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Leukocyte telomere length and marital status among middle-aged adults

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Abstract

Background: being unmarried is associated with worse health and increased mortality risk. Telomere length has emerged as a marker for biological ageing but it is unclear how telomere length relates to marital status.
Objective: to examine the relationship between telomere length and marital status in a sample of middle-aged adults.
Design and subjects: cross-sectional analysis among 321 adults aged 40–64 years.
Methods: telomere length was measured by PCR (T/S ratio). Participants provided information on healthy lifestyle activities including smoking, alcohol use, diet, exercise, obesity as well as social support.
Results: participants married or living with a partner had a mean T/S ratio of 1.70 and those widowed, divorced, separated or never married had a mean T/S ratio of 1.58 in a model adjusted for age, gender and race/ethnicity ($P < 0.001$). When the analysis was further adjusted for diet, alcohol consumption, exercise, smoking, social support, poverty and obesity, persons married or living with a partner had a higher mean T/S ratio of 1.69 than their unmarried counterparts (1.59) ($P = 0.004$).
Conclusions: these results indicate that unmarried individuals have shorter telomeres. This relationship between marital status and telomere length is independent of presumed benefits of marriage such as social support and a healthier lifestyle.

Keywords: *telomere, marital status, lifestyle, elderly*

Introduction

Chronological ageing is associated with the risk of development of a variety of diseases. Yet, chronological ageing does not exactly parallel biological ageing. Telomere length has emerged as a marker for biological ageing which may be a key to age-related morbidity [1]. Telomeres consist of TTAGGG tandem repeats, and telomere-binding proteins cap the ends of chromosomes and protect them from degradation. Telomeres become progressively shorter with each replication of somatic cells. Telomere attrition ultimately leads to a loss of replicative capacity. Systemic oxidative stress accelerates telomere shortening [2]. Further, shortened telomere length has been associated with shorter lifespan as well as a wide variety of ageing-related diseases and conditions such as cardiovascular disease, diabetes, dementia and hypertension [3–7]. It has been suggested that inflammation and oxidative stress are key to the ageing

process and telomere length provides a record of the cumulative burden of inflammation and oxidative stress rather than just the current state of one's metabolic status [8].

Data have accumulated over time that being unmarried is associated with worse health, increased mortality risk and a shorter lifespan, as well as a healthier lifestyle [9–15]. Being unmarried is also associated with the presence of systemic inflammation [12, 13]. Since systemic inflammation and oxidative stress are associated with ageing and disease as well as marital status, and telomere length can potentially function as a cumulative record of inflammatory states and oxidative stress, it is possible that telomere length may be a marker of marital benefits to health. However, it is unclear whether marital status is associated with telomere length among middle-aged individuals free of diagnosed cardiovascular disease, diabetes and cancer. Thus, the purpose of this study is to examine the relationship between telomere length and marital status in a sample of healthy middle-aged adults.

Methods

Subjects

Subjects were recruited through health fairs, flyers and advertisements posted in the local health science university. In total 321 subjects, 40–64 years of age and free of diagnosed diabetes, coronary heart disease, stroke and cancer were studied. The subjects consisted of 182 non-Hispanic Whites (56.7%), 131 non-Hispanic Blacks (40.8%) and 8 other race/ethnicity (2.5%). This is consistent with the racial/ethnic demographics of the Charleston, South Carolina area.

Variables

Telomere length

Leukocyte telomere length was measured with a quantitative PCR-based technique that compares telomere repeat sequence copy number to single-copy gene (36b4) copy number in a given sample [16]. Duplicate DNA samples are amplified in parallel 25 μl PCR reactions comprising 15 ng genomic DNA, 1× SensiMix NoRef SYBR Green master mix, 1× SYBR Green (Quantace, UK) and either 300 nmol/l of telomere-specific primers (forward: 5' GGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT3'; reverse: 5'GGCTTGCCTTACCCTTACCCT TACCCTTACCCTTACCCT3') or 300 nmol/l of the 36B4 forward primer (5'CAGCAAGTGGGAAGGTGTAATC C3') primer and 500 nM of the 36B4 reverse primer (5' CCCATTCTATCATCAACGGGTACAA3'). All PCRs were run on a Corbett Research Rotor-Gene 6000 Real-time Thermal Cycler (Corbett Research, Cambridge, UK). The thermal cycling profile begins with a 95°C incubation for 10 min to activate the *Taq* DNA polymerase followed by cycling of 15 s at 95°C and 1 min at 58°C for either 20 cycles (telomere) or 30 cycles (36B4). Prior to running samples, the linear range of the assay was determined by generating a standard curve using serially diluted DNA (200–1.56 ng in twofold dilutions) in quadruplicate. Both PCR reactions exhibited good linearity across this input range ($r^2 > 0.99$). Test samples were checked to confirm that they fall within this range, and any that did not were diluted as necessary and re-run. The specificity of all amplifications was determined by melting curve analysis. Forty-eight study samples, a calibrator sample and one no-template control sample (all in duplicate) were processed per run.

The PCR data were analysed with the comparative quantitation approach as previously described and implemented with the Corbett Research Rotor-Gene 6000 version 1.7 analysis software. During this analysis, the amplification efficiency is calculated for each sample along with the mean efficiency of the run, which is used in calculating the relative concentration of each sample relative to the calibrator sample. This calculation coupled with the use of the same calibrator samples on all runs allows for any inter-run variation. This process is done for both telomere (T) and single-copy (S) gene reactions, and telomere length expressed as a ratio of the two, the T/S ratio, of the mean data from duplicate runs. All analyses were done blinded to patient characteristics.

The telomere length assay (T/S ratio) was checked for reproducibility by re-running 76 samples on a different day. The correlation (r) between the two runs was 0.946. The minimum T/S ratio was 1.025 and the maximum T/S ratio was 2.623. Telomere length was normally distributed (Anderson–Darling test, $P = 0.104$ indicating we cannot reject the null hypothesis of a normal distribution). For our analyses, the T/S ratio was analysed as a continuous variable.

Marital status

Marital status was characterised by the subjects as married ($n = 208$), widowed ($n = 4$), divorced ($n = 57$), separated ($n = 16$), never married ($n = 34$) or living with a partner ($n = 2$). These descriptions of marital status were then collapsed into two categories as: (i) married or living with a partner ($n = 210$) or (ii) widowed, divorced, separated or never married ($n = 111$). This strategy for collapsing marital status categories has been used previously [10].

Lifestyle variables

Social support was determined using the 12 items of the Multidimensional Scale of Perceived Social Support with a seven-point rating scale ranging from very strongly disagree [1] to very strongly agree [7]. Cronbach's a was 0.88 for the total score [17]. We used the sum of the responses as a dichotomised social support variable splitting at the median (<81 and ≥81, maximum score 84 points).

Fruit and vegetable consumption was measured by self-report in answer to a series of questions that included serving cards to educate participants regarding serving size [18]. The questions included: (i) 'In general, how many servings would you say you eat of vegetables (not counting salad or potatoes)?' The subject was then asked if the number of servings

they specified was in a: 'day, week, month or year?'. (ii) 'In general, how many servings would you say you eat of fruit?' The subject was then asked if the number of servings they specified was in a: 'day, week, month or year?'. Answers to these questions were used to calculate the number servings of fruit and vegetables the subject had in a day.

Daily alcohol consumption was determined from a series of questions asking whether an individual consumed alcoholic beverages, and, if so, how many drinks per week of beer, wine or spirits. According to the American Heart Association and the American Diabetes Association moderate alcohol consumption is defined no more than one drink per day for women and two drinks per day for men [19, 20]. Thus, we used 1–14 drinks per week for men and 1–7 drinks per week for women as 'moderate' drinking for this study. More than 14 drinks per week for men and more than 7 drinks per week for women were characterised as 'exceeds moderate'.

Exercise was characterised by the answer to the question: 'Would you say that you play sports or exercise … 0 = Never, 1 = Seldom, 2 = Sometimes, 3 = Often, or 4 = Very Often?' This question is from a longer measure of exercise designed for epidemiological studies [21, 22]. The response was used as a dichotomised variable (<3 and ≥3) for the amount of exercise the subject did. Current smoking was assessed by the question 'Do you smoke cigarettes now?'. Those who did not smoke now were considered non-smokers and those that did were classified as current smokers. Body mass index (BMI) in kg/m^2 was calculated using height and weight measurements measured by laboratory personnel and used as a continuous variable.

Poverty income ratio (PIR) was determined from answers to the questions: 'How many people live in your household?' and 'Including all the people in your household, what is the total combined income (before taxes) for your household in the last 12 months … this includes income from all sources such as wages, salaries, social security or retirement benefits, help from relatives and so forth'. Using the number of persons in the household and the US Census Bureau poverty threshold table for 2007, the PIR was calculated as the total household income divided by the respective poverty threshold [23]. Values <1 are below the poverty threshold. PIR was not normally distributed and because few people were actually below the poverty threshold ($n = 15$) we thus used as a dichotomised variable (<2 and ≥2) in our analyses.

Control variables

Age, gender and race/ethnicity were used as control variables.

Analysis

Initially, we performed bivariate analyses comparing marital status to a variety of lifestyle and demographic variables using *t*-tests for means (age, fruit and vegetable consumption and BMI) and chi-square test for proportions (gender, race/ethnicity, alcohol consumption, exercise, smoking status, social support and PIR). We then conducted general linear models relating marital status to telomere length. The first analysis was conducted unadjusted for any covariates. The second analysis was adjusted for age, gender and race/ethnicity. The subsequent third analysis was adjusted for age, gender, race/ethnicity, fruit and vegetable consumption, alcohol consumption, exercise, smoking status, social support, PIR and BMI. The effect of marital status on telomere length was computed using general linear models [general linear models procedure (Proc GLM) and least squares means] in SAS 9.1.3 (SAS Institute Inc., Cary, NC, USA).

Results

Bivariate results showing the association of lifestyle and demographic variables with martial status are given in Table 1. Marital status was related to gender, race/ethnicity, smoking status, scaled social support and PIR. Of persons who were widowed, divorced, separated or never married, 69.4% were female compared with 47.6% of those married or living with a partner. Of those widowed, divorced, separated or never married, 54.0% were non-Whites or Hispanics, as opposed to 37.6% of those married or living with a partner. Similarly, 12.6% of those not married and not living with a partner smoked, whereas 4.3% of those married or living with a partner smoked. Both social support and PIR were lower in participants who were widowed, divorced, separated or never married.

Multivariate results controlling for demographic and lifestyle variables are shown in Table 2. The mean unadjusted telomere length (T/S ratio) was significantly longer for those married or living with a partner than for those widowed, divorced, separated or never married. Controlling for age, gender and race/ethnicity, the results changed slightly. Similarly, when controlling for age, gender, race/ethnicity, fruit and vegetable consumption, alcohol consumption, exercise, smoking status, social support, PIR and BMI, the results were essentially the same and continued to show that individuals who were married or living with a partner had significantly longer telomeres. When we examined marital status limiting the married group only to those who were married and excluding the two individuals who were co-habitating, the results were essentially unchanged. The fully adjusted model yielded a mean for the married group of 1.70 and a mean for the unmarried group of 1.59 ($P < 0.01$).

Stratified unadjusted analyses are shown in Table 3. Longer telomere length (T/S ratio) due to being married or living with a partner was seen among those who were older (53–64 years old), among both men and women, among those who exercised more (often or very often) and among those with low (≤81) social support.

Table 1. Association between telomere length, dietary, drinking, exercise, smoking, social support, PIR and BMI with marital status ($n = 321$)

	Married or living with a partner	Widowed, divorced, separated or never Married	P-value
Age (years)	51.3 ± 6.4[1]	51.4 ± 5.7	0.93
Female (%)	47.6	69.4	<0.01
Non-White and Hispanic (%)	37.6	54.0	<0.01
Fruit and vegetable consumption (servings per day)	3.0 ± 1.9	2.6 ± 1.9	0.08
Alcohol consumption (%)			
None	36.7	41.4	0.47
Moderate	58.1	51.4	
Exceeds moderate	5.2	7.2	
Exercise (never, seldom, sometimes) (%)	51.4	52.2	0.89
Current smoker (%)	4.3	12.6	<0.01
Body mass index (Mean $\pm$ SD)	28.6 ± 5.6	29.9 ± 6.5	0.08
Social support (low, <81) (%)	43.3	59.5	<0.01
PIR (low, <2) (%)	7.8	21.2	<0.01

[1]Mean $\pm$ standard deviation.

Table 2. Analyses examining association of telomere length with marital status

	Telomere length (T/S ratio)		
	Married or living with a partner	Widowed, divorced, separated or never married	P-value
Unadjusted telomere length (T/S ratio)	1.69	1.60	0.007
Model adjusted for age, gender and race/ethnicity	1.70	1.58	<0.001
Model adjusted for age, gender, race/ethnicity, fruit and vegetable consumption, alcohol consumption, exercise, smoking status, social support, PIR and BMI	1.69	1.59	0.004

Proc GLM was used to test for differences in telomere length by marital status (Type III sum of squares and least squares means).

Table 3. Stratified unadjusted analyses examining association of telomere length with marital status

	Telomere length (T/S ratio)		
	Married or living with a partner	Widowed, divorced, separated or never married	P-value
Age (years)			
40–52	1.73	1.65	0.077
53–64	1.64	1.54	0.043
Gender			
Male	1.65	1.50	0.008
Female	1.73	1.64	0.034
Exercise			
Never, seldom, sometimes	1.68	1.61	0.115
Often, very often	1.70	1.59	0.028
Social support			
Low (<81)	1.68	1.58	0.03
High (≥81)	1.70	1.62	0.16

Proc GLM was used to test for differences in telomere length by marital status (Type III sum of squares and least squares means).

Discussion

These findings add to the evidence of a marriage benefit to one's health. These findings suggest that unmarried individuals have shorter telomeres which have implications for increased risk for disease and mortality. When we stratified by age, although telomeres shorten with age, the impact of marital status on telomere length remains and becomes slightly larger in older cohorts. Theories about the marital benefits to health have suggested that having a spouse or partner provides positive benefits like social support as a buffer to life stressors or encouragement of a healthy lifestyle [14, 15]. Another explanation suggests that healthier individuals are selected into marriage and unhealthy individuals are in unmarried states [14]. The present findings suggest that the relationship between marital status and telomere length is independent of presumed benefits of marriage such as social support and a healthier lifestyle.

Social isolation, even among laboratory animals, is related to increased oxidative stress [24]. Social support has been shown to buffer stress [25]. The stress-buffering hypothesis would suggest that social support acts as a buffer for those under great stress [26]. When stress is high, the availability of companionship or help may reduce the stress. However, under normal circumstances, when stress is low then the level of social support may matter less.

Evidence has demonstrated a relationship between shorter telomere length and chronic psychological stress, suggesting that the benefit of marriage might be providing social support, which acts as a buffer to stress [27–29]. Our results suggest that social support does not account for the

difference in telomere length, as differences remain even after adjusting for social support. However, our lack of a demonstrated relationship may be due to the fact that the measurement of social support is based on current perceptions and telomere length can be seen as a cumulative marker of ageing and burden of oxidative stress.

Similarly, studies have shown a positive impact of marriage on health-related behaviours, which is also seen in our sample [15]. The health-related behaviours evaluated in this study also do not account for the differences in telomere length. Thus, the present results suggest that the theory that unhealthy individuals are selected into unmarried states may have some merit. Further studies are needed to identify the underlying factors that account for the marriage benefit established in the previous literature and seen in this study.

There are several limitations to this study. First, we used a commonly employed volunteer-based strategy for the recruitment of an asymptomatic sample. However, we did not recruit systematically on a community-wide basis. Consequently, we cannot entirely rule out the possibility of selection bias. Second, although there are significant data to suggest that marital status and longer telomere length are associated with better health and our cross-sectional design allows us to examine the association between telomere length and marital status, it does limit our ability to infer whether the short telomere length among unmarrieds leads to accelerated development of disease over time. Third, we are unable to evaluate participants' satisfaction with their marriages, which may impact the health benefit. There may be differences between individuals in happy marriages versus unhappy ones. This may lead to this study underestimating the benefits of being in a good marriage, but should not alter the significance of our results. Finally, the self-reported lifestyle variables may be unreliable.

In conclusion, telomere length is associated with marital status, indicating that individuals who are married or have a partner have longer telomeres than individuals who are unmarried. Unmarried individuals have been shown to be at risk for mortality and disease but these data suggest that accelerated biological ageing may also be taking place. Future research may need to focus on better understanding why this relationship exists and potential interventions to decrease the risk associated with being unmarried.

Key points

- Telomere length is a marker of biological ageing, and unmarried individuals have shorter telomeres.
- The relation between telomere length and marital status was not accounted for by positive lifestyle characteristics.
- Unmarried individuals may be experiencing accelerated biological ageing.

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Conflicts of interest

The authors of this manuscript have no conflicts of interest to disclose.

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Home-based cardiac rehabilitation is as effective as centre-based cardiac rehabilitation among elderly with coronary heart disease: results from a randomised clinical trial

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Abstract

Background: participation in centre-based cardiac rehabilitation (CR) is known to reduce morbidity and mortality but participation rates among the elderly are low. Establishing alternative programmes is important, and home-based CR is the predominant alternative. However, no studies have investigated the effect of home-based CR among a group of elderly patients with coronary heart disease with a long-term follow-up.