Association between Occupational Exposure to Tobacco Dust and Absolute Telomere Length: A Cross-sectional Study on Female Beedi Workers

Yamini Kanipakam1, Vezhavendhan Nagaraja2, Suganya Rajaram3, Vidyalakshmi Santhanam4, Sivaramakrishnan Muthanandam5, Santha Devy Arumugam6, Bala Maddileti Gandla7, Raj Kumar Chinnadurai8

ABSTRACT

Aim and objective: The main aim of the study was to assess the absolute telomere length (aTL) in female beedi workers using real-time polymerase chain reaction (RT-PCR) and to compare the aTL with female non-beedi workers.

Materials and methods: A cross-sectional study was carried out among age-matched 20 female non-beedi workers and 20 female beedi workers were enrolled for molecular analysis. The workers were in the age-group of 20–35 years and were workers exposed from 1 to 3 years. Saliva samples were collected from workers and control subjects for molecular analysis. The genomic DNA was extracted from saliva and aTL was estimated using real-time polymerase chain reaction.

Results: The mean and standard deviation of average absolute TL/each chromosome end for the control group and study group were 0.75 ± 0.94 and 1.45 ± 2.76 kb. There was no statistically significant difference between the control group and the study group (Z = −0.112, p = 0.911).

Conclusion: The present study revealed that there is no significant association in average absolute TL in early exposed female beedi workers compared with female non-beedi workers. Furthermore, horizons are to be expanded for the population to prevent any occupational health hazards.

Clinical significance: Telomere length is a biological clock that decides the lifetime of a cell and organism. Determination of TL is a better tool to detect genomic damage. Unburnt tobacco has been related to several health issues in beedi employees. The importance is to predict the genetic liability by estimating the aTL in beedi workers at early exposure to tobacco dust (TD).

Keywords: Occupational hazards, Real-time polymerase chain reaction, Telomere length, Tobacco dust.

World Journal of Dentistry (2021): 10.5005/jp-journals-10015-1854

INTRODUCTION

The term “occupational hazard” refers to both long- and short-term hazards associated with the workplace environment that can affect the health of employees. Occupational hazards have caused health problems in several cases. According to a report published jointly by the World Health Organization (WHO) and the World Economic Forum, India, will lose 236.6 billion by 2015 due to unhealthy lifestyles and poor diet. Beedi production began formally in 1902, though people in rural areas were well known for making beedis for their own consumption prior to this date. Tobacco production is a small-scale business in the majority of Indian states. It is an opportunity for household members, mostly women from the unorganized industrial sector that provides a large number of job opportunities. Unburned tobacco has been related to several health issues in beedi workers, including asthma, tuberculosis, oral cancer, nasopharyngeal cancer, and laryngeal cancer, as well as other health issues.

Beedi is a small-scale business in the majority of Indian states. It is an opportunity for household members, mostly women from the unorganized industrial sector that provides a large number of job opportunities. Unburned tobacco has been related to several health issues in beedi workers, including asthma, tuberculosis, oral cancer, nasopharyngeal cancer, and laryngeal cancer, as well as other health issues.

The term “occupational hazard” refers to both long- and short-term hazards associated with the workplace environment that can affect the health of employees. Occupational hazards have caused health problems in several cases. According to a report published jointly by the World Health Organization (WHO) and the World Economic Forum, India, will lose 236.6 billion by 2015 due to unhealthy lifestyles and poor diet. Beedi production began formally in 1902, though people in rural areas were well known for making beedis for their own consumption prior to this date. Tobacco production is a small-scale business in the majority of Indian states. It is an opportunity for household members, mostly women from the unorganized industrial sector that provides a large number of job opportunities. Unburned tobacco has been related to several health issues in beedi workers, including asthma, tuberculosis, oral cancer, nasopharyngeal cancer, and laryngeal cancer, as well as other health issues.

Beedi rollers inhale, swallow, and expose their skin and mucous membranes to harmful substances. Nicotine, nitrosamine, formaldehyde, acetaldehyde, crotonaldehyde, hydrazine, arsenic, nickel, cadmium, benzopyrene, and potassium are all toxic components of tobacco. Copper, sulfur, potassium, and organophosphates are also present in pesticides used in tobacco farming. During the beedi production process, all of these are released into the air. According to Joshi et al., 97% of beedi workers were unaware of the health risks associated with tobacco use due to occupational exposure. Unburned tobacco has been linked to various health problems in beedi workers, including asthma, tuberculosis, oral cancer, nasopharyngeal cancer, and laryngeal cancer, as well as other health issues.

1–5 Department of Oral and Maxillofacial Pathology and Oral Microbiology, Indira Gandhi Institute of Dental Sciences, Sri Balaji Vidyapeeth, Puducherry, India
6Department of Forensic Medicine and Toxicology, SRM Medical College Hospital and Research Centre, Trichy, Tamil Nadu, India
7Centre for Molecular Unitary Diagnostics Research and Advancements (C-MUDRA) Central Inter-Disciplinary Research Facility (CIDRF), Sri Balaji Vidyapeeth, Puducherry, India

Corresponding Author: Yamini Kanipakam, Department of Oral and Maxillofacial Pathology and Oral Microbiology, Indira Gandhi Institute of Dental Sciences, Sri Balaji Vidyapeeth, Puducherry, India

How to cite this article: Kanipakam Y, Nagaraja V, Rajaram S, et al. Association between Occupational Exposure to Tobacco Dust and Absolute Telomere Length: A Cross-sectional Study on Female Beedi Workers. World J Dent 2021;12(5):417–422.

Conflict of interest: None

© Jaypee Brothers Medical Publishers. 2021 Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (https://creativecommons.org/licenses/by-nc/4.0/), which permits unrestricted use, distribution, and non-commercial reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated.
Association between Occupational Exposure to TD and aTL

gynecological issues such as abortion, infection of the reproductive tract (urinary tract), infant low birth weight, and other issues such as headache, palpitations, eye, and skin problems.

Telomeres are specific DNA sequences that repeat themselves at the ends of each chromosome. Telomeres are reduced during each cell division, maintaining genomic integrity. If the telomere shortens to a certain length, the cell enters replicative senescence or apoptosis. However, if the condition is exceeded, alterations in DNA such as nucleolytic degradation, surplus recombination, repair, and interchromosomal fusion may occur, resulting in genetic instability. Diabetes, coronary heart disease, Alzheimer’s disease, abdominal aortic aneurysm, celiac disease, and interstitial lung disease, including cancer, are all associated with telomere length (TL) change. Hormonal changes, increased oxidative stress, polycystic ovary syndrome (PCOS), and premature ovarian failure (POF) can affect telomere integrity in females by causing telomere attrition. Only a few studies have reported biochemical and cytogenetic changes associated with occupational tobacco use. Kahl et al. reported shorter TL in tobacco farmers was associated with tobacco leaf exposure and pesticide use. Similar to chemical indicator monitoring, genetic changes can be suspected to occur in the early stages of working years and can be used as an indicator to release workers from duty for return to normal health status. As a result, this research aims to determine aTL as a tool for predicting genetic liability in early-stage exposed female beedi workers using real-time polymerase chain reaction (RT-PCR).

**Materials and Methods**

Before the study, a field visit was conducted and data was collected from the Beedi industry, which revealed that the majority of beedi rollers are females who are constantly exposed to tobacco inhalation. So, in particular, female beedi workers were included in the study. Telomere length was taken as a parameter because TL serves as a biological clock to determine the lifespan of a cell and an organism. Telomere length is identified as a better tool to detect genomic damage in few studies. Very short telomeres and an organism. Telomere length is identified as a better tool to determine the lifespan of a cell.

The sample size was calculated using the formula:

\[
 n = \frac{2\sigma^2 (Z_{1-\beta} + Z_{1-\alpha}/2)^2}{(\mu_1 - \mu_2)^2}
\]

The Mann–Whitney U test was used in the statistical analysis to compare the average absolute TL/each chromosome end (kb) between the control and study groups.

**Study Population**

The study included two groups: Group I—Female non-beedi workers (control group) of aged 20–35 years with no other comorbidities. Group II—Female beedi workers (study group) of aged 20–35 years with working experience of 1–3 years and no other comorbidities.

**Inclusion Criteria**

Female beedi workers of age-group ranging from 20 to 35 years, whose duration of employment should be >1 year and <3 years. Female non-beedi workers of age-group 20–35 years, their family members also should not be involved in beedi industry.

**Exclusion Criteria**

Participants with a habit history of tobacco or alcohol consumption. Patients with high temperature, stress, hypertension, diabetes, chronic obstructive pulmonary diseases (COPDs), osteoarthritis, tuberculosis, carcinoma, HIV, Parkinson’s disease, and any other systemic, infectious diseases or vascular injury/truma and individuals with a high-stress level as indicated by the perceived stress assessment scale were also excluded.

The study protocol was approved by the Institutional Review Board and Institutional Ethical Committee (IRB/IEC Ref. No. IGDSIEC2018NRP23PGKYOPM). The study involved a total of 40 individuals, 20 non-exposed and 20 exposed (beedi workers) to tobacco dust. The workers were in the age-group of 20–35 years and were workers experience from 1 to 3 years. Most studies showed that long-term tobacco exposure shortens aTL. This study was conducted to establish the relationship between aTL and short-term tobacco dust exposure. All people who took part in this study gave their informed consent. Saliva samples were collected from 20 female beedi workers in the Trichy region, Tamil Nadu, India. The age-matched 20 control samples were collected simultaneously.

**Sample Collection and Transportation**

In the morning, saliva samples were taken (8–10 am). For at least 1 hour before the saliva collection, the participants were advised not to eat, drink, or perform any oral hygiene procedures. Distilled drinking water was provided to rinse their mouth. Participants were asked to gargle well for 1 minute before being asked to expectorate. Ten milliliters of saliva were extracted in a 50 mL centrifuge tube after 5–10 minutes. After collecting the samples, they were transferred to the laboratory on dry ice for RT-PCR analysis, which was completed on the same day as the sample collection.

Extraction of genomic DNA and quantification from saliva samples (Qiagen DNA kit) DNA was extracted and quantified from saliva samples using the Qiagen DNA kit.

All of the procedures were carried out in accordance with the user manual for the Qiagen DNA kit. Due to a processing error, one of the samples in the research group has been lost.

One milliliter of saliva was taken in a 50 mL falcon tube, along with 4 mL of PBS, and centrifuged at 1,800 × g for 5 minutes at room temperature. The pallet was resuspended in 180 μL of PBS after the supernatant was thoroughly removed. To the sample, 20 μL of Qiagen protease was added, followed by 200 μL of buffer AL and vortexing immediately. The incubation time was 10 minutes at 56°C. Following the incubation period, the tubes were spun down and absolute alcohol was added and mixed by vortexing. The contents were then moved to a QIamp spin column and placed in a 2 mL collection tube without touching or wetting the rim, before being centrifuged at 6,000 × g for 1 minute. The filtrate was discarded, and the spin column was placed in a collection tube. The sample was then mixed with 500 μL of buffer AW 1 and centrifuged at 6,000 × g for 1 minute. The filtrate was discarded, and the spin column was moved to a clean collection tube with 500 μL of AW 2 buffer. Three minutes of centrifugation at full speed. After filtration, the tube was centrifuged for 1 minute at 8,000 revolutions per minute (RPM). The concentration of extracted DNA was validated using a NanoDrop...
Association between Occupational Exposure to TD and aTL

2000 c spectrophotometer. After exporting the data to Microsoft Excel, the average concentration for each purified sample was calculated. The Absolute Human Telomere Length Quantification real-time PCR Assay kit was used to determine the average TL in the samples. All of the procedures were carried out in accordance with the user manual for TL quantification. Fifty nanograms of genomic DNA was used to determine the length of telomere using real-time PCR as described by O’Callaghan and Fenech. 36B4 was used as a single copy gene to determine the number of genome copies present in the sample, and the length of telomere present in the sample was estimated from the telomere standard curve have been depicted in Figure 1. By normalizing TL with 36B4 copy number per reaction as depicted in Figure 2, the absolute quantification of TL was determined.

RESULTS AND OBSERVATIONS

Statistical Analysis

The mean value of average absolute TL/each chromosome end (kb) for the control group was 0.70 and 1.45 for the study group. The minimum mean value of average absolute TL/each chromosome end (kb) for the study group was less (0.0007) compared with the control group (0.0049). The maximum mean value of average absolute TL/each chromosome end (kb) was high among the study group (11.72) compared with the control group (4.01). The median value of average absolute TL/each chromosome end (kb) was 0.52 for the control group and 0.33 for the study group (Table 1 and Fig. 3).

Comparison of average absolute TL/each chromosome end (kb) between the control group and study group was compared using the Mann–Whitney U test and found there was no significant difference between the control group and study group (Z = −0.112, p = 0.911). The rank average of absolute TL/each chromosome end (kb) for the control group was 20.2 and that for the study group was 19.79 explains that the control group and study group had almost an equal average absolute TL/each chromosome end (kb) (Table 2).

DISCUSSION

India holds a significant position in beedi manufacturing in the world. The beedi industry is primarily a labor-intensive industry that falls into the unorganized sector category. According to the Ministry of Labour, Government of Asian Nation (2003), the Indian beedi industry employs over 4.48 million people.1 Beedi is made primarily of sun-cured tobacco (Nicotiana tabacum) flakes that are hand-rolled and packed into a dried leaf (tendu, temburni, etc.). Tobacco dust additives, nicotine, carbon monoxide, and polycyclic aromatic hydrocarbons (PAHs) exposure have all been accompanying by an increased risk of various illnesses, including cancer.1,20 According to numerous studies, tobacco use is strongly associated with the progression of various diseases, especially those affecting the mouth, lungs, and respiratory system. Certain tobacco and smokeless tobacco compounds have been classified as carcinogenic agents by the International Agency for Research on Cancer (IARC), which primarily cause cancers of the oral cavity, esophagus, and larynx. Alzheimer’s disease, arteriosclerosis, cancer, chronic liver disease/cirrhosis, COPD, diabetes, hypertension, heart attack, nephritis/CRF, and stroke are some of the common illnesses associated with occupational lifestyle.21,22 Oral diseases such as dental caries, periodontal disease, premalignant lesions, and conditions that could lead to malignancy are more common among beedi workers.13,23 According to studies among beedi workers, the effect of tobacco dust exposure and work duration is directly proportional to chromosomal aberration and chromosomal

![Fig. 1: Amplification plot—single-copy reference (SCR)—each sample reaction fluorescence intensity](image1.png)

![Fig. 2: Amplification plot—telomere (each sample reaction fluorescence intensity)](image2.png)

| Table 1: Mean value of average absolute telomere length/each chromosome end (kb) between the control group and study group |
|---------------------------------------------------------------|
| ![Table 1](image3.png) |

![Table 1](image4.png)
Table 2: Mann–Whitney U test for comparison of the mean rank of average absolute telomere length/each chromosome end (kb) between the control group and study group

| Group           | N   | Mean rank | Sum of ranks | Mann–Whitney U | Z value | p value |
|-----------------|-----|-----------|--------------|----------------|---------|---------|
| Control group   | 20  | 20.20     | 404.00       | 186.00         | −0.112  | 0.911   |
| Study group     | 19  | 19.79     | 376.00       |                |         |         |
| Total           | 39  |           |              |                |         |         |

**Fig. 3:** Mean and standard deviation value of average absolute telomere length/each chromosome end (kb) between the control group and study group.

**Table 2:** Mann–Whitney U test for comparison of the mean rank of average absolute telomere length/each chromosome end (kb) between the control group and study group.

In the present study, apart from the exogenous agents, endogenous factors play a significant role in telomere shortening. The mean and standard deviation of average absolute TL/each chromosome end (kb) for group I and group II were 0.70 ± 0.94 and 1.45 ± 2.76 kb, respectively. The Mann–Whitney U test was used to compare group I and group II. There was no statistically significant difference between group I and group II (Z = −0.112, p = 0.911). The rank average for group I was 20.2 and that for group II was 19.79. This explains that group I and group II had almost an equal average absolute TL/each chromosome end (kb).

Telomere length acts as a biological clock, determining the lifetime of a cell and an organism. Telomeres that are too short cause senescence and cell cycle arrest and it is a basic tumor suppressor mechanism that inhibits a cell’s ability to proliferate and prevents instability of the genome caused by telomere dysfunction. 

Telomeres protect DNA degradation, repair, and recombination operations by forming a unique heterochromatic structure at the end of linear chromosomes. Telomeres are therefore necessary for chromosome stability. It has been suggested that it protects the ends of chromosomes from telomerase activity, as well as degradation and DNA repair activities.

Shelterin, a specialized complex that regulates TL and protects telomeres from the DNA damage response, binds to telomeres (DDR). End-replication problems cause telomeres to shorten with each cell division as a result of incomplete replication of linear DNA molecules by traditional DNA polymerases.

Because of the end-replication problem, replication fork collapse, oxidative stress, and nucleolytic processing, telomere shortening is a natural result of cell division that occurs throughout life. Rare mutations in telomere maintenance genes like TERT, RTEL1, DKC1, and WRN can result in markedly shorter telomeres, premature aging, and an increased risk of various rare diseases. Telomere shortening causes replicative senescence, which leads to stem cell dysfunction and inflammation, which leads to aging-related diseases.

Hormonal changes and increased oxidative stress in PCOS patients may affect telomere integrity and lead to telomere attrition. The granulosa cells of POF patients had shorter telomeres. As a result, TL is a biomarker for human diseases that can be used to detect molecular genetic damage. Telomere length estimation can be done in various ways, with PCR being the most common method for making copies of particular DNA fragments.

Vega et al., in their study, stated that human telomeres repeat and can range in length from 2 to 50 kb pairs. Kahl et al. stated that TL varies between individual telomeres and cell types. In a systematic review, Buehring et al. found that TL shortens with age as a physiological process influenced by genetic and lifestyle factors. In the present study, the mean average absolute TL for group I and group II ranges from 0.70 to 1.45 kb/chromosome, which is lower than the normal values.

Natural telomere shortening can be accelerated by unhealthy lifestyles and occupational and environmental exposures, according to Kahl et al. and Kelesidis et al. Apart from exogenous agents, endogenous agents such as DNA replication errors, reactive oxygen species (ROS), and spontaneous hydrolytic reactions may cause chromosomal aberrations, translocations, point mutations, gene disruption, and telomere shortening, among other things.

In the present study, apart from the exogenous agents, endogenous
factors like genetic alterations may also influence group I and group II (as the people are residing in the same area), which can contribute to shorter TL. In a study of beedi workers, Khanna et al. found that chromosomal abrasion and DNA damage are linked to the duration of occupational tobacco dust exposure.6,34 According to Mahimkar and Bhishey, tobacco processors are subjected to significant genotoxicity as a result of their work.35 Lu et al. conducted a cross-sectional study in which they found that TL decreased more rapidly in the second-hand smoke exposure group than in the non-exposed group when compared with the non-exposed group.35 According to O’Callaghan and Fenech, buccal cells had a mean absolute TL of 211.2 kb/diploid genome, the absolute TL in their younger group was (1.14 kb/diploid genome), and the range was 45–594 kb (0.4–6.4 kb/diploid genome). According to Thomas et al., a young person’s buccal cell TL can be as long as 40 kb per diploid genome. Young controls in their study had absolute TLs of 41.98 ± 32.66 (0.45 ± 0.35 kb/diploid genome).36,37 The control group’s mean absolute TL was 0.70 ± 0.94 kb in the current study. Montpetit et al. indicated that results from PCR technique-based studies are difficult to compare between studies. This restriction is due to variations in DNA quality depending on the technique used to extract genomic DNA. One of the limitations of the qPCR approach, according to Nettle et al., is the study that it highlights variations between laboratories.38,39 In the present study, due to their shorter duration of employment, the data showed less variation between group I and group II on assessing the TL. This study also showed alterations in average absolute TL among individual beedi workers and non-beedi workers. The TL seems to be shorter (0.0007–11.72 kb) in the people of Woraiyur, Tiruchirapalli factory was found to be 1–3 years. However, aTL among beedi workers. The TL seems to be shorter (0.0007–11.72 kb) in the people of Woraiyur, Tiruchirapalli factory was found to be 1–3 years. However, aTL among beedi workers. However, aTL among beedi workers in that area was low when compared with the normal healthy individual. Overall, the evidence from this study does hint at possible health risks for tobacco dust and would warrant further in-depth studies in the future. The relatively small sample size and shorter period of tobacco exposure of the employees (1–3 years), as well as the control samples obtained from the same area people, are limitations of this research. More horizons need to be extended for the population to avoid any occupational health risks and to overcome the obstacles of a larger population.

**References**

1. Bhat P, Kumar A, Aruna C, et al. Assessment of oral mucosal conditions among beedi workers residing in beedi workers colonies in Karnataka, India. J Oral Maxillofac Pathol 2018;22(3):298. DOI: 10.4103/jomfp.JOMFP_140_18.
2. Manigandan T, Kishore K, Julius A, et al. Tobacco: an overview. Drug Vention Today 2019;12(13):532–536.
3. Karlesder J, Kachatrian L, Takai H, et al. Targeted deletion reveals an essential function for the telomere length regulator Trf1. Molecular Cell Biol 2003;23(18):6533–6541. DOI: 10.1128/MCB.23.18.6533-6541.2003.
4. Ansari MS, Raj A. Socio-economic status of women Beedi workers in Bundelkhand region of Uttar Pradesh: an empirical analysis. UTMS J Econom 2015;6(1):53–66.
5. Niakan Kalhori SR, Behzadi A, Maharlou H, et al. A burden assessment of occupational exposures in Iran, 1990–2010: findings from the global burden of disease study 2010. Int J Prev Med 2018;9:56.
6. Sundaramoorthy R, Srivinasan V, Gujar J, et al. Clinical, cytogenetic and CYP1A1 Exon-1 gene mutation analysis of Beedi workers in Vellore region, Tamil Nadu. Asian Pacific J Cancer Prevent 2013;14(12):7555–7560. DOI: 10.7314/apjcp.2013.14.12.7555.
7. Joshi K, Robins M, Parashramlau V, et al. An epidemiological study of occupational health hazards among bidi workers of Amarchinta, Andhra Pradesh. J Acade Indust Res 2013;10:561–564.
8. Bhisrey R, Bagwe A, Mahimkar M, et al. Biological monitoring of beedi industry workers occupationally exposed to tobacco. Toxicol Lett 1999;108(2-3):259–265. DOI: 10.1016/S0378-4274(99)00097-1.
9. Umadevi B, Svarna M, Padmavathi P, et al. Cytogenetic effects in workers occupationally exposed to tobacco dust. Mutat Res/Gene Toxicol Environ Mutagen 2003;535(2):147–154. DOI: 10.1016/s1383-7581(02)00291-7.
10. Kahl VF, Dhillon V, Fenech M, et al. Occupational exposure to pesticides in tobacco fields: the integrated evaluation of nutritional intake and susceptibility on genomic and epigenetic instability. Oxid Med Cell Longe 2018;2018:7017423. DOI: 10.1155/2018/7017423.
11. Basu G, Sarkar D, Pal R, et al. Morbidity audit of women Beedi workers in an urban fringe of West Bengal, India. J Clin Diagnos Res 2018;12(3):5–9. DOI: 10.7860/jcdr/2018/31226.11265.
12. Pande R, Structural Violence and Women’s Health–Work in the Beedi Industry in India. In: Violence and Health, Proceedings of WHO Global Symposium 1999 Oct pp. 192–205.
13. John P, Beedi Industry and Welfare of Workers in India. Review of policies and literature. New Delhi. 2015.
14. Shammas MA. Telomeres, lifestyle, cancer, and aging. Curr Opin Clin Nutrit Metabol Care 2011;14(1):28. DOI: 10.1097/MCO.0b013e32834121b1.
15. Kelesidis T, Schmid I. Assessment of telomere length, phenotype, and DNA content. Curr Proto Cytom 2017;79(1):7–26. DOI: 10.1002/cycy.12.
16. Kahl V, Da Silva J. Telomere length and its relation to human health, In: Telomere: a Complex End of a Chromosome. ML Larramendy; 2016. pp. 163–187.
17. Cassidy A, De Vivo I, Liu Y, et al. Associations between diet, lifestyle factors, and telomere length in women. Am J Clin Nutrit 2010;91(5):1273–1280. DOI: 10.3945/ajcn.2009.28947.
18. Nagesh A, Ugle SS. A research on effects of tobacco dust on status of total thiol in bidi industry workers. Int J Adv Med 2020;7(8):1269–1273. DOI: 10.18203/2349-3933.ijam20203127.
19. Silva F, Silva J, Nunes E. Application of the buccal micronucleus cytome assay and analysis of PON1Gln192Arg and CYP2A6*9 (-487-G) polymorphisms in tobacco farmers. Environ Molcule Mutagen 2012;53(7):525–534. DOI: 10.1002/em.21713.
20. Gustavsson P, Jakobsson R, Johansson H, et al. Occupational exposures and squamous cell carcinoma of the oral cavity, pharynx, larynx, and oesophagus: a case-control study in Sweden. Occup Environ Med 1998;55(6):393–400. DOI: 10.1136/oem.55.6.393.

21. Jethwa AR, Khariwala SS. Tobacco-related carcinogenesis in head and neck cancer. Cancer Metast Rev 2017;36(3):411–423. DOI: 10.1007/s10555-017-9689-6.

22. Cogliano VJ, Baan R, Straif K, et al. Preventable exposures associated with human cancers. J Natl Cancer Inst 2011;103(24):1827–1839. DOI: 10.1093/jnci/djr483.

23. Yasmin S, Afroz B, Hyat B, et al. Occupational health hazards in women beedi rollers in Bihar, India. Bullet Environ Contaminat Toxicol 2010;85(1):87–91. DOI: 10.1007/s00128-010-0037-6.

24. Khanna A, Gautam D, Gokhale M, et al. Tobacco dust induced genotoxicity as an occupational hazard in workers of bidi making cottage industry of central India. Toxicol Internat 2014;21(1):18. DOI: 10.4103/0971-6580.128785.

25. Singh SK, Gupta A, Rajan SY, et al. Correlation of presence of Candida and epithelial dysplasia in oral mucosal lesions. J Clin Diagnos Res 2014;8(10):31. DOI: 10.7860/JCDR/2014/9872.4956.

26. Shukla P, Khanna A, Jain SK. Working condition: a key factor in increasing occupational hazard among bidi rollers: a population health research with respect to DNA damage. Indian J Occup Environ Med 2011;15(3):139. DOI: 10.4103/0019-5278.93206.

27. Martinez P, Blasco MA. Telomere-driven diseases and telomere-targeting therapies. J Cell Biol 2017;216(4):875–887. DOI: 10.1083/jcb.201610111.

28. Barrett JH, Iles MM, Dunning AM, et al. Telomere length and common disease: study design and analytical challenges. Hum Genet 2015;134(7):679–689. DOI: 10.1007/s00439-015-1563-4.

29. Vega LR, Mateyak MK, Zakian VA. Getting to the end: telomerase access in yeast and humans. Nat Rev Mol Cell Biol 2003;4(12):948–959. DOI: 10.1038/nrm1256.

30. Kahl VF, Allen JA, Nelson CB, et al. Telomere length measurement by molecular combing. Front Cell Develop Biol 2020;8:493. DOI: 10.3389/fcel.2020.00493.

31. Buhring J, Hecker M, Fitzner B, et al. Systematic review of studies on telomere lengths in patients with multiple sclerosis. medRxiv 2020.

32. De Lange T. Shelterin: the protein complex that shapes and safeguards human telomeres. Genes Develop 2005;19(18):2100–2110. DOI: 10.1101/gad.1346005.

33. Kosebent EG, Uysal F, Ozturk S. Telomere length and telomerase activity during folliculogenesis in mammals. J Reproduct Develop 2018;64(6):477–484. DOI: 10.1262/jrd.2018-076.

34. Mahimkar MB, Bhisey RA. Occupational exposure to bidi tobacco increases chromosomal aberrations in tobacco processors. Mutat Res/Environm Mutagen Relat Sub 1995;334(2):139–144. DOI: 10.1016/0165-1161(95)90004-7.

35. Lu L, Johnman C, McGlynn L, et al. Association between exposure to second-hand smoke and telomere length: cross-sectional study of 1303 non-smokers. Int J Epidemiol 2017;46(6):1978–1984. DOI: 10.1093/ije/dyx212.

36. O’Callaghan NJ, Fenech M. A quantitative PCR method for measuring absolute telomere length. Biol Proced Online 2011;13(3). DOI: 10.1186/1480-9222-13-3.

37. Thomas P, O’Callaghan NJ, Fenech M. Telomere length in white blood cells, buccal cells and brain tissue and its variation with ageing and Alzheimer’s disease. Mechanis Age Develop 2008;129(4):183–190. DOI: 10.1016/j.mad.2007.12.004.

38. Nettle D, Seeker L, Nussey D, et al. Consequences of measurement error in qPCR telomere data: a simulation study. PLoS ONE 2019;14(5):e0216118. DOI: 10.1371/journal.pone.0216118.

39. Montpetit AJ, Alhareeri AA, Montpetit M, et al. Telomere length: a review of methods for measurement. Nurs Res 2014;63(4):289. DOI: 10.1097/NRR.0000000000000037.