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# ASSOCIATION BETWEEN TELOMERE SHORTENING AND AGEING DURING OCCUPATIONAL EXPOSURE

## VEZA IZMEĐU SKRAĆENJA TELOMERA I STARENJA USLED PROFESIONALNE IZLOŽENOSTI

*Sima Ataollahi Eshkoor[1], Fatemeh Jahanshiri[2], Patimah Ismail[1], Sabariah Abd. Rahman[1], Saidi Moin[1], Mohd Yusoff Adon[3]*

*[1]Faculty of Medicine and Health Sciences*
*[2]Faculty of Biotechnology, University Putra Malaysia (UPM), 43400 Serdang, Selangor*
*[3]Institute for Medical Research, Ministry of Health Malaysia, Kuala Lumpur*

**Summary:** Telomere length is considered as a biomarker of ageing, resulting in shortening during the process. The present investigation was an attempt to determine the relative telomere length in mechanical workshop workers. Telomere length shortening in cells during occupational exposure causes accelerated ageing. Genomic DNA was isolated from buccal epithelial cells collected from 240 individuals, comprising two groups of 120 exposed workers and 120 unexposed controls. Telomere length was measured by using real time PCR. Both telomere (T) and single copy gene (S) specific primers were used to compute the relative T/S ratio and expressed as the relative telomere length. Telomere length differed significantly between the workers and controls ($p<0.05$). The results showed an indirect and significant association (r=–0.356, p=0.001) between age and telomere length in the workers. This study showed that the difference in telomere length shortening was statistically significant ($p<0.05$) between the workers and controls. It was concluded that occupational exposure acts as a risk factor to enhance telomere length shortening and accelerate ageing.

**Keywords:** telomere, 36B4 gene, ageing, occupational exposure, buccal cells, real-time PCR

**Kratak sadržaj:** Dužina telomera smatra se biomarkerom starenja i tokom ovog procesa rezultira skraćenjem. Ovo istraživanje predstavlja pokušaj određivanja relativne dužine telomera kod radnika u mehaničarskim radionicama. Skraćenje dužine telomera u ćelijama tokom profesionalne izloženosti izaziva ubrzano starenje. Genomska DNK izolovana je iz epitelnih ćelija unutrašnje strane obraza sakupljenih od 240 osoba, koji su činili dve grupe: 120 profesionalno izloženih radnika i 120 neizloženih kontrolnih subjekata. Dužina telomera izmerena je tehnikom PCR u realnom vremenu. Specifični prajmeri telomera (T) i gena prisutnih u jednoj kopiji (S) upotrebljeni su za izračunavanje relativne razmere T/S i izraženi kao relativna dužina telomera. Dužina telomera značajno se razlikovala između radnika i kontrolnih subjekata ($p<0,05$). Rezultati su pokazali da između starosti i dužine telomera kod radnika postoji indirektna i značajna povezanost (r=–0,356, p=0,001). Ova studija je pokazala da je razlika u skraćenju dužine telomera između radnika i kontrolnih subjekata bila statistički značajna ($p<0,05$). Zaključeno je da profesionalna izloženost predstavlja faktor rizika za znatnije skraćenje dužine telomera i ubrzano starenje.

**Ključne reči:** telomera, gen 36B4, starenje, profesionalna izloženost, ćelije unutrašnje strane obraza, PCR u realnom vremenu

## Introduction

Ageing causes progressive reduction of tissue function in the body that finally results in the end of life. The diminished function can be a consequence of lost or reduced function of the cells after mitotic activity or failure in the replacement of decreased functional cells with the active and replicable cells.

*Address for correspondence:*

Patimah Ismail
patimahismail@gmail.com
Faculty of Medicine and Health Sciences,
43400 UMP Serdang, Selangor
Tel: +60389472314

Ageing is a biological process that differs among people (1). Ageing is an accumulation of mutations in the somatic cells and results in tissue atrophy, cancers and the reduced function of organs (2). Ageing seems to be due to a combination of effects of both genetic and environmental factors (3).

Telomeres are special structures at the end of chromosomes, which help to distinguish a natural and broken chromosome (4). Telomere is a TTAGGG repeat complex, located at the end of a chromosome in eukaryotic cells (5). It provides a protective mechanism to preserve the chromosomal integrity in a cell (6).

Telomeres switch between uncapped and capped states in cells (7). The end replication problems (8, 9) and produced oxidative stress (10, 11) cause erosion and shortening in telomeres. The telomere length has a major role in tissue functioning and life expectancy in organisms, and its shortening stops cell division which results in senescence (8, 9). Telomere dysfunction in advanced age acts as a biological biomarker (12).

Telomeres shorten with age in various somatic tissues (13–17). In some cells such as fibroblasts a telomere does not shorten (18) and long telomere length has been reported in newborns (19), fetuses (20), and zygotes (21). However, the individuals with longer telomere length seem to have higher life expectancy. Long telomere length or a slow rate of telomere erosion results in a longer life-span (15, 22–26). Low rate of telomere erosion is the most important factor causing greater longevity (15). One reason of erosion in the telomere is environmental exposure (27).

Hence, telomere shortening due to each cell cycle division limits cell proliferation and the number of cell divisions, which induces apoptosis or replicative senescence (28). The acquired information could be considered as an early warning about the potential risk of exposure on health, when populations are exposed for a longer time (29). This study evaluated telomere shortening in the mechanical workshop workers during occupational exposure for the first time. It is a step to explore the possible influence of occupational exposure and telomere shortening on early ageing.

## Materials and Methods

The approval and permission for the study were obtained from the Ethical Committee of the Medical and Health Sciences Faculty (Reference Number: UPM/FPSK/PADS/T7-MJKEtikaPer/F01 (JSB-Aug (08)05). The samples were collected from buccal mucosa epithelial cells. The subjects comprised 120 workers who had been exposed in the car mechanical workshops and 120 controls as the non-exposed population. The individuals were interviewed to determine their health status and lifestyles. The respondents were asked to rinse their mouth with water before sample collection.

The cells were collected by scraping the inner part of both sides of the cheeks with a cytology brush. Then, the cells were gently mixed with 0.9% sodium chloride in a micro-centrifuge tube and brought to the laboratory. After collecting, genomic DNA was extracted by using the QIAamp DNA blood MiniKit (Qiagen, Courtaboeuf, France). Real-time PCR was used to measure telomere length. Telomere length in the individuals was measured in triplicate. The used primers were telomere and 36B4, which were described by Cawthon (30).

The primer sequences were: tel1, GGTTTTT-GAGGGTGAGGGTGAGGGTGAGG-GTGAGGGT; tel2, TCC-CGACTATCCCTATCCCTATCCCTATCCC-TATCCCTA; 36B4u, CAGC-AAGTGGGAAGGTG-TAATCC; and 36B4d, CCCATTCTATCA-TCAACGGG-TACAA. The telomere repeat copy number to single gene copy number (T/S) ratio was determined using the Corbett Rotor-Gene 6000 (Corbett Life Science, Sydney, Australia) with 36 wells. For PCR reaction, a 25 μL volume of solution was prepared in PCR tubes. In each run, both gene telomere and 36B4 were settled for one sample in separate tubes. Primers were obtained from Bioline (London, UK).

The solution for PCR reaction included 0.6 μL of each primer (10 pm/μL), 1 μL Eva green, 1 μL DNA, 5 μL master mix Immomix (Bioline, London, UK), and 16.8 μL pure water. The reaction proceeded 1 cycle at 94 °C for 5 min, followed by 40 cycles at 94 °C for 30s, 56 °C for 30s, and 72 °C for 50s. Melting temperature was 70 °C to 95 °C. The obtained data were interpreted throughout the data set to assess threshold cycle values. The PCR products were identified by running 1.8% to 2% agarose gel electrophoresis, and the gel was then viewed with an AlphaImager analysis system (Alpha Innotech, San Leandro, CA). The product sizes were 76 bp for telomere and 74 bp for 36B4 (*Figure 1* and *2*).

In addition, for having a good view on the efficiency of PCR reaction, standard curve was run based on serial dilution of genomic DNA from 5 to 100 ng using genomic DNA, derived from one selected buccal cell sample (*Figure 3* and *4*). The data were interpreted to assess the threshold cycle ($C_t$) values. The T/S ratio for each sample was calculated by subtracting the average 36B4 Ct value from the average telomere $C_t$ value. One sample was used as reference in each run in triplicate to compare the results. The formula of $2^{-\Delta\Delta}$ was used to calculate the T/S ratio. For calculating, $\Delta C_T = C_t$ (telomere) – $C_t$ (36B4) formula was used. For final calculation the results of $\Delta C_t$ (target) – $\Delta C_t$ (reference) were used to compare the measurements and for taking proper T/S ratio.

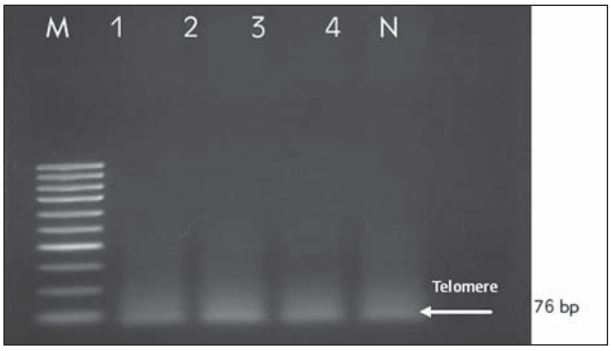


**Figure 1** Electrophoresis in samples 1–4. M represents the 100 bp by DNA Ladder. N is negative control during PCR reaction.

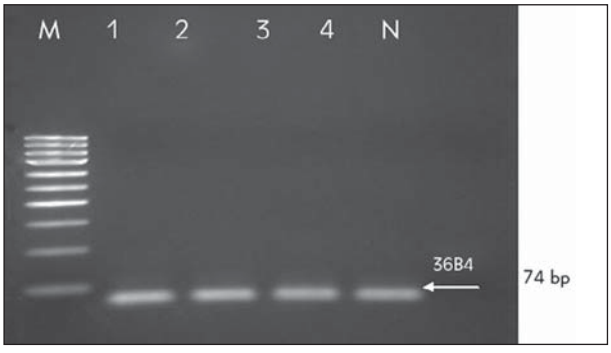


**Figure 2** PCR product of 36B4 resolved in 2% agarose gel electrophoresis in samples 1–4. M represents the 100 bp by DNA Ladder. N is negative control in PCR reaction.

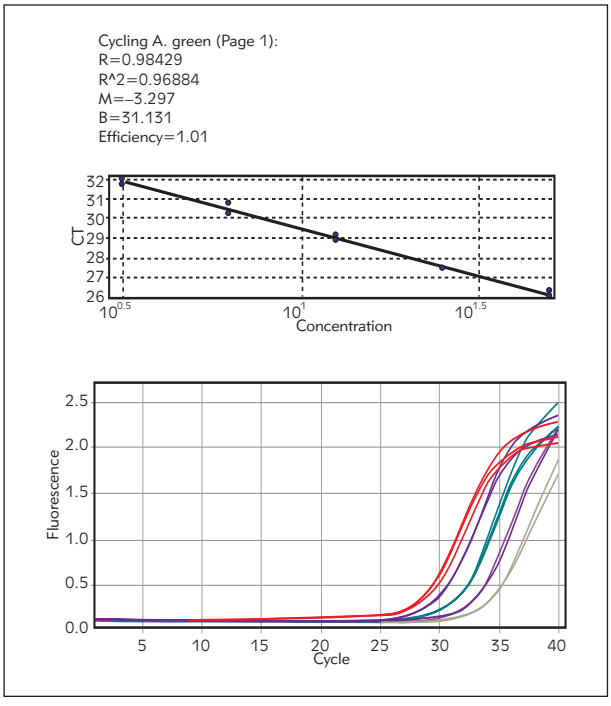


**Figure 3** 36B4 standard curve in one buccal cell sample in different dilutions.

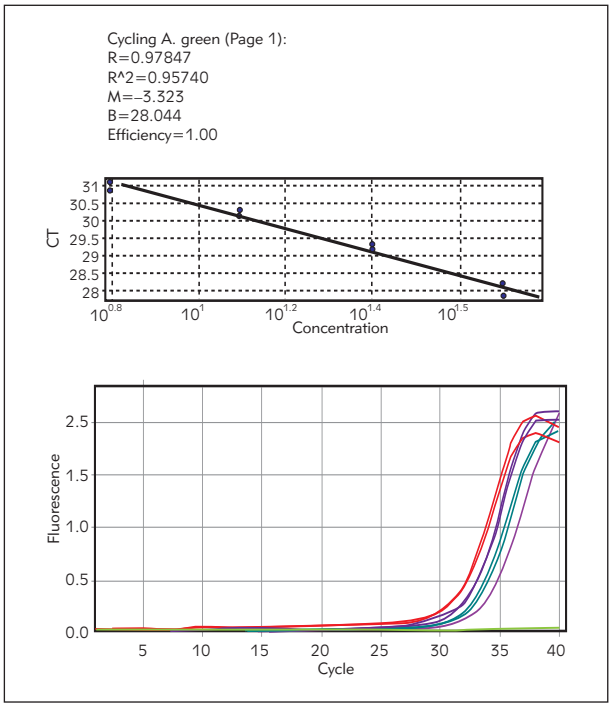


**Figure 4** Telomere standard curve in one buccal cell sample in different dilutions.

*Statistical analysis*

The normality of variables was evaluated using the Kolmogorov-Smirnov test. The independent t-test and ANOVA were used to compare the demographic characteristics of the study populations. Telomere length measurements were tested using the independent t-test. Calculation of correlation between the variables was done by Pearson rank test. The critical level for rejection of the null hypothesis (two-tailed test) was a p value of 5% (p=0.05). All analyses were performed using the Statistical Package for the Social Sciences (SPSS, Chicago, IL) software version 16.0.

## Results

The minimum of relative telomere length in the workers and controls was 0.00. The maximum of relative telomere length in the workers and controls was 4.00 and 53.08, respectively. Difference in the relative telomere length between the workers and controls was statistically significant (p=0.001) (*Table I*). Association between relative telomere length and age was statistically significant in the individuals (r=–0.131, p=0.043) and workers (r=–0.356, p=0.001), but in the controls it was not significant (*Table II*). The workers and controls were classified by age into groups below 30 years and above. Difference in the relative telomere length between the workers and controls in the older group was not statistically significant ($p>0.05$). The effect of occupational exposure

**Table I** Summary and comparison of relative telomere length between the workers and controls.

| Study groups | N | Max | Min | *p* Value |
|---|---|---|---|---|
| Workers | 120 | 4.00 | 0.00 | 0.001[a] |
| Controls | 120 | 53.08 | 0.00 | |

[a] Significant at the 0.05 level using the independent t-test.

**Table II** Correlation between age and relative telomere length.

| Groups | test | r | *p* Value |
|---|---|---|---|
| All individuals | Pearson | –0.131 | 0.043 |
| Workers | | –0.356 | 0.001 |
| Controls | | –0.056 | 0.542 |

Correlation is significant at the 0.05 level (two tailed).

**Table III** Effect of exposure and age on relative telomere length.

| Groups | ≥30 | <30 | *p* Value |
|---|---|---|---|
| | N | N | |
| All individuals | 65[a] | 175[a] | 0.001** |
| Workers | 54[c] | 66[d] | 0.001** |
| Controls | 11[e] | 109[f] | 0.613** |
| *p* value | 0.070* | 0.004* | |

Means with different superscripts are significant at p<0.05 using the independent t-test.
*p value is between the workers and controls in each column.
**p value is between age groups (<30≤) in each row.

on telomere length was statistically significant in the younger (p=0.004) and all individuals (p=0.001) groups. In the workers the difference in relative telomere length between the older and younger groups was statistically significant (p=0.001) (*Table III*).

Smoking, alcohol consumption, educational level and ethnicity showed no statistically significant effect on telomere length ($p>0.05$). Duration of employment was divided into more or less than 5 years and showed a significant effect on telomere length shortening (p=0.001) (*Table IV*).

**Table IV** Result of relative telomere length with the socio-demographic factors.

| Group | Workers | Controls | All subjects | |
|---|---|---|---|---|
| | N | N | N | *p* |
| All subjects | 120 | 120 | 240 | 0.046 |
| Smokers | 59 | 30 | 89 | 0.377 |
| Non-smokers | 61 | 90 | 151 | |
| Educated | 24 | 105 | 129 | 0.437 |
| Non-educated | 96 | 15 | 111 | |
| Drinkers | 12 | 3 | 3 | 0.683 |
| Non-drinkers | 108 | 117 | 117 | |
| Malay | 65 | 78 | 143 | |
| Chinese | 45 | 33 | 78 | 0.814 |
| Indian | 10 | 9 | 19 | |
| Working time>5Y | 37 | | | 0.001 |
| Working time<5Y | 83 | | | |

P value is significant at the significance level (p=0.05).

## Discussion

In this study, telomere length in the cells was analyzed by real-time PCR to determine the occupational exposure effects. Real-time PCR is an easy and good method to obtain reproducible results of the telomere length. It is a type of PCR method, which can be done with a lesser amount of DNA and completed in a short time (31). In this study, telomere length was shortened in the workers as compared to the controls. It indicated that the occupational exposure reduced the length of the telomere in the workers. This finding is probably due to the influence of exposure on genes involved in the telomere length maintenance (32). Correlation of age and telomere length in our study suggested a possible influence of the widespread environmental and occupational hazards exposure on ageing acceleration (33–35), through shortening the telomere length.

This study confirmed the previous investigations in showing the correlation of telomere length and age (31, 36, 37). Telomere length and its patterns in the chromosomes are inherited. Ageing could be relevant to different genes and genetic controls, which are involved in the regulation of telomere length (31, 36–40). However, the findings of this study expressed the possible early biological ageing in buccal cells reflected in telomere shortening during occupational

exposure. Still, using buccal cells as sources to measure telomere length bears some limitations. One of the reasons is the high rate of cell division and turnover rate in epithelial cell tissue, which shortens the telomeres in the cells. Other limiting factors are the mixed sample cells, containing karyorrhectic, karyolytic, pyknotic and condensed chromatin cells in the cellular senescence. Another contributing reason is the highly oxygenated buccal cells, which induce an oxidative stress effect to shorten telomere length (41).

Ageing and telomere length are influenced by genetic and socio-demographic factors. These factors contribute to genetic damage and result in synergic or additive effects to accelerate ageing (42, 43). In this investigation, the results indicated that the duration time of exposure in the workers could enhance DNA damage (44, 45) and telomere length shortening (31). Hence, the improvement of work behaviors could reduce genomic damage resulting from the environmental and occupational effects (46). However, the interpretation is influenced by different factors, including ethnicity, sample size, type of used tissue, present conditions in the laboratory and many other differences, which have been presented in the studies (31).

It was concluded that the shortening of telomere length contributes to ageing. This study was the first report of the effect of occupational exposure on telomere length shortening and ageing in the car mechanical workshop workers. However, further telomere studies need to be conducted intensively with more samples in different age groups, for many years, to derive firm conclusions.

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## Conflict of interest statement

The authors stated that there are no conflicts of interest regarding the publication of this article.

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