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ORIGINAL ARTICLE

ENUMERATION OF PERIPHERAL BLOOD LYMPHOCYTE SUBSETS IN A HEALTHY IRANIAN POPULATION

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Abstract

Objective-This study was conducted to determine the reference intervals for CD3+ (T cells), CD4+ (T helper/inducer cell, Th), CD8+ (T suppressor/cytotoxic cells, Ts/c), CD3+CD4+/ CD3+CD8+ ratio, CD19+ (B cells) and CD16++56+ (NK cells) in a healthy Iranian population.

Method-Becton-Dickinson Immunocytometry Systems (BDIS) Simultest IMK-lymphocyte (dual color) reagents were used. The samples were first stained with appropriate monoclonal antibodies and then lysed using BDIS FACS lysing solution. The samples were analysed by BDIS FACS Caliber flowcytometry using the SimulSET software, version 2.5.

Results- The study population consisted of 152 healthy, male and female non-smokers, 18 to 60 years of age. The mean CD3+ percentage was 69.95% for males and 71.46% for females, and the mean percentage of CD3+ CD4+ Th subset was 40.4% and 44.21% for males and females respectively. The mean CD3+ CD4+/CD3+ CD8+ ratio was 1.55±0.54 for male population and 1.7±0.65 for female populations. The mean percentage of B lymphocytes (CD19+) was 14.52% for the females and 13.08% for males.

Conclusion- This study indicates that race, sex and age should be a consideration in the evaluation of the immune status.

Keywords • T lymphocytes • B lymphocytes • NK cells • CD4/CD8 ratio

Introduction

Quantitation of B and T lymphocytes and natural killer cells (NK cell) in the peripheral blood has gained importance in the assessment of the immunological status in conditions such as leukemia, lymphomas, autoimmune diseases, infectious diseases, immunodeficiencies and other pathological conditions.[1-5]

The reference distribution of peripheral blood T lymphocyte subsets, B lymphocytes and NK cells has been established for healthy Caucasians and other ethnic groups.[6-8] Effects of age and sex has been examined and differences exist for certain subsets of lymphocytes.[9,10] Age–related changes have been reported and show similarities for both genders.[11] The CD4+ cells and NK cells have been reported to increase significantly with age[12,13], while the percentage of CD8+ cells remain unchanged. Since the CD3+CD4+ percentage changes, the CD3+CD4+/CD3+ CD4+ ratio will also increase with age.

Definite differences have been reported in T lymphocytes and NK cells between sexes.[9] Most studies have been performed on Caucasians but there is evidence that significant differences exist for different ethnicities.[6-9] Hence, this study examines the percentage of various T cell subsets, B cells and NK cells in order to establish appropriate reference ranges for the Iranian population.

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Materials and Methods

One-hundred and sixty-five male and female subjects aged between 18-60 years were studied. A questionnaire was administered to each study subject in order to collect demographic, lifestyle and medical information. Complete blood counts

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were performed by Coulter counter (Coulter Co, USA) and white cell differential counts were conducted on the smears of all donors. Thirteen subjects showing abnormal values by these examinations were excluded from further analysis.

Antibodies

As recommended by the manufacturers, BDIS (CA, USA) Simultest two-color direct immunofluorescence reagents were used from the vials without dilution. Fluorescein isothiocyanate (FITC) and conjugated phycoerythrin (PE) antibodies were used. The kit contains six reagents: Leukogate (CD45-FITC/CD14-PE), control (γ1-FITC/ γ2-PE), CD3-FITC/CD19-PE, CD3-FITC/CD4-PE, CD3-FITC/CD8-PE, CD3- FITC/ CD16+56-PE.

Sample preparation and flowcytometry

Whole blood samples (2ml) were obtained by venipuncture into a sterile EDTA blood collection tube. Six dual color tubes (20 µl of an appropriate monoclonal antibody) were prepared for each sample. One-hundred microliter of blood was placed in each tube, mixed thoroughly and incubated at room temperature for 20 min before lysing with the FACS lysing solution. Samples were washed twice and analyzed immediately.

Statistical analysis

All results are expressed as the mean ± SD and the student's *t-test* was used to assess the differences. Regression, coefficient of correlation (ρ) and the level of significance (p) were analyzed using the Microsoft Excel program.

Results

The range and distribution of T cell subsets, CD4/CD8 ratio, B lymphocytes and NK cells in the combined study population are shown in Table 1, which also presents the mean percentage of the mononuclear cell subsets according to gender. As shown in this Table, females had a mild increase in the proportion of $CD3^+$ T cells but this was not statistically significant. The mean percentage of $CD3^+CD4^+$ T cells exhibited statistically significant differences between the male and female populations ($p=0.001$). No significant difference existed in the mean percentage of $CD3^+CD8^+$ T cells between males and females. The mean $CD3^+CD4^+/CD3^+CD8^+$ ratio was 1.7 for females and 1.55 for males; this difference was not statistically significant ($p=0.116$). As shown in Table 1, the number of $CD19^+$ B lymphocytes appeared to be higher in the female population compared to the male population which was statistically significant ($p=0.049$). The number of NK cells are also summarized in Table 1 according to gender. Statistically, no significant difference was observed between males and females.

Table 2 presents T lymphocyte subsets, B lymphocytes and NK cells in different age groups in the combined population. Data obtained for both male and female subjects were analysed in order to determine whether differences in age were associated with changes in the proportion of mononuclear cell subsets or not. These analyses confirmed that the proportion of $CD3^+$ T lymphocytes may change by a few percentages for every decade of life, but these were not statistically significant. However, $CD3^+$ T cells were significantly less in the subjects (combined) aged 51-60 years as compared with the subjects aged 31-40 years ($p<0.005$). Moreover, the coefficient of correlation (ρ) calculated for the mean percentage of $CD3^+$ cells was $r = -0.078$ for female subjects and $r = +0.033$ for male subjects, which was not statistically significant. As shown in Table 2 the percentages of $CD3^+CD4^+$ cells was higher in the 31-40-year age group which shows an

Table 1. Mononuclear cell subsets in the combined study population according to gender (*p<0.05).

Cell type	Range	Percent mean±SD in the sample	Percent mean±SD in males	Percent mean±SD in females	significance level
$CD3^+$ T cell	56-82	70.62±7.29	69.95±6.30	71.46±6.30	0.196
$CD3^+$ $CD4^+$ T cell	28-52	42.11±6.95	40.40±6.93	44.21±6.42	0.001 *
$CD3^+$ $CD8^+$ T cell	16-48	28.41±8.58	28.41±8.33	28.38±8.94	0.996
CD4 / CD8 ratio	0.7-3	1.61±0.60	1.55±0.54	1.70±0.65	0.116
$CD19^+$ B cell	7.7-22	13.72±4.59	13.08±5.06	14.52±3.82	0.049 *
$CD16^+$+56^+ NK cell	6-33	15.81±6.97	16.68±7.68	14.74±5.86	0.079

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Table 2. Mononuclear cell subsets stratified by age.

Cell type	Age			
	18-30	**31-40**	**41-50**	**51-60**
$CD3^+$ T cell	70.58	72.50	70.19	67.29
$CD3^+$ $CD4^+$ T cell	40.98	44.03	43.86	41.93
$CD3^+$ $CD8^+$ T cell	29.50	29.25	26.86	22.21
CD4 / CD8 ratio	1.48	1.63	1.86	2.07
$CD19^+$ B cell	18.41	13.22	14.23	16.00
$CD16^+$+56^+ NK cell	16.31	13.68	15.81	17.64

age group with statistically significant difference with the 18-30 and 51-60-year age groups ($p<0.001$). However, the age-related trend was not significant with every decade of life ($r = +0.041$ for females and $r = +0.11$ for males). When a comparison is made between different age groups, the number of $CD8^+$ T cells appeared to decline with increasing age and the difference was statistically significant between the 18-31, 31-40 and 51-60-year age groups ($r = -0.16$ for female subjects and $r = -0.17$ for male subjects $p<0.001$). The ratio of CD3CD4/CD3CD8 for different age groups are summarized in Table 2. This ratio revealed statistically significant differences between the 18-30, 41-50 and 51-60-year age groups. It showed a gradual increase with advancing age and appeared to increase with every decade of life ($r = +0.017$ for females and $r = +0.018$ for males). The number of $CD16^+$+56^+ NK cells and its age-trend is shown in Table 2. Statistically, no significant difference was seen between males and females when compared across the same age group ($r = +0.43$ for males and $r = -0.05$ for females). In addition, no significant change was observed in the mean percentage of $CD19^+$ B lymphocytes among different age groups in female subjects ($r = +0.008$). However, the coefficient of correlation calculated for the mean percentage of B cells in male subjects revealed a significant increase with advancing age ($r = +0.105$).

Discussion

In spite of the increasingly common use of flow-cytometry in evaluating the cellular components of the immune system in healthy and immunocompromised patients, there have been few studies of ethnic variations in blood components. In this study we address the potential influence of ethnic background on immunological status. A comparison of T cell subsets, B cells and NK cell values in the present study is made with those published for other ethnic groups. It was noticed that the Iranian population had significantly lower mean percentage of $CD3^+$ when compared to Caucasians. It was further observed that the Iranian male population had a significant lower mean percentage of $CD3^+$ as compared to the Saudi Arabian male population (69.95±7.98 vs 76±6). In our study, there were no statistically significant differences in the percentage of $CD3^+$ T cells between the male and female population. Reichert, et al, however, reported a significant difference between male and female Caucasians. A significant decline was also seen in the percentages of $CD3^+$ T cells in the above 50 age group. Similar results have been reported by Reichert, et al and Ruiz, et al. The findings of this study also indicate that there was no significant difference in the number of $CD3^+CD4^+$ cells between Iranians, Caucasians and Saudi Arabians. As shown in Table 1, females had higher mean percentages of $CD3^+CD4^+$ cells than males. This is in accordance with the results published for Caucasians. Despite the observation presented in this study, that changes in the $CD4^+$ T lymphocytes was not associated with increasing age (which is compatible with the findings of Ruiz, et al) we have reported an increase in the percentages of $CD3^+CD4^+$ lymphocytes with increasing age.[2,6,11,15] A potential source of such conflicting results could be due to factors such as smoking.

This study also showed a significant difference in the number of $CD3^+CD8^+$ T cells in the peripheral blood of the Iranian population when compared to Caucasians ($p<0.001$). Moreover, the mean percentages of $CD8^+$ T cells for the Iranian male population was significantly less than that reported for the Saudi male population (28.41±8.33 vs 40±7; $p<0.001$). Apart from racial differences, these discrepancies could be attributed to factors such as smoking habits, diet, climate and/or hygienic conditions. The significant difference between male and female $CD3^+CD8^+$ cell population is in accordance with the results published for Caucasians. In this study, a distinct age-related decline was noticed in the percentages of $CD8^+$ T cells. This finding is compatible with

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some of the earlier reports.[2,7,14,16] The ratio of $CD4^+$/CD8 T lymphocytes in our combined population appeared greater than that reported for Caucasians ($p<0.05$). Our results also showed that the Iranian population had a lower mean CD4/CD8 ratio than the American-Asian population.[17] Furthermore, it was shown that Iranian males had a higher mean CD4/CD8 ratio than their Saudi Arabian counterparts ($p<0.005$). One reason for this difference is due to the fact that Iranian males had less CD8+ T cells when compared to Saudi males. Although statistically insignificant, a distinct age-related increase was observed in the ratio of CD4/CD8 with advancing age which is compatible with previous reports.[7] This study also confirmed that the mean percentages of $CD16^+$+56^+ NK cells of the combined Iranian population was higher than that reported for the Caucasians ($p<0.001$). A similar result has been reported by Prince, et al which shows that the Chinese have relatively more NK cells than Caucasians. It was also noticed that Iranian males have more NK cells in their peripheral blood than Saudi males ($p<0.005$). In this study, a significant difference did not exist in the number of NK cells between male and female subjects. Reichert, et al however reported a statistically significant difference between male and female Caucasians. Despite reports that NK cells increase with advancing age[7,13,16,18], the present study also showed that the mean percentages of NK cells does not change with advancing age. Finally, the number of $CD19^+$ B cells of the Iranian population did not show a significant difference when compared with other races.

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