Association between telomere length and complete blood count in US adults

Mohsen Mazidi[1,2], Peter Penson[3], Maciej Banach[4,5]

[1]Key State Laboratory of Molecular Developmental Biology, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, China
[2]Institute of Genetics and Developmental Biology, International College, University of Chinese Academy of Science, Beijing, China
[3]School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, Liverpool, UK
[4]Department of Hypertension, Chair of Nephrology and Hypertension, Medical University of Lodz, Lodz, Poland
[5]Cardiovascular Research Centre, University of Zielona Gora, Zielona Gora, Poland

Corresponding author:
Mohsen Mazidi
Key State Laboratory
of Molecular
Developmental Biology
Institute of Genetics
and Developmental Biology
Chinese Academy
of Sciences
Beijing 100101, China
E-mail: Moshen@genetics.
ac.cn

Abstract

Introduction: Telomere length (TL) is related to age-related health outcomes, but little is known about the relationship between TL and complete blood count (CBC) parameters. We aimed to determine the relationship between TL and CBC in a sample of healthy US adults.

Material and methods: Participants in the National Health and Nutrition Examination Survey (NHANES) recruited between 1999 and 2002 who had essential data on total CBC and TL were studied. We computed age- and race-adjusted mean values for total CBC using analysis of covariance (ANCOVA). All statistical analyses accounted for the survey design and sample weights by using SPSS Complex Samples v22.0 (IBM Corp, Armonk, NY).

Results: Of the 8892 eligible participants, 47.8% (n = 4123) were men. The mean age was 41.8 years overall, 41.0 years in men and 42.6 in women (p = 0.238). The sex-stratified ANCOVA showed no significant difference in the total CBC across TL quartiles (all $p > 0.05$) in both sexes. In the adjusted model, there was a significant negative relationship with monocyte count (β = –0.051, 95% CI: –0.422; –0.142), mean cell hemoglobin (β = –0.051, 95% CI: –0.038; –0.011) and red cell distribution width (β = –0.031, 95% CI: –0.054; –0.003), while there was a significant positive relationship with basophil ratio (β = 0.046, 95% CI: 0.049–0.171).

Conclusions: These results support the possibility that telomere attrition may be a marker for reduced proliferative reserve in hematopoietic progenitor cells.

Key words: telomere length, complete blood count, National Health and Nutrition Examination Survey.

Introduction

Telomeres are nucleotide sequences located at the ends of chromosomes that assist in the preservation of chromosomal mechanical reliability and genomic constancy [1]. It has been reported that telomere length (TL) of replicating somatic cells is inversely associated with age and age-related disorders, such as cardiovascular diseases (CVDs) [1, 2]. The development of bone marrow aging includes slow replacement of he-

matopoietic tissue by adipocytes and fibroblasts [3]. This process is mainly observed in subjects more than 80 years old, who have 50% less hematopoietic tissue than younger subjects [4].

The bone marrow aging process is also associated with increased DNA impairment in hematopoietic stem cells (HSCs) [5]. Although it has been stated that telomerase activity in HSCs would preserve a constant TL [6], recently, it has been reported that telomere shortening can restrict the preservation and functions of the adult stem cells [7]. It is possible that the telomerase activity in HSCs is insufficient to avert telomere destruction and, consequently, unable to avert cell senescence [8]. It has been reported that the telomerase activity varies in HSCs from very weak to a level of activity similar to that seen in immortal cell lines, with no age-dependent deterioration [9]. To date, the relationship between TL and peripheral blood count factors has been explored in middle-aged [10], combined middle-aged and elderly [11], and elderly [12] subjects. However, the findings of these studies are controversial. Hence, it is still largely unknown how telomere maintenance might be associated with blood count parameters. In this regard, we aimed to determine the association between TL and blood count parameters in healthy adults from the National Health and Nutrition Examination Surveys (NHANES) cohort.

Material and methods

Population

National Health and Nutrition Examination Surveys are ongoing repeated cross sectional surveys conducted by the US National Center for Health Statistics (NCHS) [2, 13–15]. The NHANES uses a multistage probability sampling strategy, which oversamples certain subgroups of the population, including blacks, Mexican-Americans, and those of lower socioeconomic status [2, 13, 14, 16]. The National Center for Health Statistics Research Ethics Review Board approved the NHANES protocol, and consent was obtained from all participants (informed consent was obtained from all subjects) [2, 13, 17]. Moreover, all methods were performed in accordance with the relevant guidelines and regulations [2, 13, 14]. Data collection on demographic information is conducted through in-home administered questionnaires, while anthropometric, inflammatory and biochemical data are collected by trained persons using mobile examination units. More detailed information on the NHANES is available elsewhere [13, 18]. The current study was based on analysis of data for two 2-year NHANES survey cycles: 1999–2000 and 2001–2002. Adults with a history of diabetes, coronary heart disease, angina, myocardial infarction, stroke or congestive heart failure were excluded.

We restricted our analysis to subjects more than 18 years old. The main response variables of interest were 13 types of cellular blood composition: hemoglobin (HGB), mean cell hemoglobin (MCH), mean cell volume (MCV), hematocrit (HCT), red blood cell count (RBC), platelet count (PLT), basophil number (BAS), eosinophil number (EOS), monocyte number (Mon), segmented neutrophil number (Neu), lymphocyte number (Lym), neutrophil/lymphocyte ratio (NLR), and white blood cell count (WBC). The CBC analysis was performed using the Coulter method, following the NHANES quality control and quality assurance protocols [19].

Leukocyte telomere length measurements

Briefly, aliquots of purified DNA, isolated from whole blood using the Puregene (D-50 K) kit protocol (Gentra Systems, Inc., Minneapolis, Minnesota), were obtained from participants. The leukocyte TL assay was performed using the quantitative polymerase chain reaction method to measure TL relative to standard reference DNA (also known as the telomere-to-single (T/S) ratio) [20]. Each sample was assayed 3 times on 3 different days. The samples were assayed on duplicate wells, resulting in 6 data points. Control DNA values were used to normalize between-run variability. The Centers for Diseases Control (CDC) conducted a quality control review before linking the TL data to the NHANES data files. The CDC Institutional Review Board granted human subject approval for this study [2, 17].

Statistical analysis

We conducted the analyses according to the guidelines set out by the CDS for analysis of the complex NHANES dataset, accounting for the masked variance and using the proposed weighting methodology [2, 16, 21]. We computed age- and race-adjusted values of CBC by using analysis of covariance (ANCOVA). Categorical demographic variables were compared by using analysis of variance (ANOVA) and the χ^2 test. Fully adjusted multivariate linear regression (stepwise mode) and partial correlation were used to determine the association of CBC by TL. All tests were two sided, and $p < 0.05$ was the level of significance. Results were analyzed using SPSS complex sample module version 22.0 (IBM Corp, Armonk, NY). Sample weights were applied to account for unequal probabilities of selection, nonresponse bias, and oversampling.

Results

The weighted distributions of study population characteristics are shown in Table I. Of the 8892 eligible participants, 47.8% ($n = 4123$) were men. The mean age of the total population sample was

41.8 years, 41.0 years in men and 42.6 in women (*p* = 0.238). It can be seen in Table II that whites (non-Hispanic) comprised the majority (70.3%) of the population in the study. Moreover, 55.5% (*n* = 4294) of the sample consisted of married subjects. The mean and SEM for the TL in the total population was 1.08 ±0.015, and for males and females it was 1.07 ±0.014 and 1.08 ±0.016, respectively.

In the sex-stratified ANCOVA (age and race adjusted), we found no significant difference in the values of the parameters comprising the CBC across TL quartiles (all *p* > 0.05) in either sex. In the adjusted model, we found a significant negative relationship of TL with monocyte count (β = −0.051, 95% CI: −0.422; −0.142), mean cell hemoglobin (β = −0.051, 95% CI: −0.038; −0.011) and red cell distribution width (β = −0.031, 95% CI: −0.054; −0.003), while there was a significant positive relationship with basophil ratio (β = 0.046, 95% CI: 0.049–0.171) (Table II).

Discussion

This study investigated the relationship between telomere length and complete blood count in healthy US adults. We found a positive associa-

tion between TL and the percentage of basophils, but a significant negative relationship between TL and monocyte count, mean cell hemoglobin and red cell distribution width. The association between telomere length and complete blood count has been reported in the literature [22–24]. Compte *et al.* found that telomere length was associated with total WBC count and neutrophils and CV diseases with monocytes. Moreover, the findings of a national investigation in the US revealed an inverse association between MCV and telomere length in a large urban US population, and suggested a biologic mechanism for macrocytosis of aging [23].

In contrast with our findings, De Meyer *et al.* found a positive association of TL and red blood cell count but no significant association of TL and hematocrit, hemoglobin or leukocytes in middle-aged subjects in the Asklepios study [25]. Interestingly, some of the investigations found no significant association between telomere length and complete blood count in elderly individuals [26, 27]. Den Elzen *et al.* proposed an explanation for the lack of association between telomere length and hematological parameters in elderly people – that telomere length in WBC in old age

Table I. Sample size and weighted characteristics of NHANES 1999–2002 adult participants

Parameter		*N*	Weighted distributions of the participants
Gender	Men	4123	47.8%
	Women	4769	52.6%
Age, mean ± SEM [years]		8892	41.82 ±0.28
Education level	Less than high school	2461	19.8%
	Completed high school	1815	25.4%
	More than high school	3507	54.6%
Race/ethnicity	White (non-Hispanic)	4066	70.3%
	Non-Hispanic black	1738	10.7%
	Mexican-American	2315	7.2%
	Other Hispanic and other	773	11.8%

Table II. Multivariate linear regression (stepwise mode) and partial correlation (100 bootstrap replications) for determining the relation between telomere length and CBC

Variables	Linear regression		Partial correlation
	β coefficient	95% CI	Coefficient
Monocyte count	−0.051	(−0.422, −0.142)	−0.057*
Basophils (%)	0.046	(0.049–0.171)	0.048*
Mean cell hemoglobin [pg]	−0.051	(−0.038, −0.011)	−0.43*
Red cell distribution width (%)	−0.031	(−0.054, −0.003)	−0.08

*Both models adjusted for age, race, gender, and body mass index. *p > 0.001.*

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is unstable and does not have enough predictive power for age-related morbidity and mortality at ages over 85 years [26, 28]. However, Gutmajster *et al.* found a weak and independent correlation of TL with both RBC and WBC in elderly individuals [29]. Therefore, the type of cells studied (peripheral blood mononuclear cells, hematopoietic cells, lymphocytes, etc.) and differences in studied groups should be considered when interpreting clinical studies and trials.

Interestingly, some of the recent literature stated that telomere length varies according to the type of lymphocytes studied: B cells have the longest telomere length and highest telomerase activity, in contrast to CD8+ CD28– T cells; moreover, women have longer telomeres than men and may be protected by their hormonal status [22, 30]. Shorter TL in men may explain gender-specific dissimilarities in the degree of peripheral blood cell count. Therefore, in comparison to women, it should be easier to identify associations in men [29]. In addition, other potential confounders including stress and cortisol can affect telomere length and WBC counts [22, 30, 31].

This study has several strengths. To our knowledge, it is the largest study of the association of CBC and TL. The study is sufficiently powered to test the associations. The selection of the participants was based on random sampling of the general population, and therefore the results obtained from nationally representative samples can be extrapolated to the general population.

The findings from our study have to be considered in the context of some study limitations. The cross-sectional nature does not allow inference about causality. Unfortunately, we did not have available any repeated measure of TL with quantitative polymerase chain reaction in the same subjects after several follow-up years to elucidate the temporality of these findings.

This study has important clinical and public health implications. Understanding the interplay between CBC and TL is a necessary and important step toward any application of the resulting knowledge for public health policy and action, as it has been suggested that shorter telomeres have been associated with coronary heart disease [32, 33]. Our study provides a comprehensive snapshot of the relationship of CBC with TL at the national level in the US.

In conclusion, according to the findings of the present study, it can be concluded that telomere length is associated with blood cell parameters. Further investigations with a rigorous study design allowing correction for factors known to influence telomere length are necessary in order to clarify the clinical implications of measuring this parameter.

Conflict of interest

The authors declare no conflict of interest.

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