

Research Article

The association of mean telomere length with all-cause, cerebrovascular and cardiovascular mortality

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Mean telomere length (MLT) is a marker of cell aging and may associate with age-related diseases. However, the relationship between MLT and mortality risk remains unclear. We aimed to investigate the relationship between MLT and all-cause, cerebrovascular and cardiovascular mortality among adults in United States. We analyzed data were from National Health and Nutrition Examination Survey (NHANES, 1999–2002) with follow-up data through 31 December 2015. Based on MLT, participants were categorized into low, middle and high groups. Multivariate Cox proportional hazards regression, subgroup analysis and generalized additive model (GAM) were performed by using hazard ratios (HRs) and 95% confidence intervals (CIs). A total of 7827 participants were included in analysis (48.18% male). After 158.26 months of follow-up on average, there were 1876 (23.97%), 87 (1.11%) and 243 (3.10%) onset of all-cause, cerebrovascular and cardiovascular mortality. After adjustment for potential confounders, using the low group as the reference, HRs for all-cause (0.87 and 0.86), cerebrovascular (0.75 and 0.75) and cardiovascular mortality (1.01 and 0.69) for the middle to high groups were not statistically significant (all $P > 0.05$ for trend). MLT was non-linearly related to all-cause mortality but not to cerebrovascular and cardiovascular mortality. It was the first study to demonstrate the non-linear relationship between MLT and all-cause mortality.

Introduction

With the development of life sciences and technology, a large number of experiments indicated that circulating biomarkers such as telomere length might associate with the risk of mortality [1]. Specifically, cardiovascular mortality has been an increasing burden for public health, more efforts are needed to elucidate the associated risk factors.

Telomeres are the caps and repetitive nucleotide sequences located at the end of linear chromosomes of most eukaryotic organisms [2]. Telomere is also a necessary and vital part of human cells which shortens throughout the lifespan that affect our cells age in humans [3]. Telomere length has been acting as an important biomarker of biological age and was linked to the etiology of age-related diseases, such as atherosclerosis [4], Alzheimer's disease [5], stroke [6] and coronary heart disease [7]. Short leukocyte telomere length is a hallmark characteristic of aging and might predict mortality independent of chronological age [8,9]. Though several epidemiological and clinical studies showed a relationship between shorter telomere length and the risk of morbidity and mortality, the results remained elusive and heterogeneous [11–17]. In addition, to the best of our knowledge, most of the previous studies were conducted among elderly or with small sample size. Evidence from a large sample of a well-designed cohort study among general population is lacking. Therefore, we aim to investigate the potential association between mean

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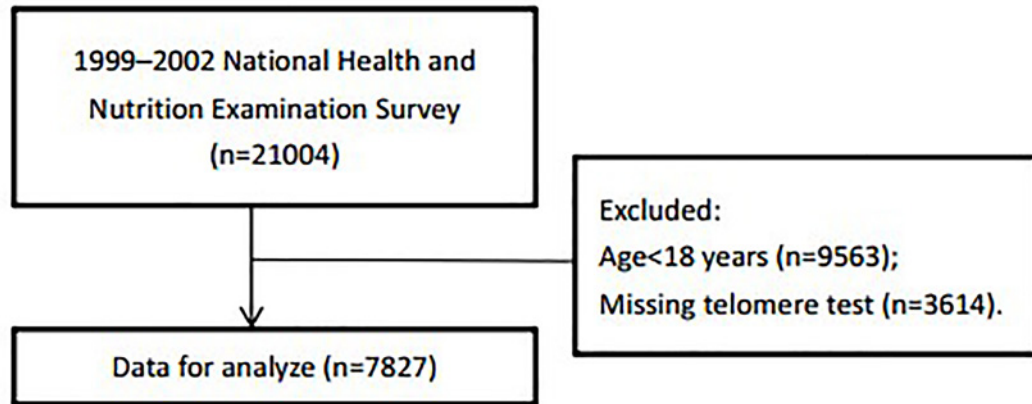


Figure 1. The research flow chart

telomere length (MTL) and all-cause, cerebrovascular and cardiovascular mortality using the data from the National Health and Nutrition Examination Survey (NHANES).

Materials and methods

Study design and study population

The NHANES is a national representative survey of the civilian, non-institutionalized U.S. population conducted by the Centers for Disease Control and Prevention (CDC) [15]. In the present study, we included 7827 participants aged ≥ 18 and had the telomere test results from NHANES (1999–2002) (Figure 1). The survey protocol was approved by the Institutional Review Board of the CDC. All participants provided written informed consent.

Measurements

Questionnaires were distributed to participants to acquire demographics information, including smoking, drinking, previous history of diseases, medication history. Physical assessments and laboratory tests were performed to examine systolic blood pressure (SBP), diastolic blood pressure (DBP), height, weight, fasting blood glucose (FBG), total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), C-reactive protein (CRP). Body mass index (BMI) was defined as mass (kg) divided by the square of height (m^2). Estimated glomerular filtration rate ($\text{ml}/\text{min}/1.73 \text{ m}^2$) (eGFR) was calculated through the formula: $186 \times (\text{SCr})^{-1.154} \times (\text{age})^{-0.203} \times [0.724 \text{ if female}] \times [1.212 \text{ if Black}]$, where SCr is serum creatinine (mg/dl) [17]. An SCr level in $\mu\text{mol}/\text{l}$ was converted into mg/dl by dividing them by 88.4. The telomere length assay was performed by using the quantitative polymerase chain reaction (qPCR) method to measure telomere length relative to standard reference DNA (T/S ratio), as described in detailed previously [18]. Detailed procedures on questionnaires and testing methods can be found on the website (<https://wwwn.cdc.gov/nchs/nhanes/Default.aspx> and https://wwwn.cdc.gov/Nchs/Nhanes/1999-2000/TELO_A.html).

Mortality

All-cause and cardiovascular mortality data were abstracted from the 1999–2002 NHANES public-use linked mortality files (LMF), which captured the vital status and cause of death of survey participants from survey participation (1999–2002) up to 31 December 2015. We examined all-cause mortality, as well as mortality due to cerebrovascular and cardiovascular diseases. Cardiovascular and cerebrovascular mortality was defined by International Classification of Diseases, 10th Edition, Clinical Modification System codes I00–I78 derived from death-certificate data. Detailed mortality variables for participants can be found on the website (<https://www.cdc.gov/nchs/data-linkage/mortality-public.htm>).

Statistical analysis

Participants were classified into three groups (low, middle, high) according to MLT. All the continuous variables were presented as mean \pm standard deviation, and categorical variables were presented in frequency or as a percentage. The One-Way Anova, Kruskal–Wallis Rank Sum Test, Fisher test and chi-square tests assessed subgroup differences.

We used multivariate Cox proportional hazards regression to estimate the risk of all-cause, cerebrovascular and cardiovascular mortality and hazard ratios (HRs) and 95% confidence intervals (CIs) were shown. Censoring was done at the date of death or 31 December 2015, whichever came first. Model I only included MLT. The model II was adjusted for age, gender and BMI. The model III was further adjusted for SBP, CRP, TC, HDL-C, alcohol, smoking, marriage, poverty income ratio, education, race, diabetes, hypertension, cardiovascular diseases, eGFR, the use of antihypertensive drugs, lipid lowering drugs and anti-diabetic drugs. Kaplan–Meier survival curves for incidence of all-cause mortality between difference blood pressure groups were plotted and compared by the log-rank test. Subgroup analyses were conducted by stratifying age (<65 and ≥ 65 years), gender (female and male), BMI (<25 and ≥ 25 kg/m²), eGFR (<90 and ≥ 90 ml/min/1.73 m²), history of diabetes (yes and no), hypertension (yes and no) or cardiovascular disease (yes and no) at baseline. In addition, we used generalized additive model (GAM) to identify the non-linear relationship and the likelihood ratio test was performed. If the non-linear correlation was observed, a two-piecewise linear regression model was performed to calculate the threshold effect of the MLT on mortality in terms of the smoothing plot. A two-sided $P < 0.05$ was considered statistically significant. All statistical analyses were performed using R version 3.3.2 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Baseline characteristics

There were total 7827 subjects in the study and 3771 (48.18%) were males. The characteristics of all participants are shown in Table 1. Briefly, median values of MLT in the lowest to highest quartiles were 0.77 ± 0.09 , 1.00 ± 0.06 and 1.32 ± 0.26 T/S ratio. During the mean follow-up period of 158.26 ± 44.71 months, there were 1876 (23.97%), 87 (1.11%) and 243 (3.10%) onset of all-cause, cerebrovascular and cardiovascular mortality, respectively. In addition, there were significant subgroup differences in age, gender, BMI, SBP, FBG, CRP, TC, TG, LDL-C, HDL-C, alcohol, smoking status, marriage, poverty income ratio, education level, race, diabetes, hypertension, cardiovascular diseases, eGFR, the use of antihypertensive drugs, lipid lowering drugs and anti-diabetic drugs (all $P < 0.05$).

The relationship between MTL and all-cause, cerebrovascular and cardiovascular mortality

As shown in Table 2, when treating MLT as a continuous variable, MLT was inversely associated with all-cause (HR = 0.08, 95% CI: 0.06, 0.10; $P < 0.0001$), cerebrovascular (HR = 0.01, 95% CI: 0.00, 0.04; $P < 0.0001$) and cardiovascular (HR = 0.05, 95% CI: 0.03, 0.09; $P < 0.0001$) mortality in Model I. However, in the fully adjusted model (Model III), MLT showed no significant association with all-cause 0.78 (95% CI: 0.55, 1.10; $P = 0.1534$), cerebrovascular 1.21 (95% CI: 0.16, 9.24; $P = 0.8547$) and cardiovascular mortality 0.45 (95% CI: 0.14, 1.41; $P = 0.1707$). When treating MLT as a categorical variable (MLT-low group as reference), in the fully adjusted model, high level of MLT did not associate with the risk of all-cause 0.86 (95% CI: 0.70, 1.05; $P = 0.1332$) ($P = 0.127$ for trend), cerebrovascular 0.75 (95% CI: 0.20, 2.81; $P = 0.6729$) ($P = 0.593$ for trend) and cardiovascular mortality 0.69 (95% CI: 0.33, 1.42; $P = 0.3077$) ($P = 0.421$ for trend). However, Kaplan–Meier survival curves demonstrated that there were significant differences in the prevalence rate of all-cause, cerebrovascular and cardiovascular mortality among MLT groups (Figure 2).

Subgroup analysis of MTL with all-cause, cardiovascular and cerebrovascular mortality

Table 3 demonstrates the multivariate Cox regression analysis association between MLT with all-cause, cardiovascular and cerebrovascular mortality in subgroups. When treating MLT as a continuous variable, we only found that MLT was inversely associated with all-cause mortality among males (HR = 0.53, 95% CI: 0.30, 0.94; $P = 0.0302$), participants with eGFR < 90 ml/min/1.73 m² (HR = 0.54, 95% CI: 0.30, 0.98; $P = 0.0417$) or age < 65 years (HR = 0.45, 95% CI: 0.28, 0.75; $P = 0.0021$) or age ≥ 65 years (HR = 0.27, 95% CI: 0.12, 0.58; $P = 0.0009$). For cardiovascular mortality, the association was significant among those aged < 65 years (HR = 0.04, 95% CI: 0.01, 0.29; $P = 0.0015$).

The analyses of non-linear relationship

As shown in Table 4, two-piecewise linear regression model suggested that the inflection point of all-cause, cerebrovascular and cardiovascular mortality were 0.94 T/S ratio, 0.92 T/S ratio and 1.22 T/S ratio, respectively. On the left and right of the inflection points, the HRs of all-cause mortality were 0.41 (95% CI: 0.21, 0.79; $P = 0.0083$) and 1.25 (95% CI: 0.74, 2.13; $P = 0.4069$), respectively. However, on both sides of the threshold, the HRs for cerebrovascular mortality were 0.08 (95% CI: 0.00, 2.36; $P = 0.1412$) and 9.90 (95% CI: 0.75, 130.17; $P = 0.0812$), while that for

Table 1 Baseline characteristics of participants

| | Low (n=2609) | Middle (n=2609) | High (n=2609) | P |
|------------------------------------|-----------------|-----------------|-----------------|--------|
| Age (years) | | | | <0.001 |
| <45 | 595 (22.81%) | 1211 (46.42%) | 1723 (66.04%) | |
| ≥45, <65 | 851 (32.62%) | 820 (31.43%) | 619 (23.73%) | |
| ≥65 | 1163 (44.58%) | 578 (22.15%) | 267 (10.23%) | |
| BMI (kg/m ²) | 28.61 ± 6.07 | 28.41 ± 6.11 | 27.97 ± 6.26 | <0.001 |
| SBP (mmHg) | 122.25 ± 15.77 | 118.91 ± 15.41 | 116.91 ± 14.45 | <0.001 |
| DBP (mmHg) | 70.36 ± 14.74 | 70.74 ± 12.74 | 70.28 ± 12.61 | 0.498 |
| FBG (mmol/l) | 109.57 ± 37.88 | 104.54 ± 38.08 | 98.93 ± 29.16 | <0.001 |
| TC (mg/dl) | 207.59 ± 42.32 | 205.22 ± 41.67 | 200.10 ± 42.50 | <0.001 |
| TG (mg/dl) | 159.99 ± 105.70 | 156.08 ± 157.46 | 138.90 ± 108.10 | <0.001 |
| LDLC (mg/dl) | 124.53 ± 33.37 | 124.79 ± 35.49 | 121.25 ± 36.33 | 0.029 |
| HDLc (mg/dl) | 50.95 ± 15.62 | 51.66 ± 15.84 | 52.25 ± 15.46 | 0.011 |
| eGFR (ml/min/1.73 m ²) | 90.19 ± 36.78 | 97.55 ± 35.98 | 106.11 ± 45.58 | <0.001 |
| CRP (mg/dl) | 0.78 ± 1.60 | 0.64 ± 1.33 | 0.65 ± 1.35 | <0.001 |
| Alcohol (g) | 8.10 ± 32.36 | 9.96 ± 32.58 | 11.13 ± 34.72 | 0.005 |
| MTL (T/S ratio) | 0.77 ± 0.09 | 1.00 ± 0.06 | 1.32 ± 0.26 | <0.001 |
| Gender (n, %) | | | | <0.001 |
| Male | 1397 (53.55%) | 1203 (46.11%) | 1171 (44.88%) | |
| Female | 1212 (46.45%) | 1406 (53.89%) | 1438 (55.12%) | |
| Smoking (n, %) | | | | <0.001 |
| Non-smoker | 1238 (47.51%) | 1337 (51.36%) | 1441 (55.36%) | |
| Ex-smoker | 878 (33.69%) | 705 (27.08%) | 518 (19.90%) | |
| Current smoker | 490 (18.80%) | 561 (21.55%) | 644 (24.74%) | |
| Marriage (n, %) | | | | <0.001 |
| Married | 1572 (61.94%) | 1478 (59.48%) | 1303 (53.58%) | |
| Single | 697 (27.46%) | 510 (20.52%) | 359 (14.76%) | |
| Never married | 176 (6.93%) | 348 (14.00%) | 600 (24.67%) | |
| Others | 93 (3.66%) | 149 (6.00%) | 170 (6.99%) | |
| Educational level (n, %) | | | | <0.001 |
| Less than high school | 1041 (39.99%) | 841 (32.28%) | 758 (29.08%) | |
| High school diploma | 586 (22.51%) | 597 (22.92%) | 630 (24.17%) | |
| More than high school | 976 (37.50%) | 1167 (44.80%) | 1219 (46.76%) | |
| Race/ethnicity (n, %) | | | | <0.001 |
| Black | 356 (13.65%) | 406 (15.56%) | 571 (21.89%) | |
| Mexican American | 637 (24.42%) | 674 (25.83%) | 565 (21.66%) | |
| Other Hispanic | 126 (4.83%) | 122 (4.68%) | 169 (6.48%) | |
| Other race/ethnicity | 1490 (57.11%) | 1407 (53.93%) | 1304 (49.98%) | |
| Diabetes (n, %) | | | | <0.001 |
| No | 2169 (84.23%) | 2318 (89.57%) | 2398 (92.44%) | |
| Yes | 406 (15.77%) | 270 (10.43%) | 196 (7.56%) | |
| Hypertension (n, %) | | | | <0.001 |
| No | 1585 (60.94%) | 1808 (69.75%) | 1994 (76.90%) | |
| Yes | 1016 (39.06%) | 784 (30.25%) | 599 (23.10%) | |
| Cardiovascular diseases (n, %) | | | | <0.001 |
| No | 2410 (93.37%) | 2509 (96.61%) | 2533 (97.35%) | |
| Yes | 171 (6.63%) | 88 (3.39%) | 69 (2.65%) | |
| Antihypertensive drugs (n, %) | | | | <0.001 |
| No | 1846 (70.76%) | 2128 (81.56%) | 2277 (87.27%) | |
| Yes | 763 (29.24%) | 481 (18.44%) | 332 (12.73%) | |
| Lipid lowering drugs (n, %) | | | | <0.001 |
| No | 2332 (89.38%) | 2445 (93.71%) | 2499 (95.78%) | |
| Yes | 277 (10.62%) | 164 (6.29%) | 110 (4.22%) | |
| Anti-diabetic drugs (n, %) | | | | <0.001 |
| No | 2388 (91.53%) | 2462 (94.37%) | 2503 (95.94%) | |
| Yes | 221 (8.47%) | 147 (5.63%) | 106 (4.06%) | |

Continued over

Table 1 Baseline characteristics of participants (Continued)

| | Low (n=2609) | Middle (n=2609) | High (n=2609) | P |
|---------------------------------|---------------|-----------------|---------------|--------|
| All-cause mortality (n, %) | | | | <0.001 |
| No | 1592 (61.02%) | 2063 (79.16%) | 2292 (87.88%) | |
| Yes | 1017 (38.98%) | 543 (20.84%) | 316 (12.12%) | |
| Cardiovascular mortality (n, %) | | | | <0.001 |
| No | 2465 (94.48%) | 2545 (97.55%) | 2574 (98.66%) | |
| Yes | 144 (5.52%) | 64 (2.45%) | 35 (1.34%) | |
| Cerebrovascular mortality | | | | <0.001 |
| No | 2551 (97.78%) | 2588 (99.20%) | 2601 (99.69%) | |
| Yes | 58 (2.22%) | 21 (0.80%) | 8 (0.31%) | |

Data are expressed as mean \pm SD of three groups. Abbreviation: Q, quartile.

Table 2 Multivariate Cox regression analysis of MTL with all-cause, cardiovascular and cerebrovascular mortality

| All-cause mortality | Model I HR (95% CI) P | Model II HR (95% CI) P | Model III HR (95% CI) P |
|---------------------------|---------------------------|---------------------------|----------------------------|
| MTL (T/S ratio) | 0.08 (0.06, 0.10) <0.0001 | 0.75 (0.60, 0.94) 0.0120 | 0.78 (0.55, 1.10) 0.1534 |
| MTL groups (T/S ratio) | HR (95% CI) P | HR (95% CI) P | HR (95% CI) P |
| Low | 1.0 | 1.0 | 1.0 |
| Middle | 0.48 (0.43, 0.53) <0.0001 | 0.90 (0.81, 1.01) 0.0755 | 0.87 (0.74, 1.03) 0.1132 |
| High | 0.26 (0.23, 0.30) <0.0001 | 0.85 (0.74, 0.97) 0.0203 | 0.86 (0.70, 1.05) 0.1332 |
| P for trend | <0.001 | 0.012 | 0.127 |
| Cardiovascular mortality | HR (95% CI) P | HR (95% CI) P | HR (95% CI) P |
| MTL (T/S ratio) | 0.05 (0.03, 0.09) <0.0001 | 0.71 (0.37, 1.37) 0.3095 | 0.45 (0.14, 1.41) 0.1707 |
| MTL groups (T/S ratio) | HR (95% CI) P | HR (95% CI) P | HR (95% CI) P |
| Low | 1.0 | 1.0 | 1.0 |
| Middle | 0.41 (0.30, 0.55) <0.0001 | 0.85 (0.62, 1.17) 0.3128 | 1.01 (0.61, 1.66) 0.9799 |
| High | 0.21 (0.15, 0.31) <0.0001 | 0.82 (0.54, 1.25) 0.3573 | 0.69 (0.33, 1.42) 0.3077 |
| P for trend | <0.001 | 0.360 | 0.421 |
| Cerebrovascular mortality | HR (95% CI) P | HR (95% CI) P | HR (95% CI) P |
| MTL (T/S ratio) | 0.01 (0.00, 0.04) <0.0001 | 0.25 (0.07, 0.87) 0.0294 | 1.21 (0.16, 9.24) 0.8547 |
| MTL groups (T/S ratio) | HR (95% CI) P | HR (95% CI) P | HR (95% CI) P |
| Low | 1.0 | 1.0 | 1.0 |
| Middle | 0.34 (0.20, 0.55) <0.0001 | 0.87 (0.51, 1.48) 0.6009 | 0.75 (0.30, 1.89) 0.5444 |
| High | 0.12 (0.06, 0.26) <0.0001 | 0.44 (0.17, 1.14) 0.0912 | 0.75 (0.20, 2.81) 0.6729 |
| P for trend | <0.001 | 0.130 | 0.593 |

Data are expressed as HR and 95% CI.

Model I adjusted for None.

Model II adjusted for age, gender and BMI.

Model III adjusted for age, gender, BMI, SBP, CRP, TC, HDL-C, alcohol, smoking, marriage, poverty income ratio, education, race, diabetes, hypertension, cardiovascular diseases, eGFR, antihypertensive drugs, lipid lowering drugs and anti-diabetic drugs.

cardiovascular mortality were 0.61 (95% CI: 0.16, 2.30; $P=0.4675$) and 0.01 (95% CI: 0.00, 83.57; $P=0.3187$), respectively. As shown in Figure 3, GAM revealed that the relationship between MTL and all-cause mortality was non-linear but had no significant relationship with cerebrovascular and cardiovascular mortality after adjustment for potential variables.

Discussion

In the present study, our main findings were as follows: (1) MTL was non-linearly related to all-cause mortality in a large, nationally representative sample of U.S. adults; (2) MTL has no statistically significant relationship with cardiovascular and cerebrovascular mortality; (3) when MTL was less than 0.94 T/S ratio, it could increase the risk of all-cause mortality by 41% for every 0.1 T/S ratio shortening of telomere. However, when it was higher than 0.94 T/S ratio, there was no statistically significant relationship between MTL and all-cause mortality.

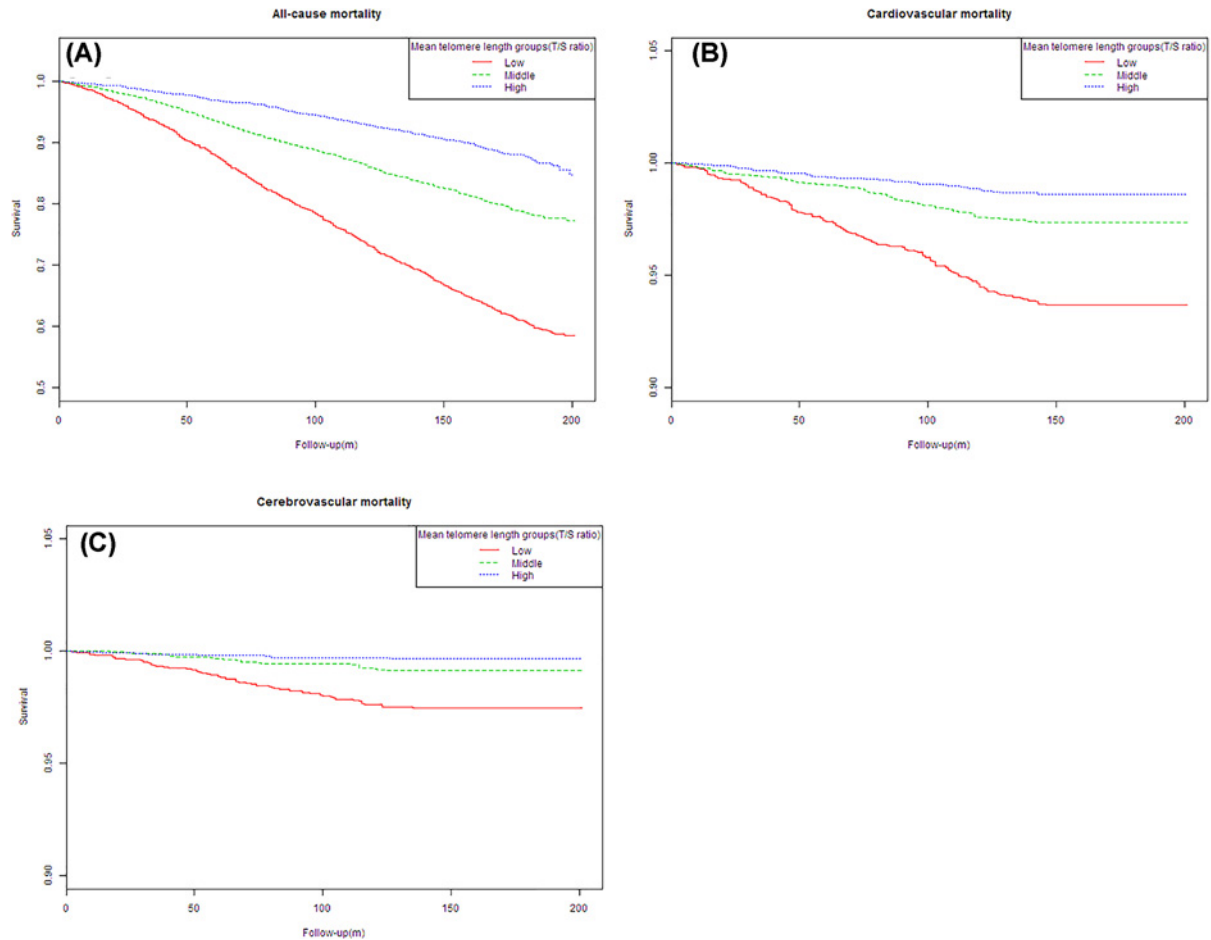


Figure 2. Kaplan–Meier estimated cumulative survival curves based on groups of MTL

In the present study, there was no statistically significant relationship between all-cause mortality when MTL was a categorical variable. A study from Swedish elderly men indicated that MTL attrition did not predict all-cause mortality [19]. Furthermore, Kotsopoulos et al. [20] provided preliminary evidence that telomere length likely did not predict mortality after a diagnosis of ovarian cancer. In addition, among a community-based cohort of African Americans, after a median follow-up of 9 years, longer telomere length was associated with a lower risk of incident ischemic stroke and total mortality in age- and sex-adjusted models, but these associations were no longer significant in fully adjusted models [21]. However, there were also a large number of studies that suggested telomere length relating to all-cause mortality in different population [22,23].

According to the present study, we also revealed that the high MTL group could have 31% reduction in the risk of cardiovascular mortality and 25% less risk of cerebrovascular mortality compared with the low MTL group, but these associations were no longer significant after adjustment for potential variables. These findings were in agreement with previous research that there was no significant association between telomere length and cardiovascular or cerebrovascular mortality [24,25]. In contrast, a previous study found a 60% decreased risk for cardiovascular mortality in those who shortened their telomeres [19]. Burnett-Hartman et al. [26] found that telomere biology and associated genes may play a role in cardiovascular disease-related death, particularly among women.

The possible reasons for the different results of our research and previous research may due to differential ethnicity background and covariates being adjusted. Most of the previous studies only adjusted for anthropometric and demographic features, but we adjusted more mortality-related additional confounding variables such as eGFR, CRP, blood lipids, FBG and taking drugs. We speculated that some of the confounding factors associated with mortality may have a certain impact on the results. Furthermore, telomere variation, telomere measurement techniques and age at measurement contributed to the heterogeneity and may also have an effect on the conclusion of some studies.

Table 3 Subgroup analysis of MTL with all-cause, cardiovascular and cerebrovascular mortality

| | Number | All-cause mortality HR (95% CI) P | Cardiovascular mortality HR (95% CI) P | Cerebrovascular mortality HR (95% CI) P |
|---------------|--------|--------------------------------------|---|--|
| Gender | | | | |
| Male | 3771 | 0.53 (0.30, 0.94) 0.0302 | 0.49 (0.11, 2.13) 0.3421 | 0.34 (0.02, 5.44) |
| Female | 4056 | 1.23 (0.67, 2.27) 0.4992 | 0.40 (0.06, 2.64) 0.3408 | 6.84 (0.53, 88.52) 0.1410 |
| P interaction | | 0.0384 | 0.8622 | 0.1130 |
| Diabetes | | | | |
| No | 6885 | 0.82 (0.51, 1.31) 0.4004 | 0.52 (0.14, 1.92) 0.3274 | 0.89 (0.10, 8.15) 0.9215 |
| Yes | 872 | 0.56 (0.18, 1.68) 0.2995 | 0.24 (0.01, 4.05) 0.3253 | 17.26 (0.18, 1629.91) 0.2195 |
| P interaction | | 0.5232 | 0.6247 | 0.2718 |
| Hypertension | | | | |
| No | 5387 | 0.76 (0.44, 1.31) 0.3229 | 0.65 (0.10, 4.22) 0.6472 | 0.67 (0.03, 13.28) 0.7951 |
| Yes | 2399 | 0.79 (0.40, 1.57) 0.5071 | 0.37 (0.09, 1.62) 0.1876 | 2.50 (0.18, 33.86) 0.4907 |
| P interaction | | 0.9215 | 0.6427 | 0.5011 |
| CVD | | | | |
| No | 7452 | 0.74 (0.47, 1.16) 0.1835 | 0.43 (0.12, 1.54) 0.1932 | 1.61 (0.21, 12.48) 0.6493 |
| Yes | 328 | 1.68 (0.30, 9.41) 0.5572 | 0.68 (0.03, 15.27) 0.8062 | 0.00 (0.00, 14373.05) 0.4724 |
| P interaction | | 0.3647 | 0.7883 | 0.3915 |
| Age | | | | |
| <65 | 5819 | 0.45 (0.28, 0.75) 0.0021 | 0.04 (0.01, 0.29) 0.0015 | 2.22 (0.20, 24.15) 0.5123 |
| ≥65 | 2008 | 0.27 (0.12, 0.58) 0.0009 | 0.79 (0.18, 3.45) 0.7552 | 0.06 (0.00, 1.30) 0.0731 |
| P interaction | | 0.2544 | 0.0160 | 0.0747 |
| BMI | | | | |
| <25 | 2402 | 0.69 (0.33, 1.43) 0.3179 | 0.48 (0.07, 3.10) 0.4420 | 3.04 (0.10, 91.94) 0.5226 |
| ≥25 | 5176 | 0.83 (0.50, 1.39) 0.4765 | 0.44 (0.10, 1.93) 0.2749 | 1.01 (0.09, 11.83) 0.9968 |
| P interaction | | 0.6664 | 0.9369 | 0.5979 |
| eGFR | | | | |
| <90 | 3683 | 0.54 (0.30, 0.98) 0.0417 | 0.47 (0.12, 1.91) 0.2932 | 0.36 (0.02, 6.50) 0.4884 |
| ≥90 | 4135 | 1.13 (0.63, 2.05) 0.6789 | 0.39 (0.05, 3.11) 0.3773 | 5.34 (0.43, 65.87) 0.1911 |
| P interaction | | 0.0685 | 0.8816 | 0.1583 |

Data are expressed as HR and 95% CI.

Adjusted for age, gender, BMI, SBP, CRP, TC, HDL-C, alcohol, smoking, marriage, poverty income ratio, education, race, diabetes, hypertension, cardiovascular diseases, eGFR, antihypertensive drugs, lipid lowering drugs and anti-diabetic drugs.

Table 4 The results of two-piecewise linear regression model between MTL and all-cause, cardiovascular and cerebrovascular mortality

| Outcome | All-cause mortality HR (95% CI) P | Cardiovascular mortality HR (95% CI) P | Cerebrovascular mortality HR (95% CI) P |
|---------------------------------|--------------------------------------|---|--|
| Cutoff value (T/S ratio) | 0.94 | 1.22 | 0.92 |
| <Cut-off value | 0.41 (0.21, 0.79) 0.0083 | 0.61 (0.16, 2.30) 0.4675 | 0.08 (0.00, 2.36) 0.1412 |
| ≥Cut-off value | 1.25 (0.74, 2.13) 0.4069 | 0.01 (0.00, 83.57) 0.3187 | 9.90 (0.75, 130.17) 0.0812 |
| P for log likelihood ratio test | 0.029 | 0.326 | 0.076 |

Data are expressed as HR and 95% CI.

Effect: all-cause, cardiovascular and cerebrovascular mortality, Cause: MTL. Adjusted for age, gender, BMI, SBP, CRP, TC, HDL-C, alcohol, smoking, marriage, poverty income ratio, education, race, diabetes, hypertension, cardiovascular diseases, eGFR, antihypertensive drugs, lipid lowering drugs and anti-diabetic drugs.

Our results highlight the need to confirm these findings and further explore the reasons behind the heterogeneous observation.

Several potential limitations should be taken into consideration. First, the analysis method of the qPCR was full of high variability, so there may be errors in the measurement of telomere length. Second, the study population was solely from United States, so the conclusions of this research may not be extrapolated to other populations. Third, both all-cause, cerebrovascular and cardiovascular mortality are multifactor-related end points, although we have adjusted

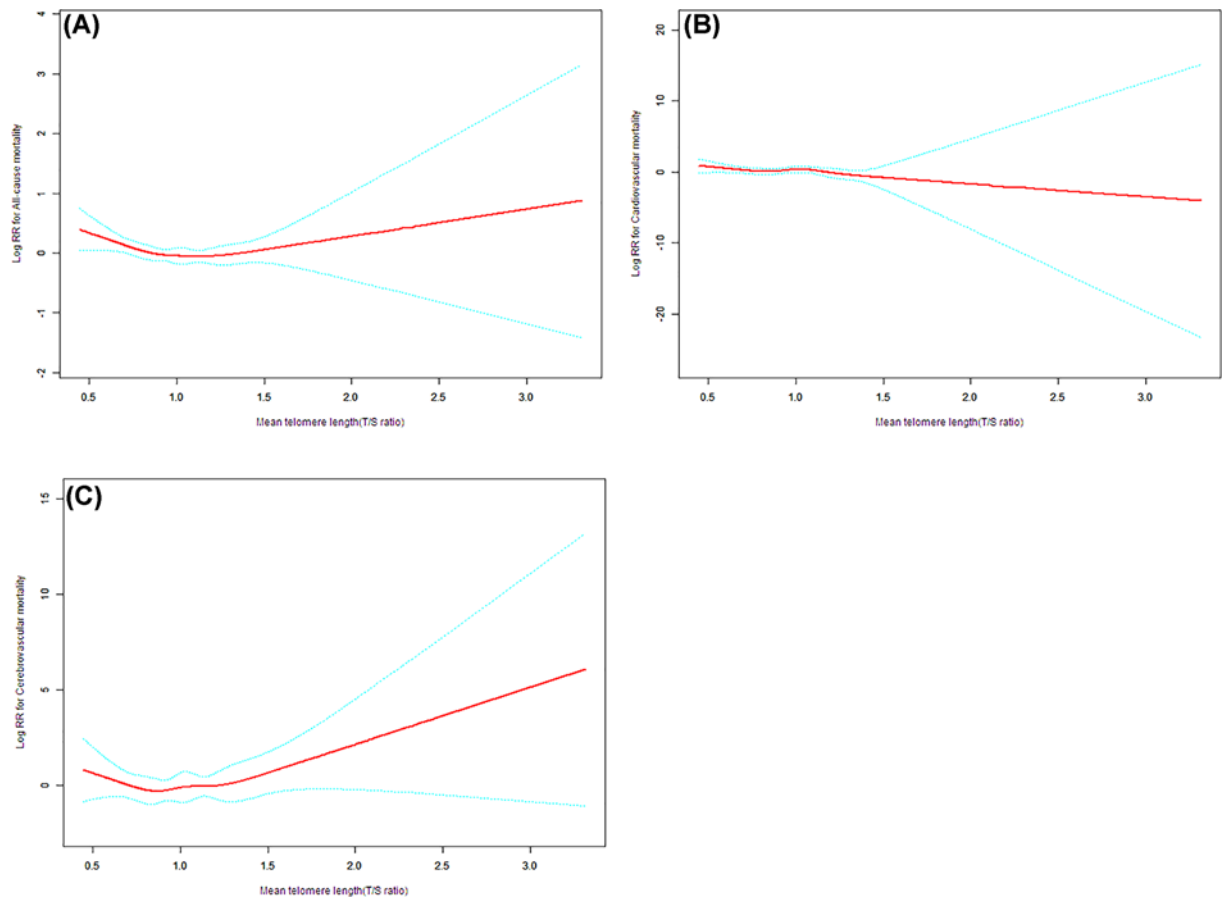


Figure 3. The association of MTL with all-cause, cerebrovascular and cardiovascular mortality

Data are expressed as mean (95% CI). The red line represents RR and the green line represents 95% CI. Abbreviation: RR, relative risk.

multiple potential confounders. The present study has not adjusted for some other death-related confounders such as tumor history, weakness, mental condition, exercise and sleep.

Despite these limitations, the present study has several strengths. First, the sample size of the present study was comparatively large from a well-designed population-based cohort study. Second, the research results have sufficient statistical effects due to the long follow-up period. Third, numerous confounding factors associated with mortality, including socio-demographic factors, lipids parameters, blood pressure, BMI, FBG, CRP, previous history of diseases, taking drugs, alcohol and smoking status were adjusted in the present study.

In the present study we show the non-linear association between MLT and all-cause mortality. MLT inversely associated with all-cause mortality when MLT was less than 0.94 T/S ratio. However, there is no significant association with MLT cerebrovascular and cardiovascular mortality.

Author Contribution

Ying-qing Feng and Bin Zhang designed the project and revised the manuscript. Yu-qing Huang wrote the manuscript. Kenneth Lo analyzed the data. All authors approved the manuscript.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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Abbreviations

BMI, body mass index; CDC, Centers for Disease Control and Prevention; CI, confidence interval; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; FBG, fasting blood glucose; GAM, generalized additive model; HDL-C, high-density lipoprotein cholesterol; HR, hazard ratio; LDL-C, low-density lipoprotein cholesterol; MLT, mean telomere length; NHANES, National Health and Nutrition Examination Survey; qPCR, quantitative polymerase chain reaction; SBP, systolic blood pressure; SCr, serum creatinine; TC, total cholesterol; TG, triglyceride.

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