CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, May 1999, p. 410–414 Vol. 6, No. 3
1071-412X/99/$04.00+0

Immunohematological Reference Ranges for Adult Ethiopians

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Received 10 August 1998/Returned for modification 25 September 1998/Accepted 19 January 1999

A cross-sectional survey was carried out with 485 healthy working adult Ethiopians who are participating in a cohort study on the progression of human immunodeficiency virus type 1 (HIV-1) infection to establish hematological reference ranges for adult HIV-negative Ethiopians. In addition, enumeration of absolute numbers and percentages of leukocyte subsets was performed for 142 randomly selected HIV-negative individuals. Immunological results were compared to those of 1,356 healthy HIV-negative Dutch blood donor controls. Immunohematological mean values, medians, and 95th percentile reference ranges were established. Mean values were as follows: leukocyte (WBC) counts, 6.1×10^9/liter (both genders); erythrocyte counts, 5.1×10^{12}/liter (males) and 4.5×10^{12}/liter (females); hemoglobin, 16.1 (male) and 14.3 (female) g/dl; hematocrit, 48.3% (male) and 42.0% (female); platelets, 205×10^9/liter (both genders); monocytes, 343/μl; granulocytes, 3,057/μl; lymphocytes, 1,857/μl; CD4 T cells, 775/μl; CD8 T cells, 747/μl; CD4/CD8 T-cell ratio, 1.2; T cells, 1,555/μl; B cells, 191/μl; and NK cells, 250/μl. The major conclusions follow. (i) The WBC and platelet values of healthy HIV-negative Ethiopians are lower than the adopted reference values of Ethiopia. (ii) The absolute CD4 T-cell counts of healthy HIV-negative Ethiopians are considerably lower than those of the Dutch controls, while the opposite is true for the absolute CD8 T-cell counts. This results in a significantly reduced CD4/CD8 T-cell ratio for healthy Ethiopians, compared to the ratio for Dutch controls.

Hematological reference values for Ethiopians have never been established, although a few attempts at determining hemoglobin and hematocrit levels in some populations have been made (1, 15, 22). The values which are currently used in the country are adopted from textbooks which refer mainly to Caucasian subjects (24).

Similarly, the immunological reference values used in Ethiopia are derived from non-Ethiopian subjects. The need to estimate Ethiopian immunological reference values, like those for total lymphocytes and their subpopulations, has increased, especially due to the importance of CD4 T cells in monitoring human immunodeficiency virus (HIV) infection progression (8, 10, 20). At the end of 1997, an estimated 2.5×10^6 Ethiopians were HIV infected, including 150,000 children (Ethiopian Ministry of Health, 1998).

Several factors, including genetics, dietary patterns, sex, age, and altitude, affect immunohematological parameters (11, 24). Since these factors differ depending on the populations and geographical areas studied, it is not surprising that sometimes radical differences have been reported for immunohematological parameters worldwide. For example, low CD4 T-cell counts in Asians (13) and Chinese (5, 6), low CD4/CD8 T-cell ratios in Saudi Arabians (19), and leucopenia in Sierra Leoneans (18) have been observed. A recent study, though the subjects were few, indicated low percentages of CD4 T cells and high percentages of CD8 T cells in Ethiopians (25). Also, low CD4 T-cell counts in Ethiopian Jews in Israel were reported (16). In contrast, the hemoglobin and hematocrit levels in Ethiopians are reportedly high (1, 15, 22), most likely due to the fact that the studied populations are living in the Ethiopian

TABLE 1. Means, medians, and 95th percentile reference ranges of hematological parameters for 485 HIV-negative adult Ethiopians

Subject group (*n*) and parameter	WBC count (10^9/liter)	RBC count (10^{12}/liter)	Hemoglobin level (g/dl)	Hematocrit (%)	Platelet count (10^9/liter)
Male (280)					
Mean ± SD	6.0 ± 1.8	5.1 ± 0.4	16.1 ± 1.1	48.3 ± 3.4	207 ± 62
Median	5.9	5.0	16.1	48.2	203
95% range	3.0–9.8	4.3–5.9	13.9–18.3	41.6–55.1	97–324
Female (205)					
Mean ± SD	6.2 ± 2.2	4.5 ± 0.4	14.3 ± 1.2	42.0 ± 3.2	202 ± 67
Median	5.9 (0.99)[a]	4.5 (0.0001)	14.4 (0.0001)	42.1 (0.0001)	193 (0.22)
95% range	3.0–12.2	3.7–5.2	12.2–16.6	35.3–48.8	98–352

[a] All values in parentheses are *P* values (Mann-Whitney U test) for comparison of medians for male and female subjects.

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TABLE 2. Means, medians, and 95th percentile reference ranges of WBC subset absolute counts for 142 HIV-negative adult Ethiopians[a]

Subject group (*n*) and parameter	Granulocyte count	Monocyte count	Lymphocyte count	CD4 T-cell count	CD8 T-cell count	CD4/CD8 T-cell ratio	CD3 T-cell count	B-cell count	NK cell count
Male (92)									
Mean ± SD	3,083 ± 1,361	359 ± 136	1,857 ± 606	753 ± 227	777 ± 362	1.1 ± 0.4	1,564 ± 485	184 ± 96	277 ± 143
Median	2,775	324	1,801	733	645	1.1	1,465	170	272
95% range	1,053–7,179	166–697	956–3,474	306–1,249	318–1,891	0.4–2.1	696–2,738	31–420	56–639
Female (50)									
Mean ± SD	3,009 ± 1,287	314 ± 120	1,856 ± 522	816 ± 218	692 ± 269	1.3 ± 0.5	1,539 ± 423	203 ± 91	258 ± 153
Median	3,093 (0.98)[b]	276 (0.05)	1,701 (0.88)	810 (0.17)	632 (0.30)	1.2 (0.03)	1,483 (0.84)	198 (0.17)	227 (0.36)
95% range	750–5,521	96–622	1,098–3,487	456–1,368	273–1,418	0.6–2.7	871–2,413	61–471	85–871

[a] Absolute counts were measured per microliter of whole blood.
[b] All values in parentheses are *P* values (Mann-Whitney U test) for comparison of medians for male and female subjects.

highlands (altitude, >2.000 m), where the major food, *injera*, has a very high iron content (22).

Thus, adopting non-Ethiopian reference values for Ethiopians might be misleading. Given this background, an extensive cross-sectional study was performed with the aim of establishing immunohematological reference values for future use in Ethiopia.

MATERIALS AND METHODS

Subjects. A total of 738 adult Ethiopians were involved in this cross-sectional study. The subjects are factory workers in Akaki (a town about 20 km southeast of the Ethiopian capital, Addis Ababa), and they are participants in a long-term cohort study on the progression of HIV type 1 infection in Ethiopia, performed by the Ethiopian-Netherlands AIDS Research Project (ENARP) at the Ethiopian Health and Nutrition Research Institute (EHNRI). All study participants were examined by a medical doctor. The purpose of this examination was to stage all study participants, regardless of their HIV status, according to the World Health Organization staging systems for HIV infection and disease (23). The conditions listed in the World Health Organization staging system include symptoms (e.g., weight loss, fever, diarrhea, and persistent generalized lymphadenopathy) or diseases (e.g., pulmonary and extrapulmonary tuberculosis, pneumonia, and recurrent respiratory tract infections). Each of the 31 conditions listed in the staging system was systematically checked for by the clinician. Only when no conditions were found and the study participant looked healthy was the subject categorized as asymptomatic.

Blood collection and HIV serology. Whole blood was collected with a Vacutainer system in 10-ml tubes containing EDTA. HIV status was determined with plasma samples by an enzyme-linked immunosorbent assay with a Vironostika HIV Uni-Form II plus O kit (Organon Teknika, Boxtel, The Netherlands). Positive results were confirmed by Western blot analysis (HIV BLOT 2.2; Genelabs Diagnostics, Singapore, Singapore).

Hematological analysis. A Coulter counter T540, which was standardized against a 4C plus blood control, was used for whole-blood analysis of hematological parameters. The machine automatically dilutes a whole-blood sample of 29.6 μl, lyses, counts, and gives a printout result of absolute numbers of leukocytes (WBC) (expressed as number of cells [10^9] per liter), erythrocytes (RBC) (expressed as number of cells [10^{12}] per liter), platelets (expressed as number of cells [10^9] per liter), and lymphocytes (expressed as number of cells [10^9] per liter). In addition, hemoglobin (in grams per deciliter) and hematocrit (in percent) and percentages of lymphocytes are measured.

Flow cytometric analysis. Lymphocyte subsets and three part differentials (percent granulocytes, lymphocytes, and monocytes) were analyzed on a FACScan flow cytometer (Becton Dickinson Immunocytometry Systems, San Jose, Calif.) with either six combinations of two monoclonal antibodies (MAbs) (aCD45-aCD14, immunoglobulin G1-immunoglobulin G2 control, aCD3-aCD19, aCD3-aCD4, aCD3-aCD8, and aCD3–aCD16-aCD56) or four combinations of three MAbs (aCD3-aCD4-aCD45, aCD3-aCD8-aCD45, aCD3-aCD19-aCD45, and aCD3–aCD16-aCD56–aCD45). In brief, 100 μl of whole blood was mixed and incubated at room temperature for 20 min with 10 μl of each MAb combination, in separate tubes. RBC were then lysed by adding 2 ml of fluorescence-activated cell sorter lysing solution (Becton Dickinson). After vortexing, tubes were incubated in the dark at room temperature for 10 min and centrifuged at 300 × *g* for 5 min. The cell pellet was washed once with 2 ml of Isoton, resuspended in 500 μl of Isoton, and analyzed with Simulset or Multiset software (Becton Dickinson) of the FACScan.

The FACScan was calibrated with fluorescent beads (CaliBrite) and AutoComp software weekly. Analyses were interpreted according to the Centers for Disease Control and Prevention criteria for quality control.

Statistical analysis. Data were entered and analyzed with the DbaseIII+ and STATA programs, respectively. Mean, median, and standard deviation were calculated for each immunohematological parameter. The 95th percentile reference ranges were determined by using 2.5 and 97.5 percentiles. The nonparametric Wilcoxon rank-sum test (Mann-Whitney U test) was used to compare the distribution of immunohematological parameters between genders.

Ethics. This study is part of a long-term cohort study on the progression of HIV-1 infection in Ethiopia, and it is approved by both the Institutional and National Ethical Clearance Committees. Informed consent was obtained from each participant.

RESULTS

A total of 738 individuals, from ages 15 to 45 years, participated in this study; 87 (11.8%) of them were HIV positive. The 87 HIV-positive and an additional 166 HIV-negative symptomatic individuals were excluded, and the remaining 485 HIV-negative asymptomatic subjects (280 males and 205 females) were included in the analysis.

TABLE 3. Means, medians, and 95th percentile reference ranges of WBC subset percentages for 142 HIV-negative adult Ethiopians

Subject group (*n*) and parameter	% of:							
	Granulocytes	Monocytes	Lymphocytes	CD4 T cells	CD8 T cells	CD3 T cells	B-cells	NK cells
Male (92)								
Mean ± SD	55.1 ± 12.3	6.7 ± 1.7	35.2 ± 10.3	38.1 ± 7.8	37.9 ± 10.0	77.6 ± 6.7	9.0 ± 3.5	13.9 ± 6.3
Median	56.0	6.0	35.5	38.0	35.0	78.0	9.0	13.0
95% range	31.6–78.7	4.0–10.7	16.0–55.4	24.7–53.7	23.0–60.7	62.0–90.7	3.0–18.0	4.0–29.0
Female (50)								
Mean ± SD	54.3 ± 12.5	6.0 ± 2.0	36.4 ± 11.1	41.3 ± 6.1	34.4 ± 7.9	77.2 ± 7.0	10.2 ± 4.4	12.8 ± 5.7
Median	58.0 (0.89)[a]	6.0 (0.04)	33.5 (0.71)	41.0 (0.01)	34.0 (0.08)	78.5 (0.89)	10.0 (0.11)	11.0 (0.24)
95% range	23.0–73.0	3.0–12.1	19.8–64.1	29.0–57.9	17.4–50.1	58.3–87.0	3.3–27.7	5.3–29.7

[a] All values in parentheses are *P* values (Mann-Whitney U test) for comparison of medians for male and female subjects.

Table 1 shows the means, medians, and 95th percentile reference ranges for the hematological parameters measured for 485 HIV-negative Ethiopians, grouped according to gender. As a result, the distributions of the RBC parameters (median hemoglobin, hematocrit, and RBC) were statistically different by gender; females had lower values than males ($P < 0.001$). No gender-specific differences were observed for WBC or platelets.

Various lymphocyte subsets and WBC differential counts were determined for 142 randomly selected HIV-negative individuals (90 males and 52 females). Tables 2 and 3 show the means, medians, and 95th percentile reference ranges for absolute counts and percentages, respectively, of WBC subsets measured for the 142 HIV-negative Ethiopians, grouped according to gender. It can be concluded that the various WBC subset values are not statistically different between males and females, except for the CD4/CD8 T-cell ratio, which is lower ($P < 0.05$) in males.

Table 4 puts the above hematological values in the context of other studies and textbooks. Low values for WBC (3.0×10^9/liter to 10.2×10^9/liter) and platelets (98×10^9/liter to 337×10^9/liter) were found in Ethiopians compared to the values found in the subjects of other studies. Table 5 shows a more detailed comparison of the hemoglobin values in Ethiopia versus those in other African countries. The hemoglobin values for Ethiopians are consistently higher than those for residents of other sub-Saharan African countries.

Table 6 shows a comparison of means, medians, and 95th percentile ranges for WBC populations between HIV-negative Ethiopians and HIV-negative Dutch blood donor controls. Compared to the Dutch blood donor controls (1997 intake of the Central Laboratory of The Netherlands Red Cross Blood Transfusion Service), Ethiopians have significantly lower means of lymphocytes, B cells, and CD4 T cells, while they have a higher mean of CD8 T cells and therefore a reduced CD4/CD8 T-cell ratio ($P < 0.001$). There is no significant difference between the number of CD3 T cells in Ethiopians and the number in Dutch subjects.

DISCUSSION

The aim of this study was to establish immunohematological reference values which may serve as Ethiopian standards for interpretation of laboratory results. The study population consisted of 485 asymptomatic HIV-negative Ethiopian adults, who are employed at a factory in the vicinity of Addis Ababa.

Compared with textbook and other reference values established in Europe and the United States but being used by hematology laboratories in Ethiopia, low values for platelets (98×10^9/liter to 337×10^9/liter) and WBC (3.0×10^9/liter to 10.2×10^9/liter) were found in this study. Low values for WBC and platelets have also been reported from other African countries (2, 9, 18). It was suggested in the studies in Nigeria and Zambia that platelet counts are lower in Africans than in Caucasians because of chronic low-grade malaria parasitemia (2, 9). However, the factory workers participating in the present study are living at an altitude of >2.000 m, and very few malaria episodes were diagnosed among them in the past years. The RBC parameters of Ethiopia are consistently higher than those of many other African countries (2, 3, 7). Altitude-induced erythropoiesis and/or dietary factors could play a role in causing these variations. Interestingly, the present values for hemoglobin are in agreement with those in previous reports from Ethiopia; they were measured by manual methods 1 to 2 decades ago (1, 15, 22).

TABLE 4. Ninety-fifth percentile reference ranges[a] of hematological parameters for HIV-negative Ethiopians compared with other reported values

Source and reference	WBC count (10^9/liter)	RBC count (10^{12}/liter)		Hemoglobin level (g/dl)		Hematocrit level (%)		Platelet count (10^9/liter)	Lymphocyte level (%)	Monocyte level (%)	Granulocyte level (%)
		Males	Females	Males	Females	Males	Females				
Present study	3.0–10.2 (6.1)	4.3–5.9 (5.1)	3.7–5.2 (4.5)	13.9–18.3 (16.1)	12.2–16.6 (14.3)	41.6–55.1 (48.3)	35.3–48.8 (42.0)	98–337 (205)	17–59 (36)	3–10 (6)	31–78 (55)
South Africa (3)	3.7–12.6 (7.2)	3.2–5.8 (4.6)	3.0–5.3 (4.2)	10.3–16.7 (14.0)	9.0–15.2 (12.4)	31.0–52.5 (42.3)	27.3–47.2 (37.6)	NA[b]	NA	NA	NA
Wintrobe, *Clinical Hematology* (24)	4.3–10.0 (7.2)	4.5–6.3 (5.4)	4.2–5.5 (4.8)	14.0–18.0 (16)	12.0–16.0 (14)	41.0–51.0 (46)	37.0–47.0 (42)	150–450	NA	NA	NA
Coulter Electronics[c]	4.8–10.8	4.7–6.1	4.2–5.4	14.0–18.0	12.0–16.0	42.0–52.0	37.0–47.0	130–400	21–51	2–9	42–75

[a] Values in parentheses are means.
[b] NA, not available.
[c] Reference values proposed by the manufacturer.

TABLE 5. Comparison of hemoglobin values with values from other studies in Africa (including Ethiopia)

Gender of subjects	Hemoglobin values by country and reference[a]							
	Present study	Ethiopia (22)	Ethiopia (1)	Ethiopia (15)	South Africa (3)	Namibia/S. Africa (7)	Nigeria (2)	Zambia (9)
Male	16.1 (1.1)	15.7 (1.1)	16.4 (1.5)	15.7 (1.2)	14.0 (1.6)	14.7 (NA[b])	13.9 (1.1)	15.3 (1.3)
Female	14.3 (1.2)	14.2 (1.1)	NA	14.1 (1.4)	12.4 (1.4)	13.8 (NA)	11.5 (1.0)	NA

[a] Values are means, in grams per deciliter; values in parentheses are standard deviations.
[b] NA, not available.

The finding of significant gender differences for the RBC parameters (RBC, hemoglobin, and hematocrit) agrees with the well-established fact that males have higher values for RBC, hemoglobin, and hematocrit than females, partly due to the influence of the hormone androgen on erythropoiesis and also due to menstrual loss. No differences between the genders with regard to WBC and platelet counts were observed. The general absence of gender differences for WBC counts agrees with other reports (3, 18).

It should be emphasized that the above hematological reference values were established on Ethiopian highland subjects (86% of them are of Amhara or Oromo origin). Care should be taken if these standards are used for interpreting the hematological results for Ethiopians of lowland areas and other ethnic origins.

Ethiopian mean CD4 T-cell counts and CD4/CD8 T-cell ratios are lower than those of the Ugandans (21) and Tanzanians (14). Also, compared to the Dutch blood donor controls, Ethiopians had significantly lower mean absolute CD4 T-cell counts (775 versus 993), CD4/CD8 T-cell ratios (1.2 versus 2.2), and B-cell counts (191 versus 313). The opposite was true for CD8 T cells (747 versus 506). However, Ethiopian CD4 T-cell values and CD4/CD8 T-cell ratios were comparable to those reported for Chinese adults (12). In general, this study confirms and extends previous reports of low CD4 T-cell counts in Ethiopians (16, 25). High prevalence of infections and nutritional factors have been indicated as possible contributors to the reduced CD4/CD8 T-cell ratios (25). Mycobacterial infections and/or subclinical hepatitis has also been mentioned as a possible factor in accounting for low CD4 T-cell counts in the Chinese population (12). However, in our study population, there was no difference between the CD4 T-cell counts of HIV-negative individuals with positive tuberculin tests and the counts of those with negative tests (data not shown).

There are reports on significant age-related changes for lymphocyte subsets (4, 12). A multicenter study on adult Caucasians in Europe showed a significant increase per decade of CD4 T cells (1.2%), NK cells (0.9%), and CD4/CD8 T-cell ratios (0.07%) (4). Similarly, in China a significant increase per decade of CD4 T cells (1.6%) and CD4/CD8 T-cell ratios (0.11%) was observed (12). In Ethiopia, as also reported from Romania (17), no age-dependent increase of CD4 T cells was found. However, too few subjects might have been included in this study to detect a change of CD4 T-cell counts by age.

Absolute CD4 and CD8 T-cell counts, as well as CD4/CD8 T-cell ratios, which are well-established HIV disease progression markers, might have to be quantitatively reestablished for use as prognostic markers in HIV-infected Ethiopians. These values can be established only in a long-term prospective cohort study aimed at describing the progression of HIV infection in an Ethiopian context, a study which has been undertaken by ENARP at the EHNRI.

With regard to gender differences for lymphocyte subsets, Ethiopian females were found to have significantly higher CD4/CD8 T-cell ratios and relatively higher CD4 T-cell counts than males, whereas males had higher NK cell counts. Similar observations have been reported from Uganda (21), China (12), Asia (13), and Europe (4).

The comparison of the immunological results for Ethiopian subjects with those for Dutch blood donor controls has its limitations. Factors such as environmental differences, dietary patterns, and prevailing infections could contribute to the observed differences. Genetic differences, if any, would have been ruled out if the study had been done on Ethiopians living in The Netherlands or vice versa. Although the total number of subjects included is comparable to that in similar studies (17, 18, 21), the immunological reference values will need to be updated by testing a larger number of subjects in the future.

In the absence of established immunohematological reference values for Ethiopians, the present reference ranges could be used for the clinical management of Ethiopian patients and the interpretation of laboratory data in research.

TABLE 6. Comparison of means, medians, and 95th percentile reference ranges of lymphocyte subset absolute counts for HIV-negative adult Ethiopians with those of HIV-negative adult Dutch subjects[a]

Subject group (*n*) and parameter	Lymphocyte count	CD4 T-cell count	CD8 T-cell count	CD4/CD8 T-cell ratio	CD3 T-cell count	B-cell count	NK cell count
Ethiopians (142)							
Mean ± SD	1,857 ± 576	775 ± 225	747 ± 333	1.2 ± 0.5	1,555 ± 463	191 ± 94	250 ± 137
Median	1,781	761	637	1.2	1,471	178	226
95% range	1,032–3,432	366–1,235	311–1,618	0.4–2.4	854–2,556	51–419	75–581
Dutch (1,356)							
Mean ± SD	2,054 ± 573	993 ± 319	506 ± 220	2.2 ± 1.0	1,525 ± 458	313 ± 147	NA[c]
Median	1,985 (0.0001)[b]	950 (0.0001)	460 (0.0001)	2.0 (0.0001)	1,460 (0.47)	290 (0.0001)	NA
95% range	1,120–3,390	509–1,761	200–1,042	0.9–4.8	819–2,591	110–670	NA

[a] Absolute counts were measured per microliter of whole blood.
[b] All values in parentheses are *P* values (Mann-Whitney U test) for comparison of medians for Ethiopian and Dutch subjects.
[c] NA, not available.

ACKNOWLEDGMENTS

This study is a collaborative effort of the EHNRI, the Amsterdam Municipal Health Service, the Central Laboratory of The Netherlands Red Cross Blood Transfusion Service, and the Academic Medical Center of the University of Amsterdam. ENARP is financially supported by The Netherlands Ministry of Foreign Affairs and the Ethiopian Ministry of Health as a bilateral project.

We thank the study participants for their kind collaboration.

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