

Impact Of E-Cigarette/Vape Use And Cytotoxic Damage In Oral Epithelial Cells: A Review

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Abstract

Background: In India, there has been a notable increase in the use of electronic cigarettes despite the government's ban. This study aims to review the cytotoxic & genotoxic damage in vape users. There are existing reports on the use of electronic cigarettes and their potential impact on developing oral squamous cell carcinoma. We aimed to assess the association between e-cigarettes and oral cancers. Additionally, the paper addresses the composition of E-juice and safety considerations of electronic cigarettes compared to conventional cigarette smoking.

Conclusion: Regular monitoring using this non-invasive assay could be crucial in risk assessment and implementing preventive strategies in at-risk populations. It's essential that responses are evidence-based and carefully crafted, with robust enforcement by federal and state governments. Policymakers should focus on reducing youth vaping while also providing support for smokers seeking to quit.

Key Word: Vape; E-Cigarettes; ENDS (Electronic nicotine delivering systems); Micronuclei; E-juice.

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I. Introduction

Electronic nicotine delivery systems (ENDS), commonly known as e-cigarettes or vape, have gained popularity as alternatives to traditional tobacco products. These battery-powered devices vaporize a flavored liquid that typically contains nicotine, an addictive substance. The rapid absorption of nicotine into the bloodstream, especially when inhaled, significantly contributes to its addictive potential. Factors such as the age of first exposure and the amount of nicotine consumed play crucial roles in determining a person's risk of developing long-term addiction. Studies have found that youths using ENDS or e-cigarettes are more likely to use regular cigarettes later. E-cigarettes increase the likelihood to experiment with regular tobacco products and increase intention to indulge in cigarette smoking. They also increase the risk of dual use due to lack of awareness about the harmful effects of ENDS.¹

In a Developing country like India, despite the ban implemented, different e-cigarette brands with varied configuration of nicotine delivery available in the market, for example, cigarlikes (first generation), tank systems (second generation) and personal vaporizers (third generation) with over 7700 flavours.² The rise of vape among youth is increasingly viewed as an adoption of trends from Western cultures. Many teenagers are drawn to vaping not just for the nicotine but also for the social status it can confer. The appeal lies in the variety of flavors, stylish device designs, and the perception that vaping is a more modern or trendy alternative to smoking traditional cigarettes.³

II. Discussion

Origin of Vape and Its Evolution

Vape origin refers to the invention of the modern e-cigarette by Chinese pharmacist Hon Lik in 2003. He developed the device to serve as an alternative to conventional smoking. Vaping products were first introduced

in China in 2004, and then to the rest of the world within a few years.⁴ Later Hon Lik started the dual use of conventional cigarette and e-cigarette that may lead to detrimental effects.⁵

E-cigarettes have evolved over time, and the different designs are classified in generations. First-generation e-cigarettes tend to look like traditional cigarettes and are called "cigarlikes".⁶ Second-generation devices are larger and look less like traditional cigarettes.⁷ Third-generation devices include mechanical mods and variable voltage devices.⁶ The fourth-generation includes sub-ohm tanks (meaning they have electrical resistance of less than 1 ohm) and temperature control. There are also pod mod devices that use protonated nicotine, rather than free-base nicotine found in earlier generations providing higher nicotine yields.^{8,9}

Major tobacco companies have started to buy or develop electronic nicotine delivery systems (ENDS) to expand their product lines while promoting these products as less harmful alternatives to cigarettes. Cigarette smokers who may have otherwise given up the habit are thereby retained as nicotine-addicted customers, while those who may have never attempted to experiment with cigarettes are drawn into the nicotine addiction web. As traditional cigarette use declines in many countries, the marketing approach of these companies aims to maintain a customer base.³ Aguilar, Carmen (11 June 2018) conducted a study in which respondents in Euro barometer poll had to give at least three reasons why they started using e-cigarettes.¹⁰

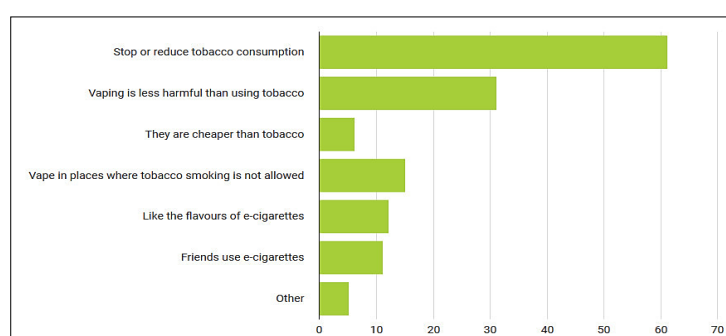


Fig 1: Aguilar, Carmen (11 June 2018). "Lung cancer deaths on the rise in two thirds of European countries". VoxEurop/The European Data Journalism Network.¹⁰

E-Juice content & Nicotine delivery

The functioning of electronic cigarettes (e-cigarettes) involves heating a liquid mixture that typically includes propylene glycol, vegetable glycerin, nicotine, and various flavoring agents.¹¹ When vaporized, this mixture produces aerosol that users inhale. While e-liquids have a simpler composition than traditional tobacco products, they still present health risks. E-cigarettes can emit harmful substances, including:

- Tobacco-specific nitrosamines: These are known carcinogens derived from tobacco.¹² Tobacco alkaloids: These compounds can have various effects on health.
- Nicotine decomposition products: Heating nicotine can produce harmful byproducts.
- Aromatic amines: These substances can be toxic and are associated with cancer risk.
- Heavy metals: Found in some e-cigarette components, such as coils, these can leach into the vapor.¹³
- Carbonyl compounds: These include substances like formaldehyde and acetaldehyde, which have proved potential of being carcinogenic when inhaled.

The presence of these toxicants raises concerns about the potential adverse oral health outcomes associated with e-cigarette use, such as gum disease, tooth decay, and other oral conditions. A typical cartridge contains about as much nicotine as a pack of 20 regular cigarettes and can act as a potential source for nicotine addiction. Furthermore, the amount of nicotine and other chemicals in these products varies widely, and thus, the consumer remains unaware of the actual contents of the products they use. Studies on these nicotine solvents had shown a varied degree of release of potential carcinogens – which includes acetaldehyde, formaldehyde and acetone – depending on the battery output voltage. Interestingly, flavoured e-liquids, particularly menthol, seem to be more harmful compared to e-liquids without flavours.^{14, 15} E-cigarettes can deliver significant levels of nicotine depending on various factors. Higher nicotine concentrations in e-liquids, advanced devices used at greater power, and longer puffs enhance nicotine delivery. Flavoring can also influence nicotine exposure by affecting user preferences and behavior. Additionally, other components like pH, alcohol, sweeteners, and minor nicotine alkaloids play a role. Because many factors—ranging from e-liquid composition to user habits—affect nicotine delivery, regulatory measures focused solely on nicotine concentration may be insufficient to effectively manage user exposure.¹⁶ More importantly, our data suggest that EC aerosols may cause cytotoxicity to human oral keratinocytes via oxidative stress response.¹⁷

Studies investigating cytotoxicity of e- cigarette aerosol ¹⁸

Ji et al (2016)	Normal human oral keratinocytes (NHOKs)	E-cigarette aerosol	Cytotoxic to cells due to oxidative stress induced by substrates
Hwang et al (2016)	Human keratinocytes (HaCaTs)	E-cigarette aerosol (7 brands)	Cytotoxic to cells
Yu et al (2016)	Human keratinocytes (HaCaTs), human HN squamous cell carcinoma cells	E-cigarette aerosol (V2, VaporFi)	Cytotoxic to cells; DNA strand break-inducing agent; potentially
Reuther et al (2016)	Human buccal mucosa	E-cigarette aerosol (with and without nicotine)	Increased capillary perfusion of buccal mucosa with nicotine-con

Cytotoxic effects

Welz et al ¹⁵ treated primary oropharyngeal mucosal cells with e-liquids and confirmed a significant reduction in cell viability as well as increased DNA damage following incubation with fruit-flavoured e-liquids. More importantly, literature suggest that EC aerosols may cause cytotoxicity to human oral keratinocytes via oxidative stress response.¹⁸

The investigation of chromosomal aberrations, sister chromatid exchanges, and micronucleus tests are methods used to detect DNA damage and assess genotoxic effects in tobacco users. Among these, the micronucleus test is favored due to its simplicity and efficiency; it does not require complex procedures like cell culture or DNA staining. This assay operates on interphase cells, allowing it to effectively reveal mitotic interference and chromosomal mutations or breakages. Micronuclei (MN) are small, nuclei-like structures that arise when lagging chromosomes or chromosome fragments fail to be properly incorporated into the primary nucleus during cell division (mitosis or meiosis). These structures form when a nuclear envelope surrounds these extraneous chromosomal materials, resulting in their persistence during interphase. The presence of micronuclei can serve as a biomarker for genomic instability and is often used in studies to assess genotoxic damage caused by various agents, including chemicals and radiation. Monitoring micronuclei can provide valuable insights into the underlying mechanisms of diseases such as cancer, as well as help in evaluating the genotoxic potential of substances in toxicological assessments.^{19, 20}

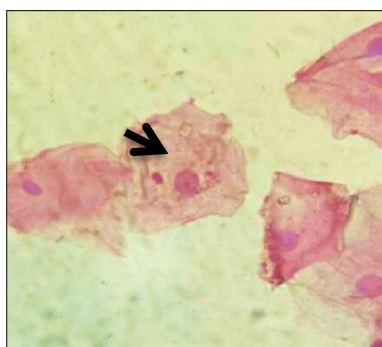


Fig 2: Epithelial cells containing 1 MN (black arrows) from e-cigarette user (Pap stain, ×40)

Carcinogenic Potential

Vaping is a relatively new phenomenon, there is a lack of studies and reports investigating the long-term health effects of e-cigarette aerosols. Further molecular studies are needed to elucidate the relationship between e-cigarette aerosols and oral cavity cancer. Darren Klawinski,²¹ et al reported a case, supporting the hypothesis that vaping can cause cancer and there should be heightened awareness about both the short- and long-term health risks of vaping. We hypothesize that this patient’s frequent vaping through nicotine-delivery systems increased his risk for developing SCC through the direct carcinogenic effects of inhaled toxins present in nicotine solutions on oral cavity cells. Bustamante et al²² in his Case-control study, on Human saliva, with E-cigarette chronic use, Endogenous formation of carcinogenic N'-nitrosornicotine inside oral cavity. There is substantial evidence that chemicals present in the aerosols are capable of inducing DNA damage and mutagenesis, supporting the hypothesis that e-cigarette aerosols could increase the risk for cancer.²²

Only very few studies have investigated e-cigarettes in the context of HN cancers in the clinical environment. A case series by Nguyen et al ²³ described two patients with a positive history of chronic e-cigarette use who developed oral cancers, indicating a link between the long-term consumption of e-cigarettes and this type of cancer Korrapati et al ²⁴ (2016) studied Human epithelial and HN squamous cell carcinoma cells and found out Cytotoxicity to cells; DNA double-strand break induction; increased migration of HN cells.

The effects of e-cigarettes are not limited to HN cancers, and there is growing evidence to suggest that they may play active roles in the pathogenesis of other malignancies.^{23,24} Measurement of bladder carcinogens in the urine of e-cigarette users demonstrated greater concentrations of carcinogenic aromatic amines, suggesting a potential role of e-cigarette in the pathogenesis of bladder cancer. Lee et al reported that e-cigarette aero- sol exposure promotes DNA damage and impairs DNA repair in human lung and bladder cells, suggesting susceptibility of these cells to oncogenic transformation and carcinogenesis.²⁶ Current research on the role of e-cigarettes in cancer focuses primarily on HN, bladder and lung cancers, and the majority of evidence in the literature is limited to either in vitro or in vivo studies.²⁵

Local and systemic effects

The study by Bardellini et al²⁶ explored the prevalence of oral mucosal lesions (OMLs) in two groups: former tobacco smokers and current e-cigarette users. While the results indicated a higher prevalence of OMLs among e-cigarette smokers, the lack of statistical significance suggests that the difference might not be strong enough to draw definitive conclusions. The presence of these toxicants raises concerns about the potential adverse oral health outcomes associated with e-cigarette use, such as gum disease, tooth decay, and other oral conditions. Ganesan et al²⁷ revealed that the changes that appeared in the micro biome and the differences in bacterial biofilm production and architecture are more likely to be caused by the glycerol and propylene glycol present in e-liquids and not by nicotine. These sugar alcohols can become a source of nutrients for bacteria. They also discovered high virulence signatures, and proinflammatory signals in clinically healthy e-cigarette users, and they emphasize the fact that pathogenetic mechanisms associated with e-cigarette use might be different from conventional cigarettes

Vaping-associated pulmonary injury (VAPI), also called e-cigarette or vaping product use-associated lung injury (EVALI), is an acute or subacute respiratory illness characterized by a spectrum of clinicopathologic findings mimicking various pulmonary diseases. According to the CDC criteria, EVALI is a clinical diagnosis that requires the use of an e-cigarette in the 90 days preceding the appearance of initial symptoms; pulmonary infiltrates on a plain chest radiograph or chest CT, and the absence of any other possible etiology, such as infection. Although the etiology remains unclear, several causes are under investigation. Of these, vitamin E acetate is the most recognized agent associated with an e-cigarette or vaping product use-associated lung injury (EVALI). Welz et al¹⁵ treated primary oropharyngeal mucosal cells with e-liquids and confirmed a significant reduction in cell viability as well as increased DNA damage following incubation with fruit-flavoured e-liquids.²⁸

Vape versus smoking

ENDS or e-cigarettes are popularly perceived as a smoking cessation aid, but their efficacy and safety as a quitting aid have not yet been firmly established. Although some smokers claim to have cut down smoking while using ENDS, the total nicotine consumption seems to remain unchanged. E-cigarettes seem to have rather similar or even weaker efficacy as a cessation aid when compared to nicotine patches, due to different sizes of e-liquid vials, variable amount of nicotine in each vial, uncontrolled number of vapes and variable amount of nicotine in each vape puff. Moreover, a considerable number of ex-smokers who have reported stopping cigarette use with the aid of ENDS continue using the latter product, thus sustaining nicotine dependence.²⁹ While "The ACS does not recommend the use of e-cigarettes as a cessation method. No e-cigarette has been approved by the Food and Drug Administration (FDA) as a safe and effective cessation product."³⁰

Future directions

Because of the potential relationship with tobacco laws and medical drug policies, e-cigarette legislation is being debated in many countries. The companies that make e-cigarettes have been pushing for laws that support their interests. E-cigarette companies commonly promote that their products contain only water, nicotine, glycerin, propylene glycol, and flavoring but this assertion is misleading as researchers have found differing amounts of heavy metals in the vapor, including chromium, nickel, tin, silver, cadmium, mercury, and aluminum. The widespread assertion that e-cigarettes emit "only water vapor" is not true because the evidence demonstrates e-cigarette vapor contains possibly harmful chemicals such as nicotine, carbonyls, metals, and volatile organic compounds, in addition to particulate matter. They are marketed to men, women, and children as being safer than traditional cigarettes. They are also marketed to non-smokers. E-cigarette marketing is common. There are growing concerns that e-cigarette advertising campaigns unjustifiably focus on young adults, adolescents, and women.³

While "The ACS does not recommend the use of e-cigarettes as a cessation method. No e-cigarette has been approved by the Food and Drug Administration (FDA) as a safe and effective cessation product. The rising rates of vaping among young people have created urgent challenges for policymakers. It's essential that responses are evidence-based and carefully crafted, with robust enforcement by federal and state governments. Policymakers should focus on reducing youth vaping while also providing support for smokers seeking to quit.

Additionally, policies must be forward-thinking, as the rapidly evolving e-cigarette market allows companies to adapt more quickly than regulators.³⁰

III. Conclusion

Oral tissues are particularly vulnerable to the harmful effects of electronic cigarettes, and while progress has been made, significant research gaps remain regarding the short- and long-term effects of e-vapors. Current evidence indicates that vaping impacts the oral keratinocytes, periodontal ligament and fibroblasts, E-cigarette use adversely affects the cardiovascular system, impairs respiratory, immune cell function and airways in a way similar to cigarette smoking and is responsible for severe respiratory disease contributing to serious health issues oral cancer and EVALI. Understanding the toxicity mechanisms of e-cigarettes is crucial, as various factors, such as the chemical composition of e-liquids, can influence health outcomes. The vast diversity in e-liquid formulations complicates research and makes it challenging to quantify exposure accurately.

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