REVIEW

Editorial Process: Submission:01/17/2025 Acceptance:07/11/2025

Systemic and Salivary Cytokine Levels among Adult E-Cigarette Users: A Systematic Review and Meta Analysis

Aishwarya Lakshmi Billa[1], Jagadeeswara Rao Sukhabogi[1], Dolar Doshi[1], Ramesh Athe[2]*

Abstract

Objective: This systematic review and meta-analysis aimed to evaluate the effects of e-cigarette use on systemic and salivary cytokine levels among adults. **Methods:** This systematic review and meta-analysis, registered under PROSPERO (Prospective Register of Systematic Reviews, CRD42024571203), was conducted in compliance with Cochrane and PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) standards. Studies assessing systemic and salivary cytokines (e.g., IL-6 [Interleukin-6], TNF-α [Tumor Necrosis Factor-alpha], IL-10 [Interleukin-10]) among adult e-cigarette users, traditional smokers, mixed smokers, and non-smokers were included based on Population-Intervention-Comparators-Outcomes (PICO) criteria. Data were extracted, risk of bias was assessed using the Joanna Briggs Institute tool, and the quality of evidence was graded with GRADE (Grading of Recommendations Assessment, Development, and Evaluation). Meta-analysis was performed using SPSS (Statistical Package for the Social Sciences) and R software, reporting standardized mean differences (SMD) with 95% confidence intervals (CI); $p \leq 0.05$ was considered significant. **Results:** A total of 286 studies were screened, with 10 meeting the inclusion criteria. The studies, conducted in the U.S., Saudi Arabia, Kuwait, Latvia, and India, included 48 to 3,614 participants. Cytokines such as, TNF-α (SMD 0.88, 95% CI 0.23–1.13; $p = 0.003$) and IL-1RA (SMD 0.31, 95% CI 0.10–0.52; $p = 0.004$), were significantly elevated in e-cigarette users compared to conventional smokers and non-smokers. INF-γ and CRP levels did not significantly differ between groups ($p = 0.81$ and 0.29, respectively). Meta-analyses showed elevated levels of pro-inflammatory cytokines in e-cigarette users, with substantial heterogeneity across studies. Sensitivity analyses and publication bias tests were also conducted. **Conclusion:** Systemic and salivary cytokine levels were significantly elevated among e-cigarette users compared to non-smokers and conventional smokers, indicating a heightened inflammatory response associated with e-cigarette use.

Keywords: Electronic Nicotine Delivery Systems- Vaping; Cytokines- Saliva- Interleukins

Asian Pac J Cancer Prev, **26 (7)**, 2327-2338

Introduction

E-cigarettes, a popular type of Electronic Nicotine Delivery System (ENDS) and Electronic Non-Nicotine Delivery System (ENNDS), are battery-operated devices that heat a liquid to create aerosols for inhalation. These e-liquids, which may or may not contain nicotine, are tobacco-free but often contain harmful substances such as formaldehyde, acrolein, heavy metals, and volatile organic compounds [1]. These chemicals have been linked to adverse health effects ranging from nicotine poisoning to exposure to toxic metals and carcinogenic compounds leading to inflammation, oxidative stress, and DNA damage [2].

Despite the growing body of literature on ENDS, considerable debate persists regarding their primary effects. While some researchers [3, 4] suggest that e-cigarettes could serve as an effective tool in reducing traditional cigarette use, others view them as an alternative method of nicotine consumption that may contribute to novel public health challenges [5, 6]. World Health Organization (WHO) has expressed significant concern over these devices, which also encompass products like e-cigars and e-pipes [7]. The primary issue stems from their aggressive marketing strategies aimed at young people, featuring over 16,000 enticing flavors, eye-catching designs, and cartoon-themed packaging. Alarmingly, 88 countries have no minimum age restrictions for purchasing e-cigarettes, and 74 lack regulations to control their use [8]. This regulatory gap has contributed to a surge in e-cigarette use among youth, often surpassing adult usage rates. Exposure on social media further exacerbates the issue,

*[1]Department of Public Health Dentistry, Government Dental College and Hospital, Hyderabad, Telangana, India. [2]Department of Data Science and Artificial Intelligence, Indian Institute of Information Technology, Dharwad, Karnataka, India. *For Correspondence: dr.athe9@gmail.com*

promoting positive perceptions and increased interest in these harmful products among young people worldwide [9-11]. Although there is a common belief that e-cigarettes are less harmful than traditional smoking, the long-term health effects remain uncertain and insufficiently studied, raising critical questions about their true safety and public health implications [3, 4].

E-cigarette use has been increasingly linked to adverse health outcomes, including an elevated risk of oral cancer [12]. A key factor in this association is the modulation of systemic and salivary cytokine levels, particularly interleukins, which are key proteins involved in immune responses and inflammation. Cytokines play a pivotal role in cancer development, as they regulate processes such as cell proliferation, survival, and invasion, all of which are hallmarks of malignancy [13]. Exposure to the harmful chemicals found in e-cigarette aerosols triggers an inflammatory cascade that stimulates the production of pro-inflammatory cytokines, notably interleukin-6 (IL-6) and interleukin-8 (IL-8) [14, 15]. These cytokines produce a microenvironment conducive to cancer progression by promoting cellular proliferation and survival. In the oral cavity, prolonged inflammation resulting from e-cigarette exposure can lead to cellular alterations that heighten the risk of carcinogenesis. E-cigarettes contain a range of noxious substances, including nicotine, formaldehyde, acrolein, and volatile organic compounds, all of which contribute to cytokine dysregulation and exacerbate inflammation [16, 17].

Elevated levels of key pro-inflammatory cytokines, including IL-6, IL-8, and Tumor Necrosis Factor-α (TNF-α), alongside a reduction in anti-inflammatory cytokine like IL-10, are indicative of a dysregulated immune response, akin to the inflammatory damage caused by traditional tobacco smoking [18, 19]. Moreover, studies have documented that e-cigarette users exhibit altered cytokine profiles in their saliva, which reflect persistent inflammatory processes in the oral cavity potentially serving as early biomarkers for oral cancer [20-22]. E-cigarettes are often marketed as a benign alternative to conventional tobacco products, yet the chemicals they release can still pose significant health risks. Although the long-term effects of e-cigarettes remain unclear, the evidence linking their use to cytokine alterations and oral cancer risk is evident, suggesting that prolonged use may increase the likelihood of developing oral malignancies, similar to traditional smoking. Therefore, this systematic review and meta-analysis aimed to evaluate the effects of e-cigarette use on systemic and salivary cytokine levels among adults.

Materials and Methods

Study design

This systematic review will be performed following the Cochrane Handbook for Systematic Reviews of Interventions (Version 6.5) [23] and reported following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement [24] (Supplementary file 1). This protocol is listed in the International Prospective Register of Systematic Reviews (PROSPERO) - CRD42024571203.

The research question was "Do electronic cigarettes (e-cigarettes) affect systemic and salivary cytokines among adult e-cigarette smokers?"

Eligibility criteria

Studies was assessed for inclusion in the review according to the Population-Intervention-Comparators-Outcomes-(PICO) Type of studies criteria.

PICO Framework

- Population: Adults >18 years
- Intervention: Use of Electronic cigarette
- Comparators: Conventional cigarette smokers, Non-smokers, Mixed smokers
- Outcomes: Specific cytokines (e.g., IL-6, TNF-α, IL-10) in saliva and plasma

Inclusion and Exclusion criteria

Peer-reviewed original studies published in English language (inception to December 2024) and approved by an institutional ethics committee was included. Studies which compared cytokine levels among e-cigarette users, traditional smokers, mixed smokers, and non-smokers and reported cytokine levels in saliva or systemic circulation as a primary or secondary outcome was included in this review. Narrative reviews, case series, case reports, in vitro and animal studies was excluded from the review. Studies that do not distinguish e-cigarette users from traditional smokers or non-smokers and studies that lacked numerical data on inflammatory markers or cytokine levels related to e-cigarette use (missing data) was excluded.

Search Strategy

An extensive electronic literature search was conducted in these engines: PubMed, Embase, Web of Science, Scopus, Science Direct and the Cochrane Library and secondary references of included studies. All databases was searched from inception to December 31, 2024. For PubMed, the following search strategy was employed: (((“E-cigarettes” [MeSH]) AND “Cytokines” [MeSH]) AND) AND “Saliva”. For the rest, the keywords as “e-cigarettes,” “cytokines,” “saliva,” and “systemic inflammation” was used, along with the use of Boolean operators “OR” and “AND”. Moreover, the reference lists of all included articles were manually reviewed, and a citation analysis was conducted to identify any additional studies that may have been potentially missed in the initial search.

Screening process

Two independent reviewers conducted title and abstract screening of the identified studies using predefined inclusion and exclusion criteria, facilitated by Rayyan software (Rayyan Systems Inc., Version 4.0). Duplicates and irrelevant studies were excluded. Discrepancies were resolved through discussion with a third reviewer to achieve consensus. Full-text articles meeting the initial screening criteria were subsequently reviewed in detail, with reasons for exclusion systematically documented.

Data Extraction

Relevant information from selected articles were systematically collected and recorded in a standardized data extraction form using Microsoft Excel, Microsoft Corporation (Redmond, WA, USA). Data included: Name of the first author and year of publication, country of origin, study design, study duration, gender, mean age, sample size, mean value and standard deviation of salivary and systemic cytokine levels. Corresponding authors were contacted for studies with missing, incomplete, or suppressed data.

Risk of bias assessment

Two reviewers assessed the risk of bias using Joanna Briggs Institute's risk of bias tool [25]. Disagreement was resolved by discussion with a third reviewer to reach a consensus.

Assessment of heterogeneity

Heterogeneity was assessed using Cochran's Q test and quantified with the Higgins and Thompson I^2 statistic [26]. The I2 statistic of <25% represented low heterogeneity, 25%-50%, moderate heterogeneity and >50%, high heterogeneity.

Grading quality of evidence

The Grading of Recommendations Assessment, Development and Evaluation (GRADE) method was utilized to evaluate the degree of certainty in the evidence [27]. Quality of evidence were scored as "high", "moderate", "low," and "very low".

Data Analysis

Quantitative analysis on systemic and salivary cytokine levels was assessed in ng/ml, between E-cigarette users, conventional cigarettes smokers, mixed smokers, and non-smokers. All computations was performed using Statistical Package for Social Sciences software (SPSS V. 29, Chicago, IL., USA). The standardized mean difference (SMD) method with a 95% Confidence Interval (CI) was used. The Meta-analysis was performed using R software (version 4.3.1). A p value of ≤ 0.05 was considered statistically significant. Forest plots were constructed to visualize estimates with 95% CI. Funnel plot was used to assess publication bias.

Results

Search results

A total of 286 studies were initially identified, of which 135 duplicated pieces of literature were excluded. Additionally, 56 records were marked as ineligible by automation tools and 15 were removed for other reasons. Remaining 80 studies underwent screening based on titles and abstracts, excluding 32 studies. Additionally, 48 were left for full-text screening. After reading the full text, 20 studies were unable to retrieve, leaving 28 potentially eligible articles. Further, 18 studies were excluded that lacked blinding assessments or data. Finally, 10 studies were included. A PRISMA flow diagram illustrating the process of study selection is presented in Figure 1.

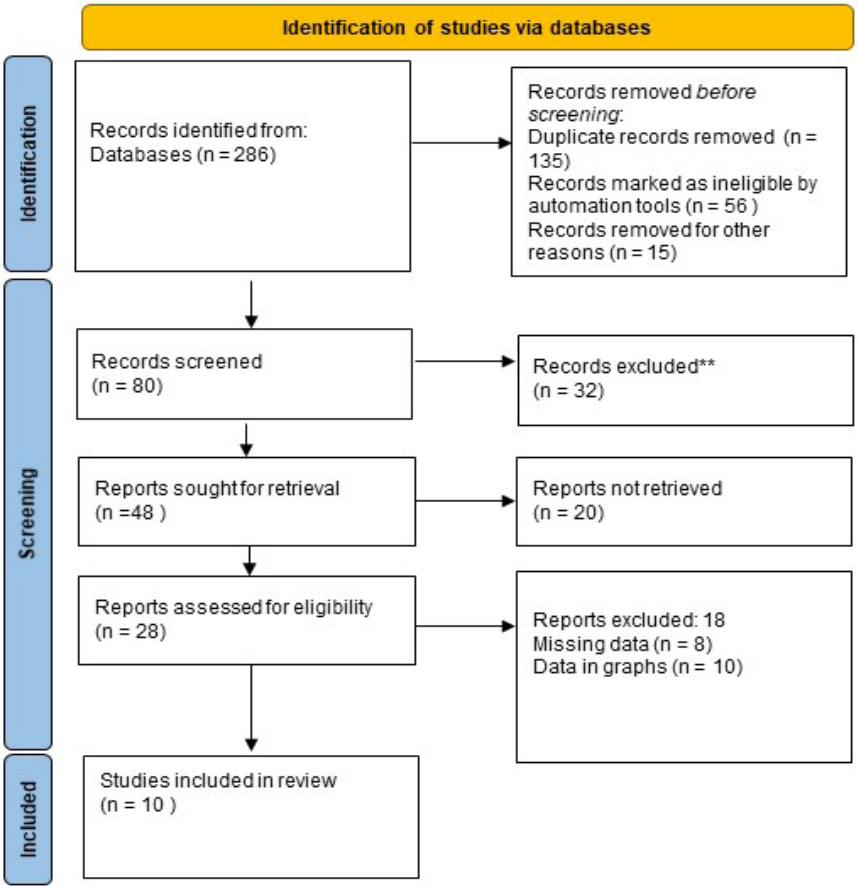

Figure 1. PRISMA Flow Diagram

Characteristics of studies

The characteristics such as author, year of publication, study design, intervention, region, age, sample size, gender of the 10 included studies are described in Table 1. Of all the included eligible studies, 5 studies were conducted in the U.S., 2 studies were conducted in Saudi Arabia, and remaining 3 study were conducted in Kuwait, Latvia, and India, respectively. All the participants were within the age group 18-80 years. The sample size of studies included ranges from the minimum of 48, a study conducted by Almubarak et al. [28] to a maximum of 3614, a cohort study conducted by Christensen et al. [29]. The studies were published between 2018 and 2022. Most of the studies were conducted in hospital-based settings. One study was conducted at the dental unit of a tertiary healthcare center in Riyadh, Saudi Arabia [30]. Another study focused on college students [31], while one study utilized data from the Population Assessment of Tobacco and Health (PATH) Study, a nationally representative longitudinal cohort study involving 45,971 U.S. adults and youth (Christensen et al.) [29].

Quality assessment

All included studies passed quality assessment based on the Joanna Briggs Institute Meta-Analysis for Statistics Assessment and Review Instrument for cross-sectional studies (JBI_MAStARI).

Systemic and Salivary Cytokines among study populations

Results of each included study are summarized in tabular and narrative form. Systemic and salivary biomarkers and parent compounds among all the groups were categorized according to the International Agency for Research on Cancer (IARC) monograph on human carcinogenic risk assessment [32]. These compounds were then cross-referenced using the Health and Environment, Toxicology and Disease Collaboration (HEDTC) database to identify associations with oral cancer and grouped according to the strength of the evidence [33].

Analysis of systemic and salivary cytokines among e-cigarette users and placebo group

Interferon – Gamma (INF- γ)

Seven studies reported INF- γ with a cumulative sample of 618 patients. After observing significant heterogeneity between studies, a random effects model was used (I2 = 96%; p>0.05). Results of the meta-analysis showed that INF- γ was higher in e-cigarette users than placebo group (SMD -0.16, 95% CI -1.47-1.15; p = 0.81) (Figure 2).

Tumour Necrosis Factor-Alpha (TNF- α)

Fifteen studies reported TNF-α levels, encompassing a total of 1,118 patients. Due to significant heterogeneity among the studies, a random effects model was applied (I^2 = 84%; p < 0.05). The meta-analysis revealed a significant difference in TNF-α levels between e-cigarette users and the placebo group, with higher levels observed in e-cigarette users (SMD 0.88, 95% CI 0.23–1.13; p = 0.003) (Figure 3).

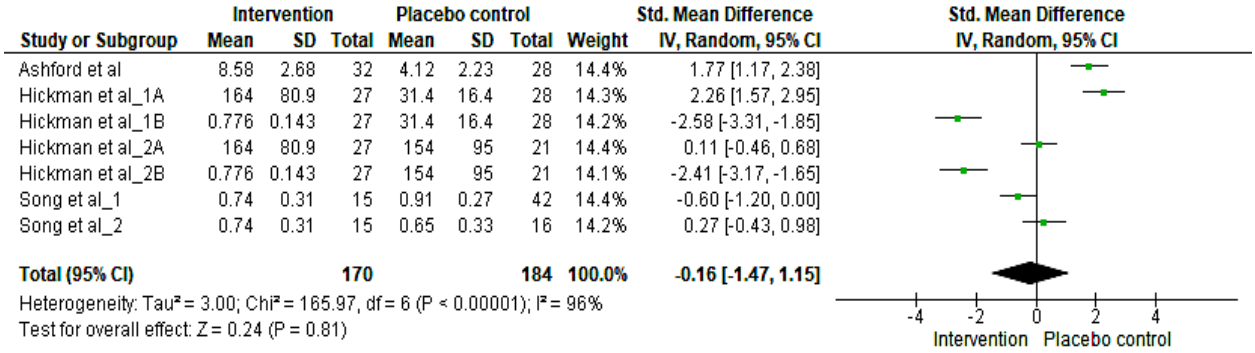

Figure 2. INF-Gamma Analysis and Forest Plot

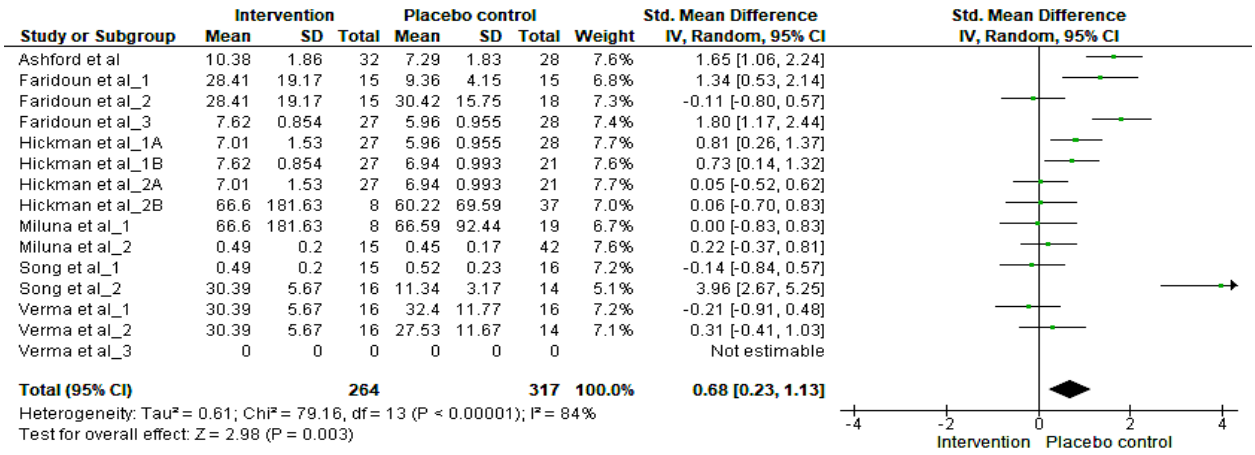

Figure 3. TNF-Alpha Analysis and Forest Plot

Table 1. Characteristics of Included Studies

S.No.	Author	Year	Country	Study Design	Sample	Sample Size	Mean Age/Age Range	Male and Female%	Study Groups
1.	Verma et al.	2021	India	Cross-sectional study	Saliva	60	34.4	63.4% - Male 36.6% - Female	1 E-cigarette users 2 Conventional smokers 3 Mixed smokers 4 Non-smokers
2.	Ashford et al.	2020	United States	Cross-sectional study	Saliva	61	18-25 years	47.5% - Male 52.5% - Female	1 E-cigarette users 2 Non-smokers
3.	Song et al.	2020	United States	Cross-sectional study	Plasma	73	21-30 years	53% - Male 47% - Female	1 E-cigarette users 2 Conventional smokers 3 Non-smokers
4.	Hickman et al.	2022	United States	Cross-sectional study	Plasma	103	27.2±7.42	53.4% - Males 46.6% - Females	1 Gen III & IV E-cigarette users 2 Conventional smokers 3 Non-smokers
5.	Miluna et al.	2022	Latvia	Cohort study	Saliva	76	24.46	50% - Male 50% - Female	1 E-cigarette users 2 Conventional smokers 3 Non-smokers
6.	Ali et al.	2022	Kuwait	Cross-sectional study	Saliva	75	49.5 ± 2.3	72% - Male 28% - Female	1 E-cigarette users 2 Conventional smokers 3 Non-smokers
7.	Faridoun et al.	2021	United States	Cross-sectional study	Saliva	64	51.66± 16.81	57.8% - Male 42.2% - Female	1 E-cigarette users 2 Conventional smokers 3 Mixed smokers 4 Non-smokers
8.	AlMubarak et al.	2022	Saudi Arabia	Cross-sectional study	Saliva	48	25.2± 3.2	58.4% - Male 41.6% - Female	1 E-cigarette users 2 Non-smokers
9.	Christensen et al.	2021	United States	Cross-sectional study	Plasma	3712	>18 years	38.8% - Male 61.2% - Female	1 E-cigarette users 2 Conventional smokers 3 Mixed smokers 4 Non-smokers
10.	Alhumaidan et al.	2022	Saudi Arabia	Cohort Study	Saliva	54	41.3±1.8	66.6% - Male 33.4% - Female	1 E-cigarette users 2 Conventional smokers 3 Non-smokers

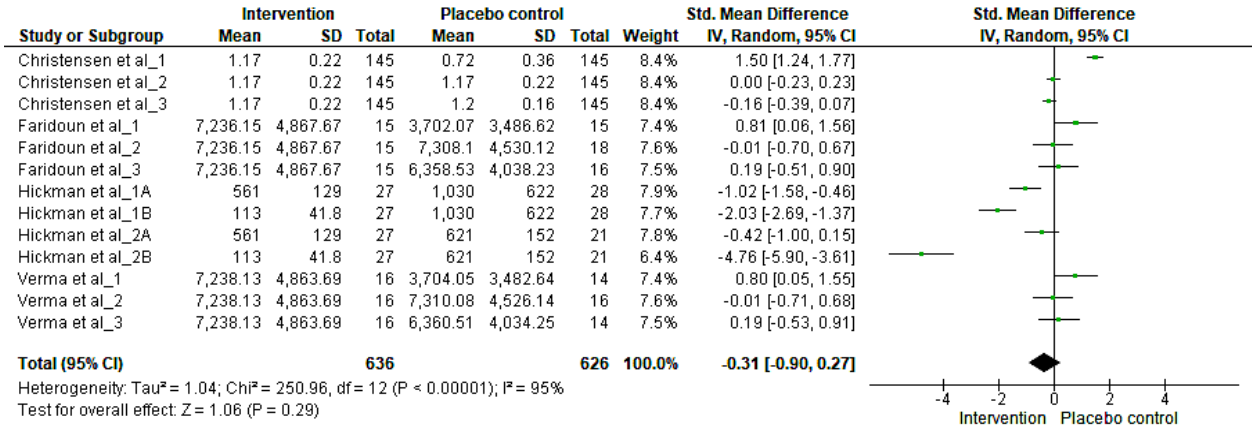

Figure 4. CRP Analysis and Forest Plot

C-Reactive Protein (CRP)

Thirteen studies, including a total of 11,626 patients, assessed CRP levels. Given the significant heterogeneity across studies, a random effects model was utilized (I^2 = 95%; p > 0.05). The meta-analysis showed a difference in CRP levels between e-cigarette users and the placebo group, with higher levels generally reported among e-cigarette users, except in the study by Hickman et al. [34] (SMD -0.31, 95% CI -0.90 to 0.27; p = 0.29) (Figure 4).

Interleukins (IL)Analysis among e-cigarette users and conventional smokers

Systemic and salivary cytokine levels were compared between e-cigarette users and conventional smokers. Due to substantial heterogeneity among studies, a random effects model was applied (I^2 = 93%; p < 0.05). The analysis revealed that most biomarkers, including IL-1A, IL-1B, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-13, IL-15, IL-16, IL-17, and IL-18, were significantly higher among e-cigarette users compared to traditional tobacco smokers. However, IL-RA levels were higher among conventional cigarette smokers, as reported by Verma et al. [35] and Faridoun et al. [36] (SMD 0.31, 95% CI 0.10 to 0.52; p = 0.004) (Figure 5).

Interleukins (IL)Analysis among e-cigarette users and non-smokers

Systemic and salivary cytokine levels were analyzed in e-cigarette users and non-smokers. Due to significant heterogeneity across studies, a random effects model was employed (I^2 = 94%; p < 0.05). The analysis showed that most biomarkers, including IL-1A, IL-1B, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-13, IL-15, IL-16, IL-17, and IL-18, were significantly elevated in e-cigarette users compared to non-smokers. However, IL-RA levels were higher among non-smokers, as noted by Verma et al. [35] and Faridoun et al. [36] (SMD 0.91, 95% CI 0.57–1.24; p < 0.00001) (Figure 6).

Subgroup analysis

Systemic and salivary cytokine levels were compared across various groups, including non-smokers versus traditional tobacco users, non-smokers versus e-cigarette users, The analysis indicated that all biomarkers were significantly elevated in e-cigarette users compared to non-smokers, as well as in traditional tobacco users compared to non-smokers except for IL-RA.

Sensitivity analysis

High heterogeneity between studies could not be avoided, despite the fact that all included studies received high quality scores after a rigorous assessment of the quality of the literature. Sensitivity analyses were performed to track the heterogeneity of each outcome metric. In the cases of outcomes with high heterogeneity, the included studies were individually excluded so that statistical merging and heterogeneity tests could be performed again to clarify the changes. Sensitivity analyses were also conducted for other subgroups to track the heterogeneity of each outcome indicator.

Publication bias

The funnel plots of individual studies in the meta-analysis appeared symmetrical (Supplementary Figures 1-5). Studies that evaluated systemic and salivary cytokine levels among e-cigarette users were plotted with their Standardized Mean Difference (SMD) on the x-axis and corresponding standard error of the SMD along the y-axis.

Discussion

This systematic review and meta-analysis provides significant insights into the impact of e-cigarette consumption, emphasizing the pronounced alterations in systemic and salivary cytokine levels among users. Elevated levels of cytokines were consistently observed among e-cigarette users compared to both conventional smokers and non-smokers. These results highlight the potential for e-cigarettes to provoke immune dysregulation and inflammatory responses comparable to or exceeding those caused by traditional tobacco products.

Despite the rising prevalence of e-cigarette use, particularly among younger adults, the long-term health impacts of these devices remain insufficiently understood. One critical area of research lies in their influence on inflammatory biomarkers, particularly cytokines. Cytokines are integral to immune responses and serve as

Study or Subgroup	Intervention Mean	SD	Total	Placebo control Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI
Alhumaidan (IL-1B) et al_1	67.3	5.1	18	42.8	3.7	18	0.6%	5.38 [3.91, 6.85]
Alhumaidan (IL-1B) et al_2	67.3	5.1	18	72.2	9.3	18	0.9%	-0.64 [-1.31, 0.03]
Ali (IL-15) et al_1	174.7	19.2	18	4.1	0.2	19	0.3%	12.47 [9.40, 15.55]
Ali (IL-15) et al_2	174.7	19.2	18	189.5	26.7	19	0.9%	-0.62 [-1.28, 0.04]
Ali (IL-18) et al_1	2,793.1	196.4	18	100.3	2.5	19	0.2%	19.25 [14.56, 23.93]
Ali (IL-18) et al_2	2,793.1	196.4	18	2,869.8	285.6	19	0.9%	-0.30 [-0.95, 0.34]
Almubarak (IL-1B) et al	26.2	6.4	24	0.12	0.005	24	0.7%	5.67 [4.36, 6.98]
Ashford (IL-10) et al	3.3	2.25	32	3.13	1.94	28	0.9%	0.08 [-0.43, 0.59]
Ashford (IL-13) et al	9.36	1.68	32	8.75	1.85	28	0.9%	0.34 [-0.17, 0.85]
Ashford (IL-2) et al	2.12	1.87	32	1.27	1.99	28	0.9%	0.44 [-0.08, 0.95]
Ashford (IL-4) et al	0.08	3.51	32	0.16	2.92	28	0.9%	-0.02 [-0.53, 0.48]
Ashford (IL-6) et al	10.81	2.23	32	8.27	1.95	28	0.9%	1.19 [0.64, 1.74]
Ashford (IL-8) et al	989.14	1.44	32	892.34	1.54	28	0.0%	64.24 [52.33, 76.14]
Christensen (IL-6) et al_1	1.19	0.12	145	0.95	0.27	982	0.9%	0.94 [0.76, 1.12]
Christensen (IL-6) et al_2	1.19	0.12	145	1.19	0.12	1891	0.9%	0.00 [-0.17, 0.17]
Christensen (IL-6) et al_3	1.19	0.12	145	1.15	0.08	596	0.9%	0.45 [0.26, 0.63]
Faridoun (IL-10) et al_1	28.44	28.8	15	28.62	34.31	15	0.9%	-0.01 [-0.72, 0.71]
Faridoun (IL-10) et al_2	28.44	28.8	15	23.15	26.01	18	0.9%	0.19 [-0.50, 0.88]
Faridoun (IL-10) et al_3	28.44	28.8	15	27.26	28.73	16	0.9%	0.04 [-0.66, 0.74]
Faridoun (IL-1B) et al_1	2.84	1.51	15	1.43	1.54	15	0.8%	0.90 [0.14, 1.66]
Faridoun (IL-1B) et al_2	2.84	1.51	15	1.39	1.64	18	0.9%	0.89 [0.17, 1.62]
Faridoun (IL-1B) et al_3	2.84	1.51	15	1.21	1.44	16	0.8%	1.08 [0.32, 1.84]
Faridoun (IL-6) et al_1	1.68	1.48	15	2.04	1.71	15	0.9%	-0.22 [-0.94, 0.50]
Faridoun (IL-6) et al_2	1.68	1.48	15	1.81	1.61	18	0.9%	-0.08 [-0.77, 0.60]
Faridoun (IL-6) et al_3	1.68	1.48	15	1.65	1.52	16	0.9%	0.02 [-0.68, 0.72]
Faridoun (IL-8) et al_1	1.27	0.8	15	1.2	0.67	15	0.9%	0.09 [-0.62, 0.81]
Faridoun (IL-8) et al_2	1.27	0.8	15	1.28	0.86	18	0.9%	-0.01 [-0.70, 0.67]
Faridoun (IL-8) et al_3	1.27	0.8	15	1.22	0.74	16	0.9%	0.06 [-0.64, 0.77]
Faridoun (IL-RA) et al_1	215.39	86.09	15	243.19	96.32	15	0.9%	-0.30 [-1.02, 0.42]
Faridoun (IL-RA) et al_2	215.39	86.09	15	245.58	107.46	18	0.9%	-0.30 [-0.99, 0.39]
Faridoun (IL-RA) et al_3	215.39	86.09	15	27.26	28.73	16	0.8%	2.90 [1.85, 3.94]
Hickman (IL-10) et al_1A	0.656	0.062	27	0.636	0.0709	28	0.9%	0.30 [-0.24, 0.83]
Hickman (IL-10) et al_1B	0.857	0.0963	27	0.636	0.0709	28	0.9%	2.58 [1.86, 3.31]
Hickman (IL-10) et al_2A	0.656	0.062	27	0.731	0.096	21	0.9%	-0.94 [-1.54, -0.34]
Hickman (IL-10) et al_2B	0.857	0.0963	27	0.731	0.096	21	0.9%	1.29 [0.66, 1.92]
Hickman (IL-13) et al_1A	154	22.3	27	133	24.2	28	0.9%	0.89 [0.33, 1.45]
Hickman (IL-13) et al_1B	155	23.7	27	133	24.2	28	0.9%	0.91 [0.35, 1.46]
Hickman (IL-13) et al_2A	154	22.3	27	191	27.9	21	0.9%	-1.46 [-2.11, -0.82]
Hickman (IL-13) et al_2B	155	23.7	27	191	27.9	21	0.9%	-1.38 [-2.02, -0.74]
Hickman (IL-15) et al_1A	0.89	0.32	27	0.882	0.321	28	0.9%	0.02 [-0.50, 0.55]
Hickman (IL-15) et al_1B	0.364	0.137	27	0.882	0.321	28	0.9%	-2.06 [-2.72, -1.39]
Hickman (IL-15) et al_2A	0.89	0.32	27	1.15	0.454	21	0.9%	-0.67 [-1.25, -0.08]
Hickman (IL-15) et al_2B	0.364	0.137	27	1.15	0.454	21	0.8%	-2.44 [-3.21, -1.68]
Hickman (IL-16) et al_1A	1,250	150	27	911	172	28	0.9%	2.07 [1.40, 2.73]
Hickman (IL-16) et al_1B	1,220	296	27	911	172	28	0.9%	1.26 [0.68, 1.85]
Hickman (IL-16) et al_2A	1,250	150	27	807	165	21	0.8%	2.78 [1.97, 3.59]
Hickman (IL-16) et al_2B	1,220	296	27	807	165	21	0.9%	1.64 [0.97, 2.31]
Hickman (IL-17) et al_1A	3.99	1.62	27	5.4	1.69	28	0.9%	-0.84 [-1.39, -0.29]
Hickman (IL-17) et al_1B	9.85	3.55	27	5.4	1.69	28	0.9%	1.59 [0.98, 2.20]
Hickman (IL-17) et al_2A	3.99	1.62	27	9.58	3.44	21	0.9%	-2.14 [-2.86, -1.41]
Hickman (IL-17) et al_2B	9.85	3.55	27	9.58	3.44	21	0.9%	0.08 [-0.49, 0.65]
Hickman (IL-1A) et al_1A	609	104	27	401	65	28	0.9%	2.37 [1.67, 3.08]
Hickman (IL-1A) et al_1B	414	71.3	27	401	65	28	0.9%	0.19 [-0.34, 0.72]
Hickman (IL-1A) et al_2A	609	104	27	697	122	21	0.9%	-0.77 [-1.36, -0.18]
Hickman (IL-1A) et al_2B	414	71.3	27	697	122	21	0.8%	-2.88 [-3.71, -2.05]
Hickman (IL-1B) et al_1A	82.7	13.9	27	61	10.4	28	0.9%	1.75 [1.12, 2.38]
Hickman (IL-1B) et al_1B	77.2	18.2	27	61	10.4	28	0.9%	1.08 [0.51, 1.65]
Hickman (IL-1B) et al_2A	82.7	13.9	27	91.9	14.9	21	0.9%	-0.63 [-1.22, -0.05]
Hickman (IL-1B) et al_2B	77.2	18.2	27	91.9	14.9	21	0.9%	-0.86 [-1.46, -0.26]
Hickman (IL-2) et al_1A	2.04	0.289	27	1.63	0.249	28	0.9%	1.50 [0.90, 2.10]
Hickman (IL-2) et al_1B	2.3	0.275	27	1.63	0.249	28	0.9%	2.52 [1.80, 3.24]
Hickman (IL-2) et al_2A	2.04	0.289	27	2.82	0.521	21	0.9%	-1.89 [-2.58, -1.19]
Hickman (IL-2) et al_2B	2.3	0.275	27	2.82	0.521	21	0.9%	-1.28 [-1.91, -0.65]
Hickman (IL-4) et al_1A	4.63	3.81	27	0.376	0.108	28	0.9%	1.57 [0.96, 2.18]
Hickman (IL-4) et al_1B	0.156	0.0313	27	0.376	0.108	28	0.9%	-2.71 [-3.45, -1.96]
Hickman (IL-4) et al_2A	4.63	3.81	27	4.78	3.12	21	0.9%	-0.04 [-0.61, 0.53]
Hickman (IL-4) et al_2B	0.156	0.0313	27	4.78	3.12	21	0.9%	-2.21 [-2.94, -1.48]
Hickman (IL-5) et al_1A	1.25	0.336	27	1	0.255	28	0.9%	0.83 [0.28, 1.38]
Hickman (IL-5) et al_1B	0.758	0.236	27	1	0.255	28	0.9%	-0.97 [-1.53, -0.41]
Hickman (IL-5) et al_2A	1.25	0.336	27	0.47	0.341	21	0.9%	2.27 [1.53, 3.01]
Hickman (IL-5) et al_2B	0.758	0.236	27	0.47	0.341	21	0.9%	0.99 [0.38, 1.60]
Hickman (IL-6) et al_1A	61.4	14.8	27	27.5	5.72	28	0.8%	3.00 [2.21, 3.79]
Hickman (IL-6) et al_1B	47.6	10.1	27	27.5	5.72	28	0.9%	2.43 [1.72, 3.13]
Hickman (IL-6) et al_2A	61.4	14.8	27	127	35	21	0.8%	-2.52 [-3.29, -1.74]
Hickman (IL-6) et al_2B	47.6	10.1	27	127	35	21	0.8%	-3.21 [-4.10, -2.33]
Hickman (IL-7) et al_1A	15.4	3.51	27	13.7	3.56	28	0.9%	0.47 [-0.06, 1.01]
Hickman (IL-7) et al_1B	7.12	2.11	27	13.7	3.56	28	0.9%	-2.21 [-2.89, -1.53]
Hickman (IL-7) et al_2A	15.4	3.51	27	12.8	2.31	21	0.9%	0.84 [0.24, 1.44]
Hickman (IL-7) et al_2B	7.12	2.11	27	12.8	2.31	21	0.8%	-2.54 [-3.32, -1.76]
Hickman (IL-8) et al_1A	3,930	503	27	3,550	683	28	0.9%	0.62 [0.08, 1.17]
Hickman (IL-8) et al_1B	4,240	957	27	3,550	683	28	0.9%	0.82 [0.27, 1.37]
Hickman (IL-8) et al_2A	3,930	503	27	3,730	702	21	0.9%	0.33 [-0.25, 0.90]
Hickman (IL-8) et al_2B	4,240	957	27	3,730	702	21	0.9%	0.59 [0.00, 1.17]
Miluna (IL-1) et al_1	78.71	92.37	8	21.32	26.53	37	0.8%	1.27 [0.46, 2.08]
Miluna (IL-1) et al_2	78.71	92.37	8	49.69	82.17	19	0.8%	0.33 [-0.50, 1.16]
Miluna (IL-6) et al_1	107.39	70.92	8	40.54	78.34	37	0.8%	0.85 [0.06, 1.64]
Miluna (IL-6) et al_2	107.39	70.92	8	59	94.59	19	0.8%	0.53 [-0.31, 1.37]
Miluna (IL-8) et al_1	445.09	135.12	8	281.85	249.13	37	0.8%	0.68 [-0.09, 1.46]
Miluna (IL-8) et al_2	445.09	135.12	8	355.52	120.67	19	0.8%	0.70 [-0.15, 1.55]
Song (IL-10) et al_1	0.09	0.02	15	0.09	0.02	42	0.9%	0.00 [-0.59, 0.59]
Song (IL-10) et al_2	0.09	0.02	15	0.09	0.029	16	0.9%	0.00 [-0.70, 0.70]
Song (IL-13) et al_1	1.59	0.96	15	1.8	1.78	42	0.9%	-0.13 [-0.72, 0.46]
Song (IL-13) et al_2	1.59	0.96	15	1.36	0.42	16	0.9%	0.31 [-0.40, 1.02]
Song (IL-1B) et al_1	1.51	1.48	15	0.82	0.39	42	0.9%	0.83 [0.22, 1.44]
Song (IL-1B) et al_2	1.51	1.48	15	6.08	5.37	16	0.8%	-1.11 [-1.88, -0.35]
Song (IL-2) et al_1	0.35	0.09	15	0.38	0.12	42	0.9%	-0.26 [-0.85, 0.33]
Song (IL-2) et al_2	0.35	0.09	15	0.31	0.15	16	0.9%	0.31 [-0.40, 1.02]
Song (IL-4) et al_1	0.04	0.008	15	0.04	0.009	42	0.9%	0.00 [-0.59, 0.59]
Song (IL-4) et al_2	0.04	0.008	15	0.04	0.01	16	0.9%	0.00 [-0.70, 0.70]
Song (IL-6) et al_1	1.56	1.02	15	0.99	0.56	42	0.9%	0.80 [0.19, 1.40]
Song (IL-6) et al_2	1.56	1.02	15	4.21	4.93	16	0.9%	-0.71 [-1.44, 0.02]
Song (IL-8) et al_1	66.09	115.71	15	28.37	37.2	42	0.9%	0.56 [-0.04, 1.16]
Song (IL-8) et al_2	66.09	115.71	15	88.52	114.52	16	0.9%	-0.19 [-0.90, 0.52]
Verma (IL-10) et al_1	26.42	24.82	16	30.6	30.33	14	0.9%	-0.15 [-0.87, 0.57]
Verma (IL-10) et al_2	26.42	24.82	16	25.13	22.03	16	0.9%	0.05 [-0.64, 0.75]
Verma (IL-10) et al_3	26.42	24.82	16	29.24	24.71	14	0.9%	-0.11 [-0.83, 0.61]
Verma (IL-1B) et al_1	2.82	1.53	16	1.41	1.52	14	0.8%	0.90 [0.14, 1.66]
Verma (IL-1B) et al_2	2.82	1.53	16	1.37	1.66	16	0.9%	0.89 [0.15, 1.62]
Verma (IL-1B) et al_3	2.82	1.53	16	1.19	1.46	14	0.8%	1.06 [0.29, 1.83]
Verma (IL-6) et al_1	1.66	1.46	16	2.02	1.69	14	0.9%	-0.22 [-0.94, 0.50]
Verma (IL-6) et al_2	1.66	1.46	16	1.79	1.59	16	0.9%	-0.08 [-0.78, 0.61]
Verma (IL-6) et al_3	1.66	1.46	16	1.63	1.5	14	0.9%	0.02 [-0.70, 0.74]
Verma (IL-8) et al_1	1.25	0.78	16	1.19	0.59	14	0.9%	0.08 [-0.63, 0.80]
Verma (IL-8) et al_2	1.25	0.78	16	1.26	0.85	16	0.9%	-0.01 [-0.70, 0.68]
Verma (IL-8) et al_3	1.25	0.78	16	1.21	0.72	14	0.9%	0.05 [-0.67, 0.77]
Verma (IL-RA) et al_1	217.37	82.11	16	245.17	92.34	14	0.9%	-0.31 [-1.03, 0.41]
Verma (IL-RA) et al_2	217.37	82.11	16	247.56	103.48	16	0.9%	-0.32 [-1.01, 0.38]
Verma (IL-RA) et al_3	217.37	82.11	16	243.4	116.24	14	0.9%	-0.25 [-0.98, 0.47]
Total (95% CI)			**2886**			**6086**	**100.0%**	**0.31 [0.10, 0.52]**

Heterogeneity: Tau² = 1.20; Chi² = 1710.47, df = 117 (P < 0.00001); I² = 93%
Test for overall effect: Z = 2.88 (P = 0.004)

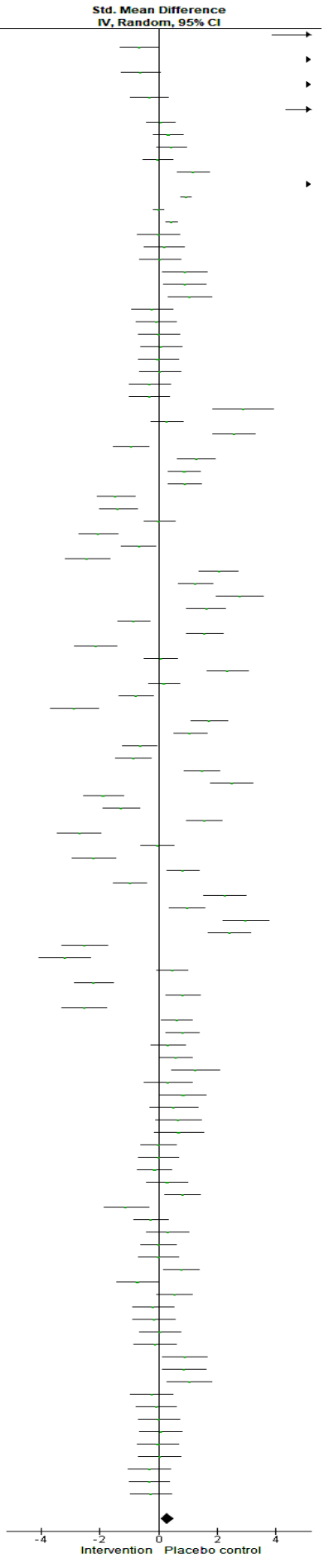

Figure 5. IL Analysis and Forest Plot

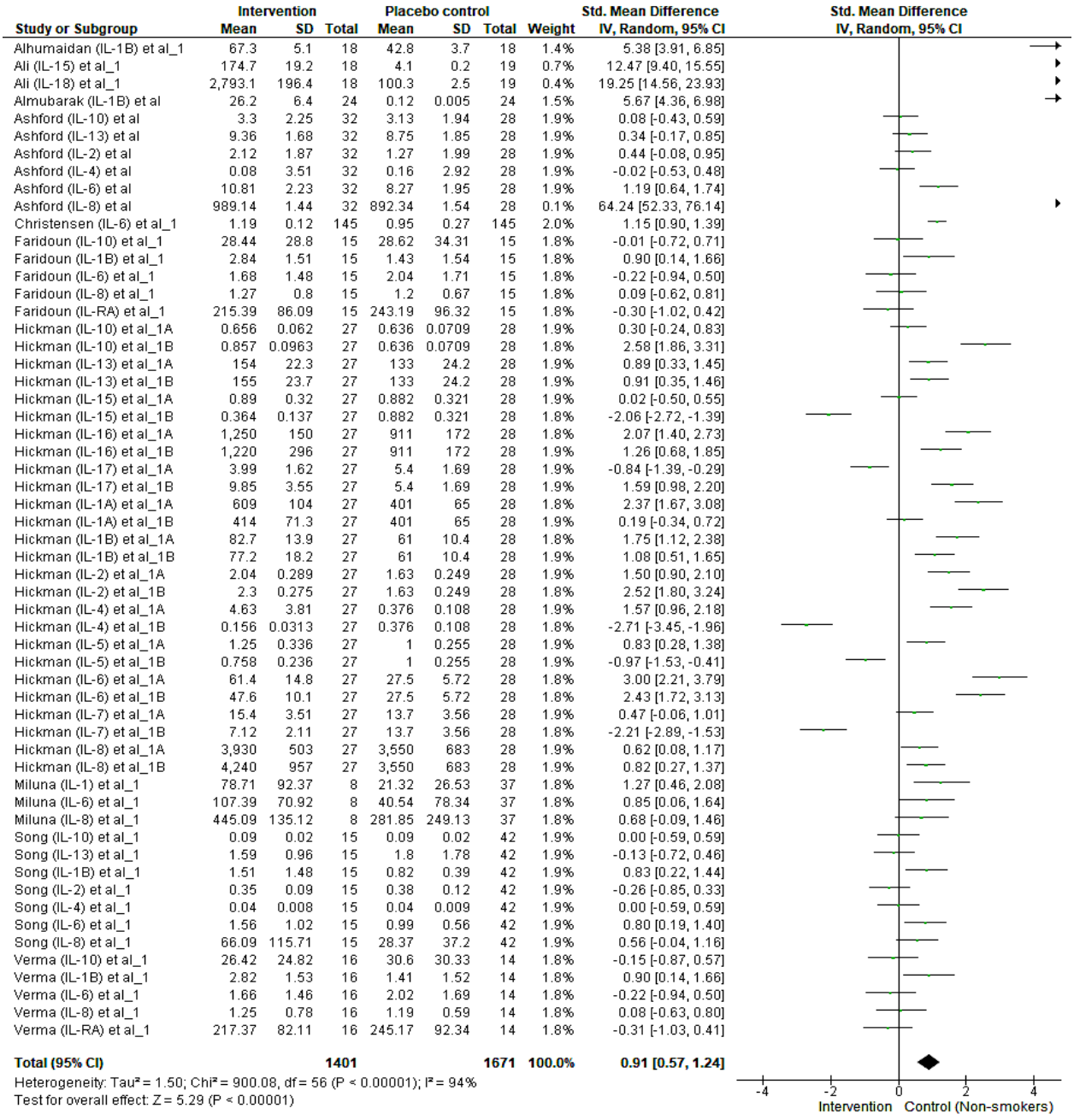

Figure 6. IL Analysis among Intervention and Non-Smokers with Forest Plot

indicators of both systemic and localized inflammation. The oral cavity, being the initial site of exposure to inhaled toxins, is particularly susceptible to the inflammatory effects of e-cigarettes. Traditional tobacco smoking has long been known to induce the release of various pro-inflammatory cytokines, contributing to a range of oral and systemic health issues, including periodontal disease and oral cancer [37]. Elevated levels of cytokines such as IL-1β, IL-2, IL-4, IL-6, IL-8, TNF-α, and Transforming Growth Factor-β (TGF-β), coupled with reduced levels of anti-inflammatory cytokines like IL-10, have been observed in the saliva of both conventional and e-cigarette smokers, signaling a dysregulated immune response [38, 39]. Similar alterations in cytokine profiles have been identified in studies of e-cigarette users, suggesting that their inflammatory effects mirror those of traditional smoking [40, 41].

Pro-inflammatory and anti-inflammatory cytokines play critical roles in modulating the immune response and are released by tumor and immune cells within the tumor microenvironment. In this context, various inflammatory cytokines have been evaluated, including pro-inflammatory markers such as IL-1, IL-1α, IL-1β, IL-6, IL-8, and TNF-α, and anti-inflammatory markers like IL-RA, IL-2, IL-4, IL-10, IL-12, IL-13, and IFN-γ. Among these, TNF-α, IL-8, and IL-6 have been the most extensively studied. Elevated levels of interleukins, including IL-1A, IL-1B, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-13, IL-15, IL-16, IL-17, and IL-18, have been observed in e-cigarette users compared to traditional smokers and non-smokers, whereas IL-RA levels were higher in conventional smokers and non-smokers, as

reported by Verma et al. [35] and Faridoun et al. [36].

Elevated Cytokine Levels and Inflammatory Response

The increase in cytokine levels among e-cigarette users suggests a dysregulated immune response likely induced by exposure to toxic chemicals present in e-cigarette aerosols, such as formaldehyde, acrolein, nicotine, and heavy metals [20]. The elevation of pro-inflammatory cytokines like IL-1α and IL-1β, which are crucial in initiating and amplifying immune responses, may result from epithelial cell damage caused by Reactive Oxygen Species (ROS) in e-cigarette vapor [19]. Similarly, IL-6, a key mediator in acute and chronic inflammation, is elevated due to oxidative stress and tissue damage, linking e-cigarette use to systemic inflammation and chronic conditions such as cardiovascular disease and cancer. IL-8, a potent chemokine responsible for neutrophil recruitment, increases in response to airway irritation and epithelial damage caused by e-cigarette aerosols, contributing to localized inflammation. The heightened levels of IL-17 and IL-18, which promote inflammatory responses and recruit immune cells, further underscore the immune dysregulation induced by e-cigarette exposure, possibly through the activation of T-helper cells and epithelial cell damage [42, 43].

Anti-inflammatory cytokines also exhibit altered levels among e-cigarette users. For instance, IL-2, which supports T-cell growth and activation, shows an increase, potentially reflecting an immune system attempt to regulate inflammation. IL-4 and IL-13, mediators of humoral immunity and anti-inflammatory responses, may rise as a compensatory mechanism to counteract the inflammatory effects of e-cigarette aerosols or due to allergic-type reactions. Elevated IL-10 levels indicate an attempt by the immune system to mitigate inflammation and oxidative stress, although this anti-inflammatory response may be insufficient to counterbalance the damage caused by e-cigarette toxicants [17, 41, 42]. Interestingly, IL-RA, which inhibits IL-1 receptor activation to reduce inflammation, was found to be higher in conventional smokers and non-smokers than in e-cigarette users, suggesting that e-cigarette exposure may suppress certain anti-inflammatory pathways, leading to an imbalance in the immune response [43].

Other cytokines, such as IL-5, IL-7, IL-12, IL-15, and IL-16, also show elevated levels in e-cigarette users, reflecting diverse immune responses to the toxicant exposure. IL-5, associated with eosinophil activation, may rise due to airway irritation, while IL-7 supports T- and B-cell survival in response to tissue damage. IL-12, which promotes T-helper cell differentiation and IFN-γ production, increases due to oxidative stress and immune activation. IL-15, enhancing natural killer and T-cell activity, and IL-16, which attracts T cells and promotes inflammation, further highlight the broad impact of e-cigarette aerosols on immune regulation [42-44]. Collectively, these findings emphasize the systemic and localized inflammatory effects of e-cigarettes, highlighting the potential health risks associated with their use.

Comparison with Conventional Smokers and Non-smokers

When e-cigarette users were compared to conventional smokers and non-smokers, distinct patterns in cytokine levels emerge, shedding light on the differential impacts of these habits on the immune system and inflammatory responses. E-cigarette users exhibited significantly higher levels of several pro-inflammatory cytokines, including IL-1A, IL-1B, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-13, IL-15, IL-16, IL-17, and IL-18, compared to both conventional smokers and non-smokers. Conversely, a study conducted by Ali et al. indicated that the salivary levels of inflammatory cytokines IL-15 and IL-18 were higher in cigarette smokers compared to ENDS users, indicating more pronounced inflammatory responses associated with traditional cigarette use [45]. This heightened inflammatory profile suggests that e-cigarette aerosols, despite being marketed as a safer alternative, may induce a pronounced immune response similar to that of traditional cigarette smoke. On other hand, IL-RA levels were notably higher in conventional smokers and non-smokers than in e-cigarette users, as reported by Verma et al. [35] and Faridoun et al. [36]. This indicates that while traditional smoking triggers anti-inflammatory mechanisms to counteract inflammation, e-cigarette use might suppress such protective responses, leading to a sustained pro-inflammatory state. However, ENDS users exhibited intermediate levels of inflammatory biomarkers, indicating reduced but still notable exposure and effects relative to smokers as reported by Song et al. [46].

In comparing e-cigarette users to conventional smokers, both groups demonstrated elevated levels of inflammatory markers, yet the profiles differed. Conventional smokers exhibited higher IL-RA levels, which act to inhibit IL-1 receptor activation and reduce inflammation, potentially as an adaptive response to prolonged exposure to a broader array of toxicants in cigarette smoke [47]. On the other hand, e-cigarette users experienced a distinct cytokine elevation pattern, likely influenced by the unique constituents of e-cigarette aerosols, including particulate matter, heavy metals, and volatile organic compounds. These constituents might provoke localized inflammation, particularly in the respiratory and oral cavities, more intensely than systemic effects seen in conventional smokers [48, 49].

When compared to non-smokers, e-cigarette users showed a dramatic increase in cytokine levels, indicating that even the absence of traditional tobacco exposure does not shield users from significant inflammatory responses. Non-smokers typically exhibit low baseline cytokine levels, so the substantial increase observed in e-cigarette users underscores the harmful potential of e-cigarettes, even in individuals with no prior smoking history as indicated by Miluna et al. [50]. This finding highlights the need to challenge the perception of e-cigarettes as a safe or benign alternative to smoking, as the inflammatory profiles suggest otherwise.

This systematic review and meta-analysis possess several strengths that bolster its validity and relevance. The comprehensive approach to data collection, including an extensive search of multiple databases and citation

analyses, ensured the inclusion of relevant studies from various regions and settings. Adherence to rigorous guidelines, such as the Cochrane Handbook and PRISMA protocols, reinforced the methodological robustness. The use of standardized tools like the Joanna Briggs Institute's risk of bias assessment and the GRADE method enhanced the credibility of the findings. The inclusion of both systemic and salivary cytokines provided a comprehensive analysis of inflammatory responses, offered valuable insights into the health risks posed by e-cigarette use.

However, certain limitations must be acknowledged. Significant heterogeneity across studies posed challenges in synthesizing results and interpreting findings. Variability in study designs, populations, and cytokine measurement methodologies contributed to this heterogeneity, necessitating the use of random-effects models. Additionally, the limited number of eligible studies may restrict the generalizability of findings. The review's reliance on published data introduces potential publication bias, as indicated by asymmetrical funnel plots. The inability to access some full-text articles and the exclusion of studies with incomplete cytokine data may have introduced selection bias. Furthermore, the cross-sectional nature of most studies limits causal inferences, necessitating caution in interpreting results. Future research, particularly longitudinal studies, is essential to address these gaps and provide a comprehensive understanding of the long-term inflammatory effects of e-cigarette use.

Future research in the field of e-cigarette use and its impact on immune function should focus on several key areas. Longitudinal studies are needed to track cytokine levels and health outcomes over extended periods, helping to understand the long-term risks associated with e-cigarette use. Additionally, studies exploring the molecular mechanisms by which e-cigarette components influence cytokine expression and immune pathways could provide essential insight at a cellular level. Finally, intervention studies examining the effects of cessation or reduction in e-cigarette use on cytokine levels could help inform public health strategies and clinical guidelines for managing the health risks associated with these products.

In conclusion, this review highlighted the significant inflammatory burden associated with e-cigarette use, challenging the notion of these devices as harmless alternatives to traditional smoking. In this review, we analyzed studies evaluating the systemic and salivary cytokine profiles among e-cigarette users, conventional smokers, and non-smokers. IL-6, TNF-α, and IL-8 emerged as the most studied pro-inflammatory cytokines, consistently found at elevated levels in e-cigarette users compared to both traditional smokers and non-smokers. A meta-analysis of the included studies revealed that e-cigarette users exhibited significantly higher levels of multiple pro-inflammatory cytokines, including IL-1A, IL-1B, IL-6, and IL-8, compared to non-smokers, while IL-RA was elevated among conventional smokers and non-smokers. These findings suggest that e-cigarette use induces a distinct inflammatory response, which may contribute to systemic inflammation and disease risk.

These findings challenge the notion that e-cigarettes are harm reduction tools to conventional smoking and highlight the need for robust public health interventions to mitigate these risks. Further research is essential to fully elucidate the long-term health consequences of e-cigarette use and to inform evidence-based policy and regulatory actions.

Author Contribution Statement

All authors have read the manuscript and gave their final approval and agree to be accountable for all aspects of the work. Each author believes that the manuscript represents honest work. ALB: Study concept and design; analysis and interpretation of data; drafting of the manuscript; critical revision of the manuscript. DD: Study concept and design; analysis and interpretation of data; drafting of the manuscript. JRS: Drafting of the manuscript; critical revision of the manuscript. RA: Analysis and interpretation of data; Meta-analysis and critical revision of the manuscript.

Acknowledgements

This manuscript is not part of any student thesis and hence is not submitted for any scientific body for approval.

Ethics approval and consent to participate

This was a systematic review and no ethical approval is required.

Availability of data and materials

The datasets are available from the corresponding author on reasonable request.

Protocol registration

This study protocol is listed in the International Prospective Register of Systematic Reviews (PROSPERO) - CRD42024571203. Available from: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42024571203

Conflict of interest

The authors declare that they have no competing interests.

Abbreviations

E-cigarettes - Electronic cigarettes

PICO - Population-Intervention-Comparators-Outcomes

GRADE - Grading of Recommendations Assessment, Development and Evaluation

PROSPERO - International Prospective Register of Systematic Reviews

ENDS - Electronic Nicotine Delivery System

ENNDS - Electronic Non-Nicotine Delivery System

WHO - World Health Organization

IL - Interleukin

TNF-α - Tumor Necrosis Factor-α

PRISMA - Preferred Reporting Items for Systematic Review and Meta-Analyses

SPSS - Statistical Package for Social Sciences

SMD - Standardized Mean Difference
CI - Confidence Interval
PATH - Population Assessment of Tobacco and Health
JBI_MAStARI - Joanna Briggs Institute Meta-Analysis for Statistics Assessment and Review Instrument for cross-sectional studies
IARC - International Agency for Research on Cancer
HEDTC - Health and Environment, Toxicology and Disease Collaboration
INF- γ – Interferon Gamma
CRP - C-Reactive Protein
IL-RA - Interleukin-1 Receptor Antagonist
TGF-β - Transforming Growth Factor-β
ROS - Reactive Oxygen Species

References

1. National Center for Chronic Disease Prevention and Health Promotion (US) Office on Smoking and Health. E-Cigarette Use Among Youth and Young Adults: A Report of the Surgeon General [Internet]. Atlanta (GA): Centers for Disease Control and Prevention (US); 2016.
2. DeVito EE, Krishnan-Sarin S. E-cigarettes: Impact of E-Liquid Components and Device Characteristics on Nicotine Exposure. Curr Neuropharmacol. 2018;16(4):438-59. https://doi.org/10.2174/1570159X15666171016164430.
3. Chhina MS. Are e-cigarettes a safer alternative to reduce incidences of oral cancer? Evid Based Dent. 2024;25(1):13-4. https://doi.org/10.1038/s41432-023-00956-7.
4. Peralta AR, Guntur VP. Safety and efficacy of electronic cigarettes: a review. Mo Med. 2014;111(3):238-44.
5. Cameron A, Meng Yip H, Garg M. e-Cigarettes and Oral Cancer: what do we know so far? Br J Oral Maxillofac Surg. 2023;61(5):380-2. https://doi.org/10.1016/j.bjoms.2023.03.013.
6. Amalia B, Kapoor S, Sharma R, Singh RJ. E-cigarette retailer storefront availability following a nationwide prohibition of e-cigarettes in India: A multicentric compliance assessment. Tob Prev Cessat. 2020;6:42. https://doi.org/10.18332/tpc/123822.
7. WHO global report on trends in prevalence of tobacco use 2000–2030. Geneva: World Health Organization; 2024.
8. Smith MJ, Hilton S. Youth's exposure to and engagement with e-cigarette marketing on social media: a UK focus group study. BMJ Open. 2023;13(8):e071270. https://doi.org/10.1136/bmjopen-2022-071270.
9. Vrinten C, Parnham JC, Radó MK, Filippidis FT, Vamos E, Laverty AA. Associations of social media use with smoking and e-cigarettes: a national longitudinal study. Lancet. 2023;402 Suppl 1:S91. https://doi.org/10.1016/S0140-6736(23)02125-6.
10. Heidt C, Dal MS, Graen L, Ouédraogo N, Schaller K. Tobacco and e-cigarette promotion on social media: the case of German rap music. Tob Control. 2024:tc-2024-058683. https://doi.org/10.1136/tc-2024-058683.
11. Smith MJ, Buckton C, Patterson C, Hilton S. User-generated content and influencer marketing involving e-cigarettes on social media: a scoping review and content analysis of YouTube and Instagram. BMC Public Health. 2023;23(1):530. https://doi.org/10.1186/s12889-023-15389-1.
12. Auschwitz E, Almeda J, Andl CD. Mechanisms of E-Cigarette Vape-Induced Epithelial Cell Damage. Cells. 2023;12(21):2552. https://doi.org/10.3390/cells12212552.
13. Danella EB, Costa de Medeiros M, D'Silva NJ. Cytokines secreted by inflamed oral mucosa: implications for oral cancer progression. Oncogene. 2023;42(15):1159-65. https://doi.org/10.1038/s41388-023-02649-y.
14. Martin EM, Clapp PW, Rebuli ME, Pawlak EA, Glista-Baker E, Benowitz NL, et al. E-cigarette use results in suppression of immune and inflammatory-response genes in nasal epithelial cells similar to cigarette smoke. Am J Physiol Lung Cell Mol Physiol. 2016;311(1):L135-44. https://doi.org/10.1152/ajplung.00170.2016.
15. Khan NA, Lawyer G, McDonough S, Wang Q, Kassem NO, Kas-Petrus F, et al. Systemic biomarkers of inflammation, oxidative stress and tissue injury and repair among waterpipe, cigarette and dual tobacco smokers. Tob Control. 2020;29(Suppl 2):s102-9. https://doi.org/10.1136/tobaccocontrol-2019-054958.
16. Wilson C, Tellez Freitas CM, Awan KH, Ajdaharian J, Geiler J, Thirucenthilvelan P. Adverse effects of E-cigarettes on head, neck, and oral cells: A systematic review. J Oral Pathol Med. 2022;51(2):113-25. https://doi.org/10.1111/jop.13273.
17. Chiamulera MMA, Zancan CB, Remor AP, Cordeiro MF, Gleber-Netto FO, Baptistella AR. Salivary cytokines as biomarkers of oral cancer: a systematic review and meta-analysis. BMC Cancer. 2021;21(1):205. https://doi.org/10.1186/s12885-021-07932-3.
18. Roxlau ET, Pak O, Hadzic S, Garcia-Castro CF, Gredic M, Wu CY, et al. Nicotine promotes e-cigarette vapour-induced lung inflammation and structural alterations. Eur Respir J. 2023;61(6):2200951. https://doi.org/10.1183/13993003.00951-2022.
19. Lee YH, Na HG, Choi YS, Bae CH, Song SY, Kim YD. E-cigarettes exacerbate allergic inflammation via cytokine induction and MUC5AC/5B expression in a murine asthma model. Environ Toxicol Pharmacol. 2024;107:104395. https://doi.org/10.1016/j.etap.2024.104395.
20. Gallagher KP, Vargas PA, Santos-Silva AR. The use of E-cigarettes as a risk factor for oral potentially malignant disorders and oral cancer: a rapid review of clinical evidence. Med Oral Patol Oral Cir Bucal. 2024;29(1):e18-e26. https://doi.org/10.4317/medoral.26042.
21. Alqutaibi AY, Alharbi AT, Alaydaa RM. Risks of E-cigarettes on oral health and increased susceptibility to oral cancer. J Taibah Univ Med Sci. 2024;19(6):1098-9. https://doi.org/10.1016/j.jtumed.2024.11.005.
22. Escobar YH, Morrison CB, Chen Y, Hickman E, Love CA, Rebuli ME, et al. Differential responses to e-cig generated aerosols from humectants and different forms of nicotine in epithelial cells from nonsmokers and smokers. Am J Physiol Lung Cell Mol Physiol. 2021;320(6):L1064-73. https://doi.org/10.1152/ajplung.00525.2020.
23. Higgins JPT, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, et al. Cochrane Handbook for Systematic Reviews of Interventions version 6.5. Cochrane, 2024. Available from www.training.cochrane.org/handbook.
24. 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. https://doi.org/10.1136/bmj.n71.
25. Systematic reviews of etiology and risk. JBI Manual for Evidence Synthesis - JBI Global Wiki [Internet]. [cited 2024 Sep 30]. Available from: https://jbi-global-wiki.refined.site/space/MANUAL/4687372/Chapter+7%3A+Systematic+reviews+of+etiology+and+risk
26. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med. 2002;21(11):1539-58. https://doi.org/10.1002/sim.1186.
27. Schünemann H, Brożek J, Guyatt G, Oxman A. GRADE Handbook [Internet]. [cited 2024 Sep 30]. 2013; Available

from: https://gdt.gradepro.org/app/handbook/handbook.html.
28. AlMubarak AM, Alqutub MN, Javed F, Vohra F, Abduljabbar T. Whole Salivary Cotinine Levels and Interleukin 1-β Levels among Young Adults Involuntarily Exposed to Vapor from Electronic Nicotine Delivery Systems. Oral Health Prev Dent. 2022;20:127-32. https://doi.org/10.3290/j.ohpd.b2805483.
29. Christensen CH, Chang JT, Rostron BL, Hammad HT, van Bemmel DM, Del Valle-Pinero AY, et al. Biomarkers of Inflammation and Oxidative Stress among Adult Former Smoker, Current E-Cigarette Users-Results from Wave 1 PATH Study. Cancer Epidemiol Biomarkers Prev. 2021;30(10):1947-55. https://doi.org/10.1158/1055-9965.EPI-21-0140.
30. Alhumaidan AA, Al-Aali KA, Vohra F, Javed F, Abduljabbar T. Comparison of Whole Salivary Cortisol and Interleukin 1-Beta Levels in Light Cigarette-Smokers and Users of Electronic Nicotine Delivery Systems before and after Non-Surgical Periodontal Therapy. Int J Environ Res Public Health. 2022;19(18):11290. https://doi.org/10.3390/ijerph191811290.
31. Ashford K, McCubbin A, Rayens MK, Wiggins A, Dougherty K, Sturgill J, Ickes M. ENDS use among college students: Salivary biomarkers and persistent cough. Addict Behav. 2020;108:106462. https://doi.org/10.1016/j.addbeh.2020.106462.
32. Samet JM, Chiu WA, Cogliano V, Jinot J, Kriebel D, Lunn RM, et al. The IARC Monographs: Updated Procedures for Modern and Transparent Evidence Synthesis in Cancer Hazard Identification. J Natl Cancer Inst. 2020;112(1):30-7. https://doi.org/10.1093/jnci/djz169.
33. Dooley EE. EHPnet: Collaborative on Health and the Environment Toxicant and Disease Database. Environ Health Perspect. 2006;114(9):A523.
34. Hickman E, Payton A, Duffney P, Wells H, Ceppe AS, Brocke S, et al. Biomarkers of Airway Immune Homeostasis Differ Significantly with Generation of E-Cigarettes. Am J Respir Crit Care Med. 2022;206(10):1248-58. https://doi.org/10.1164/rccm.202202-0373OC.
35. Verma A, Anand K, Bhargava M, Kolluri A, Kumar M, Palve DH. Comparative Evaluation of Salivary Biomarker Levels in e-Cigarette Smokers and Conventional Smokers. J Pharm Bioallied Sci. 2021;13(Suppl 2): S1642-5. https://doi.org/10.4103/jpbs.jpbs_393_21.
36. Faridoun A, Sultan AS, Jabra-Rizk MA, Weikel D, Varlotta S, Meiller TF. Salivary biomarker profiles in e-cigarette users and conventional smokers: a cross-sectional study. Oral Dis. 2021;27(2):277-9. https://doi.org/10.1111/odi.13533.
37. Ganapathy V, Jaganathan R, Chinnaiyan M, Chengizkhan G, Sadhasivam B, Manyanga J, et al. E-Cigarette Effects on Oral Health: A Molecular Perspective. Food Chem Toxicol. 2024:115216. https://doi.org/10.1016/j.fct.2024.115216.
38. Tzortzi A, Kapetanstrataki M, Evangelopoulou V, Beghrakis P. A Systematic Literature Review of E-Cigarette-Related Illness and Injury: Not Just for the Respirologist. Int J Environ Res Public Health. 2020;17(7):2248. https://doi.org/ 10.3390/ijerph17072248.
39. Thomas SC, Xu F, Pushalkar S, Lin Z, Thakor N, Vardhan M et al. Electronic Cigarette Use Promotes a Unique Periodontal Microbiome. mBio. 2022;13(1):e0007522. https://doi.org/10.1128/mbio.00075-22.
40. Dolatabadi A, Noori F, Raee A. Effect of conventional cigarettes and e-cigarettes on salivary biomarkers: A systematic review. J Adv Periodontol Implant Dent. 2024;16(1):44-48. https://doi.org/10.34172/japid.2024.006.
41. Czekala L, Simms L, Stevenson M, Tschierske N, Maione AG, Walele T. Toxicological comparison of cigarette smoke and e-cigarette aerosol using a 3D in vitro human respiratory model. Regul Toxicol Pharmacol. 2019;103:314-324. https://doi.org/10.1016/j.yrtph.2019.01.036.
42. Chen IL, Todd I, Tighe PJ, Fairclough LC. Electronic cigarette vapour moderately stimulates pro-inflammatory signalling pathways and interleukin-6 production by human monocyte-derived dendritic cells. Arch Toxicol. 2020;94(6):2097-112. https://doi.org/10.1007/s00204-020-02757-8.
43. Niklander SE. Inflammatory Mediators in Oral Cancer: Pathogenic Mechanisms and Diagnostic Potential. Front Oral Health. 2021;2:642238. https://doi.org/10.3389/froh.2021.642238
44. Caruntu A, Scheau C, Codrici E, Popescu ID, Calenic B, Caruntu C, Tanase C. The Assessment of Serum Cytokines in Oral Squamous Cell Carcinoma Patients: An Observational Prospective Controlled Study. J Clin Med. 2022;11(18):5398. https://doi.org/10.3390/jcm11185398.
45. Ali D, Kuyunov I, Baskaradoss JK, Mikami T. Comparison of periodontal status and salivary IL-15 and -18 levels in cigarette-smokers and individuals using electronic nicotine delivery systems. BMC Oral Health. 2022;22(1):655. https://doi.org/10.1186/s12903-022-02700-6.
46. Song MA, Freudenheim JL, Brasky TM, Mathe EA, McElroy JP, Nickerson QA et al. Biomarkers of Exposure and Effect in the Lungs of Smokers, Nonsmokers, and Electronic Cigarette Users. Cancer Epidemiol Biomarkers Prev. 2020;29(2):443-51. https://doi.org/10.1158/1055-9965.EPI-19-1245.
47. Bastías D, Maturana A, Marín C, Martínez R, Niklander SE. Salivary Biomarkers for Oral Cancer Detection: An Exploratory Systematic Review. Int J Mol Sci. 2024;25(5):2634. https://doi.org/10.3390/ijms25052634.
48. Shinriki S, Jono H, Ota K, Ueda M, Kudo M, Ota T et al. Humanized anti-interleukin-6 receptor antibody suppresses tumor angiogenesis and in vivo growth of human oral squamous cell carcinoma. Clin Cancer Res. 2009;15(17):5426-34. https://doi.org/10.1158/1078-0432.CCR-09-0287.
49. Yuan SF, Wang YM, Chan LP, Hung AC, Nguyen HDH, Chen YK et al. IL-1RA promotes oral squamous cell carcinoma malignancy through mitochondrial metabolism-mediated EGFR/JNK/SOX2 pathway. J Transl Med. 2023;21(1):473. https://doi.org/10.1186/s12967-023-04343-9.
50. Miluna S, Melderis R, Briuka L, Skadins I, Broks R, Kroica J, Rostoka D. The Correlation of Swedish Snus, Nicotine Pouches and Other Tobacco Products with Oral Mucosal Health and Salivary Biomarkers. Dent J (Basel). 2022;10(8):154. https://doi.org/10.3390/dj10080154.