Reference ranges for lymphocyte subsets in healthy adult male Omanis

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ABSTRACT

الهدف: يهدف البحث الحالي إلى قياس المعدلات الطبيعية للخلايا الليمفاوية بالنسبة للأفراد الذكور الأصحاء من العمانيين.

الطريقة: تم دراسة عدد 118 من الذكور المتبرعين بالدم من الفئة العمرية بين 18 إلى 51 سنة ومعدل العمر لدى المجموعة التي درست هو 25 سنة. تم الحصول على عينات من الدم من أفراد الفئة المذكورة و تم قياس المعدلات الطبيعية للخلايا الليمفاوية باستخدام جهازمعداد الخلايا. تم انجاز هذا البحث خلال عام 2006 في مختبرات المناعة في كلية الطب و العلوم الصحية– جامعة السلطان قابوس.

النتائج: أشارت الدراسة إلى أن المعدلات الطبيعية للخلايا الليمفاوية للعمانيين الذكور هي:
CD3: 68.53±7.5%، 1701±489cells/µl؛ CD4: 40.4±6.5%، 1006±319 cells/µl؛ CD8: 25.8±5.9%، 638±225 cells/µl؛ CD19: 13.7±4.7%، 349±158cells/µl and CD56: 12.2±6.7%، 308±204 cells/µl.

خاتمة: تم الحصول على المعدلات الطبيعية للخلايا الليمفاوية للعمانيين الذكورالاصحاء ووجد أن هذه المعدلات تختلف بعض الشيء عن تلك التي كانت تستخدم سابقا كمعدلات طبيعية في مختبرات المناعة.

Objective: To determine the reference ranges of lymphocyte subsets in serologically HIV-seronegative healthy male adults in Oman.

Methods: A cohort, of 118 healthy male blood donors ranging in age from 18-51 years, was included in the study. The average age was 25 years. Blood samples collected into tubes containing ethylene-diamine-tetra acetic acid were investigated for lymphocyte subsets using flow cytometer. This study was conducted in the Immunology Laboratory of the Sultan Qaboos University, College of Medicine and Health Sciences, Muscat, Oman during the year 2006.

Results: For the 118 males investigated, the mean percentage and absolute values of the lymphocyte subsets were as follows: CD3: 68.53 ± 7.5%, 1701 ± 489 cells/µl; CD4: 40.4 ± 6.5%, 1006 ± 319 cells/µl; CD8: 25.8 ± 5.9%, 638 ± 225 cells/µl; CD19: 13.7 ± 4.7%, 349 ± 158 cells/µl, and CD56: 12.2 ± 6.7%, 308 ± 204 cells/µl. The ratio of CD4/CD8 was 1.6.

Conclusion: Immunophenotyping has been used to establish reference values of lymphocyte subsets in normal healthy adult males in Oman. The Omani male reference values obtained in this study show wide variations compared with kits values previously used as a reference.

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Quantitative analysis of lymphocyte subsets (T cell subsets, B cells, and natural killer [NK] cells) in the peripheral blood has gained importance in the assessment of the immunological status in conditions such as leukemia, lymphomas, autoimmune diseases, infectious diseases, immunodeficiency's, and other pathological conditions.[1,2] The reference distribution of peripheral blood lymphocyte subsets has been established for healthy Caucasians, and other ethnic groups.[3] There is very little data available on the normal levels of lymphocytes subsets in the healthy adult population of the Gulf, and Arabs in general. In many instances, the normal ranges for lymphocytes subsets, which are used by many hospitals within the Arab world are those that are supplied by the manufacturer of the equipment or

reagents, and these values may not reflect the normal values of the local population. Therefore, it is essential that each population establish its own normal values that can be used locally.[3] The objectives of this study were to examine the percentage, and absolute values of various T lymphocyte subsets, B lymphocytes, and NK cells in order to establish appropriate reference ranges for the Omani adult male population.

Methods. This study was conducted in the Immunology Laboratory of the Sultan Qaboos University, College of Medicine and Health Sciences, Muscat, Oman during the year 2006. A cohort, consisting of 118 healthy blood donors aged between 18-51 years, was studied. All were blood donors at Sultan Qaboos University Hospital (SQUH), Muscat, Sultanate of Oman. A questionnaire was administered to each individual in order to collect demographic, lifestyle, and medical information. Individuals who are taking medications for any acute or chronic illness, those who have been refused to donate blood previously, and cigarette smokers were excluded from this study. All participants were non-related-Omani healthy volunteers coming to SQUH as blood donors, and consented to participate in this study before a blood sample was taken. All individuals were found to be negative for HIV-1 and 2, hepatitis B and C virus. Ethical approval was obtained, from the College of Medicine and Health Sciences Medical Research and Ethics Committee before starting this project. Whole blood was collected into ethylene-diamine-tetra acetic acid vacutainer tubes. Complete blood counts were performed by Coulter counter (Coulter Co, USA) and white cell differential counts were conducted on the smears of all donors. Lymphocyte subsets were analyzed on FACScalibur (Becton Dickinson) with the following monoclonal antibody combinations (BD Bioscience, USA): immunoglobulin G1-immunoglobulin G1 control (with different fluorescent dyes), CD2 (phycoerythrin [PE])-CD19 (PerCP), CD3 (PerCP)-CD4 (FITC), CD3 (PerCP)-CD8 (PE), CD4 (FITC)-CD8 (PE), and CD3 (PerCP)-CD16/CD56 (PE). In brief, 50 µl of whole blood was mixed, and incubated in the dark, with 20 µl of each monoclonal antibody in separate tubes, at room temperature. Red blood cells were then lysed by adding 2 ml of lysing solution (Becton Dickinson), and the tubes were vortexed, and incubated in the dark at room temperature for 10 minutes, and finally centrifuged at 2,500 rpm for 5 minutes. The pellet was then washed once with 2 ml of phosphate-buffered saline (PBS), re-suspended in 500 µl of PBS, and finally analyzed with CellQuest software (Becton Dickinson). The FACScalibur was calibrated with Calibrite beads (Becton Dickinson), and AutoComp weekly.

For statistical analysis all data were analyzed using statistical software SPSS version 10. Descriptive analysis for variables was used to calculate mean values, and standard deviations. The student t-test was used for comparing groups. Frequencies were expressed as percentages, and p-values of <0.05 were considered significant.

Results. A total of 118 Omani men aged between 18-51 years (mean 25 years) were recruited for this study. The majority of participants were aged between 18-25 years (81/118, 68.6%) **Figure 1**. **Table 1** shows the percentages of the different lymphocyte subsets. An average CD4/CD8 ratio of 1.6 was obtained. A mean total white blood cell (WBC x 10^9/L) count of 5.5 was obtained with a range of 2.9-9.3 (**Table 2**). **Table 3** shows the lymphocyte subsets ranges obtained in this study compared with those provided by the kit (BD Multitest IMK kit) that are normally used for routine reporting of diagnostic tests in the Immunology Laboratory. Wide variations were demonstrated between the local data obtained, and those suggested by the kit.

Discussion. Establishing reference ranges for the different immunological parameters in the Omani population is essential for many laboratory clinical investigations of Omani patients. This will lead to many immunological, and hematological disorders in Omanis being diagnosed accurately, and therefore appropriate treatment can be achieved. In the present study, we have investigated the expression of T lymphocytes markers (CD3, CD4, and CD8), B lymphocytes (CD19), and NK cells (CD16 and CD56) in male Omani individuals. **Table 1** illustrates the mean of lymphocyte subpopulations, and subpopulations in absolute counts of CD3, CD4, CD8, CD19, and NK cells, for Omani males.

Environmental factors such as infection, smoking,[1,4] and poor nutrition have been suggested as possible causes of the ethnic differences between populations in lymphocyte subsets.[5] Our results are wider in ranges

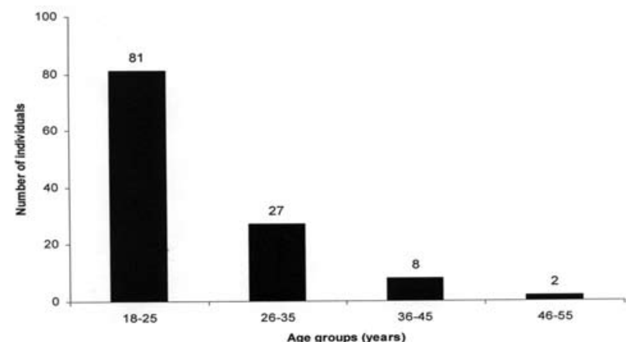

Figure 1 - Age distribution of the male Omani volunteers.

Table 1 - Reference ranges for lymphocyte subsets, in the Omani healthy male adult population (N=118).

Lymphocyte subsets		Mean	SD	Median	Reference range		95% CI for mean
					Minimum	Maximum	
CD3	%	68.5	7.5	55	34	89	67.1-69.9
	Cells/µL	1701.2	489.2	3011.1	682.5	3015.6	1612.5-1790.4
CD8	%	25.8	5.9	37	14	51	24.7-26.9
	Cells/µL	638.0	225.4	1231.8	262.5	1494.3	596.9-679.1
CD4	%	40.4	6.5	35	23	58	39.2-41.6
	Cells/µL	1005.8	319.3	1805.7	381.3	1868.4	947.6-064.0
NK	%	12.2	6.7	37	3	37	11.0-13.5
	Cells/µL	308.1	204.4	1098.9	71.1	1098.9	270.9-345.3
CD19	%	13.7	4.7	24	4	28	13.1-14.8
	Cells/µL	348.8	157.5	716.9	348.8	818.4	320.1-377.5

Cells/µL - absolute count, SD - standard deviation, CI - confidence interval, NK - natural killer, CD - cluster differentiation

Table 2 - The descriptive statistics of white blood cells (WBC) and total lymphocytes in male adult healthy Omanis.

Cells	Mean	SD	Median	Reference range		95% CI for mean
				Minimum	Maximum	
WBC ($x10^9$/L)	5.5	1.5	6.6	2.9	9.3	5.2 - 5.8
Lymphocytes ($x10^9$/L)	2.5	0.7	3.5	1	4.5	2.4 - 2.6

SD - standard deviation, CI - confidence interval

Table 3 - Comparing the kit (BD MultiTEST IMK Kit) lymphocyte subsets reference ranges as percentages with results obtained from the present study.

Lymphocyte Subset	BD MultiTEST IMK Kit (n=164)		Present study (n=118)		
	Mean	Reference ranges	Mean	Reference ranges	*P*-value
Total T lymphocytes (CD3)	72	56-86	68.5	34-89	0.001
Suppressor/Cytotoxic T lymphocytes (CD8)	24	13-39	25.8	14-51	0.002
Helper T lymphocytes (CD4)	45	33-58	40.4	23-58	0.001
NK lymphocytes	13	5-26	12.2	3-37	0.223
B lymphocytes (CD19)	14	5-22	13.7	4-28	0.906

NK - natural killer

than the kit (BD MultiTEST IMK Kit) reference ranges (**Table 3**), which are normally used in our immunology laboratory previously, confirming that local values need to be established for each local population. We have grouped the donors in this study into 4 groups according to age (18-25, 26-35, 36-45, and 46-55 years). Most of the donors in this study were in their 20's (in the group of 18-25, **Figure 1**), whereas only 2 were in the 46-55 years group. We could not demonstrate any statistical significant differences of any of the lymphocyte subpopulations between the different age groups in this study. It has been demonstrated previously that the CD4 cells increase with age, while CD8 cells decrease with age.[6] Other studies, however, showed that neither CD4 nor CD8 cells increase or decrease with age or both CD4, and CD8 cells may decrease or increase with age.[5,7] These contradictory results may be attributed partly to factors, such as genetic, race, and environmental influences.[3,7] Some studies did not consider possible bias by age, and gender related to variations of lymphocyte subsets, thus attributing diverting results to ethnic rather than age, and gender associated variations.[6,8]

Previous studies comparing the data for lymphocyte subpopulations for males, and females within the same population reported no differences within the 2 genders.[8] Taking this fact into consideration, the data obtained in this study may be used as a reference for Omani females within the same age group, although we would strongly recommend another study be performed on females.

Comparing our findings with the results of a study performed on Kuwaiti individuals,[8] we noticed no significant differences in most of the lymphocyte subpopulation. In fact, our data looks almost identical to that obtained for Kuwaitis despite the fact that in the Kuwaiti study they have used a different instrument (Coulter EPICS Profile II flow cytometer) from the one we used in the present study to measure lymphocyte subpopulations. This could be attributed to similarities in geographic region, and similar habitat between Omanis, and Kuwaitis. However, despite using a similar instrument for measuring the lymphocyte subpopulations, our data are significantly different from those reported for Saudis.[9] The variations may be due to the larger sample size in the Saudi study (209 males). The variations of our results, and those reported for Europeans,[10,11] Ethiopian,[12,13] and Asians[5] could be due to difference in race,[3] ethnic origin,[5,12] age, and gender,[7,14] habitats, and the different geographic distribution.

Despite the fact that this study has its own limitations, such as the smaller sample size, and no females were included due to difficulties in recruiting females, this study is still valid, if compared with other similar studies. Therefore, we would recommend that local populations in general, and within the Arab world, should establish their own local data for the different lymphocyte subpopulation, and should not depend on the data established for a different population, which is normally given by the instrument manufacturer.

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