Genotoxic, Cytotoxic, and Inflammatory Alterations of the Oral Mucosa Caused by the Use of Electronic Cigarettes: Literature Systematic Review

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Alterações Genotóxicas, Citotóxicas e Inflamatórias da Mucosa Oral Induzidas pelo Uso de Cigarro Eletrônico: Revisão Sistemática da Literatura

Alteraciones Genotóxicas, Citotóxicas e Inflamatorias de la Mucosa Oral Causadas por el Uso de Cigarrillos Electrónicos: Revisión Sistemática de la Literatura

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ABSTRACT

Introduction: The increased use of electronic cigarettes (vapes) has been identified as a new public health problem. **Objective:** To find evidence on the effects of vaping on the establishment of cellular and tissue damage and to identify carcinogenic pathways related to head and neck cancer and oral cancer. **Method:** Systematic review research in the PubMed, LILACS, SciELO, and Cochrane databases, with a ten-year time frame (2014-2024). Descriptors: e-cigarettes, vaping, oral cancer, squamous carcinoma, and head & neck cancer. Fourteen original *in vitro* and *in vivo* studies that investigated alterations at the molecular and clinical level resulting from exposure to and consumption of vapes were included and analyzed. The studies were classified according to the risk of bias using the OHAT and ROBINS-E tools. **Results:** Findings were grouped by the predominance of genotoxic and cytotoxic effects, as well as alterations in inflammatory pathways and cell progression and tissue lesions in the oral mucosa. *In vitro* and *in vivo* results showed DNA damage, micronucleus formation, reduced cell viability, increased LDH and ROS, activation of oncogenic pathways such as NF-κB and STAT3, and inflammatory pathways (increased interleukins) and progression pathways (epithelial-mesenchymal transition phenotype). **Conclusion:** Together, the findings demonstrate that vapes are capable of activating carcinogenic pathways in head and neck cells and oral mucosa. In addition, we consider activating and accelerating inflammatory processes, creating a microenvironment favorable to neoplastic initiation, promotion, and progression. These findings highlight the need for more robust clinical studies and mechanistic investigations focused on agent-specifictradic pathways.

Key words: Electronic Nicotine Delivery Systems; Genotoxicity; Carcinogenesis; Inflammation; Mouth Neoplasms.

RESUMO

Introdução: O aumento do uso de cigarros eletrônicos (*vapes*) tem sido apontado como um novo problema de saúde pública. **Objetivo:** Identificar evidências sobre efeitos do *vape* no estabelecimento de danos celulares e teciduais, e identificar vias carcinogênicas relacionadas ao câncer de cabeça e pescoço e câncer bucal. **Método:** Revisão sistemática, nas bases de dados PubMed, LILACS, SciELO e Cochrane, com recorte temporal de dez anos (2014-2024). Os descritores foram: *e-cigarretes, vaping, oral cancer, squamous carcinoma* e *head & neck cancer*, sendo incluídos e analisados 14 estudos originais *in vitro* e *in vivo*, que investigaram alterações em nível molecular e clínico decorrentes da exposição e consumo dos *vapes*. Os estudos foram classificados conforme o risco de viés pelas ferramentas OHAT e ROBINS-E. **Resultados:** Os achados foram agrupados pela predominância dos efeitos genotóxicos, citotóxicos e de alterações de vias inflamatórias e de progressão de células e lesões teciduais de mucosa oral. Os resultados *in vitro* e *in vivo* evidenciaram danos ao DNA, formação de micronúcleos, redução da viabilidade celular, aumento de LDH e ROS, ativação de vias oncogênicas como NF-κB e STAT3 e de vias inflamatórias (aumento de interleucinas) e de progressão (fenótipo de transição epitélio-mesenquimal). **Conclusão:** Em conjunto, os achados demonstram que *vapes* são capazes de ativar vias carcinogênicas de células de cabeça e pescoço e da mucosa oral, além de ativar e acelerar quadros inflamatórios, configurando um microambiente favorável à iniciação, à promoção e à progressão neoplásica e reforçando a necessidade de mais estudos clínicos e investigação de vias próprias ao agente.

Palavras-chave: Sistemas Eletrônicos de Liberação de Nicotina; Genotoxicidade; Carcinogênese; Inflamação; Neoplasias Bucais.

RESUMEN

Introducción: El aumento del uso de cigarrillos electrónicos (vapeadores) se ha identificado como un nuevo problema de salud pública. **Objetivo:** Identificar evidencias sobre los efectos del vapeo en el daño celular y tisular, así como las vías carcinogénicas relacionadas con el cáncer de cabeza y cuello y el cáncer oral. **Método:** Revisión sistemática con búsqueda en las bases de datos PubMed, LILACS, SciELO y Cochrane, dentro del período de diez años (2014-2024). Los descriptores fueron: *e-cigarretes, vaping, oral cancer, squamous carcinoma* e *head & neck cancer*. Se incluyeron y analizaron catorce estudios originales *in vitro* e *in vivo* que investigaron alteraciones a nivel molecular y clínico derivadas de la exposición y el consumo de vapeadores. Los estudios se clasificaron según el riesgo de sesgo mediante las herramientas OHAT y ROBINS-E. **Resultados:** Los hallazgos se agruparon según el predominio de efectos genotóxicos y citotóxicos, así como alteraciones en las vías inflamatorias, la progresión celular y las lesiones tisulares en la mucosa oral. Los resultados *in vitro* e *in vivo* mostraron daños en el ADN, formación de micronúcleos, reducción de la viabilidad celular, aumento de LDH y ROS, activación de vías oncogénicas como NF-κB y STAT3, y vías inflamatorias (aumento de interleucinas) y vías de progresión (fenotipo de transición epitelio-mesenquimal). **Conclusión:** En conjunto, los hallazgos demuestran que los cigarrillos electrónicos son capaces de activar vías carcinogénicas en células de cabeza y cuello y mucosa oral, además de activar y acelerar procesos inflamatorios, creando un microambiente favorable para la iniciación, promoción y progresión neoplásica, y reforzando la necesidad de realizar más estudios clínicos e investigar las vías específicas de este agente.

Palabras clave: Sistemas Electrónicos de Liberación de Nicotina; Genotoxicidad; Carcinogénesis; Inflamación; Neoplasias de la Boca.

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INTRODUCTION

Created as an alternative to traditional cigarettes, electronic cigarettes emerged to help reduce or even drop conventional smoking[1]. By removing tobacco combustion, these devices reduce exposure to widely known carcinogens. However, there are actually adverse effects and damage to health that contradict the initial objectives.

Despite being designed for chronic smokers, electronic cigarettes quickly overcame this target audience and became a trend among young people[2]. Popularly called vape, the use of vaping pods became frequent among non-smokers. Attractive flavors, appealing colors, and designs were some of the factors that made it popular, especially among individuals aged 15-35 years, as shown by a USA-based cohort study from 2021, which portrayed the use of electronic cigarettes as an epidemic[3].

The growing number of evidence correlating the use of vaping pods to several pathologies reinforces even more the concern and need for studies. In 2020, a systematic review revealed significant associations between the device and diseases, including acute pulmonary lesions and severe intoxications[4]. Additionally, another research from the same period found damage to DNA and inhibition of repair proteins in mice exposed to electronic cigarettes, reinforcing that the initial perception of safety around this device was equivocal and contributed to new public health problems[5].

The impact of electronic cigarettes is particularly relevant in the head and neck areas, and, within them, the epithelial tissues that cover the oral mucosa, since these give rise to a great part of malignant neoplasms in these body parts, which are directly exposed to aerosols during use. This context justifies this study, which aims to gather molecular, cellular, and clinical data to build evidence about the mechanisms of cellular damage caused by electronic cigarettes in the head, neck, and oral cavity areas.

Thus, the main objective of this review is to analyze the cellular and molecular effects of the use of electronic cigarettes (vaping pods) in the head and neck area, emphasizing genotoxicity, cytotoxicity, and tissue inflammation mechanisms in the oral mucosa. The hypotheses were based on investigating the relationship between exposure to or use of electronic cigarettes and inflammatory processes and their influence on the progression of inflammatory or malignant oral tissue lesions.

METHOD

The articles were searched in August-November 2024. The primary research initially used keywords in three languages according to the inclusion criteria and a time frame of ten years (2014-2024).

The inclusion criteria were original articles from the last ten years in English, Portuguese, or Spanish, with experimental data in animal models and analyses *in vitro* or *in vivo*, in addition to cross-sectional observational studies on exposure, which compared patients who used vaping pods and non-users.

The exclusion criteria were unoriginal articles, case reports, and systematic reviews, studies that did not encompass the head and neck or oral cavity regions, tangential studies, incomplete articles, or articles in languages other than English, Portuguese, or Spanish.

The screened and selected articles were taken from the following public platforms: PubMed, LILACS, SciELO and Cochrane. The search was conducted using the following controlled terms of the *Medical Subject Headings* (MeSH): "e-cigarettes and Head & Neck Cancer", "vaping and Head & Neck cancer", "e-cigarettes and oral cancer", "vaping and oral cancer", "e-cigarettes and squamous carcinoma", and "vaping and squamous carcinoma".

The primary descriptor search leveraged 162 articles, and after reading titles and abstracts and applying the inclusion criteria, 14 articles were selected for analysis, tabulation, and complete review. This study was registered in the International Prospective Register of Systematic Reviews (PROSPERO)[6] database, according to the journal's guidelines, ID number: 1244636.

The methodology and selection flowchart can be observed in detail in Figure 1[7], while the organization of the PICO research questions is detailed in Chart 1.

The whole sample was submitted to risk of bias analysis using the OHAT[8] tool for experimental studies and the ROBINS-E[9] tool for observational studies on exposure to vaping pods. The categorization of the research domains was done by two independent reviewers, and upon a discrepancy regarding one of the criteria, a third reviewer set the score.

Of the sample articles analyzed by OHAT (n=9), no study was assessed as having a high risk of bias; all the studies presented low or moderate risk in most applicable study domains.

Regarding the studies analyzed by ROBINS-E (n=5), the observational studies showed heterogeneous designs and samples, presenting moderate to severe risk and at least one domain. However, no critical risk was found in any of the articles. The result of this analysis can be found in the Supplementary Material. No article was excluded in this phase of the manuscript elaboration.

RESULTS

The 14 studies analyzed in depth enable the definition of 2 evidence axes: genotoxic, mutagenic, and cytotoxic

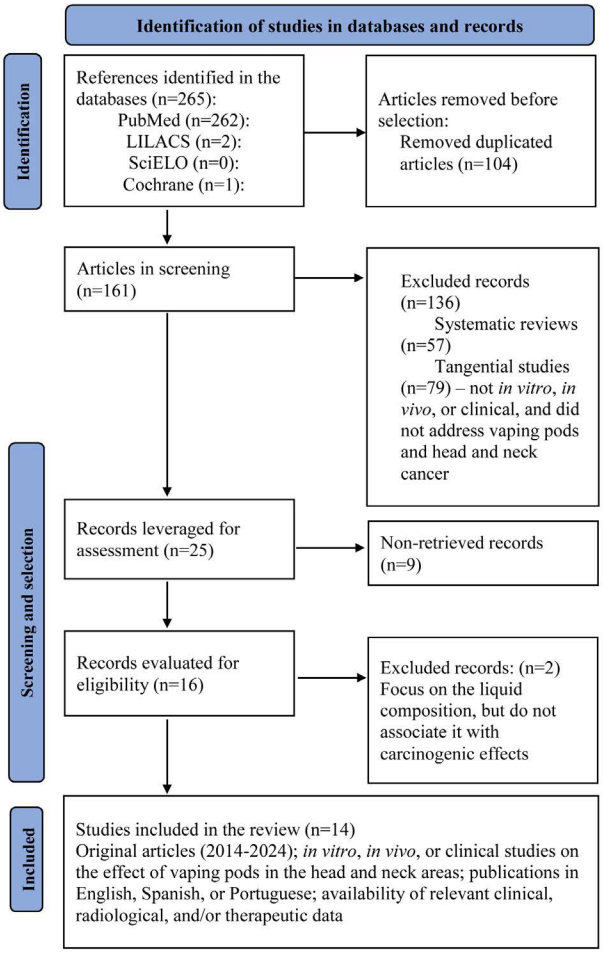

Figure 1. Flowchart of selection and inclusion criteria of articles according to the PRISMA[7] model

Chart 1. Structure of the research question according to the PICO strategy[10]

Component (PICO)	Description
(P) Population/ Sample	Experimental or clinical studies on the carcinogenesis of the head and neck or oral mucosa
(I) Intervention	Exposure to electronic cigarettes and their products
(C) Comparator	Exposed and not exposed samples
(O) Outcome	Evidence of genotoxicity, mutagenicity, cytogenicity, inflammation, and carcinogenesis

effects; and association with inflammatory events and progression of lesions in the oral mucosa. Thus, the results were described following this line of tumoral progression.

Genotoxic agents are those capable of interacting with DNA, producing changes to their structure and function. When these changes are set, a mutation ensues. The proof of genotoxicity and mutagenicity constitutes an important part of cancer research[11].

The present review has five studies that directly investigate this genotoxicity from vaping, occurring through MAPK pathways, protein ubiquination, AhR (aryl hydrocarbon) receptor signaling, tRNA loading, Rho family GTPases signaling, and the Wnt/Ca^{2+} pathway. Three articles suggest that, although electronic cigarettes may present lower levels of carcinogens compared to conventional cigarettes, their use is far from being harmless, especially regarding the genotoxic effects on the oral cavity's cells[12-16].

Hamad et al.[12] assessed gene expression after acute exposure to vaping pods, observing differential regulation of dozens of genes related to DNA damage response and cancer, with a highlight to overexpression of the TP53 gene — acknowledged by its function in the regulation of the cellular cycle and in DNA damage response — and MPG repression, an essential glycosylase in the basal excision repair pathway. Additionally, the genotoxic effects were dependent on the volume and intensity of inhalations, showing that user behavior directly influences molecular impact.

In line with that, Tellez et al.[13] also confirmed the genotoxic effects of vaping in oral epithelial cell lines of the tongue exposed to different e-liquids, observing a significant increase in the formation of micronuclei, lipid peroxidation, and oxidative stress — even in devices without nicotine. Products with specific flavors, like *Blue Pucker* and *Love Potion*, presented greater genotoxic potential, suggesting that flavoring additives can also play a critical role in DNA damage.

With inconclusive results, Guo et al.[14] analyzed the formation of apurinic/pyrimidine sites (AP sites), which indicate lesions to DNA and oral cells of electronic cigarette users. Vaping pod users presented significantly lower levels of these damages (median 3.3 per 10^7 nucleotides) in comparison with conventional smokers (5.7). These paradoxical data were associated with the bactericidal action of propylene glycol on the oral microbiota, suggesting a possible reduction in inflammation. However, the authors warn that this reduction does not imply, in itself, lower genotoxic risk, since other DNA-damage mechanisms could be present.

At a transcriptomic level, Tommasi et al.[15] demonstrated that regular users of electronic cigarettes presented over a thousand differentially expressed transcripts in the oral mucosa cells. Although the number was lower than that of conventional smokers, the proportion of regulatory non-codifying RNAs was higher among the *vapers*, with a potential to alter pathways like Wnt/Ca^{2+} and Rho family GTPases — both related to carcinogenesis.

Regarding cytotoxicity, which refers to the ability of a substance or agent to cause cell damage or death[17], this review observed direct and predominant effects of cytotoxicity by electronic cigarettes in three articles,

which evidenced reductions in cellular viability through different trials.

In the study by Omaiye et al.[18], the drop in cellular viability was analyzed through the classic MTT assay, a colorimetric test that works by reducing the MTT compound through mitochondrial dehydrogenases, forming purple formazan crystals; thus, the amount of coloring is directly proportional to the number of viable cells. The authors observed a decrease in the formation of formazan, therefore, a reduction in mitochondrial and cellular activity. As a complement, the NRU assay was conducted, which assesses the capacity of living and metabolically active cells to incorporate and retain the NR neutral red coloring in their lysosomes. Reduced absorption was found, which prompted the conclusion that there was also a decrease in the integrity of the lysosomal membrane, therefore cellular damage**.**

Complementing these findings, two studies have analyzed tissue lesion biomarkers, observing a significant increase in lactate dehydrogenase (LDH) under the use of electronic cigarettes[18,19]. Pandarathodiyil et al.[19] analyzed the increase in LDH levels in the users' saliva, confirming the cytotoxic and harmful effects of electronic cigarettes in the oral cavity and mucosa.

In addition to these findings, Yu et al.[20] observed that cell survival was affected by the use of electronic cigarettes regardless of the nicotine amount involved and concluded on a significant reduction in cellular viability and clonogenic survival of HaCaT (immortalized human epidermal keratinocyte lineage), UMSCC10B (squamous cell carcinoma cell line of the head and neck derived from metastatic lymph node), and HN30 (squamous cell carcinoma cell line of the head and neck derived from a primary laryngeal tumor) cells, increasing the necrosis and apoptosis rate, in addition to the cellular DNA damage due to the increase in the length of the comet tail, a biomarker used to detect double-strand breaks in DNA.

Finally, illustrating the molecular effects with great clinical association, Manyanga et al.[16] verified that oral cancer cells exposed to electronic cigarette aerosols presented resistance to the action of cisplatin, a widely used drug in the treatment of oral cavity cancers. The analyzed mechanism found alterations in the expression of drug influx and efflux transporters (like CTR1, ATP7A, and ABCG2) and does not rely on the presence of nicotine, which reinforces the contribution of other compounds present in the aerosols of these electronic devices.

Therefore, the results from these articles demonstrate through different means that the use of electronic cigarettes produces genotoxic and cytotoxic effects, resulting in evident damage to DNA (increase in the length of the comet tail and accumulation of γ-H2AX hotspots, increase in DNA strand breaks, oxidative stress, formation of micronuclei) and decrease in cellular viability by the following parameters: inhibitory concentration of 70% (CI 70), LDH increase, increased necrosis rates, as well as the aforementioned increase in the length of the comet tail an accumulation of γ-H2AX hotspots, that is, increase in DNA strand breaks.

The eight articles with predominance of molecular analyses, the main cells utilized in the *in vitro* or *in vivo* studies, the genotoxic pathways analyzed, as well as the assays and findings directly associated with cytotoxicity of this sample, were summarized in Chart 2.

Gradually, considering a carcinogenic progression post initiation and promotion, one of the contexts in which the association between electronic cigarettes and head and neck cancer has been investigated is inflammation — a process already known as a risk condition for carcinogenesis. Literary evidence at cellular and clinical levels pointed to seven studies that partially elucidate this correlation.

In vitro studies, such as that by Tsai et al.[21], observed that electronic cigarette liquids, even without vaporization, already increase the expression of RAGE receptor — a central actor in chronic inflammation associated with the progression of several types of cancer. This process is enhanced with the introduction of nicotine present in most devices. Additionally, another inflammatory marker analyzed in the study, IL-1α, shows that the chemical components present in these liquids are biologically active.

Reinforcing this activation of inflammatory pathways, Robin H. et al.[22] demonstrated that the vapor generated from electronic cigarette liquids increases the expression of TNF-α (tumor necrosis factor alpha) and proliferative pathways, such as extracellular signal-regulated kinases (ERK).

In the same direction, Lima et al.[23] demonstrated that the e-liquid is capable of inducing important phenotypic alterations in oral epithelial cells, including an increase in proliferation, migration, and invasiveness, in addition to activation of markers of the epithelial-mesenchymal transition (EMT), like the reduction of E-cadherin and increase of vimentin and β-catenin — classical and central processes in tumoral progression. Such alterations have been observed even in pre-malignant and malignant cellular lineages, reinforcing the role of electronic cigarettes not only in the initiation, but also in the acceleration of progression of pre-existing lesions.

In a complementary way, Ganesan et al.[24] demonstrated that the exposure of human epithelial cells to aerosols derived from electronic cigarettes containing propylene glycol, glycerol, and nicotine resulted in a significant

Chart 2. Studies that analyzed genotoxicity and cytotoxicity resulting from exposure to e-cigarette components

Article/Type of study	Sample	Assay	Regulatory genes	Cellular signaling pathways analyzed	Genotoxic alterations	Cellular viability	Study conclusions
Hamad et al.[12] *In vivo* study	Internal cheek epithelium of vaping pod users	Ingenuity Pathway Analysis and RT-PCR	TP53, MAP2K6, and MAPK12	Cellular cycle, cancer pathways, and MAPK pathways	—	—	Electronic cigarette inhalations significantly alter the expression of TP53
Tellez et al.[13] *In vitro* study	MOE1A, MOE1B, and MSK-LEUK1	NRU, ROS-Glo, TBAR, micronuclei, and comet	hTERT, CDK4R2C, cyclin D1, and P53C234	Proliferative pathways	—	—	E-liquids caused ≥20% cellular toxicity, induced significant levels of oxidative stress, and micronuclei formation up to 5 times greater
Guo et al.[14] *In vivo* study	Oral cells of vaping pod users, conventional smokers, and non-smokers	Quantification of AP sites	Unspecified	Proliferative pathways	—	—	Levels of AP sites in electronic cigarette users were significantly lower than in smokers and non-smokers. Propylene glycol, present in the vapor of electronic cigarettes, can inhibit the inflammation induced by bacteria in the oral cavity
Tommasi et al.[15] *In vivo* study	Oral cells of vaping pod users and tobacco smokers	RNA-seq and differential expression analysis	NOTCH1 and HERC2	Protein ubiquination, AhR signaling, tRNA loading, GTPases signaling, and Wnt/Ca^{2+}	—	—	A deregulation of critically important genes and associated molecular pathways in the oral epithelium of electronic cigarette users was identified
Manyanga et al.[16] *In vitro* study	UM-SCC-1, WSU-HN6, and WSU-HN30	MTT, clonogenicity, apoptosis, and RT-qPCR	XPA, MMS19, and ERCC1	Proliferative pathways	—	—	Oxidative stress and metalloproteins
Omaiye et al.[18] *In vitro* study	BEAS-2B (human bronchial epithelium)	Chemical analysis of e-liquids by GC-MS, with quantification of nicotine and flavorings, associated with *in vitro* exposure of cells to the pods' content and cytotoxicity assessment by MTT, neutral red uptake, and LDH release	—	—	Not directly assessed, but inferred by LDH release and cellular damage	MTT, NRU, LDH Reduced cellular viability, acute cytotoxicity in every JUUL pods	The concentrations of nicotine and some flavoring chemical substances in electronic cigarettes are cytotoxic. Cancer cells exposed to e-cigarette aerosol extracts and treated with cisplatin presented a significant decrease in cellular death, increased viability, increased clonogenic survival, and increased CI 50
Pandarathodiyil et al.[19] *In vivo* study	Saliva of vaping pod users, conventional smokers, and non-smokers	Comparative study with collection of total non-stimulated saliva and measurement of salivary LDH activity through colorimetric assay, with later statistical comparison between groups	—	—	—	Higher LDH levels in the saliva of vaping pod users when compared to non-smokers	Cytotoxic and harmful effects of electronic cigarettes on the oral mucosa based on the LDH level increase
Yu et al. (2015)[20] *In vitro* study	HaCaT, UMSCC10B, and HN30	Exposure of cellular cultures to electronic cigarette vapor extract with and without nicotine, followed by assessment of cytotoxicity and cellular death by Annexin V, *trypan blue*, and clonogenic assay, in addition to DNA damage analysis by neutral comet assay and immunomarking for γ-H2AX	—	—	Increase in DNA breaks (comet assay, γ-H2AX)	Reduced cellular viability and survival, and increased necrosis/ apoptosis	The vapor of electronic cigarettes is cytotoxic for lineages of epithelial cells and induces breaks to the DNA chain

Captions: UM-SCC-1, WSU-HN6, and WSU-HN30: Cell lineages of human head and neck squamous cell carcinoma; Via MAPK: mitogen-activated protein; NOTCH1 and HERC2: Tumor suppressor genes; oxidative stress and metalloprotein: interfere with cisplatin detoxification and transport; MOE1A and MOE1B: Oral epithelial cells lineage; MSK-LEUK1: Lineage established from a leukoplakia dysplastic lesion adjacent to a squamous cell carcinoma of the tongue; CI 50: inhibitory concentration of 50%; LDH: lactate dehydrogenase.

increase in the production of IL-6, IL-8, and reactive oxygen species (ROS), indicating an acute inflammatory response. This sustained inflammation and oxidative stress are intimately connected to the activation of oncogenic pathways, such as Nuclear Factor kappa B (NF-κB) and Signal Transducer and Transcription Activator 3 (STAT3), in addition to promoting alterations in the tumoral microenvironment that favor angiogenesis, immune evasion, and resistance to programmed cellular death.

Added to these processes, Escobar et al.[25] verified that the inflammatory response is not limited to the local damage to the epithelium, since data obtained from the analysis of gum fluid and saliva of electronic cigarette users also showed significantly higher levels of interleukins (IL6, IL8, IL10, TNFα, IFNγ, and GMCSF) in comparison with non-smokers. Such immunologic alterations reflect the systemic and persistent action of vaping pods that contribute to the establishment of a pro-tumoral microenvironment.

The main findings and inflammatory pathways analyzed in this review are summarized in Chart 3.

Connecting these inflammatory findings with the clinic, the present sample highlights the study by Bardellini et al.[26], conducted with 90 patients — 45 former conventional smokers and 45 electronic cigarette users, for at least six months. These authors demonstrated that, despite no greater global prevalence of oral mucosa lesions (OML) being observed among electronic cigarette users in comparison with former conventional smokers, there was evidence of three types of inflammatory lesions, with an increase in their occurrence: nicotinic stomatitis, hairy tongue, and angular cheilitis. These findings raise concerns, given that these lesions are rare in a young population such as vaping pod users, and suggest that, rather than simply representing a simple parallel with traditional smoking, electronic cigarettes may be associated with a more complex and aggressive mechanism, considering the components present in e-liquids — such as propylene glycol, glycerol, flavorings, and alternative forms of nicotine — not to mention the metallic components of the device itself; capable of provoking cellular responses distinct from those caused by conventional cigarette smoke.

A summary of the pathways found in the articles from this review and the carcinogenic correlation is represented in Figure 2.

DISCUSSION

This literature review was able to find and delineate some biological effects in experimental and observational studies of head and neck cells, and direct exposure regions of the oral mucosa. To deepen the discussion about this carcinogenic evidence, we chose to group genotoxic and cytotoxic findings as molecular carcinogenic evidence and inflammatory events as clinical signs of progression.

The article by Guo et al.[14] was the only controversy found in this review, since the analysis of AP sites in the DNA of vaping pod users presented lower rates when compared to conventional cigarettes; however, the authors themselves highlighted that this isolated data does not exclude the presence of other genotoxic mechanisms in action. Thus, Hamad et al.[12] confirmed and reinforced the complexity of the damage when demonstrating significant changes in the expression of genes involved in DNA repair and cellular apoptosis, like TP53 and MPG, after acute exposure to electronic cigarette aerosol. These findings support the idea that vaping pods can trigger relevant molecular imbalance even in the short term. Corroborating this perspective of multiple pathways, Al-Otaibi et al.[27] observed that some electronic cigarette liquids expressed mutagenic activity after simulated metabolic activation (fraction S9), showing that non-initially toxic components can become mutagenic after liver biotransformation.

Complementing this mutagenic evidence through *in vivo* studies, the experiments conducted by Tellez et al.[13] with oral epithelial cells demonstrated that several e-liquids lead to the formation of micronuclei and oxidative stress. The presence of these damages, especially with specific flavorings, suggests that the aromatic additives used in vaping pods are significant contributors to cellular damage, contributing to the carcinogenic microenvironment. In this sense, a recent systematic review[12] demonstrated that the smoke of hookahs also induces genotoxicity in human cells, with positive results in micronuclei assays, comet assay, and oxidative stress markers, reinforcing that different alternative forms of tobacco intake share common deleterious mechanisms, although it is not yet possible to suggest exclusive pathways.

Tommasi et al.[15] and Manyanga et al.[16] suggested that electronic cigarettes interfere with molecular pathways crucial to cellular control, promoting deregulated proliferation, apoptosis evasion, and even resistance to chemotherapy drugs. Tommasi et al.[15] found the dysregulation of genes and non-coding RNA associated with tumor progression, while Manyanga et al.[16] demonstrated the reduced efficacy of cisplatin in oral cancer cell lines due to the modulation of cellular transporters. Consolidating these findings, the review by Jitareanu et al.[28] reinforces that electronic cigarettes induce oxidative stress, DNA damage, and chronic inflammation to the oral mucosa — conditions that favor tumoral microenvironment. Moreover, the

Chart 3. Studies that analyzed inflammatory activity and neoplastic progression

Article/Type of study	Sample	Analyses/Assay	Associated inflammatory mediators	Observed progression markers	Observed lesion type
Pandarathodiyil et al.[19] *In vivo* study	Saliva of vaping pod users, conventional smokers, and non-smokers	Compares LDH and pH in the saliva of smokers, vapers, and non-users	Salivary LDH levels of groups of smokers and vapers were higher than in the control group (p>0.01). There were no differences in the salivary LDH between vapers and conventional smokers (p=0.234)	—	Not recorded
Tsai et al.[21] *In vitro* study	Ca9-22 and CAL-27	Assesses cellular invasion, inflammation, and RAGE protein, comparing the control group (with no exposure to the e-liquid) with the exposure group	Green Apple: Increased IL-1α and IL-8. With nicotine, a lower increase. Red Hot: IL-1α increased 580 times; with nicotine, 28 times. IL-8 rose 2.2 times but was reduced by 3.3 times with nicotine. RAGE increase in cells	MMP-13 decreased (Green Apple)	Not recorded
Robin et al.[22] *In vitro* study	CA9-22 (oral squamous carcinoma) and CAL27 (tongue squamous carcinoma)	Exposure of carcinoma cells to e-liquids with different flavorings/nicotine, followed by cellular invasion assessment, analysis of inflammatory mediators, and associated proteins by western blot	Ca9-22 cells: NF-κB and ERK increased with Apple EVE and nicotine. Unchanged with Red Hot EVE. JNK and TNF-α increased in all of them. Cal27 cells: NF-κB and ERK decreased with Apple EVE and with Red Hot EVE. JNK: Decreased with Apple EVE and nicotine but increased with Red Hot EVE and nicotine. TNF-α: Increased with Apple EVE and with Red Hot EVE in the presence of nicotine	In Ca9-22: MMP-9 decreased with and without nicotine in Red Hot EVE. MMP-13 only increased with Red Hot EVE with nicotine. Cal27 cells: MMP-9 was not altered and MMP-13 decreased with Red Hot EVE alone and increased with nicotine	Not recorded
Lima et al.[23] *In vitro* study	Normal tongue cells and squamous cell carcinoma of the tongue	Exposure to e-liquid in concentrations and in vitro assessment by HPLC, viability/ cytotoxicity assays, and analysis of EMT markers by immunocytochemistry, protein expression, and qRT-PCR	IL-6, IL-8, TNF-alfa	Reduction of E-cadherin, increase in vimentin and β-catenin	Not recorded
Ganesan et al.[24] *In vivo* study	Subgingival plaque on teeth and gingival crevicular fluid (GCF)	Compared the inflammatory profile of 5 groups: smokers, non-smokers, electronic cigarette users, former smokers who use vaping pods, and users of both	Interleukins (IL-2, IL-4, IL-6, IL-8, IL-10), IFN-γ, GM-CSF, and TNF-α.	—	Not recorded
Escobar et al. (2021[25]**)** *In vitro* study	Flushing of superficial nasal scraping biopsies (hNECS) from smokers and non-smokers	Statistical analysis after dosage of mucin and cytokines with previous exposure of cells to e-cig components.	Propylene glycol aerosol: glycerol: increased IL-1β, IL-15, and IL-10, IFN-γ and IL-4; Nicotine salt: increased IL-12p70, IL-1b, IL-2, IL-4, IL-8; Freebase nicotine: increased IL-12p70, IL-7, IL-8, VEGF. Increased VEGF and decreased GM-CSF.	Glycerol aerosol: Increased MUC5AC. Freebase nicotine: Increased MUC5AC and MUC5B	Not recorded
Bardellini et al.[26] *In vivo* study	Two groups: former smokers and e-cig users	Each patient was examined to detect possible oral lesions, and, if necessary, a swab or biopsy of the lesion was collected	—	—	Inflammatory: nicotine stomatitis, hyperplastic candidiasis, median rhomboid glossitis, and erythematous candidiasis. Reactive: melanosis, hairy tongue, hyperkeratosis. Pre-malignant: lichen planus. Malignant: squamous cell carcinoma

Captions: MUC5AC and MUC5B: secretory mucins that make up the mucus of the airways, acting in epithelial protection and signaling inflammatory changes and dysfunctions of the mucosal barrier; MCP-1, MIP-1β, and TARC: chemokines involved in the regulation of the immune and inflammatory response; GM-CSF: granulocyte-macrophage colony-stimulating factor; MMP-9 and MMP-13: enzymes from the metalloproteinase family that degrade components of the extracellular matrix, promoting cell invasion and tumor metastasis; LDH: lactate dehydrogenase; EMT = epithelial-mesenchymal transition.

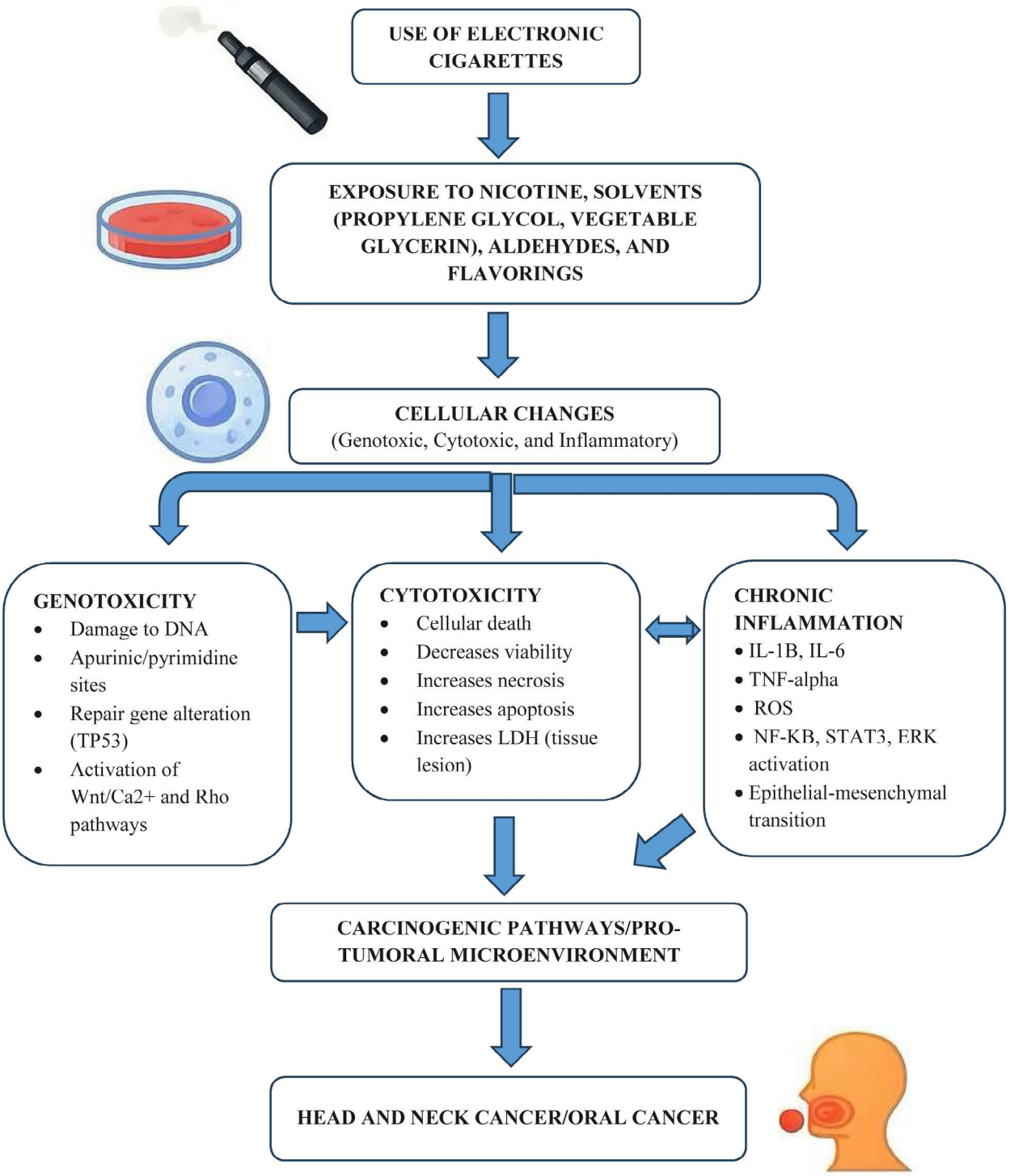

Figure 2. Carcinogenic pathways identified in the *in vivo* and *in vitro* studies in this review
Captions: ROS: oxygen reactive species, ERK: signal-regulated extracellular kinases, LDH: lactate dehydrogenase.

activation of nicotinic acetylcholine receptors (nAChR) promotes apoptosis inhibition, exacerbated cellular proliferation, and immune response evasion, which directly contributes to oral cancer progression and reduced therapeutic efficacy. This data supports the view that vaping pods exert biological effects beyond direct exposure, favoring oncogenesis.

The analysis of results on cytotoxicity corroborates the growing evidence that electronic cigarettes are not harmless, given their effects on decreased cellular viability. The studies by Omaiye et al.[18] and Pandarathodiyil et al.[19] demonstrated significant tissue damage, indicated by the increase in LDH levels. Additionally, it was suggested that the damage is not restricted to a single pathway, given

that electronic cigarette exposure compromised both mitochondrial metabolic activity and the integrity of the cellular membrane. Anda et al.[29] reinforce these findings, as they observed that the aerosols from electronic cigarettes induce a dose-dependent reduction of cellular viability in oral epithelium, regardless of the nicotine amount, in addition to causing oxidative stress and double-strand DNA break, contributing to the observed cytotoxicity.

Dose-dependence reinforces a point seen by Yu et al.[20] that cytotoxicity occurs regardless of the nicotine amount present in most electronic devices. Therefore, the other aerosol components might be the main toxic agents related to electronic cigarettes, which challenges the popular perception that the danger of these devices would be solely in nicotine, reinforcing the idea that the initial "safety" of the device fails. In agreement, Silva et al.[30] approached the different and new ways of nicotine presentation that may be dissolved in several compounds, like propylene glycol, glycerol, and benzoic acid, which alter the concentration rates of nicotine, as well as alter absorption rates and irritate the mucosa.

Finally, justifying the findings of increased ROS concentration in some studies, Jelic et al.[31] suggested that high levels of ROS are essential to cellular proliferation, survival, and adaptation, fundamental processes in carcinogenesis. ROS can induce cellular senescence mediated by p53, leading to p21 expression, which can impact treatment response and tumoral resistance. Therefore, in addition to reproducing the pro-inflammatory role observed in the other discussed mediators, ROS present a capacity to modulate treatment response, contributing to both therapeutic resistance and tumoral adaptation in chronic aggression contexts, like that induced by the use of electronic cigarettes.

Together, the *in vitro, in vivo*, and observational exposure studies show evidence and suggest that the use of electronic cigarettes is not free of genotoxicity and cytotoxicity risks, and the deleterious effects in head and neck mucosa are multifactorial, involving from epigenetic alterations to impacts in pharmacology efficacy. These findings signal that, despite being often promoted as "less harmful" alternatives to conventional cigarettes, vaping pods induce potentially carcinogenic cellular and molecular changes. The variability in devices, the utilized liquids, and use standards reinforces the need for further studies with larger samples and methodological standardization.

As to the findings referring to inflammation, these reveal special importance in the increase of RAGE expression observed in electronic cigarette smokers. Li et al.[32] demonstrated that the ISG15–RAGE axis promotes NF-κB/STAT3 activation, intensifying inflammation and tumoral progression, especially in head and neck squamous cell carcinoma.

Connecting this same pathway and receptor, Plemmenos et al.[33] demonstrated how RAGE, activated by HMGB1 binder, triggers the NF-κB pathway, resulting in a reduction of E-cadherin and an increase in vimentin, changes characteristic of EMT. Such correlation is essential, given that it represents a key event in tumoral progression by favoring more invasiveness, metastatic capacity, and resistance to therapies in head and neck squamous cell carcinoma. Thus, RAGE overexpression can be interpreted not only as a vaping pod-induced inflammation marker but also as a potential mediator of a malignant phenotype progression.

Underscoring this progression associated with EMT, Liu et al.[32] demonstrated that macrophages ($SPP1^+$) from the tumoral microenvironment secrete NF-α, activating the NF-κB pathway and promoting proliferation and expression of osteopontine (OPN) — markers associated with invasion and metastasis. The mechanism suggests that TNF-α increase is not limited to a local inflammatory phenomenon, but works as a functional mediator, reinforcing the creation of a pro-tumoral microenvironment by favoring events such as EMT, stroma remodeling, and potential dissemination of malignant cells[34].

Parallel to EMT, most articles in the sample observed an expressive increase of several interleukins, with IL-6 and IL-8 being recurring. From this perspective, Xu et al.[35] demonstrated that a high expression of IL-8 is associated with cellular proliferation, migration, and invasion both *in vitro* and *in vivo*. This effect is mediated by the activation of receptors CXCR1 and CXCR2 (chemokine receptors involved in pro-inflammatory signaling and cell migration), which triggers the signaling pathway STAT3, inactivating PTEN (tumor suppressor protein) and activating metalloproteinases (MMP2, MMP9), vimentin, and Snail — classic EMT markers.

The inhibition of this pathway with CXCR1/2 antagonists or STAT3 inhibitors would block these effects, suggesting that IL-8 acts as a functional mediator for tumoral progression. In addition, according to Španko et al.[36], the persistent activation of the signaling pathway measured by IL-6 contributes to a chronic inflammatory state that favors both tumor initiation and maintenance. Together, the increase in IL-6 and IL-8 observed in this study reinforces the role of these cytokines as key modulators of the tumoral microenvironment in head and neck cancers, with the exacerbated production of these interleukins creating a pro-inflammatory and favorable setting for the development of neoplasms, highlighting their potential as therapeutic targets and prognosis biomarkers[37].

Some of these hypotheses have been recently corroborated in the study by Pérez-Jardón et al.[38] which, in a paired sample of electronic cigarette users, was able to analyze clinical parameters (presence and absence of lesions), cytological alterations (through exfoliative cytology and pap smear coloration) and the expression of genes *p16*, *IL1-beta*, *CXCL8*, *TNF*, and *KRT13*, important for inflammation, cellular cycle regulation, and epithelial differentiation. Despite the results not being fully conclusive, the authors delineated a possible clinical study model and observed the consistency of cytological changes and molecular suppression of markers such as p16 and TNF, which pointed to a potentially deleterious effect of electronic cigarettes on oral health.

CONCLUSION

This review demonstrated that the use of electronic cigarettes provokes expressive effects in head and neck cells, especially in oral cavity cells, manifested by genotoxic, cytotoxic, and inflammatory changes. It was possible to observe a drop in cellular viability, DNA damage, and activation of inflammatory processes, which participate in tumoral initiation and progression. The results also highlight that these effects are not exclusively dependent on nicotine, but include the action of solvents, flavorings, and other components present in the aerosol, in addition to the likely association with device voltage and temperature, which reinforces the complexity of the mechanisms involved and impairs the search for exclusive signaling pathways and tissue damage.

In the genotoxic scope, studies show alterations to DNA integrity, formation of apurinic/pyrimidine sites, differential expression of genes that regulate the cell cycle and repair (like TP53 and MPG), and modulation of pathways associated with carcinogenesis, like Wnt/Ca^{2+} and RHO family GTPases. Such alterations configure real oncogenic potential, especially under prolonged use.

Regarding cytotoxicity, results from different assays (MTT, NRU, LDH, and Annexin V) showed a significant drop in cellular viability, increased necrosis, and increased LDH levels, indicating direct tissue lesion and reduced repair capacity. Notably, such effects occurred even in the absence of nicotine, highlighting the toxic role of solvents and flavorings present in e-liquids.

Furthermore, despite the scarcity of controlled studies, which limit these results, it was demonstrated that the inflammatory component emerges as a key axis in tumoral progression associated with electronic cigarettes. Exposure to vaping pod vapors stimulates the production of pro-inflammatory cytokines (IL-1α, IL-6, IL-8, TNF-α), increases ROS liberation, and activates oncogenic pathways like NF-κB, STAT3, and ERK. This persistent inflammatory condition was able to establish inflammatory oral lesions and sustain tumor progression through phenotypic alterations of greater migration, invasiveness, and activation of the epithelial-mesenchymal transition (reduction of E-cadherin and increase in vimentin and β-catenin).

Together, these mechanisms configure a favorable microenvironment for neoplastic initiation, promotion, and progression. Therefore, the use of electronic cigarettes must not be seen as a safe alternative to traditional smoking, but as an emerging new risk factor for head and neck cancer and, more specifically, oral cancer, requiring more studies, strong sanitary regulations, and prevention and health education public policies.

Longitudinal clinical studies that enable us to measure the magnitude of damage and clarify all the biological mechanisms involved are essential. Such evidence would support prevention strategies, strengthen public policies, and guide the population on the risks associated with the use of electronic cigarettes.

CONTRIBUTIONS

Thiago Queiroz Moreira and Maria Isabel Domingo Santos have substantially contributed to the conception and planning of the study, and in data collection, analysis, and interpretation. Beatriz Reberte Miyagui contributed to the design and planning of the study, and wording. Isabela Hatisuka de Carvalho contributed to the design and planning of the study; in data interpretation; and wording. Isabela Maria Lisboa da Silva contributed to data interpretation and research; and wording. Nayara Bezerra de Assis contributed to the study design and planning; and research data collection. Fernanda Salgueiredo Giudice, Janaina Pereira Dina Torelli, and Veronica Quispe Yujra contributed to the study design and planning; and to critical review with intellectual contribution. All the authors approved the final version for publication.

DECLARATION OF ARTIFICIAL INTELLIGENCE USE

The authors used ChatGPT (OpenAI, version GPT-5.3) in this article only to support the initial data organization, draft illustrations, and review language. The analysis, interpretation, or synthesis of results is the sole responsibility of the authors, with no artificial intelligence intervention.

DECLARATION OF CONFLICT OF INTERESTS

There is no conflict of interest to declare.

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