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Genotoxicity by micronucleus assay of exfoliated oral buccal cells in cigarette smokers

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Abstract
Smoking cigarettes can lead to a number of health issues or fatal diseases including cancer. The numerous lethal carcinogens found in cigarettes cause DNA damage in a variety of organs. The frequency and length of exposure directly correlate with this harm. Micronuclei arise primarily as a result of genetic damage to living cells. The micronucleus (MN), a nuclear body that can easily be seen in exfoliated buccal cells, is created when chromosomes break or segregate during cell division. This study was done to estimate the DNA damage in smokers' exfoliated buccal cells. So, utilizing MN assay of exfoliated buccal cells from nonsmokers (Comparison group) and smokers with respect to duration and frequency of use, the current study was conducted to assess the genetic damage. Two groups of people were taken, 50 for smokers, and 30 nonsmokers. Results were noted, In Controls and Smokers, the mean and variance of the percentage of nuclear abnormalities overall were, respectively. (25.967±0.681), (46.240±0.700*). The results of an independent sample t-test indicated a significant difference between the means of the two groups. Statistically significant results were seen for both smokers and controls.

Keywords: Buccal mucosa, micronucleus, micronucleus assay, smokers

Introduction
Smoking has been known for thousands of years, and Christopher Colombo was the first to do so. It was first cultivated by Native Americans in the late fifteenth century AD, and when a French sailor (Nicotte) brought it to Europe in 9551 AD, it came to be known as the primary ingredient in smoking. Cigarette rolling machines and matchboxes were created in the year 9889 AD along with nicotine, which aided in the spread of these substances. Typically, the governments of Denmark, Sweden, and the Netherlands passed smoking ban laws throughout the seventeenth century. On average, 10.3% of women and 47.5% of males currently smoke. The second leading cause of death worldwide remains smoking. If current trends continue, the annual death toll from smoking will reach 9 million by 2030 [1].

Smoking can have negative effects on the digestive system, such as a tongue-taste gland imbalance, stomach ulcers, indigestion, which can lead to pancreatic, stomach, and duodenal cancer, as well as stomach and duodenal cancer. Scientific study and health statistics back up the duodenal and stomach ulcer rates for smokers being three times higher than for non-smokers. The smoker secretes nearly twice as much acids as the average person [2]. Cytogenetic damage appears to be a good biomarker for assessing the effects of exposure to chromosome-damaging compounds in smoke since the chemicals in tobacco smoke are genotoxic [3]. The most significant fundamental cause of developmental and degenerative diseases is likely genomic damage. Furthermore, it is well known that environmental exposure to genotoxins, medical procedures (such as radiation and chemicals), micronutrient deficiencies (such as a lack of folate), lifestyle factors (such as alcohol, smoking, drug use, and stress), and genetic factors, such as inherited flaws in DNA metabolism and/or repair, all contribute to genomic damage [4].

The MN test, which may identify chromosomal loss or mitotic spindle dysfunction brought on by aneugenic pathways, is a strong candidate to act as such a biomarker [5]. By removing the cells from the afflicted tissues, MN was found in buccal mucosa cells employed to explore preneoplastic effects. However, it is still unknown if an increased frequency of MN in specific tissues, such as oral epithelia, would indicate a higher chance of developing cancer in the future, or may indicate a range of cancers in other sections of the body. MN in buccal mucosa has been linked to cancer risk for the upper aerodigestive tract, including early stages such mouth leukoplakia (4). Many cytogeneticists underlined the significance of assessing MN caused by the activity of genotoxic substances.

MN staining was carried out initially with a straightforward Giemsa stain, followed by slide scoring [6].

Chromosome fragments from interphasic cells produce micronuclei. The micronuclei are cytoplasmic entities that are 1/5 to 1/3 the size of the nucleus and have the same stain as the nucleus. The average proportion of cells containing micronuclei in general populations ranges from 0 to 0.9%. Any increase in the number of micronuclei reflects chromosomal changes. The degree of carcinogenic effects has been correlated with the number of micronuclei [7]. Lymphocytes, fibroblasts, and exfoliated epithelial cells are just a few of the different cell types that can be scored with the MNi. Although MNi scoring in the exfoliated oral buccal epithelial cells is noninvasive, the mouth's normal bacteria can interfere with it. This study's primary goal is to assess micronuclei (MNi) score as a biomarker for the early diagnosis and screening of cigarette smoking's genotoxic effects. This approach has been suggested as a potential future strategy for the identification and initial prevention of human cancer risks among cigarette smokers [8]. The first goal of this study is to evaluate the micronuclei (MN) that have been caused by cytogenic damage in patients who smoke tobacco. Comparing the MN score between patients who use tobacco and those who do not is the study's secondary goal.

Materials and methods

Study design: Buccal cells were taken. This study included 30 samples from healthy (non-smokers) people and 50 patients who consume cigarettes.

Sampling collection: Samples were taken between 12 January and 15 March, 2023. For the MN assay, subjects were instructed to properly rinse their mouths with tap water for two minutes. Both the control groups and the patients' exfoliated buccal cells for micronuclei (MNi) analyses were taken from one or both cheeks. Using a wooden spatula, the buccal mucosa cells were dispersed across spotless glass slides. Geimsa-stained slides were used to calculate the average number of micronuclei. Calculations were made and comparisons between the two groups of smokers and non-smokers about the number of micronuclei per 2000 cells [9].

Results

In the current investigation, oral mucosa samples from 80 samples (50 smokers and 30 nonsmokers) were separated into two groups and subjected to an MN assay. Healthy people and patients ranged in age from 23 to 30, When correlation between smoking and non-smoking use and MN count per 2000 cells, cells was calculated, significant positive correlation with all Anomalies was observed (p<0.05). Observed the abnormal cells as shown in the following table (1) and figure (1).

Table 1: Anomalies of Micronucleus in the buccal mucosa cells

Anomalies	Non-smokers	Smokers
Binucleation	4.367±0.237	3.760±0.170*
Condensed chromatin	8.633±0.360	10.360±0.410*
Broken egg	1.833±0.220	2.760±0.173*
Karyolysis	9.700±0.366	27.74±0.499*
Micronucleus	1.567±0.189	2.520±0.198*
Percentage of nuclear anomalies in total	25.967±0.681	46.240±0.700*

*This indicates that there are significant differences between each of the two horizontal variables at a significant level P<0.05.

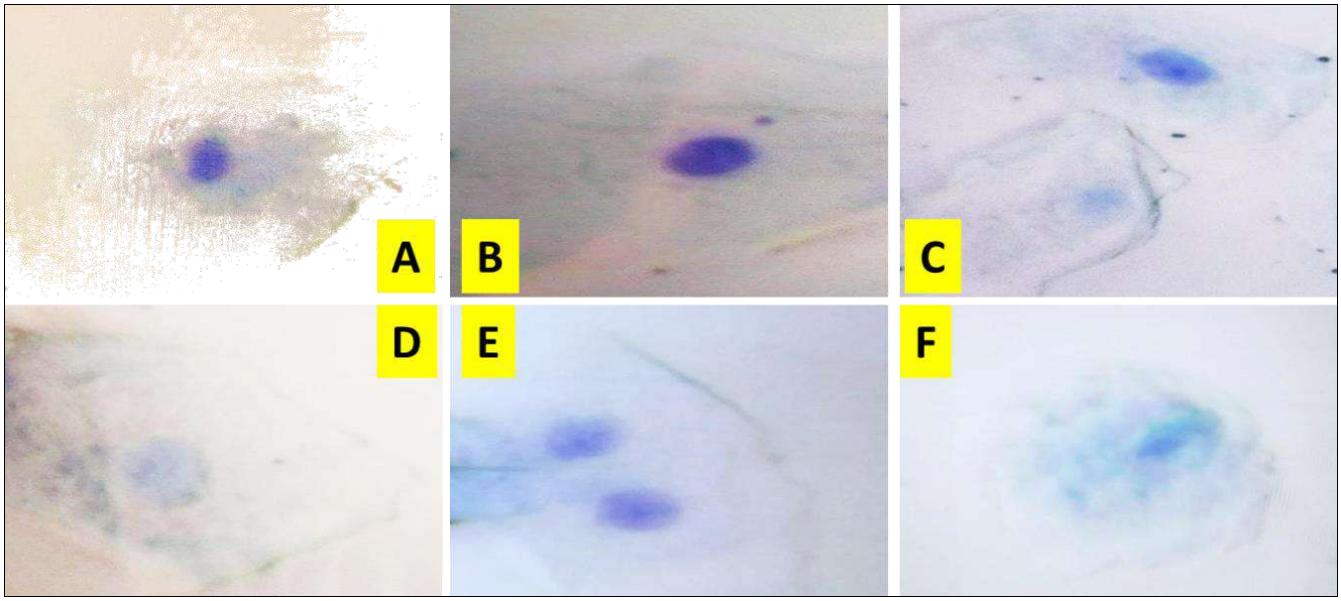

Fig 1: Cellular DNA genetics damaged in the exfoliated Oral cells of epithelial cells were evaluated, (A) normal cell, (B) micronucleus, (C, D) Karyolysis cell (E) Binucleated, and (F) Condensed Chromatin.

Discussion

Smoking is one of the key risk factors for the development of cancer in several organs and is currently the most avoidable cause of illnesses and deaths in the world. Patients who smoke should therefore be closely examined in light of the variety of changes that tobacco might bring

about. According to the study, smokers had a considerably larger mean number of micronuclei in their buccal mucosa cells than non-smokers. Micronucleus testing (MNT) has been used to assess the genotoxicity of tobacco on human buccal mucosal cells. Because MNT is one of the most often used short-term tests in genetic toxicology and has grown to be one of the most significant assays utilized by regulatory agencies across the world to assess xenobiotic mutagenicity and sensitivity [10].

The presence of micronuclei indicates a genotoxic exposure because the production of micronuclei in eukaryote cells is the result of chromosomal damage or segregation abnormalities. It was discovered that the micronucleated cells had one or more micronuclei. Micronucleated cells rather than the quantity of micronuclei were the study's scoring criteria. In the typical karyokinesis, micronuclei are either entire chromosomes that are lagging or acentric chromosomal fragments [11].

These chromosome laggards or fragments are kept out of the post-mitotic daughter cells and develop into nuclei. As a result, during cell division, these micronucleated cells (MNCs) are either characterized by chromosomal breakage (clastogenicity) or a malfunction in the spindle fiber mechanism (tubragenicity). The presence of genotoxic substances in tobacco appears to be the cause of the induction of micronuclei in oral mucosal cells. Saliva-soluble tobacco compounds may diffuse into the basal cell layer and disrupt the reproductive system of the underlying population of proliferating cells, resulting in genotoxicity and the formation of micronuclei.

Nagler and Dayan likewise reported “Saliva may lose its antioxidant function and turn into a robust pro-oxidant milieu if it interacts with redox active metals in saliva and low reactive free radicals produced by chewing and smoking tobacco to increase the intensity of genotoxicity. This causes oral mucosal cells to become abnormal and encourages the development of oral cancer” [12]. Micronuclei (MN) analysis in oral cells is thought to be a sensitive tool for identifying genetic damage in humans, hence assessment of MN in human epithelial cells is a novel methodology for assessing genetic toxicity. Human cells exposed to hereditary toxicity factors or receiving preventive therapies can be vitally investigated employing epithelial cells. In addition to the fact that more than 90% of cancer cases start in epithelial tissues, which are frequently the real targets of carcinogens due to direct exposure to chemicals, including those found in tobacco, this is because it is simple to collect from the mouth, nose, and bladder using non-invasive methods of action [13].

Conclusion

Both smoked and smokeless tobacco usage have cytotoxic and genotoxic consequences. The micronucleus assay is a great biomarker to identify those who are most vulnerable to oral mutations that may be brought on by smoking, which also has a negative impact on the oral cavity, especially the periodontium. The fact that there hasn't been a rise among moderate smokers doesn't mean that tissues like the lung or other organs aren't suffering from genotoxic consequences. Further research should be done to determine the extent to which lymphocytes may substitute for DNA damage brought on by breathed toxins in lung cells. If the subject is aware of risk signs found in the clinically healthy oral mucosa, behavior intervention to quit smoking may be considerably aided.

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