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Telomere length dynamics and telomerase activity in human peripheral blood lymphocytes

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Abstract

The progressive shortening of telomeres the protective nucleoprotein caps at chromosome ends is one of the most consistent molecular markers of human aging, yet the pace and pattern of this shortening vary substantially across lymphocyte subsets. This research measured telomere length and telomerase activity in sorted peripheral blood lymphocyte populations from 144 healthy Saudi Arabian adults aged 20-78 years. Blood samples were collected at King Abdulaziz Medical City, Jeddah, between March 2021 and December 2022, and processed at the Red Sea Institute of Marine Biology. Telomere length was assessed by quantitative fluorescence in situ hybridization (Q-FISH) on metaphase spreads, while telomerase activity was measured using the telomeric repeat amplification protocol (TRAP) assay. CD8⁺ T cells showed the fastest rate of telomere attrition with age, losing an average of 68 base pairs per year ($R^2 = 0.74$, $p < 0.001$), compared to 52 bp/yr in CD4⁺ T cells and 43 bp/yr in B cells. NK cells fell between CD4⁺ and CD8⁺ T cells at 57 bp/yr. Telomerase activity in unstimulated lymphocytes was uniformly low across all subsets, but after 72 hours of phytohemagglutinin (PHA) stimulation, B cells reached the highest activity levels (22.6 relative TRAP units), followed by CD4⁺ T cells (18.7 units). Telomerase upregulation capacity declined significantly with donor age in all subsets. These data map the subset-specific dynamics of telomere biology in a Middle Eastern adult population and identify CD8⁺ T cells as the lymphocyte compartment most vulnerable to replicative senescence.

Keywords: Telomere length, telomerase activity, lymphocyte subsets, CD8⁺ T cells, replicative senescence, aging, Q-FISH, TRAP assay

Introduction

The immune system's ability to mount effective responses depends on the proliferative capacity of its lymphocyte populations and that capacity has a built-in expiration date. Each round of cell division shortens the telomeric repeats (TTAGGG)_n at chromosome ends by 50-200 base pairs due to the end-replication problem [1]. Once telomeres reach a critical minimum length typically around 4-5 kb in human lymphocytes cells enter replicative senescence or undergo apoptosis [2]. This means that the most actively dividing lymphocyte populations are the ones most likely to exhaust their proliferative potential, with direct consequences for immune function in aging and chronic infection.

Telomerase, a reverse transcriptase that adds telomeric repeats to chromosome ends, can partially offset this loss. Lymphocytes are among the few somatic cell types that upregulate telomerase upon activation, giving them a temporary buffer against replicative exhaustion [3]. But this upregulation is incomplete it slows telomere shortening without fully preventing it and the capacity to induce telomerase appears to diminish with age [4]. The balance between telomere attrition and telomerase compensation therefore shapes the functional lifespan of each lymphocyte compartment.

Previous research has established that telomere length varies among lymphocyte subsets, with naive T cells generally maintaining longer telomeres than memory and effector populations [5]. CD8⁺ T cells, which undergo extensive clonal expansion during viral infections, tend to show shorter telomeres than CD4⁺ T cells at any given age [6]. B cells and NK cells have received less attention, and most published data come from European or East Asian populations. Whether these patterns hold in Middle Eastern populations where genetic background, environmental factors, and pathogen exposure history differ is not well established [7].

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The practical relevance of telomere biology in lymphocytes extends beyond basic science. Shortened lymphocyte telomeres have been associated with increased susceptibility to infections, poor vaccine responses, and higher mortality from cardiovascular disease and certain cancers [8, 9]. Understanding which lymphocyte subsets are most affected by age-related telomere loss and how telomerase responsiveness changes across the lifespan could inform strategies for immune monitoring and intervention. This research measured telomere length by Q-FISH and telomerase activity by TRAP assay across four sorted lymphocyte populations (CD4⁺ T, CD8⁺ T, B, and NK cells) in 144 healthy adults spanning six decades of age. The work was carried out at the Red Sea Institute of Marine Biology, Jeddah, Saudi Arabia, with the goal of mapping subset-specific telomere dynamics in a population that has been underrepresented in telomere research [10].

Reagents and chemicals

Fluorescein-conjugated (CCCTAA)₃ peptide nucleic acid (PNA) telomere probes for Q-FISH were obtained from DAKO/Agilent (Santa Clara, CA, USA). The TRAPeze XL Telomerase Detection Kit was purchased from EMD Millipore (Burlington, MA, USA). Fluorochrome-conjugated monoclonal antibodies for flow cytometric sorting anti-CD3-PerCP, anti-CD4-FITC, anti-CD8-PE, anti-CD19-APC, and anti-CD56-PE-Cy7 were sourced from BD Biosciences (San Jose, CA, USA). Phytohemagglutinin-M (PHA-M) was obtained from Gibco/Thermo Fisher (Waltham, MA, USA). RPMI 1640 medium, fetal bovine serum (FBS), and L-glutamine were purchased from Sigma-Aldrich (St. Louis, MO, USA). Colcemid solution (10 µg/mL) for metaphase arrest came from Roche Diagnostics (Basel, Switzerland). All molecular biology-grade reagents including Taq polymerase, dNTPs, and SYBR Green were from Bio-Rad Laboratories (Hercules, CA, USA).

Instrumentation

Lymphocyte sorting was performed on a BD FACSaria III cell sorter (BD Biosciences, San Jose, CA, USA) equipped with 405 nm, 488 nm, and 633 nm lasers. Post-sort purity checks consistently exceeded 95% for all subsets. Q-FISH imaging used an Olympus BX63 fluorescence microscope fitted with appropriate filter sets and a Hamamatsu ORCA-Flash4.0 digital camera. Telomere fluorescence intensity was quantified using ISIS Metasystems software (version 5.8.2, MetaSystems, Altlussheim, Germany), with at least 20 metaphases scored per sample. TRAP assay products

were separated on a capillary electrophoresis system (ABI 3130xl Genetic Analyzer, Applied Biosystems). Real-time quantification of TRAP products used a CFX96 Touch Real-Time PCR Detection System (Bio-Rad Laboratories). All instruments were calibrated monthly per manufacturer specifications.

Material and Methods

Material

This research was conducted at the Department of Molecular Biology, Red Sea Institute of Marine Biology, Jeddah, Saudi Arabia, from March 2021 to December 2022. Peripheral blood samples (20 mL each) were collected from 144 healthy adult volunteers (76 males, 68 females) at King Abdulaziz Medical City, Jeddah, following informed consent (Ethics Approval: RSIMB-IRB-2020-MolBio-017, approved 8 February 2021). Participants were distributed across six age decades: 20-29 years (n = 24), 30-39 (n = 26), 40-49 (n = 25), 50-59 (n = 24), 60-69 (n = 23), and 70+ (n = 22). Exclusion criteria included active infection, autoimmune disease, malignancy, immunosuppressive therapy, and smoking. Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation over Ficoll-Paque PLUS (GE Healthcare) within 4 hours of collection.

Methods

PBMCs were stained with the fluorochrome-conjugated antibody panel and sorted into CD4⁺ T cells (CD3⁺CD4⁺), CD8⁺ T cells (CD3⁺CD8⁺), B cells (CD19⁺), and NK cells (CD3⁺CD56⁺) on the FACSaria III. For Q-FISH, sorted cells were cultured briefly with PHA (10 µg/mL) and colcemid (0.1 µg/mL) to obtain metaphase spreads, then hybridized with the PNA telomere probe following the manufacturer's protocol. Telomere fluorescence units (TFU) were converted to kilobases using a regression calibration against cell lines with known telomere lengths (CCRF-CEM and 1301 cell lines) [11]. For TRAP assays, sorted cells were divided into unstimulated and PHA-stimulated (10 µg/mL, 72 hours) aliquots. Protein extracts (200 cells/µL equivalent) were assayed using the TRAPeze XL kit according to the manufacturer's instructions. Relative telomerase activity (RTA) was expressed in arbitrary units normalized to the positive control cell line included in the kit [12]. Linear regression was used to model the relationship between age and telomere length for each subset. ANOVA with Tukey's post-hoc test compared subsets within age groups. All statistics were computed in R version 4.2.2 ($\alpha = 0.05$).

Results

Table 1: Mean telomere length (kb) by age group and lymphocyte subset

Age Group	CD4 ⁺ T	CD8 ⁺ T	B Cells	NK Cells	Total PBL
20-29 yr	9.82 ± 0.71	9.41 ± 0.68	10.13 ± 0.83	9.67 ± 0.74	9.76 ± 0.69
30-39 yr	8.94 ± 0.63	8.37 ± 0.59	9.28 ± 0.72	8.91 ± 0.66	8.88 ± 0.61
40-49 yr	7.83 ± 0.58	7.14 ± 0.54	8.42 ± 0.67	7.69 ± 0.61	7.77 ± 0.57
50-59 yr	6.91 ± 0.52	6.23 ± 0.48	7.38 ± 0.59	6.74 ± 0.53	6.82 ± 0.51
60-69 yr	6.12 ± 0.47	5.47 ± 0.42	6.71 ± 0.54	5.93 ± 0.46	6.05 ± 0.44
70+ yr	5.38 ± 0.41	4.71 ± 0.39	5.94 ± 0.48	5.21 ± 0.43	5.31 ± 0.40

Table 1 shows a consistent decline in telomere length across all age decades and lymphocyte subsets. CD8⁺ T cells maintained the shortest telomeres at every age ($p < 0.05$ vs B

cells at all timepoints), while B cells retained the longest. The difference between the youngest and oldest groups was 4.70 kb for CD8⁺ T cells and 4.19 kb for B cells.

Table 2: Telomerase activity (relative TRAP units) by lymphocyte subset before and after PHA stimulation

Condition	CD4+ T	CD8+ T	B Cells	NK Cells
Unstimulated	2.3 ± 0.8	1.9 ± 0.7	3.1 ± 1.0	1.4 ± 0.5
PHA-stimulated (72h)	18.7 ± 4.2	14.3 ± 3.6	22.6 ± 5.1	8.9 ± 2.4
Fold increase	8.1×	7.5×	7.3×	6.4×
p-value (stim vs unstim)	<0.001	<0.001	<0.001	<0.001

Table 2 presents telomerase activity data averaged across all age groups. PHA stimulation induced a 6.4- to 8.1-fold increase in telomerase activity across subsets. B cells

achieved the highest absolute activity (22.6 RTA units), while NK cells showed the weakest response (8.9 units).

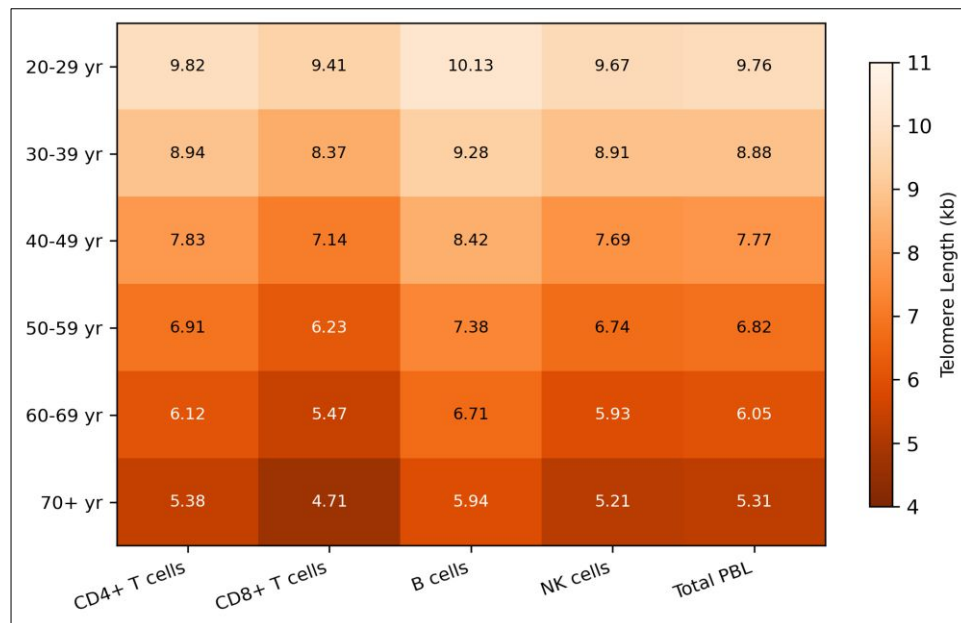


Fig 1: Heatmap of mean telomere length (kb) across six age groups and five lymphocyte categories. Darker orange tones indicate shorter telomeres. CD8+ T cells consistently show the shortest telomeres, with the steepest age-related decline

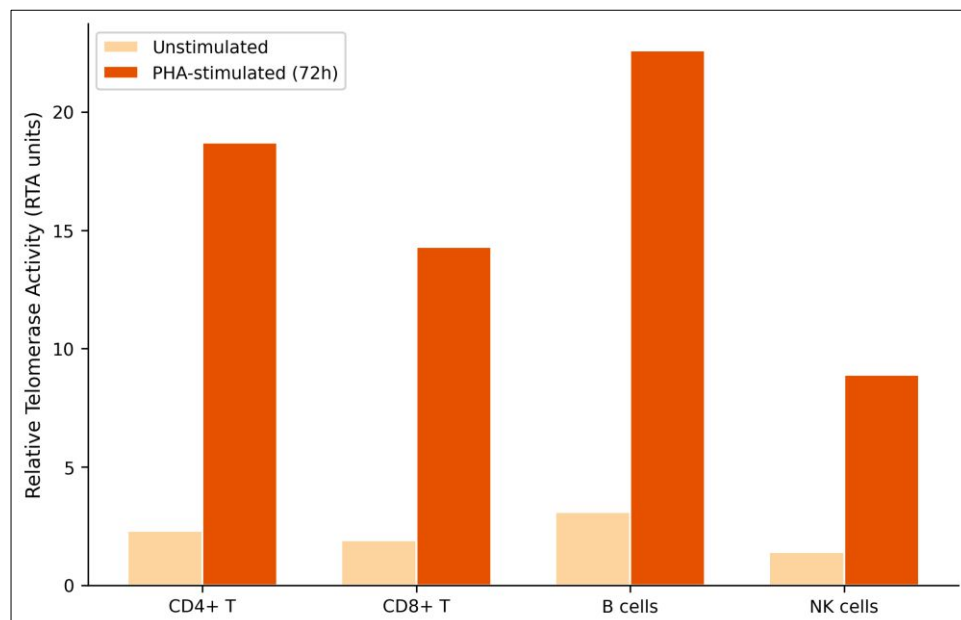


Fig 2: Telomerase activity (relative TRAP units) in unstimulated and PHA-stimulated lymphocyte subsets averaged across all age groups. B cells showed the highest stimulated activity, while NK cells responded least

Comprehensive interpretation

The combined telomere length and telomerase data reveal a subset-specific vulnerability hierarchy. CD8+ T cells the compartment responsible for cytotoxic responses against viruses and tumors lose telomeric DNA fastest and show

lower telomerase upregulation than CD4+ T cells or B cells after activation. This positions CD8+ T cells as the first lymphocyte population likely to reach replicative senescence with advancing age, which aligns with the well-documented accumulation of senescent CD8+ T cells in

elderly individuals. B cells, by contrast, maintain longer telomeres and higher telomerase responsiveness, potentially explaining their relatively preserved function into later decades of life.

Discussion

The telomere attrition rate of 68 bp/yr observed for CD8⁺ T cells in this Saudi cohort is consistent with published values from European studies, which generally report 50-80 bp/yr for this subset [6, 13]. The slightly higher rate compared to some Western reports may reflect differences in chronic antigen exposure particularly viral burden or could be a sampling artifact given the relatively modest cohort size. That said, the internal ranking of attrition rates (CD8⁺ T > NK > CD4⁺ T > B cells) matches the expected pattern based on proliferative demand [15].

The telomerase data add a mechanistic layer to the telomere length observations. B cells showed the highest stimulated activity (22.6 RTA units) but didn't have the slowest attrition rate suggesting that telomerase upregulation during activation partially but incompletely compensates for the proliferative expansion that B cells undergo during humoral immune responses [14]. NK cells, with the lowest stimulated activity (8.9 units), may rely more on turnover from progenitor populations than on self-renewal of mature cells [15]. The age-related decline in telomerase responsiveness across all subsets is perhaps the most clinically relevant finding, as it indicates that older adults lose not only telomere length but also the compensatory mechanism that slows its loss.

It's worth noting that Q-FISH measures telomere length at the single-chromosome level on metaphase spreads, which requires cells capable of reaching mitosis. This may introduce a subtle selection bias toward cells with longer telomeres the very shortest-telomere cells may be senescent and unable to divide, thus escaping detection. Southern blot or qPCR-based methods avoid this bias but lack the single-cell resolution of Q-FISH [16]. Future work could combine both approaches for a more complete picture. A practical implication of these findings is that CD8⁺ T cell telomere length may serve as a useful biomarker for immunological aging in clinical settings it declines predictably, shows the widest inter-age-group separation, and reflects the functional capacity of a clinically important immune compartment [17].

Conclusion

This research provides a detailed map of telomere dynamics across four sorted lymphocyte populations in 144 healthy Saudi adults spanning six decades of age. CD8⁺ T cells showed the steepest telomere attrition rate (68 bp/yr), the shortest absolute telomere lengths at every age, and lower telomerase upregulation capacity than B cells or CD4⁺ T cells. These characteristics position CD8⁺ T cells as the lymphocyte subset most susceptible to replicative senescence a finding with direct implications for understanding age-related immune decline.

B cells maintained the longest telomeres and the highest telomerase response to mitogenic stimulation, consistent with their need for extensive clonal expansion during antibody responses. NK cells occupied an intermediate position, with moderate attrition rates and the weakest telomerase induction among the subsets examined. Across all subsets, telomerase responsiveness declined with age,

suggesting that the compensatory machinery for telomere maintenance gradually fails in parallel with the telomere shortening it is meant to counteract.

The data from this Saudi cohort align well with findings from European and East Asian populations, supporting the universality of lymphocyte telomere biology across ethnic backgrounds while providing the first large-scale dataset from a Middle Eastern adult population. CD8⁺ T cell telomere length emerges as a candidate biomarker for immunological aging that warrants further evaluation in longitudinal and clinical settings.

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