Comparison of Telomere Length Between Buccal Cells and Blood Cells

Lu Xu
Hangzhou Normal University
Zhuoziqi Qiu
Hangzhou Normal University
Yu-Sheng Cong (yscong@hznu.edu.cn)
Hangzhou Normal University

Research Article

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Abstract

Background

Telomere length (TL) in blood has been extensively studied as a biomarker of aging and aging-associated disease. TL in blood cells is commonly used as a proxy for TL in other tissue types. The source of DNA of adequate quality and quantity is an important consideration in telomere length analysis. Compared to blood cells, buccal cells easy for genomic DNA preparation would facilitate the rapid and reliable telomere length analysis. However, the feasibility of buccal cells for TL analysis remains yet unestablished.

Methods

A total of 52 participants ranged in age from 18 to 80 years including 24 males and 28 females were included in this study. Both buccal and blood samples were taken at the same time by using buccal cell swabs and fingertip stick from each participant. Relative telomere length (RTL) was analyzed using the quantitative real-time polymerase chain reaction (qPCR) method.

Results

The results indicate that there is a strong positive correlation between buccal RTL and blood RTL and negative correlation between both buccal RTL and blood RTL with age.

Conclusion

The validity of sampling using buccal cell swabs provides simple operation and good reproducibility for telomere length analysis, which overcomes the discomfort and risk of infection caused by blood sampling.

Background

Telomere is a repetitive DNA sequence located at the end of a chromosome, which plays an important role in maintaining the stability of the chromosome [1]. Due to the “end replication problem” [2, 3], the length of telomeres gradually decreases with cell division. When telomeres are shortened to a critical length, irreversible cellular senescence is induced. Therefore, TL is considered to be an important biological indicator of individual aging. Meanwhile, decades of research have shown that telomere shortening critically involved in the occurrence and development of aging-related diseases, including cancer [4]. The length of telomeres is closely related to the individual aging and disease occurrence. Therefore, detection of telomere length could help to monitor individual physiological and pathological conditions and provides scientific basis for public health management.

Epidemiologic studies of TL predominantly use blood as a source of DNA. Recent studies have shown that TL in blood cells is used as a proxy for telomere length in many tissue types [5]. TL in blood cells has been widely studied as a biomarker of human aging and risk factors for age-related diseases. Unfortunately, the blood collection process is invasive with potential safety issues and uncomfortable. In addition, although the method of extracting DNA from peripheral blood is commonly used, it can only be tested in places with medical facilities [6, 7], which limits the scope of TL measurement. Consequently, establishing an easy and convenient method of telomere length detection in a wider scale is of necessity.

Recently saliva has used as source of genomic DNA for the detection of TL [8, 9]. Saliva collection has the advantages of convenience and painlessness for genomic DNA extraction. However, the previous reports mainly analyzed the relationship between TL in saliva and TL in venous blood in children or newborns, changes in telomere length with age have not been characterized. Buccal swabs and saliva are the two most commonly used oral sampling methods in medical research. Studies have shown that buccal swabs contain a higher proportion of epithelial cells than saliva, which is conducive to genomic DNA preparation [10]. Here, we assessed feasibility of using buccal cells as alternative and convenient approach for telomere length analysis.

Materials And Methods

Study Participants.

The current study was conducted in a Chinese cohort from Guilin City of Guangxi Province in February 2021. The study protocols and consent procedures had been approved by the Ethics Committee of Hangzhou Normal University. All participants have signed the informed consent forms. A total number of 52 participants (24 males and 28 females) between 20 and 80 years old were recruited into this study.

Sample collection, DNA isolation, and qPCR.

Both buccal and blood samples were taken at the same time by using buccal cell swabs and fingertip stick from each participant. Genomic DNA was extracted by TIANamp Micro DNA Kit (TIANGEN) and quantified using a NanoDrop One (Thermo Fisher Scientific). Relative telomere length (RTL) was analyzed using the quantitative real-time polymerase chain reaction (qPCR) method previously described[11]. The reaction mix contained 10 ng genomic DNA, iTaq Universal SYBR Green Supermix (Bio-Rad), primers for telomeres and the single copy gene 36B4, and nuclease-free water to complete the final volume (10ml). The primer sequences are Tel-F, 5’-CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT-3’; Tel-R, 5’-GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT-3’; 36B4-F, 5’- CAGCAAGTGGGAAGGTGTAATCC-3’; 36B4-R, 5’-CCCATTCTATCATCAACGGGTACAA-3’. Standard curves were made by serial dilutions of known amounts of human genomic DNA and generated by the Bio-Rad CFX Manager software. The $2^{-\Delta\Delta Ct}$ equation has been adopted for relative telomere length measurement, which uses the difference of cycle

thresholds (CT) of the telomere signal (T) and a single copy gene (S) to calculate the relative gene expression levels (T/S ratio).

Statistical analyses.

Statistical analyses were conducted using the Excel, SPSS 25.0 software package (IBM), and GraphPad Prism 7.0 (https://www.graphpad.com). Buccal RTL, blood RTL and age were used as continuous variables while sex as a nominal variable. We conducted Pearson correlations to assess the concordance of buccal RTL and blood RTL. Statistical significance was defined as *P < 0.05, **P < 0.01 or ***P < 0.001.

Results

A total of 52 participants, 24 males and 28 females, ranged in age from 18 to 80 years were included in this study. The genomic DNA of participants were collected by buccal cell swabs and fingertip sticks. We have examined the quality of DNA samples through measurement of the OD260/OD230 and OD260/OD280 ratios. The quality of DNAs extracted by the two methods were comparable.

Relative telomere length (RTL) was analyzed using the method of quantitative real-time polymerase chain reaction (qPCR) method. The results showed a highly positive correlation between buccal RTL and blood RTL ($r=0.877$, $p<0.001$) (Fig. 1), which suggests the feasibility that TL measurement in buccal cells may be used as an alternative to blood TL.

To assess whether buccal RTL is associated with age as blood RTL does. We analyzed the correlation between RTL and age in the two sources of genomic DNAs (Figure 2) and results showed that both buccal RTL ($r=-0.832$, $p<0.001$) and blood RTL ($r=-0.866$, $p<0.001$) are negatively correlated with age, respectively. The results suggest that buccal RTL, like blood RTL, can be used as biomarker of aging or aging associated disease.

Discussion

This study provides evidence of the concordance of RTL in buccal cells and in blood cells, validating the feasibility of using buccal cells for TL analysis with easy access, simple operation, and good reproducibility. We recruited 52 volunteers ranging from 18 to 80 years old and prepared genomic DNAs from buccal and blood cells by methods of buccal cell swabs and fingertip stick using for each participant. The results indicate that there is a strong positive correlation between buccal RTL and blood RTL and negative correlation between both buccal RTL and blood RTL with age, which not only supports the feasibility of using buccal cells for TL analysis and the validity of buccal RTL but also validates the feasibility of using buccal cells for TL analysis as aging biomarker like blood RTL.

Declarations

Ethics approval and consent to participate: Research involving human subjects complied with all relevant national regulations, institutional policies and is in accordance with the tenets of the Helsinki Declaration (as revised in 2013), and has been approved by the authors' Institutional Review Board (approval number 2019064).

Consent for publication Not applicable.

Availability of data and material The data supporting the conclusions of this article are included within the article.

Competing interests: The authors declare that they have no competing interests

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Authors' contributions: L.X., Z.Q., and Y.-S.C. conceived of and designed the experiments. L.X., Z.Q. performed the experiments and data analyses. L.X., Z.Q., and Y.-S.C. wrote the paper. All authors have read and approved the manuscript.

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Figures

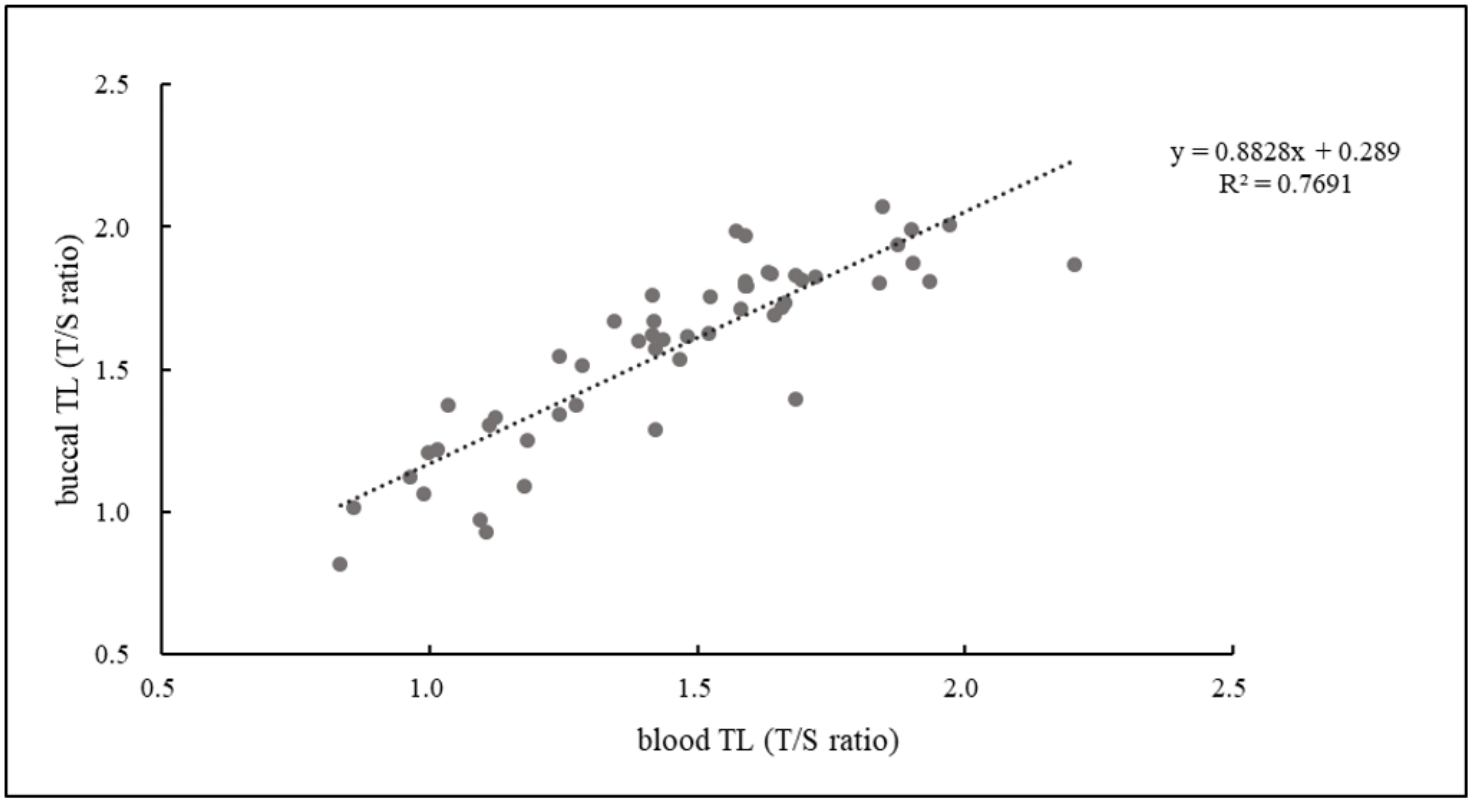

Figure 1

The relationship between buccal RTL and blood RTL. A total number of 52 participants were recruited into this study. Relative telomere length (RTL) was analyzed using the quantitative real-time polymerase chain reaction (qPCR) method. The primer sequences are Tel-F, 5'-CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT-3'; Tel-R, 5'-GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT-3'; 36B4-F, 5'- CAGCAAGTGGGAAGGTGTAATCC-3'; 36B4-R, 5'-CCCATTCTATCATCAACGGGTACAA-3'. Pearson correlation was used to assess the consistency of buccal RTL and blood RTL. The results show that buccal RTL and blood RTL are highly positively correlated ($r=0.877$, $p<0.001$). Circles represent individual data points; the line represents linear fit.

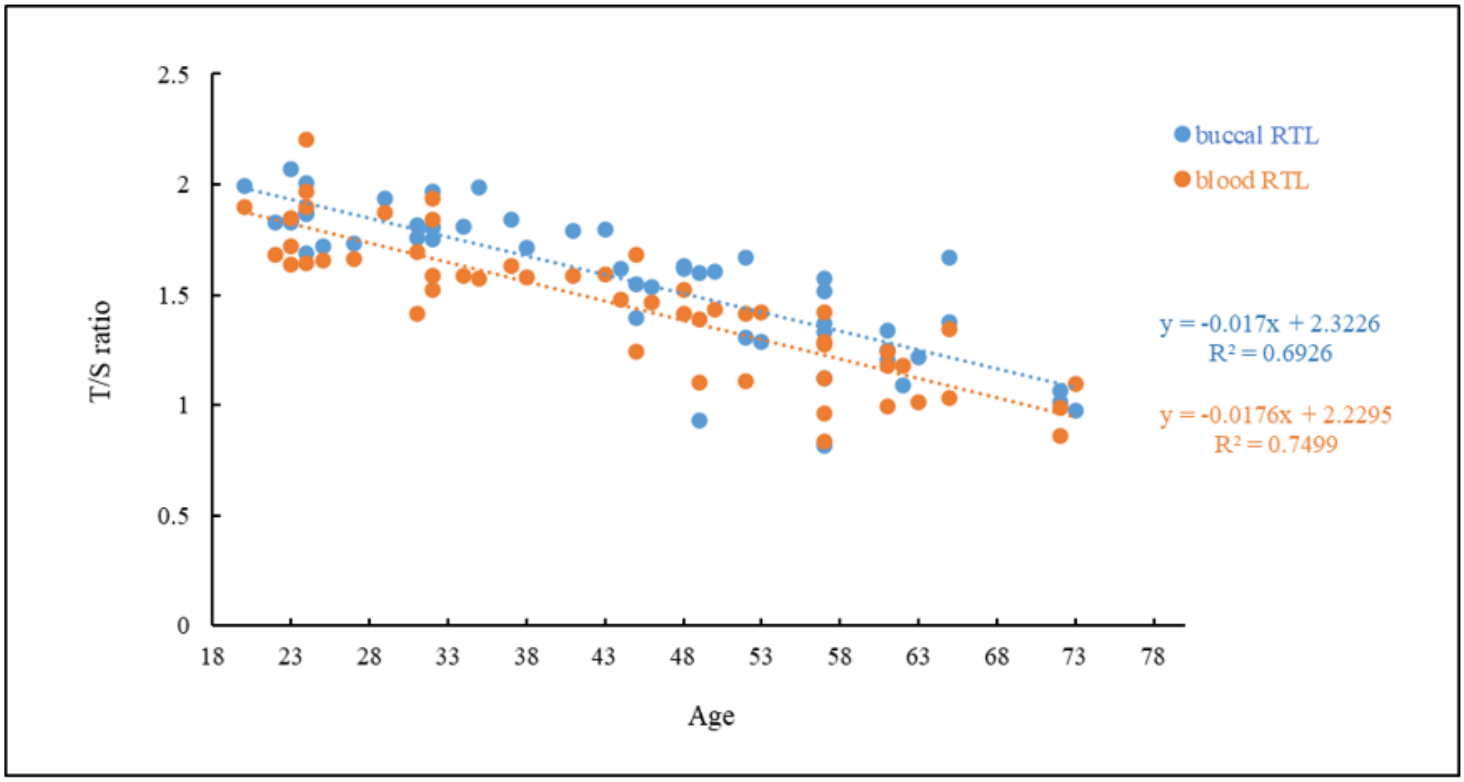

Figure 2

The relationship between RTL and age. Data plotted was derived from the dataset of 52 participants between 18 and 80 years old. Both buccal RTL (r=-0.832, p<0.001, blue) and blood RTL (r=-0.866, p<0.001, orange) are negatively correlated with age respectively. Circles represent individual data points; lines represent linear fits.