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ORIGINAL RESEARCH

Reference Intervals of Haematological Parameters for Apparently Healthy Adults in Northeast Ethiopia

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Background: Clinical laboratory reference intervals play a vital role in evaluating overall well-being, tracking the progression of diseases, and detecting potential harmful effects and complications. Despite evidence revealing disparities, many African nations currently rely on reference intervals for blood analysis obtained mainly from Western populations. This practice increases the risk of misidentifying and misdiagnosing healthy individuals. The aim of this study was to establish common hematological parameters reference intervals for healthy adults in Northeast Ethiopia.

Methods: This community-based cross-sectional study consisted of 328 individuals who were presumed to be in good health. To assess their blood-related characteristics, blood samples were collected and analyzed using the advanced Dirui BF-6500 analyzer, along with serological testing. In accordance with guidelines provided by the Clinical and Laboratory Standards Institute, the study employed a non-parametric approach to calculate the medians and 95% confidence intervals. To explore potential variations between males and females, a statistical test known as the Mann–Whitney *U*-test was used to compare the reference intervals.

Results: The established reference intervals were: white blood cells 3.5–11.3×10^9/L; red blood cells 4.0–6.1×10^{12}/L; hemoglobin 11.2–17.5g/dL; hematocrit 35.4–52.0%; MCV 77.9–93.8fl; MCH 24.7–32.0pg; MCHC 306–349g/L; RDW-CV 12.1–13.8% and platelet 131–391×10^9/L. The reference values of monocytes, eosinophils, red blood cells, hemoglobin, hematocrit and RDW-CV in males were higher than females, while females had significantly higher platelet counts compared to males. The reference intervals discovered differed from the reference intervals now in use, those mentioned in earlier research in Ethiopia or other African nations, as well as those conducted on Western populations.

Conclusion: In the adult demographic of Northeast Ethiopia, specific reference intervals for commonly observed hematological parameters were established, tailored to the local community. Consequently, these reference intervals hold the potential to enhance informed decision-making within this population, by providing valuable guidance when interpreting laboratory test outcomes.

Keywords: reference intervals, hematological parameters, healthy adults, Northeast Ethiopia

Introduction

Clinical laboratory reference intervals are necessary resources for proper test result interpretation.[1,2] They can be used for health status evaluation, disease progression tracking, and reporting of potential toxicity and adverse events in clinical treatment and clinical trials. International recommendations encourage the use of reference intervals pertinent to the population of interest for which a particular test will be applied for the correct and trustworthy interpretation of laboratory test findings.[3–5]

The most crucial components for health assessment, diagnosis, staging, prognostication, and event monitoring are hematology reference intervals. Most African countries now use reference intervals for these characteristics that are primarily derived from Western populations.[6] Research has shown that there are discrepancies between the reference intervals for hematological parameters used in Western populations and those applicable to Africans. Consequently, this disparity leads to the incorrect classification and misdiagnosis of healthy individuals of African descent.[7–13] Age, gender, race or ethnic origin,

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nutrition, geographic location, ambient altitude, seasonal patterns, pathogen exposure, and analytical methods or instruments utilized are some examples of the variables that affect the reference values of hematological parameters.[14–19] It is of utmost importance for laboratories across African nations to establish and adhere to their own reference intervals for these parameters. This becomes even more critical considering the expected substantial growth in the number of clinical trials and patients receiving clinical services in sub-Saharan Africa.[6,14]

Presently employed reference intervals in Ethiopia are derived from diverse sources such as published literature and manufacturer studies, which predominantly rely on samples obtained from Western populations. Nevertheless, there exists a significant lack of information concerning locally generated, reliable reference intervals.[20] According to the few investigations that have been done, hematology reference values differ from Western-based values.[13,21–24] These discrepancies have the potential to complicate the interpretation of laboratory test results and impact clinical decision-making. Hence, to ensure precise evaluation of patient well-being and to facilitate safe execution of medical studies, it is imperative to establish population-specific hematological reference intervals in Ethiopia. The objective of this research was to determine reference intervals for commonly observed hematological parameters specifically tailored to the adult population in Northeast Ethiopia.

Materials and Methods

Study Design and Setting

Between April 2019 and January 2021, a community-based cross-sectional study was carried out in Dessie town and the surrounding communities of Tita, Gerado, and Borumeda in the South Wollo Zone of Northeast Ethiopia. Dessie, positioned approximately 401 kilometers northeast of Addis Ababa, the capital city of Ethiopia, is a city situated within the Amhara Regional State. Geographically, it is located at 11°8′ N and 39°38′ E, with an elevation ranging from 2470 to 2550 meters above sea level. Dessie experiences a subtropical highland climate, characterized by an average annual temperature of 22.26°C, resembling a temperate oceanic climate. Tita, Gerado, and Borumeda are rural communities within the South Wollo Zone of Northeast Ethiopia, encompassing the hospital's catchment area.

Sample Size and Sampling Techniques

The sample size for this study was determined based on the guidelines provided by the Clinical and Laboratory Standards Institute (CLSI). According to the CLSI recommendation, in order to establish a reliable reference interval, a minimum of 120 individuals who are deemed to be in good health should be included in each subgroup (such as based on sex). The analysis employed a non-parametric method with a statistical power of 90%.[25] However, prior extensive study conducted in other African nations[26] found that approximately 30% of the participants did not meet the inclusion criteria for reference interval determination for a variety of reasons. Consequently, a comprehensive group of 344 individuals who were presumed to be in good health were enlisted to ensure the attainment of the recommended sample size of 240, as suggested by the CLSI for determining the reference intervals. The study population was selected utilizing a convenient sampling technique based on predetermined criteria from the community residing in Dessie town and the neighboring areas of the South Wollo Zone.

Dessie town and Tita, representing urban communities, along with Gerado and Borumeda, representing rural communities, were intentionally chosen from the study area based on differences in altitude and residence. The sample size was determined in a manner that proportionally reflected the population size of each selected community. Within these communities, sub-communities were further selected based on their accessibility and suitability for safely transporting blood samples to the hospital laboratory. Ultimately, individuals from each community who met the eligibility criteria of the study were included until the desired sample size was achieved.

Study Participants

Participants for the study were meticulously selected and included based on stringent inclusion and exclusion criteria. Each participant underwent a comprehensive evaluation that included a review of their medical history, a thorough physical examination, and tests to assess pregnancy (for females), C-reactive protein (CRP), HIV, hepatitis, syphilis, hemoparasites, intestinal parasites, and other relevant conditions. Ultimately, apparently healthy adults aged 18 years and above, who had

resided in the designated areas for at least 5 years, were included in the study. The reference group, however, excluded individuals with the following conditions: known chronic diseases (such as diabetes mellitus, hypertension, chronic renal failure, ischemic heart disease, anemia, thyroid disorders, and liver diseases), high blood pressure (140/90mmHg or higher), obesity (body mass index 30 kg/m2 or higher), a recent history of blood donation within the past six months, blood transfusion within the past year, the use of pharmacologically active substances or prescription drugs, and positive screening test results (including CRP, HIV, hepatitis B surface antigen (HBsAg), anti-hepatitis C virus (HCV) antibody, syphilis, hemoparasite, intestinal parasite infection, and pregnancy).

Data Collection and Laboratory Methods

The study participants were invited to visit a nearby healthcare facility, where they underwent individual consenting, screening, and blood collection procedures. After being informed about the study's objectives and the associated risks, individuals who willingly provided written consent underwent comprehensive medical history assessments and physical examinations conducted by trained nurses. Socio-demographic and medical history data were collected using a carefully designed questionnaire that had been pre-tested for accuracy and relevance. The physical examinations were conducted on-site using calibrated equipment and standardized techniques.

Under sterile conditions, aseptically, between 8:00 and 11:00 am in the morning, eligible study participants underwent the collection of venous blood from the antecubital vein, with a volume of five milliliters. This procedure took place following an overnight fast of at least eight hours. Two milliliters of the whole blood were carefully dispensed into an Ethylene Diamine Tetra Acetic acid (EDTA) Vacutainer tube (manufactured by Becton Dickinson, USA) for hematologic analysis. The remaining three milliliters were dispensed into a plain tube, left to clot for 60 minutes at room temperature, and then centrifuged for five minutes at 2500 revolutions per minute (rpm) to facilitate serologic tests. Stool specimens were collected using a clean container for the detection of intestinal parasites, while urine samples were collected from female participants using a sterile plastic container, and on-site HCG testing was conducted.

The whole blood samples were analysed within two hours after blood collection using Dirui BF-6500 automated hematology analyzer (Dirui Industrial Company, China), which performs 24 hematologic parameters, for in vitro diagnosis use in clinical laboratories. The measured hematology parameters for full blood count and differential cell count were white blood cells (WBC), neutrophil (NEU#), lymphocyte (LYM#), monocyte (MON#), eosinophil (EOS#), basophil (BAS#), red blood cell (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width-coefficient of variation (RDW-CV) and platelets.

Data Quality Assurance and Management

The quality of the data was assured by using a carefully designed semi-structured questionnaire, and then it was translated into the local language (Amharic), and re-translated into the English version to check the accuracy and consistency. It was pre-tested on 5% of participants in Kombolcha town. Appropriate modifications were made based on information obtained from the pre-testing questionnaire. In addition, data collectors were trained before beginning data collection to reduce technical and observer bias.

All stages of pre-analysis, analysis, and post-analysis adhered to the principles of Good Clinical Laboratory Practices (GCLP) and followed standardized operating procedures. Prior to sample analysis, the instrument underwent thorough calibration using calibrators and internal controls to ensure accurate results. Internal quality control (QC) measures were implemented utilizing three levels (low, normal, and high) of commercial QC materials, and the analyzer was calibrated in accordance with the manufacturer's recommendations. Daily QC runs were recorded on Levy Jennings charts, and the results for all hematologic parameters fell within ± 2 standard deviations from their target values. Additionally, the hospital laboratory actively participates in the External Quality Assessment (EQA) program facilitated by the Ethiopian Public Health Association (EPHI).

Statistical Analysis and Interpretation

The collected data were meticulously reviewed for completeness, thoroughly checked, and subsequently entered into EpiData version 3.1 software, developed by the Epidata Association based in Odense, Denmark. Data analyses were carried out using

SPSS version 25 software, developed by SPSS Inc. located in Chicago, IL, USA, as well as MedCalc version 20.027 software from Ostend, Belgium. To assess the normal distribution of the data, the Kolmogorov–Smirnov test was employed. Any extreme values deemed outliers were identified and eliminated using the Dixon method.[27] The study performed comprehensive calculations for each hematological parameter, including the determination of the mean, median, and non-parametric reference intervals at the 2.5th and 97.5th percentiles. Furthermore, to establish the boundaries of the reference intervals with a 90% confidence level, the study also determined the corresponding confidence intervals (CI), aligning with the guidelines prescribed by the CLSI.[25] The Mann–Whitney *U*-test was employed to examine potential disparities in reference values based on gender. A significance level of less than 0.05 ($P < 0.05$) was deemed statistically significant.

Ethical Considerations

The Declaration of Helsinki's ethical guidelines were followed to conduct the study. The study received ethical approval from the Institutional Review Board of the College of Medicine and Health Sciences at Wollo University, under reference number CMHS130/13/2019. Before participating in the study, each participant provided written informed consent. Participants who were diagnosed with any illness were referred to appropriate healthcare facilities for proper treatment. Throughout the study, all collected information was handled with strict confidentiality to ensure privacy and data security.

Results

Out of the initial sample of 344 apparently healthy adults selected using a convenient sampling technique, a total of 16 participants were excluded from the study due to positive serological test results. Consequently, the final analysis included test values from 328 participants (164 males and 164 females) to establish reference intervals for common hematological parameters. The study participants had a mean age of 29.2 ± 8.2 years, with males having a mean age of 29.8 ± 8.1 years and females having a mean age of 28.6 ± 8.2 years. The age range of the participants varied from 18 to 57 years.

Table 1 present the mean, median, 95% reference intervals, and the corresponding 90% confidence intervals (CI) for the lower and upper limits of the hematological parameters. The combined median and reference intervals for both males and females were as follows: 6.49 (3.49–11.3 × 10^9/L) for WBC, 3.63 (1.22–7.04 × 10^9/L) for neutrophil count, 2.16 (1.07–4.23 × 10^9/L) for lymphocyte count, 0.52 (0.1–1.01 × 10^9/L) for monocyte count, 0.08 (0.02–1.05 × 10^9/L) for

Table 1 The Mean, Median, and 95% Reference Intervals for Hematological Parameters in Healthy Adults from Northeast Ethiopia

Parameters	Sex	Mean	Median	95% RIs	95% RIs		*P*-value
					90% CI (Lower Limit)	90% CI (Upper Limit)	
WBC (x10^9/L)	C	6.59	6.49	3.49–11.3	3.34, 3.71	10.43, 11.92	0.607
	M	6.52	6.46	3.54–10.9	2.72, 3.87	9.47, 11.96	
	F	6.65	6.49	3.47–11.8	3.34, 3.65	10.43, 11.92	
Neutrophil (x10^9/L)	C	3.60	3.63	1.22–7.04	0.53, 1.27	6.27, 7.97	0.734
	M	3.54	3.68	0.74–6.47	0.44, 1.50	5.83, 7.97	
	F	3.65	3.62	1.25–7.87	1.22, 1.27	6.27, 9.11	
Lymphocyte (×10^9/L)	C	2.31	2.16	1.07–4.23	0.80, 1.21	4.10, 4.28	0.362
	M	2.23	2.18	1.07–3.84	1.07, 1.18	3.81, 4.43	
	F	2.38	2.16	0.88–4.25	0.79, 1.51	4.23, 4.28	
Monocyte (×10^9/L)	C	0.52	0.52	0.10–1.01	0.08, 0.11	0.95, 1.16	0.009
	M	0.56	0.57	0.12–1.00	0.10, 0.19	0.95, 1.24	
	F	0.48	0.49	0.08–1.05	0.05, 0.10	0.81, 1.23	

(*Continued*)

Table 1 (Continued).

Parameters	Sex	Mean	Median	95% RIs	95% RIs		*P*-value
					90% CI (Lower Limit)	90% CI (Upper Limit)	
Eosinophil (×10^9/L)	C	0.14	0.08	0.02–0.46	0.01, 0.02	0.39, 0.57	0.019
	M	0.15	0.09	0.02–0.48	0.02, 0.03	0.41, 0.82	
	F	0.12	0.07	0.01–0.41	0.01, 0.02	0.39, 0.47	
Basophil (×10^9/L)	C	0.03	0.03	0.00–0.09	0.00, 0.00	0.08, 0.10	0.238
	M	0.04	0.03	0.00–0.09	0.00, 0.01	0.08, 0.10	
	F	0.03	0.02	0.00–0.09	0.00, 0.01	0.08, 0.10	
RBC (×10^{12}/L)	C	5.05	4.95	3.98–6.12	3.70, 4.08	5.96, 6.48	< 0.001
	M	5.22	5.40	3.81–6.38	3.65, 4.05	6.12, 6.54	
	F	4.87	4.79	4.06–5.85	3.65, 4.27	5.72, 6.08	
HGB (g/dL)	C	14.3	14.3	11.2–16.8	10.5, 11.8	16.5, 18.1	< 0.001
	M	14.7	15.1	11.3–17.5	10.5, 11.8	16.8, 19.5	
	F	13.9	14.0	10.8–16.1	10.1, 12.2	15.9, 18.1	
HCT (%)	C	43.7	43.4	35.4–52.0	34.4, 36.2	50.5, 55.8	< 0.001
	M	45.0	46.1	35.2–53.9	34.7, 36.1	51.6, 63.9	
	F	42.5	42.1	35.4–49.8	32.7, 37.1	48.9, 55.2	
MCV (fl)	C	86.9	86.7	77.9–93.8	76.6, 80.6	93.6, 96.4	0.098
	M	86.1	85.9	77.0–93.6	76.5, 80.6	92.0, 93.6	
	F	87.6	87.7	78.5–96.4	76.8, 81.7	93.8, 96.4	
MCH (pg)	C	28.6	28.6	24.7–32.0	24.6, 25.7	31.1, 33.2	0.069
	M	28.3	28.5	24.7–32.4	24.4, 24.8	31.1, 33.3	
	F	28.9	28.8	25.7–32.0	24.6, 26.4	31.2, 33.7	
MCHC (g/L)	C	328	328	306–349	304, 307	347, 353	0.235
	M	327	328	304–349	301, 306	347, 353	
	F	328	329	307–349	304, 316	348, 354	
Platelet (×10^9/L)	C	264	269	131–391	117, 144	375, 436	< 0.001
	M	251	249	130–395	79, 141	361, 391	
	F	276	274	144–434	89, 158	375, 441	
RDW-CV (%)	C	13.0	12.9	12.1–13.8	11.8, 12.3	13.6, 14.5	< 0.001
	M	13.1	13.1	12.3–14.4	12.3, 12.5	13.8, 14.6	
	F	12.8	12.8	11.9–13.6	11.5, 12.1	13.4, 13.8	

Abbreviations: WBC, white blood cells; RBC, red blood cells, HGB: Hemoglobin; HCT: Hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW-CV, red cell distribution width-coefficient of variation; C, combined; M, males; F, females.

eosinophil count, 0.03 (0.0–0.09 × 10^9/L) for basophil count, 4.95 (3.98–6.12 × 10^{12}/L) for RBC, 14.3 (11.2–17.5 g/dL) for hemoglobin, 43.4 (35.4–52.0%) for hematocrit, 86.7 (77.9–93.8 fl) for MCV, 28.6 (24.7–32.0 pg) for MCH, 328 (306–349 g/L) for MCHC, 269 (131–391 × 10^9/L) for platelet and 12.9 (12.1–13.8%) for RDW-CV. Significantly higher median values were observed in males compared to females for monocytes, eosinophils, RBC (red blood cell) count, hemoglobin level, hematocrit level, and RDW-CV (red cell distribution width coefficient of variation) ($P < 0.05$). Conversely, females exhibited significantly higher platelet counts compared to males.

Tables 2 and 3 provide a comparison of the hematological reference intervals established for healthy adults in this study with reference intervals reported in previous studies conducted in Ethiopia, other African countries, and the United States (US) populations. The findings reveal notable differences between the reference intervals derived in this study and those reported in previous studies within Ethiopia and other African countries. Specifically, in Table 2, there are significant differences (10%) observed in the lower and/or upper limits of the reference intervals for WBC (white blood cell), RBC (red blood cell), and platelet counts when compared to the currently employed laboratory reference ranges in the area. Comparing the reference intervals obtained in this study with those from US populations, it is evident that the lower limits of the intervals for WBC counts, hemoglobin, hematocrit, and platelet counts were significantly lower (<10%). Moreover, the upper reference limits for RBC and platelet counts in this study were significantly higher (>10%) than the limits observed in the US ranges (Table 3).

Table 2 Comparison of the Hematological Reference Intervals Obtained from This Study with the Currently Employed Reference Intervals and Other Ethiopian Studies

Parameters	Sex	Present Study	Northwest Ethiopia (Gondar) (21)	Northwest Ethiopia (Gojjam) (22)	Amhara State (13)	Southwest Ethiopia (23)	Currently in Use
WBC (x10^9/L)	C	3.4–11.3	3.2–8.8	3.5–11.5	3–11.2	NA	4–10
	M	3.4–10.9	NA	3.5–10.9	NA	3.3–11.6	
	F	3.3–11.9	NA	3.6–11.9	NA	3.2–10.1	
Neutrophil (x10^9/L)	C	1.2–6.7	1.6–5.1	Na	1.1–6.7	NA	
	M	1.4–6.4	NA	NA	NA	1.01–7.22	
	F	1.2–6.6	NA	NA	NA	1.08–6.69	
Lymphocyte (×10^9/L)	C	1.2–4.2	1–3.5	NA	1.1–4.5	NA	
	M	1.2–3.8	NA	NA	NA	1.1–3.84	
	F	0.9–4.3	NA	NA	NA	1.2–3.98	
Monocyte (×10^9/L)	M	0.12–1.00	NA	NA	NA	0.24–0.8	
	F	0.08–1.03	NA	NA	NA	0.27–0.87	
Eosinophil (×10^9/L)	M	0.02–0.57	NA	NA	NA	0.05–1.21	
	F	0.01–0.40	NA	NA	NA	0.04–1.12	
Basophil (×10^9/L)	M	0.01–0.09	NA	NA	NA	0.01–0.05	
	F	0.01–0.09	NA	NA	NA	0.0–0.05	
RBC (×10^{12}/L)	C	3.8–6.2	NA	3.93–6.1		NA	3.5–5.5
	M	4.1–6.5	3.53–6.93	3.9–6.2	4.0–6.0	4.3–6.7	
	F	3.7–6.0	3.45–6.25	3.9–5.8	3.5–5.6	4.0–6.2	

(*Continued*)

Table 2 (Continued).

Parameters	Sex	Present Study	Northwest Ethiopia (Gondar) (21)	Northwest Ethiopia (Gojjam) (22)	Amhara State (13)	Southwest Ethiopia (23)	Currently in Use
HGB (g/dL)	C	11.1–17.0	NA	12–19	NA	NA	12–18
	M	11.8–17.5	11.5–18.0	13.6–19.8	12–18.9	12.1–18.8	
	F	10.4–16.2	11.0–16.7	12–18	10.7–17.5	12.3–17.9	
HCT (%)	C	35.5–53.5	NA	40–58.2	NA	NA	37–54
	M	35.7–55.8	36.2–58.6	45–59	35.7–55.3	36.7–54.5	
	F	34.4–50.2	32.1–56.6	39–55	32.2–50.1	36.9–51.6	
MCV (fl)	C	77.6–93.8	85–100	89.5–107.5	81–100	NA	80–100
	M	76.6–92.3	NA	90–109	NA	74.8–93.9	
	F	78.6–96.3	NA	90.3–106	NA	77.3–98.8	
MCH (pg)	C	24.7–31.9	NA	28–34	25.3–34.6	NA	27–34
	M	24.3–31.7	26.6–33.3	28–34.4	NA	24.9–32.8	
	F	25.7–33.4	25.8–32.8	28–34	NA	26.3–33.6	
MCHC (g/L)	C	303–350	NA	30–332	288–369	NA	320–360
	M	298–353	295–344	30–333	NA	321–365	
	F	307–350	285–344	30–332	NA	320–360	
Platelet ($\times 10^9$/L)	C	131–391	128–432	NA	90–399	NA	150–350
	M	126–390	NA	106–352	NA	164–403	
	F	144–434	NA	120–379	NA	202–445	
RDW-CV (%)	C	12–13.8	12–17	NA	11.6–15.4	NA	
	M	12.4–14.4	NA	NA	NA	12.5–17.6	
	F	11.8–13.6	NA	NA	NA	12.4–15.6	

Abbreviation: NA, not available.

Table 3 Comparison of the Hematological Reference Intervals Obtained from This Study with Reference Intervals from Other Selected Studies Conducted in Africa, as Well as Reference Intervals from the United States

Parameters	Sex	Present study	Ghana (10)	Kenya (28)	Uganda (29)	Tanzania (11)	USA (30)
WBC ($\times 10^9$/L)	C	3.4–11.3	3.4–9.2	2.8–8.2	2.8–8.2	3.0–7.9	4.5–11.0
	M	3.4–10.9	3.5–9.2	2.7–7.5	2.8–8.2	2.8–7.9	NA
	F	3.3–11.9	3.4–9.3	3.0–9.1	3.2–9.0	3.2–8.0	NA
Neutrophil ($\times 10^9$/L)	C	1.2–6.7	1.5–5.6	0.9–4.7	0.9–3.9	1.1–4.7	1.8–7.7
	M	1.4–6.4	1.5–5.9	0.9–4.3	0.9–3.8	1.1–4.8	NA
	F	1.2–6.6	1.4–5.5	1.0–5.6	1.1–4.4	1.2–5.4	NA

(*Continued*)

Table 3 (Continued).

Parameters	Sex	Present study	Ghana (10)	Kenya (28)	Uganda (29)	Tanzania (11)	USA (30)
Lymphocyte ($\times 10^9$/L)	C	1.2–4.2	1.2–4.4	1.1–3.5	1.2–3.7	1.1–3.0	1.0–4.8
	M	1.2–3.8	1.2–5.2	1.1–3.2	1.2–3.6	1.1–2.8	NA
	F	0.9–4.3	1.2–4.4	1.3–4.0	1.3–3.7	1.1–3.1	NA
RBC ($\times 10^{12}$/L)	C	3.8–6.2	3.39–5.83	4.0–6.2	3.5–6.1	4.0–6.1	NA
	M	4.1–6.5	3.79–5.96	4.4–6.3	3.8–6.1	4.4–6.3	4.5–5.9
	F	3.7–6.0	3.09–5.30	3.7–5.6	3.3–5.3	3.8–5.6	4.0–5.2
HGB (g/dL)	C	11.1–17.0	9.8–16.0	6.7–11.1	10.8–17.1	11.7–17.2	NA
	M	11.8–17.5	11.3–16.4	8.3–11.3	11.6–17.1	13.7–17.7	13.5–17.5
	F	10.4–16.2	8.8–14.4	5.9–10.0	9.8–16.2	11.1–15.7	12.0–16.0
HCT (%)	C	35.5–53.5	28.9–48.7	30–50	31.2–49.5	36.5–52.7	NA
	M	35.7–55.8	33.2–50.5	40–50	33.8–49.5	40.2–53.7	41.0–53.0
	F	34.4–50.2	26.4–45.0	30–50	28.3–46.8	36.2–46.8	36.0–46.0
MCV (fl)	C	77.6–93.8	72–97	68.8–97.2	71–97	77.6–98.1	80–100
	M	76.6–92.3	70–98	71.4–98.2	71–97	76.4–98.8	NA
	F	78.6–96.3	73–96	66.0–95.7	74–94.5	77.7–97.9	NA
MCH (pg)	C	24.7–31.9	22.6–33.5	22.4–33.5	23.5–33.7	23.6–33.1	26.0–34.0
	M	24.3–31.7	22.7–33.5	23.3–33.8	23.0–33.8	23.1–33.2	NA
	F	25.7–33.4	22.3–33.6	21.3–33.0	24.8–32.7	24.2–33.1	NA
MCHC (g/L)	C	303–350	305–362	322–353	325–353	306–349	310–370
	M	298–353	306–360	324–353	324–353	306–351	NA
	F	307–350	30.4–36.5	322–352	330–355	304–348	NA
Platelet ($\times 10^9$/L)	C	131–391	89–380	120–411	109–384	150–395	NA
	M	126–390	88–352	115–366	106–362	147–356	150–350
	F	144–434	89–403	124–444	138–457	151–425	150–350
RDW-CV (%)	C	12.0–13.8	12.6–23.0	NA	11.0–16.8	NA	11.5–14.5
	M	12.4–14.4	12–23.4	NA	10.9–16.8	NA	NA
	F	11.8–13.6	12.6–22.9	NA	11.0–17.3	NA	NA

Abbreviation: NA, not available.

Table 4 shows the proportion of apparently healthy individuals whose haematology test results would have been described as abnormal when the reference intervals produced by the laboratory reports in the study area and those the US-based ranges[28] are used. By use of the reference ranges supplied by the laboratory reports in the study area, up to 31.0% of the RBC values in males and 23.9% of the platelet values in females were out of range. When applying the US-based ranges to the total study population, 17.7% of males and 33.3% of females had abnormal RBC values. These proportions are: platelet count, 20.3% in males and 23.9% in females; hemoglobin, 18.4% in males and 8.2% in females; and 19.9% for WBC values.

Table 4 Out of the Values Obtained, a Certain Proportion Fell Outside the Range When Compared to the Reference Values Currently in Use and Those from the United States

Parameters	Sex	Present Study	Currently in Use		Out of Range Comparison USA	
			n (%)	95% Reference Interval	n (%)	95% Reference Interval
WBC (x10^9/L)	Combined	3.4–11.3	56 (17.7)	4–10	63 (19.9)	4.5–11.0
RBC (×10^{12}/L)	Male	4.1–6.5	49 (31.0)	3.5–5.5	28 (17.7)	4.5–5.9
	Female	3.7–6.0	19 (11.9)	3.5–5.5	53 (33.3)	4.0–5.2
HGB (g/dL)	Male	11.8–17.5	10 (6.3)	12–18	29 (18.4)	13.5–17.5
	Female	10.4–16.2	13 (8.2)	12–18	13 (8.2)	12–16
HCT (%)	Male	35.7–55.8	11 (7.0)	37–54	39 (24.7)	41–53
	Female	34.4–50.2	15 (9.4)	37–54	7 (4.4)	36–46
MCV (fl)	Combined	77.6–93.8	16 (5.0)	80–100	16 (5.0)	80–100
MCH (pg)	Combined	24.7–31.9	55 (17.4)	27–34	23 (7.3)	26–34
MCHC (g/L)	Combined	303–350	51 (16.1)	320–360	21 (6.6)	310–370
Platelet (×10^9/L)	Male	126–390	32 (20.0)	150–350	32 (20.3)	150–350
	Female	144–434	38 (23.9)	150–350	38 (23.9)	150–350
RDW-CV (%)	Combined	12.0–13.8	1 (0.6)	11.5–14.5	1 (0.6)	11.5–14.5

Discussion

The absence of precise and dependable reference intervals for the Ethiopian population has led numerous clinical laboratories to rely on reference values provided by in-vitro diagnostic company kit inserts, textbooks, or published literature. This practice can potentially lead to erroneous interpretation of laboratory test results, resulting in misdiagnosis, patient safety concerns, and unnecessary exclusions during participant screening. Recognizing the significance of addressing these crucial gaps, this study was conducted to establish reference intervals for commonly utilized hematological parameters in healthy adult populations from Northeast Ethiopia.

The lower limit of the WBC reference interval in this study was comparable to reports from Gojjam, Northwest Ethiopia[22] and Ghana.[10] On the other hand, it was higher than reports from Amhara Regional State,[13] Gondar, Northwest Ethiopia,[21] Uganda,[29] Tanzania[11] and Kenya,[30] and lower than from USA.[28] The upper limit of WBC count in this study was comparable to reports from previous studies in Ethiopia,[13,22] but higher than reports from other African settings[10,11,21,29,30] and the USA.[28] In this study, there was no significant difference between males and females in the WBC count, which is consistent with previous reports from different parts of Ethiopia,[13,21–23] Nigeria[12] and USA.[28] The upper limits of the intervals for neutrophil and lymphocyte counts in this study are comparable with reports from Amhara Regional State;[13] but higher than those from other African countries[10,11,21,29,30] and lower than those from USA.[28] These variations could be due to differences in population genetics, environmental altitude, seasonal patterns, pathogen exposure and dietary factors or the use of different methods/instruments.[14,18,31,32] No significant sex differences were found in neutrophil and lymphocyte counts across our study populations as supported by previous studies in Ethiopia,[13,21–23] Ghana,[10] Nigeria[12] and USA.[28]

Regarding eosinophil, monocyte and basophil counts, our reference values were higher than those reported from the US population; this finding is consistent with previous studies in other African regions.[10,12,29,30,33] These differences have been largely attributed to socio-economic status, genetic factors and/or the geographic distribution of parasites including helminths, schistosomiasis and malaria.[14,34] However, future region-specific research is needed as there is some evidence to suggest a lower eosinophil and monocyte counts for African adults compared with Caucasians populations.[35] The significant sex-differences in

eosinophil and monocyte counts in this study are consistent with other studies in Africa.[10,29,30] The reasons for these differences are still unclear, but there is evidence indicating that this is attributed to sex differences in immune system and the pathogenesis of inflammatory and immune diseases due to the effects of sex hormones.[36]

The lower reference limits for RBC count in this study are comparable with those reported from Ethiopian studies;[13,22] but lower than reports from Kenya,[30] Tanzania[11] and USA,[28] and higher than those from Gondar, Ethiopia,[21] Ghana[10] and Uganda.[29] The upper reference limit of RBC count in this study was comparable with reports from Gojjam, Ethiopia,[22] Kenya,[30] Uganda[29] and Tanzania,[11] but lower than those from Gondar, Ethiopia,[21] and higher than those from Amhara Regional State,[13] Ghana[10] and USA.[28] The difference observed in RBC counts is possibly related to genetic factors, environmental altitude, seasonal, nutritional, chronic exposure to parasites and/or analytical variations.[14,16,33] Significant sex-difference in RBC count was observed in this study, with males having higher values than females. This was consistent with previous reports from studies in Ethiopia,[13,21–23] Tanzania,[11] Uganda[29] and Kenya,[30] Nigeria[12] and USA.[28] The sex-related difference in RBC count could be due to the effects of androgens on erythropoiesis and to menstrual blood loss in females.[14,37]

The lower limits of the intervals for hemoglobin and hematocrit in this study are lower than those reported from Gojjam,[22] Amhara Regional State,[13] Southwest Ethiopia,[23] Tanzania[11] and USA,[28] but higher than those from Gondar, Ethiopia,[21] Uganda,[29] Ghana,[10] Kenya.[30] The upper limits of hemoglobin and hematocrit in this study are comparable with those reported from Uganda,[29] Tanzania[11] and USA,[28] but lower than those from other studies in Ethiopia[13,21–23] and higher than those from Ghana[10] and Kenya.[30] The differing reference intervals may be explained by genetic variation, dietary role, altitude, sessional variation and exposure to parasites or due to the analytical variability.[14,16,17,33] The significant sex differences observed in the RBC parameters is a well-established fact that females have lower hemoglobin and hematocrit levels than males and has been similarly reported in studies from Ethiopia,[13,21–23] Africa[10–12,29,30] and USA.[28] These sex-related differences in hemoglobin and hematocrit levels are attributed to a direct effect of the sex hormones on erythropoiesis, gender-based hormonal effects on the erythropoietin gene, or to the menstrual bleeding, which can lead iron loss in females.[37–40]

The reference intervals for the red cell indices (MCV, MCH and MCHC) in this study showed obvious differences compared with those reported from previous studies in Ethiopia,[13,21–23] other African countries[10,11,29,30] and USA,[28] probably due to genetic, racial/ethnic, nutritional, environmental factors and/or method and instrument variations.[14,17] Sex-dependent differences in the reference values of MCV, MCH and MCHC were not observed in this study, consistent with previous reports from Ethiopia[13,21,23] and other African country studies.[10,12,29] However, significant sex-differences were reported in African populations for the reference values of MCH and MCHC.[11,22,30] The reference interval for RDW-CV in this study was lower than reports from Northwest Ethiopia[21] and Ghana,[10] but higher than those from Uganda[29] and USA.[28] RDW values vary based on race/ethnic origin, instrumentation and method of calculation.[17,41] RDW-CV reference values were significantly higher in males as compared to females as supported by previous reports that RDW values differ by sex.[23,42]

Our platelet count reference intervals are comparable to those reported from Amhara Regional State,[13] Uganda[29] and Tanzania,[11] but lower than those from Northwest Ethiopia[21] and Kenya[30] and higher than those from Ghana[10] and USA.[28] Differences observed in the platelet counts could be attributed to variations in sex, age, race/ethnicity, genetic factors and/or biological variations such as geographical, seasonal, and lipid variations.[18,43–46] We have found median platelet counts to be significantly higher in females than in males, which may be related to different hormonal profiles or a compensatory mechanism associated with menstrual blood loss.[47,48] The observed sex-dependent differences in platelet count reference values align with previous reports in African populations,[10–12,22,23,29,30] highlighting the need for separate reference intervals to be employed for males and females.

Reference intervals provided in clinical laboratory reports play a crucial role in assisting clinicians with test result interpretation, patient health assessment, and clinical decision-making. However, it is important to note that clinical laboratories commonly rely on reference ranges proposed by test manufacturers, which are often derived from non-Ethiopian populations. Our findings indicate that a significant proportion of hematological test results, specifically 31.0% of RBCs in males and 23.9% of platelets in females, fall outside the currently employed reference ranges. This highlights the crucial role of the local reference population in establishing normal hematology reference values and emphasizes the necessity of utilizing laboratory-specific ranges. The absence or improper use of reference intervals can lead to detrimental consequences, including misdiagnosis, inappropriate treatments, and increased patient risks, all of which significantly impact the quality of patient care. Hence, it aligns

with the recommendations of international regulatory bodies that emphasize the importance of clinical laboratories establishing their own reference intervals tailored to the specific local population they serve.[4,5,25]

Comparing the reference values obtained with the US reference range data, used in most medical research studies, also revealed significant variations for most of the parameters. Indeed, up to 20.3% of male and 33.3% of female healthy participants would have demonstrated abnormalities and require further monitor and investigation, if the US-based values are used as the standards for interpretations of normality of laboratory test results. This finding is consistent with other studies in the region, suggesting that Western derived laboratory reference values used during participant screening for study enrollment may not be applicable to Africans.[10,11,29,30,49] In one of these studies; for example, using the US derived hematological reference values, up to 53% of potential study participants would have been declared as having abnormal laboratory parameters and would be excluded.[10] Locally established population-specific reference intervals are therefore critical in the assessment of participant health and in decision-making. This becomes even more critical considering the anticipated significant increase in the number of clinical trials in sub-Saharan Africa.[6,14] The present study has limitations in that it did not evaluate the participants for their iron profile, haemoglobinopathies, or benign ethnic neutropenia to determine whether these factors should have led to their exclusion.

In conclusion, this study successfully established reference intervals for commonly used hematological parameters in apparently healthy adults from Ethiopia. The findings revealed significant differences between males and females in monocytes, eosinophils, RBC, hemoglobin, hematocrit, RDW-CV, and platelet counts. These results support previous studies indicating that hematology reference values derived from Western populations may not be suitable for African populations in routine clinical care and medical studies. While further research is warranted, the reference intervals established in this study hold promise in facilitating the interpretation of laboratory test results and aiding decision-making processes within this specific population.

Abbreviations

HBsAg, Hepatitis B surface antigen; HBV, hepatitis B virus; CRP, C-reactive protein; HCV, hepatitis C virus; HIV, human immune deficiency virus; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; NA, not available; QC, quality control; RBC, red blood cells, RDW-CV, red cell distribution width-coefficient of variation; WBC, white blood cells.

Data Sharing Statement

The data analyzed in this study are available from the manuscript.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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