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PII: S2212-4403(26)00044-1
DOI: https://doi.org/10.1016/j.oooo.2026.01.018
Reference: OOOO 5673

To appear in: *Oral Surg Oral Med Oral Pathol Oral Radiol*

Received date: 12 September 2025
Revised date: 25 January 2026
Accepted date: 25 January 2026

Please cite this article as: Reuber Mendes Rocha DDS, M.Sc , Felipe Mesquita Araújo DDS , Felipe Assis Mercadante DDS , Alana Castilho de Souza DDS , Túlio Eduardo Nogueira DDS, M.Sc, Ph.D , Daniela de Melo e Silva M.Sc, Ph.D , Fernanda Paula Yamamoto Silva DDS, M.Sc, Ph.D , Brunno Santos Freitas Silva DDS, M.Sc, Ph.D. , Genotoxicity in Oral Mucosal Cells Associated with E-Cigarette Use: A Systematic Review and Meta-Analysis, *Oral Surg Oral Med Oral Pathol Oral Radiol* (2026), doi: https://doi.org/10.1016/j.oooo.2026.01.018

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Conflict of interest: none

Word Count (Abstract): 200

Word Count (Manuscript): 4.321

Number of references: 43

Number of figures: 04

Number of tables: 01

Appendix: 04

Funding: This study was partially financed by the Goiás Research Foundation – FAPEG - Brazil grant – [AUX2024361000137].

Abstract

Objectives: To evaluate the effects of electronic smoking devices (e-cigarettes) on the genotoxicity of oral mucosal cells compared with conventional cigarette smokers and non-smokers, and to critically assess the methodological frameworks generating this evidence. **Study Design:** Searches were conducted up to July 2025 in PubMed, EMBASE, Scopus, Web of Science, CINAHL, Cochrane Library, LILACS, and grey literature. Eligible studies were observational designs assessing genotoxic damage in oral mucosal cells of adult e-cigarette users through the buccal micronucleus cytome assay (BMNcyt). Data extraction and risk of bias assessment (JBI checklists) were performed independently by two reviewers. Meta-analyses used random-effects models. **Results:** Nine cross-sectional studies (n=711) were included. Meta-analysis showed that e-cigarette users had significantly lower micronucleus (MN) frequency than smokers (mean difference: 2.75; 95% CI: 0.02–5.47; p=0.048), but no significant difference compared with non-smokers. Subgroup analysis indicated higher MN frequencies in e-cigarette users versus non-smokers when ≥1,000 cells were analyzed per sample. Risk of bias ranged from low to high, and the certainty of the pooled evidence was rated as “very low”. **Conclusions:** E-cigarettes induce less genotoxicity than conventional smoking but may increase DNA damage compared with non-smokers under standardized BMNcyt protocols. Stronger prospective studies with methodological rigor are needed.

Introduction

Cigarette smoking represents a major public health concern, accounting for the deaths of more than 7 million people annually worldwide and causing the death of up to half of its users who do not quit.[1] Therefore, smoking cessation is strongly associated with substantial health benefits. Numerous interventions have been investigated to support individuals in quitting smoking.[2] One such intervention that has gained prominence in recent years is the use of electronic nicotine delivery systems (ENDS), commonly known as e-cigarettes, which deliver nicotine through liquid vaporization.[3] While nicotine is a primary factor in tobacco dependence, other behavioral and ritualistic elements also contribute to the addiction associated with conventional cigarette smoking.[4]

E-cigarettes provide both nicotine and sensory cues, which may make them a potentially effective tool for smoking cessation by delivering nicotine in a non-combustible manner. Indeed, there is evidence suggesting that nicotine-containing e-cigarettes can increase smoking quit rates.[4] However, concerns remain regarding their safety, as emerging evidence indicates that their use may lead to various health issues[5-8], including the potential for increasing the risk of certain types of cancer[9], thereby raising questions about their suitability as an alternative to combustible cigarettes.

With respect to oral health, e-cigarettes appear to affect periodontal health by increasing plaque levels among users[10], raising the incidence of dental caries[11], disturbing the oral microbiome[12], causing oral dryness[13], triggering aphthous ulcers[13], and inducing genotoxic alterations in oral mucosal cells.[14] Evidence from prospective studies remains insufficient to establish e-cigarette use as a definitive risk factor for oral cancer.[15] Nevertheless, potentially carcinogenic substances have been identified in e-liquid aerosols, including formaldehyde, acetaldehyde, acrolein, and certain nitrosamine compounds .[16] In addition, some studies have detected heavy metals such as nickel and chromium.[16] These findings, combined with previously reported genotoxic alterations in oral mucosal cells associated with e-cigarette use[14], do not rule out the possibility that these devices may contribute to an increased risk of oral cancer development. Therefore, further research is warranted.

One widely used parameter to investigate potential genotoxic damage and carcinogenic risk in oral mucosal cells is the buccal micronucleus cytome assay (BMNcyt).[17] The BMNcyt is a quantitative method for assessing DNA damage through the counting of micronuclei (MN) in exfoliated oral mucosal cells.[17] It is a low-cost, painless, and non-invasive technique with strong potential for biomonitoring large populations[18], including e-cigarette users. Indeed, it is currently employed to investigate the potential harmful effects of e-cigarettes on oral mucosal cells.[14,19-22] Although some studies have observed negative effects through MN counts, findings are not entirely consistent regarding this potential risk. [14,19-22]

A recent meta-analysis[17] synthesized evidence on the occurrence of MN in oral mucosal cells associated with tobacco use, alcohol consumption, and pesticide exposure, also including pooled data on e-cigarettes. For e-cigarette use, measured by the frequency of MN, significantly higher values were reported compared with control groups. However, the review addressed a non-focused research question, hindering the interpretation and dissemination of its findings. Additionally, their search was restricted to studies available up to 2023. An updated review is warranted, as our preliminary search identified additional primary studies published after the cited systematic review. Moreover, technical parameters in MN counting, such as staining methods and the number of cells counted per sample, and other methodological aspects, including sample size and control of confounding variables[23], should be considered when evaluating current evidence on the harmful effects of e-cigarettes on oral mucosal cells.

The present systematic review was therefore conducted to address the focused research question: “What are the effects of using electronic smoking devices on the genotoxicity of oral mucosal cells compared with conventional cigarette smokers and non-smokers?” Additionally, it aims to examine the methodological framework in which this evidence has been generated.

Material and methods

Protocol and registration

This systematic review was reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020

guidelines.[24] The review protocol was developed following PRISMA-P recommendations and registered in the Prospective Register of Systematic Reviews (PROSPERO) under registration number CRD420251130013.

Eligibility criteria

The eligibility (inclusion and exclusion) criteria adopted in this systematic review were structured according to the acronym PECOS (Population, Exposure, Comparison, Outcome, and Study design):

P - Participants: Adults (>18 years).

E – Exposure: Use of Electronic Smoking Device (e-cigarette)

C - Comparison: Non-users of electronic smoking devices or users of combustible cigarettes.

O - Outcome: Quantitative analysis of genotoxic damage using the micronucleus cytome assay or comet assay from exfoliated oral mucosal cells.

S – Study design: Observational cross-sectional and cohort studies.

This review included observational studies that evaluated the presence of MN in oral mucosal cells of adults (>18 years) who had used e-cigarettes for at least four months, with MN detection performed using the BMNcyt.

The studies were excluded of the present review if they were based on: (1) Individuals with systemic decompensation, a history of squamous cell carcinoma (SCC), or previous cancer treatment (including surgery, radiotherapy, or chemotherapy) in any organ or system; (2) Pediatric patients, animals, in vitro models, or ex vivo models; (3) Use of antibiotics or anti-inflammatory drugs within the past 30 days; (4) Electronic smoking device use for less than four months; (5) Concomitant use of other tobacco products, including hand-rolled cigarettes, country-style cigarettes, cigars, or pipes; (6) Absence of a comparator group; (7) Genotoxicity testing performed on cells from anatomical sites other than the oral cavity; (8) Assessment of genotoxicity using methods other than the micronucleus cytome assay; (9) Studies in which the data cannot be used to assess the association between electronic smoking device use and genotoxicity in oral mucosal cells; (10) Studies involving individuals inherently predisposed to DNA damage, such as those with congenital immunodeficiencies, or acquired immune deficiency syndrome

(AIDS); (11) Reviews, letters, personal opinions, book chapters, conference abstracts, case reports, and case series; (12) Duplicate publication.

Information sources and search strategy

All searches were performed up to July 2025. The detailed electronic strategies with specific word combinations and truncations were elaborated with support of a Health Sciences librarian for 7 main databases (PubMed (Medline), EMBASE, Scopus, Web of Science, CINAHL (via EBSCO), Cochrane Library, and Latin American and Caribbean Health Sciences (LILACS) (via BVS) (Appendix 1). Additional searches in the grey literature (ProQuest Dissertations & Theses Citation Index and Google Scholar) were performed, restricted to the first 100 results. No restrictions regarding language and date of publication were applied. To identify the studies in the databases, terms referring to “electronic cigarette”, “cell damage”, “micronucleus”, and “oral cells” were used selected from the Health Sciences Descriptors (DeCS), Medical Subject Headings (Mesh), and Embase controlled vocabulary (EMTREE). Free terms were also selected form the previous published literature. Alerts for newly published articles containing the combination of the selected terms were set up. An additional hand search in the reference list of the selected articles was performed to prevent missing studies from the platform searches. Two experts in the theme were reached to prevent missing studies from the platform searches.

Selection process

After retrieving references from each electronic database, they were stored and managed using EndNote Web (Thomson Reuters, Toronto, Canada). Duplicate records were removed in two steps: first using EndNote Web, and subsequently with the Rayyan application (Qatar Computing Research Institute, Doha, Qatar).[25] Study selection was conducted in two phases by two independent reviewers (R.M.R. and F.M.A.). A third reviewer (B.S.F.S.), with expertise in oral pathology and systematic review methodology, participated in the selection process only when consensus between the first two reviewers could not be reached. Rayyan software was employed to ensure a blinded selection process. In both phases, reviewers were calibrated on the

application of the eligibility criteria by screening the first ten articles retrieved in the search. In Phase 1, references were screened by title and abstract according to the predefined eligibility criteria. In Phase 2, the full texts of the selected articles were independently assessed by the same reviewers, applying the same criteria. A detailed description of the selection process is provided in Figure 1, and the reasons for study exclusion in Phase 2 are presented in Appendix 2.

Data collection process

Data from the selected articles were extracted independently by the two reviewers (R.M.R. and F.M.A.) involved in the study selection process and cross-checked by a third reviewer (B.S.F.S.). The collected variables included: study characteristics (first author's name, year of publication, country), study design, sample characteristics (size, sex distribution, mean age), smoking habits (daily e-cigarette use, duration of e-cigarette use, number of conventional cigarettes smoked daily, duration of conventional cigarette use), general findings (analysis of additional tissues and organs, presence of oral manifestations), oral cell analysis (cell collection technique, micronucleus frequency [mean, standard deviation], number of cells counted, staining methods), and main conclusions. The extracted data are summarized in Table 1. When essential information for quantitative analysis was missing or could not be derived from the reported values, study authors were contacted to obtain the missing data. No imputation methods were applied to handle missing data.

Risk of Bias Assessment (Methodological Quality)

The methodological quality of the included studies was independently assessed by two reviewers (R.M.R. and F.M.A.) using the Joanna Briggs Institute (JBI) Critical Appraisal Checklists.[26] Any disagreements were resolved by consultation with a third reviewer (B.S.F.S.). For cohort studies, the JBI Checklist comprises 11 items to evaluate methodological quality. For studies with a cross-sectional design, the corresponding JBI Checklist was applied, which includes 8 items. Each domain in both checklists was rated as "yes," "no," "unclear," or "not applicable," based on the guiding questions. The risk of bias was classified as "high" (<50% of items rated "yes"), "moderate" (50–69%), or

"low" (≥70%). "Not applicable" items were excluded from the denominator when calculating percentages. This scoring approach was adopted to facilitate risk of bias classification, as previously described.[27]

Effect measures

The main outcome was the association between e-cigarette use and the frequency of micronuclei (MN). This outcome was evaluated using mean differences, calculated from the sample size, mean values, and standard deviations reported in each study, along with their 95% confidence intervals.

Synthesis methods

The meta-analysis was conducted using Jamovi (version 2.6) (The jamovi project, 2025; Retrieved from https://www.jamovi.org). Standardized mean differences were used as the effect size measure, and a random-effects model was applied. Between-study heterogeneity was estimated using the restricted maximum-likelihood (REML) estimator. In addition to the estimate of τ^2 (Tau^2), Cochran's Q test and the I^2 statistic were reported. When heterogeneity was detected ($\tau^2 > 0$, regardless of Q-test results), a prediction interval for the true effect size was also provided. Separate meta-analyses were conducted for comparisons of e-cigarette users versus conventional cigarette smokers and e-cigarette users versus controls (non-users/former users).

Sensitivity analyses were performed by sequentially excluding individual studies with potential methodological biases to identify those that might overestimate or underestimate the pooled estimates. A subgroup analysis was conducted to evaluate whether the number of cells counted influenced the frequency of MN in oral mucosal cells, as recommended by the Human MicroNucleus Project.[28] Additional subgroup analyses were not feasible due to insufficient information on e-cigarette usage patterns, device types, or complete data on participants ages. The subgroup meta-analysis was carried out using OpenMeta [Analyst] software. All statistical tests were two-tailed, with a significance level of 0.05, and results were reported with 95% confidence intervals (CIs).

Bias of publication assessment

Publication bias was assessed using funnel plots, in which standardized mean differences were plotted against their standard errors to visually inspect asymmetry, indicative of potential publication bias.[29-31] In addition, reporting bias was assessed using the Fail-safe N method (Rosenthal's approach), the rank correlation test, and the regression test for funnel plot asymmetry, with a significance threshold of $p < 0.05$.

Certainty Assessment

The quality of evidence from the pooled studies was evaluated using the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) approach and classified as "high", "moderate", "low", or "very low" based on factors including risk of bias, inconsistency, indirectness, imprecision, and potential publication bias.[32]

Results

Study selection

In Phase 1, a search of seven major electronic databases, as well as the grey literature, identified 1,402 references. After removing duplicates, 1,143 references were screened by title and abstract. In Phase 2, 17 studies underwent full-text assessment. Of these, 8 were excluded for specific reasons, which are detailed in Appendix 2, leaving 9 studies for further analysis. Additional hand-searching of the reference lists of the included studies did not identify any further eligible studies. The complete study selection process is illustrated in the flow diagram in Figure 1.

Study characteristics

Of the 9 studies included in the qualitative analysis, all were cross-sectional and published between 2016 and 2024. The primary objective across studies was to evaluate the genotoxicity of e-cigarettes in buccal mucosal cells by assessing MN frequency.[14,19-22,33-36] Two studies additionally examined other non-combustible alternatives[34,35], and 1 also investigated the increase in DNA strand breaks in the blood of e-cigarette users.[33] Two studies included former smokers in their samples. One compared MN counts between former smokers,

conventional cigarette smokers, and e-cigarette users[14], while the other included former smokers as a control group for comparison with e-cigarette users.[20]

The studies were conducted in Brazil[14,36], Croatia[35], Indonesia[21], Italy[19,20], Romania[22], Saudi Arabia[33], and Ukraine.[34] Sample sizes ranged from 28 to 160 participants, totaling 711 individuals. Based on data available from 8 studies, the pooled male-to-female ratio was estimated at 1.24:1. Participant ages ranged from 15 to 80 years, with a pooled mean age of 34.9 years. Four studies primarily included young adults (≤30 years, mean age)[21,22,34,36], while 5 analyzed populations with mean ages above 30 years.[14,19,20,33,35]

Regarding the intensity of e-cigarette use, most studies did not provide sufficient or comparable data. Franco et al. (2016)[19] reported nicotine consumption estimated according to the classification of nicotine content in the charging liquid, ranging from 0.4 to 1.6 mg, whereas Menicagli, Marotta, and Serra (2020)[20] evaluated nicotine concentrations in salivary mucins, which represent indirect biomarkers rather than direct measures of consumption. Schwarzmeier et al. (2021)[14] was the only study to report daily intake in milliliters, describing heterogeneous subgroups that included three individuals consuming 4–5 mL/day, one consuming 12 mL/day, and seven consuming 5–30 mL/day, without a consolidated mean. The remaining studies did not provide information on e-cigarette intensity.[21,22,33-36] The reported duration of e-cigarette use varied between approximately 4 months and 7 years. Due to inconsistencies in reporting units, the absence of standardized measures, and insufficient data, it was not possible to calculate a reliable pooled estimate of daily e-cigarette use or the duration of e-cigarette use.

With respect to conventional cigarette use, the reported daily consumption ranged from 2 to 30 cigarettes per day across the included studies, while the duration of smoking ranged from 1 to 33 years. Data on either daily cigarette consumption or smoking duration were not provided in two studies.[20,34] Overall, the findings indicate predominantly moderate-to-heavy smoking histories; however, the absence of standardized measures and incomplete reporting limited comparability across studies.

Oral manifestations among e-cigarette users were reported only by Franco et al. (2016)[19], who identified gingivostomatitis in two patients. In

contrast, several studies did not observe oral alterations in e-cigarette users[14,20-22,33-35], while one study did not provide information on this outcome.[36]

For MN analysis, oral mucosal cells were primarily obtained from the buccal mucosa. Four studies reported that samples were collected bilaterally[19,20,22,33], whereas 3 studies described sampling from the buccal mucosa without specifying whether it was unilateral or bilateral.[21,34,35] Only 1 study included additional oral sites, collecting cells from the lateral border of the tongue and the floor of the mouth.[14]

Regarding the MN counting methodology, the number of cells evaluated varied from 100 to 2,000 across studies, with most studies counting 1,000 cells per sample.[19,21,22,33] Two studies counted more than 1,000 cells[14,35], while three studies counted fewer than 1,000 cells.[20,34,36]

The predominant staining methods were Feulgen[14,33,35], Giemsa[19,21], and Papanicolaou.[20,22] Oral mucosal cells were obtained using scraping[19,34,36], smear[14,20-22], or cytobrush techniques.[33,35] Detailed information from individual studies is provided in Table 1.

Risk of bias within studies

Among the selected cross-sectional studies, the main concerns were related to the domains “Were confounding factors identified?” and “Were strategies to deal with confounding factors stated?”. Some studies did not report alcohol consumption[20,21,34], prior use of tobacco products[20,21,34], or age restrictions[20,21,34] as potential confounding factors. Moreover, these studies did not specify how such well-established risks for increased MN frequency were addressed when evaluating their impact on MN occurrence in e-cigarette users. Overall, most studies were judged to have a “low” risk of bias, while 2 were classified as “moderate” and another 2 as “high” risk of bias. Detailed information regarding the risk of bias assessment is presented in Figure 2.

Synthesis of results

The pooled mean and standard deviation for the frequency (total count) of MN were estimated using a random-effects model. A total of eight studies were included in the quantitative analysis. Incomplete data on MN total counts in Bruschi et al. (2024)[36] precluded its inclusion in the pooled analysis. When

comparing conventional cigarette smokers with e-cigarette users, the estimated average mean difference in MN frequency was 2.7461 (95% CI: 0.0215–5.4708; Z = 1.9754, p = 0.0482) (Figure 3, A), indicating a statistically significant difference between the two groups. However, the Q-test suggested substantial heterogeneity (Q = 188.7781, p < 0.0001; Tau^2 = 15.1522; I^2 = 99.14%). Studentized residuals indicated that Al-qudaihi et al. (2023)[33] may represent a potential outlier, with values exceeding ±2.7344. Cook's distances further suggested that this study might exert undue influence on the model. Sensitivity analysis, excluding studies one at a time, did not reveal any significant effect on effect measure or heterogeneity.

For e-cigarette users compared with controls (non-users/former users), the pooled mean difference in MN frequency was -0.9725 (95% CI: -5.8677–3.9226; Z = -0.3894, p = 0.6970), indicating no statistically significant difference. Nonetheless, heterogeneity was again substantial (Q = 203.8136, p < 0.0001; Tau^2 = 49.3803; I^2 = 99.75%) (Figure 3, B). Studentized residuals once more flagged the study of Al-qudaihi et al. (2023)[33] as a potential outlier, and Cook's distances suggested excessive influence. Sensitivity analysis confirmed that this study markedly affected effect measure and heterogeneity. After its exclusion, the pooled mean difference shifted to 1.3836 (95% CI: -0.0439–2.8111; Z = 1.8996, p = 0.0575) (Figure 3, C).

A subgroup meta-analysis was conducted based on the number of cells evaluated in the micronucleus assay. Studies analyzing ≥1,000 cells showed a significant increase in the mean micronucleus frequency among e-cigarette users compared with controls (SMD = 0.551; 95% CI: 0.152–0.950; I^2 = 56.83%; p < 0.001). In contrast, studies analyzing ≤1,000 cells showed no significant difference (SMD = 3.619; 95% CI: –0.614 to 7.852; I^2 = 97.44%; p = 0.000). Overall, a non-significant trend toward higher micronucleus frequency was observed in e-cigarette users (SMD = 1.384; 95% CI: –0.044 to 2.811; I^2 = 97.09%; p = 0.000) (Figure 4).

Reporting Biases

With respect to publication bias, both the rank correlation test and the regression test indicated potential funnel plot asymmetry (p = 0.0017 and p < 0.0001, respectively). However, as this meta-analysis included only eight

studies, these tests have low statistical power; therefore, the funnel plot was not presented.

Certainty of evidence

Using the GRADE approach, evidence was evaluated for two comparisons: (1) MN frequency in conventional cigarette smokers versus e-cigarette users, and (2) MN frequency in e-cigarette users versus controls (non-users/former users). In both comparisons, the certainty of evidence was rated “very low”, due to “very serious” inconsistency, “serious” indirectness, and “very serious” imprecision. Details are provided in Appendix 4.

Discussion

Current evidence on the effects of e-cigarettes on oral cancer risk remains limited[15], underscoring the importance of investigating their genotoxic impact on oral mucosal cells using the BMNcyt assay.[14] However, existing studies report conflicting results[14,19-21], particularly when comparing e-cigarette users with conventional cigarette smokers and non-smokers. These inconsistencies raise concerns about the safety of e-cigarettes as a smoking cessation strategy.

In this systematic review and meta-analysis, e-cigarette users exhibited a lower frequency of micronuclei (MN) compared with conventional cigarette smokers, consistent with the findings of Caponio et al. (2024)[17], suggesting a potentially lower genotoxic impact on the oral mucosa. Moreover, MN frequencies in e-cigarette users did not differ significantly from those in non-smokers. However, subgroup analyses demonstrated that these results were strongly influenced by the number of cells analyzed in the BMNcyt assay, highlighting the importance of methodological accuracy in genotoxicity studies.

The number of cells evaluated plays a critical role in generating reliable estimates of MN frequency. Standardized protocols, such as those recommended by HUMNxl[28], specify analyzing more than 1,000 cells per individual to enhance accuracy and reduce variability. Pooled evidence from studies that analyzed more than 1,000 cells[14,19,21,22,35] consistently reported higher mean MN frequencies in e-cigarette users compared with controls, whereas studies analyzing fewer than 1,000 cells[20,34] yielded inconsistent

findings. Additional evidence from OECD guidelines (#487)[37] and simulation studies[38,39] indicates that evaluating approximately 2,000 cells per individual provides the best balance between laboratory effort and statistical robustness. HUMNxl further recommends analyzing a minimum of 4,000 cells to reduce variability in MN mean estimates. This recommendation is justified by the low baseline MN frequency in buccal cells, which can amplify statistical variability but also provide a low background against which genotoxic effects can be more readily detected.[28] Therefore, the lack of adherence to standardized protocols in the included studies may have compromised the reliability and comparability of their findings.

The observed increase in MN frequency among e-cigarette users compared with non-smokers is biologically plausible. In vitro studies have shown that e-cigarette liquids can induce DNA damage, and clinical evidence links e-cigarette use to elevated levels of acrolein-DNA adducts, metanuclear anomalies, and lactate dehydrogenase expression, along with reduced apurinic/apyrimidinic sites compared with non-smokers.[40,41] Additionally, potentially carcinogenic substances, such as formaldehyde, acetaldehyde, acrolein, and certain nitrosamine compounds, have been detected in e-liquid aerosols.[16] Collectively, these findings support the hypothesis that e-cigarette aerosols may exert measurable genotoxic effects on the oral mucosa.

Despite these findings, the pooled results of this review should be interpreted with caution due to significant methodological limitations. Many studies enrolled participants across a wide age range [14,19,20,33,35], with several including individuals over 50 years of age. This could introduce bias, as DNA damage naturally become more frequent after this age.[42] However, due to missing data, a subgroup meta-analysis to investigate this factor could not be performed. There was also considerable variability in the duration of e-cigarette use, ranging from four months to several years, which could potentially influence MN frequency. Additionally, the composition of the comparison groups may have introduced bias, as some conventional smoker groups included heavy smokers or even former smokers as controls .[20] Notably, only one study[14] adequately reported the daily level of e-cigarette consumption among users. Furthermore, the inclusion criteria for e-cigarette users in some studies required abstinence from conventional cigarettes for only three months[22] or six months[19],

intervals that may be insufficient to eliminate residual genotoxic effects from prior tobacco use, as no validated washout period for such damage currently exists. In addition, only cross-sectional designs were employed, limiting the ability to establish cause-and-effect relationships.

Additional heterogeneity arose from variations in device types, nicotine concentrations, e-liquid compositions, and flavoring agents, none of which were evaluated in a standardized manner. Only one study reported the composition of the e-liquid used by the e-cigarette users group.[20]

Geographic representation was also limited, as populations from countries of high e-cigarette prevalence, such as the United States[43], were not included. Recruitment strategies posed another source of bias; for instance, some studies recruited e-cigarette users from undergraduate health student populations [21,22,36], hospital-based populations[14,19] or members of vaping associations[21], potentially limiting external validity.

Future research should address these issues by including larger, more representative populations and employing prospective cohort designs, particularly in regions with high e-cigarette use. Studies should adopt standardized BMNcyt protocols, such as those recommended by HUMNxl, analyze more than 1,000 cells per sample, ideally 4,000, and control for confounding factors, including prior tobacco use, device type, e-liquid composition, and intensity of use. The BMNcyt assay remains a valuable tool for monitoring the genotoxic effects of e-cigarettes and has potential for large-scale screening, but consistent methodologies are crucial to ensure data quality and comparability.

Conclusions

Overall, the available evidence suggests that while the genotoxic effects of e-cigarettes on oral mucosal cells may be lower than those of conventional cigarettes, they remain higher than in non-smokers when standardized protocols of BMNcyt are applied. Given the very low quality of the cumulative evidence and the substantial methodological heterogeneity among studies, e-cigarettes should not be regarded as a safe alternative to conventional tobacco products. More rigorous and standardized research is needed to clarify their true impact on oral mucosal cells.

Conflict of interest

The authors deny any conflicts of interest related to this study.

Acknowledgements

This study was partially financed by the Goiás Research Foundation – FAPEG - Brazil grant – [AUX2024361000137].

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used Grammarly to check grammar, spelling, and text fluency. No AI tools were used for data interpretation, analysis, or to generate or modify the scientific content of the manuscript. After using this tool, the authors thoroughly reviewed and edited the text as needed and take full responsibility for the content of the publication.

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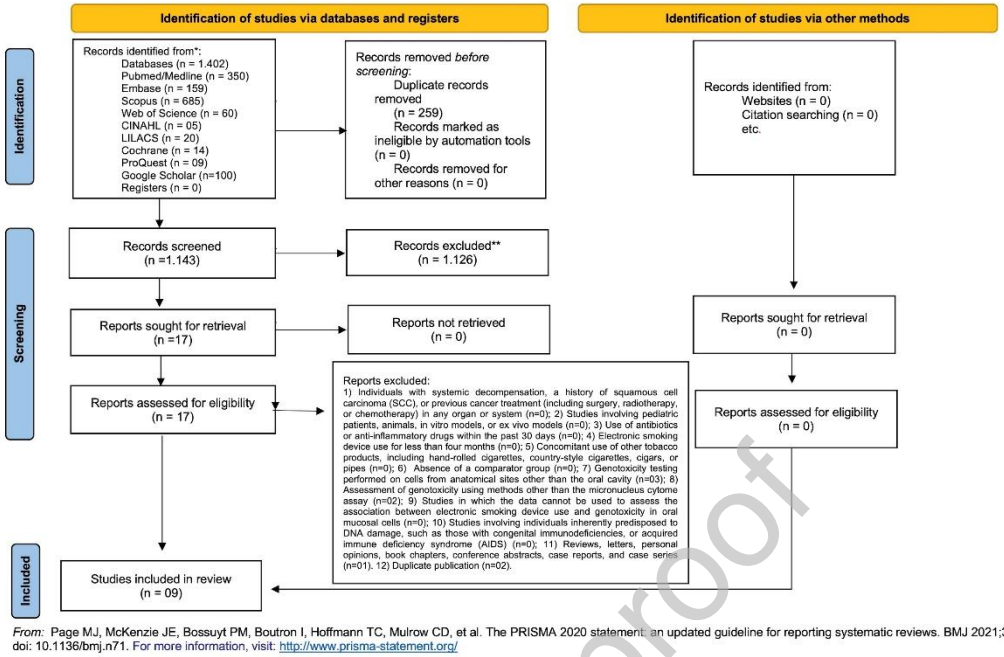

From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71. For more information, visit: http://www.prisma-statement.org/

Figure 1. Flowchart illustrating the literature search and study selection process - adapted from the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.

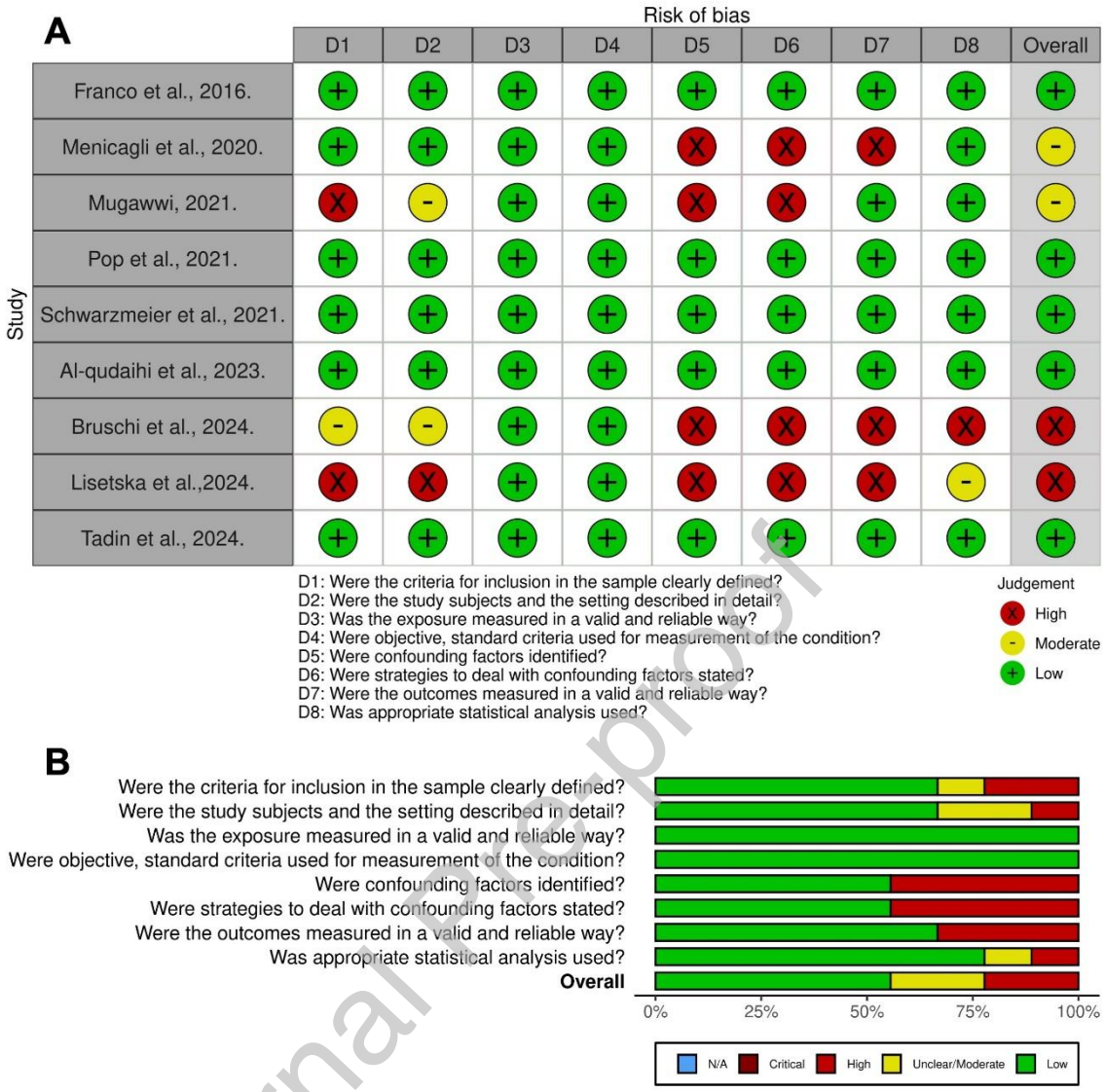

Figure 2. Assessment of risk of bias (methodological quality) for individual studies using the Joanna Briggs Institute (JBI) Critical Appraisal Checklist for Cross-sectional Studies: (A) Risk of bias graph; (B) Risk of bias summary.

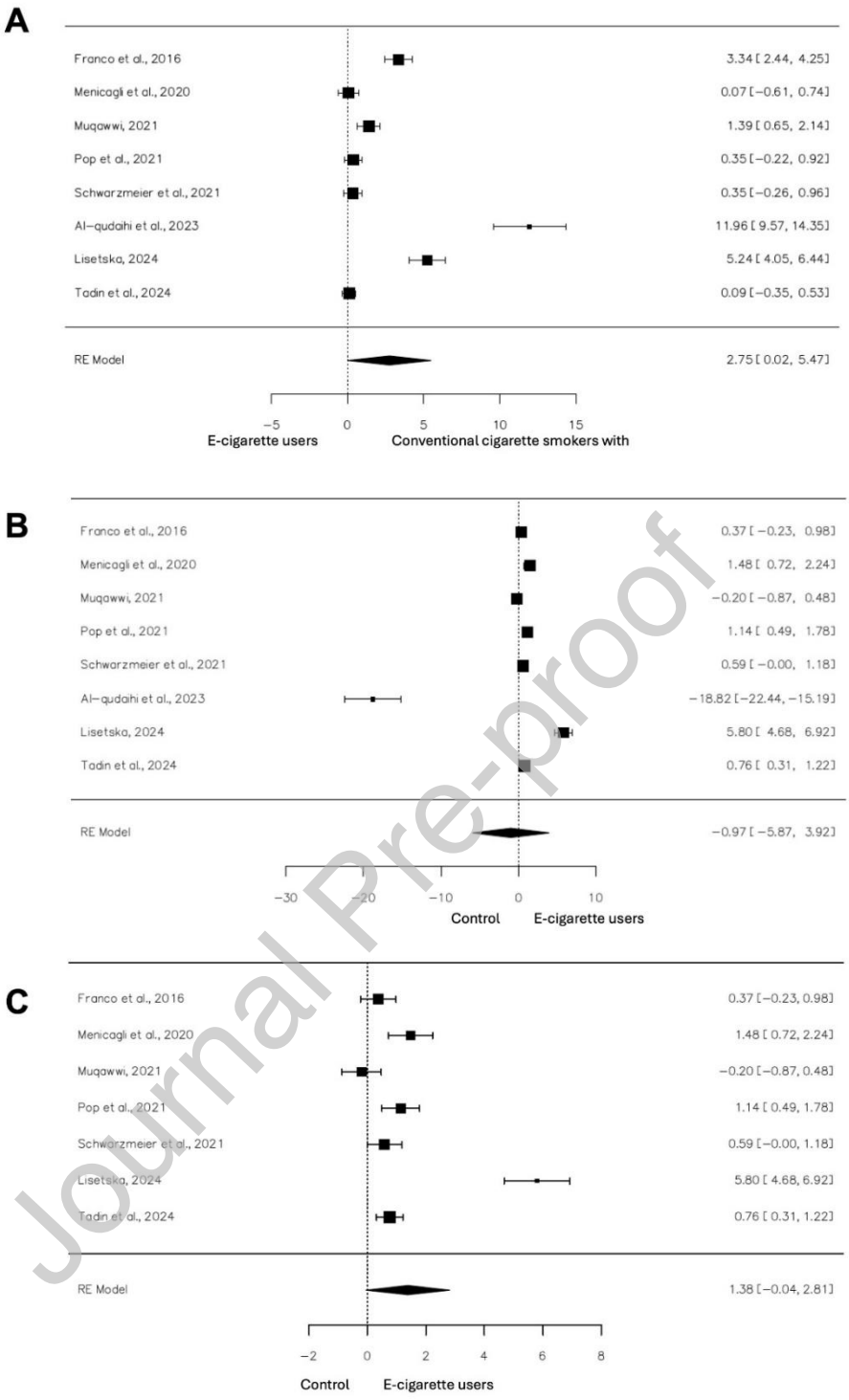

Figure 3. Forest plot of the overall mean micronucleus (MN) frequency comparing: (A) conventional cigarette smokers with e-cigarette users; (B) e-cigarette users with controls; and (C) e-cigarette users with controls (non-users or former users) after sensitivity analysis.

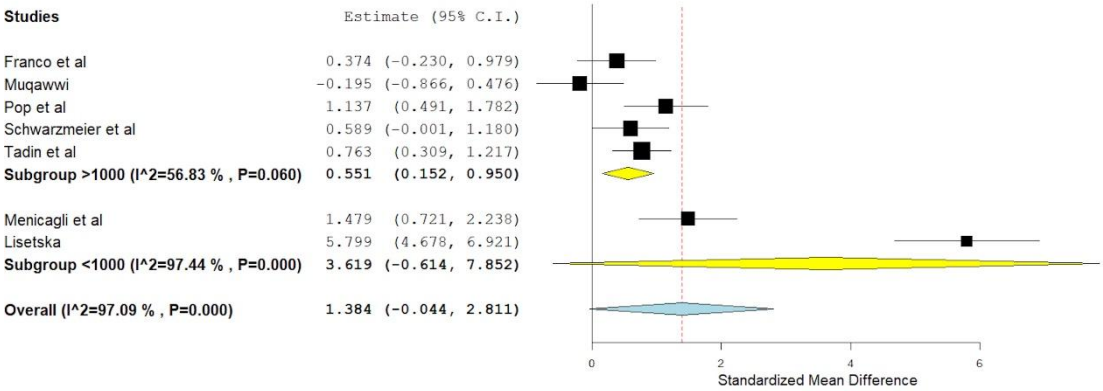

Figure 4. Forest plot of the subgroup meta-analysis based on the number of cells counted for MN evaluation (≥1,000 cells vs. ≤1,000 cells).

Table 1. Summary of descriptive characteristics of included articles.

Author, Year	***Country***	***Study Design***	***Sample Size (N)***	***Sex (Male/Female)***	***Mean Age (years; range) (range)***	***E-Cigarette Use (mL per day or concentration of nicotine on blood or saliva mL/d)***	***Duration of E-Cigarette Use***	***Number of Conventional Cigarettes Smoked Daily***	***Duration of Conventional Cigarette Use***	***Analysis of Additional Tissues or Organs (Yes/No/ Description)***	***Presence of Oral Manifestations (Yes/No/ Description)***	***Anatomical Site of Oral Mucosa Cell Sampling***	***Cell Collection Technique (smear, brush, scraping)***	***Micronucleus Frequency (mean; SD)***	***Number of Cells Counted***	***Staining Method***	***DNA tail Length – Comet Assay (%; mean; SD)***	***Main conclusions***
Franco et al., 2016	Italy	Cross-sectional	65	Male: 33 Female: 32	Smokers 23-73 (47.6) E-cigarettes smokers 27-65 (57.8) Non-smokers 23-74 (46.7)	0.4-0.16 mg (nicotine)	≥6 months	16 (10-30)	--	No	Yes Gengivostomatitis (n=2)	Bilateral buccal mucosa	Scraping	Smokers: 0.088 (range 0.02-0.3, SD ± 0.0058 E-cigarettes Smokers: 0.028 (range 0.016-0.084,	1000	Giemsa	--	Our results show that e-cigarettes cause no harm in the oral cavity and, therefore, should be suggested as

																		SD ±0.024) Non-smokers: 0.012 (range 0.008-0,023 SD ± 0.056)	a reliable aid to smoking cessation compared to alternative methods.
Menicagli, et al., 2020	Italy	Cross-sectional	51	Male: 51	32-41	Salivary mucins: E-nicotine: 30.0-36.1 E-vapor: 30-33 Control: 31-33.8	--	No (former-smokers)	--	No	No	Bilateral buccal mucosa	Smear	E-nicotine: 1.81 (SD± 0.80) E-vapor: 1.75 (SD± 0.96) Control: 0.72 (SD± 0.05)	500	Papanicolaou	--	Even the low number of MN indicates a negligible genotoxic alteration by the components of the various typ

Muqawwi, 2021	Indonesia	Cross-sectional	55	Male: 44 Female: 11	Smokers: 28.8 E-cigarettes smokers :26.3 Non-smokers: 21.7	--	E-cigarettes smokers: 2.5 years (SD: 0.7)	12	Smokers: 10.9 (SD 4.4)	No	No	Buccal mucosa	Smear	Smokers: 253.9 (range: 128-659; SD ± 129.4) E-cigarettes smokers: 109.9 (range: 69-187; SD ± 35.7) Non-smokers: 128.3 (range: 55-520; SD ± 117.4)	1000	Giemsa	--	Vape users have lower numbers of micronucleous compared to smokers (positive controls) and even the frequency is below the non-vape-smokers (negative controls).

Pop et al., 2021	Romania	Cross-sectional	68	Male: 38 Female: 30	Smokers: 21.52 (SD: 1.65) E-cigarette smokers: 21.7 (SD: 1.59) Non-smokers: 22.36 (SD: 1.46)	--	≥ 1 year	≥ 10 cigarettes	≥1 year	No	No	Bilateral buccal mucosa	Smear	Smokers: 3.6 (SD± 1.08) e-cigarettes smokers: 3.21 (SD± 1.12) Non-smokers: 1.95 (SD± 1.05)	100	Papanicolaou	--	Clinically healthy young tobacco smokers and e-cigarette users presented an increased number of MN in the oral epithelial cells, compared to nonsmoking individuals, sugges

																		ting the presence of cytologic changes.
Schwarzmeier et al., 2021	Brazil	Cross-sectional	91	Male: 50 Female: 41	Smokers: 51.45 (SD: 11.27, range: 29-70) E-cigarette smokers: 41.5 (SD: 13.03, range: 17-60) Former smokers	3 individuails: 0 and 18 mg nicotine concentration in e-cig-frequency (mL/d): 4 and 5 mL/d 1 individual: 6 mg nic	≥ 5 months	E-cigarette smokers: 23.26 ±15.53 (Years, median 20) Smokers: 17.05 ±9.844 (Years, median 15) Former smokers:	E-cigarette smokers 21.58 ± 14.17 (Years, median 20) Smokers: 33.09 ± 11.86 (Years, median 33.5) Former smokers: 39.	No	No	Lateral border of the tongue and floor of the mouth	Smear	Smokers: 4.00 (SD± 3.65) E-cigarette smokers: 2.95 (SD± 3.06) Former smokers: 2.26 (SD± 2.75) Non-smok	150 0	Fulgen	--	In summary, e-cig users presented genotoxicity and cytotoxicity in the oral mucosa cells.

: 58.91 (SD: 10.24, range: 38-73) Non-smokers: 56.48 (SD: 12.93, range: 30-80)	otine concentration in e-cig-frequency (mL/d): not could quantify 1 individual: 1.2 mg nicotine concentration in e-cig-frequency (mL/d): 12 mL/d 7 individuals: 2 and 4 mg nicotine co	18.55 ± 11.38 (Years, median 16.5)	86 ± 13.22 (Years, median 40)	ers: 1.33 (SD± 2.41)

						nc entrati on in e-cig - feque ncy (m L/ d): 5,1 0 an d 30 m L/ d												
Al-qu daihi et al., 202 3	S au di A ra bi a	C ro ss - se ct io n al	7 9	Ma le: 51 Fe ma le: 28	19 - 59	--	A l m os t 1 ye ar	>1	>2 ye ars	No	No	Bi lat er al bu cc al m uc os a	C yt ol og ic al br us h	Sm ok ers : 29 (S D± 0.7 28) E-cig are tte sm ok ers : 20 (S D± 0.7 61) No n-sm ok ers : 30 (S D± 0.3 05)	1 0 0 0	Fe ulg en	C ig ar et te s m o k er s: 2 3, 9 1 7 E - ci g ar et te s m o k er s: 1 6, 1 7 3 N o n-s m o k er s:	Thi s pil ot stu dy rev eal ed tha t e-cig are ttes are ass oci ate d wit h inc rea sed DN A bre aka ge in blo od and mi cro nuc leu s ind uct ion in buc

Buschi, et al., 2024	Brazil	Cross-sectional	28	Male: 2 Female: 26	18-36	--	4-7 years	2-15/day	2-25 years	No	--	Bilateral buccal mucosa	Scrapping	Not related	500	Violet crystal	--	12, 633	calcells, with these effects being similar to those for regular cigarette smokers. These results suggest that exposure to tobacco smoke, regardless of the type of cigarette, is associated with an inc

Lisetska, 2024.	Ukraine	Cross-sectional	114	--	15-24	--	--	--	--	No	No	Buccal mucosa	Scrapping	Smokers: 2.74 (SD± 0.18) E-cigarette smokers: 1.85 (SD± 0.15) Users of tobacco heating devices: 1.91	100	Aceto-orcein	--	The obtained results of the study of cytogenetic status in adolescents and young adults indicate the presence of cyt

rease in the frequency of micronuclei, indicating a greater potential for cellular damage.

												(SD± 0.17) Non-smokers: 0.93 (SD± 0.16)					oge net ic dis ord ers in sm oke rs. Mo reo ve, the se ind icat ors are mo re pro no unc ed in peo ple wh o sm oke tra diti ona l cig are ttes .
Tadin, et al., 2024	Croatia	Cross-sectional	160	Male: 61 Female: 99	20-71 Mean: 41.6: SD: 14.6	--	5.1 years SD: 3,4	--	13.3± 13.9	No	No	Buccal mucosa	Cytological brush	Number of Cell with micronuclei: Smokers: 1.95	2000	Fulgen	--	Non smokers demonstrated significantly lower levels

(SD± 1.46) E-cigarette: 1.85 (SD± 0.58) IQOS (tobacco heater): 1.40 (SD± 1.17) Non-smokers: 1.22 (SD± 1.00)	of damage compared to individuals using traditional combustible tobacco products cigarettes and non-combustible alternatives heated tobacco products and e-cigarettes.

(--) Not reported. % percentage. N/A – Not applicable. SD – Standard deviation.

Statement of Clinical Relevance

E-cigarettes appear less genotoxic to oral mucosal cells than conventional smoking but may still pose risks compared with non-smokers; clinicians should recognize these potential oral health risks given the very low certainty of evidence.