Reviewing the oral carcinogenic potential of E-cigarettes using the Bradford Hill criteria of causation

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Abstract: The past decade has seen a surge in the use of e-cigarettes, which has prompted the medical community to assess any associated potential health hazards. A major concern was the risk of cancer. Chemical analysis of e-cigarettes has shown the presence of volatile organic compounds with the potential for carcinogenicity. Comparative toxicology analysis has shown e-cigarette to have relatively lower dosages of toxins than conventional combustible cigarettes. Based on comparative analysis, e-cigarettes have been increasingly advocated as a safe alternative to conventional cigarettes. It is vital to recognize that presence of relatively lower toxin level does not preclude carcinogenic potential. The nicotine present in the e-cigarette was presumed to be the major cytotoxic agents, thus nicotine-free e-cigarette was considered as inert. On the contrary, experimental studies on oral cell lines have shown DNA strand breaks on exposure to e-cigarette vapors with or without nicotine. In addition, dysregulations of genes associated with carcinogenic pathways have also been demonstrated in oral tissues exposed to e-cigarette vapors. Despite alarming molecular data, the oral carcinogenic potential of e-cigarette remains unclear, which can be attributed to the lack of long-term prospective and large-scale case-control studies. As e-cigarette users often have other well-established risk factors (conventional cigarette smoking, alcohol, etc.) as associated habits, it is difficult to assess e-cigarette as an independent risk factor for oral cancer. Thus, the present manuscript aims to review the published literature using the Brad Ford Hill criteria of causation to determine the oral carcinogenic potential of e-cigarettes.

Keywords: Carcinogens; cigarette smoking; electronic nicotine delivery systems; mouth neoplasms; volatile organic compounds

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Introduction

Tobacco is a well-established independent risk factor for oral cancer (1-7). Tobacco when consumed in combination with alcohol, increases the oral cancer risk exponentially (8-13). Tobacco is mainly consumed by smoking or chewing. The most common forms of smoking tobacco are cigarettes, pipes, shisha, and bidis, while pan masala, mawa, gutka, zarda, toombak, khaini, Shammah and naswar represent the common forms of smokeless/chewed tobacco (14-17). Given the overwhelming evidence of cancer risk associated with both smokeless and smoking form of tobacco, large scale restrictions are sanctioned by health agencies all over
the globe (18,19). Tobacco products are mandated to carry warnings indicating the health hazards associated with their use. Like alcohol, the sale of tobacco products has been forbidden to individuals under the legal age (20). Further, smoking in public is restricted to prevent health hazards associated with secondary smoking (21,22). Governments have constantly increased the tax on tobacco products, to increase its base price with the intention of reducing its sales (23,24). Health agencies have regularly conducted tobacco cessation and awareness programs to deter present and future tobacco users (25,26).

Based on the above-mentioned large-scale strategies employed by government and health agencies, one may think that the tobacco industry may have significantly reduced its productivity due to the decreasing sales. On the contrary, the tobacco industry has found innovative means to rejuvenate its sales. The major strategy employed by the tobacco industry included the introduction of newer products, marketed to be safe alternatives to conventional tobacco products (27,28). One such innovation is the electronic cigarette (e-cigarette). The introduction of e-cigarettes ensured a steady product flow. Unlike most commercial tobacco products, there is not much information as to the effects of e-cigarettes on health, especially with respect to cancer. Thus, without sufficient evidence, implementing anti-tobacco regulations is problematic. Sustained widespread marketing of e-cigarettes as a safe alternative to conventional tobacco products has large scale repercussions. In addition to conventional smokers shifting to e-cigarettes, even previous non-smokers are predisposed to vaping (28,29). E-cigarettes have been advocated along with nicotine patches as conventional tobacco replacement products, which in turn created a false sense of safety among its users (30-32).

Given the increasing global use of e-cigarettes, researchers have focused on understanding their short and long-term effects. Examination of the components of various brands of e-cigarettes has shown the presence of several well-established carcinogenic agents (33-41). In addition to this, studies have also investigated the molecular changes induced by e-cigarettes on the oral mucosa. The results have been alarming with reports ranging from reduced anti-oxidants levels, gene dysregulations to DNA strand breaks (42-46). There were a growing number of oral health hazards associated with e-cigarettes, with potential links to oral cancer (42-63). To implement preventive measures, conclusive evidence of e-cigarette induced oral carcinogenesis is required. Thus, the present review uses the Bradford Hill criteria of causation to assess the oral carcinogenic potential of e-cigarette based on published literature. Bradford Hill's criteria of causation consist of a total of 9 parameters (64-66). All 9 parameters must be satisfied to infer a causal designation to e-cigarette for oral cancer.

Strength of association

Epidemiological studies are considered to be preliminary evidence for a causal relationship. Based on the prevalence of a potential risk factor in a specific disease, a hypothesis for causal inference is generated (64). The major limitation in evaluating the strength of association in multifactorial diseases like cancer is the presence of confounding factors (66). E-cigarette users are often current or past users of conventional cigarettes and have also shown to exhibit other known risk factors such as alcohol consumption (67). Thus, in such cases, the strength of association (statistical significance) from the epidemiological data must be evaluated only after accounting for the potential confounders. Longitudinal observational studies on e-cigarettes have a relatively shorter follow up period, given that research focus on e-cigarette began to rise just a decade ago (60,61). Most observational studies on e-cigarettes involve comparative analysis of toxic products released and their potential health impacts (52,68-73). The comparison groups have ranged from only e-cigarette users, only combustible cigarette users, to dual users (both e-cigarette and combustible cigarette users) (52,68-70). In some studies, instead of having multiple comparison groups, the comparison is made in the same individual as their habit is changed from conventional cigarette to e-cigarette (71-73). Although most of these observational studies varied in their methodologies, the common consensus was that the toxic product dosage and health hazards were relatively less in e-cigarettes than combustible cigarette users and dual users (52,68-70). In studies involving a shift in smoking habits, health hazards, and toxicity decreased after replacing combustible smoking with e-cigarettes (71-73). Some of the assessed toxic products are proven or potential carcinogenic agents, and thus although their dosages have shown to be less in e-cigarette users than combustible cigarette users, they still could pose a cancer risk.

Table S1 summarizes the published data on the in-vitro, in-vivo, and clinical assessment of e-cigarettes, along with their various comparison groups (74-84). In-vitro studies on e-cigarettes have largely focussed on assessing the
carcinogenic constituents of the cigarette and analyzing their effects on the oral cell lines. The mutagenic potential of e-cigarette was examined by Thorne et al. (74) on Salmonella typhimurium strains TA98 and TA100. The results showed that the aerosol collected matter from the e-cigarette did not have any mutagenic effect in either of the test strains. In comparison, the conventional cigarette (3R4F cigarette) exhibited mutagenicity within 24 hours.

Although most of the above-mentioned studies have shown that the toxicity of e-cigarette is substantially lower than conventional cigarettes, it is not clear if the reduced toxicity of e-cigarette could be carcinogenic to the oral mucosa. In addition, it was believed that the nicotine in the e-cigarette vapor was the major cause for toxicity, and thus a nicotine-free e-cigarette could potentially be inert. To test this hypothesis, Yu et al. (43) examined the effects of vapor from e-cigarettes with and without nicotine on cell lines of normal oral cells and head and neck squamous cell carcinoma. The results showed that irrespective of the presence or absence of nicotine, the vapors from the e-cigarettes induced cell death through apoptosis or necrosis and caused DNA strand breaks. Evidence of DNA strand breaks from Yu et al. study was supported by Kadimisetty et al. (39), and Holliday et al. (44). Although it is unclear if the DNA strand breaks were sufficient for carcinogenic transformation, there is sufficient evidence to indicate that e-cigarettes with or without nicotine cannot be considered to be inert.

Unlike a combustible cigarette, there is a lack of literature on the effects of e-cigarette on the oral mucosa. Tommasi et al. (42) compared the gene regulation and pathways of molecular pathogenesis on the oral cells of users of only conventional cigarette and only e-cigarette with non-smokers. The results showed that similar to conventional cigarette smokers, even e-cigarette users showed dysregulation of genes involved in carcinogenic pathways. Although the e-cigarette has shown to be relatively less harmful than a combustible cigarette, most of these studies just evaluated the levels of toxic exposure. Studies analyzing the effects of these toxins on the oral cells including that of Yu et al. (43), Holliday et al. (44) and Tommasi et al. (42) provide a strong case for the carcinogenic potential of e-cigarettes. The evidence of DNA strand breaks in-vitro cell line study and dysregulation of genes involved in carcinogenesis provides the necessary strength of association for considering e-cigarette to be a potential oral carcinogenic risk factor (42-44). Further studies required to strengthen the association would include decoding the genomic, proteomic, and secretome profile of oral keratinocytes exposed to e-cigarette vapors. In addition, longitudinal studies including patients with only e-cigarette habits with no prior history of any other oral cancer-associated risk habits could aid in assessing e-cigarettes as an independent risk factor.

**Consistency**

Consistency according to Bradford Hill refers to similar results obtained from different techniques in different populations that will enhance the causal relationship (64). As mentioned above, the preliminary evidence for the cause is derived from epidemiological data. The advent of molecular biology has allowed data integration between preliminary epidemiological evidence with molecular carcinogenic pathways, which provides comprehensive evidence for a causal relationship. Although individual case reports/series do not carry the same weight as long term prospective or large-scale case-control studies, in the absence of large-scale epidemiological data, preliminary evidence from case reports could be used to formulate a hypothesis. Nguyen et al. (48) reported 2 cases of oral squamous cell carcinoma (OSCC) associated with the chronic use of e-cigarettes. In both cases, there has been no other associated habits/ factor.

Despite the lack of convincing clinicopathological evidence, studies have shown considerable molecular data. Tommasi et al. (42) observed upregulation of 857 transcripts (74.4%) and downregulation of 295 transcripts (25.6%) in oral epithelium exposed to e-cigarette vapors. Molecular pathways and functional analysis revealed a 62% association of e-cigarettes with oral cancer. Wnt/Af pathway was indicated as a major route for E-cigarettes induced oral carcinogenesis. Gene dysregulation and the DNA strand breaks induced by e-cigarettes on oral cells provide additional molecular evidence for the toxic nature of e-cigarettes on the oral mucosa (39,43,44). In addition, e-cigarette vapors have shown to contain variable levels of aldehyde carbonyls which are known to cause oxidative stress, DNA adduct damage, stress-induced cellular senescence which in turn have shown to induce carcinogenesis (45,46). E-cigarette exposure mediated upregulation of RAGE increases prostaglandins and COX2 in gingival epithelial cells have also been implicated in carcinogenesis (85). Sundar et al. (45) reported e-cigarette induced oxidative stress, carbonyl stress, DNA damage, HDCA 3 reduction, increased inflammatory response in gingival epithelial cells which could potentially lead to
malignant transformation. Based on the above data, there is consistent evidence for e-cigarette induced carcinogenicity to the oral mucosa.

Specificity

Due to the lack of long-term clinical studies on the use of e-cigarettes, there is a scarcity of direct evidence for oral malignant transformation. The added difficulty in specifically associating e-cigarettes to oral cancer is due to the wide range of newer generations of e-cigarettes being produced. The newer generation products have different designs patterns from existing models, with adjustable voltages and flavorings (86). In such cases, the best way to assess the carcinogenic potential of the different e-cigarette designs would be to assess the common ingredient present in their vapor. Over 115 volatile components have been found in e-cigarette aerosol (87). Potential e-cigarette carcinogens include metals (cadmium, chromium, etc.), carbonyls (acrolein), propylene oxide, and especially flavoring additives. The concentration of chemicals depends on many factors. Higher voltage is associated with a 10-fold increase in the release of inflammatory cytokines, which in turn have shown to increase the reactive oxygen species (ROS) causing significant DNA damage (88). Flavoring chemicals like vanillin, ethyl vanillin, ethyl maltol, and menthol and toxic aldehydes (acetaldehyde, formaldehyde) have been found to cause DNA strand breaks as demonstrated by increased comet tail length and γ-H2AX foci numbers. These effects are regardless of nicotine concentrations and are observed in both short term and long term (40,43). It is increasingly accepted that ROS formed due to the vaporization of such liquids are probably responsible for carcinogenesis (37,46). Another laboratory assay showed that refill solutions were cytotoxic, especially to stem cells, irrespective of voltage (41). A rat model study showed phase I carcinogen bio-activation and DNA damage at both chromosomal and molecular levels (50). Based on the presence of known carcinogenic agents, there is sufficient proof for specificity. Future studies must focus on long term prospective studies on individuals with only e-cigarette habits. Malignant oral mucosal changes in such individuals would in turn provide additional support for specificity.

Temporality

The scarcity of longitudinal studies on e-cigarettes renders temporal causation analysis a tedious job. An alternative approach would be to analyze the association between e-cigarettes and oral potentially malignant disorders (OPMDs) (66). Since there is an abundance of literature and conventional acceptance regarding the temporal link of OPMDs and oral cancer, an analysis of e-cigarette and OPMDs would hold relevance (89). However, studies with OPMDs and e-cigarettes are also scarce. A Google Scholar and PubMed search using he terms “oral potentially malignant disorders/premalignant lesions and e-cigarettes” did not reveal any relevant results. A possible link between e-cigarette use and oral submucous fibrosis (OSMF) was discussed by Javed et al. (53). The combined exposure of nicotine and arecoline (from associated chewing of areca nut) was shown to induce keratinocyte senescence and fibroblast proliferation. Based on the results, the authors implied that malignant transformation of OSMF may be further induced/accelerated by nicotine from the e-cigarettes.

Apart from OPMDs, there are other less established oral mucosal changes with an increased risk for oral cancer. Some of these oral mucosal changes have shown to be induced by the components of e-cigarettes which in turn could be used as evidence for temporality. Renne et al. (54) assessed the effect of aerosolized glycerol inhalation in rats. The exposure to propylene glycol resulted in the development of squamous metaplasia of the epiglottis. A similar study in rats revealed that exposure to acrolein, acetaldehyde, and formaldehyde caused sensory irritation (55). A crossover trial by Bullen et al. (90) reported that the most frequent adverse effects of e-cigarette included mouth and throat irritation. Persistent local irritation may result in pathological mucosal reactions that may predispose to the development of potentially malignant oral disorders. A systematic review by Farsalinos et al. (57) found that even though such clinical adverse effects have been found, they were considerably lower than conventional smoking. Lack of carcinogenic evidence on e-cigarettes was supported by data from another systematic review by Pisinger et al. (58). Based on the 76 studies analyzed in their systematic review, Pisinger et al. (58) stated that there were no significant adverse effects in e-cigarette smokers that might lead to oral cancer. Although there is evidence to indicate that e-cigarette contains potentially carcinogenic agents, its presence seems to be relatively lower than conventional cigarettes as supported by the lack of oral cancer association from the systematic reviews (57-59). On the contrary, there is also evidence to show e-cigarette induced molecular
changes (39,42-44) could potentially lead to cancer. As e-cigarettes are a recent innovation, it might be too soon to comment on a temporal association with cancer, but the evidence of molecular dysregulation and DNA strand breaks cannot be unnoticed. Long term perspective research in individuals with only e-cigarette habit is needed for gaining temporal association for the e-cigarette with oral cancer.

**Biological gradient**

In epidemiology, increased exposure of a potential risk factor results in an increased incidence of the disease, which in turn can be used for establishing a causal inference (64). In multifactorial diseases like cancer, the dose-response curve is non-linear due to the presence of confounding factors (66). Biological gradients are difficult to characterize in such cases due to individual susceptibility (genetic predisposition) and synergistic or antagonistic effects of cumulative exposures. In cases of oral cancer with e-cigarette usage, there is often a concomitant use of other known risk factors including combustible cigarettes, thus, estimating a biological gradient in such cases would not reveal the true dose-response pattern. Modern analytics have shown that molecular changes can occur even in the low-dose range of environmental chemical exposures, although these changes may not lead to observable disease. Thus, even though studies have shown e-cigarette to have relatively lower dosages of toxic components than conventional cigarettes (52,68-73), even these lower dosages of toxin could induce molecular changes in the oral mucosa with no apparent clinical change. Future studies on e-cigarette exposure must include a complete molecular panel of proteomic, genomic, and, secretome analysis to elicit the subtle changes induced by e-cigarettes. At present, there is a paucity of research related to the direct physiological effect of the e-cigarette vapors on oral tissues. Apart from studies demonstrating e-cigarette induced DNA strand breaks (43,44) in oral cells and eliciting the presence of few dysregulated genes (39,42), there are no comprehensive biological gradient studies to elicit a dose-response relationship between e-cigarettes and oral cancer.

**Biological plausibility**

For a potential causal agent to be implicated in a specific disease, there must be a defined etiopathogenesis (64). Since the advent of molecular biology, several carcinogenic pathways have been delineated for several cancers, including oral cancer. Thus, establishing an e-cigarette mediated pathway for oral carcinogenesis would aid in supporting a causal inference. The first step in assessing biological plausibility would be to establish the presence of carcinogens in e-cigarettes. As mentioned earlier, a chemical analysis of e-cigarette aerosol has revealed more than 115 volatile components (87) including metals (cadmium, chromium, etc.), carbonyls (acrolein), propylene oxide, flavoring additives. The second part would be to establish that the carcinogenic compounds come in contact with the area of interest and that the area of interest has reported evidence of cancer. Over 90% of all human cancers are of epithelial origin (91,92) and the oral epithelium is the first site of exposure to carcinogens present in e-cigarette vapor. The third part would be to confirm that the agents released by the e-cigarette vapors have a detrimental effect on the oral mucosa at the clinical, histopathological and molecular levels. At a clinical and histopathological level, there is not much evidence, other than that e-cigarette users develop oral mucosal changes which in turn may carry a risk for oral cancer (49,56,93). In contrary to clinicopathological evidence, there is a relatively greater number of studies assessing the molecular changes induced by e-cigarettes in the oral cells. Several studies including that of Yu et al. (43) have confirmed the e-cigarette induced DNA strand breaks in oral cells. In addition, whole transcriptome analysis of oral cells from e-cigarette users, have shown deregulation of key genes, the majority of which converging on cancer-related pathways and functions (94-96). The final part of biological plausibility would require decoding the e-cigarettes mediated molecular pathways of oral carcinogenesis. Based on the above evidence, it is biologically plausible that e-cigarette causes molecular changes in oral cells, but it is not clear if the changes lead to malignant transformation.

**Coherence**

For causal inference, the risk factor in question must be associated with the disease consistently across studies with different research designs (64). Thus, data from epidemiological studies must be coherent with data from other research design including in-vivo, in-vitro experimental studies. The E-cigarette has shown to have carcinogenic components and their vapor on exposure to oral cells has shown to cause changes DNA strand breaks and gene dysregulations (33-45). DNA double-strand breaks are the most lethal form of DNA damage, and if left
unrepaired, they can result in chromosomal rearrangement and could potentially lead to carcinogenesis. The major cause of conflict in the evidence for an e-cigarette mediated oral carcinogenesis is the studies eliciting that replacing the conventional combustible cigarette with e-cigarettes reduces the overall toxicity (52,68-73). Although e-cigarette carries a lower toxin level than a combustible cigarette, even the lower levels of e-cigarettes toxins could potentially carry an oral cancer risk. Based on the evidence, there is coherence in the evidence for the carcinogenic potential of e-cigarettes.

**Experiment**

The mere presence of a potential risk factor in a disease entity does not confer a causal status. It is possible that the disease predisposes accumulation of the agent in question, which could, in turn, explain its high prevalence in the disease entity. Thus, even a strong epidemiological association is not sufficient for causal inference. Conclusive evidence would require experimental studies exposing the potential risk factor to a physiological tissue and inducing the disease status. In addition, removal of the potential risk factor must result in regression of the disease, unless the disease has progressed to an irreversible state as in malignancy. Although the presence of carcinogenic components in e-cigarettes and their detrimental effects (DNA strand breaks and gene dysregulation) (39-43) on oral cells provides considerable evidence of carcinogenicity, there are no longitudinal studies that have shown that e-cigarette cessation reverses or reduces the disease state. Further, in multifactorial diseases like cancer, the associated e-cigarette might be a coincidental factor and the other known risk factors (combustible cigarettes, alcohol, etc.) could have been the causal agent (17,27,67). Thus, clinical experimental studies need to be specific in their inclusion criteria wherein patients with only e-cigarette history are included. Such patients should be observed for carcinogenic molecular changes during and post-cessation of e-cigarette usage.

**Analogy**

In circumstances where accurate measurement of the confounding factors and quantification of exposure may be difficult, clear cut analogy could shed light to of evidence for potential causative factors, that are otherwise considered as weak association, provided such factors possess similar property/condition as that of the factor with a stronger association with the disease (64). In other words, when there is enough evidence to substantiate causal relationship with a specific agent and disease, a second agent that is similar in some aspects with weaker evidence of association with the disease can also be established as a causal association. It is well-established that combustion of tobacco in regular cigarettes releases toxins and carcinogenic molecules which can cause oral cancer. In comparison, e-cigarette also possesses such carcinogenic agents, although at a lesser concentration than combustible cigarettes (52,68-73). Exposure of oral cells to combustible cigarettes have shown to cause carcinogenic molecular changes. Similar molecular changes (DNA strand breaks and gene dysregulation) have also been observed in oral cells exposed to e-cigarette vapor (39-43). The additional information, which is missing in e-cigarettes studies and, which confirms the carcinogenicity of combustible cigarette is molecular data delineating the various oral carcinogenic pathways and clinical data eliciting reduction of potentially malignant lesions following habit cessation. Applying the concept of analogy, it can be hypothesized that e-cigarettes having carcinogenic agents and capable of inducing DNA strand breaks (43,44) and gene dysregulation (39,42) in oral cells could also be implicated in oral cancer.

**Conclusions**

Lack of long term prospective and large-scale case-control studies is a major limiting factor in assessing the association of e-cigarettes with oral cancer. In addition, comparative analysis suggesting a relatively lower toxic level in e-cigarette than combustible cigarettes, in turn, creates a misconceived notion on the safety of e-cigarette use. It is important to acknowledge that a relatively lower toxic dosage, does not mean it is risk-free. Evidence of the presence of carcinogenic agents in e-cigarettes and their vapor inducing DNA strand breaks and gene dysregulation is compelling evidence, but not sufficient to infer a causal relationship. Experimental studies are needed to assess if the relatively lower dosage of toxin released by the e-cigarette is capable of inducing malignant transformation in oral epithelial cell lines and mouse models. Further delineating the molecular pathway of e-cigarette induced oral mucosal changes could provide valuable insight into e-cigarettes’ oral carcinogenic potential and would also aid in identifying diagnostic markers and therapeutic targets. In addition, longitudinal studies with
longer follow up periods are needed, wherein the included cases must be only e-cigarette users, with no history any other associated risk factor. Evidence of malignant mucosal changes in such cases would provide the necessary clinical evidence for a causal association, although, still genetic predisposition would be a confounding factor. To conclude, based on the evidence from the published literature, the e-cigarette can be considered as a potential risk factor for oral cancer due to the presence of carcinogenic components and due to their ability to induce detrimental changes to oral cells, although there is insufficient evidence for causal inference.

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References

1. International Agency for Research on Cancer (IARC). IARC Monographs on the Evaluation of Carcinogenic Risk in Humans. Tobacco Smoke and Involuntary Smoking. Volume 83. Lyon, France: IARC Press; 2004.
2. Gandini S, Botteri E, Iodice S, et al. Tobacco smoking and cancer: a meta-analysis. Int J Cancer. 2008;122:155-64.
3. Guha N, Warnakulasuriya S, Vlaanderen J, et al. Betel quid chewing and the risk of oral and oropharyngeal cancers: a metaanalysis with implications for cancer control. Int J Cancer 2014;135:1433-43.
4. Gupta B, Johnson NW. Systematic review and meta-analysis of association of smokeless tobacco and of betel quid without tobacco with incidence of oral cancer in South Asia and the Pacific. PLoS One 2014;9:e113385.
5. Merchant AT, Pitiphat W. Total, direct, and indirect effects of paan on oral cancer. Cancer Causes Control 2015;26:487-91.
6. Song H, Wan Y, Xu YY. Betel quid chewing without tobacco: a meta-analysis of carcinogenic and precarcinogenic effects. Asia Pac J Public Health 2015;27:NP47-57.
7. Patil S, Arakeri G, Alamir AWH, et al. Is Toombak a risk factor for oral leukoplakia and oral squamous cell carcinoma? A systematic review. J Oral Pathol Med 2020;49:103-9.
8. Blot WJ, McLaughlin JK, Winn DM, et al. Smoking and drinking in relation to oral and pharyngeal cancer. Cancer Res 1988;48:3282-7.
9. Mashberg A, Boffetta P, Winkelman R, et al. Tobacco smoking, alcohol drinking, and cancer of the oral cavity and oropharynx among U.S. veterans. Cancer 1993;72:1369-75.
10. De Stefani E, Boffetta P, Deneo-Pellegrini H, et al. The effect of smoking and drinking in relation to oral and pharyngeal cancers: A case-control study in Uruguay. Cancer Lett 2007;246:282-9.
11. Znaor A, Brennan P, Gajalakshmi V, et al. Independent and combined effects of tobacco smoking, chewing and alcohol drinking on the risk of oral, pharyngeal and esophageal cancers in Indian men. Int J Cancer 2003;105:681-6.
12. International Agency for Research on Cancer (IARC). Section 2.2. Cancer of the oral cavity and pharynx. In: IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, editors. IARC Monographs on the
Evaluation of Carcinogenic Risks to Humans. Alcohol Consumption and Ethylcarbamate. Volume 96. Lyon, France: IARC Press; 2010:237-329.

13. Raj AT, Patil S, Awan KH, et al. Odds Ratio for Oral Cancer is directly Proportional to the Number of associated Habits. World J Dent 2017;8:351.

14. Asthana S, Labani S, Kailash U, et al. Association of smokeless tobacco use and oral cancer: A systematic global review and meta-analysis. Nicotine Tob Res 2019;21:1162-71.

15. Raj AT, Patil S, Sarode SC, et al. Systematic reviews and meta-analyses on smokeless tobacco products should include Shammah. Nicotine Tob Res 2019;21:1147.

16. Raj AT, Patil S, Awan KH. Waterpipe Smoking: A Traditional Health Hazard Passed Through Generations. Dent Med Res 2019;7:1-2.

17. Kong G, Creamer MR, Simon P, et al. Systematic review of cigars, cigarillos, and little cigars among adolescents: Setting research agenda to inform tobacco control policy. Addict Behav 2019;96:192-7.

18. Lempert LK, Glantz SA. Heated tobacco product regulation under US law and the FCTC. Tob Control 2018;27:s118-25.

19. Combes RD, Balls M. A critical assessment of the scientific basis, and implementation, of regulations for the safety assessment and marketing of innovative tobacco-related products. Altern Lab Anim 2015;43:251-90.

20. Morain SR, Malek J. Minimum Age of Sale for Tobacco Products and Electronic Cigarettes: Ethical Acceptability of US "Tobacco 21 Laws". Am J Public Health 2017;107:1401-5.

21. Cummings KM, Proctor RN. The changing public image of smoking in the United States: 1964-2014. Cancer Epidemiol Biomarkers Prev 2014;23:32-6.

22. Centers for Disease Control and Prevention (CDC). State smoking restrictions for private-sector worksites, restaurants, and bars--United States, 2004 and 2007. MMWR Morb Mortal Wkly Rep 2008;57:549-52.

23. Brock B, Choi K, Boyle RG, et al. Tobacco product prices before and after a statewide tobacco tax increase. Tob Control 2016;25:166-73.

24. Marsh L, Cameron C, Quigg R, et al. The impact of an increase in excise tax on the retail price of tobacco in New Zealand. Tob Control 2016;25:458-63.

25. Biener L, Nyman AL, Stepanov I, et al. Public education about the relative harm of tobacco products: an intervention for tobacco control professionals. Tob Control 2014;23:385-8.

26. Odukoya OO, Chife JO, Odeyemi KA, et al. Young peoples awareness and support for tobacco control legislation: A study among in-school youth in Lagos, Nigeria. Nig Q J Hosp Med 2015;25:193-201.

27. Hirschhorn N. Evolution of the tobacco industry positions on addiction to nicotine. Tobacco free initiative Available from: Geneva: World Health Organization; 2008.

28. Raj AT, Patil S, Gupta AA, et al. Flavored tobacco to E-cigarette’s: How the tobacco industry sustains its product flow. Oral Oncol 2018;85:110.

29. Hammond D, Reid JL, Rynard VL, et al. Prevalence of vaping and smoking among adolescents in Canada, England, and the United States: repeat national cross sectional surveys. BMJ 2019;365:l2219.

30. McRobbie H, Bullen C, Hartmann-Boye J, et al. Electronic cigarettes for smoking cessation and reduction. Cochrane Database Syst Rev 2014;(12):CD010216.

31. Bullen C, Howe C, Laugesen M, et al. Electronic cigarettes for smoking cessation: a randomised controlled trial. Lancet 2013;382:1629-37.

32. Bullen C, Williman J, Howe C, et al. Study protocol for a randomised controlled trial of electronic cigarettes versus nicotine patch for smoking cessation. BMC Public Health 2013;13:210.

33. Cheng T. Chemical evaluation of electronic cigarettes. Tob Control 2014;23 Suppl 2:i11-7.

34. Goniewicz ML, Knysak J, Gawron M, et al. Levels of selected carcinogens and toxicants in vapour from electronic cigarettes. Tob Control 2014;23:133-9.

35. Kosmider L, Sobczak A, Fik M, et al. Carbonyl compounds in electronic cigarette vapors: effects of nicotine solvent and battery output voltage. Nicotine Tob Res 2014;16:1319-26.

36. Jensen RP, Luo W, Pankow JF, et al. Hidden formaldehyde in e-cigarette aerosols. N Engl J Med 2015;372:392-4.

37. Lerner CA, Sundar IK, Yao H, et al. Vapors produced by electronic cigarettes and e-juices with flavorings induce toxicity, oxidative stress, and inflammatory response in lung epithelial cells and in mouse lung. PLoS One 2015;10:e0116732.

38. Williams M, Villarreal A, Bozhilov K, et al. Metal and silicate particles including nanoparticles are present in electronic cigarette cartomizer fluid and aerosol. PLoS One 2013;8:e57987.

39. Kadimisetty K, Malla S, Rusling JF. Automated 3-D Printed Arrays to Evaluate Genotoxic Chemistry: E-Cigarettes and Water Samples. ACS Sens 2017;2:670-8.

40. Khlystov A, Samburova V. Flavoring compounds dominate
toxic aldehyde production during E-cigarette vaping, Environ Sci Technol 2016;50:13080-5.

41. Behar RZ, Wang Y, Talbot P. Comparing the cytotoxicity of electronic cigarette fluids, aerosols and solvents. Tob Control 2018;27:325-33.

42. Tommasi S, Caliri AW, Caceres A, et al. Deregulation of Biologically Significant Genes and Associated Molecular Pathways in the Oral Epithelium of Electronic Cigarette Users. Int J Mol Sci 2019;20:1-18.

43. Yu V, Rahimy M, Korrapati A, et al. Electronic cigarettes induce DNA strand breaks and cell death independently of nicotine in cell lines. Oral Oncol 2016;52:58-65.

44. Holliday R, Kist R, Bauld L. E-cigarette vapour is not inert and exposure can lead to cell damage. Evid Based Dent 2016;17:2-3.

45. Sundar IK, Javed F, Romanos GE, et al. E-cigarettes and flavorings induce inflammatory and pro-senescence responses in oral epithelial cells and periodontal fibroblasts. Oncotarget 2016;7:77196-204.

46. Lerner CA, Sundara IK, Watsonb RM, et al. Environmental Health Hazards of e-Cigarettes and their Components: Oxidants and Copper in e-cigarette aerosols. Environ Pollut 2015;198:100-7.

47. Sultan AS, Jessri M, Farah CS. Electronic nicotine delivery systems: Oral health implications and oral cancer risk. J Oral Pathol Med 2018. [Epub ahead of print].

48. Nguyen H, Kitzmiller JP, Nguyen KT, et al. Oral Carcinoma Associated with Chronic Use of Electronic Cigarettes. Otolaryngol (Sunnyvale) 2017;7:304.

49. Bardellini E, Amadori F, Conti G, et al. Oral mucosal lesions in electronic cigarettes consumers versus former smokers. Acta Odontol Scand 2018;76:226-8.

50. Canistro D, Vivarelli F, Cirillo S, et al. E-cigarettes induce toxicological effects that can raise the cancer risk. Sci Rep 2017;7:2028.

51. Cravo AS, Bush J, Sharma G, et al. A randomised, parallel group study to evaluate the safety profile of an electronic vapour product over 12 weeks. Regul Toxicol Pharmacol 2016;81:S1-14.

52. Goniewicz ML, Gawron M, Smith DM, et al. Exposure to Nicotine and Selected Toxicants in Cigarette Smokers Who Switched to Electronic Cigarettes: A longitudinal Within-Subjects Observational Study. Nicotine Tob Res 2017;19:160-7.

53. Javed F, Kellesarian SV, Sundar IK, et al. Recent updates on electronic cigarette aerosol and inhaled nicotine effects on periodontal and pulmonary tissues. Oral Dis 2017;23:1052-7.
69. Lorkiewicz P, Riggs DW, Keith RJ, et al. Comparison of Urinary Biomarkers of Exposure in Humans Using Electronic Cigarettes, Combustible Cigarettes, and Smokeless Tobacco. Nicotine Tob Res 2019;21:1228-38.
70. Shahab L, Goniewicz ML, Blount BC, et al. Nicotine, Carcinogen, and Toxin Exposure in Long-Term E-Cigarette and Nicotine Replacement Therapy Users: A Cross-sectional Study. Ann Intern Med 2017;166:390-400.
71. O’Connell G, Graff DW, D’Ruiz CD. Reductions in biomarkers of exposure (BoE) to harmful or potentially harmful constituents (HPHCs) following partial or complete substitution of cigarettes with electronic cigarettes in adult smokers. Toxicol Mech Methods 2016;26:443-54
72. D’Ruiz CD, Graff DW, Robinson E. Reductions in biomarkers of exposure, impacts on smoking urge and assessment of product use and tolerability in adult smokers following partial or complete substitution of cigarettes with electronic cigarettes. BMC Public Health 2016;16:543.
73. Pulvers K, Emami AS, Nollen NL, et al. Tobacco Consumption and Toxicant Exposure of Cigarette Smokers Using Electronic Cigarettes. Nicotine Tob Res 2018;20:206-14.
74. Thorne D, Crooks I, Hollings M, et al. The mutagenic assessment of an electronic-cigarette and reference cigarette smoke using the Ames assay in strains TA98 and TA100. Mutat Res 2016;812:29-38.
75. Taylor M, Carr T, Oke O, et al. E-cigarette aerosols induce lower oxidative stress in vitro when compared to tobacco smoke. Toxicol Mech Methods 2016;26:465-76
76. Misra M, Leverette RD, Cooper BT, et al. Comparative In Vitro Toxicity Profile of Electronic and Tobacco Cigarettes, Smokeless Tobacco and Nicotine Replacement Therapy Products: E-Liquids, Extracts and Collected Aerosols. Int J Environ Res Public Health 2014;11:11325-47.
77. Breheny D, Oke O, Pant K, et al. Comparative Tumor Promotion Assessment of e-Cigarette and Cigarettes Using the In Vitro Bhass 42 Cell Transformation Assay. Environ Mol Mutagen 2017;58:190-8.
78. Taylor M, Jaunky T, Hewitt K, et al. A comparative assessment of e-cigarette aerosols and cigarette smoke on in vitro endothelial cell migration. Toxicol Lett 2017;277:123-8.
79. Tommasi S, Bates SE, Behar RZ, et al. Limited mutagenicity of electronic cigarettes in mouse or human cells in vitro. Lung Cancer 2017;112:41-6.
80. Cuadra GA, Smith MT, Nelson JM, et al. A Comparison of Flavorless Electronic Cigarette-Generated Aerosol and Conventional Cigarette Smoke on the Survival and Growth of Common Oral Commensal Streptococci. Int J Environ Res Public Health 2019;16:1-14.
81. Clemens MM, Cardenas VM, Fischbach LA, et al. Use of electronic nicotine delivery systems by pregnant women II: Hair biomarkers for exposures to nicotine and tobacco-specific nitrosamines. Tob Induc Dis 2019;17:50.
82. Lee HW, Parka SH, Wenga M, et al. E-cigarette smoke damages DNA and reduces repair activity in mouse lung, heart, and bladder as well as in human lung and bladder cells. Proc Natl Acad Sci U S A 2018;115:E1560-9.
83. Bustamante G, Ma B, Yakovlev G, et al. Presence of the Carcinogen N’-Nitrosonornicotine in Saliva of E-cigarette Users. Chem Res Toxicol 2018;31:731-8.
84. Ganapathy V, Manyanga J, Brane L, et al. Electronic cigarette aerosols suppress cellular antioxidant defenses and induce significant oxidative DNA damage. PLoS One 2017;12:e0177780.
85. Nasry WHS, Rodriguez-Lecompte JC, Martin CK. Role of COX-2/PGE2 Mediated Inflammation in Oral Squamous Cell Carcinoma. Cancers (Basel) 2018;10:E348.
86. Seidenberg AB, Jo CL, Ribisl KM. Differences in the design and sale of e-cigarettes by cigarette manufacturers and non-cigarette manufacturers in the USA. Tob Control 2016;25:e3-5.
87. Jones DM, Majeed BA, Weaver SR, et al. Prevalence and Factors Associated with Smokeless Tobacco Use, 2014-2016. Am J Health Behav 2017;41:608-17.
88. Wallis SP, Stafford ND, Greenman J. Clinical relevance of immune parameters in the tumor microenvironment of head and neck cancers. Head Neck 2015;37:449-59.
89. Speight PM, Khurram SA, Kujan O. Oral potentially malignant disorders: risk of progression to malignancy. Oral Surg Oral Med Oral Pathol Oral Radiol 2018;125:612-27.
90. Bullen C, McRobbie H, Thornley S, et al. Effect of an electronic nicotine delivery device (e cigarette) on desire to smoke and withdrawal, user preferences and nicotine delivery: randomised cross-over trial. Tob Control 2010;19:98-103.
91. Ali J, Sabiha B, Jan HU, et al. Genetic etiology of oral cancer. Oral Oncol 2017;70:23-8.
92. Peng M, Pang C. MicroRNA-140-5p inhibits the tumorigenesis of oral squamous cell carcinoma by targeting p21 activated kinase 4. Cell Biol Int 2019. [Epub ahead of print].
93. Etter JF. Electronic cigarettes: a survey of users. BMC...
Public Health 2010;10:231.
94. Kupfer DM, White VL, Jenkins MC, et al. Examining smoking-induced differential gene expression changes in buccal mucosa. BMC Med Genomics 2010;3:24.
95. Boyle JO, Gumus ZH, Kacker A, et al. Effects of cigarette smoke on the human oral mucosal transcriptome. Cancer Prev Res (Phila) 2010;3:266-78.
96. García-Closas M, Egan KM, Abruzzo J, et al. Collection of genomic DNA from adults in epidemiological studies by buccal cytobrush and mouthwash. Cancer Epidemiol Biomarkers Prev 2001;10:687-96.

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In-vitro assessment of the cellular stress response of the human bronchial epithelial cells to the comparative groups

E-cigarette aqueous aerosol extract (AAE); tobacco smoke AAE

E-cigarette AAE concentration up to 0.5 µg/mL did not elicit any cellular stress-related response. Tobacco smoke AAE concentration of 0.065 µg/mL elicited cellular stress-related responses.

In-vitro assessment of toxicity induced on Salmonella typhimurium by the comparative groups

E-cigarette vapor

The toxic effect induced by the e-cigarette aerosol in the form of polycyclic aromatic hydrocarbons activation increased production of oxygen free radical production, DNA strand breaks, micronucleus formation, point mutations.

A clinical study comparing tobacco-related toxicant concentration of the comparative groups

E-cigarette users; combustible cigarette users; past users of combustible cigarettes and NRT replacement therapy (NRT); dual users of combustible cigarettes and NRT

The switch did not cause change in levels of nicotine and some polycyclic aromatic hydrocarbons activation increased production of oxygen free radical production, DNA strand breaks, micronucleus formation, point mutations.

A clinical study comparing the tobacco-related toxicant concentration between the comparative groups

E-cigarette users; combustible tobacco cigarette users; dual users; never smokers

The highest toxicity was observed for dual users followed by combustible tobacco users, and e-cigarette users. E-cigarette users had significantly lower levels of volatile organic compounds (VOC) nicotine, metal, tobacco-specific nitroamines, and polycyclic aromatic hydrocarbons.

A clinical study comparing the potentially harmful constituents in the comparative groups

E-cigarette; combustible cigarette; smokeless tobacco; never users

Compared to never users, e-cigarette users had a higher level of cyanide, sylphox, styrine, and ethylbenzene. VOC, nicotine levels of E-cigarette users were lower than combustible cigarette users. E-cigarette users had a higher level of sylphox, N-nitrodimethylamine, and acrylonitrile than smokeless tobacco users.

A clinical study comparing the BOE in the comparative groups

Past users of combustible cigarette switched to e-cigarettes but was higher for individuals switched to E-cigarettes

In-vitro assessment of genotoxicity on oral streptococci by the comparative groups

E-cigarette electron-dense (e-)nicotine; cigarette smoke

Mutagenicity up to 2,400 µg/plate and 1 L/min dilution (for 3 h) respectively.

A clinical study assessing the gene expression of the Bhas 42 mouse fibroblast cells by the comparative groups

E-cigarette aqueous extract; E-cigarette smoke extract

E-cigarette aerosol caused DNA damage and suppressed the antioxidant defenses of the cells. The extract increased reactive oxygen species and reduced total antioxidant capacity. The level of presence of 8-oxo-dG (highly oxidized guanine) was higher for the aerosol than the extract.