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Telomere length and periodontal attachment loss: a prospective cohort study

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Abstract

Aim: To examine the association between telomere erosion and periodontitis in a longstanding prospective cohort study of New Zealand adults. Specific hypotheses tested were: (1) that exposure to periodontitis at ages 26 and 38 was associated with accelerated leucocyte telomere erosion; and (2) that accelerated leucocyte telomere erosion was associated with higher rates of periodontitis by ages 26 and 38.

Materials and Methods: Periodontal attachment loss data were collected at ages 26 and 38. Blood samples taken at the same ages were analysed to obtain estimates of leucocyte telomere length and erosion over a 12-year period.

Results: Overall, mean telomere length reduced by 0.15 T/S ratio (adjusted) from age 26 to 38 among the 661 participants reported on here. During the same period, the mean attachment loss increased by 10%, after adjusting for sex, socio-economic status and smoking. Regression models showed that attachment loss did not predict telomere length, and that telomere erosion did not predict attachment loss.

Conclusions: Although both periodontitis and telomere length are age-dependent, they do not appear to be linked, suggesting that determination of leucocyte telomere length may not be a promising clinical approach at this age for identifying people who are at risk for periodontitis.

Key words: epidemiology; periodontitis; cell ageing; telomere shortening

Introduction

Adult periodontitis is a chronic, progressive condition which is the result of a complex interplay among bacterial, genetic, inflammatory and behavioural influences. Its cumulative nature means that its occurrence is strongly age-related, consistent with observations made about other chronic, cumulative conditions, such as cardiovascular disease and diabetes (Reynolds, 2013). Reports of the considerably higher prevalence and extent of periodontitis among middle-aged and older adults than among younger people (Eke et al, 2012; Ministry of Health, 2010) have led, unsurprisingly, to the assertion that periodontal attachment loss increases with age. This has been confirmed in a number of reports from prospective cohort studies of population samples in which the same individuals have been followed over time (Thomson et al, 2004; Thomson et al, 2014). Whether age is itself a risk factor for periodontitis is a moot point. On the one hand, the robust and invariably-observed age gradient provides strong circumstantial evidence that it may be so; on the other hand, that gradient may result from ongoing, cumulative exposure to the condition's true risk factors. After all, periodontitis embodies the risk accumulation life-course model (Nicolau et al, 2007), whereby adverse and beneficial exposures exert their effects gradually through time to produce the disease status which is observable at any given point in the life course. This means that, other factors being equal, older adults will have more periodontal attachment loss. In short, we currently do not know for sure age is a risk factor for periodontitis.

Telomere length is a marker which is associated with both ageing and adverse exposures through life. Telomeric repeat sequences define the ends of chromosomes in eukaryotic cells. Each division of a cell erodes telomere length, with the ultimate outcome being intracellular dysfunction and an end to cell division (that is, senescence). Leucocyte telomere length represents telomere length at birth and the subsequent replicative history of hematopoietic stem cells and progenitor cells (Aviv, 2008). Shorter telomere length has been implicated in greater susceptibility to age-related chronic disease and early death (Weischer et al, 2013); it has also been proposed that it is an outcome of oxidative stress and the inflammatory burden associated with age-related disorders (Masi et al, 2014).

Accordingly, shorter telomere length has been proposed as both a biomarker for—and a consequence of—early ageing (Shalev et al, 2013). In considering periodontitis in the context of telomere erosion, there are three possibilities. First, the chronic inflammatory burden associated with periodontitis could contribute to telomere erosion; second, if periodontitis is indeed a disease of ageing, accelerated telomere erosion could contribute to the condition's occurrence; third, both periodontitis and telomere erosion could be influenced by a third variable (such as smoking) which confounds the association between them.

Interest in telomeres and periodontitis has been longstanding, with the first such investigation reported in 1984; in that study, Takahashi et al compared gingival fibroblast telomere length in patients with aggressive periodontitis and age-matched non-cases, and observed no difference. Masi et al (2011) compared leucocyte telomere length (LTL) in dental school clinical patients with and without periodontitis. They observed shorter LTL (on average) in the former than in the latter, although the utility of their findings was somewhat compromised by their exclusion of anyone taking any medication or having a chronic medical condition, and by their use of a clinical sample rather than a population-based one. A recent review highlighted a need for more investigation of the LTL-periodontitis association (Steffens et al, 2013). Most recently, Sanders et al (2015) used a case-control study design to investigate whether telomere erosion over a six-year period was associated with chronic periodontitis, and found no association. To date, it is unclear whether telomere erosion and periodontitis are associated. It is arguable that, if such an association does exist, it is more likely to be observed in a prospective longitudinal study of a population-based cohort. Accordingly, the aim of this study was to examine the association between telomere erosion and periodontitis in a longstanding prospective cohort study of New Zealand adults. Specific hypotheses tested were: (1) that exposure to periodontitis at ages 26 and 38 was associated with accelerated leucocyte telomere erosion; and (2) that accelerated leucocyte telomere erosion was associated with higher rates of periodontitis by ages 26 and 38.

Methods

The Dunedin Multidisciplinary Health and Development Study (DMHDS) is a longitudinal study of a complete birth cohort born at Queen Mary Hospital, Dunedin, New Zealand between 1 April 1972 and 31 March 1973 (Poulton et al, 2015). Perinatal data were obtained and the sample for the longitudinal study (N = 1037) was defined at age 3 years. Participants were assessed within a month of their third birthdays and again at ages 5, 7, 9, 11, 13, 15, 18, 21, 26, 32 and 38, when 961 (95% of the living cohort) were assessed. Cohort families represented the full range of socioeconomic status in New Zealand in the early 1970s, as compared to the New Zealand census. Cohort members were primarily white; 7.5% self-identify as being Māori (which matches the ethnic distribution of the South Island of New Zealand). We have published evidence that long-term participation in the longitudinal study has not improved Study members' mental or physical health over that of same-aged participants in the New Zealand National Health and Nutrition (one-off) surveys (Poulton et al, 2006).

Ethical approval for each assessment phase was obtained from the Otago Research Ethics Committee, and Study members gave informed consent before participating. Each underwent a full day of assessments (of, for example, oral health, mental health, cardiovascular health) presented as standardised modules in counterbalanced order and conducted by a different assessor/examiner who is kept blind to all other study data.

The periodontal assessments

Periodontal examinations were conducted at ages 26, 32 and 38; only half-mouth periodontal examinations were possible at age 26, but full-mouth examinations were undertaken at 32 and 38. Third molars and implants were not included in the periodontal examinations. The current analysis uses the periodontal data from ages 26 and 38. At age 26, dental examinations were conducted by three examiners who had been previously calibrated and who examined 85%, 10% and 5% (respectively) of the cohort. Periodontal measurements were made in two quadrants (quadrants 1 and 3 for those whose study ID number was odd; quadrants 2 and 4 for those with an even ID number; the mix of odd and

even ID numbers was approximately 50:50) because of time constraints in the busy assessment day. Three sites (mesiobuccal, buccal, and distolingual) per tooth were examined, and gingival recession (GR; the distance in millimetres from the cemento-enamel junction to the gingival margin) and probing depth (PD; the distance from the gingival margin to the base of the pocket) were recorded, using the Hu-Friedy PCP-2 periodontal probe. Midbuccal measurements for molars were made at the midpoint of the mesial root. All measurements were rounded down to the nearest whole millimetre at the time of recording. Where the gingival margin was situated more than 1mm coronally to the cemento-enamel junction, a negative value for GR was recorded. Periodontal measurements were not conducted on those with a history of cardiac valvular anomalies or rheumatic fever. A full-mouth examination was undertaken at age 38; the clinical procedures were identical, except that all teeth (excluding third molars) were assessed. Two examiners undertook 58% and 39% of the age-38 examinations respectively, and a third examiner completed 3% of them. For each age, the attachment loss (AL) for each site was computed by summing the GR and PD measurements, and the means of those were calculated for each person at the analysis stage.

Determination of mean relative leucocyte telomere length (LTL)

Standard procedures (Bowtell, 1987; Jeanpierre, 1987) were used to extract leucocyte DNA from blood samples taken at ages 26 and 38, and the extracted DNA was stored at -80°C until assayed, to prevent sample degradation. These analyses were not undertaken for Maori participants. All DNA samples were assayed for LTL at the same time, and the laboratory technician undertaking the assays was unaware of the periodontal status of the individuals from whom the blood had been obtained. LTL was measured using a validated quantitative PCR method (Cawthon, 2002) which determines mean telomere length across all chromosomes for all of the sampled cells, as previously described (Shalev et al., 2014a; 2014b). Two quantitative PCR reactions are involved for each participant; one involves a single-copy gene (S) and the other involves the telomeric repeat region (T). All DNA samples were run in triplicate for telomere and single-copy reactions at both ages 26 and 38, making 12 reactions per Study member, in total. To eliminate the risk of measurement artifacts affecting comparisons of LTL measured on the same individual at different ages,

DNA triplicates from the same individual, from both ages 26 and 38, were assayed on the same plate (as further described for the Dunedin Study cohort by Shalev at al, 2014). The mean coefficient of variation (CV) for the triplicate Ct values was 0.81% for the telomere (T) and 0.48% for the single-copy gene (S), indicating low measurement error. LTL, as measured by the T/S ratio, was normally distributed at each age. The mean % coefficient of variation (%CV) was 9.68%, as calculated from measurements of the standard samples used to create the standard curve. However, this value was likely inflated since the precision of the assay declines as DNA concentration is reduced, such as in the case of the low concentration standard samples. Because inter-assay reliability is greater than intra-assay reliability, we elected to minimize error in longitudinal assessments by assaying a participant's DNA samples taken at ages 26 and 38 within the same plate. To further minimize type II error, participant DNA-sample pairs were randomly distributed across assay plates, blind to periodontal status.

Covariates

A set of smoking exposure and sociodemographic variables were considered in this study as confounding factors when assessing the association between AL and telomere length. Tobacco smoking status was determined at ages 26 and 38 using the question "Have you smoked every day for one month or more of the previous 12 months?". Cannabis smoking was determined by asking at ages 26 and 38 how many times they had used cannabis in the previous year, in order to identify those who had smoked it at least weekly. In New Zealand, cannabis smokers do not usually mix it with tobacco.

Socio-economic status (SES) was measured in childhood using standard New Zealand occupationally-based indices[12,13] which employ a 6-category classification (where, for example, a doctor scores 1 and a labourer scores 6). This measure was based on both parents, and is the mean of the SES from the assessments at birth through age 15 years (Poulton et al, 2002). For adult SES measurement, participants were classified using the same occupational scale. They were then allocated to one of the following two groups according to their childhood SES or adult SES score: (1) low (score 4 to 6) or (2) high

(score 1 to 3). Those childhood and adult SES categorizations were used to allocate each participant to one of four SES trajectory groups: (1) those who were in the high SES group to the age of 15 and then in adulthood (at 26 and 38 years of age separately) were categorized as the "high-high" group; (2) those in the low SES group at both stages were designated the "low-low" group; and the (3) "high-low" and (4) "low-high" groups comprised those who were downwardly and upwardly mobile, respectively.

Statistical analyses

Because the association between telomere length and periodontitis remains unclear, it could be argued that shorter telomere length leads to higher rates of periodontitis or exposure to periodontitis reduces the telomere length. We therefore tested for the effects both ways. At each age, telomere length and AL were treated as the dependent variable and independent variable in successive regression models which were used to assess the associations between telomere length and AL at ages 26 and 38 separately. Linear regressions were used to model telomere length using AL. However, since AL data are highly skewed (as commonly known), we modelled it using a Gamma distribution with log link; the resulting estimates should be interpreted as odds ratios. We then adjusted the associations by considering a set of confounders, most notably sex and SES. Age was not included in the models, because all study members were the same age (1972–1973 births). The pooled associations between telomere length and AL were also assessed using GEE, which allows the observations from the same individual over time to be correlated. The GEE modelling was conducted using an unstructured correlation structure, and robust standard errors were used to obtain the confidence intervals of the estimates. All statistical analyses were undertaken in Stata 13.

Results

Of the original 1037 Study members, 734 had complete data on all variables at age 26; that number was 785 for age 38. These participants were included in the analyses for assessing the association at ages 26 and 38 respectively. Among these, 665 participants had complete data at both age 26 and 38. Comparisons were made between those included and those excluded at each of those ages, and there was no sex difference, although more of those in the "Low-Low" SES trajectory group (and more smokers) were excluded from the study at either age. Of those included in the analysis at age 26, 19% were in the "Low-Low" SES group, and 7%, 38% and 36% of the participants were in the "Low-High", "High-Low" and "High-High" groups, respectively. Those proportions were 30%, 9%, 38% and 23% (respectively) for the participants who were excluded. Similar patterns were observed at age 38. The GEE approach for assessing the pooled assocations was based on 854 participants, where the model included the participants who had complete data on all variables for at least one age.

Telomere measures were available for 758 participants at both ages. We expected the LTL measures on the same individual measured at ages 26 and 38 to be correlated, and the GEE approach took such correlation into account[1].

The sociodemographic and clinical characteristics of the analytic sample are summarised in Table 1. Males, smokers and those in the "Low-Low" SES group had higher AL, but telomere length did not differ significantly by any of those characteristics.

Table 2 presents data on the impact of LTL on AL at age 26 and 38 separately. The data show no association between telomere length and AL, and this holds after controlling for confounding factors. Males had 6% and 11% greater AL than females at ages 26 and 38, respectively, while smokers had 6% greater AL than non-smokers at age 26, and that had increased to 11% by age 38. Those in the "Low-Low" SES transition group had higher AL scores than those in the other groups at age 38. This association was not apparent at age 26, where only the "High-High" group showed less AL (by 4%) than the "Low-Low" group.

[1]As expected, the estimated correlation given the GEE approach was 0.64, which suggested that the two measures were moderately correlated.

Linear regression models for telomere length at both ages showed that AL did not predict telomere length (Table 3). None of the other factors predicted telomere length either.

Unadjusted and adjusted pooled associations between telomere erosion and AL over ages 26 and 38 are presented in Table 4 (for the effect of the former on the latter) and Table 5 (for the effect of the latter on the former). Once again, there was no evidence to suggest that the two were associated. Overall, the mean telomere length among the participants reduced by 0.15 T/S ratio (adjusted) from age 26 to 38. During the same period, the mean AL increased by 10%, after adjusting for the other characteristics.

Discussion

We examined the association between telomere erosion and periodontitis in a longstanding prospective cohort study of New Zealand adults. Neither of the specific hypotheses we tested were supported; that is, exposure to periodontitis at ages 26 and 38 was not associated with accelerated leucocyte telomere erosion; and accelerated leucocyte telomere erosion did not lead to more periodontitis experience by ages 26 or 38.

This study presents methodological advances in the investigations of telomere erosion and periodontitis: the data were collected prospectively; the sample was a complete birth cohort rather than a clinical convenience sample; the measurements were made over a period of 12 years; and appropriate multivariate modelling was undertaken. The outcome was a null finding. On the other hand, there were also some limitations. First, we used partial recording protocols for the periodontal examinations. There are two issues here. First, for the examinations at 26, 32 and 38, we measured periodontal attachment loss at only three sites (instead of six) per tooth, although the particular three-site combination used here was associated with the least misclassification relative to estimates from the use of all six sites per tooth (Susin et al, 2005). Caution should be exercised in interpreting findings from any periodontal study which has not used full-mouth recordings from six sites per tooth (Papapanou, 2012). Second, periodontal status at age 26 was determined using half-mouth data, whereas full-mouth data were collected at 32 and 38. We repeated the analyses using

half-mouth data for all three ages (the same two quadrants at each age), and there was no change to the study outcome. Third, we could have included other covariates in our analyses but, because no association between telomere erosion and periodontitis was found, such adjustment was not needed. Fourth, the coefficient of variation in our samples was higher than that reported by Cawthon (2002) in his original description of the LTL PCR assay approach, meaning that perhaps a degree of caution is needed in interpreting the LTL data.

Turning to the findings, how do we explain the lack of association (in either direction) with periodontal attachment loss in this study? The null finding is consistent with the outcome of the only other analytical epidemiological study to have examined the periodontitis-LTL erosion association using longitudinal LTL data, whereby Sanders et al (2015) also found no association in a nested case-control study using a sample of middle-aged and older adults. They speculated on whether their decision to maximise the phenotypic separation between cases (severe periodontitis) and controls (no disease) by omitting those with moderate periodontitis (which was the norm in the age groups being studied) affected the power of their study to find a difference. Our study used a prospective cohort study design, younger adults and the full range of periodontitis experience and still found no association with telomere erosion. Of course, a limitation of the study may be that the cohort involved here is still too young (even at 38) for there to have been enough disease experience to affect telomere erosion. Future follow-up assessments and analyses should clarify whether this is indeed the case, but those are likely to be a decade away.

If telomere erosion is indeed a marker of ageing, the current study's null finding suggests that periodontitis is not a disease of ageing *per se*, and that the ubiquitous age gradient is indeed the outcome of ongoing, cumulative exposure to the condition's true risk factors (such as smoking and poor plaque control). Thus, although both periodontitis and telomere length are age-dependent, they do not appear to be linked.

However, there is controversy over what telomere erosion actually represents and how it should be determined (Sanders and Newman, 2013). Notwithstanding a degree of

heterogeneity in findings from epidemiological studies of telomere erosion and aspects of health, the evidence is firming up (Codd et al, 2013; Blackburn et al, 2015). There are also methodological concerns: the measurement of LTL is method-dependent, and the quantitative PCR approach used in the current study—while efficient—has been reported to be less accurate than alternative approaches. Accordingly, an important consideration is whether the estimates of leucocyte telomere erosion in the Dunedin study were valid. There have been three recent reports of positive associations from that study. Shalev et al (2014a) reported that persistent internalising disorders from ages 11 to 38 were associated with greater rates of leucocyte telomere erosion. Belsky et al (2014) described shorter LTL by age 38 in those with lifelong chronic asthma. Finally, Shalev et al (2014b) reported an association between perinatal complications and LTL by age 38. These three reports provide evidence for the robustness of the Dunedin study telomere erosion data (and suggest not only a role for chronic stress and/or morbidity in telomere erosion, but also an influence of perinatal programming).

It may be that LTL is inappropriate for epidemiological investigations of the putative association between telomere erosion and periodontitis, and that appropriate cells should be sampled directly from the periodontal tissues (whether by punch biopsy or curettage of periodontal pockets, or some other method). A Japanese study comparing telomere length in 20 patients with aggressive periodontitis and 51 healthy controls found no association with disease status and moderate correlation in TL between leukocytes and cultured gingival fibroblasts (Takahashi et al, 2004). Such an approach would present ethical and practical challenges for those using larger, population-based samples, however. Investigation of the concordance between LTL and telomere length in cells from periodontal tissues in a study with adequate power would be a useful first step in determining whether sampling the latter would be justified in those studies.

In conclusion, although both periodontitis and telomere length are age-dependent, they do not appear to be linked (at least in this cohort), suggesting that determination of leucocyte telomere length may not be a promising clinical approach for identifying people who are at risk for periodontitis. Further investigation in follow-up studies of other representative

samples—and of a wider age range—is required before a definitive picture of the association and its clinical utility can be achieved.

Conflicts of interest

The authors declare no conflicts of interest in relation to the work presented in this article.

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Clinical relevance

Scientific rationale for the study: Leucocyte telomere length reflects cellular age, and periodontitis is known to be strongly age-related. There have been few investigations of their association, however, and the findings have been inconclusive. A prospective study of a complete birth cohort of adults offers a unique opportunity to investigate this relationship. *Principal findings*: Exposure to periodontitis at ages 26 and 38 was not associated with accelerated leucocyte telomere erosion, and neither was accelerated leucocyte telomere erosion associated with more periodontitis experience by ages 26 or 38. *Practical implications*: The findings suggest that determination of leucocyte telomere length is not a promising clinical approach for identifying people who are at risk for periodontitis.

Table 1. Mean Leucocyte Telomere Length (LTL) and Attachment Loss (AL) by sample characteristics at age 26 and 38 (brackets contain standard deviations unless otherwise indicated).

	Age 26			Age 38		
	N (%)	LTL	AL	N (%)	LTL	AL
Total sample	734 (100)	1.18 (0.40)	1.49 (0.31)	785 (100)	1.05 (0.32)	1.60 (0.74)
Sex						
Male	395 (54)	1.18 (0.40)	1.53 (0.30)	397 (51)	1.04 (0.32)	1.72 (0.79)
Female	339 (46)	1.19 (0.39)	1.43 (0.31)	388 (49)	1.06 (0.32)	1.47 (0.67)
Smoking						
Yes	265 (36)	1.15 (0.37)	1.55 (0.34)	186 (24)	1.04 (0.32)	2.12 (1.14)
No	469 (64)	1.20 (0.41)	1.45 (0.29)	599 (76)	1.05 (0.32)	1.44 (0.45)
SES trajectory group						
Low-Low	138 (19)	1.19 (0.39)	1.53 (0.33)	121 (15)	1.07 (0.34)	1.99 (1.06)
Low-High	51 (7)	1.15 (0.39)	1.54 (0.39)	89 (11)	1.06 (0.31)	1.46 (0.39)
High-Low	276 (38)	1.16 (0.39)	1.51 (0.30)	194 (25)	1.01 (0.31)	1.73 (0.93)
High-High	269 (36)	1.21 (0.41)	1.43 (0.28)	381 (49)	1.06 (0.32)	1.44 (0.47)
Weekly Cannabis Use						
Yes	100 (14)	1.20 (0.46)	1.55 (0.32)	67 (9)	1.02 (0.30)	2.35 (1.51)
No	634 (86)	1.18 (0.38)	1.48 (0.31)	718 (91)	1.05 (0.32)	1.53 (0.58)

Table 2. Unadjusted and adjusted estimates for the effects of LTL on AL at ages 26 and 38.

		Age 26		Age 38	
	Variable	Estimate	95% C.I.	Estimate	95% C.I.
Unadjusted	LTL	1.00	(0.97, 1.04)	1.00	(0.90, 1.10)
Adjusted	LTL	1.00	(0.97, 1.04)	1.03	(0.95, 1.11)
	Male sex	**1.06**	**(1.03, 1.09)**	**1.11**	**(1.05, 1.16)**
	Smoking[a]	**1.06**	**(1.03, 1.10)**	**1.34**	**(1.26, 1.42)**
	SES trajectory group[b]				
	Low-High	1.03	(0.96, 1.10)	**0.83**	**(0.75. 0.91)**
	High-Low	0.99	(0.95, 1.03)	**0.90**	**(0.83. 0.97)**
	High-High	**0.96**	**(0.92, 0.99)**	**0.83**	**(0.77, 0.89)**
	Weekly cannabis use[c]	1.00	(0.96, 1.05)	**1.22**	**(1.11, 1.34)**

[a]Reference = Non-smoker
[b]Reference = High SES group
[c]Reference = Less than weekly cannabis use

Table 3. Unadjusted and adjusted estimates for the effects of AL on LTL at ages 26 and 38.

		Age 26		Age 38	
	Variable	Estimate	95% C.I.	Estimate	95% C.I.
Unadjusted	AL	0.01	(-0.08, 0.10)	0	(-0.03, 0.03)
Adjusted	AL	0.01	(-0.09, 0.10)	0.01	(-0.02, 0.04)
	Male sex	-0.02	(-0.08, 0.04)	-0.02	(-0.07, 0.02)
	Smoking[a]	-0.06	(-0.12, 0.01)	-0.01	(-0.06, 0.05)
	SES trajectory group[b]				
	Low-High	-0.05	(-0.18, 0.08)	-0.01	(-0.10. 0.08)
	High-Low	-0.03	(-0.11, 0.05)	-0.06	(-0.13. 0.01)
	High-High	0.01	(-0.07, 0.10)	-0.01	(-0.08, 0.06)
	Weekly cannabis use[c]	0.06	(-0.03, 0.15)	-0.02	(-0.11, 0.07)

[a]Reference = Non-smoker
[b]Reference = High SES group
[c]Reference = Less than weekly cannabis use

Table 4. Pooled unadjusted and adjusted estimates for the effects of leucocyte telomere length (LTL) on periodontal attachment loss (AL) using GEE.

	Variable	Pooled Estimate	95% C.I.
Unadjusted	LTL	0.98	(0.93, 1.03)
	LTL	1.02	(0.97, 1.07)
	Male sex	**1.08**	**(1.04, 1.12)**
	Smoker[a]	**1.17**	**(1.12, 1.22)**
Adjusted	SES trajectory group[b]		
	Low-High	**0.91**	**(0.86, 0.97)**
	High-Low	**0.93**	**(0.88, 0.99)**
	High-High	**0.88**	**(0.83, 0.93)**
	Weekly cannabis use[c]	**1.10**	**(1.02, 1.18)**
	Age 38[d]	**1.10**	**(1.06, 1.13)**

[a]Reference = non-smoker
[b]Reference = Low-Low SES trajectory group
[c]Reference = Less than weekly cannabis use
[d]Reference = Age 26

Table 5. Pooled unadjusted and adjusted estimates for the effects of periodontal attachment loss (AL) on leucocyte telomere length (LTL) using GEE.

	Variable	Pooled Estimate	95% C.I.
Unadjusted	AL	-0.01	(-0.04, 0.01)
Adjusted	AL	0.02	(-0.01, 0.05)
	Male sex	-0.03	(-0.07, 0.02)
	Smoking[a]	-0.02	(-0.06, 0.02)
	SES trajectory group[b]		
	Low-High	0.01	(-0.05, 0.07)
	High-Low	-0.01	(-0.07, 0.05)
	High-High	-0.01	(-0.06, 0.05)
	Weekly cannabis use[c]	0.02	(-0.04, 0.08)
	Age 38[d]	**-0.15**	**(-0.18,-0.13)**

[a]Reference = non-smoker
[b]Reference = Low-Low SES trajectory group
[c]Reference = Less than weekly cannabis use
[d]Reference = Age 26