

Reference Values of Lymphocyte Sub-Populations in Healthy Human Immunodeficiency Virus-Negative Iranian Adults

Atefeh Kamallou¹, Mahbobeh Haji Abdolbaghi², Minoos Mohraz², Mernaz Rasolinejad², Ehsan Karbasi¹, Bitas Ansaripour¹, Samaneh Soltani¹, Arezou Rezaei¹, Neda Khalili¹, Aliakbar Amirzargar^{1*}

¹Molecular Immunology Research Center and Department of Immunology, ²HIV Research Center and Department of Infectious Diseases, Imam Khomeini Hospital, Tehran University of Medical Sciences, Tehran, Iran

ABSTRACT

Background: Lymphocyte subsets enumeration is considered prominent in the management of primary and acquired immunodeficiency disorders. Because of local variations due to race, age, gender, and environmental conditions on lymphocyte subsets, and to improve the accuracy of interpretation of laboratory findings, reference intervals must be determined in every population. **Objective:** To establish a normal reference range for CD3⁺, CD4⁺, CD8⁺, CD19⁺ and CD56⁺ lymphocytes in a healthy Iranian adult population using flowcytometry. **Method:** Blood samples were collected from 221 HIV seronegative individuals, including 112 females and 109 males, with ages ranging from 20 to 40 years old. The percentage of lymphocytes expressing either of CD3, CD4, CD8, CD19 and CD56 surface markers were determined by flowcytometry assay. **Result:** Total mean percentage and absolute count of lymphocyte subsets were as follows: CD3⁺: 70.90 ± 7.54%, 1800.87 ± 471.09 cells/μl; CD4⁺: 41.04 ± 7.86%, 1039.99 ± 338.02 cells/μl; CD8⁺: 31.11 ± 6.60%, 783.95 ± 234.87 cells/μl; CD19⁺: 12.77 ± 4.56%, 328.37 ± 153.17 cells/μl; CD56⁺: 15.53 ± 6.34%, 388.62 ± 176.17 cells/μl, respectively. The ratio of CD4⁺/CD8⁺ lymphocytes for the studied population was 1.39 ± 0.48. Significant differences were observed between male and female subjects indicating that the average percentage of CD3⁺ cells (p=0.017) and CD4⁺ T cells (p=0.003) were higher in the female population, whereas the average percentage of CD19⁺ cells (p=0.02) tended to be higher among males. However, investigations on the CD56⁺ NK cell and CD8⁺ T cell sub-populations did not show any statistical differences between the two genders. In comparison with reports of other populations, we were confronted with different results. **Conclusion:** Establishing reference values of lymphocyte subsets for each population is helpful in achieving standard criteria for the prognosis of HIV infection. Therefore, normal ranges established by this survey can be used as a reference for decisions made in clinical practice.

Kamallou A, et al. Iran J Immunol. 2014; 11(4): 221-232

Keywords: HIV Seronegativity, Immunophenotyping, Lymphocyte Subsets, Reference Value

*Corresponding author: Dr. Aliakbar Amirzargar, Department of Immunology, Medical School, Tehran University of Medical Sciences, Tehran, Iran, Tel: (+) 98 21 88953009, Fax: (+) 98 2166422337, e-mail: amirzara@tums.ac.ir

INTRODUCTION

Immunohematological parameters are affected by several factors including genetics, age, gender, environment (e.g. nutrition, drug intake, smoking, dietary patterns), immunological disorders (such as autoimmune disorders, infection, lymphoma, leukemia, immunodeficiency) and so on. As the differentiation of these factors depends on the population and geographical region studied, racial differences and variations of immunohematological parameters exist worldwide. On the other hand, ethnic and racial differences are remarkable elements which influence the level of immune cells such as lymphocyte subsets. With this in mind, it is considerable that these parameters could be used as alternative markers for the assessments of immune status in various diseases (1). In this context, the notable example is Human Immunodeficiency Virus syndrome (HIV). The stated parameters could be used for the management of diagnosis and disease progression as well as decisions regarding prophylactic therapies against opportunistic infections and response to antiretroviral treatment in HIV infected patients (2). Attempting to understand the pathogenesis of HIV infection has prompted careful study of immune cells, demonstrating that the prominent changes in lymphocyte subpopulations including T cells, B cells and NK cells are associated with HIV infection (3-6). These lymphocyte subpopulations are distinguishable by their surface CD markers (clusters of differentiation) (7). B cells are defined by the expression of either CD19 or CD20; Natural Killer (NK) cells are determined by the presence of CD16 or/and CD56 and negativity for CD3; T cells are characterized as the cells expressing CD3 antigen and T cell subpopulations (Helper, Suppressor/Inducer) are identified by the coexpression of CD4 or CD8. With this background, it is beneficial to develop reference values of immunohematological parameters for each population individually in order to improve the quality of health care. Currently, flowcytometry is considered as the standard technology for quantification of lymphocyte subsets. Thus, lymphocyte immunophenotyping classified on the basis of their CD markers could be used as an important tool for the evaluation of immunological status (8). Therefore, this cross sectional study was performed to define normal reference ranges of both percentage and absolute count of peripheral blood lymphocyte subsets in HIV seronegative Iranian males and females with age of 20 to 40 years using flowcytometry. With regard to immunodeficiency syndromes such as HIV, the present reference ranges could be advantageous for diagnosis, evaluation of disease stage and progression, and therapeutic monitoring in Iranian individuals with HIV infection. Finally, we compared our results with other similar studies in order to point out the disparities of normal values of lymphocyte subsets in different populations.

MATERIALS AND METHODS

Subjects. Between December 2010 and December 2012, a total of 221 medical students of Tehran University of Medical Sciences and University-affiliated hospital employees were recruited to this study. Participants, who were comprised of 109 males and 112 females, were grouped into four age categories: 20-24 years, 25-29 years, 30-34 years and 35-40 years. HIV testing was done for all consenting individuals using the same kits and procedures. Subsequently, subjects who were seronegative for HIV-1 and had no history of smoking, alcohol consumption, and drug abuse were involved in our study.

The following exclusion criteria were considered: any sickness within the last month (including viral infection or parasitic infection); any chronic disease; any vaccination (within the last 4 weeks); any major surgery (within the last 6 months); pregnancy/lactation (within the last 6 months). The characteristics of individuals are listed in Table 1. The study was approved by the Ethics Committee of Tehran University of Medical Sciences and its related hospitals and all subjects gave an informed written consent prior to enter this study.

Sample Collection and HIV Serology. Two blood samples were drawn from each participant. One specimen was collected in a 5 ml anticoagulant-free tube for HIV testing and the second one in a 5 ml EDTA-containing tube for determination of hematological indices. In order to determine the seronegativity for HIV, serum samples were analyzed using enzyme-linked immunosorbent assay (Virinostika HIV Ag/Ab ELISA, BioMerieux, France) based on a one-step sandwich test. Specific Microelisa wells were coated with HIV-1 gp160, HIV-1 ANT70 peptide, HIV-2 env peptide (amino acids 592-603) and anti-HIV-1 p24. All samples were analyzed on the day of collection.

Hematological Analysis. A Hemacytometer (Sysmex KX21, Japan) device was used for the whole-blood analysis of hematological parameters. After automatic dilution of the whole-blood sample, lysis, and count the device gave a printout result of absolute numbers of leukocytes (WBC), platelets, neutrophils, eosinophils, monocytes, Bands (all expressed as number of cells*[10⁹]/lit), lymphocytes (expressed as number of cells*[10⁹]/lit and percentages), and hemoglobin level (gram per deciliter). Besides, ESR (mm/hour) was reported using the Westergren method.

Flowcytometric Analysis. Lymphocyte cells were analyzed using a FACSCalibur Flowcytometer (Becton Dickinson Immunocytometry Systems). Specimens were tested to determine the proportion of T lymphocytes identified by the expression of CD3, T-cell subpopulations (T Helper Cells and Cytotoxic T Cells) by the co-expression of CD4 or CD8, B lymphocytes by the expression of CD19 and Natural Killer (NK) lymphocytes by the positivity of CD16 and/or CD56 markers and lack of CD3 expression. This was performed by incubating anticoagulated whole blood with monoclonal antibodies (MAb) which were purchased from Becton Dickinson: CD3-FITC/CD19PE, CD4-FITC/CD8-PE and CD56-PE. Briefly; 100µl of whole blood was mixed with 10µl of the appropriate MAb combination and then incubated at room temperature for 20 mins in the dark. RBCs were then lysed by adding 1 ml of fluorescence activated cell sorter lysing solution (Becton Dickinson). After vortexing, tubes were incubated in the dark at room temperature for 10 mins and then centrifuged at 252 RCF of G for 5 min. The cell pellet was washed once with 1 ml of Facs Flow®, resuspended in 500 µl of Facs Flow, on forward and side scatter the lymphocytes were acquired, gated and then analyzed (Figure 1) using the Cell Quest Pro software (Becton Dickinson). Using cell blood count, absolute values of lymphocyte subsets were obtained.

Statistical Analysis. Statistical analysis was performed using SPSS software (version 14.0). One-sample Kolmogorov-Smirnov test was performed to find out whether the variables were normally distributed or not. Since the T cell subsets had a normal distribution, the T cell subset proportions and hematological parameters were compared between males and females using Student's *t-test*. The age-related differences were also analyzed by ANOVA test. For each immunohematological parameter the average value

and standard deviation (SD) was calculated and expressed as the mean \pm SD. Results with a p value less than 0.05 were considered statistically significant.

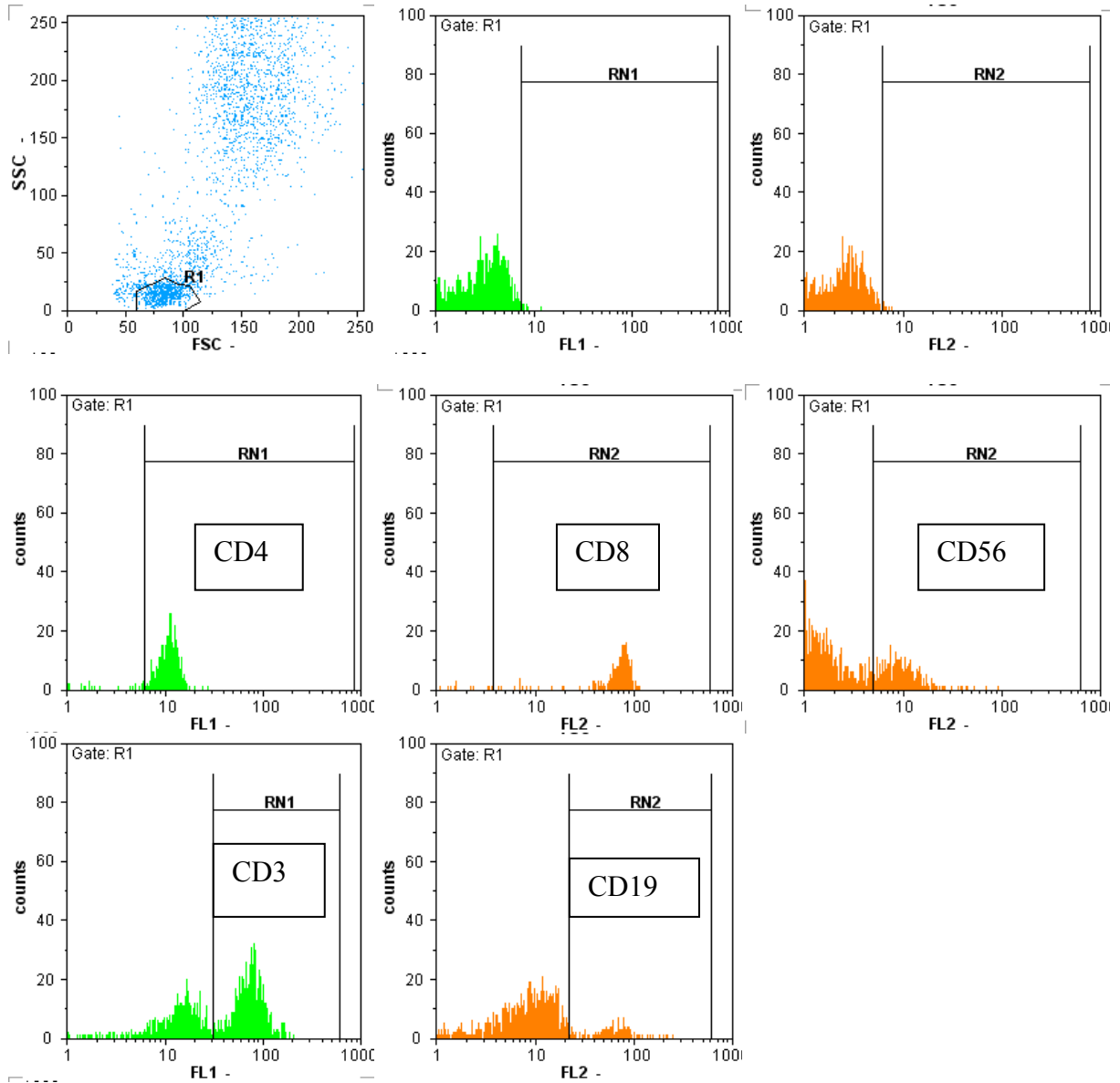


Figure 1. Example of CD3, CD4, CD8, CD19 and CD56 analysis FITC-RPE negative control.

RESULTS

Characteristics of Studied Subjects. Blood samples from a total of 221 healthy Iranians, including 109 males (49.3%) and 112 females, (50.7%) were collected and screened for HIV infection. Afterwards, the effect of age and gender of the HIV seronegative participants on immunohematological indices was survived by categorizing each gender group into four age subgroups including 20-24 years, 25-29 years, 30-34 years and 35-40 years. Using immunophenotyping, specimens were tested for the proportion of lymphocyte subsets comprised of CD3⁺ T cells, sub-populations of T cells (CD4⁺ T helper and CD8⁺ T suppressor/inducer), CD19⁺ B cells and CD56⁺ NK cells, considering sex and age group. Distribution in each age group according to gender is presented in Table 1. The mean age range for females and males was 29.07 ± 6.1 and 28.74 ± 6.1 , respectively.

Table 1. Age distribution of subjects for both genders according to age groups.

Gender	Male		Female		Total	
	No.	Mean \pm SD	No.	Mean \pm SD	No.	Mean \pm SD
20-24 years	32	22.28 \pm 1.67	33	21.87 \pm 1.49	65	22.07 \pm 1.58
25-29 years	28	26.85 \pm 1.62	29	25.55 \pm 0.68	57	26.19 \pm 1.39
30-34 years	27	31.59 \pm 1.27	26	30.84 \pm 1.25	53	31.22 \pm 1.31
35-40 years	22	38.68 \pm 1.61	24	38.25 \pm 2.0	46	38.45 \pm 1.84
Total	109	29.07 \pm 6.1	112	28.41 \pm 6.2	221	28.74 \pm 6.1

Hematological Analysis in HIV-Seronegative Iranian Subjects. The results of the hematological parameters according to gender were as follows: Platelets: 242.83 ± 52.79 (females) and 218.92 ± 43.01 cells/ μ l (males); Neutrophils: 3588.79 ± 971.07 (females) and 3619.18 ± 956.87 (males) cells/ μ l; Monocytes: 291.098214 ± 93.417494 (females) and $313.899083 \pm 108.195805$; Band: 44.348214 ± 61.918387 (females) and 54.541284 ± 78.765655 (males); Eosinophil: $136.696429 \pm 130.089771$ (females) and $172.862385 \pm 120.142159$ (males); hemoglobin: 13.10 ± 1.12 (females) and 14.68 ± 1.31 (males) g/dl; ESR: 8.16 ± 5.35 (females) and 5.66 ± 3.25 (males) mm/hr. According to this gender-related data it was confirmed that the average Platelet count ($p=0.02$) and ESR ($p=0.01$) were higher in female subjects, whereas the average count for Eosinophil ($p=0.03$) and hemoglobin level ($p=0.05$) were statistically higher in males. None of the other studied hematological indices represented a significant difference between the two genders ($p>0.05$).

The mean value of total lymphocyte count among different age groups is given in Table 2. As a result, no gender-specific difference was observed for total lymphocyte enumeration ($p>0.05$). Moreover, no apparent age-dependent differences for lymphocyte subpopulation were noted in the data.

Table 2. The mean \pm SD of lymphocyte counts for both genders among age groups.

Gender	Lymphocyte count		
	Female	Male	P-value
Age Group	Mean \pm SD	Mean \pm SD	P-value
20-24 years	2530.06 \pm 663.45	2493.71 \pm 622.46	NS
25-29 years	2414.00 \pm 615.79	2572.64 \pm 599.54	NS
30-34 years	2660.11 \pm 572.90	2508.29 \pm 529.92	NS
35-40 years	2482.87 \pm 617.49	2694.22 \pm 623.48	NS
Total	2520.08 \pm 619.14	2558.07 \pm 591.56	NS

NS: non-significant

Immunophenotyping of Lymphocyte Sub-Populations in HIV-Negative Iranian Subjects. Table 3 displays the average of absolute counts and percentages of lymphocyte subsets in 112 female and 109 male subjects. Regarding gender-specific comparison, the mean percentages of CD3⁺ T cells and CD4⁺ T cells were significantly higher in females but not supported by absolute values, whereas the mean percentage of CD19⁺ B cells tended to be higher in males.

Table 3. The mean \pm SD percentages and absolute values of lymphocyte subsets in 112 female and 109 male healthy adults.

Gender	Female (112)		Male (109)		Total
	Mean \pm SD	Mean \pm SD	P-value	Mean \pm SD	
CD3 ⁺ T cells	Absolute value	1816.73 \pm 482.21	1784.57 \pm 461.4	NS	1800.87 \pm 471.09
	%	72.09 \pm 7.54	69.68 \pm 7.37	0.017*	70.90 \pm 7.54
CD4 ⁺ T cells	Absolute value	1072.05 \pm 384.90	1007.05 \pm 279.85	NS	1039.99 \pm 338.02
	%	42.56 \pm 8.69	39.48 \pm 6.59	0.003*	41.04 \pm 7.86
CD8 ⁺ T cells	Absolute value	777.42 \pm 223.77	790.67 \pm 246.61	NS	783.95 \pm 234.87
	%	31.20 \pm 6.51	31.01 \pm 6.72	NS	31.11 \pm 6.60
CD19 ⁺ B cells	Absolute value	309.91 \pm 143.99	347.33 \pm 160.51	NS	328.37 \pm 153.17
	%	12.11 \pm 3.89	13.45 \pm 5.08	0.02*	12.77 \pm 4.56
CD56 ⁺ NK cells	Absolute value	387.57 \pm 183.30	389.69 \pm 169.37	NS	388.62 \pm 176.17
	%	15.64 \pm 6.80	15.42 \pm 5.87	NS	15.53 \pm 6.34
CD4 ⁺ /CD8 ⁺ ratio		1.44 \pm 0.53	1.35 \pm 0.42	NS	1.39 \pm 0.48

NS:non-significant

Table 4. The mean \pm SD percentages and absolute values of lymphocyte subsets according to age groups.

	20-24 years		25-29 years		30-34 years		35-40 years	
	Female	Male	Female	Male	Female	Male	Female	Male
	Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD
CD3 [#]	1805.92 \pm 547.83	1706.16 \pm 455.24	1763.12 \pm 438.74	1814.50 \pm 466.42	1890.22 \pm 485.36	1768.34 \pm 431.34	1816.75 \pm 452.03	1880.46 \pm 506.60
%	70.96 \pm 7.12	68.62 \pm 8.38	73.31 \pm 5.66	70.48 \pm 6.92	70.90 \pm 9.96	70.17 \pm 6.15	73.47 \pm 7.02	69.63 \pm 8.02
CD4 [#]	1000.24 \pm 450.96	999.50 \pm 304.34	1086.36 \pm 315.36	967.51 \pm 259.14	1160.91 \pm 349.86	990.95 \pm 286.07	1057.21 \pm 401.37	1088.12 \pm 262.68
%	40.07 \pm 8.71	39.98 \pm 6.14	45.32* \pm 8.45	37.69 \pm 5.35	43.14 \pm 7.46	39.36 \pm 7.07	42.01 \pm 9.62	41.16 \pm 7.82
CD8 [#]	826.75 \pm 230.50	744.43 \pm 220.80	755.30 \pm 199.12	858.36 \pm 266.79	754.80 \pm 233.19	803.57 \pm 238.23	760.80 \pm 235.35	755.94 \pm 261.11
%	33.07* \pm 5.67	29.98 \pm 6.03	31.90 \pm 6.72	33.48 \pm 6.63	28.49 \pm 6.7	32.05 \pm 6.83	30.73 \pm 6.48	28.07 \pm 6.64
CD19 [#]	311.19 \pm 126.98	354.04 \pm 182.69	315.98 \pm 161.60	353.26 \pm 145.18	328.30 \pm 159.14	315.09 \pm 158.50	280.92* \pm 130.39	369.59 \pm 151.83
%	12.18 \pm 3.23	13.97 \pm 5.6	12.67 \pm 4.13	13.72 \pm 4.96	12.26 \pm 4.51	12.31 \pm 5.23	11.17* \pm 3.79	13.75 \pm 4.32
CD56 [#]	429.29 \pm 146.56	359.09 \pm 175.08	340.52 \pm 132.37	422.42 \pm 178.42	375.90 \pm 197.96	406.04 \pm 124.17	399.68 \pm 250.49	372.49 \pm 196.85
%	17.58* \pm 5.79	14.41 \pm 5.77	14.56 \pm 5.92	16.62 \pm 5.95	14.30 \pm 7.58	16.60 \pm 5.42	15.71 \pm 7.92	13.93 \pm 6.2
CD4/CD8 ratio	1.22 \pm 0.45	1.38 \pm 0.34	1.51* \pm 0.55	1.19 \pm 0.39	1.63* \pm 0.61	1.31 \pm 0.46	1.43 \pm 0.44	1.55 \pm 0.47

*: P-value less than 0.05

#: Absolute value,

However, no significant differences in the average percentages and absolute counts of other lymphocyte subsets (CD8⁺ T cells and NK cells) were observed ($p>0.05$). Similarly, no statistically significant difference was noted for the mean value of CD4/CD8 ratio between the two genders ($p>0.05$).

Table 4 represents a comparison between the average percentages and absolute values of lymphocyte sub-populations for each studied age group. According to age-group comparisons between the two genders, some parameters were higher in female subjects including: the average percentage of CD8⁺ T cells ($p=0.037$) and CD56⁺ NK cells ($p=0.031$) in 20-24 years age group; mean percentage of CD4⁺ T cells ($p=0.00$) in 25-29 years age group. Absolute value and average percentage of CD19⁺ B cells ($p=0.04$) in 35-40 years age group was higher in males in comparison to females. In addition, the mean value of CD4⁺/CD8⁺ ratio in the 25-29 years ($p=0.015$) and 30-34 years ($p=0.037$) age groups was higher in females compared to males. According to the population's age classification, our results did not show any significant variation of lymphocyte subsets among the considered age groups.

DISCUSSION

This study aimed to establish age and gender specific immunohematological reference values which may be considered as the Iranian standard for the follow-up of HIV-positive patients. Generally, analysis of lymphocyte subsets is a crucial aspect in the management of patients with HIV. With more details, the importance of establishing absolute counts and percentage values of lymphocyte subsets and, especially, the ratio of CD4⁺/CD8⁺ T cells is considerable in manipulating the progression of HIV infection, guiding the initiation of treatment, and monitoring responses to antiviral therapy. Region-specific reference values of peripheral lymphocyte subtypes have been determined by several studies throughout the world and have exhibited some discrepancies based on geographical region and methodology. Additionally, it is suggested that these variations could also be attributed to ethnical diversity, genetics, environmental factors such as nutrition, climate, smoking, exposure to different antigens and methods of enumeration. The accurate interpretation of immunophenotyping data, including T cell sub-populations' counts, is reliant on age-specific lymphocyte subsets' reference values of healthy subjects in the relevant population (9,10,11).

Therefore, we have enumerated lymphocyte subsets in our population using venous blood obtained from 221 healthy HIV-negative volunteers stratified by age, ranging from 20 to 40 years. It is evident that the results are not creditable for children and the elderly. Immunophenotyping by flowcytometry was used for the evaluation of lymphocyte subsets including T, B and NK cells.

Based on our hematological results, it was shown that both average platelet count ($p=0.00$) and rate of ESR ($p=0.00$) were increased in females. Nevertheless, the average eosinophil count ($p=0.03$) and hemoglobin level ($p=0.00$) were statistically higher in males. Besides, with respect to age-dependent changes, differences among groups were not worthy of reflection. Similarly, no age-related differences between males and females were identified for total lymphocyte count among the four studied age groups ($p>0.05$).

With respect to gender-specific differences in lymphocyte subsets, Iranian females were found to have significantly higher percentages of CD3⁺ T and CD4⁺ T cells, whereas the

percentage of CD19⁺ B cells was higher in males. However, comparison of absolute values failed to show any statistically significant difference for the mentioned lymphocyte subsets ($p>0.05$). The mean percentage and absolute values of NK cells and CD8⁺ T cell subsets were also found to be gender-independent ($p>0.05$). We observed no differences in the mean CD4⁺/CD8⁺ ratio between males and females ($p>0.05$). One possible explanation for the observed gender differences in the average percentage of CD4⁺, CD3⁺ and CD19⁺ cells, is that it may be secondary to the differential effects of sex hormones (12,13).

Considering the studied age groups, we identified the average percentage and absolute count of each sub-type among four groups in each sex. As a summary, in the 20-24 years age group: the average percentage of CD8⁺ T cells ($p=0.037$) and the average percentage of CD56⁺ NK cells ($p=0.031$); in the 25-29 years age group: the average percentage of CD4⁺ T cells ($p=0.00$) were higher in females than males and in the 35-40 years age group: the absolute value and average percentage of CD19⁺ B cells ($p=0.04$) were higher in males than female subjects. Also mean percentage of CD4⁺/CD8⁺ ratio, the so-called helper/suppressor ratio, for the 25-29 years ($p=0.015$) and the 30-34 years age groups ($p=0.037$) was higher in females compared to males.

As a whole, our investigated immunohematological parameters did not increase or decrease in ages between 20 to 40. The influence of age on lymphocyte sub-populations is not yet well established, since different studies report different results; various studies have illustrated that the number of CD4⁺ T cells increases, whereas the number of CD8⁺ T cells decreases with age (13), while others have demonstrated that the number of both CD4⁺ and CD8⁺ T cells either increase (12,15) or decrease (16) with age, or do not change at all (17,18).

Comparison of immunophenotyping data between Asian and non-Asian populations has indicated that the Asian population tend to have lower levels of CD3⁺ and CD4⁺ T cells and CD4⁺/CD8⁺ ratio (19).

Comparing our data with previous studies in other regions of Asia, we point out some differences as depicted in Table 5 (20-25).

Comparisons of T and B lymphocytes among different adult population in Iran, Turkey, Kuwait and China did not show any significant difference. We observed higher levels for the mean percentage of CD3⁺, CD4⁺ and CD8⁺ T cells and lower values for the mean percentage of CD19⁺ T cells than the previously mentioned report in Iran (19,20). Another noticeable finding was a lower mean CD4⁺/CD8⁺ ratio in our study compared to the pervious study. This was reflected by a higher CD8⁺ count in the population of our study. Although our current study showed that the absolute number of CD3⁺ T lymphocytes was 1800/mm³, which was much higher than the reported values in previous studies, the difference was insignificant. Indeed, the number of B lymphocytes in this study was quite similar to previous studies done in Iran and Kuwait, which were all insignificantly higher than of those studies in Turkey and China. Methodological features, environmental factors (smoking, poor nutrition, infection), and genetics could also contribute to the differences revealed among these populations.

In conclusion, this investigation leads to the establishment of gender-related reference ranges of immunohematological parameters for the Iranian population with 20 to 40 years of age. Since the age of our study population is between 20 to 40 years, which is considered to be a narrow age-range, it would be difficult to come to a definitive conclusion about the impact of age on lymphocyte subsets. Therefore, we recommend recruiting volunteers of a wider age range for future studies.

Table 5. Comparison of T-lymphocyte subsets in different populations.

Population Year of study	CD3 count	CD3(%)	CD4 count	CD4(%)	CD8 count	CD8(%)	CD19 count	CD19(%)	CD56 count	CD56(%)	Ratio
Our study	1800.87 ± 471	70.9 ± 7.54	1039.99 ± 338.02	41.04 ± 7.86	783.95 ± 234.87	31.11 ± 6.60	328.37 ± 153.17	12.7 ± 4.56	388.62 ± 176.17	15.53 ± 6.34	1.39 ± 0.48
Iran 2011(19)	1504 ± 505	67.66 ± 7.76	827 ± 313	39.22 ± 6.7	522 ± 185	25.42 ± 5.4	332 ± 186	14.41 ± 5.09	NA*	NA*	1.6 ± 0.46
Malay 1993(20)	NA*	69.14 ± 10.30	NA*	34.14 ± 8.41	NA*	36.23 ± 9.96	NA*	11.14 ± 3.62	NA*	NA*	1.07 ± 0.43
India 2004(21)	NA*	68.65 ± 8.24	NA*	37.10 ± 7.83	NA*	34.04 ± 8.79	NA*	14.67 ± 5.42	NA*	12.44 ± 7.55	1.16 ± 0.41
China 1996(22)	1370 ± 400	69.0 ± 7.7	725 ± 256	36.4 ± 7.5	589 ± 205	29.7 ± 7.2	221 ± 108	11.1 ± 3.9	394 ± 194	19.8 ± 8.1	1.0 ± 0.4
Turkey 2005 (23)	1680 ± 528	72.70 ± 8.44	1095 ± 391	47.37 ± 9.10	669 ± 239	28.99 ± 5.99	254 ± 122	10.96 ± 4.44	161 ± 92	7.03 ± 3.26	1.68 ± 0.43
Kuwait 2002 (24)	1770 ± 470	NA*	1050 ± 300	NA*	730 ± 220	NA*	330 ± 140	NA*	320 ± 130	NA*	1.50 ± 0.35

*NA= Not available

Nevertheless, these reference ranges are applicable for immunodeficient patients such as HIV-positive patients in the mentioned age range and also individuals who have a high risk of HIV infection.

It should be kept in mind that these results may be reliable for the follow-up of HIV-positive patients as well as for a variety of diseases that involve alterations in lymphocyte sub-populations such as lymphoma, leukemia, etc.

ACKNOWLEDGEMENTS

This research (student thesis for MD graduation) was financially supported by the research deputy of Tehran University of Medical Sciences.

REFERENCES

1. Chng WJ, Tan GB, Kuperan P. Establishment of adult peripheral blood lymphocyte subset reference range for an Asian population by single-platform flow cytometry: influence of age, sex, and race and comparison with other published studies. *Clinical and diagnostic laboratory immunology*. 2004; 11:168-73.
2. Fahey JL, Taylor JM, Detels R, Hofmann B, Melmed R, Nishanian P, et al. The prognostic value of cellular and serologic markers in infection with human immunodeficiency virus type 1. *N Engl J Med*. 1990; 322:166-72.
3. Giorgi JV, Detels R. T-cell subset alterations in HIV-infected homosexual men: NIAID Multicenter AIDS cohort study. *Clin Immunol Immunopathol*. 1989; 52:10-8.
4. Streeck H, Nixon DF. T cell immunity in acute HIV-1 infection. *J Infect Dis*. 2010; 202(Supplement 2):S302-S8.
5. Ibarondo F, Wilson S, Hultin L, Shih R, Hausner M, Hultin P, et al. Preferential depletion of gut CD4-expressing iNKT cells contributes to systemic immune activation in HIV-1 infection. *Mucosal immunol*. 2013; 6:591-600.
6. Malaspina A, Moir S, Kottlilil S, Hallahan CW, Ehler LA, Liu S, et al. Deleterious effect of HIV-1 plasma viremia on B cell costimulatory function. *J Immunol*. 2003; 170:5965-72.
7. Zola H, Swart B, Nicholson I, Aasted B, Bensussan A, Boumsell L, et al. CD molecules 2005: human cell differentiation molecules. *Blood*. 2005; 106:3123-6.
8. Hannel I, Erkeller-Yuksel F, Lydyard P, Deneys V, DeBruyère M. Developmental and maturational changes in human blood lymphocyte subpopulations. *Immunol today*. 1992; 13:215-8.
9. Das BR, Bhanushali AA, Khadapkar R, Jeswani KD, Bhavsar M, Dasgupta A. Reference ranges for lymphocyte subsets in adults from western India: influence of sex, age and method of enumeration. *Indian journal of medical sciences*. 2008; 62:397-406.
10. Maini M, Gilson R, Chavda N, Gill S, Fakoya A, Ross E, et al. Reference ranges and sources of variability of CD4 counts in HIV-seronegative women and men. *Genitourin med*. 1996; 72:27-31.
11. Raheleh Shokouhi Shoormasti, Anahita Azimdoost Shiva Saghafi, Masood Movahhedi, et al. Normal Range Determination of Lymphocytes Subsets in Normal Adults in Iran. *Iran J Allergy Asthma Immunol* December. 2011; 10: 295-8.
12. Bartlett JA, Schleifer SJ, Demetrikopoulos MK, Delaney BR, Shiflett SC, Keller SE. Immune function in healthy adolescents. *Clin Diagn Lab Immunol*. 1998; 5:105-13.
13. Rudy BJ, Wilson CM, Durako S, Moscicki A-B, Muenz L, Douglas SD. Peripheral blood lymphocyte subsets in adolescents: a longitudinal analysis from the REACH project. *Clinical and diagnostic laboratory immunology*. 2002; 9:959-65.
14. Tollerud DJ, Ildstad ST, Brown LM, Clark JW, Blattner WA, Mann DL, et al. T-cell subsets in healthy teenagers: transition to the adult phenotype. *Clin Immunol Immunopathol*. 1990; 56:88-96.
15. Wiener D, Rowlands DT, Lowell N, Shah S, Malone J, Lowitt S. Multiparametric analysis of peripheral blood in the normal pediatric population by flow cytometry. *Journal of clinical laboratory analysis*. 1990; 4:175-9.

16. Sansoni P, Cossarizza A, Brianti V, Fagnoni F, Snelli G, Monti D, et al. Lymphocyte subsets and natural killer cell activity in healthy old people and centenarians [see comments]. *Blood*. 1993; 82:2767-73.
17. Stulnig T, Maczek C, Böck G, Majdic O, Wick G. Reference intervals for human peripheral blood lymphocyte subpopulations from 'healthy' young and aged subjects. *International archives of allergy and immunology*. 1995;108:205-10.
18. Uppal S, Verma S, Dhot P. Normal values of CD4 and CD8 lymphocyte subsets in healthy Indian adults and the effects of sex, age, ethnicity, and smoking. *Cytometry B Clin Cytom*. 2003;52:32-6.
19. Howard RR, Fasano CS, Frey L, Miller CH. Reference intervals of CD3, CD4, CD8, CD4/CD8, and absolute CD4 values in asian and non-asian populations. *Cytometry*. 1996; 26:231-2.
20. Shoormasti R, Saghafi S, Ashtiani MTH, Eslami MB. Normal range determination of lymphocytes subsets in normal adults in Iran. *Iran J Allergy Asthma Immunol*. 2011; 10:259-8.
21. Chin SF, Cheong SK, Lim YC, Ton SH. The distribution of immunoregulatory cells in the peripheral blood of normal Malaysian adults. *Malays J Pathol*. 1993; 15:49-52.
22. Saxena R, Choudhry V, Nath I, Das S, Paranjape R, Babu G, et al. Normal ranges of some select lymphocyte sub-populations in peripheral blood of normal healthy Indians. *CURRENT SCIENCE-BANGALORE*. 2004; 86:969-74.
23. Kam KM, Leung WL, Kwok MY, Hung MY, Lee SS, Mak WP. Lymphocyte subpopulation reference ranges for monitoring human immunodeficiency virus-infected Chinese adults. *Clin Diagn Lab Immunol*. 1996; 3:326-30.
24. Yaman A, n u, Cetiner S, Kibar F, Taşova Y, Şeydaoğlu G, et al. Reference ranges of lymphocyte subsets of healthy adults in Turkey. *Med Princ Pract*. 2005; 14:189-93.
25. Kaaba SA, Al Fadhli S, Khamis A. Reference values of lymphocyte subsets in the normal healthy adult Kuwaiti Arab population. *Immunol lett*. 2002; 81:199-203.