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Nuclear Abnormalities and Apoptotic Index of Buccal Cells in Tobacco and Electronic Cigarette Smokers

Dody Novrial[1]✉, Laksmita Tanjung[2], Rinawati Satrio[3]

[1]Department of Anatomical Pathology, Faculty of Medicine, Universitas Jenderal Soedirman, Purwokerto, Indonesia
[2]Faculty of Medicine, Universitas Jenderal Soedirman, Purwokerto, Indonesia
[3]Department of Dentistry, Faculty of Medicine, Universitas Jenderal Soedirman, Purwokerto, Indonesia

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Abstract

High prevalence of smoking continues to become a global public health hazard, including oral health. Meanwhile, e-cigarettes have become increasingly popular as alternatives to smoking. This study was conducted in 2022 and aimed to investigate cytotoxic damage to the buccal mucosa caused by smoking. Sixty male participants were divided into 3 groups of 20 each: tobacco cigarette (TC), e-cigarette (EC), and non-smokers (NS). Buccal mucosal smears were processed with a modified Papanicolaou stain. Duration of smoking history was assessed using the Mann-Whitney test. Kruskal-Wallis test followed by post hoc Dunn test was used to analyze nuclear abnormalities. Apoptotic index was assessed using one-way ANOVA followed by Tukey post hoc test. P-value < 0.05 was set as significant. The duration of smoking was significantly longer in the TC than in the EC group. The frequency of nuclear abnormalities was significantly higher in both TC and EC than in the NS group. Conversely, apoptotic index was significantly lower in TC and EC than in the NS group. There were no significant differences in nuclear abnormalities or the apoptotic index between TC and EC. Marked increase in nuclear abnormalities and lower decrease of apoptotic index over a much shorter smoking duration suggest greater potential toxicity and damage from e-cigarette use.

Introduction

One of the greatest hazards to global public health, the tobacco pandemic claims the lives of about 8 million people annually. Of those deaths, almost 7 million are directly related to tobacco use, and over 1.2 million are caused by secondhand smoke exposure for nonsmokers. Over 80% of the 1.3 billion tobacco users globally reside in low- and middle-income nations, where tobacco-related sickness and mortality are most prevalent. In Indonesia, since 1990, the high prevalence of smoking has not substantially decreased. Male smoking rates, which are still among the highest in the world, have been the main cause of this gain. According to the survey, 70.2 million adults, or 34.5% of the population, were tobacco users. Tobacco use is approximately 3.3% among females and 65.5% among males. From 0.3% in 2011 to 3% in the last 2021 report, the use of electronic cigarettes has expanded tenfold over the past 10 years (Ministry of Health Republic of Indonesia & World Health Organization Indonesia Centers for Disease Prevention and Control, 2021).

Worldwide, there is ample evidence of smoking's detrimental impacts on human health. Smoking not only directly impairs lung function but also affects the body's levels of nutrients such as magnesium and vitamin

✉ Correspondence Address:
Department of Anatomical Pathology, Faculty of Medicine, Universitas Jenderal Soedirman, Purwokerto, Indonesia
Email: dodynovrial@unsoed.ac.id

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D (Laksono *et al*., 2025; Lorensia *et al*., 2025; Mohammed *et al*., 2025). The adverse effects of smoking on human health are also well-documented globally. Its consequences include tooth discoloration, inflammation, increased tooth plaque, tooth loss, reduced success of oral surgery and implant procedures, leukoplakia, and oral cancer (Gajendra *et al*., 2023). Since the implementation of the WHO Framework Convention on Tobacco Control (FCTC) starting in 2003, cigarette consumption has fallen in most countries. However, tobacco consumption in the South and Southeast Asian countries remains high and tends to increase. China and Indonesia, for example, showed higher increases in tobacco consumption of 0.75 million metric tonnes (MMT) and 0.1 MMT, respectively, compared with a decade earlier (Hoffman *et al*., 2019). This condition is suspected to be the leading cause of the increasing incidence of oral cancer in this region (Filho & Warnakulasuriya, 2024).

In 2003, the electronic cigarette (EC) was discovered in China and introduced as a cessation aid for smokers' addiction to tobacco, since this battery-operated device is tobacco-free (Voos *et al*., 2019). EC companies claimed that their products are less harmful and can even improve oral health by providing alternatives to smoking. EC smoking has reached significant popularity in the last two decades. It has experienced a fantastic increase in sales after entering the US market in 2007, and continues to rise exponentially every year (Besaratinia & Tommasi, 2020). Few studies were available on EC's effects on oral health, and some reported conflicting results. An in vitro study revealed that exposure to ECs vapor with flavorings increased oxidative stress, pro-inflammatory, and pro-senescence responses in periodontal cells (Sundar *et al*., 2016). Conversely, another study found inflammation and self-perceived oral symptoms were significantly higher in tobacco cigarette (TC) smokers (Javed *et al*., 2017), and EC does no harm in the oral cavity (Franco *et al*., 2016). Thus far, the direct effects of ECs' vapor on the oral mucosa have been little evaluated. This present study aims to assess genotoxic damage and risk of tumor development on the oral mucosa of EC smokers and TC smokers with a minimum of 2 years of smoking experience, using micronucleus, binucleus, broken egg, and apoptosis assay. .

Method

This historical cohort study was conducted in the Anatomical Pathology Laboratory, Faculty of Medicine, Universitas Jenderal Soedirman between January and May 2022. This study was approved by the Health Research Ethics Committee of the Faculty of Medicine, Universitas Jenderal Soedirman, Purwokerto (Ref. 168/KEPK/VII/2021). Informed consent was obtained from all subjects involved in the study. The study population comprised tobacco cigarette (TC) smokers, electronic cigarette (EC) smokers, and non-smokers (NS). Sixty participants (20 for each group) were recruited for this study based on the following inclusion criteria: male, age between 20-40 years old, usage mix diet, exclusive conventional tobacco cigarettes consumption at least in the last 2 years for TC group, exclusive user of ECs minimum in the last 2 years for EC group, no dental procedures in the last 6 months, no alcoholism, no history of malignancy and concomitant diseases, and no chronic carcinogen exposure. The NS group was matched to the TC and EC groups for sex, age, and diet.

Before the sampling procedure, the study participants underwent an oral examination to assess the condition of the oral cavity. Participants were then asked to rinse their mouths twice with a 0.9% NaCl isotonic solution. The exfoliated bilateral buccal mucosa cells were obtained using a cytobrush, which was then spread over the glass slides. The prepared slides were fixed in 95% ethyl alcohol for 10-15 minutes and then washed with running tap water. A modified Papanicolaou (Pap) staining method was used to determine nuclear cell abnormalities (Sinkar & Arakeri, 2017). The micronucleus (MN), binucleus (BN), broken egg (BE), and apoptotic index (sum of pyknosis, karyorrhexis, and karyolysis) frequencies were counted in 1,000 exfoliated buccal cells from each subject by pathologists under a light microscope. Comparison of the duration of smoking history between the TC and EC groups was assessed using the Mann-Whitney test. Kruskal-Wallis test followed by

post hoc Dunn test was used to compare nuclear abnormalities (MN, BN, and BE) frequencies between groups. Apoptotic index was assessed using one-way ANOVA followed by Tukey post hoc test. A p-value < 0.05 was set as significant.

Result and Discussion

The characteristics of participants are summarized in Table 1. The average age ranged from 23 to 26 years and was relatively similar across groups. The duration of smoking history in the TC group was significantly longer compared to the EC group (p = 0.000). All participants met the inclusion criteria based on diet, alcohol use, exposure to carcinogens, history of dental procedures, chronic diseases, and malignancy. Nonetheless, during the oral examination, we found gingival melanosis and teeth staining in most of the TC group's participants; leukoplakia (1 participant in the TC group); and no clinical significance was

TABLE 1. Characteristics of Participants

	Groups		
	TC Smokers	**EC Smokers**	**Non-Smokers**
Age (Mean ± SD)	26.65 ± 2.43	25.30 ± 3.64	23.75 ± 2.22
Duration of smoking (Mean ± SD)*	6.15 ± 1.89	3 ± 0.92	0
Mixed diet	Yes	Yes	Yes
Alcohol consumption	No	No	No
Carcinogens exposure	No	No	No
Dental procedure within the last 6 months	No	No	No
Malignancy or chronic disease	No	No	No
Clinical finding in oral examination	Gingival melanosis, Teeth stain, Leukoplakia	None	None

*Statistically significant (p=0.000).

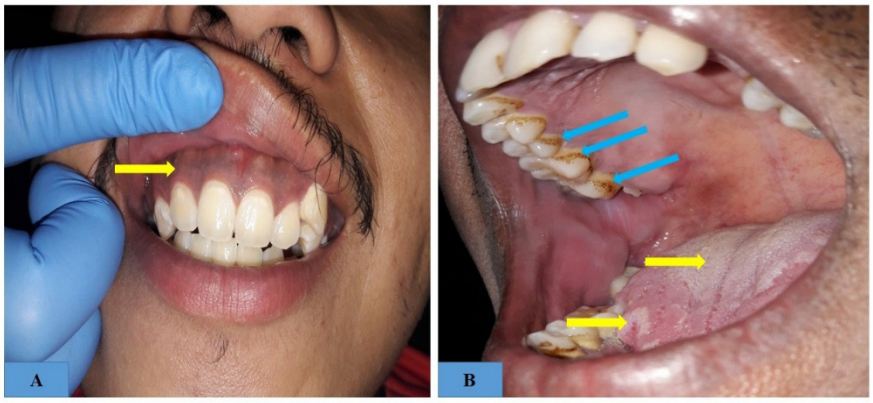

FIGURE 1. Clinical Findings in Oral Examination in the TC Group. A. Gingival Melanosis (Yellow Arrow); B. Leukoplakia on Tongue Mucosa (Yellow Arrows) and Teeth Staining (Blue Arrows).

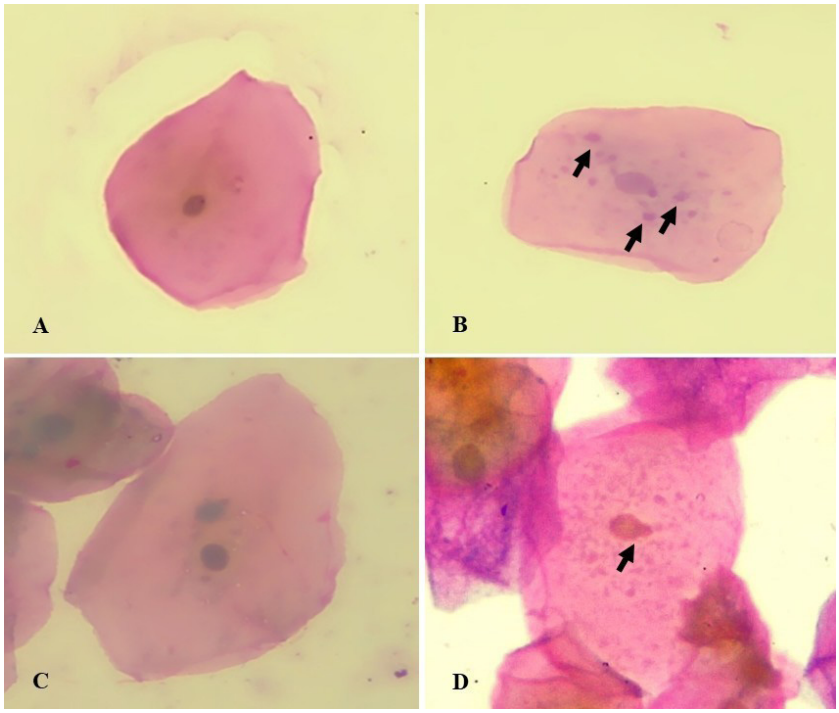

FIGURE 2. A. A Typical Cell Has a Single Round to Oval Nucleus, and No Other DNA-Containing Structures are Observed. B. Cell Containing Both a Nucleus and a Micronucleus, With A Smaller Nucleus Sized Between 1/3 and 1/16 Of the Primary Nucleus Diameter (Black Arrow). C. A Binucleated Cell Has Two Nuclei That Have the Same Morphology as That Observed in Normal Cells. D. Cell Containing a Nucleus and Nuclear Bud (Broken Egg Nuclei) Sized Between 1/4 and 1/2 Of The Primary Nucleus Diameter (Black Arrow). Modified Pap Stain, 400x Magnification.

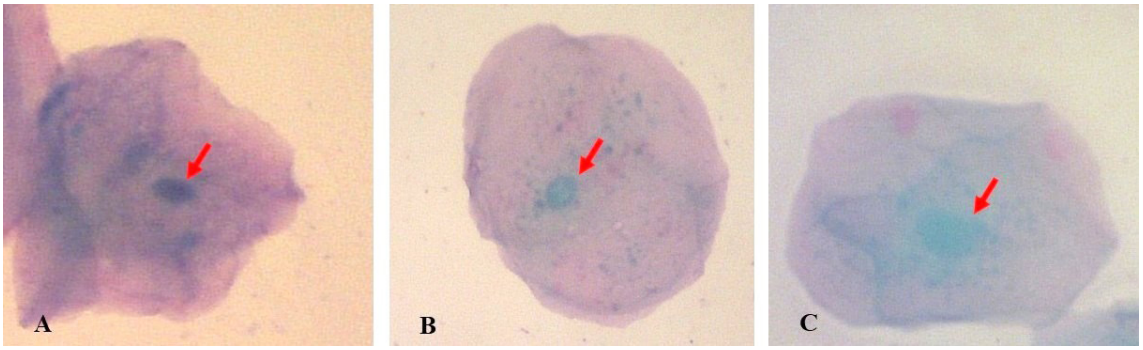

FIGURE 3. Appearance of Cells Undergoing Apoptosis. A. Picnotic Nuclei Marked by Condensed Chromatin; B. Fragmented Nuclei (Karyorrhexis); and C. Dissolution of Nuclear Components (Karyolysis). Modified Pap Stain, 400x Magnification.

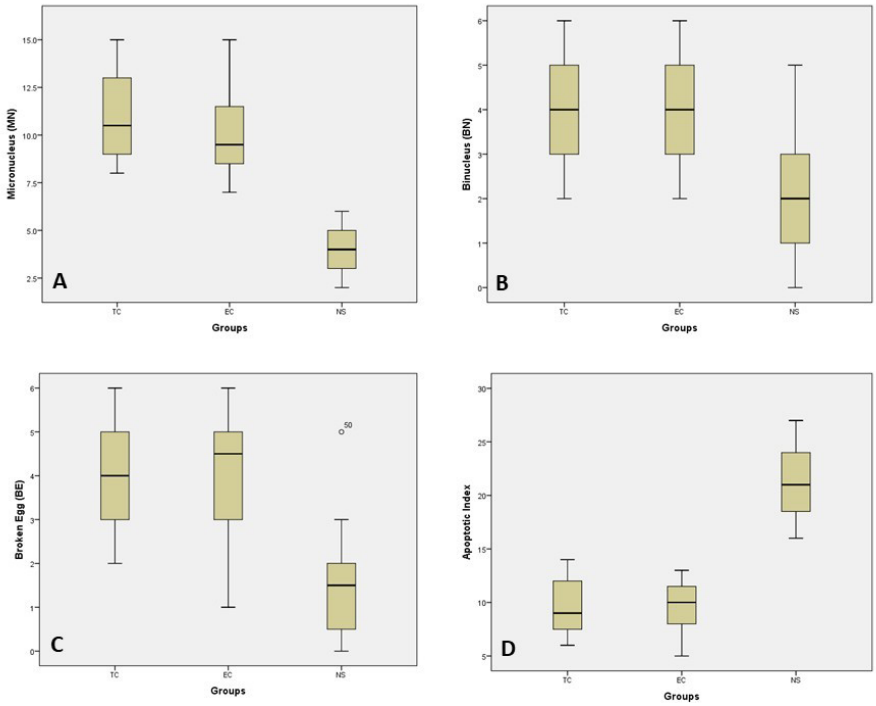

FIGURE 4. A. Mean of MN in TC (11.05±2.28), EC (9.95±2.28), NS (3.95±1.23); B. Mean of BN in TC (4.1±1.21), EC (3.7±1.22), NS (1.95±1.43); C. Mean of BE in TC (3.9±1.37), EC (4.1±1.41), NS (1.55±1.32); D. Mean of apoptotic index in TC (9.6±2.7), EC (9.65±2.18), NS (21.25±3.29).

found in the EC and NS groups (Fig 1).

A cytological study was performed to assess nuclear abnormalities and the apoptosis index of oral mucosa cells. Characteristics of MN, BN, and BE are shown in Fig 2, and Fig 3 shows pyknosis, karyorrhexis, and karyolysis nuclei. The distribution of MN, BN, BE, and apoptotic index in the different groups is depicted in Fig 4. The frequencies of MN, BN, and BE were significantly higher in both the TC and EC groups than in the NS group ($p = 0.000$). Conversely, apoptotic index was lower in the TC and EC groups than in the NS group, significantly ($p = 0.000$). However, there were no significant differences in the nuclear abnormalities and apoptosis index between the TC and EC groups.

Tobacco usage has become one of the main global public health issues regarding the growth and popularity of more recent tobacco and nicotine products. (Gajendra *et al*., 2023). Electronic cigarettes are one of the most popular nicotine products nowadays. It is a portable device that heats a liquid typically containing flavorings and nicotine. Instead of inhaling smoke, EC users can inhale nicotine as vapor. EC does not expose users to the exact quantities of toxins that can make conventional cigarette smokers sick, since they do not burn tobacco (Lindson *et al*., 2024). EC is a popular way for people to give up smoking. The sensory, behavioral, and/or social aspects of smoking that former smokers miss when they quit—such as holding a cigarette, inhaling, enjoying smoking, and feeling a part of a group—are not sufficiently addressed by any of the current smoking addiction treatments, even though they deliver nicotine more slowly and at lower levels than smoking. EC may provide a means to get around these restrictions (Notley *et al*., 2018).

The discussion of the safety of heated tobacco products and electronic cigarettes has become more prevalent in the scientific literature in recent years. These substitutes for traditional tobacco were first thought to offer smokers a way out. However, new scientific data have begun to raise serious questions about the dangers of using these products compared to traditional tobacco, including those related

to oral health (Liu *et al*., 2024). In this study, we found significantly increased nuclear abnormalities in buccal mucosal epithelial cells in both EC and TC smokers compared with non-smokers. Our results are consistent with previous studies that revealed harmful effects of tobacco on health, including oral illnesses. Compared to non-smokers, smokers have a much higher risk of oral diseases (Natarajan *et al*., 2024; Zhang *et al*., 2019).

In exfoliated buccal mucosal cells, the presence of micronuclei (MN) and other nuclear abnormalities (NAs) is a sensitive indicator of cytotoxic effects, chromosomal damage, and mitotic disruptions (Sánchez-Alarcón *et al*., 2026). Exposure to known genotoxic chemicals, such as those that have been classified as carcinogens by the International Agency for Research on Cancer (IARC), is consistently linked to increased MN frequencies (Hopf *et al*., 2019). Following exposure to a genotoxic substance, additional nuclear changes may be observed, including broken egg, cytoplasmic bridges, pyknosis, karyorrhexis, an increase in binucleated cells, and karyolysis. Although the rise in these changes' frequency is unrelated to cancer directly, it is a helpful indicator of the genotoxicity of exposure to numerous carcinogens (Sánchez-Alarcón *et al*., 2021). The rate of binucleated cells is substantially associated with the prevalence of disorders such as autoimmune diseases, infection, and cancer, reflecting the cumulative damage process underlying MN formation (Abdalwahab *et al*., 2025). In addition, BE and nuclear extensions, which may generate MN, share a common source with MN and are connected to the process of gene amplification (Gómez-Meda *et al*., 2016).

Our research findings revealed that EC smokers had a higher number of BE than TC smokers. Conversely, the number of MN and BN was higher in TC smokers than in EC smokers. However, these nuclear abnormalities were not significantly different between TC and EC smokers. These results are consistent with a previous study that reported similar cellular effects in both TC and EC (Ahmedah *et al*., 2025). Our results also reinforce other studies with larger participants on the dangers of EC and TC, particularly on the buccal mucosa (Fuoad Al Bayati *et al*., 2025; Pop *et al*., 2021). However, another study showed that NAs are more prevalent in TC smokers than in EC smokers, although it demonstrates a strong association between both and changes in the buccal mucosa's cytomorphology (Salih *et al*., 2025).

By introducing over 4,000 chemical substances, including nitrosamines and polycyclic aromatic hydrocarbons (PAHs), tobacco smoke promotes genotoxicity by directly damaging DNA, inducing oxidative stress, and interfering with cellular repair processes. This damage results in chromosomal abnormalities, the development of micronuclei, and genetic changes that promote carcinogenesis (Pinto *et al*., 2025). On the other hand, EC liquid aerosols have been shown to contain potentially carcinogenic chemicals, such as formaldehyde, acetaldehyde, acrolein, and certain nitrosamine compounds (Rocha *et al*., 2026). In vitro, EC liquids have been shown to cause DNA damage, and there is clinical evidence that EC usage is associated with increased levels of acrolein-DNA adducts, metanuclear abnormalities, and lactate dehydrogenase expression, as well as decreased apurinic/apyrimidinic sites, as compared to non-smokers (Tomar *et al*., 2019; Yang *et al*., 2020). All of these results lend credence to the theory that the oral mucosa may experience quantifiable genotoxic effects from EC aerosols.

Apoptosis is a well-regulated, programmed form of cell death that tightly controls both cell survival and death. It has a signal-regulated process in which cells undergo self-destruction in response to internal or external stimuli. By removing damaged or superfluous cells, it plays a crucial part in preserving tissue homeostasis (Mustafa *et al*., 2024). Previous research indicates that smoke's harmful cytotoxic effects may actually raise cell death indicators in both tobacco and electronic cigarettes. Exposure to various environmental substances, such as cigarette smoke, can cause non-lethal DNA damage, which is the core of carcinogenesis (Alanazi *et al*., 2018; Rouabhia *et al*., 2017). However, we found a significantly lower apoptotic index in the TC and EC groups than in the NS group. This result is in line with another study between tobacco smokers,

smokeless tobacco smokers, and normal controls (Devadoss *et al*., 2021).

Nicotine and NNK (4-methylnitrosamino)-1-(3-pyridyl)-1-butanone, which are found in cigarette smoke, are responsible for the reduction in the number of cells undergoing apoptosis. These compounds bind to nicotinic acetylcholine receptors (nAChRs) and, in turn, stimulate the phosphorylation of pro-apoptotic proteins (BAD, BAX) and anti-apoptotic proteins (BCL2, MCL-1) by activating multiple protein kinase pathways. Their phosphorylation will enhance the anti-apoptotic role of BCL2 and MCL-1. In contrast, its function as a pro-apoptotic protein will be diminished by phosphorylation of BAD and BAX proteins (Firdausa *et al*., 2023). Nicotine also inhibits caspase-mediated apoptosis by increasing the PI3K-Akt pathway, a signal transduction cascade that supports cellular survival and proliferation (Aspera-Werz *et al*., 2018). Similar physiological effects to those of cigarette smoking, such as the activation of genes responsive to oxidative stress, nicotine-mediated prevention of apoptosis, and stimulation of Akt cell signaling pathways, may be induced by nicotine vaporization through e-cigarettes (Rigg & Gielda, 2019).

The most important regulator in the smokers' buccal mucosa is the tumor protein 53 (TP53) gene, which triggers apoptosis. P53 mutations are among the most prevalent genetic changes in human malignancies, including oral cancer (Khaleel *et al*., 2022). In addition, it was observed that the number of apoptotic cells continues to increase at mild and moderate dysplasia levels. When dysplasia and cancer reach a severe stage, fewer cells undergo apoptosis (Firdausa *et al*., 2023). Because of this decrease in cell death, damaged cells—such as micronuclei, which indicate chromosomal damage—are not eliminated, increasing the risk of developing cancer. The mutation of apoptotic regulatory genes is likely to reduce the apoptotic index in the TC and EC groups, and leukoplakia, a pre-cancerous lesion found in the TC group, might be at a severe stage of dysplasia, which would explain the decreased apoptosis.

Conclusion

The substantial effects of electronic cigarettes on oral health are comparable to those of tobacco cigarettes. In comparison to NS, the buccal mucosal epithelium of the EC and TC groups showed a considerable decrease in the apoptotic index and a significant rise in nuclear abnormalities. However, the significant incidence of cytological abnormalities during a considerably shorter smoking time points to increased potential harm and toxicity from using electronic cigarettes. Given the population's persistently high smoking incidence, which influences public health, the repercussions of encouraging alternative tobacco use methods, such as electronic cigarettes, should be carefully considered. According to our study, exfoliative cytology is a non-invasive, effective, and efficient method for identifying genotoxicity. However, we recognize limitations of this study, including the small number of participants available and the absence of molecular testing to confirm the cellular changes found. We recommend conducting further research with a comprehensive molecular investigation in a more extensive population.

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