Telomere length and cognitive function in southern Chinese community-dwelling male elders

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Abstract

Background: telomere attrition has been associated with an increased risk of different age-related diseases and is widely accepted as a marker of cellular ageing. On the other hand, it is well known that cognitive function declines with age. The telomere length may therefore act as a marker for the pathway associated with cognitive function.

Methods: we examined telomere length and cognitive functions in a community-dwelling Chinese male population aged 65 years and above living in Hong Kong. The telomere length was measured by quantitative real-time PCR in 976 men. Cognitive function was assessed by Chinese (Cantonese) version of Mini-Mental State Exam and Community Screening Interview for Dementia.

Results: our result showed there was a significant association between telomere length, delayed recall ($P = 0.007$) and category verbal fluency ($P = 0.048$). These associations remained significant after adjustment for age and education. Further analysis using a cut-off score for MMSE, three-item recall and word list generation tests suggested that the telomere length was positively correlated with performance in these areas ($P = 0.015$).

Conclusion: the findings support the association of telomere length and cognitive function and suggested that the telomere length may serve as a biological marker for cognitive decline.

Keywords: cognitive decline, telomeres, genetics, elderly, older people

Introduction

Inflammation has been widely recognised to contribute in the pathogenic mechanism in Alzheimer’s disease (AD). Certain polymorphisms in immunoregulatory genes (TNF, IL-10, COX-2) were associated with the predisposition of AD [1, 2]. Although the association is well established, it remains uncertain how an exaggerated immune response leads to neuronal cell loss, decline of cognitive functions and AD. Supporting cells in the CNS (microglial and astrocytes) would have a high turnover rate and exacerbated the process of cellular ageing as a consequence of inflammation. A hypothesis suggests that the chronic process of exacerbated cellular senescence due to high cell turnover
associated with inflammation may account for the neuronal and other insult associated with inflammatory response.

Telomeres are nucleo-protein complexes at the end of mammalian chromosomes, comprising of a short repetitive DNA sequence TTAGGG, which is important in protecting the structural integrity of chromosome and preventing abnormal fusion between chromosome ends. The telomere is shortened by 30–200 nucleotides each time a somatic cell replicates [3] as the DNA polymerase is unable to completely replicate them. Therefore, the measurement of telomere length reflects the replication history of cells, and the telomere length is associated with senescence and apoptosis [4]. The telomere length has been proposed as a biomarker for ageing [5] and associated with lifespan [6] and various age-related conditions such as cancer [7], cardiovascular disease [8], osteoporosis [9], vascular dementia [10] and AD [11]. AD patients had shorter telomere in peripheral blood lymphocytes, which was correlated with higher serum TNF concentration and higher percentage of heat-induced apoptosis in T cells [11]. In addition, shorter telomere in AD also predicted higher mortality [12]. Therefore, these studies suggested that inflammation exacerbates the biological ageing and cell turnover resulting in cellular senescence, which may account for the loss of cognitive function. However, contradictory results on the association between telomere length and life span were reported [13]. The use of the telomere length as a biomarker for ageing remains inconclusive.

There is high inter-individual variability in an age-related cognitive decline, as it is significantly modulated by genetic and environmental factors [14]. Oxidative stress and individual variation may influence the rate of telomere attrition [15]. It is hypothesised that telomere length attrition is associated with the cognitive decline, which might reflect the consequences of increased oxidative stress. There were a few studies investigating the association of the telomere length and the cognitive function or rate of the cognitive decline in non-demented elders [16, 17] or other age groups of healthy individuals [18]. However, such investigation was not performed in populations other than Caucasians. In view of the possible difference in the genetic makeup between Chinese and other populations, we examined the association between telomere length and cognitive parameters in a large cohort of 976 healthy Chinese male elders in this study.

**Materials and methods**

**Study participants**

The subjects were part of a community elderly cohort recruited for a health survey between 2001 and 2003 in Hong Kong. They were invited to attend a health check carried out in the School of Public Health of the Chinese University of Hong Kong, by placing recruitment notices in community centres for the elderly and housing estates. Several talks were also given at these centres explaining the purpose, procedures and investigations to be carried out. The study has been approved by the Clinical Research Ethics Committee of the Chinese University of Hong Kong, which requires informed consent to be obtained. The present study included data from 976 men for whom both telomere length and cognitive function data are available.

Participants were screened with Cantonese version of the Mini-Mental State Examination (CMMSE) [19] and Community Screening Interview for Dementia [20]. The sections on three-item recall and category verbal fluency test (number of animals named in 1 min) were adopted for episodic memory and executive function, respectively.

**Measurement of telomere length**

The principle of the laboratory method has been previously described [21]. In short, DNA was extracted in the peripheral blood by the phenol–chloroform method and stored at −80°C. Telomere length measurement followed the method published by Cawthon [22] with modification [23] by obtaining a corrected Ct ratio of telomere and control gene (36B4) from quantitative real-time PCR (T/S ratio).

Quantitative real-time PCR was performed by Roche LightCycler 480 (Roche, Mannheim, Germany). The efficiency of the experiment was calculated by generating a curve of Ct values with four serially diluted control samples. The T/S ratio (−dCt) for each sample was calculated by the following equation:

$$
\frac{(PCR \ efficiency \ of \ the \ T \ assay \ plate)^\Delta Ct \ of \ sample \ in \ T \ assay}{(PCR \ efficiency \ of \ the \ S \ assay \ plate)^\Delta Ct \ of \ sample \ in \ S \ assay}
$$

The T/S ratio was plotted against the standard curve produced from five control subjects with the telomere length quantified by terminal restriction fragment analysis with the commercial kit TeloTAGGG Telomere Length Assay (Roche, USA), to obtain the telomere length in kilo base pairs (kb). The detailed methodology on the calibration was described previously [21, 24]. The average coefficient of correlation between the two parameters was $r^2 = 0.63$, which is not different from that reported in the original article by Cawthon [22], $r^2 = 0.68$. Calibration of the telomere length in kb was carried out independently for each batch, partially corrected for between-batch variation in the determination of the T/S ratio and the telomere length. The within-batch and between-batch CV% of the T/S ratio was 8.5 and 7.5%, respectively.

**Statistical analysis**

The independent $t$-test was used to assess the correlation between age and telomere length in the tertile data. Non-parametric Spearman’s correlation was used to study the association between the telomere length and the parameters for cognitive function. Linear regression was performed to identify the relationship between telomere
lengths and global cognitive function as measured by CMMSE, three-item recall and word list generation tests. All analyses were adjusted to age, and the significant level was set to 0.05 (two-sided). SPSS 17.0 was used for all the analyses.

Results

The age of the subjects included in this study was 65–91 (mean = 72.6, SD = 5.0). The telomere length of the study participants ranged between 3.75 and 13.17 kb, and the mean telomere length was 8.81 kb (Figure 1). The participants were divided into three groups based on the telomere length. The ‘short’ telomere group was composed of 335 individuals with the mean telomere length of 7.20 kb. The ‘medium’ telomere group was composed of 356 individuals with the mean telomere length of 8.85 kb and the ‘long’ telomere group was composed of 251 individuals with the mean telomere length of 11.00 kb. No significant difference in the education level was found among different telomere groups (Table 1). There was a trend towards negative correlation between telomere length and age in the three telomere length tertile groups, but this trend did not reach statistical significance (P = 0.058). The telomere length was negatively associated with age (Pearson’s correlation coefficient r = –0.085, P = 0.008).

No association between the telomere length and the MMSE score was found (P > 0.05). We divided the subjects according to the generally accepted MMSE cut-off of 24 for cognitive impairment [25], MMSE ≤ 24 and MMSE > 24. Subjects with MMSE ≤ 24 had on average 251 base pairs shorter in the telomere length than those with MMSE scored > 24 (P = 0.04) (Figure 2). The episodic memory of the subjects was reflected by the three-item recall test. A significant correlation was identified between the telomere length and episodic memory recall (r = 0.086, P = 0.007). Subjects in ‘long’ or ‘medium’ telomere groups performed significantly better in three-item recall (mean = 1.03, SD = 1.09) when compared with those in the ‘short’ telomere group (mean = 0.84, SD = 1.03) (P = 0.006) (Table 2A). Executive function of the subjects was tested by the verbal fluency test. Significant association between the telomere length and the score in the executive function test was found. A significant correlation between verbal fluency and telomere length was identified (r = –0.053, P = 0.048) (Table 2B). The correlation between telomere length and CMMSE, episodic memory or executive function remained significant in a linear regression model adjusted for age and education (P = 0.02 and P = 0.03, respectively). However, no significant association between telomere length and logical memory was identified (P > 0.05).

Subjects were classified of having better cognitive performance with the following cut-off score: MMSE > 24, three-item recall > 1 and verbal fluency > 12. The cut-off scores were selected based on the generally accepted cut-off score or results from local validation study [25–27]. According to this classification system, subjects were divided into groups having 0 to 3 tests with scores above the cut-off value. The more the score above the cut-off score, the better the performance. A significant correlation was found between the summative score of these three tests and the telomere length (r = 0.078, P = 0.015) (Table 2). Our results showed that the performance of the subjects was positively correlated with the telomere length. Subjects with all three tests scored above the cut-off score had a significantly longer mean telomere length, 9.03 kb when compared with those scored below the cut-off score, with the telomere length of 8.67 kb (Table 3).

Discussion

The telomere length has been linked to lifespan [6], and it has been proposed as the biomarker of ageing [5]. The result of the present study is consistent with previous studies reporting a negative correlation between telomere length and age [3, 5, 16]. Previous studies reported an association between telomere length and risk of dementia [10],

Table 1. Descriptive statistics of the study cohort by telomere tertile group

<table>
<thead>
<tr>
<th>Telomere tertile group</th>
<th>Short (n = 352)</th>
<th>Medium (n = 360)</th>
<th>Long (n = 255)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean, SD)</td>
<td>73.3 (5.1)</td>
<td>72.5 (4.8)</td>
<td>72.5 (5.2)</td>
<td>0.058</td>
</tr>
<tr>
<td>Education &lt; high school</td>
<td>61.0</td>
<td>60.9</td>
<td>63.6</td>
<td>0.356</td>
</tr>
</tbody>
</table>

SD, standard deviation.
and the mean telomere length is shorter among AD patients when compared with control subjects [12]. However, the association between telomere length and cognitive outcomes in non-demented population was controversial [16, 18].

The central finding of the current study identified cognitive performance, specifically episodic memory and executive function correlated significantly with telomere length adjusted for age, among healthy community-dwelling elderly men. Ageing is known to be accompanied by episodic memory decline [28] and executive dysfunction [29]. Our finding suggested that the telomere length was associated with these two areas of cognitive function in addition to the effect of age. One of the possible mechanisms for this association would be the increased oxidative stress [30]. Previous studies suggested that increased oxidative stress is not only limited to neurodegenerative diseases such as AD, but also found in normal ageing with decline in cognitive performance [31]. Oxidative stress plays an important role in modulating both telomere length [15] and cognitive decline [17]. Besides, the telomere length may also be negatively correlated with the status of inflammation [9]. Therefore, inflammation and oxidative stress might both modulate the telomere length and regulating the degree of the cognitive decline.

Interestingly, our results suggested that the telomere length was not directly correlated with the MMSE score but subjects with better performance in MMSE have significantly longer telomere length. On the other hand, the summative score representing the overall performance in MMSE, three-item recall and verbal fluency was positively correlated with the telomere length. Since MMSE is a test for the global cognitive function including orientation, repetition, attention, recall, language and design copying [32], the telomere length might not be implicating in all areas of cognition and direct correlation between the telomere length and MMSE performance might not be observed. Using three-item recall and verbal fluency, the subjects’ episodic memory and executive function were assessed; a strong association between these cognitive areas and the telomere length was revealed. It is possible that these areas of cognitive function are strongly influenced by similar genetic factors or pathways as for telomere length regulation.

Our study identified the correlation between the telomere length and specific areas of cognition including episodic memory and executive function. We note the potential limitation of our study. On the basis of the result of the current study, we were unable to identify whether the telomere length regulates the initiation of the cognitive decline or it affects the rate of decline. A longitudinal study measuring the rates of change in the telomere length will provide further information on this issue. Despite the limitation of the study, one of the strengths of our study was the inclusion of a large cohort of community elderly subjects. Therefore, this study has the power to detect any association between cognitive function and telomere length.

Based on our findings and others’ previous studies, it is concluded that there is strong correlation between the telomere length and the cognitive decline. Development of markers to predict the cognitive decline will be important for early detection and treatment. Biomarkers should be non-invasive, inexpensive and readily available; the telomere length can be easily measured by routine blood withdrawal, and therefore, it has good potential to be developed as a prognostic marker for the cognitive decline to identify individuals at risk.
Key points

- Telomere length is widely accepted as a marker of cellular ageing.
- Significant association between telomere length, delayed recall and category verbal fluency was observed in a community-dwelling Chinese male population aged 65 years and above in Hong Kong.
- Our finding may provide further support for the development of using a telomere length as a marker for a cognitive decline.

Conflicts of interest

None declared.

Funding

The study was funded by the Direct Grant from the Chinese University of Hong Kong, School of Public Health, and the Hong Kong Jockey Club Charities Foundation.

References

Living and dying with dignity in Chinese society: perspectives of older palliative care patients in Hong Kong

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Abstract

Background: the empirical Dignity Model has profoundly influenced the provision of palliative care for older terminally ill patients in the West, as it provides practical guidance and intervention strategies for promoting dignity and reducing distress at the end-of-life.

Objective: to examine the concept of ‘living and dying with dignity’ in the Chinese context, and explore the generalisability of the Dignity Model to older terminal patients in Hong Kong.

Methods: using qualitative interviews, the concept of dignity was explored among 16 older Chinese palliative care patients with terminal cancer. Framework analysis with both deductive and inductive methods was employed.

Results: the three major categories of themes of the Dignity Model were broadly supported. However, the subtheme of death anxiety was not supported, while two subthemes of generativity/legacy and resilience/fighting spirit manifested differently in the Chinese context. Furthermore, four new emergent themes have been identified. They include enduring pain, moral transcendence, spiritual surrender and transgenerational unity.

Conclusion: these findings highlight both a cultural and a familial dimension in the construct of dignity, underline the paramount importance of cultural awareness and competence for working with ethnically diverse groups, and call for a culturally sensitive and family oriented approach to palliative care interventions with older Chinese terminal patients.

Keywords: Chinese, elderly, dignity, palliative care, qualitative research, older people