Immunohematological Reference Ranges for Adult Ethiopians

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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 leucocyte 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: leucocyte (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 |
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| 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)* | 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 |

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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 system 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 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 system 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.

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.

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

| Subject group (n) and parameter | Granulocyte count | Monocyte count | Lymphocyte count | CD4 T-cell count | CD8 T-cell count | CD3 T-cell ratio | 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      | 1.1          | 645          |
| Median                        | 2,775            | 324           | 1,801           | 733             | 645            | 1.1           | 1.1         | 645          |
| 95% range                     | 1,053–7,179      | 166–697       | 956–3,474       | 306–1,249       | 318–1,891      | 0.4–2.1       | 1.1         | 696–2,738    |
| Female (50)                   |                  |               |                 |                 |                 |                |             |              |
| Mean ± SD                     | 3,099 ± 1,287    | 314 ± 120     | 1,856 ± 522     | 816 ± 218       | 692 ± 269       | 1.3 ± 0.5      | 1.3         | 654          |
| Median                        | 3,093 (0.98³)    | 276 (0.05⁴)   | 1,701 (0.88⁵)   | 810 (0.17⁶)     | 632 (0.30⁷)     | 1.2 (0.03⁸)    | 1.2         | 1,483 (0.84⁹)|
| 95% range                     | 750–5,521        | 96–622        | 1,098–3,487     | 456–1,368       | 273–1,418       | 0.6–2.7       | 0.6         | 871–2,413    |

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³ Absolute counts were measured per microliter of whole blood.
⁴ 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 3. Means, medians, and 95th percentile reference ranges of WBC subset percentages for 142 HIV-negative adult Ethiopians**

| Subject group (n) and parameter | Granulocyte | Monocyte | Lymphocyte | CD4 T-cell | CD8 T-cell | CD3 T-cell | B-cell | NK cell |
|--------------------------------|-------------|----------|------------|------------|------------|-----------|--------|---------|
| Male (92)                      |             |          |            |            |            |           |        |         |
| Mean ± SD                      |             |          |            |            |            |           |        |         |
| Median                         |             |          |            |            |            |           |        |         |
| 95% range                      |             |          |            |            |            |           |        |         |
| Female (50)                    |             |          |            |            |            |           |        |         |
| Mean ± SD                      |             |          |            |            |            |           |        |         |
| Median                         |             |          |            |            |            |           |        |         |
| 95% range                      |             |          |            |            |            |           |        |         |

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² Absolute values are expressed as 10⁹/ml.
³ Absolute counts were measured per microliter of whole blood.
⁴ 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 \((3.0 \times 10^9/\text{liter to } 10.2 \times 10^9/\text{liter})\) and platelets \((98 \times 10^9/\text{liter to } 337 \times 10^9/\text{liter})\) 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 \((98 \times 10^9/\text{liter to } 337 \times 10^9/\text{liter})\) and WBC \((3.0 \times 10^9/\text{liter to } 10.2 \times 10^9/\text{liter})\) 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 \( \geq 2000 \text{ m} \), 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 5. Comparison of hemoglobin values with values from other studies in Africa (including Ethiopia)

| Gender of subjects | Present study Ethiopia (22) | Present study Ethiopia (1) | Present study 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)  | 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.

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

| 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            |
| 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 Ethiopians and Dutch subjects.

NA, not available.
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