

Normal Range Determination of Lymphocytes Subsets in Normal Adults in Iran

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ABSTRACT

Immunophenotyping of lymphocytes is very essential for evaluation of immune system. Due to the effect of environmental factors and ethnical diversity on immune system, establishment of an internal normal range of lymphocyte subsets is a necessity for each population. The aim of this study was to determine the normal range of T and B lymphocytes, and NK cells in normal Iranian adults.

Two hundred and thirty three Iranian normal adult volunteers took part in this study. Complete Blood Count (CBC) was performed for them with Sysmex (KX21) and cells with CD3, CD4, CD8, CD19 and CD16/56 surface markers were simultaneously detected by flow cytometry method with FACstar system. Their percentile and absolute count were determined.

The volunteers were 150 male and 83 female. Mean percentages of lymphocyte subpopulation were: CD3 (67.66 ± 7.76), CD19 (14.41 ± 5.09), CD4 (39.22 ± 6.7), CD8 (25.42 ± 5.4) and CD16/56 (10.14 ± 6.42). Also, their mean absolute count of lymphocyte bearing CD3, CD19, CD4 and CD8 were $1,504 \pm 505/\mu\text{l}$, $332 \pm 186/\mu\text{l}$, $827 \pm 313/\mu\text{l}$ and $522 \pm 185/\mu\text{l}$, respectively.

Our results are comparable with similar Asian results from other Asian population, but are different from European population, we therefore conclude that it is necessary for each laboratory to establish an internal normal range for the lymphocytes bearing above-mentioned markers.

Keywords: Immunophenotyping; CD Marker; Flow Cytometry; Lymphocyte; Normal Range; NK cell

INTRODUCTION

A reliable and accurate system for quantitative determination of cells or cellular components with high

speed is flow cytometry.¹ It is an acceptable technique for the diagnosis and monitoring of blood components of patients with immunodeficiency syndromes, leukemia, lymphoma disorders and evaluation of immune status.²⁻⁴

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The application of monoclonal antibodies and flow cytometry in clinical laboratory has been considerable, especially using immunophenotyping of various samples including blood and bone marrow.^{3,5} Immunophenotyping of lymphocytes is performed with the use of a number of fluorescent conjugated monoclonal antibodies that are made specifically versus surface and intracellular antigens of lymphocytes.⁶

The results from different countries not only indicate the presence of variation in quantities of lymphocytes and their subsets but also show that reference ranges obtained from studies in one population are not applicable for another population although similar methods were used.⁷ These reference ranges are crucial for the establishment of the precise diagnosis and prognosis. It is suggested that different factors such as environmental factors (infections, smoking and nutrition), sex, age and race may account for the variation between populations in lymphocyte subsets.^{4,8} The objective of the present study was to establish reference values for lymphocyte subsets in Iranian normal adults.

SUBJECTS, MATERIALS AND METHODS

Two hundred and thirty three healthy adult volunteers (20-45yrs) took part in this study during a 5 years period (2000- 2005). They were 150 males and 83 females. Following taking history and demographic data via interview, informed consent was obtained. All participants did not have any acute illness or acute infection that required medications and women were

not pregnant at the time of study.⁹ Furthermore, no smoker was included.

Blood Cell Count and Flow Cytometry Analysis

One milliliter of whole blood sample was collected in tubes containing EDTA for conducting Complete Blood Count (CBC) which was performed for each person with Sysmex (KX21). Also, one milliliter of whole blood was collected in heparinized tubes. A standardized method was used to determine the expression of CD3, CD19, CD4, CD8 and CD16/56 markers on lymphocytes.¹⁰ Ten μ l of each monoclonal antibody (purchased from Becton Dickinson Immunocytometry Systems, San Jose, CA, USA) was added to 100 μ l of prepared whole blood in the test tubes. After vortexing, tubes were incubated for 20 min at room temperature (RT) in darkness. Two ml of lysing solution (Becton Dickinson Immunocytometry systems, San Jose, CA, USA) was added and the mixture was vortexed and was incubated for 10 min at RT in darkness. The mixture centrifuged for 1 min at RT and the supernatant was aspirated. Two ml of PBS was added to resuspend the pellet and was centrifuged for 1 min at RT and supernatant was aspirated again. Next, five hundred μ l of staining buffer was added and then cells were analyzed by FACStar flow cytometer (Becton Dickinson Immunocytometry Systems, San Jose, CA, USA). The flow cytometer was calibrated using CaliBrite beads (Becton Dickinson Immunocytometry Systems, San Jose, CA, USA) using Lysis II software. Data were analyzed by SPSS. Mean, 10-90% percentile and absolute count were calculated for each marker.

Table 1. The mean \pm SD, means \pm 2SD, 95% Confidence and absolute counts of lymphocyte subsets in Iranian adult population in Tehran

	WBC	Lymphocyte	CD3	CD19	CD4	CD8	CD16/56	CD4/CD8 Ratio
Mean \pm SD			67.66 \pm 7.76	14.41 \pm 5.09	39.22 \pm 6.7	25.42 \pm 5.4	10.14 \pm 6.42	1.61 \pm 0.46
Mean \pm 2SD			67.66 \pm 15.52	14.41 \pm 10.18	39.22 \pm 13.4	25.42 \pm 10.8	10.14 \pm 12.84	1.61 \pm 0.92
95% Confidence			[66.66, 68.67]	[13.75, 15.07]	[37.51, 40.92]	[24.06, 26.79]	[8.09, 12.20]	
Absolute counts*	6503 \pm 3282/ μ l	2256 \pm 692/ μ l	1504 \pm 505/ μ l	332 \pm 186/ μ l	827 \pm 313/ μ l	522 \pm 185/ μ l	248 \pm 141/ μ l	
Adult's percentage ¹ (%)			57.94-77.56	8.80-21.64	30.45-50.13	18.92-33.64	3.15-17.36	
Adults Absolute Count ²	4600-8300	1344-3212	854-2232	159-568	410-1257	250-788	81-377	

*Number /per microliter

¹ Normal values 10 -90% percentile for percentage

² Normal values 10 -90% percentile for absolute count

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Table 2. The mean \pm SD, means \pm 2SD, 95% Confidence and absolute counts of lymphocyte subsets in 233 normal adults in two groups, female and males participants

	CD3	CD19	CD4	CD8	CD16/56
Male(150)					
Mean \pm SD	67.63 \pm 7.74	14.50 \pm 5.17	38.49 \pm 6.7	24.94 \pm 5.2	9.91 \pm 7.08
Mean \pm 2SD	67.63 \pm 15.48	14.50 \pm 10.34	38.49 \pm 13.4	24.94 \pm 10.4	9.91 \pm 14.16
95%Confidence	[64.81, 70.14]	[10.99, 13.51]	[36.20, 40.76]	[23.16, 26.73]	[7.27, 12.55]
Absolute Count	1515 \pm 522/ μ l	341 \pm 206/ μ l	785 \pm 277/ μ l	497 \pm 188/ μ l	234 \pm 131/ μ l
Female(83)					
Mean \pm SD	67.73 \pm 7.23	14.25 \pm 4.95	40.19 \pm 6.8	26.06 \pm 5.6	10.83 \pm 4.06
Mean \pm 2SD	67.73 \pm 14.46	14.25 \pm 9.9	40.19 \pm 13.6	26.06 \pm 11.2	10.83 \pm 8.12
95%Confidence	[62.65, 67.63]	[10.75, 16.90]	[37.48, 42.91]	[23.83, 28.30]	[7.92, 13.74]
Absolute Count	1484 \pm 476/ μ l	314 \pm 144/ μ l	876 \pm 350/ μ l	551 \pm 180/ μ l	287 \pm 167/ μ l
P value	NS	NS	NS	NS	NS

P values were calculated with t test for comparison of mean for male and female subjects.

NS: Not Significant

RESULTS

The percentage and the result of absolute counts of the different lymphocyte subsets were presented in table 1 and the results of percentage and absolute counts of lymphocytes subsets in male and female adults separately are presented in table 2

There was no significant difference (p value > 0.05) between absolute counts of CD markers in male and female adults.

DISCUSSION

The aim of the present study was to establish normal range of T and B lymphocytes and NK cells in normal adults in Iran which may be used as normal adult standard values.

Immunophenotyping of T cell, helper and cytotoxic T cell, B cell and NK cell were determined by expression of CD3(the protein tyrosin kinase-linked T-cell receptor), CD4 , CD8, CD19 (protein tyrosine kinase-linked receptors) and CD16/56 , respectively.¹¹ The interpretation of flow cytometric immunophenotyping depends on comparison of patient information to established reference values from normal persons.¹²

Infections and poor nutrition could also be as possible reasons of low CD4 T cell numbers. Practical aspects, environmental and genetic constitute could also influence outcomes.⁴ Also, it is obvious that inter laboratory difference may be considerably reduced

during utilization of both lymphocyte-gating and lyse-no-wash procedures.¹¹

Our data provide Iranian-specific reference values for lymphocytes. Howard et al study found that Asian donors had lower mean percentages for CD3, CD4 and absolute CD4 lymphocytes than other population.¹³ Also, in our study, mean percentage and absolute count for CD4 were lower than Dutch⁸ and Turkish.¹⁴ The difference between the results obtained from our population with other studies, indicate that it is necessary for each country to establish an internal normal range for these markers. No study has been performed in this area in Iran until now and unfortunately all related performed studies also are not new in other parts of world.

Different studies have showed diversity in reference range for lymphocyte subsets with regard to gender, age, race and other factors.¹⁵

A demographic issue that associated with variations in lymphocyte subsets is gender.¹⁶ In our study, females had slight increase of CD4+ T lymphocyte absolute counts than in males but it was not significant. According to the study of Jentsch-Ullrich K, it could be due to acceleration of thymocyte apoptosis by androgens in males and it's effect on profile of peripheral T cell collection.⁴ As Rudy BJ stated, variation in immune cell counts may be due to gender and sex hormones. Sex hormones can affect the immune cell population in the indirect pathways such as thymic pathways.¹⁶

In this study, we noticed no effect of age on the T-cells subsets because we had subjects in limited age range (20- 45y) and we did not study subjects >50y but results of another study showed a statistically significant decrease in CD3, CD8 T cells and B cell counts and an increase in NK cell numbers and CD4/CD8 ratio over 50 years.⁴ Therefore, it is necessary to determine these reference values in adults>50 and children in Iranian population and adolescence because of the exceptional duration of development, differentiated by intense physiologic and psychological alterations.¹⁶

In conclusion, this study determined normal range of T and B lymphocytes and NK cells in healthy adults in Iran that may be useful in interpretation of laboratory and clinical findings. In the end, our study was limited by its small sample size. Although our study was in a referral center and our population was from different cities of Iran but the lack of samples throughout Iran remain another limitation of this study.

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