Hematological Reference Ranges for Apparently Healthy Blood Donors in Debre Markos, North West Ethiopia, 2016

Mekuriaw Mesfin Birhan (✉ mekuriawm@bdu.edu.et)

<https://orcid.org/0000-0002-1208-9319>

Tigist Tadele

Bahir Dar University

Diresbachew Haile

Addis Ababa University

Dessalegn Demeke

Bahir Dar University

Biruk Getahun

Bahir Dar University

Sefialem Assefa

Bahir Dar University

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Abstract

Objective The aim of this study was to determine hematological reference ranges for apparently healthy blood donors in Debre Markos, North West Ethiopia. **Result** A total of 250 adults with the median age of 20 (range 18 to 48) years were recruited. The mean $\pm$ standard deviation of hematological values were white blood cells (WBC) count $6.34 \pm 1.82 \times 10^3/\mu\text{l}$ (males) and $6.96 \pm 1.89 \times 10^3/\mu\text{l}$ (females); absolute neutrophil count $3.57 \pm 1.47 \times 10^3/\mu\text{l}$ (male) and $3.99 \pm 1.38 \times 10^3/\mu\text{l}$ (females); absolute lymphocyte count, $2.09 \pm 0.64 \times 10^3/\mu\text{l}$ (males) and $2.27 \pm 0.68 \times 10^3/\mu\text{l}$ (females); absolute mid-sized cell count $0.68 \pm 0.36 \times 10^3/\mu\text{l}$ (males) and $0.70 \pm 0.37 \times 10^3/\mu\text{l}$ (females); red blood cell (RBC) count $5.13 \pm 0.49 \times 10^6/\mu\text{L}$ (males) and $4.57 \pm 0.36 \times 10^6/\mu\text{L}$ (females) platelets, $255.86 \pm 65.96 \times 10^3/\mu\text{l}$ (male) and $280.67 \pm 65.71 \times 10^3/\mu\text{l}$ (female).

Introduction

The International Federation of Clinical Chemistry defines "reference value" as the value obtained by observation or measurement of a particular type of quantity on a reference individual where the individual is selected using defined criteria¹.

For appropriate diagnosis, treatment, and follow-up of patients, correct interpretation of the laboratory results is mandatory. This is attained by knowing the normal reference intervals that have been established at local setting than using values from other areas of the world which have different climate, socioeconomic status, living style and genetic makeup².

In most African countries, however, reference intervals have not been adequately addressed. Instead, clinicians in those countries adopt the textbook reference intervals or from instrument manuals or publications^{3, 4} that were mainly obtained from European and American Caucasian populations⁵. Moreover, Published literatures have confirmed that many of the reference values obtained from the developed countries differ significantly from what pertains in most African localities^{2, 6, 7}.

The hematological values which are currently in use in Ethiopia are also adopted from textbooks which refer mainly to Caucasian subjects. Adopting non-Ethiopian reference values for Ethiopians might be misleading.

Hence the present study was carried out to establish normal reference intervals for the hematological parameters in healthy blood donors in Debre Markos and compare the results against hematological values being used currently. The result can be used as reference values in the future evidence-based practices.

Materials And Methods

Facility based cross sectional study design and convenient sampling technique was employed. Apparently healthy blood donors, age between 18 and 65 years, residents of Debre Markos, consented to

participate in the study and those with negative serological tests for viral infections and malaria were included in the study.

Data was collected from 250 subjects by questioner and observing medical records of the donors. Three ml of venous blood was taken with appropriate aseptic technique. The blood sample was collected by EDTA coated vacutainer tube. Within 5–8 hours of blood collection, samples were transported at room temperature in sealed boxes to Debre Markos Referral Hospital. Complete blood count analysis was performed using Auto Hematology Analyzer BC–2800 (Mindray). White blood cells, red blood cells and platelets were counted and sized by the impedance method. In order to maintain quality of the data, the performance of the hematology analyzer were controlled by running quality control measurements before analysis of the sample. The result was collected within six hours and filled on the data collection format.

Data was cleaned, edited, checked for completeness and entered into Epi Data version 3.1 and then transferred to SPSS version 22 for statistical analysis. In all the cases p-value of less than 0.05 was considered as statistically significant and 95% confidence level was accepted.

Result

A total of 270 donors participated in this study. Of this 8(3%) were excluded due to infection and 12(4.4%) were excluded due to sampling error. Thus data of 250, 192 male and 58 female, were included for final analysis. The age range of donors was between 18 to 48 years with mean age of 22.11 ± 5.63 years.

Blood group

ABO and Rhesus (RhD) blood groupings were determined for the study subjects. The most common blood type was O+ (32.4%) followed by blood type A+ (30.8%) and B+ (23.2%). AB+ was found 10%. Only 9 (3.6%) of the blood donors were RhD negative with blood group A (1.6%), O (0.8%), B (0.8%) and AB (0.4%).

Hematological parameters of the study subjects

To obtain reliable results both parametric and non-parametric analysis were performed. The mean standard deviation, median and the 95th percentile reference ranges were determined. The distributions of RBC were statistically different by gender; females had lower values than males ($p < 0.05$). Statistically significant higher WBC ($p = 0.027$), platelet ($p = 0.008$), absolute neutrophil ($p = 0.023$), and absolute lymphocyte count ($p = 0.026$), were found in females (Table 1).

One sample t-test was conducted to compare the mean hematological parameters of study participant with that of a reference value in current clinical use. In almost all cases absolute value of calculated- t value is greater than tabulated- t value; indicating that there was statistically significant variation between values in current study and text book reference range at 95% confidence interval (table 2).

Mann-Whitney Test was conducted if there is significant variation in blood parameters in males between two age groups. There was no significant variation observed across the age groups ($p>0.05$) (table 3).

Discussion

In this study, O positive blood group was predominant followed by A and B blood groups while AB was the least frequently occurred. This is in line with other studies done in United States, Hispanics⁸ and Tanzania⁹. Rh negative blood group is documented as 2% in Tanzania⁹, 2.9 in Ethiopia¹⁰. In this study 3.6 % of the subjects were Rh negative.

The WBC and neutrophil reference intervals in the current study was consistent with previously reported values from Addis Ababa, and United States based reference interval^{7,11,12}, while higher than the values described for African population^{6,13,14}. Lymphocyte count in the current study was comparable with Africa reference values, while lower than Caucasians¹². The difference between the current result and other findings might be due to geographical differences, diet and ethnic background.

Regarding platelet counts, high reference range values were observed as compared with the findings of earlier studies in Ethiopia⁷, Kenya⁶ and Uganda¹⁵. However, the values are comparable with United States based reference value. The reason for the differences observed with the other studies is still unclear and may require additional studies but may be due to dietary, environmental and genetic factors. Such differences indicate the need to develop reference values that are appropriate for the applicable population.

Results of this study showed that there were significant differences in the values of WBC, RBC, lymphocyte, neutrophil and platelet count between male and female population. The mean and median RBC values were higher for men than women. Such difference by gender is in line with the findings of Ethiopian studies^{7, 10, 13, 16, 17, 18} as well as other African studies^{20, 21, 22}. The differences noted for RBC may be attributed to gender based hormonal variations. The higher values in males may be due to the influence of the hormone androgen on erythropoiesis, and females having lower levels is partly because of menstrual blood loss.

Compared to males, females had significantly higher WBC, neutrophil, and lymphocyte counts than males. This result is in agreement with the West Kenyan²³ report which showed as there were significant differences in WBC, neutrophil, and lymphocyte counts between male and female adolescents²³. Similarly gender differences in WBC, neutrophil and lymphocyte counts were found with females having higher values than males and this increase in neutrophil counts observed in women may be related to estrogen since a decrease in counts has been reported after menopause^{14, 24}. In contrary to the current study, a study done in Togo noticed that there were no gender differences in WBC count^{7, 25}, absolute neutrophil count, and absolute lymphocyte count²⁶. This could possibly be attributed to environmental, genetic, and dietary variation.

This study showed that platelet counts were significantly higher in females compared to male population. Such differences by gender are seen on previous reports from Ethiopia^{16, 17} and different parts of the world^{21, 22, 27}. This is in line with an observation that was reported in a Nigerian study conducted at the University of Port Harcourt Teaching Hospital²⁸, as well as a study done in Western Kenya²³. Similarly gender-based statistically significant differences in platelet count were observed from Tanzanian study. The females had significantly higher platelet count, whereas the males had lower platelet count¹⁴. Higher platelet counts in females compared to males might be due to the variations in hormone types where estradiol has been demonstrated to trigger platelet formation in megakaryocytic cell. Our study was not in agreement with study by Menard²⁵ and Tsegaye et al⁷.

In the present study no significant variation was observed in hematological parameters between different age groups (Table 3). This is in agreement with other study which also reported no change in blood cells with aging²⁹. However Odhiambo et al.²³ reported significant differences in the hematological indices among males by age, with the young adults having a higher median of RBC as compared to adolescents as a result of accelerated growth²³. In addition, a previous study also observed a significant decrease in RBC count³⁰. A study from Germany also compared individuals below and beyond the age of 60, and found statistically significant age-dependent decline in RBC counts for both genders. Reduced numbers of hematopoietic stem cells, a defect in progenitor cell proliferation³¹ and the lack of hormonal stimulation or the reduced response to hormonal stimulation have been described as some of the factors affecting hematological parameters in the elderly³². It should be noted that the maximum age of the participants in our study was 48 and this may be the reason for not finding difference in hematological parameters between different age groups.

Conclusion

The hematological reference values established in this study was significantly different from those reported in other countries as well as text books. Thus our data confirms the importance of population specific hematological parameters and supports the need for local guidelines rather than adoption of generalized reference values.

Limitations of the study

Although this study meets the minimum Clinical and Laboratory standards Institute requirements for establishing valid reference ranges, there are few limitations in this study that could be cited such as data on socioeconomic status, use of multivitamins and contraceptives were not available for all reference population. Despite our efforts to only include healthy subjects, it was not feasible to screen for all medical conditions that might have influenced the laboratory results.

Declarations

Ethical approval and consent to participate

Ethical clearance was obtained from Research and Ethics Review Committee of the Department of Medical Physiology, School of Medicine, College of Health Sciences, Addis Ababa University, Ethiopia. Detailed explanations were given about the objectives of the study to the study subjects. Strict confidentiality was maintained during the study. Data was collected after obtaining written informed consent from the study subjects. Only subjects who signed the consent were participated in the study.

Consent for publication

Not applicable

Availability of data and material

We can provide the data if reasonably needed.

Competing interest

The authors do not have any conflict of interest regarding this article.

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Authors' contributions

All authors are participated in the design of the study, collecting and analyzing the data. Mr. Mekuriaw, Mr. Dessalegn, Mr. Biruk and Mr. Sefialem prepared the manuscript. All authors read and approved the final manuscript.

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Tables

Table 1: Mean, standard deviation and 95th percentile reference ranges of hematological parameters of male and female blood donors in Debre Markos, North West Ethiopia, 2016.

Parameter	Male(n=192)		Female (n=58)		P-value of median
	Mean ± SD	Median (2.5th -97.5th)	Mean ± SD	Median (2.5th -97.5th)	
WBC (×103/μL)	6.34±1.82	6.20 (3.47-10.40)	6.96±1.89	6.75 (3.25-12.12)	0.027*
Lymphocyte(abs) (×103/μL)	2.09±0.64	2.00 (1.20-3.65)	2.27±0.68	2.20 (1.04-4.36)	0.026*
Mid(abs) (×103/μL)	0.68±0.36	0.60 (0.20-1.70)	0.70±0.37	0.60 (0.30-2.07)	0.429
Neutrophil(abs) (×103/μL)	3.57±1.47	3.40 (1.20-6.72)	3.99±1.38	3.95 (1.44-8.22)	0.023*
Lymphocyte (%)	34.37±9.58	33.15 (15.80-56.75)	33.36±8.15	32.10 (19.80-53.71)	0.423
Mid (%)	10.81±4.45	9.40 (5.68-23.37)	10.14±3.63	9.00 (5.59-21.50)	0.38
Neutrophil (%)	54.83±10.67	56.10 (30.75-76.02)	56.50±9.24	57.45 (34.55-71.56)	0.206
RBC (×106/μL)	5.13±0.49	5.14 (4.21-6.17)	4.57±0.36	4.58 (3.85-5.32)	0.000*
Platelet (×103/μL)	255.86±65.96	247.50 (154.78-418.88)	280.67±65.71	276.50 (175.23-425.68)	0.008*
MPV (fL)	9.12±0.95	9.05 (7.60-11.02)	9.48±1.11	9.30 (8.04-13.44)	0.022*

Abs =absolute, Mid =mid- sized cells (including monocyte, eosinophil and basophil), SD- standard deviation, MPV- mean platelet volume

* $P < 0.05$: statistically significant difference between males and females *Mann-Whitney test for differences between males and females.

Table 2: Comparison of the mean of hematological parameters of the present study with that of a reference value in current clinical use.

Parameter	Mean present	Mean reference (Wintrobe and Greer, 2009)	t-tabulated	t-calculated
Hgb (g/dL)				
Male	16.3	14.3	0.000	15.6
Female	14.6	14.0	0.001	3.6
Hct (%)				
Male	46.7	46.0	0.04	2.0
Female	41.9	42.0	0.08	-0.2
Rbc($\times 10^6/\mu\text{L}$)				
Male	5.1	5.4	0.000	-7.6
Female	4.6	4.8	0.000	45.4
MCV (fL)	91.3	91	0.2	1.2
MCH (pg)	31.8	31	0.000	6.5
MCHC (g/dL)	34.8	34	0.000	9.8
RDW (%)	12.6	12.8	0.03	-2.2
WBC ($\times 10^3/\mu\text{L}$)	6.3	7.2	0.000	-6.2
PLT ($\times 10^3/\mu\text{L}$)	249	280	0.000	-4.4
MPV	9.2	8.9	0.000	4.8

* $|\text{Calculated- } t| > |t\text{-tabulated}|$ is assumed significant, mean reference= mean of a reference value, mean present = mean of present study

Table 3: Median and 95th percentile reference ranges of male blood donors between age group1 and Group 2 in Debre Markos, North West Ethiopia, 2016.

Parameter	Median (2.5th -97.5th)		
	Age G1(18-35) N=181	Age G2(36-48) N=11	P value
WBC (×103/μL)	6.10 (3.41-10.40)	7.60 (4.50-)	0.099
Lymphocyte (abs) (×103/μL)	2.00 (1.20-3.74)	1.90 (1.40-)	0.531
Mid (abs)(×103/μL)	0.60 (0.20-1.70)	0.60 (0.30-)	0.835
Neutrophil (abs) (×103/μL)	3.30 (1.20-6.65)	4.90 (2.50-)	0.190
Lymphocyte (%)	33.50 (15.80-57.30)	27.20 (18.50-)	0.370
Mid (%)	9.50 (5.66-23.48)	8.80 (5.90-)	0.144
Neutrophil (%)	55.20 (30.67-76.05)	63.10 (52.70-)	0.061
RBC (×106/μL)	5.13 (4.23-6.20)	5.36 (3.87-)	0.383
Platelet (×103/μL)	248.00 (152.85-420.25)	222.00 (162.00-)	0.146
MPV (fL)	9.10 (7.60-11.05)	8.80 (7.90)	0.228

abs =absolute, Mid =mid- sized cells (including monocyte, eosinophil and basophil), MPV- mean platelet volume, G1- group 1, G2- group 2.