrator and low, medium and high control samples were run during each day of analysis as defined in the instrument by the manufacturer's manual.

Statistical Analysis:
Data were entered and analyzed using SPSS version 16 software and variables were interpreted by mean, median and 95% range. Analysis of variance (ANOVA) and χ^2 were used to generate p values wherein p valued <0.05 were considered statistically significant.

Ethical Clearance:
The study was ethically approved by the Research and Ethical Committee of Bahir Dar University and written informed consents were obtained from each study participant.

Results

Study Population:
A total of 405 adults (238 males and 167 females) participated in the study. The median age of the participants was 24 years (males=25 and females=22), which ranged from 18 to 60 years. Regarding ethnicity, 382 (94.3%) of the study participants were: Amhara, 3.4% Tigray, 1.2% Oromo, and 1.1% others. BMI was calculated to determine nutritional status: 47 (11.6%) of the participants had BMI of less than 18.5 and 358 (88.4%) had normal BMI ($\geq$18.5). There was no statistically significant difference between males and females with regard to BMI (p=0.394) (Table1). Bivariate analysis showed that there was no statistically significant association between BMI and any of the immuno-hematologic parameters (p>0.057).

Immuno-haematological Parameters:
The distributions of values of CD4+ T cells, CD8+ T cells, CD4/CD8 ratio, WBC, hematocrit and platelets by age are shown with box and whisker plots (Figure 1). The median, mean (±SD) and 95% percentile range of leukocytes, lymphocytes, granulocytes, MID (monocytes, eosinophils and basophils), $CD4^+$ and $CD8^+$ T cells and haematological indices are presented in Table 1. Females had a significantly higher mean absolute $CD4^+$ T cell counts and mean CD4 to CD8 ratio (p=0.0001) than males but no significant difference was observed for $CD8^+$ T cells (Table 1). However, mean values of hemoglobin (Hb), haematocrit (Hct) and red blood cell (RBC) were significantly lower in females than in males (p=0.001). There was no statistically significant difference between the sex for hematological indices of mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC) (Table 1).

Table 1**: Median, mean (± SD) and 95% percentile ranges of immunological and hematological reference values, Bahir Dar Town**

Parameters	Female			Male			P value	Both (female & male)		
	Median	Mean (±SD)	95% range	Median	Mean(±SD)	95% range		Median	Mean (±SD)	95% range
$CD4^+$ T cells /	780	799 ± 218	390-1191	643	676 ± 235.6	307-1081	0.001	700	721±200	307-1105
$CD8^+$ T cells	532	582 ±247	206-1099	659	659.5 ± 343	214-1272	0.016	550	623±305	206-1186
CD4/CD8	1.44	1.53±0.59	0.47-2.6	1.09	1.19 ± 0.49	0.26-2.2	0.007	1.25	1.33±0.56	0.26-2.4
Lymphocyte (10^9/liter)	2.2	2.2 ± 0.54	1.2-3.14	2.0	2.11 ± 0.6	0.9-3.25	0.183	2.1	2.15±.59	0.9-3.21
WBC (10^9/liter)	6.3	6.4 ± 1.86	3.3-9.8	6.3	6.7 ± 4.4	2.9-9.8	0.500	6.3	6.6±3.6	2.9-9.8
Neutrophils (10^9/liter)	3.5	3.7± 1.57	1.3-6.6	3.5	3.7±1.57	0.9-6.6	0.789	3.5	3.7±1.6	0.9-6.6
MID (10^9/liter)	0.5	0.57±0.27	0.4-0.9	0.5	0.6±0.35	0.3-1.12	0.161	0.5	0.60±0.32	0.2-1.02
RBC (10^{12}/ liter)	4.9	4.9±0.4	3.0-5.6	5.6	5.4±0.5	3.1-6.2	0.001	5.27	5.25±0.57	3.1-6.1
Hb (g/dl)	14.8	14.7±2	11.1-16.5	16.7	16.5±1.8	11.6-18.8	0.001	15.8	15.7±2	11.6-18.6
Hct %	44.7	44±4	30.6-49.8	49.5	49±4.5	33.1-56.0	0.001	47.5	47.1±5	30.6-54.6
MCV (Fl)	98.6	89±5	65.2-95.6	89.8	90±6.1	68-98.3	0.143	89	89±5.7	68-97.8
MCH(pg)	29.7	29.7±3	20.4-32	30.3	30.4±4.5	24-33	0.094	30	30±4	18.7-32.8
MCHC (g/dl)	33	33.3±2.7	19.7-34.3	35.5	34.7±2	28.2-34.8	0.384	33.3	33.3±2.5	28-34.6
Platelet (10^9/liter)	284	280 ± 92	84-425	243	274± 64	64-405	0.703	257	277 ± 20	84-418
BMI (kg/m2	20.2	20.6 ± 3	16.8-25.5		28.5 ± 1.2	16.85-25.4	0.394	20.3	25.2± 9.1	16.7-25.4

MID: include: monocytes, eosinophils, basophils and some precursor cells; Hct: haematocrit; RBC: red blood cells; Hb: hemoglobin; MCV: Mean corpuscular volume;

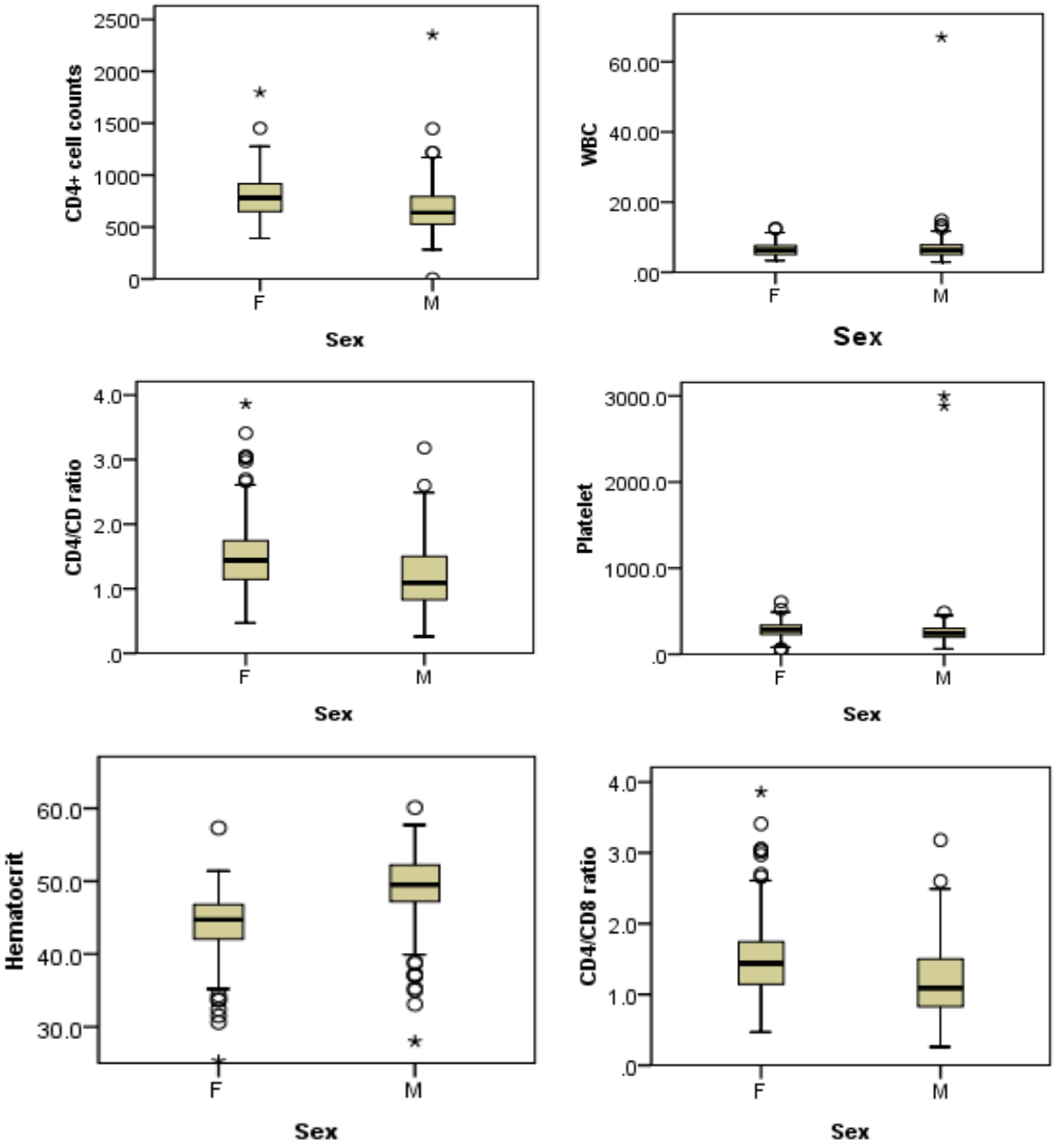

Figure 1. Box and whisper plot showing the median, quartile and range. CD4+ and CD8 T cell counts (cell/mm^3), WBC (10^9/l), platelet (10^9/l), hematocrit ((%) by sex. The central bar in each box is the media, asterisks indicate extreme values and circles indicate outliers.

Table 2 shows a comparison of the study participants' reference values by sex with normal values, which Ethiopia has adopted and is presently being used by the National ART Laboratory of the Federal Ministry of Health for Monitoring adult HIV/AIDS patients. Compared with accepted reference value of the National ART Laboratory, 62 (21.4%), 30 (7.4%) and 36 (8.8%) of the participants had lower than the lower ranges of CD4+ T cells, platelets and leucocytes, respectively. As shown in Table 2, a statistically significant difference was noted in this study between males and females, where 21.5% males and 6.6% females had CD4+ T cells below the normal values (<500 cells/mm^3) (P=0.001). Table 3 shows comparison of the medians and means of the present study with results of earlier studies in Ethiopia, Kenya and Tanzania.

Table 2: **Frequency of females and males whose reference values were below the reference values of the national ART guideline**

Parameters		**Sex**			
	FMOH ART Lab. References (16)	Male (n=238) N (%)	Female (n=167) N (%)	p-value	Both (female & male) N (%)
$CD4^+$ T cells /mm3	<500 (500-1300 cells/mm3)	51 (21.4)	11 (6.6)	0.002	62 (15.3)
	≥ 500	187 (78.6)	156 (93.4)		343 (84.2)
$CD8^+$ T cells /mm3	< 320 (320-1800 cells/mm3)	24 (10)	17 (10)	0.974	41 (10)
	≥ 320	214 (90)	150 (90)		364 (90)
CD4/CD8 ratio	< 0.5 (0.5-2.1)	5 (2.1)	1 (0.6)	0.001	6 (1.5)
	≥0.5	233 (97.9)	166 (99.4)		399 (98.5)
WBC (10^9/liter)	< 4.0 (10.5)	26 (11)	10 (6)	0.085	36 (8.8)
	≥ 4.0	212 (89)	157 (94)		369 (91.2)
Platelet (10^9/liter)	< 140 (140-415 cells/mm3)	19 (7.9)	11 (6.5)	0.034	30 (7.4)
	≥ 140	219 (92.1)	156 (93.5)		375 (92.6)
RBC (10^{12}/ liter	< 3.8 (3.8-5.1 cells/mm3)	4 (1.7)	4 (2.3)	0.61	8 (2)
	≥ 3.8	234 (98.3)	163 (97.7)		397 (98)
Hb g/dl	<11.5 (11.5-15g/dl)	4 (1.7)	6 (3.6)	0.22	10 (2.5)
	≥ 11.5	234 (98.3)	161(96.4)		395 (97.5)
HCT (%)	< 34 (34-44%)	2 (0.9)	6 (3.6)	0.05	8 (2)
	≥ 34	236 (99.1)	161(96.4)		397 (98)
Lymphocytes (10^9/liter)	< 1.0 (1.0-4.0 cells /mm3)	1 (0.4)	-	NA	1 (0.4)
	≥ 1.0	237 (99.6)	167 (100)		404 (99.6)
Neutrophils * (10^9/liter)	< 2.0 (2-8.4 cells/mm3)	11 (4.6)	8 (4.8)	0.25	19 (4.7)
	≥ 2.0	227 (95.4)	159 (95.2)		386 (95.3)

*: Lymphocytes and neutrophils were expressed in percentage in FMOH ART. Lab, but converted into absolute reference range

Table 3: **Comparison of immunological and hematological mean ± SD of values with other studies in Ethiopian and Africa, Bahir Dar Town**

Parameters	This study (mean)		Ethiopia (mean) [2]		Kenya (median) [10]		Tanzania (mean) [13]	
	Female	Male	Female	Male	Female	Male	Female	Male
$CD4^+$ T cells /µl	799 ± 218	676 ± 235.6	816 ± 218	753 ± 227	982	744	802± 250	665.6± 246
$CD8^+$ T cells /µl	582 ±247	659.5 ± 343	692± 269	777 ± 362	549	454	551.0±215.4	438±208.4
CD4:CD8 ratio	1.53±0.59	1.19 ± 0.49	1.3 ± 0.5	1.1 ± 0.4	1.7	1.6	1.5±0.3	1.6±0.3
WBC (10^9/liter)	6.4 ± 1.86	6.7 ± 4.4	6.2 ±2.2	6.0 ± 1.8	4.9	4.3	5.3 ±1.7	4.9 ±1.6
Lymphocytes	2.2 ± 0.54	2.11± 0.6	1.85±0.5	1.85 ± 0.6	2.16	1.86	1.9± 5.3	1.6 ±4.9
Neutrophils	3.7± 1.57	3.7±1.57	3.0±0.12	3.8±0.13	1.96	1.78	NA	NA
MID	0.57±0.27	0.6±0.35	NA	NA	NA	NA	NA	NA
RBC (10^{12}/L)	4.9±0.4	5.4±0.5	4.5 ± 0.4	5.1 ± 6 0.4	4.8	5.3	3.8–5.6	4.4–6.3
Platelets (10^9/L)	280 ± 92	274±64	202±67	207±62	251	218	NA	NA
Hb (g/dl)	14.7±2	16.5±1.8	14.3±1.2	16.1±1.1	8.44	9.9	12.6±1.9	14.1± 2.7
Hct (%)	44±4	49±4.5	42.0±3.2	48.3±3.4	40	47	36.2–46.8	40.2–53.7
MCV (fl)	89±5	90±6.1	NA	NA	84.9	80.0	NA	NA
MCH (pg)	29.7±3	30.4±4.5	NA	NA	28.9	30.1	NA	NA
MCHC (g/dl)	33.3±2.7	34.7±2	NA	NA	339	342	NA	NA

NA: not available, values are in means

Discussion

In Ethiopia, $CD4^+$ T cell counts have been used to assess immune impairment in HIV/AIDS patients to initiate antiretroviral therapy (ART) and antimicrobials as prophylaxis against opportunistic infections (9). Therefore, establishing national reference values is importance for clinical assessment.

The mean absolute $CD4^+$ T cell counts of females obtained in the present study are similar to previous values obtained in Ethiopia (2) and Tanzania (12). However, it was lower than those in Kenya (13) and Botswana (14). Particularly, males had a low median CD4+ T cell count which was also reported from rural Tanzania (12). These variations in CD4 T cells have been shown to be associated with ethnicity, genetic, diet, geographical and environmental factors (1-3).

With respect to gender, females had a significantly higher $CD4^+$ T cell counts compared to males (p=0.001), which is similar to findings of earlier studies (2, 12-15). This higher absolute value of $CD4^+$ T cells in females could be associated with the effect of sex hormones as peripheral lymphocytes have receptors to estrogen and androgen (21). However, males had a significantly higher median $CD8^+$ T cells compared to females, with a mean increment of 127 (p=0.034). Similarly, a study conducted in Botswana (14) and in Ethiopia (2) reported that males had significantly higher median CD8+ T cell than females (14). However, contrary to our finding, a study from Ethiopia reported no significant difference between the sexes in CD8+ T cell counts (2). A significant variation in $CD4^+$ and $CD8^+$ T cell counts between females and males was observed. However, there is one $CD4^+$ T cell reference value for both males and females in the national ART laboratory guideline. Therefore, interpretation of patients' $CD4^+$ T cells based on this guideline may not be valid for initiation of ART and monitoring for both male and female HIV/AIDS patients.

Attempts have been made to compare the present reference values with those currently used in the national

ART guideline for monitoring of adult HIV/AIDS patients. Compared with the national ART guideline (16), 62 (15.3%) of the study participants had $CD4^+$ T cell values below the lower normal range (<500 cells/mm^3). Moreover, the 95% normal reference value of $CD4^+$ T cell found in this study (307-1105 cell/mm^3) was lower than the reference values approved for the national guideline (16). A statistically significance difference was noted between males and females, where 51 (21.4%) males and 11 (6.6%) females had $CD4^+$ T cell counts that are below the lower normal range of $CD4^+$ T cell that are prescribed in the national ART guideline (p= 0.002).

Therefore, according to Sara et al. (2001), a 95% normal reference value is considered valid if no more than 10% of the tested subjects' values fall outside the adopted normal reference value (17). Therefore, based on this result, the national ART laboratory reference values for adult $CD4^+$ T cells need to be reconsidered with respect to gender. However, the reference values of hemoglobin, haematocrit and red blood cells in this study were perfectly aligned with the national ART laboratory reference ranges.

Regarding platelet counts, high mean values were observed as compared with the findings of earlier studies in Ethiopia (2), Kenya (13) and Uganda (15). However, the values are comparable with that of Wintrobes' standard (18). The differences observed with the other studies could be attributed to environmental and genetic factors (19). No difference between the genders was observed with regard to the median values for platelet counts (2).

The adult normal range of erythrocyte counts, hemoglobin and haematocrit levels are consistent with previous Ethiopian values (2, 20) but higher than those of other African countries (12, 13). High altitude induced erythropoiesis could account for the differences. The significant difference between females and males for reference range of RBC, Hb and Hct levels were in agreement with the known fact that males have higher values due to the influence of androgen on their erythropoiesis as well as due to the loss of blood in menstrual cycles in females (10).

The strength of this study was that blood samples were collected exactly at the same time of the day for all subjects (from 9:00-12.00 a.m. in the morning) and analyzed on the same day. Samples were analyzed with the recommended methods of FACS Count and automated Cell-DYN 1800. The study participants were included after screening for observable morbid conditions by clinical screening and by anti-HIV antibody test for HIV. However, latent tuberculosis, HIV infection in window periods, hepatitis B and C virus infections and intestinal helminthic infections might have been missed.

In conclusion, the reference values of $CD4^+$ and $CD8^+$ T cells found in this study were different from the normal values Ethiopia is currently using. Females had significantly higher $CD4^+$T cell counts than males. It is imperative to consider sex differences for interpretation of $CD4^+$T cell counts for monitoring HIV/AIDS patients. The hematological reference values in this study are consistent with national ART guideline values. The findings of this study provide local reference values which can be used to prepare and update guidelines and for interpretation of laboratory data in further studies.

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