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Evaluation of the peripheral T cell subsets and NK cells within age in the Erbil city population

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ABSTRACT

Immunosenescence, or immunological aging, is a condition in which the immune system's ability to operate declines with age. One important indicator of a diseased or healthy state is the shift in immunological phenotype. This study aimed to investigate how aging affects human peripheral lymphocyte subsets and to estimate the normal absolute percentages and range of these lymphocyte groups in healthy individuals (both male and female) in Erbil city. Peripheral blood samples were collected from 75 healthy individuals with ages ranging from 20 years to 80 years in the EDTA tube. The examination of immunophenotype was carried out by using flow cytometry. Ranges of absolute percentages of lymphocyte subpopulations were CD3 T cells, 60.75 (35–79); CD4+ T cells, 49.5 (33–69.4); CD8+ T cells, 32.8 (11–67); CD4:CD8 ratio, 1.7 (1.0–2.4); and NK cells, 12.6 (3–36). There was a significant decrease in $CD3^+$ T cells, $CD8^+$ T cells, and naïve T cell populations (naïve $CD4^+$, naïve $CD8^+$) within age. However, there was a significant increase in the $CD4^+$ T cells in males and the ratio of CD4:CD8 T cells in females. On the other hand, memory T cells (memory $CD4^+$, memory $CD8^+$) and NK cells increased significantly within age. In conclusion, this study shows how aging dynamically alters T lymphocyte subsets and NK cells; these alterations revealed impairment of cellular immunity in the elderly population and increased susceptibility of the aged body to developing diseases.

1.Introduction

Immunophenotyping of peripheral lymphocytes has emerged as a crucial diagnostic technique for hematologic and immunologic conditions, including autoimmune illnesses, immunodeficiencies, and lymphoproliferative disorders (Jia et al., 2015).

One of the most significant lymphocyte subset, T cells are essential for the emergence of age-related immunological dysfunction (Mittelbrunn and Kroemer, 2021). Another lymphocyte cells that is significantly impacted by aging is natural killer (NK) cells (Solana et al., 2014).

Immunosenescence, or immunological aging, is the term used to describe the loss of lymphoid tissue that occurs with deterioration in immune response and involves many changes at the cellular and molecular levels. It is typified by the dysregulation of various physiological systems from their ideal homeostatic state, including the endocrine, metabolic, central and peripheral neurological, immunological, and other systems (Xu and Larbi, 2018, Masters et al., 2017). This decrease in the quality and quantity of immune responses has been linked to older adults' heightened vulnerability to a variety of illnesses, such as heart disease, autoimmune disorders, cancer, and compromised immunity to immunizations and infections (Valiathan et al., 2016, Yan et al., 2010). Within aging, there is persistent oxidative stress that specifically affects the cells of these core regulatory systems, altering their interactions. This affects how they function, throws off their equilibrium, and could shorten their lifespan (Müller et al., 2019, Hemn Jameel et al., 2021), as well as higher morbidity and mortality rates in older persons are linked to age-related immunological decline (Pawelec, 2017).

The reference or typical values of lymphocyte subpopulations observed in several countries are correlated with environmental factors like exposure to infectious agents, sociodemographic characteristics like age and sex, and ethnicity. Normal ranges for local populations must be established in order to interpret lymphocyte subpopulation data for clinical practice (Zhang et al., 2016). There are no established ranges for lymphocyte subpopulations among the residents of Erbil city and how aging affects these cells. Therefore, this study aimed to determine the typical absolute percentages and range of lymphocyte subpopulations in healthy individuals (both male and female) in Erbil city, as well as to examine the effects of aging on human peripheral lymphocyte subsets.

2. Materials and methods

2.1 Subjects and samples

The current study has been authorized and approved by the Salahaddin University/Erbil College of Science Human Ethics Committee (Approval No: 45/114). January 08, 2024, is the date. 75 healthy individuals (male and female) with ages ranging from 20 years to 80 years were included in this study, Table 1. The participants were free of long-term conditions such systemic lupus erythematosus and rheumatoid arthritis. Additionally, none of the subjects had a mental handicap, a diagnosis of depressive syndrome, or acute infections like colds or viruses.

The study excluded participants with serious cardiac disease, anemia, renal failure, autoimmune illnesses, and other conditions that could compromise the immune system. The use of immunosuppressive drugs or corticosteroids was an additional exclusion criterion.

Basic biochemical tests, such as blood sugar, lipid profile, renal function tests and complete blood count, are requested for the physical examination in order to better evaluate each person. Following this assessment, 75 out of 200 older participants who were in good physical health and had normal test results were added to the study.

Table 1 Age and sex of participants in the study.

Age groups	Total	Male	Female
20	8	5	3
30	16	6	10
40	15	4	11
50	12	6	6
60	11	4	7
70	9	3	6
>80	4	1	3
Total	**75**	**29**	**46**

2.2 subpopulation analysis of lymphocytes using flow cytometry

The FACSDiva software of the FACSCanto flow

cytometer device (Becton Dickinson, USA) was used to conduct the immunophenotypic analysis (Figure1). Samples of peripheral blood were drawn from each group and placed in an EDTA tube. The cell in the samples were initially labeled with the fluorescence-labeled-conjugated monoclonal antibodies from Becton Dickinson (BD Bioscience), USA, Table 2. And then incubated for half an hour at ambient temperature in a dark environment. Finally, the "ammonium chloride-containing lysing solution" was used to lyse the erythrocytes in the environment. As negative controls, fluorochrome-conjugated isotype-matched antibodies were employed. The prepped cells were passed in front of the flow cytometer's laser light.

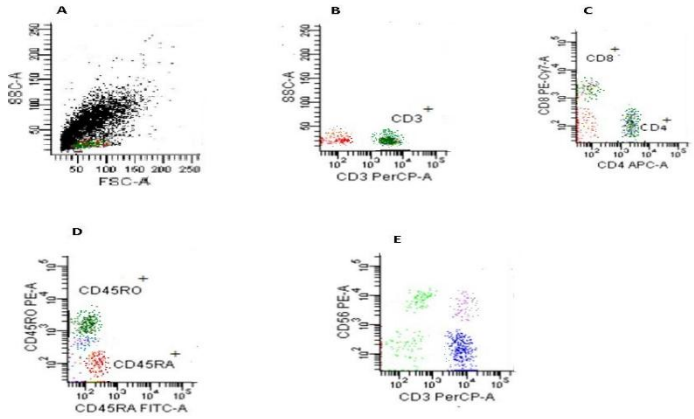

Figure 1: Flow cytometry analysis of lymphocyte sub population. A: Total cell population gate. B: Gate set on $CD3^+$ T cell. C: Gate set on $CD4^+$T cell and $CD8^+$ T cell. D: Gate set on memory & naïve T cell. E: Get set on NK cells.

Table 2. List of flow cytometry antibodies

Marker	Fluroescence	Catalog Number
CD4	APC (allophycocyanin)	561841
CD8	PE-CyTM7 (phycoerythrin-cyanin7)	557746
CD3	PerCP (peridinin-chlorophyll-protein	300428
CD45RA	FITC (fluorescein isothiocyanate)	649458
CD45RO	PE (phycoerythrin)	555493
CD56	PE	345812

2.3 Statistical analysis

The statistical analysis and graphic creation were done using GraphPad Prism (version 6.0). To determine if the data were normally distributed, the Shapiro-Wilk normality test, Kolmogorov-Smirnov test, and D'Agostino-Pearson omnius test were used. For data that was normally distributed, a t-test was employed, and the results were shown as means ± SE (standard error); The Wilcoxon test was performed to compare the results between the male and female and was displayed as the median (range) if the data were not normally distributed. The percentage of cells with various cell surface markers and age were compared using linear regression analysis to see if there was a linear relationship. R2, the regression coefficient, was used to evaluate how the regression models fit the observed data. P value of less than 0.05 for every analysis was deemed statistically significant.

3.Results

3.1Percentages of lymphocyte subpopulations in healthy Erbil adults.

Table 3 shows the absolute percentages of lymphocyte subpopulations in healthy Erbil adults, male and female. There was a notable distinction between male and female CD3+ and CD4+ T cells, the percentage of $CD3^+$ T cells were higher in women (P =0.005), and percentage of $CD4^+$ T cells were higher in male (P=0.01). Table 4 represents the range of peripheral lymphocyte subpopulations in healthy Erbil adults.

Table 3: Absolute percentages of lymphocyte subpopulations in healthy Erbil adults by gender

Lymphocyte subsets	Male Median (range)	Female Median (range)	P value
$CD3^+$ T cells	56.50 (40-71.5)	63.5 (35-79)	0.005
$CD4^+$ T cells	50.9 (33-69.4)	48 (42-66.4)	0.01
$CD8^+$ T cells	32.6 (11-49)	33.7 (16-67)	0.8
CD4:CD8 T cells	1.8 (1.3-2.4)	1.7 (1.0- 2.3)	0.3
naïve $CD4^+$ ($CD4^+CD45RA^+$)	10.7 (0.3-31)	7 (0.5-25)	0.09
naïve $CD8^+$ ($CD8^+CD45RA^+$)	4 (0.3-21)	3.5(0.1-14)	0.3
memory $CD4^+$ ($CD4^+CD45RO^+$)	18 (2 -49)	15(3 -46)	0.9
memory $CD8^+$ ($CD8^+CD45RO^+$)	2.4 (0.2 -21)	3.4(0.4-11)	0.6
NK cells ($CD3^-CD56^+$)	11.6(4 -29)	13.5 (3 -36)	0.2

Table 4: Range of peripheral lymphocyte subpopulations in healthy Erbil adults.

Lymphocyte subsets	Median (range)
$CD3^+$ T cells	60.75 (35 -79)
$CD4^+$ T cells	49.5 (33- 69.4)
$CD8^+$ T cells	32.8 (11- 67)
CD4:CD8 T cells	1.7 (1.0 – 2.4)
naïve $CD4^+$ ($CD4^+CD45RA^+$) cell	8.8 (0.3 – 31)
naïve $CD8^+$ ($CD8^+CD45RA^+$)	3.7 (0.1 -22)
memory $CD4^+$ ($CD4^+CD45RO^+$)	16 (2 – 49)
memory $CD8^+$ ($CD8^+CD45RO^+$)	3.1 (0.2 -21)
NK cells ($CD3^-CD56^+$)	12.6 (3 – 36)

3.2- T cell subsets within age in male and female.

Figure 2, Figure 3 and Table 5 represent the regression analysis of T cell subsets for all individuals with age. The phenotypic analysis of lymphocyte subpopulations revealed a significant decline in the $CD3^+$ T cells percentage within age in both male and female (P = 0.001, R^2= 0.5; P = 0.01, R^2 = 0.2; Figure 2A). There were a significant an increase in the frequency of $CD4^+$ cells with age in male P =0.01, R^2 = 0.21 but not in female P= 0.7 (Figure 2 B). The percentage of $CD8^+$ T cells declined significantly in both groups (P=0.005, R^2 = 0.3 for male and P = 0.003, R^2 = 0.26 for female; Figure 2C). This was accompanied by a significant increase in the ratio of CD4:CD8 T cells with aging in female (P=0.03, R^2 = 0.1; Figure2 D).

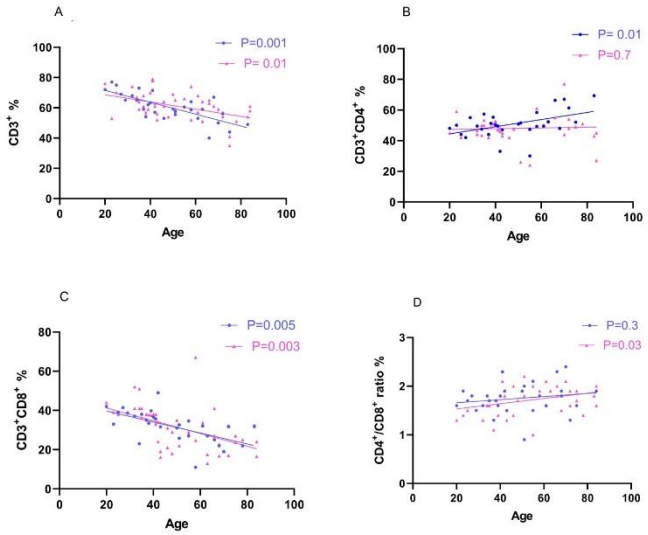

Figure 2 (A-D): The T cells subsets in healthy individuals, linear regression results for all individuals (male blue line and female pink line). A: percentage of $CD3^+$T cells, B: percentage of $CD4^+$ T cells, C: percentage of $CD8^+$ T cells, D: the ratio CD4/CD8.

The naïve $CD4^+$ ($CD4^+CD45RA^+$) cells significantly decreased with age in male and female (P= 0.04, R^2 = 0.25; P= 0.001, R^2 =0.51; Figure 3A), while naïve $CD8^+$ ($CD8^+CD45RA^+$) decrease significantly in female P=0.04, R^2= 0.09 , figure 3 B). An expansion in the memory $CD4^+$ ($CD4^+CD45RO^+$) and $CD8^+$ ($CD8^+CD45RO^+$)

cells with age. The proportion of memory $CD4^+$ cells were increased significantly in both male and female (P = 0.001, R^2 = 0.48; P= 0.005, R^2 = 0.26; Figure 3 C), and the proportion of memory $CD8^+$ raised significantly only in female (P = 0.02, R^2 = 0.1; Figure 3 D).

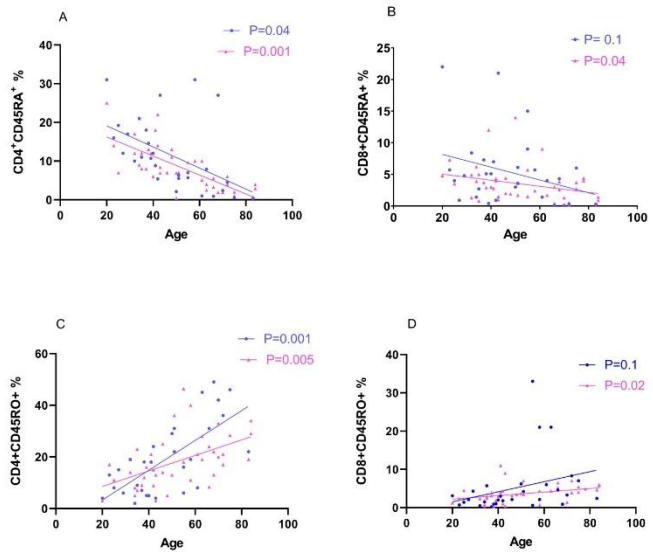

Figure 3 (A-D): The T cells subsets in healthy individuals, linear regression results for all individuals (male blue line and female pink line). A-B percentage of naïve T cell ($CD4^+CD45RA^+$) & ($CD8^+CD45RA^+$), C-D percentage of memory T cells ($CD4^+CD45RO^+$) & ($CD8^+CD45RO^+$).

Table 5: Regression coefficient (R^2) of lymphocyte subpopulation with age.

Lymphocyte subsets	Male (R^2)	Female (R^2)
$CD3^+$ T cells	0.5	0.2
$CD4^+$ T cells	0.21	0.002
$CD8^+$ T cells	0.3	0.26
CD4:CD8 T cells	0.03	0.1
naïve $CD4^+$ ($CD4^+CD45RA^+$)	0.25	0.51
naïve $CD8^+$ ($CD8^+CD45RA^+$)	0.096	0.09
memory $CD4^+$ ($CD4^+CD45RO^+$)	0.48	0.26
memory $CD8^+$ ($CD8^+CD45RO^+$)	0.09	0.1
NK cells ($CD3^-CD56^+$)	0.19	0.45

3.3 NK cells within age in male and female

Figure 4 and Table 5 represent the regression analysis of NK cell subsets for all individuals with age. In this study there is a rapid rise in the proportion of NK cells in circulation ($CD3^-CD56^+$) within age (P= 0.01, R^2 = 0.19 in male and P= 0.001, R^2 = 0.45 in female).

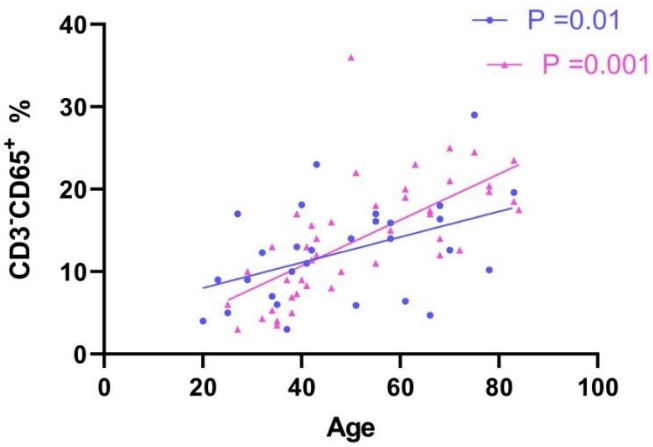

Figure 4: Total NK cells in healthy individuals, linear regression results for all individuals (male blue line and female pink line).

4.Discussion

The process of aging is intricate and includes many biochemical and physiological changes. The immune system is significantly affected, changing immune subtypes and function, and gradually losing its capacity to react to diseases and foreign pathogens as well as to remember and improve memory responses (Jia et al., 2023).

Numerous research studies have examined the processes underlying the deterioration of immune function as well as the relationships between various immunological subtypes and the beginning of various diseases and clinical outcomes (Sun et al. 2022). However, little is currently known about the composition of immune cells across age groups and how they evolve over time.

The three main types of lymphocytes are T cells, B cells, and NK cells. According to other research, B cells do not significantly change as people age (Frasca et al., 2022, Mogilenko et al., 2022).

This is the first study conducted in Erbil City to demonstrate normal percentages of T lymphocytes subpopulation and NK cells, in healthy adults' peripheral blood.as well as evaluate the percentage of these lymphocytes groups within age. In this study the ranges of absolute percentages of lymphocyte subpopulations were CD3 T cells, 60.75 (35–79); CD4+ T cells, 49.5 (33–69.4); CD8+ T cells, 32.8 (11–67); CD4:CD8 ratio, 1.7 (1.0–2.4); and NK cells, 12.6 (3–36). There was a significant difference in the $CD3^+$ T cells and $CD4^+$ T cells in male and female. When lymphocyte subgroups were compared between males and females, there was only a significant difference in CD3 T cells and CD4+ T cells. Numerous factors influence the lymphocyte levels, including environmental factors like exposure to infectious agents, air pollution, and lifestyle choices, in addition to demographic factors like age, gender, and ethnicity (Zhang et al., 2016).

As was observed in the present study; there was a variety of immunophenotypes within age. There was a marked decline in the percentage of $CD3^+$T cells and $CD8^+$ T cells in both genders, which is in agreement with the finding in other studies (Yan et al., 2010, Amadori et al., 1995, Tavares et al., 2014, Jia et al., 2023). The $CD4^+$ T cells increased significantly only in male, but there was no discernible change in the $CD4^+$ T cells in the female.

This study found that the $CD4^+$ T cells percentage is much less affected by age than that of $CD8^+$ T cells. One explanation could be that CD8 T cells are more exhausted than CD4 cells; there has not yet been any investigation of the basic mechanisms (Jia et al., 2023). The ratio of $CD4^+$ to $CD8^+$ cells rose in this study as a result of the sharp decline in $CD8^+$ T cells with aging.

Prior research has shown that cells with more complicated functions and rapid differentiation are more prone to developing mutations and DNA damage. Hematopoietic stem cells, which have a particular structure, are the source of T cells. Over time, T cells may collect mutations and damage to their DNA. On the other hand, more DNA damage and mutations could cause T cell failure even more (Barnett and Barnett, 1998).

The proportion of naïve T cells significantly decreased, while the proportion of memory T cells increased. Age is known to cause a decrease in thymus production, which is most likely the direct cause of the decline in naive T cells (Minato et al., 2020). One explanation for the increase in effector memory cells is chronic antigenic stimulation (Pita-Lopez et al., 2009).

T cells are among the immune system's most significant subsets, and they are essential for the emergence of age-related immunological dysfunction. Mittelbrunn and Kroemer put several characteristics of T cell aging. These include phenotypic alterations such as an imbalance in naive-memory T cells, a reduction in T cell receptor (TCR) repertoire, senescence of T cells, and a lack of effector plasticity. T cell depletion is another phenomenon that is seen as people age (Mittelbrunn and Kroemer, 2021). Chronic exposure and activation by foreign or viral antigens, which is often typified by the accumulation of terminally developed, dysfunctional T cells (Gustafson, 2021, Huang et al., 2021). Notably, disease-associated immunological dysfunction frequently results in T cell exhaustion, while biological aging typically causes T cell senescence (Mogilenko et al., 2022).

In a part from T cells, NK cell alterations are a significant predictor of immune age. In the course of anti-tumor and anti-infectious immune responses, NK cells primarily carry out killing tasks and mediate cytotoxic capacities (Poli et al., 2009).

The percentage of NK cells has significantly increased in the current investigation. This is in line with earlier research (Yan et al., 2010, Jia et al., 2023). While the quantity of NK cells had grown, another study found that their ability to function had declined (Facchini et al., 1987).

The aging-related alterations could be partially to blame for the decline in the cellular immune response, decreased lymphocyte cytotoxicity, and decreased proliferative activity (Tavares et al., 2014). Changes in the percentage of these cell types were the primary basis for determining immune age, the findings of this study revealed which immune cells are most impacted as people

age.

5.Conclusion

The percentages of lymphocyte subpopulations in Erbil city were CD3 T cells, 60.75 (35–79); CD4+ T cells, 49.5 (33–69.4); CD8+ T cells, 32.8 (11–67); CD4:CD8 ratio, 1.7 (1.0–2.4); and NK cells, 12.6 (3–36). This work shows how T lymphocyte subsets and NK cells dynamically alter with age. These changes demonstrated that the elderly population's cellular immunity was compromised, and that their bodies were more vulnerable to disease.

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Conflict of interest

The author has no conflicts of interest to declare and there has been no significant financial support for this work.

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