SUBMITTED 21 FEB 22
REVISIONS REQ. 10 MAY & 1 JUN 22; REVISIONS RECD. 12 MAY & 12 JUN 22
ACCEPTED 15 JUN 22
ONLINE-FIRST: JUNE 2022
DOI: https://doi.org/10.18295/squmj.6.2022.043

Cytotoxic and Genotoxic Effects of Waterpipe on Oral Health Status

A systematic review and meta-analysis

***Ricardo Grillo,[1] Mehdi Khemiss,[2] Yuri Slusarenko da Silva[3]**

[1]Department of Oral and Maxillofacial Surgery, Faculdade São Leopoldo Mandic, Campinas, Brazil; [2]Department of Dental Medicine, Fattouma Bourguiba University Hospital, University of Monastir, Monastir, Tunisia; [3]Department of Oral & Maxillofacial Surgery, UniFG University Center, Guanambi, Brazil.
**Corresponding Author's e-mail: doutorgrillo@uol.com.br*

Abstract

A worldwide increase in waterpipe consumption can be observed. The present systematic review aims to assess cytotoxic and genotoxic impacts on oral health related to waterpipe smoking. We searched MEDLINE, Cochrane Library, and Dimensions evaluating if waterpipe smokers (P) have any cytotoxic or genotoxic effects on oral cells (I) compared to non-smokers (C) regarding mouth neoplasms (O). PRISMA guidelines were adopted for the current systematic review. Review Manager was utilized for statistical analysis ($p < 0.05$). A risk of bias and summary were performed to assess the grade of the 20 included articles. With some of the articles included, a forest plot was created in different levels. Waterpipe smoking is harmful to oral health, causing cytotoxic and genotoxic effects on oral cells with a Risk Difference of 0.16. It causes a series of detrimental cellular and genetic modifications such as acanthosis, epithelial dysplasia, and hyperparakeratosis. Changes in DNA methylation and p53 expression were assessed among others. In addition, waterpipe has a bunch of carcinogenic compounds. Even with few publications on the subject, articles are very devastating in confirming the carcinogenicity of waterpipe smoking. Waterpipe smoke is cytotoxic and

genotoxic. Due to the release of many organic compounds, it increases the incidence of oral cancer.

Keywords: Mouth Neoplasms; Oral Health; Smoking Water Pipes; Tobacco Use; Toxicity Measure.

Introduction

Tobacco may be smoked in different ways. Waterpipe is one form of tobacco use that has been gaining popularity during the last decades. A systematic review conducted in 2018 showed that waterpipe use prevalence was alarmingly high in the Eastern Mediterranean and European regions, especially among youth.[1]

The waterpipe smoke contains a wide range of carcinogens such as naphthylamines, tobacco-specific nitrosamines, polycyclic aromatic hydrocarbons, primary aromatic amines, and carbon monoxide carbonyls like formaldehyde, acetaldehyde, or acrolein [2]. Waterpipe use has been associated with DNA damage and cell death, and these genotoxicity and cytotoxicity are involved in oral carcinogenesis [3]. Laboratory-based investigations have shown various genomic and transcriptomic alterations previously categorized in various cancers [4]. In fact, Walters et al. [5] observed changes in DNA methylation at 727 locations in the genome. DNA methylation may predispose the cells to cancer by activating specific genes and repressing others [6]. It also plays a significant role in metastasis [7]. In addition, nuclear changes in the oral mucosa cells of waterpipe smokers (WS) were reported [8]. These changes occur in the early stages of cancer and may be used as biomarkers to screen oral dysplastic and malignant lesions [9].

However, the contribution of waterpipe use to the development of oral cancer is not well-established [2]. Furthermore, the few available studies on this topic were not focused explicitly on oral cancers [10]. Systematic reviews determine whether scientific evidence is consistent and can be generalized across populations [11]. Therefore, this study aimed to systematically review the scientific literature regarding the cytotoxic or genotoxic effects of waterpipe smoking on oral mucosal cells.

Materials and Methods

A systematic review and meta-analysis were conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [12]. This study protocol was registered in the PROSPERO database.

Cytotoxic and genotoxic original studies were considered in this systematic review. In addition, inclusion criteria were considered: a) inclusion of waterpipe usual users; b) any cytotoxic or genotoxic effects; c) comparison to group control.

The first hit was conducted online by two independent reviewers (RG and MK) in MEDLINE (via PubMed), Cochrane Library, Health Virtual Library (BVS) and Dimensions from inception until Dec 12th of 2021. Terms selected in the primary articles selected to justify this review were combined with Boolean operators (OR / AND), answering the acronym PICO (population, intervention, control, and outcome). The following strategy was used: ((((hookah) OR (shisha) OR (waterpipe) OR ("waterpipe") OR (narghile)))) AND (((oral) OR (oral health) OR (dental) OR (buccal))) NOT (systematic review). PECO acronym to be answered was: Do waterpipe smokers (P) have any cytotoxic or genotoxic effects on oral cells (I) compared to non-smokers (C) regarding mouth neoplasms (O)?

Exclusion criteria were: 1. clinical changes, and 2. radiographic modifications, 3. Studies performed out of head, face and neck region, and 4. Animal studies. Comparative studies but with no conclusion specific to waterpipe toxicity were excluded. Studies that met the inclusion criteria or those with doubtful information either in the title or abstract were selected for full-text assessment in this review's second round.

Two different reviewers (RG and MK) independently extracted the following data from the included studies for analysis: year of study, demographic data, cytotoxic and/or genotoxic effect, waterpipe, and control group sizes. Any discrepancies were resolved by consensus. In the case of persistence, arbitration was performed by a third author (Y.S.S.). Alteration such as micronuclei, pyknosis, karyorrhexis and karyolysis were discussed.

To assess the studies' quality, the risk of bias was assessed according to the Quality Assessment Tool for Diagnosis Accuracy Studies (QUADAS-2) [13]. The results were used in Review Manager Software 5.4 (Review Manager (RevMan) [Computer program]. Version 5.4. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014). Results were considered statistically significant with a 95% confidence interval.

The QUADAS-2 Tool was assessed through risk of bias and risk of applicability across studies to evaluate the following questions: (1) Patient selection: description of patient selection and inclusion; (2) Index text: description of the index test, its conduction and interpretation; (3) Reference standard: description of the reference standard, its conduction and interpretation; (4) Flow and timing of ach included article: description of the patient who did not receive the index test or reference standard and who were excluded.

Results

The first bibliographic search redeemed 346 records from databases. BVS have returned no result. Duplicates were removed, remaining 181 articles. After screen reading and excluding paper unrelated to our search, 38 remained. Reports from the same authors/co-authors or same study center were excluded [14–19] as such reviews, comments, letters, hypotheses, and expert opinions [3,20–26]. Two exclusive microbiological studies were removed [27,28], same to animal studies [29,30]. One study was not found [31]. Manual search has retrieved no additional paper. The searched records distribution and the number of studies finally selected are shown in the flow diagram (Fig. 1).

The full text of all data sets viewed from the first round was independently checked for the same reviewers'eligibility. A total of 20 articles were included in this review. Studies included authors, year of publication, demographic data, cytotoxic or genotoxic evaluation, and the number of patients in waterpipe and control groups are in Table 1.

Three studies were in vitro [4,32,33], and biological samples were obtained from patients in 17 studies [8,31,33-37,39-48]. In addition, the levels of pro-inflammatory cytokines, the receptor activator of nuclear factor-κB (RANKL), and osteoprotegerin were evaluated [34–36].

Six studies investigated the genotoxic effect of waterpipe smoke [4,32,33,37–39]. A comet assay was performed in one study [33]. Cell line-based models were used to understand the mechanisms of action of waterpipe smoke on oral cells [4,32]. Immortalized non-transformed normal human oral keratinocytes (OKF6/TERT1) chronically (eight months) exposed to waterpipe smoke were developed by Patil et al. [4]. When phenotypic alterations were studied, they revealed genomic anomalies in OKF6/TERT1-waterpipe cells, with some overexpressed and some downregulated genes. In the other study that developed a cell line-based model, two human normal oral epithelial were treated with 100g/L of waterpipe smoke solution for two days [32]. When examined, both cells became more elongated and showed decreased cell-cell contact compared to untreated ones. This epithelial-mesenchymal transition was accompanied by the deregulation of a set of genes related to oncogenesis [32].

On the other hand, eight studies evaluated nuclear changes in cytology samples from the buccal mucosa of patients. Some pathological assessments were performed, including micronuclei (DNA aggregates separate from the primary nucleus), karyorrhexis (nuclear fragmentation), karyolysis (complete dissolution of nuclear components), pyknosis (shrinkage or condensation of a cell), acanthosis (benign abnormal thickening of the stratum spinosum), hyperparakeratosis (abnormal keratinization of the epidermal stratum coreum), and epithelial dysplasia (architectural and cytological epithelial changes).

The mean of micronuclei, cell nucleus perimeter, and area was contrasting in the WS group compared to NS one [8,38–42]. In addition, the mean percentages of karyorrhexis, karyolysis, and pyknosis had substantial changes [43–45]. Other histopathologic changes like acanthosis, hyperparakeratosis, and epithelial dysplasia were associated with waterpipe use. An increased oral cancer incidence were related to different types of tobacco use [46,47].

Waterpipe smoke was associated with changes in DNA methylation [37]. In fact, about 64% of global DNA methylation was detected in DNA samples isolated from WS compared to NS. In addition, promoter methylation of the *MLH1* gene was observed in the oral epithelium of the WS group [37].

The tumor suppressor protein p53 mutations were also associated with waterpipe use [48,49]. This alteration could lead to apoptosis, suppression of the cell cycle, senescence, differentiation, and DNA repair [48].

A meta-analysis was carried out with RevMan 5.4. A forest plot created only with RevMan was possible with different levels of variation. This happened because articles use different cells to assess cytotoxicity and genotoxicity in different ways (Fig.2). Of the 20 articles included, nine rated genotoxicity and 11 rated cytotoxicity. The evidence on literature is that waterpipe smoke causes several cytotoxic and genotoxic effects on oral cells with a risk difference (RD) of 0.16 (95% CI 0.09-0.23, $P < 0.00001$).

The graph for the risk of bias (Fig. 3) was created with RevMan 5.4 using the QUADAS-2 protocol. The high quality of the items can be seen in this picture. Articles come from all over the world, mainly from the Middle East (13), three multicentre studies, two from Africa and one each from Europe and South America. This can be explained by the higher and more frequent consumption of waterpipe in the Middle East.

Discussion

Although waterpipe use is a world-spread epidemic, several included studies are possible to note a colossal concern in Middle East countries where waterpipe is smoke is very usual [32,40,48].

Waterpipe smoke condensate reveals many organic compounds like nicotine, tar, heavy metals, polycyclic aromatic hydrocarbons (naphthalene, phenanthrene, fluoranthene), aldehydes (5-hydroxymethyl-5-furancarboxaldehyde, 3-ethoxy-4-hydroxybenzaldehyde), moreover carbon monoxide, well-known substances for their genotoxic and carcinogenic properties [33,40,43]. Formaldehyde was detected in waterpipe five times higher than in one 2R4F cigarette [33]. A 2R4F cigarette is a standard reference cigarette. The tobacco industry and academic laboratories uses this reference to standardize test items and inhalation toxicity research.

High values for all critical comet assay parameters (a sensitive technique for DNA damage detection) in buccal cells were found, suggesting waterpipe use is composed of

DNA-damaging ingredients [32,33,37,38,44]. For example, DNA methylation could reach increases of 64% [37]; samples with 10% methylation are considered significantly methylated.

One technique to evaluate the impact of environmental factors on genetic stability is the investigation of micronucleus, products of early events in human carcinogenic processes, especially on the oral cavity; it is considered a biomarker of genotoxicity [41]. Total micronuclei (TMN) and cells with micronuclei (CMN) were significantly higher among waterpipe users than never smokers and very similar to cigarette-smokers [8,41,49]. Furthermore, there was no association between TMN and CMN with lifetime duration of use, time to first waterpipe smoke of the day, and the number of hagars per day or week [8]. Waterpipe use was also related to chromosomal aberrations [33] and enhanced level of micronuclei [8,33,38–41].

In waterpipe smoke mixtures, mutagenic and genotoxic contaminants are present on low levels, but they are challenging to be detected since a few components are in high concentrations [33]. In addition, genotoxicity is not related to a specific compound but a set of properties and chemical interactions of the sample as a whole [33]. Waterpipe use is related to genomic and gene expression alterations, both RNA [4,32] and DNA [33,37,44].

Waterpipe use increased the risk of histopathologic changes, including acanthosis, epithelial dysplasia, hyperparakeratosis, and the development of abnormal rete ridges [46]. Acanthosis and epithelial dysplasia in WS were similar to cigarette-smokers (CS) [46]. Cytomorphometric quantitative analysis showed higher values for waterpipe users than in NS, including nuclear and cell perimeter, cytoplasm size, cell area, nuclear-cytoplasmic ratio, and relation big diameter of nucleus/small diameter of nucleus ratio [42,45] besides induction of heterochromatinization of cell nuclei, a situation caused by different stress factors [42,44]. There is an increase in many multinucleated cells, pyknosis, karyorrhexis, karyolysis than in NS [38,39,43–45] and slightly higher than in CS [38,43]. Higher incidence of vacuolization of cytoplasm concerning NS and even CS [45]. Malignant and pre-malignant lesions have a nuclear-cytoplasmic ratio increase.

Pro-inflammatory cytokine levels (Interleukin-1β, Interleukin-6, Interleukin-3, and Tumor Necrosis Factor-α) were statistically higher among waterpipe-users when

compared to NS and similar to another kind of tobacco users [32,34–36]. Cell necrosis and apoptosis have a higher relation to carcinomas.

Protein p53 expression has a relation to regulation of apoptosis and genomic stability, a crucial role in tumor suppression, named "guardian of the genome". WS have a significantly higher p53 mutation than non-smokers [48,49] in samples with malignant, pre-malignant, or even normal oral epithelium. This correlation is similar to CS group [49]. In addition, the repair index of oral mucosa cells of WS is significantly lower than in NS [39]. It must be said that the cytotoxic effects of waterpipe are more correlated to time exposure than cigarette smoking [50].

There is no peak incidence in oral cancer on WS regarding age or gender [43,47] although few papers included female samples due to oral mucosa alterations concern hormonal changes, and waterpipe use is much more common in male than female [45,46].

Waterpipe and cigarette users had similar effects on oral mucosa [42,43,46], including a substantial association increase with oral squamous cell carcinoma (OSCC) development [37,43,47–49]. The combination between waterpipe and shammah (Arabian snuff) or waterpipe and cigarettes led to a higher incidence of oral cancer than just one kind of tobacco use [47,49]. The use of waterpipe has more unfavorable effects than smoking cigarettes [38,42]. Waterpipe and Shammah combined use increased the risk of developing OSCC by nearly 35 times [47]. Khat chewing did not show significant impact on the development of oral cancer [47] . However, when associated with waterpipe use, there was an increase of the risk [46]. The cytotoxic effect of waterpipe smoke is more correlated to time exposure than cigarette smoking [41,43,44].

In addition to more restrictive legislation and interventional policy aspects, tobacco cessation programs must be a priority in some regions, consisting of education, psychological therapy, and pharmacological aid [47] especially for young [40,44,48], who believe waterpipe use is a safe addict.

Conclusions

Waterpipe use is genotoxic and cytotoxic with a Risk Difference of 0.16 ($P < 0.05$). It seems to increase the incidence of oral cancer, contrary to popular belief. Furthermore, its carcinogenicity is similar to cigarette smoke.

Conflict of Interest

No conflict to disclose

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Authors' Contribution

RG was involved in conceptualization, design, data collect and analysis and drafting the manuscript. MK and YSS contributed to the design, data collect and analysis and drafting the manuscript. All authors approved the final version of the manuscript.

References

1. Jawad M, Charide R, Waziry R, Darzi A, Ballout RA, Akl EA. The prevalence and trends of waterpipe tobacco smoking: A systematic review. PLoS One. 2018;13(2):e0192191.
2. Bou Fakhreddine HM, Kanj AN, Kanj NA. The growing epidemic of water pipe smoking: Health effects and future needs. Respir Med. 2014;108(9):1241–53.
3. Souza ACF, Galvani MG, de Souza DV, Ribeiro DA. Cytogenetic biomonitoring on oral mucosa cells of Hookah users: Is it possible? Asian Pacific J Cancer Prev. 2020 Jul 1;21(7):1849–1849.
4. Patil S, Patel K, Advani J, Subbannayya T, Rajagopalan P, Babu N, et al. Multiomic analysis of oral keratinocytes chronically exposed to shisha. J Oral Pathol Med. 2019;48(4):284–9.
5. Walters MS, Salit J, Ju JH, Staudt MR, Kaner RJ, Rogalski AM, et al. Waterpipe smoking induces epigenetic changes in the small airway epithelium. PLoS One. 2017;12(3):e0171112.
6. Ringh M V., Hagemann-Jensen M, Needhamsen M, Kular L, Breeze CE, Sjöholm LK, et al. Tobacco smoking induces changes in true DNA methylation,

hydroxymethylation and gene expression in bronchoalveolar lavage cells. EBioMedicine. 2019;46:290–304.

7. Szyf M, Pakneshan P, Rabbani SA. DNA methylation and breast cancer. In: Biochemical Pharmacology. 2004. p. 1187–97.

8. El-Setouhy M, Loffredo CA, Radwan G, Rahman RA, Mahfouz E, Israel E, et al. Genotoxic effects of waterpipe smoking on the buccal mucosa cells. Mutat Res - Genet Toxicol Environ Mutagen. 2008;695(1–2):36–40.

9. Kamboj M, Mahajan S. Micronucleus - An upcoming marker of genotoxic damage. Clin Oral Investig. 2007;11(2):121–6.

10. Maziak W. The waterpipe: An emerging global risk for cancer. Cancer Epidemiol. 2013;37(1):1–4.

11. Mulrow CD. Rationale for systematic reviews. BMJ. 1994;309(6954):597–9.

12. Moher D, Liberati A, Tetzlaff J, Altman DG, Altman D, Antes G, et al. Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. PLoS Med. 2009;6(7):e10000097.

13. Whiting PF, Rutjes AWS, Westwood ME, Mallett S, Deeks JJ, Reitsma JB, et al. Quadas-2: A revised tool for the quality assessment of diagnostic accuracy studies. Ann Intern Med. 2011;155(8):529–36.

14. Patil S, Babu N, Subbannayya T, Mohan S V., Sathe G, Solanki HS, et al. Secretome analysis of oral keratinocytes chronically exposed to shisha. Cancer Biomarkers. 2019;25(1):29–41.

15. Patil S, Rajagopalan P, Patel K, Subbannayya T, Babu N, Mohan S V., et al. Chronic shisha exposure alters phosphoproteome of oral keratinocytes. J Cell Commun Signal. 2019;13(3):281–9.

16. Patil S, Subbannayya T, Mohan S V., Babu N, Advani J, Sathe G, et al. Proteomic Changes in Oral Keratinocytes Chronically Exposed to Shisha (Water Pipe). Omi A J Integr Biol. 2019;23(2):86–97.

17. Dehghannezhad M, Jalayer Naderi N, Semyari H, Naderi NJ, Semyari H. Micronucleus Assay of Buccal Mucosa Cells in Waterpipe (Hookah) Smokers: A Cytologic Study. Iran J Pathol. 2020;15(2):75–80.

18. Ibraheem WI, Fageeh HI, Preethanath RS, Alzahrani FA, Al-Zawawi AS, Divakar DD, et al. Comparison of RANKL and osteoprotegerin levels in the gingival crevicular fluid of young cigarette- and waterpipe-smokers and individuals using electronic nicotine delivery systems. Arch Oral Biol. 2020;115:104714.

19. Patil S, Awan KH, Arakeri G, Aljabab A, Ferrari M, Gomes CC, et al. The relationship of "shisha" (water pipe) smoking to the risk of head and neck cancer. J Oral Pathol Med. 2019 Apr 1;48(4):278–83.
20. Chaouachi K. Micronuclei and shisha/goza smoking in Egypt. Mutat Res - Genet Toxicol Environ Mutagen. 2009;675(1–2):81–2.
21. Yakin M, Gavidi RO, Cox B RA. Oral cancer risk factors in New Zealand. N Z Med J. 2017;130(1451):30–8.
22. Dar NA. Narghile smoking is associated with the development of oral cancer at early age. J Evid Based Dent Pract. 2015 Sep 1;15(3):126–7.
23. Warnakulasuriya S. Waterpipe smoking, oral cancer and other oral health effects. Evid Based Dent. 2011;12(2):44–5.
24. Albeitawi S, Hamadneh J, Al-Shatanawi TN, Al mehaisen L, Al-Zubi M. Effect of hookah (water pipe) smoking on semen parameters. Andrologia. 2020 Nov 1;52(10):e13723.
25. Chaouachi K, Sajid KM. A critique of recent hypotheses on oral (and lung) cancer induced by water pipe (hookah, shisha, narghile) tobacco smoking. Med Hypotheses. 2010;74(5):843–6.
26. Rastam S, Li FM, Fouad FM, Kamal HMA, Akil N, Moustafa AE Al. Water pipe smoking and human oral cancers. Med Hypotheses. 2010;74(3):457–9.
27. Shakhatreh MAK, Khabour OF, Alzoubi KH, Masadeh MM, Hussein EI, Bshara GN. Alterations in oral microbial flora induced by waterpipe tobacco smoking. Int J Gen Med. 2018;11:47–54.
28. Vallès Y, Inman CK, Peters BA, Ali R, Wareth LA, Abdulle A, et al. Types of tobacco consumption and the oral microbiome in the United Arab Emirates Healthy Future (UAEHFS) Pilot Study. Sci Rep. 2018;8(1):11327.
29. Nemmar A, Al-Salam S, Beegam S, Yuvaraju P, Oulhaj A, Ali BH. Water-Pipe Smoke Exposure-Induced Circulatory Disturbances in Mice, and the Influence of Betaine Supplementation Thereon. Cell Physiol Biochem. 2017;41(3):1098–112.
30. Imran S, Shan M, Muazam S. A comparative histological study of submucosal gland hypertrophy in trachea of mice exposed to cigarette and shisha smoke. J Coll Physicians Surg Pakistan. 2018 Mar 1;28(3):192–5.
31. Babu N, Patil S, Mohan S V., Subbannayya T, Advani J, Datta KK, et al. Signaling alterations in oral keratinocytes in response to shisha and crude tobacco extract. J Oral Pathol Med. 2020; 50(5):459-469.

366 32. López-Ozuna VM, Gupta I, Kiow RLC, Matanes E, Kheraldine H, Yasmeen A, et
367 al. Water-pipe smoking exposure deregulates a set of genes associated with
368 human head and neck cancer development and prognosis. Toxics. 2020;8(3):73.
369 33. Al-Amrah HJA, Aboznada OA, Alam MZ, Elassouli MZM, Mujallid MI, Elassouli
370 SM. Genotoxicity of waterpipe smoke in buccal cells and peripheral blood
371 leukocytes as determined by comet assay. Inhal Toxicol. 2014 Dec 1;26(14):891–
372 6.
373 34. AlQahtani MA, Alayad AS, Alshihri A, Correa FOB, Akram Z. Clinical peri-
374 implant parameters and inflammatory cytokine profile among smokers of
375 cigarette, e-cigarette, and waterpipe. Clin Implant Dent Relat Res. 2018 Dec
376 1;20(6):1016–21.
377 35. Abduljabbar T, Akram Z, Vohra F, Warnakulasuriya S, Javed F. Assessment of
378 interleukin-1β, interleukin-6, and tumor necrosis factor-A levels in the peri-
379 implant sulcular fluid among waterpipe (narghile) smokers and never-smokers
380 with peri-implantitis. Clin Implant Dent Relat Res. 2018;20(2):144–50.
381 36. Mokeem SA, Alasqah MN, Michelogiannakis D, Al-Kheraif AA, Romanos GE,
382 Javed F. Clinical and radiographic periodontal status and whole salivary cotinine,
383 IL-1β and IL-6 levels in cigarette- and waterpipe-smokers and E-cig users.
384 Environ Toxicol Pharmacol. 2018 Jul 1;61:38–43.
385 37. Sabi SH, Khabour OF, Alzoubi KH, Cobb CO, Eissenberg T. Changes at global and
386 site-specific DNA methylation of MLH1 gene promoter induced by waterpipe
387 smoking in blood lymphocytes and oral epithelial cells. Inhal Toxicol. 2020 Feb
388 23;32(3):124–30.
389 38. Rajabi-Moghaddam M, Haji Mirzamohammad M, Yahyazadeh E, Gholinia H,
390 Abbaszadeh H. Comparison of Genotoxic Effect in Buccal Exfoliated Cells
391 between Cigarette and Waterpipe Smokers. Acta Cytol. 2020;64(5):471–6.
392 39. Taghibakhsh M, Farhadi S, Babaee A, Sheikhi M. The effect of hookah use on
393 buccal mucosa: Evaluation of repair index. Asian Pacific J Cancer Prev.
394 2019;20(4):1109–12.
395 40. Eker ED, Koyuncu H, Şahin NÖ, Yüksel A, Berköz M, Diler SB, et al.
396 Determination of genotoxic effects of hookah smoking by micronucleus and
397 chromosome aberration methods. Med Sci Monit. 2016;22:4490–4.
398 41. Prasad P, Hamed MS, Nahar P. Micronucleus Assay in Waterpipe Tobacco and
399 Cigarette Smokers: A Comparative Study - PubMed. J Contem Dent Pr.

2019;20(1):101–7.

42. Volkova O, Ryabokon E, Magda I, Shckorbatov Y. Impact of smoking habits on the state of chromatin and morphology of buccal epithelial cells among medical students. Georgian Med News. 2017 Jan 1;(262):111–5.

43. Naderi NJ, Pasha MP. Comparison of cytotoxic effect of cigarette and waterpipe smoking on human buccal Mucosa. Int J Prev Med. 2017;8:98.

44. Silveira MAD, Antonelli AS, Fiorelli BO, D'Arce LPG. Cytological multimarker screening using BMCyt test in waterpipe smokers: an integrative study of cell damage, toxicological and cancer risk. J Genet. 2018 Jun 1;97(2):399–404.

45. Seifi S, Feizi F, Mehdizadeh M, Khafri S, Ahmadi B. Evaluation of cytological alterations of oral mucosa in smokers and waterpipe users. Cell J. 2014;15(4):302–9.

46. Ali AA. Histopathologic changes in oral mucosa of Yemenis addicted to water-pipe and cigarette smoking in addition to takhzeen al-qat. Oral Surgery, Oral Med Oral Pathol Oral Radiol Endodontology. 2007;103(3):e55-9.

47. Alharbi F, Quadri MFA. Individual and integrated effects of potential risk factors for oral squamous cell carcinoma: A hospital-based case- control study in Jazan, Saudi Arabia. Asian Pacific J Cancer Prev. 2018 Mar 1;19(3):791–6.

48. Zaid K, Azar-Maalouf E, Barakat C, Chantiri M. p53 Overexpression in oral mucosa in relation to Shisha smoking in Syria and Lebanon. Asian Pacific J Cancer Prev. 2018 Jul 1;19(7):1879–82.

49. Amer HW, Waguih HM, El-Rouby DH. Development of field cancerization in the clinically normal oral mucosa of shisha smokers. Int J Dent Hyg. 2019;17(1):39–45.

50. WHO Study Group on Tobacco Product Regulation (TobReg). Waterpipe tobacco smoking: health effects, research needs and recommended actions for regulators. Geneva; 2015.

428 **Table 1:** Included studies in chronological order

Author	year	Locality (Country)	Cytotoxic/ Genotoxic	waterpipe group	control group
Ali [46]	2007	- (Yemen)	Cytotoxic	11	11
El-Setouhy et al. [8]	2008	Cairo (Egypt)	Genotoxic	128	78
Al-Amrah et al. [33]	2014	Jeddah (Saudi Arabia)	Genotoxic	20	0
Seifi et al. [45]	2014	Babol (Iran)	Cytotoxic	40	40
Eker et al. [40]	2016	Mersin (Turkey)	Genotoxic	30	30
Naderi, Pasha [43]	2017	Tehran (Iran)	Cytotoxic	25	25
Volkova et al. [42]	2017	Krakiv (Ukraine)	Cytotoxic	13	38
Abduljabbar et al. [35]	2018	Riyadh (Saudi Arabia)	Cytotoxic	41	44
Alharbi et al. [47]	2018	Jazan (Saudi Arabia)	Cytotoxic	70	140
AlQahtani et al. [34]	2018	Multicenter	Cytotoxic	40	40
Mokeem et al. [36]	2018	Riyadh (Saudi Arabia)	Cytotoxic	40	38
Silveira et al. [44]	2018	Cascavel (Brazil)	Genotoxic	40	40
Zaid et al. [48]	2018	Syria (Lebanon)	Cytotoxic	52	53
Amer et al. [49]	2019	Cairo (Egypt)	Cytotoxic	16	16
Patil et al. [4]	2019	Multicenter	Genotoxic	-	-
Prasad et al. [41]	2019	Ajman (United Arab Emirates)	Genotoxic	100	100
Taghibakhsh et al. [39]	2019	Tehran (Iran)	Cytotoxic	36	36
López-Ozuna et al. [32]	2020	Multicenter	Genotoxic	-	-
Rajabi-Moghaddam et al. [38]	2020	Birjand (Iran)	Genotoxic	30	30
Sabi et al. [37]	2020	Irbid (Jordan)	Genotoxic	150	150

429

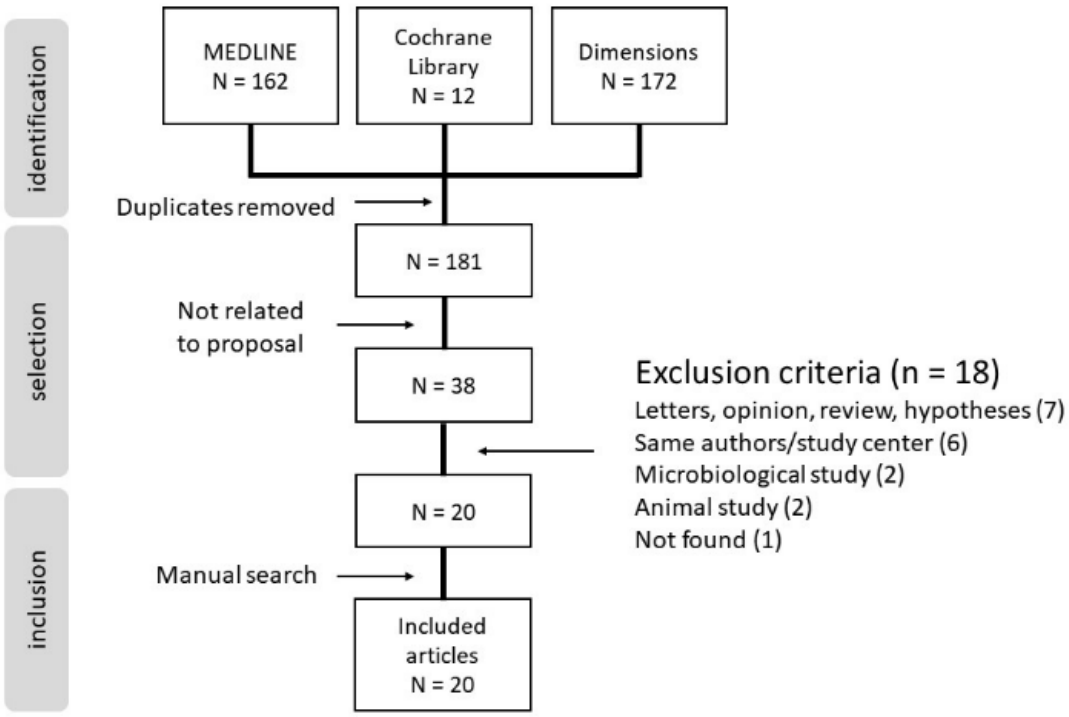

Figure 1: Flow diagram of included articles.

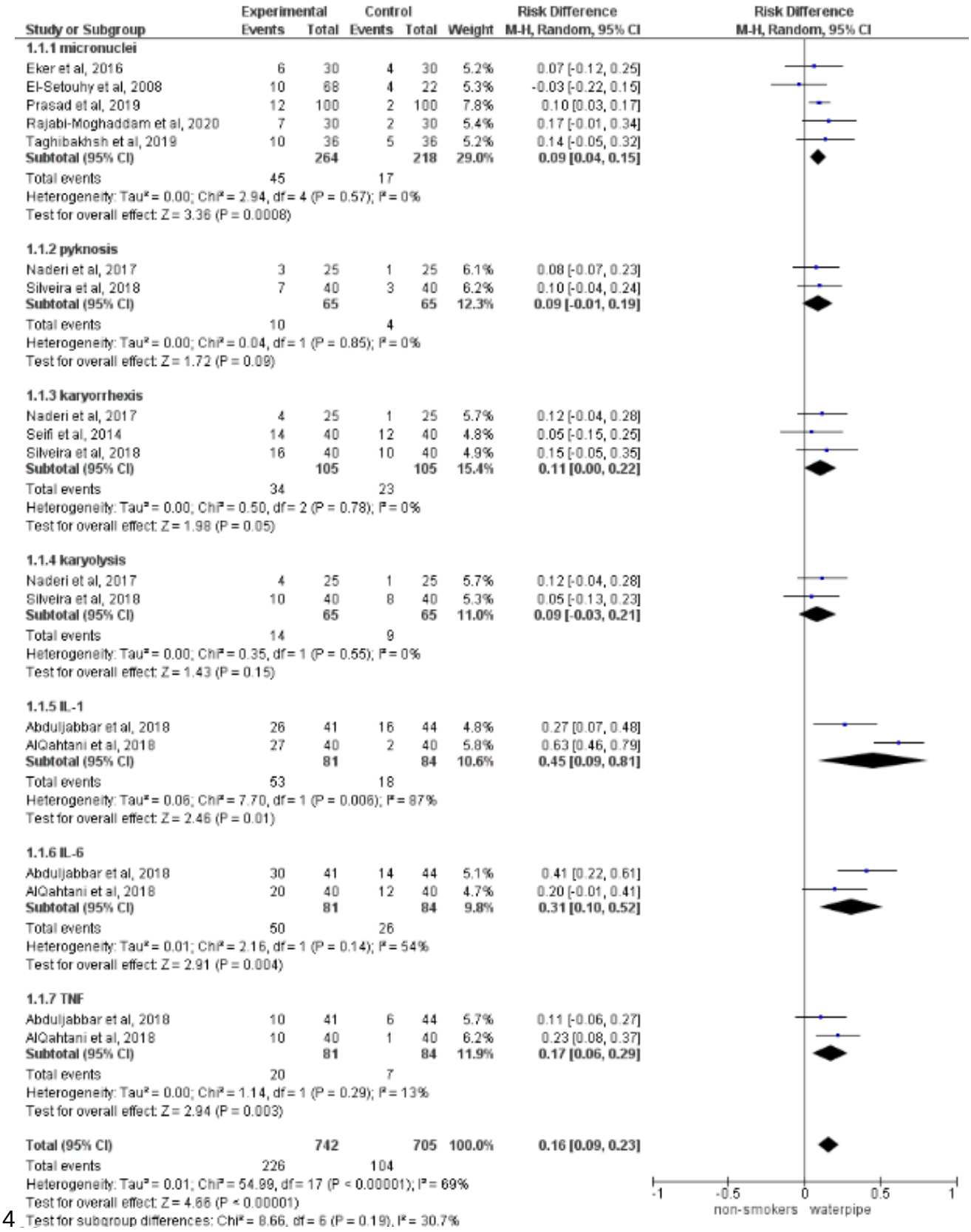

Figure 2: Forest plot generated through RevMan 5.4

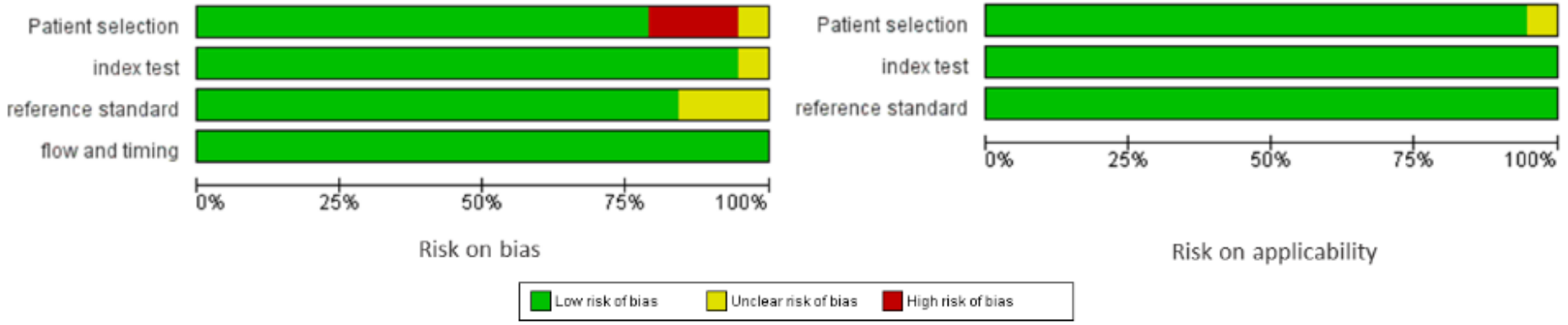

Figure 3: Risk of bias graph generated through RevMan 5.4