https://doi.org/ 10.33472/AFJBS.6.7.2024.616-623

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Micronuclei analysis among the tobacco smokers in Dharmapuri (Tamil Nadu)

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Article History

Volume 6,Issue 7, 2024

Received: 29 Mar 2024

Accepted : 05 May 2024

doi: 10.33472/AF5BS.6.7.2024.616-623

ABSTRACT:

Although it is widely acknowledged that tobacco smoke includes carcinogens, there is some disagreement in the scientific literature on the relationship between smoking habits and the incidence of micronuclei. In the Tamil Nadu district of Dharmapuri, a study was conducted to evaluate and compare the prevalence of micronuclei in long-term beedi smokers and non-smokers. Of the subjects, 70 were long-term smokers of beedi, and the remaining 70 did not smoke. The average age of the controls was 46.38±5.3, while the smokers' average age was 48.77±6.5. There was a significant relationship in both the subjects and the controls between the frequency of micronucleated cells and tobacco use, as well as between radiation exposure and the presence of micronuclei ($p>0.05$). Based on the frequency of habit and age, subjects who regularly smoked tendu leaves had a greater incidence of MN within the smokers group. While smoking is a significant factor in the creation of micronuclei, other factors that affect their frequency include ionising radiation and personal habits. In addition, cytogenetic modifications such pyknosis and karyolysis can be included in future studies to increase the specificity.

Keywords: Micronucleus, Tobacco, smoker, genetical abnormality

1. Introduction

More than 4,000 distinct chemical components can be found in cigarette smoke, and many of these combine easily to create new reactive chemicals that harm DNA and have cytogenetic effects (Husgafvel-Pursiainen 2004; Angelieri et al., 2010). Low-grade tobacco is manually rolled onto tendu (Diospyros melanoxylon) leaves and knotted with cotton thread to create the traditional beedi (Bidi) cigarette (Suhas et al., 2004). There is substantial debate in the scientific literature regarding the connection between smoking habits and the occurrence of micronuclei, despite the general consensus that tobacco smoke contains carcinogens (Bonassi et al., 2003).

Smoking harms the immune system and respiratory system both immediately and over time. It poses a significant risk for lung conditions such as cancer, pulmonary fibrosis, chronic obstructive pulmonary disease, and asthma. The harmful flavouring ingredient in tobacco products is nicotine. Additionally, the main substance influencing immunity and lung health is nicotine. It increases the inflammatory response of neutrophils and macrophages, but it also stops these two cell types from phagocytosing other species. Moreover, nicotine profoundly impacts epithelial cell function; it inhibits lymphocyte growth and cytotoxic effect (Tolbert et al., 1991; Stich et al., 1992; Majer et al., 2001).

Many assays have been suggested as possible biomarkers in biomonitoring investigations (Angelieri et al., 2010; Elhajouji et al., 2011; Crott et al., 2011). Buccal epithelial cells offer a quick and simple way to sample in addition to serving as an alternate source.5. Acentric segments or entire chromosomes absent from the descendant cells' major nuclei combine to generate the micronucleus. Medication that alters the spindle machinery (aneugens) or promotes chromosomal breakage (clastogens) can produce micronuclei. Endogenous causes can include pathological alterations, DNA abnormalities, deficiencies in vital nutrients (like folic acid), and damage from harmful metabolic products (like reactive oxygen species) (Ban et al., 2011).

An essential part of lung defence is played by respiratory epithelial cells. They serve as a barrier to prevent the entry of dangerous and infectious materials. In addition, mucus and peptides that fight microbes are released by epithelial cells to aid in host defence. Long-term nicotine exposure has been linked to goblet cell hyperplasia and effects on mucociliary transport (Petrozzi et al., 2002; Mateuca et al., 2006; Lorge et al., 2006; Grieshaber et al., 2007; Vral et al., 2011; Bull et al., 2011 Huang et al., 2011; Srivastava et al., 2012). As a result, a study was carried out in a few towns in the Dharmapuri District of Tamil Nadu to assess and compare the incidence of micronuclei among tobacco users.

2. Materials and Methods

2.1. Sample collection

One hundred and forty individuals took part in the study: seventy were long-term beedi users, while the remaining seventy served as the control group. Following a thorough mouth rinse, samples were taken from the buccal tissue using a wooden spatula within

three to four hours. They were then spread out on sterile glass slides and sent to the laboratory in a wooden box for additional processing.

2.2. Sample processing

To fix the cells and eliminate debris, slides were fixed in a freshly made, refrigerated fixative (3:1 methanol, glacial acetic acid) for 20 to 25 minutes. After hydrolyzing (IN HCl) at 60°C, the slides were left to air dry, cleaned with distilled water, and then given one more chance to air dry. 2% aceto-orcein was applied to the slides and left for 25 minutes at 40°C. They were then cleaned using water and ethanol. Using a binocular microscope, the DPX mounted slides were inspected at a 40x magnification.

2.3. Micronucleus Scoring

When 2,000 buccal cells were analysed at a 40x magnification, micronuclei (MN) were discovered. By employing an oil immersion method with a 100x magnification, their presence was verified. The information was gathered and ready for SPSS (19.0 version) compatibility. The mean, SD, Pearson chi square, p value, and cross tabulations of the frequency and presence of micronuclei in habit users are examples of descriptive and inferential statistics.

3. Results

Once the baseline data for each respondent was gathered, numerous parameters were examined. Occupation, education, brushing habits, age, eating habits, duration of smoking, smoking behaviours, frequency of smoking, and frequency of brushing were among these factors. The MN cells were determined using buccal mucosa scrapings from the smoker and control groups. Every person's total MN cells were also measured. The unique buccal epithelial cells with a squamous form were supplied by the nonsmoking control group (Figure 1A).

Figure 1B displayed the nucleus (N) and binucleated cells (BN), which were in charge of forming micronuclei. Two age categories of the subjects were created: (i) those under 30 and (ii) those beyond 30. Within the nonsmoking (control) group, 41 individuals are classified as being under 30 years old, while 29 individuals are classified as being over 30 years old. There are 45 individuals in the smokers group who are under 30 years old and 25 individuals who are over 30 years old (Figure 2). The average age of the smokers was 48.77±6.5, according to the study, whereas the average age of the controls was 46.38±5.3.

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

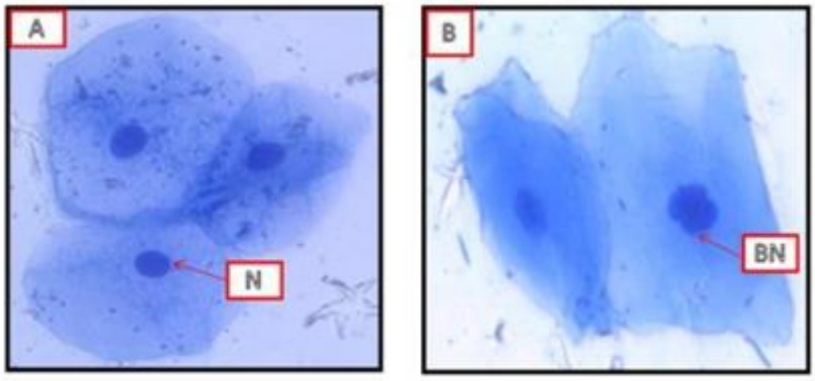

Figure 2. Age distribution of non-smoker (A) and smoker (B) group.

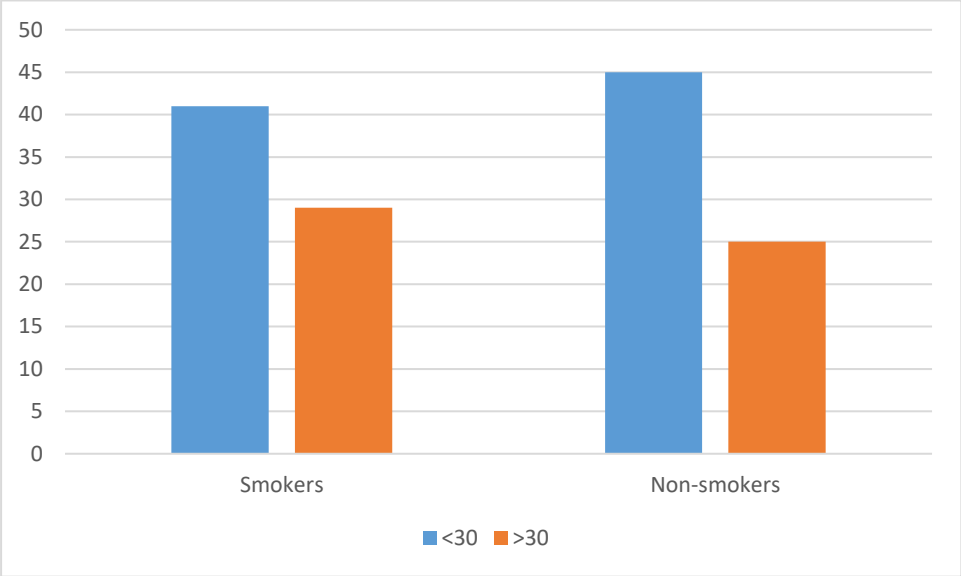

Table 1. Mean+SD frequency of smoking habits and MN scoring among the testing subjects.

Frequency of smoking (per day)	Control group (non-smoker)	Smoker (Beedi) group	Micronuclei scoring for Smoker group
0	70	NA	NA
<5	NA	6.48 ± 1.48^{e}	2.08 ± 0.06^{e}
6-10	NA	36.36 ± 3.37^{a}	6.36 ± 0.11^{d}
11-15	NA	16.27 ± 2.09^{c}	7.36 ± 0.57^{c}
16-20	NA	19.05 ± 1.99^{b}	$8.36\pm0.47^{a,b,c}$
>20	NA	11.27 ± 0.99^{d}	8.61 ± 0.43^{a}
			Chi square – 5.47 ($P<0.05$)

The overall number of MN cells and the subjects' tobacco-using behaviours were shown to be strongly correlated (beedi). The Pearson chi-square (5.47) was used with degrees of freedom (Df=2) and $p<0.05$ (Table 1). Cross-tabulating the age of the respondents (smokers and non-smokers) showed a significant association ($p<0.05$) between the two groups, with the smokers having a higher incidence of MN than the non-smokers. The smokers group had a greater incidence of micronuclei ($p<0.05$) in the number of micronucleated cells from the buccal mucosa scrapings, according to a cross-tabulation analysis. This association was shown to be significant.

Results for both smokers and controls were significantly impacted by the frequency of habit (Table 1). In both smokers and controls, a number of frequency groups (0, <5, 6 to 10, 11 to 15, 16 to 20, 21 to 25, >25) were investigated. Four degrees of freedom were examined using the Pearson chi-square test (5.47). The presence of micronuclei was shown to be significantly correlated with frequency of habit.

4. Discussion

The micronucleus (MN) test has been widely employed in numerous exploratory and mechanistic studies in an attempt to comprehend the fundamental mechanisms behind genotoxicity. Eight variables, such as food habits, climatic shifts, and physical and chemical genotoxins, might affect genetic damage. MN is a consistent and practical marker for chromosomal loss and breakage that is also visible in rapidly proliferating tissues following irradiation (Fenech 1993; Evans et al., 1959; Fenech 2000).

Saliva from humans has been shown to contain tobacco-specific nitrosamines (TSNA). MN rates rise as a result of the chromosomal and chromatid abnormalities they cause. They have strong carcinogenic effects.1. Drinkers who smoked three packs or more a day had eight times more MNd mucosa cells in their tissues than individuals who smoked one to two packs a day (Stich and Rosin 1983).

Tobacco and alcohol together result in chromosomal abnormalities that lead to the development of MNi. In a different study, Stich et al. (1983) found a substantial correlation between reverse cigar smokers and controls because of the tobacco's strong mutagenic and clastogenic qualities. A study by Suhas et al. (2004) found a substantial correlation between smoking and the frequency of MN in buccal mucosa cells. This is because tobacco, which is found in beedi smoke, includes TSNA, which is a powerful carcinogen that promotes the development of MN. However, it was shown that the MN frequency in exfoliated buccal mucosa cells and tongue lateral edges did not differ between smokers and non-smokers.

As a result of tobacco's clastogenic and aneugenic effects on buccal mucosa cells, our study found a statistically significant difference between the smoked participants' and the control group's MN cell counts. However, there was no discernible difference in the total MNd cells between smokers and controls ($P=0.14$).

The findings are consistent with a study conducted by Nersesyan et al. (2006) which found that staining techniques significantly alter the micronuclei tests in exfoliated oral mucosa cells from smokers and non-smokers. When utilising non-DNA-specific stains such as Giemsa, smokers exhibited higher rates of micronuclei; however, no significant changes were observed when employing Feulgen, AO, etc. However, aceto-orcein stain, which largely reacts with histone proteins in the nucleus, was the nuclear stain employed in our investigation. When the interaction with occupational exposure is considered, heavy smokers were the only group demonstrating a significant increase in genotoxic harm as assessed by the micronucleus assay in lymphocytes. Citing Bonassi et al., overall MN frequency in lymphocytes is not enhanced in smokers (2003). When compared to nonsmokers, there was a little drop in MN frequency in current smokers (frequency ratio (FR) = 0.97) and former smokers (FR = 0.96 with 95% confidence limit).

This is to shield lymphocytes from the direct effects of chromosomal changes caused by carcinogens found in tobacco smoke (Bonassi et al., 2003). Between smokers and controls, the frequency of habit had a significant (p=0.00014) impact. Because of the accompanying growth in the possibly cancer-causing environment, the number of micronucleated cells in the buccal mucosa grows as habit frequency increases, that is, as the number of beedis smoked per day increases.

Bloching et al. (2000) showed a strong correlation between increasing tobacco use (packs smoked annually and daily cigarettes) and an increase in the number of MNs because of the cytogenetic damage that results. The amount and duration of tobacco abuse are directly correlated with increased exposure to the carcinogenic qualities of tobacco. Gabriel et al. (2006) were unable to find a dose impact since only a small percentage of individuals (n=8) showed higher habit frequency. In particular, the micronucleus frequency did not significantly differ between individuals who smoked 20 cigarettes a day (9.3±0.4 and 10.14±0.8, respectively). A higher sample size was required in order to confirm the test results.

Acknowledgment

Authors are thankful to the authorities at PG and Research Department of Zoology, Government Arts College, Dharmapuri for the support during this study.

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