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Study on the Buccal Epithelial Cells in Beedi Smokers and Non Smokers in Dharmapuri District (Tamilnadu)

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KEYWORDS

Buccal smear, Beedi smokers, Micronucleus, Tobacco

ABSTRACT:

Introduction: Even though it is commonly recognised that tobacco smoke contains carcinogens, findings in the scientific literature that relate smoking behaviours to the frequency of micronuclei are not quite clear-cut.

Objectives: A study was carried out to compare and assess the prevalence of micronuclei in chronic beedi smokers and non-smokers in the Dharmapuri district of Tamil Nadu.

Methods: The study had one hundred male subjects who were recruited at random. 50 participants were the chronic beedi smokers and the other 50 were non-smokers. The mean age of the smokers was 44.48±4.2, while the mean age of the controls was 42.11±4.9. Buccal smears were collected and used for micronucleus analysis.

Results: In both controls and subjects, there was a significant correlation between radiation exposure and the existence of micronuclei ($p>0.05$) and between tobacco use and the frequency of micronucleated cells ($p>0.05$). Subjects who smoke tendu leaves frequently had a higher incidence of MN within the smokers group based on the frequency of habit and age.

Conclusion: This study attempted to quantify the cytogenetic damage to the oral mucosa in individuals who have been accustomed to consuming tendu leaves. Although tobacco is a major contributor to the formation of micronuclei, ionising radiation and individual habits also influence the frequency of micronuclei. Some of the contributing aspects are the smear site, sample size, and use of nuclear specific dye. Furthermore, to improve the specificity, cytogenetic changes such as pyknosis, karyolysis, etc., can be added in subsequent research.

1. Introduction

The intricate blend of more than 4,000 chemical components found in cigarette smoke easily combines to create additional reactive chemicals that harm DNA and have cytogenetic effects.[1, 2] Low-grade tobacco is hand-rolled into tendu (*Diospyros melanoxylon*) leaves and knotted with cotton thread to create the traditional beedi (Bidi) cigarette.3. Even though it is commonly recognised that tobacco smoke contains carcinogens, findings in the scientific literature that relate smoking behaviours to the frequency of micronuclei are not quite clear-cut.4

Numerous assays have been suggested as possible biomarkers in biomonitoring investigations; however, these techniques are usually time-consuming and labor-intensive, requiring highly skilled personnel to

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appropriately read and interpret slides. To this end, the application of the micronucleus test to uncultured exfoliated cells generated a great lot of enthusiasm.2, 5, 6 Buccal epithelial cells have the benefit of quick and simple sampling in addition to offering an alternate source.5

Acentric fragments or entire chromosomes that are absent from the daughter cells' major nuclei give rise to the micronucleus. Both drugs that alter the spindle machinery (aneugens) and those that promote chromosomal break age (clastogens) can induce the creation of micronuclei. Micronuclei can arise due to a variety of endogenous and external causes. Radiation, chemicals, and the infiltration of microorganisms are examples of exogenous influences. Genetic flaws, pathological alterations, a lack of vital nutrients (like folic acid), and wounds brought on by harmful metabolic products (like reactive oxygen species) are examples of endogenous causes.7–19 Thus, a study was carried out to assess and compare the prevalence of micronuclei in chronic beedi smokers and non-smokers in Dharmapuri District, Tamil Nadu.

2. Objectives

Both short-term and long-term effects of smoking are detrimental to the immune system and respiratory system. It is a significant risk factor for lung conditions such as asthma, ARDS, idiopathic pulmonary fibrosis (IPF), chronic obstructive pulmonary disease (COPD), and cancer. The harmful ingredient that adds flavour to tobacco products is nicotine. The main factor influencing immunity and lung health is also nicotine. It increases the inflammatory response of neutrophils and macrophages, but it also inhibits both of these cell types' ability to phagocytose other species. Nicotine also has a significant impact on the functions of epithelial cells, and it inhibits both the proliferation of lymphocytes and cytotoxic action.

An essential part of lung defence is played by respiratory epithelial cells. They provide a barrier that keeps pathogenic and dangerous substances out. Furthermore, mucus and antimicrobial peptides are secreted by epithelial cells to aid in host defence. An integral aspect of lung health is the preservation of epithelial cell health. Chronic nicotine exposure has been linked to an increase in goblet cell hyperplasia and effects on mucociliary transport. Nicotine's enhancement of lung inflammation and prevention of apoptosis is the primary source of respiratory epithelium damage and cancer-causing survival.

3. Methods

Sample collection

The study included one hundred participants: Fifty chronic beedi smokers and fifty non-smokers who served as the control group. Following a thorough mouth rinse, samples were taken from the buccal mucosa using a wooden spatula and spread out on sterile glass slides and transported in a wooden box to the laboratory for additional processing in a matter of three to four hours.

Sample processing

For the purpose of clearing debris and fixing the cells, slides were fixated in a freshly made, refrigerated fixative (3 methanol:1 glacial acetic acid) for 20 to 25 minutes. Slides were hydrolysis (1N HCl) at 60°C, these were air-dried and then cleaned with distilled water before being air-dried again. After staining the slides for 25 minutes at 40°C with 2% aceto-orcein, they were cleaned with ethanol and water. A binocular microscope was used to examine the DPX mounted slides at a magnification of 40×.

Micronucleus Scoring

Micronuclei (MN) were detected in 2,000 cells at a magnification of 40×. Their presence was verified by an additional scorer at random and by magnification of 100× (oil immersion).

Statistical Analysis

Data were compiled and ready for interoperability with the statistical programme Statistical Package for the Social Sciences (SPSS) version 19.0 after being entered into Microsoft Excel using a Windows 7 computer. The mean, medium median, Pearson chi square, p value, and cross tabulations of the frequency and existence of micronuclei in habit users are examples of descriptive and inferential statistics. The values of mean, variance, standard error, and standard deviation were computed.

4. Results

Following the collection of baseline data from each respondent, a variety of factors were examined,

Journal of Chemical Health Risks
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including age, education, occupation, dietary habits, brushing habits, frequency of brushing, smoking habits, length of smoking, and frequency of smoking. The buccal mucosa scrapings of the control and smoker groups were used to compute the Micronuclei (MN) cells. For every subject, the total MN cells were also determined. Figure 1A showed the definite squamous shaped buccal epithelial cells collected from the non-smokers i.e., control group. Figure 1B depicted the presence of nucleus (N) and binucleated cells (BN) which were responsible for the formation of micronuclei. Age of the subjects were categorized into two types as (i) <30 and (ii) <30 years. In non-smokers (control) group, 31 individuals belong to category I (<30 years) and 19 individuals belongs to category II (>30years). In smokers group, 35 individuals belong to category I (<30 years) and 15 individuals belongs to category II (>30years) (Figure 2). The mean age of the smokers was 44.48±4.2, while the mean age of the controls was 42.11±4.9 were observed.

Figure 1. Microscopic observation of buccal epithelial cells of non-smoker and smoker subject

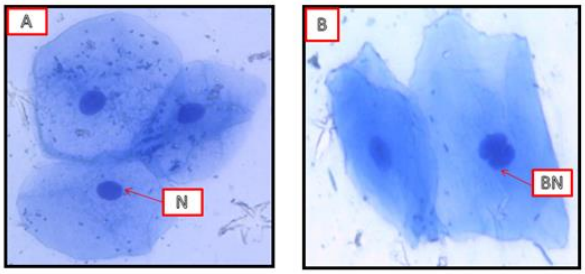
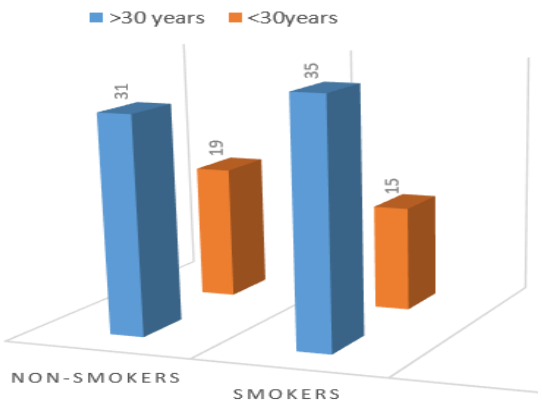


Figure 2. Age distribution of non-smoker (A) and smoker (B) group.



The subjects tobacco (beedi) smoking habits and the total number of MN cells were cross-tabulated, and the results demonstrated a significant correlation. The Pearson chi-square (6.99) was used, with degrees of freedom (df=2) and $p<0.05$ (Table 1). Age of respondents (smokers/controls) was cross-tabulated, and the results showed significant connection ($p<0.01$) as the exposure of beedi smoke increased the incidence of MN in beedi smoker group than control i.e. non-smoker group. In the smokers group, a cross-tabulation of the micronucleated cell count from the buccal mucosa scrapings revealed the increased incidence of micronuclei ($p<0.05$) the connection was determined to be significant. Among

Table 1. Mean±SD frequency of smoking habits and MN scoring among the testing subjects.

Frequency of smoking (per day)	**Control group (non-smoker)**	**Smoker (Beedi) group**	**Micronuclei scoring for Smoker group**
0	50	NA	NA
<5	NA	3.57 ± 0.99^{e}	1.35 ± 0.78^{e}
6-10	NA	28.84 ± 1.67^{a}	3.46 ± 0.23^{d}
11-15	NA	11.68 ± 1.55^{c}	5.03 ± 0.47^{c}
16-20	NA	13.85 ± 1.87^{b}	$5.99\pm0.97^{a,b,c}$
>20	NA	8.45 ± 0.53^{d}	6.24 ± 0.37^{a}
			Chi square – 6.99*

NA-not applicable; Subscripts indicated the homogenous subset between the test groups; *P<0.05

smokers and controls, the frequency of habit also significantly affected outcomes (Table 1). In smokers and controls, different frequency groups (0, <5, 6 to 10, 11to 15, 16 to 20, 21 to 25, >25) were compared. Four degrees of freedom were evaluated using the Pearson chi-square test (6.93). There was a noteworthy correlation observed between the occurrence of micronuclei and frequency habit.

Journal of Chemical Health Risks

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5. Discussion

A wide range of exploratory and mechanistic studies have made considerable use of the micronucleus (MN) test in an effort to comprehend the fundamental mechanisms behind genotoxicity. Eight factors such as dietary habits, climatic shifts, and physical and chemical genotoxins, can affect genetic damage. In actively proliferating tissues, MN can also be observed following irradiation which offers a practical and trustworthy index for loss and breakage of chromosomes.[20–22]

Human saliva has been shown to contain nitrosamines (TSNA) peculiar to tobacco use. They induce chromatid and chromosomal abnormalities, which raise the rates of MN. They are strong carcinogenic agents.1. Alcohol drinkers who smoked three or more packs of cigarettes a day had eight times more MNd mucosa cells than those who smoked one to two packs 23. Tobacco and alcohol combine to create chromosomal abnormalities that lead to the development of MNi. A different study by Stich et al. (6) also found a strong correlation between reverse cigar smokers and controls because the tobacco's TSNA acts as a strong mutagenic and clastogenic agent.

As TSNA, which are strong carcinogens that cause MN development, are present in beedi smoke, which contains tobacco, a study by Suhas et al. [3] found a substantial correlation between smoking and the frequency of MN in buccal mucosa cells. Angilieri et al. on the other hand, showed that there was no difference in MN frequency in exfoliated buccal mucosa cells and tongue lateral borders between smokers and non-smokers. Because tobacco has clastogenic and aneugenic effects on buccal mucosa cells, our study found a significant relationship between the number of MN cells in subjects and controls who smoked. However, there was no significant correlation (P=0.14) between the total MNd cells obtained from smokers and controls.

The findings are consistent with the research conducted by Nersesyan et al., which found that staining techniques have a significant impact on the micronuclei tests in exfoliated oral mucosa cells of smokers and nonsmokers. Using non-DNA-specific stains such as Giemsa, smokers had higher micronuclei rates; however, no discernible changes were observed when using Feulgen, AO, etc.24

Nevertheless, aceto-orcein stain, which mostly reacts with histone proteins in the nucleus, was employed as the nuclear stain in our investigation. However, when the interaction with occupational exposure is taken into consideration, heavy smokers were the only group showing a significant increase in genotoxic injury as measured by the micronucleus assay in lymphocytes. Overall, smokers do not experience an increase in MN frequency in lymphocytes, according to Bonassi et al. When compared to nonsmokers, there was a slight drop in MN frequency in current smokers with frequency ratio (FR)=0.97 and in former smokers FR=0.96 with 95% confidence limit.

This is so that cytogenetic changes caused by tobacco carcinogens cannot directly affect lymphocytes.4 Between smokers and controls, the frequency of habit had a significant (p=0.00014) bearing. Because of the corresponding rise in the potentially carcinogenic environment, the number of micronucleated cells in the buccal mucosa grows as the frequency of habit increases, that is, as the number of beedis smoked per day increases.

Bloching et al. [25] showed a highly significant correlation between increasing tobacco consumption (daily cigarettes and pack per years) and a higher MN count because of increased resultant cytogenetic damage. The amount and duration of tobacco abuse is directly correlated with increased exposure to carcinogenic contents in tobacco.[25] Gabriel et al could not detect dose effect, because there was no significant difference in the micronucleus frequency between those who smoked 20 cigarettes per day (9.3±0.4 and 10.1±0.8 respectively) as only a small number of subject (n=8) showed increased habit frequency. However, a higher sample size was required to confirm the test results.[26]

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Journal of Chemical Health Risks

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