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

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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 when 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.


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.1–5 It is an occupation that has expanded at the cost of smaller family farms and is a small-scale business in the majority of Indian states.1–5 It is an occupation that has expanded at the cost of smaller family farms and is an industry employing a large number of people, mostly women. In India, more than 5 million people are employed in the beedi manufacturing process. The harmful effects of tobacco use on health are well known. The dangers of unburned tobacco during occupational exposure, on the other hand, are less well known.6 Tobacco processing has a lot of fine dust and tobacco particulates in the working environment.7–10 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.,7 97% of beedi workers were unaware of the health risks associated with tobacco use due to occupational exposure.7

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...
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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.11–13

Telomeres are specific DNA sequences that repeat themselves at the ends of each chromosome. Telomeres are reduced due to each cell division, maintaining genomic integrity. If the telomere shortens to a certain length, the cell enters a 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.14–17 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.18,19 Kahl et al. reported shorter TL in tobacco farmers was associated with tobacco leaf exposure and pesticide use.10 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 lead to senescence and cell cycle arrest. It is a fundamental tumor suppressor mechanism that limits the proliferative capacity of the cell. Prevents the genome instability that may arise from telomere dysfunction.

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/trauma 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. IGIDSIEC2018NRP23PGKYOPM). 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 pellet 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 Qiapm 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, discarded the collection tube and added 100 μL of buffer AE to the Qiapm spin column in a clean 1.5 mL microcentrifuge tube. After 1–2 minutes of room temperature incubation, the tube was centrifuged for 1 minute at 8,000 revolutions per minute (RPM). The concentration of extracted DNA was validated using a NanoDrop
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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).

**Results and Observations**

**Statistical Analysis**

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

![Amplification plot—single-copy reference (SCR)—each sample reaction fluorescence intensity](image1)

![Amplification plot—telomere (each sample reaction fluorescence intensity)](image2)

**Table 1:** Mean value of average absolute telomere length/each chromosome end (kb) between the control group and study group

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Mean</th>
<th>Std. deviation</th>
<th>Minimum</th>
<th>Maximum</th>
<th>25th</th>
<th>50th (median)</th>
<th>75th</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>20</td>
<td>0.705</td>
<td>0.947</td>
<td>0.0049</td>
<td>4.01</td>
<td>0.09</td>
<td>0.52</td>
<td>0.66</td>
</tr>
<tr>
<td>Study group</td>
<td>19</td>
<td>1.45</td>
<td>2.76</td>
<td>0.0007</td>
<td>11.72</td>
<td>0.02</td>
<td>0.33</td>
<td>1.59</td>
</tr>
<tr>
<td>All</td>
<td>39</td>
<td>1.07</td>
<td>2.05</td>
<td>0.0007</td>
<td>11.72</td>
<td>0.06</td>
<td>0.45</td>
<td>1.07</td>
</tr>
</tbody>
</table>
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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

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mean rank</th>
<th>Sum of ranks</th>
<th>Mann–Whitney U</th>
<th>Z value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>20</td>
<td>20.20</td>
<td>404.00</td>
<td>186.00</td>
<td>−0.112</td>
<td>0.911</td>
</tr>
<tr>
<td>Study group</td>
<td>19</td>
<td>19.79</td>
<td>376.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>39</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

damage. Only a few articles have evaluated the genetic modification, which includes chromosomal aberration and comet tail length, according to a review of the literature. Telomere length is thought to be a useful biomarker for genetic risk. Considering this fact the current study estimated average absolute TL in female non-beedi workers and female beedi workers as a tool for determining genetic liability by using real-time PCR. The Institutional Review Board and Institutional Ethical Committee had approved the study protocol.

A field visit was performed before the report, and data was obtained from the beedi industry, which revealed that the majority of beedi rollers are females who are regularly exposed to tobacco inhalation, so the study focused on female beedi workers in particular. Long-term tobacco use or exposure decreases telomere lifespan, according to the majority of studies. The purpose of this research was to determine if there was a connection between aTL and short-term tobacco dust exposure. The current research was carried out in Tiruchirappalli beedi sector. Participants consent was obtained after volunteers were recruited and the study was explained to them in their own language. A total of 40 females were split into two groups: Group I—Female non-beedi workers (control group) aged 20–35 years with no other comorbidities or diseases that could affect genetic alterations, and Group II—Female beedi workers (study group) aged 20–35 years with 1–3 years of experience and with no other comorbidities or diseases that could affect genetic alterations. For TL estimation, the collected samples were subjected to real-time PCR.

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 statistical significant difference between group I and group II (Z = −0.112, p = 0.911). The mean rank 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 is 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.
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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. According to Mahimkar and Bhisey, tobacco processors are subjected to significant genotoxicity as a result of their work. 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. 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).

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 that it highlights variations between laboratories. In the present study, due to their shorter duration of employment, the data showed less variation between group I and group II 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 Worajiyur, Tiruchirapalli when compared with normal healthy individuals (2–50 kb).

Recently, TL has been introduced as a biomarker in occupational exposure evaluations. In the present study, the minimal duration of a worker engaged in a beedi factory was found to be 1–3 years. Our study aimed at assessing the association effect of tobacco dust on early exposed individuals. We would like to hypothesize that the alteration in aTL, when compared with normal individuals, could be due to:

- The residents invariable, their past generation have been associated with the beedi factories, which could have caused hereditary genetic damage.
- As there are many factors, the air and water would have been contaminated with tobacco particles, which could be a passive exposure to individual residents who are not working in the tobacco industry.

The present study showed no significant results when compared with beedi workers and non-beedi workers in that area. In the future, studies might selectively recruit participants based on the longer duration of employment.

**Conclusion**

The present study showed that there is no association in average absolute TL in female beedi workers when compared with female non-beedi workers. It might be the fact that short duration of tobacco dust exposure in beedi workers. However, aTL among beedi and non-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**

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