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Reference intervals for CD4 and hemoglobin among apparently healthy pregnant women in Addis Ababa, Ethiopia

Dinkenesh Chalchisa[1*], Yohannes Belay[1], Melkitu Kassaw[3], Endalkachew Befekadu[4], Letebrhan G/Egzeabher[5], Gebremedhin Gebremicael[1], Boki Lengiso[1], Dawit Chala[1], Zewdineh Sahlemariam[6], Estifanos Kebede[6], Ebba Abate[7] and Aster Tsegaye[2]

Abstract

Background Most African countries, including Ethiopia, have not developed local well-defined reference intervals (RIs) for immuno-hematological testes in terms of pregnant women. As a result, we were using reference intervals derived from non-Africans. This is not appropriate because CD4 + T cell counts (CD4 count) are affected by several factors including ethnic and environmental factors. Therefore, this study aimed to develop reference interval for CD4 count for apparently healthy pregnant women in Addis Ababa, Ethiopia.

Results After excluding six pregnant women who did not pass the screening tests, 156 apparently healthy pregnant women who were 18–49 years old were included in the final analysis. The medians of CD4 absolute counts and CD4% with inter-quartile ranges [IQR] were 757.5 [611.3-925.5] cells/μL and 43.6% [39.9–47.3] respectively while the median and IQR hemoglobin values were 14.3 g/dL [13.4–15.1]. The respective reference intervals for absolute CD4 cell count and % CD4 were 416.9-1218.4 cells/μL and 32.1–57.3%, respectively. Significant changes were observed in hemoglobin level between trimesters ($P < 0.05$).

Conclusion The results of this study showed a decrease in both percentage and absolute CD4 + T cell counts when compared to those of non-African and African countries. Establishing local reference values for diverse groups is therefore crucial.

Keywords CD4 count, %CD4, Hemoglobin, Reference interval, Ethiopia

*Correspondence:
Dinkenesh Chalchisa
dinkeneshc@gmail.com
[1]National HIV Reference Laboratory, Ethiopian Public Health Institute, Addis Ababa, Ethiopia
[2]Department of Medical Laboratory Sciences, College of Health Sciences, Addis Ababa University, Addis Ababa, Ethiopia
[3]Food Science and Nutrition Research Directorate, Ethiopian Public Health Institute, Addis Ababa, Ethiopia
[4]Department of Medical Laboratory, Amanuel Mental Specialized Hospital, Addis Ababa, Ethiopia
[5]Department of Medical Laboratory, Yekatit 12 Medical College Hospital, Addis Ababa, Ethiopia
[6]School of Medical Laboratory Sciences, Faculty of Medicine, Institute of Health Science, Jimma University, Jimma, Ethiopia
[7]Ethiopian Public Health Institute, Addis Ababa, Ethiopia

Introduction

CD4 + T cells are a subset of T-lymphocytes that act as helper or inducer T-cells. Instead of killing the pathogen directly, they trigger an immune response by sending a signal to other T cells and macrophages. The cell is used to mediate adaptive immunity for different pathogens, induce immunological memory and also participate in autoimmunity, asthma, and allergic responses [1, 2].

During pregnancy, some physiological changes occur that influence immunological and hematological activities that change the CD4 + T cell count (CD4 count) and hemoglobin (Hgb) level. CD4 count provides a figure of the immune system in health; a CD4 count within the range of the 2.5th and 97.5th percentile of apparently healthy population typically indicates the wellness of the immune system [3–6].

CD4 count and Hgb level can be affected by different factors starting from sample collection, up to analysis as well as pathological and physiological factors such as the technique and time of blood collection, transport, and storage of the specimens, posture, exercise, methodology, and instrument used to obtain the result [7, 8].

Many laboratories lack reference interval (RI) for pregnant women, even though the physiological and biochemical changes in pregnancy influence many of laboratory tests. Without adequate RI, there is an increased risk of missing significant changes due to pathological conditions and incorrectly interpreting normal changes as pathological events [9].

Certain laboratories have identified this condition and provide RI for pregnant women, even though pregnancy itself causes the same changes in routine laboratory tests. The majority of laboratories are actually unaware of the typical RI for pregnant women [10]. In comparison to populations in some other African nations and the West, adult Ethiopian populations have higher Hgb levels. Conversely, during pregnancy, which is marked by numerous changes in practically all organ systems to accommodate the growing and developing fetal placenta, they have a lower CD4 count [11, 12].According to Clinical and Laboratory Standards Institute (CLSI) recommendation, each laboratory shall establish its RI because the RI is affected by several factors including ethnicity, nutrition, altitude, geographic location, and pregnancy [13].

The RI used in clinical practice in Ethiopia are derived from non-local populations. Therefore, this study aimed to develop RI for CD4 count in apparently healthy pregnant women in Addis Ababa which was important as a guide for clinical support and treatment monitoring of patients in disease conditions and clinical trials. The study also provided Hgb values by gestational age.

Materials and methods

Study design, period, and setting

A community-based cross-sectional study was conducted on apparently healthy pregnant women from January to June 2019 in Addis Ababa, Ethiopia.

Addis Ababa is situated between 2200 and 2500 m above sea level. It is the biggest capital city of the nation. According to the 2007 census, it has a population of 3,384,569 and occupies 527 square kilometers in Ethiopia [14]. The city was divided into ten sub-cities during the study period. Four sub-cities, Yeka, Akaki Kality, Arada, and Kirkos, were selected based on the population estimate.

Healthy pregnant women in Addis Ababa between the ages of 18 and 49 who met the eligibility standards were included in the study: Eating a healthy diet, including consuming dietary supplements (taking pills of folic acid and iron), active exercise, vaccination (receiving the tetanus toxoid vaccine), and getting examined frequently with ultrasound early and free from any illness.

Ineligibility (Exclusion) criterion, Participants with chronic illness like anemia, chronic renal failure, hypertension, coronary heart disease, diabetes, thyroid disease, liver disease, or any cancer are excluded. Additionally, people who use pharmacologically active substances aside from iron, and who have received a blood transfusion within the last year, infected with intestinal parasites, malaria, have high C-Reactive Protein (CRP) levels, smoking, drinking, HIV, hepatitis B, and supplementing with folic acid were excluded.

The sampling strategy used in this study was a probability ratio. The sample size was established following the CLSI recommendation that a good way to develop RI is to collect samples from a sufficient number of different individuals to obtain at least 120 samples for analysis. Probability Proportional to Size (PPS) is used to determine the number of households in the city's *Woredas* (previously *Kebeles*, the smallest administrative units). Due to the size of Addis Ababa, four sub cities namely Arada, Kirkos, Akaki, and Yeka were chosen based on PPS with a support from the Ethiopian Statistical Agency. Using *Woreda* as the sampling frame, systematic random sampling was used to select study participants. Informed consent was obtained before a blood sample was taken for laboratory analysis, and structured questionnaires were utilized to gather socio-demographic information.

Specimen collection and laboratory analysis

About 4 ml of blood was collected using a vacationer tube containing ethylenediaminetetra acetic acid (EDTA).Samples were taken prior to 11:00 a.m. to prevent daily variations and were identified with distinct codes. At the National HIV Reference Laboratory of the Ethiopian Public Health Institute, a point-of-care (POC)

instrument, BD FACSPresto (BD, San Jose, CA, USA) was used to analyze the absolute CD4 count, CD4% and Hgb level within eight hours of sample collection. Since 2016, Ethiopia has been using BD FACSPresto^{TM}CD4 POC technology, which has acceptable agreement for CD4absolute counts and CD4%with FACSCalibur [15]. The laboratory analyses were conducted strictly adhering to laboratory standard operating procedures and manufacturer's instructions by trained laboratory technologists. Inbuilt quality-control system is used in FACSPrestoPOC instrument. Furthermore, the National HIV Reference Laboratory participates in external quality assessment scheme.

Statistical analysis

SPSS v20 was used to enter and analyze the data. Initially, the data distribution was evaluated. Then, the data were compiled, summarized, and shown as absolute numbers with percentages for categorical variables and mean ± SD and medians with IQR values for continuous variables. The nonparametric Spearman correlation test was used to measure correlation, and the nonparametric Mann-Whitney U and Kruskal-Wallis tests were used to compare groups. A non-parametric test was used to calculate RIs, estimating percentages of 2.5 (the lower limit), and 97.5 (the upper limit) with a 95% confidence interval. The RI was determined to be the 95% interval. P-value of 0.05 was used to declare statistical significance.

Results

Socio-demographic characteristics

A total of 162 apparently healthy pregnant women aged 18–49 years were included in the study. Of these, 6 participants were excluded due to chronic diseases, parasitic infections, HIV, and high CRP. A total of 156 participants participated in the final analysis. The median age of the study participants was 27 (IQR = 24–29) years. More than half (58.33%) of the participants were 25–34 years old. Most of the participants (88.4%) were married, 37.2% had primary school education and 51.9% were housewives (Table 1).

Table 1 Socio-demographic characteristics of study participants ($n = 156$)

Variables		Frequency	Percentage (%)
Age(years)	18–24	57	36.54
	25–34	91	58.33
	35 and above	8	5.13
Marital status	Single	16	10.3
	Married	138	88.4
	Divorced	2	1.3
Educational status	Illiterate	12	7.7
	Read and write	5	3.2
	Primary	58	37.2
	Secondary	57	36.5
	College and above	24	15.4
Occupation	Housewife	81	51.9
	Government employee	14	9.0
	Private employee	61	39.1

CD4 + T-cells and hemoglobin by study participant's characteristics

The median value of CD4 count and Hgb concentration by different groups of study participant's characteristics is presented in Table 2. There was no statistically significant difference in CD4 count and Hgb concentration with participant's characteristics except Hgb concentration variation by gestation stage (trimester). The median value of absolute CD4 value was 779 (640–937) cells/μl, 725 (594-927.5) cells/μl and 779(619.5–916) cells/μl in the first, second, and third trimesters respectively. The Hgb concentration of participants in the first trimester (median: 14.9, IQR: 14.3–15.4) is significantly higher than that of participants in the second (median: 14.1, IQR: 13.3–14.9) and third ((median: 14.1, IQR: 13.2–15.0) trimesters ($p < 0.05$). The Hgb concentration of pregnant women is inversely proportional to gestational age. The mean gestational age was 24 weeks ranging from 3-4weeks.

Correlation of CD4 count and hemoglobin concentration with gestational age

We assessed the correlation of absoluteCD4 count, CD4% and Hgb concentration with gestational age (Fig. 1). No significant correlation was seen between gestational age and CD4 absolute count (r=-0.042; $p = 0.605$) and CD4% (r=-0.006; $p = 0.944$); while Hgb concentration had weak negative correlation with gestational age (r=-0.160; $p = 0.048$).

Absolute and percentage CD4cells count and hemoglobin reference intervals

AbsoluteCD4 count ranged between 394 and 1699 cells/μL while CD4% ranged 22–63% and Hgb 11–18 g/ dL. The medians of absolute CD4counts and CD4%with inter-quartile ranges [IQR] were 757.5 [611.3-925.5] cells/μLand 43.6% [39.9–47.3] respectively while the median and IQR hemoglobin values were 14.3 g/dL [13.4–15.1]. The RI for CD4% and absolute CD4 + T-cell counts were 32.1–57.3% and 416.9–1218.4 cells/μL, respectively (Table 3). Since hemoglobin showed significant difference by gestation week, overall median and IQR was presented and no reference interval was determined.

Table 2 Median (IQR) value of CD4 count and Hgb concentration by different characteristics of study participants

Characteristics (*N* = 156)		AbsoluteCD4 count (cells/μL)	CD4%	Hgb (g/dL)
		Median (IQR)	Median (IQR)	Median (IQR)
Age (Years)	18–24	682 (594–875)	42.6 (39.4–46.0)	14.3(13.2–15.1)
	25–34	794 (621.5–961)	44.2(40.1–48.8	14.3(13.5–15.2
	35–49	730 (590–925)	41.8(38.1–44.8	15.2(13.4–15.4)
Gestation stages (Trimester)	1st (*n* = 33)	779 (640–937)	43.2(39.0-47.7)	***14.9(14.3–15.4)
	2nd (*n* = 49)	725(594-927.5)	43.4(40.2–48.8)	14.1(13.3–14.9)
	3rd (*n* = 74)	779(619.5–916)	43.8(40.0-46.8)	14.1(13.2–15.0)
Education status	Illiterate	726 (606–859)	43.9(41.1–46.4)	16.7(13.6–15.4)
	Read and write	931(750.5-1101.5)	46(37.0-47.4)	15.3(14.6–16.4)
	Primary	791.5(619.0-934.0)	44(40.1–47.1)	14.4(13.7–15.2)
	Secondary	723(565.5–899.0)	42.5(39.1–47.0)	12.1(13.2–14.9)
	College and above	804.5(719.3-922.8)	45.3(41.3–50.7)	14.5(13.2–15.3)
Occupational status	Housewife	750.5(608–930)	43.5(40.2–46.4)	14.3(13.5–15.2)
	Government employee	804.5(701–1029)	44.2(40.2–50.7)	14.7(13.4–15.4)
	Private employee	761(601–911)	43.8(39.3–48.8)	14.3(13.3–15.1)
BMI	Underweight	731.5(629.3-867.5)	37.2(36.0-52.3)	13.1(12.9–14.0)
	Healthy Weight	736.5(596.3–897)	43.5(40.1–47.7)	14.2(13.4–15.1)
	Overweight	775(627.8–983)	43.4(39.8–46.4)	14.5(13.6–15.2)
	Obesity	918(705.5–1118)	45.9(41.8–53.3)	15.0(13.9–15.6)
Folate supplementation	Supplemented	768.5(594.8-929.3)	43.1(39.7–46.3)	14.0(13.2–14.8)
	Not Supplemented	746.5(615.0-926.3)	44.2(40.0-48.2)	14.4(13.5–15.2)
Iron supplementation	Supplemented	768.5(613.0-925.5)	43.7(40.2–47.4)	14.3(13.4–15.2)
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Notes: *** P-value computed using non-parametric Kruskal-Wallis test comparing the median of Hgb level based on gestation stage (Trimester).H(2) = 8.056, P-value = 0.018 with a mean rank Hgb measurement of 98.15 for 1st Trimester, 75.03 for 2nd Trimester and 72.03 for 3 rd Trimester

Discussion

This study focuses on the values of absolute and percentage CD4cell counts to determine RI in 156 apparently healthy pregnant women from Addis Ababa, Ethiopia. Pregnancy has a physiological change that leads to the suppression of immunological functions for the survival of the fetus [16]. Thus, separate RI for tests that indicate immune status like CD4 count are required during pregnancy to find out the pathological complication of maternal and fetal during the pregnancy period.

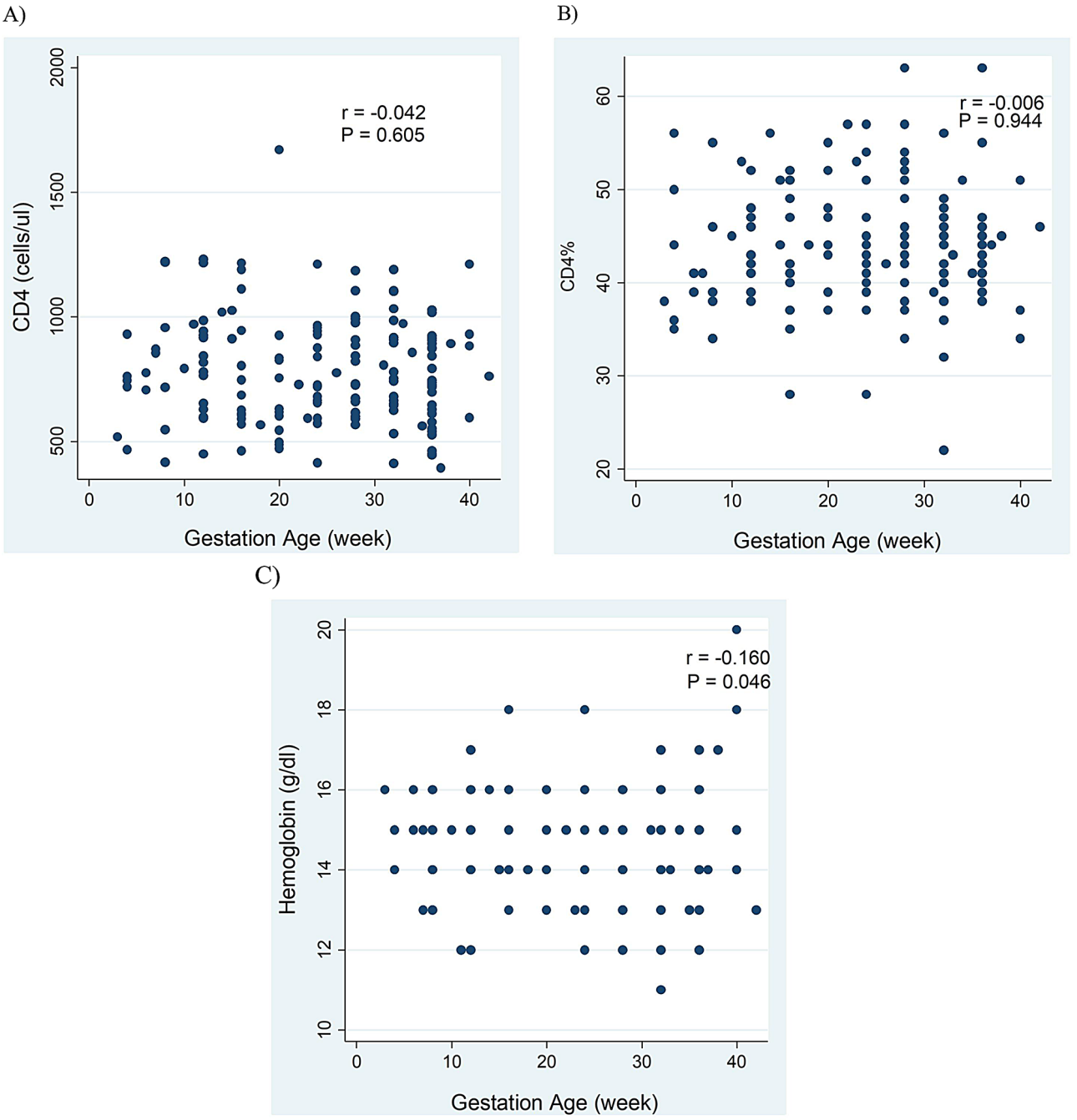

Fig. 1 Correlation of CD4 absolute count (cells/μL), CD4% and Hgb concentration (g/dL) with gestational age (week) in pregnant women. Correlation of (**A**) absoluteCD4 count Vs gestational age, (**B**) CD4% Vs gestational age and (**C**) Hgb concentration V gestational age. Non-parametric spearman correlation test was used to assess correlation

Significant difference was not observed in this study for absolute CD4 count (p-value: 0.160) andCD4% (p-value: 0.337) across the participant's age. Moreover, there was no difference in absoluteCD4count (p-value: 0.558) and CD4% (p-value: 0.916) across the gestational age (trimester).Contrary to our findings, a study from Hawassa, southern part of Ethiopia reported a significant differences between CD4 count and gestational age. CD4 count value declines from first to third trimester [17].

The current study's median absolute CD4 count was 757.5cells/μL, which was less than the 932.0 cells/μL seen in non-pregnant women in the same study setting [18]. This implies that the need to establishing RI specifically in pregnant women which is physiologically at immunocompromised state during pregnancy.

Table 3 Mean, median, range and 95% reference interval of CD4 and Hgb for healthy pregnant women ($N = 156$)

Parameters	Mean ± SD	Median (IQR)	Range	95% RI(2.5th-97.5th Percentile	Lower limit 95%CI	Upper limit 95%CI
Absolute CD4 count (Cells/μL)	782.9 ± 216.7	757.5(611.3-925.5)	394–1669	416.9-1218.4	410.7–463.0	1192.6-1263.9
CD4 (%)	44.1 ± 6.5	43.6(39.9–47.3)	22–63	32.1–57.3	27.9–34.9	55.5–62.7
Hgb (g/dL)	14.4 ± 1.3	14.3 (13.4–15.1)	11–18	NA	NA	NA

Note: NA: Not applicable, refers to RI for Hgb concentration which is not determined due to difference in Hgb concentration across the gestation stage (trimester)

The median CD4 cell count value of our study was comparable with studies conducted in Gondar, Ethiopia (738cells/μL) [11]. But; higher than the study reported from Hawassa, Ethiopia (602 cells/μL) [17] and lower than findings from Cameroon which was 831cells/μL [5].Repeated infections may cause physiological leukocytosis, which could account for the higher counts. Furthermore, temporary immune suppression is normally observed during a healthy pregnancy.

Moreover, the RI found in this study for absolute CD4 T-cell count (416.9-1218.4 cells/μL) was lower than other studies from Ethiopia (712.47–760.67) [11], and Cameroon (438–1532) [5] with range estimate of the 95% confidence limits.

Significant variations in Hgb concentration were found in this study between the gestational stages (trimesters), with the concentration being greater in trimester 1 (median 14.9 g/dL) than in trimesters 2 and 3 (median 14.1 g/dL). This result supports findings from population-based studies in England [19]. Hgb levels decrease due to the increase in plasma volume is greater than the rise in red cell mass during pregnancy [6].

The overall median value of Hgb in the present study (14.3 g/dL) is higher than studies conducted in north east 11.9 g/dL [7], southern 12.0 g/dL [17], northwest 13.2 g/dL [19], and eastern part of Ethiopia which was 11.7 g/dL [20].This might be due to the geographical differences that the study participants located at a high altitude which is one of the factors affecting Hgb value. So, study findings indicate that developing of local reference intervals is crucial for the good management of fetal and maternal health status during pregnancy. Factors that could partly contribute to the observed differences could be dietary pattern among populations since Ethiopia has a heterogeneous population of more than 80 Ethnic groups with diverse culture. Instrument variation is among the factors affecting RIs. However, field evaluation study from the same laboratory has demonstrated comparable performance between BD FACSPresto and Sysmex XT-1800 [15].

Because of the insufficient sample size, trimester-specific RI cannot be estimated for hemoglobin which shows statistically significant difference by trimester while both absolute and percent CD4 did not. The participants might not be entirely representative of the whole country, because they were recruited from the highlands. Furthermore, our data solely includes the healthy Addis Ababa pregnant women, notwithstanding Ethiopia's ethnic variety. However, health care professionals and academics working in this field can benefit from the reference intervals defined in this specific study area. Besides, hemoglobin is one of the tests routinely requested to assess anemia among pregnant women and to monitor drug toxicity in HIV positive pregnant women on antiretroviral therapy. The study findings, thus, highlights the need for trimester specific reference intervals for pregnant women.

Limitations

This study's primary limitation was small number of samples recruited due to financial limitations for this study and most participants did not meet the inclusion criteria during the pre-examination and post-examination procedures during the study period, making it impossible to estimate trimester-specific RI within the allotted time frame. Additionally, due to their highland origins, the participants may not be fully representative of the entire nation.

Conclusions

The results of this study showed a decrease in both percentage and absolute CD4 + T cell counts when compared to those of non-African and African countries. Establishing local reference values for diverse groups is, therefore, crucial. It is preferable to use locally generated reference intervals for CD4 count because significant discrepancies is seen between the values in this study and other studies from Ethiopia and other nations. Moreover, Hgb concentrations varied significantly between trimesters ($P < 0.05$), indicating that RI should be determined for each stage of the trimester in the study area.

Abbreviations

CD	Cluster Differentiation
Hgb	Hemoglobin
RI	Reference Interval
HIV	Human Immuno-Deficiency Virus
CLSI	Clinical and Laboratory Standards Institute

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Author contributions
Dinkenesh Chalchisa, YohannesBelay, G/Medin G/Michael were engaged in the study's design, execution, data analysis, writing of the draft and final versions of the manuscript; Boki Lengiso, Dawit Chala, Melkitu Kassaw, Endalkachew Befekadu, Letebrhan G/Egzeabher took part in sample collection and lab analysis; Zewdineh Sahlemariam, Estifanos Kebede, Ebba Abate, Aster Tsegaye engaged in the study design, supervision, data analysis and writing during the draft, interim, and final versions. Every author has reviewed and approved the completed manuscript.

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Data availability
All data that support the findings of this study are available on request from the corresponding author.

Declarations

Ethics approval and informed consent
The Helsinki Declaration was followed in the conduct of this study. The Departmental Research and Ethics Review Committee of Addis Ababa University's Department of Medical Laboratory Sciences examined and approved the study protocol. At the time of enrollment, all volunteering participants gave written informed consent for participation and publication. Participants received their CD4 count and Hgb test results upon request.

Publication consent
All volunteering participants gave written informed consent for publication.

Competing interests
The authors declare no competing interests.

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