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REVIEW

Cytotoxic and Genotoxic Effects of Waterpipe on Oral Health Status

Systematic review and meta-analysis

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ABSTRACT: This systematic review and meta-analysis aimed to assess the cytotoxic and genotoxic impacts of waterpipe smoking on oral health. The databases MEDLINE, Cochrane Library and Dimensions were searched to find studies evaluating whether waterpipe smokers exhibited any cytotoxic or genotoxic effects on their oral cells compared to non-smokers, with regard to mouth neoplasms. Particularly, changes in DNA methylation and p53 expression were assessed. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines were adopted for the systematic review. Review Manager was utilised for statistical analysis with a significance level at $P<0.05$. To assess the grades of the included articles, a risk of bias analysis was summarised. A forest plot, including some of the included articles included, was created regarding the different grades. A total of 20 studies were included in this review. The results showed that waterpipe smoking has cytotoxic and genotoxic effects on oral cells, with a risk difference of 0.16. Although the published articles are few in number, all confirm the devastating effects of waterpipe smoking related to the carcinogenicity. Waterpipe smoking is harmful to oral health. It causes a series of detrimental cellular and genetic modifications such as acanthosis, epithelial dysplasia and hyperparakeratosis. In addition, waterpipe smoke contains several carcinogenic compounds. As it releases many harmful organic compounds, waterpipe smoking increases the incidence of oral cancer.

Keywords: Mouth Neoplasms; Oral Health; Smoking Waterpipes; Tobacco Use; Toxicity Tests.

Tobacco is smoked in many different ways. Waterpipe smoking is one form of tobacco use that has gained popularity in the past decades. In this light, a systematic review conducted in 2018 showed that the prevalence of waterpipe use was alarmingly high in the Eastern Mediterranean and European regions, especially among the youth.[1]

Waterpipe smoke contains a variety of carcinogens such as naphthylamines, tobacco-specific nitrosamines, polycyclic aromatic hydrocarbons, primary aromatic amines, along with carbon monoxide carbonyls (such as formaldehyde, acetaldehyde or acrolein).[2] Moreover, waterpipe use for smoking is associated with DNA damage and cell death; these kinds of genotoxicity and cytotoxicity are also involved in oral carcinogenesis.[3] Indeed, laboratory-based investigations on waterpipe smoking showed various genomic and transcriptomic alterations previously observed in various types of cancer.[4] In fact, Walters *et al*. observed concomitant changes in DNA methylation at 727 locations in the genome.[5] Thus, DNA methylation may predispose cells to cancer (by activating specific genes and repressing others) and it also plays a significant role in metastasis.[6,7] In addition, nuclear changes in the oral mucosa cells of waterpipe smokers (WS) were previously 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 adequately established.[2] Furthermore, the few available studies on this topic do not explicitly focus on oral cancers.[10] With regard to addressing this knowledge gap, this study aimed to systematically review the scientific literature regarding the cytotoxic or genotoxic effects of waterpipe smoking on oral mucosal cells.[11]

Methods

The 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 (CRD42021238867).

Only original studies regarding the cytotoxicity and genotoxicity of waterpipe use were considered in the systematic review. Additionally, the following inclusion criteria were considered: (1) studies on regular waterpipe users; (2) studies demonstrating cytotoxic or genotoxic effects of waterpipe smoking; and (3) studies that included comparisons with a control group.

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This study's search was conducted online by two researchers (RG and MK) in MEDLINE (via PubMed), Cochrane Library, Health Virtual Library (BVS) and Dimensions, with no date restriction until December 2021. The terms chosen in the primary articles selected to justify this review were combined with Boolean operators (OR/AND) within the population, intervention, control and outcome (PICO) framework. Here, 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). As per the PICO framework, the following questions were to be answered: Do waterpipe smokers (P) demonstrate any cytotoxic or genotoxic effects on oral cells (I) compared to non-smokers (C) regarding mouth neoplasms (O)?

The exclusion criteria were as follows: (1) studies with clinical changes; (2) studies with radiographic modifications; (3) studies performed on the head, face and neck; and (4) animal studies. Moreover, comparative studies without conclusions specific to waterpipe toxicity were excluded. In addition, studies that met the inclusion criteria or those with doubtful information either in their titles or abstracts were selected for full-text assessments in this review's second round.

Two researchers (RG and MK) independently extracted the following data from the included studies for analysis: year of study, demographic data, cytotoxic and/or genotoxic effects, waterpipe use and control group sizes. Any discrepancies were resolved by arriving at a consensus. In the case of persisting discrepancies, arbitration was performed by a third researcher (YSS). Notably, alterations related to micronuclei, pyknosis, karyorrhexis and karyolysis were discussed.

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

The QUADAS-2 Tool assessed the risks of bias and applicability across the selected studies to evaluate the following aspects: (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; and (4) flow and timing of each included article: description of patients who did not receive the index test or reference standard and who were excluded.

Results

The first bibliographic search found 346 records from the previously mentioned databases; however, BVS returned no results. Thereafter, duplicate studies were excluded, leading to a remainder of 181 articles. After screening and excluding papers that were unrelated to the study topic, 38 robust studies remained relevant. Then, reports from the same authors/co-authors or the same study centre were excluded, (e.g. reviews, comments, letters, hypotheses and expert opinions).[3,14–26] Additionally, two exclusively microbiological studies were removed, and two were considered as animal studies.[27–30] However, one study was found but could not be retrieved properly.[31] Furthermore, manual search retrieved no additional papers [Figure 1].

The full-text of all studies viewed from the first round were independently checked to ensure each reviewer's eligibility. Finally, a total of 20 articles were included in this review. The selected articles included information on authors, year of publication, demographic data, cytotoxic or genotoxic evaluation and the number of patients in the waterpipe and control groups [Table 1].

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

Six studies were found to have investigated the genotoxic effect of waterpipe smoke.[4,32,33,37–39] A comet assay was performed in one of these

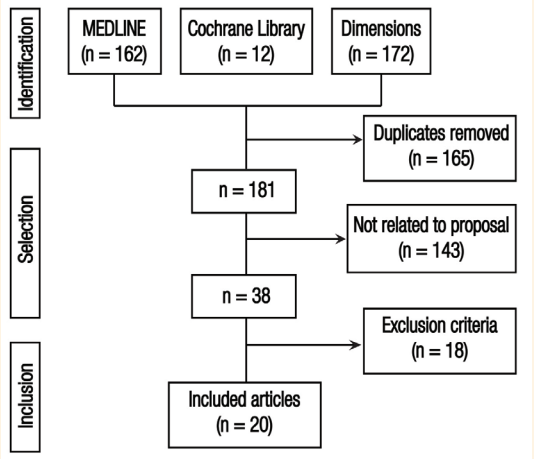

Figure 1: Flow diagram of included articles.

Table 1: Included studies in chronological order[4,8,32–49]

Author and year of publication	City (country)	Cytotoxic/genotoxic	Waterpipe group	Control group
Ali[46] (2007)	NM (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 and 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)	Riyadh (Saudi Arabia)	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)	Multicentre*	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)	Multicentre*	Genotoxic	–	–
Rajabi-Moghaddam *et al.*[38] (2020)	Birjand (Iran)	Genotoxic	30	30
Sabi *et al.*[37] (2020)	Irbid (Jordan)	Genotoxic	150	150

**The study was conducted on human cell lines.*

studies.[33] Cell-line models were used to understand the mechanisms of action of waterpipe smoke on oral cells.[4,32] In the study by Patil *et al.*, immortalised non-transformed normal (human) oral keratinocytes (OKF6/TERT1) chronically (i.e. for eight months) exposed to waterpipe smoke were developed.[4] When the phenotypic alterations were studied, they revealed genomic anomalies in OKF6/TERT1-waterpipe cells, with some overexpressed and some downregulated genes. In another study that developed a cell-line model, two normal (human) oral epithelial cells were treated with 100 g/L of waterpipe smoke solution for two days.[32] Upon examination, it was observed that both cells became more elongated and showed decreased cell-cell contact compared to the untreated cells. This epithelial-mesenchymal transition was also accompanied by the deregulation of a set of genes related to oncogenesis.[32]

On the other hand, eight studies evaluated the nuclear changes in cytology samples from the buccal mucosa of patients.[8,38–40,41,43–45] In these studies, some pathological assessments were performed, including micronuclei (DNA aggregates separating 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 keratinisation of the epidermal stratum coreum), and epithelial dysplasia (architectural and cytological epithelial changes).

The mean of micronuclei, cell nucleus perimeter, and area was found to be contrasting in the WS group compared to the non-smoker (NS) group.[8,38–42] In addition, the mean percentages of karyorrhexis, karyolysis and pyknosis showed substantial changes.[43–45] Other histopathologic changes such as acanthosis, hyperparakeratosis and epithelial dysplasia were found to be associated with waterpipe use. Therefore, an increased incidence of oral cancer was related to different types of tobacco use.[46,47]

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

The mutations of tumour suppressor protein p53 were also found to be associated with waterpipe

Study or subgroup	Experimental Events	Experimental Total	Control Events	Control Total	Weight in %	Risk difference (95% CI)
1.1.1 micronuclei						
Eker *et al.*[40] (2016)	6	30	4	30	5.2	0.07 (-0.12, 0.25)
El-Setouhy *et al.*[8] (2008)	10	68	4	22	5.3	-0.03 (-0.22, 0.15)
Prasad *et al.*[41] (2019)	12	100	2	100	7.8	0.10 (0.03, 0.17)
Prasad *et al.*[41] (2019)	7	30	2	30	5.4	0.17 (-0.01, 0.34)
Prasad *et al.*[41] (2019)	10	36	5	36	5.2	0.14 (-0.05, 0.32)
Subtotal (95% CI)		**264**		**218**	**29.0**	**0.09 (0.04, 0.15)**
Total events	45		17			
Heterogeneity: Tau² = 0.00; Chi² = 2.94, df = 4 (*P* = 0.57); I² = 0%						
Test for overall effect: Z = 3.36 (*P* = 0.0008)						
1.1.2 pyknosis						
Naderi and Pasha[43] (2017)	3	25	1	25	6.1	0.08 (-0.07, 0.23)
Silveira *et al.*[44] (2018)	7	40	3	40	6.2	0.10 (-0.04, 0.24)
Subtotal (95% CI)		**65**		**65**	**12.3**	**0.09 (-0.01, 0.19)**
Total events	10		4			
Heterogeneity: Tau² = 0.00; Chi² = 0.04, df = 1 (*P* = 0.85); I² = 0%						
Test for overall effect: Z = 1.72 (*P* = 0.09)						
1.1.3 kayyorrhexis						
Naderi and Pasha[43] (2017)	4	25	1	25	5.7	0.12 (-0.04, 0.28)
Seifi *et al.*[45] (2014)	14	40	12	40	4.8	0.05 (-0.15, 0.25)
Silveira *et al.*[44] (2018)	16	40	10	40	4.9	0.15 (-0.05, 0.35)
Subtotal (95% CI)		**105**		**105**	**15.4**	**0.11 (0.00, 0.22)**
Total events	34		23			
Heterogeniety: Tau² = 0.00; Chi² = 0.50, df = 2 (*P* = 0.78); I² = 0%						
Test for overall effect: Z = 1.98 (*P* = 0.05)						
1.1.4 karyolysis						
Naderi and Pasha[43] (2017)	4	25	1	25	5.7	0.12 (-0.04, 0.28)
Silveira *et al.*[44] (2018)	10	40	8	40	5.3	0.05 (-0.13, 0.23)
Subtotal (95% CI)		**65**		**65**	**11.0**	**0.09 (-0.03, 0.21)**
Total events	14		9			
Heterogeneity: Tau² = 0.00; Chi² = 0.35, df = 1 (*P* = 0.55); I² = 0%						
Test for overall effect: Z = 1.43 (*P* = 0.15)						
1.1.5 IL-1						
Abduljabbar *et al.*[35] (2018)	26	41	16	44	4.8	0.27 (0.07, 0.48)
AlQahtani *et al.*[34] (2018)	27	40	2	40	5.8	0.63 (0.46, 0.79)
Subtotal (95% CI)		**81**		**84**	**10.6**	**0.45 (0.09, 0.81)**
Total events	53		18			
Heterogeneity: Tau² = 0.06; Chi² = 7.70, df = 1 (*P* = 0.006); I² = 87%						
Test for overall effect: Z = 2.46 (*P* = 0.01)						
1.1.6 IL-6						
Abduljabbar *et al.*[35] (2018)	30	41	14	44	5.1	0.41 (0.22, 0.61)
AlQahtani *et al.*[34] (2018)	20	40	12	40	4.7	0.20 (-0.01, 0.41)
Subtotal (95% CI)		**81**		**84**	**9.8**	**0.31 (0.10, 0.52)**
Total events	50		26			
Heterogeneity: Tau² = 0.01; Chi² = 2.16, df = 1 (*P* = 0.14); I² = 54%						
Test for overall effect: Z = 2.91 (*P* = 0.004)						
1.1.7 TNF						
Abduljabbar *et al.*[35] (2018)	10	41	6	44	5.7	0.11 (-0.06, 0.27)
AlQahtani *et al.*[34] (2018)	10	40	1	40	6.2	0.23 (0.08, 0.37)
Subtotal (95% CI)		**81**		**84**	**11.9**	**0.17 (0.06, 0.29)**
Total events	20		7			
Heterogeneity: Tau² = 0.00; Chi² = 1.14, df = 1 (*P* = 0.29); I² = 13%						
Test for overall effect: Z = 2.94 (*P* = 0.003)						
Total (95% CI)		**742**		**705**	**100.0**	**0.16 (0.09, 0.23)**
Total events	226		104			
Heterogeneity: Tau² = 0.01; Chi² = 54.99, df = 17 (*P* <0.00001); I² = 69%						
Test for overall effect: Z = 4.66 (*P* <0.00001)						
Test for subgroup difference: Chi² = 8.66, df = 6 (*P* = 0.19); I² = 30.7%						

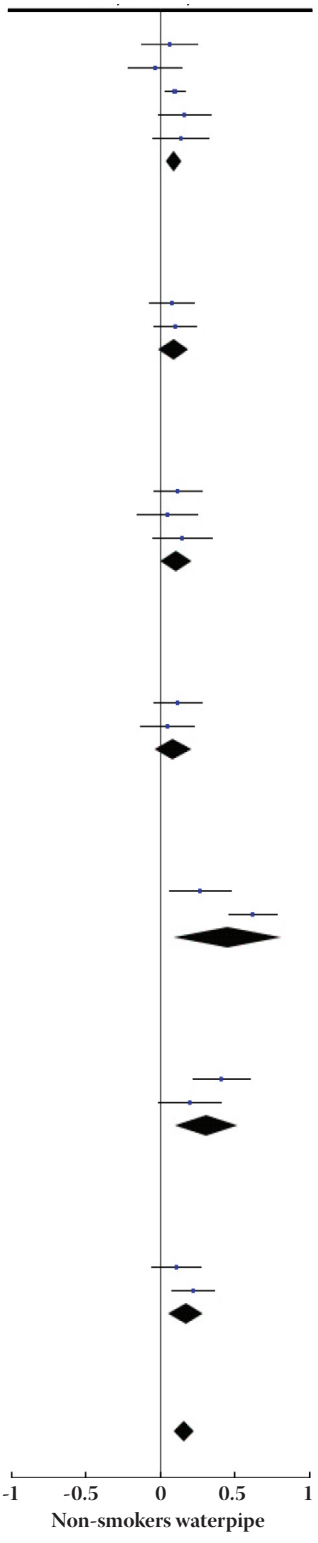

Figure 2: Forest plot generated through RevMan 5.4.

use.[48,49] This alteration could lead to apoptosis as well as the suppression of the cell cycle, senescence, differentiation and DNA repair.[48]

Furthermore, a meta-analysis was carried out using RevMan 5.4 (Cochrane.org, London, UK). Indeed, a forest plot could only be created with RevMan

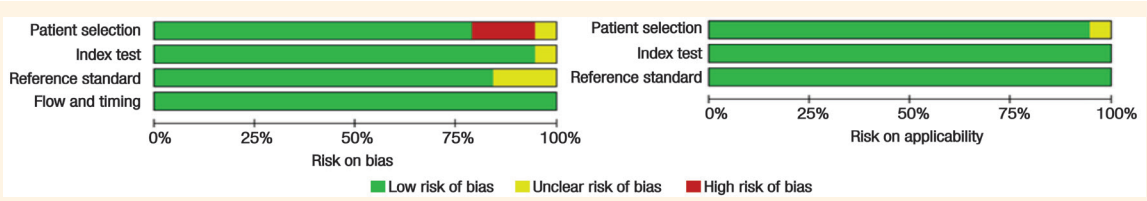

Figure 3: Risk of bias graph generated through RevMan 5.4.

in accordance with the different levels of variation regarding the aims and methods of the selected studies. This was because the included articles used different cells to assess cytotoxicity and genotoxicity in different ways [Figure 2]. Of the 20 articles included, nine rated genotoxicity while 11 rated cytotoxicity. As per evidence from the literature, waterpipe smoke was believed to have several cytotoxic and genotoxic effects on oral cells, with a risk difference of 0.16 (95% CI: 0.09–0.23; P <0.00001).

A graph depicting the risk of bias was created by RevMan 5.4 using the QUADAS-2 protocol [Figure 3]. Indeed, the high quality of the study articles can be observed in this graph. The articles came from all over the world, mainly from the Middle East (n = 14); out of the two multicentre studies, two were conducted in Africa and the other two came from Europe and South America, respectively.[4,8,32,42,44,49] The predominance of studies from the Middle East can be explained by the higher and more frequent consumption of waterpipe in the region.

Discussion

Although waterpipe use is a worldwide phenomenon, several included studies noted an urgent concern with waterpipe smoking in Middle Eastern countries, where it is widespread.[32,40,48]

Such concern is appreciable, as waterpipe smoke condensate reportedly revealed many organic compounds that are well-known for their genotoxic and carcinogenic properties—such as nicotine, tar, heavy metals, polycyclic aromatic hydrocarbons (naphthalene, phenanthrene, fluoranthene), aldehydes (5-hydroxymethyl-5-furancarboxaldehyde, 3-ethoxy-4-hydroxybenzaldehyde) and also carbon monoxide.[33,40,43] In the included studies, the volume of formaldehyde detected in waterpipe smoke was five times higher than its volume in one 2R4F cigarette (a 2R4F cigarette is a standard reference cigarette; tobacco industries as well as academic laboratories use this reference cigarette to standardise test items and conduct inhalation toxicity research).[33]

Notably, in the selected studies, high values were found for all critical comet assay parameters (a sensitive technique of DNA damage detection) in buccal cells, suggesting that waterpipe use comprises DNA-damaging ingredients.[32,33,37,38,44] For example, DNA methylation due to waterpipe use could reach up to a 64% level;[37] this should be alarming, as samples with only 10% methylation are considered as significantly methylated.

An effective technique to evaluate the impact of environmental factors on genetic stability is the investigation of the micronuclei—the products of early events in human carcinogenic processes, especially in the oral cavity; they are considered biomarkers of genotoxicity.[41] According to the included studies, total micronuclei (TMN) and cells with micronuclei (CMN) were significantly higher among waterpipe users (very similar to the same values for cigarette-smokers) compared to NS groups.[8,41,49] Furthermore, there was no association between TMN and CMN regarding lifetime duration of use, time of first waterpipe smoke of the day and number of times per day/week.[8] Waterpipe use was also related to chromosomal aberrations and an increased level of micronuclei.[8,33,38–41]

Moreover, in the waterpipe smoke mixtures, mutagenic and genotoxic contaminants were present at low levels; however their detection was challenging since a few components were in available high concentrations.[33] In addition, genotoxicity was not related to a specific compound but to a set of properties and chemical interactions of the entire sample of one selected study.[33] In other words, waterpipe use was related to genomic and gene expression alterations, for both RNA and DNA.[4,32,33,37,44]

Furthermore, 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 also similar to those of cigarette smokers (CS).[46] In addition, cytomorphometric quantitative analysis showed higher values for WS groups than NS groups concerning nuclear and cell perimeter, cytoplasm size, cell area, nuclear-cytoplasmic ratio and the big diameter of nucleus/small diameter of nucleus ratio, besides the induction of heterochromatinisation in cell nuclei,

a situation caused by different stress factors.[42,44,45] Additionally, some studies observed increases in multinucleated cells, pyknosis, karyorrhexis, karyolysis in WS groups compared to NS groups, the former groups' values being slightly higher than those of the CS groups.[38,39,43–45] There was also a higher incidence of cytoplasm vacuolisation concerning NS as well as CS groups.[45] Malignant and pre-malignant lesions were found to facilitate an increase in the nuclear-cytoplasmic ratio.

Pro-inflammatory cytokine levels (interleukin-1β, interleukin-6, interleukin-3, and tumour necrosis factor-α) were statistically found to be higher among WS groups compared to NS, a result similar to other kinds of tobacco users, such as CS, E-cigarettes, and cigars.[32,34–36] Additionally, cell necrosis and apoptosis were found to be closely related to carcinomas.

Protein p53 expression was found to have a relation to the regulation of apoptosis and genomic stability, along with playing a crucial role in tumour suppression (it was named the 'guardian of the genome'). WS groups had a significantly higher p53 mutation than NS groups in samples with malignant, pre-malignant, or even normal oral epithelium.[48,49] This correlation was similar to that of the CS groups.[49] In addition, the repair index of the oral mucosa cells of WS groups was significantly lower than that of the NS groups.[39] Evidently, the cytotoxic effects of waterpipe smoke were more correlated to time exposure than those of cigarette smoke.[41,43,44,50]

However, there was no peak incidence of oral cancer in WS groups regarding age or gender.[43,47] Although a few papers included female samples due to oral mucosa alterations concerning hormonal changes, waterpipe use was found to be much more common in males than females.[45,46]

Furthermore, waterpipe and cigarette users demonstrated similar effects on oral mucosa, including a substantial increase of association with oral squamous cell carcinoma (OSCC) development.[37,42,43,46–49] The combination of waterpipe and shammah (Arabian snuff) or waterpipe and cigarettes led to a higher incidence of oral cancer compared to only one kind of tobacco use.[47,49] In fact, the use of waterpipe was found to have more unfavourable effects than cigarette smoking.[38,42] On the other hand, the combined use of waterpipe and shammah increased the risk of developing OSCC by nearly 35 times.[47] Khat chewing did not demonstrate any significant impact on the development of oral cancer.[47] However, when associated with waterpipe use, it lead to an increased risk of oral cancer.[46]

In this light and in addition to more restrictive legislation and interventional policy aspects, tobacco cessation programmes must become a priority in some concomitantly affected regions. These programmes can consist of education, psychological therapy and pharmacological aid, especially for young people who believe that waterpipe smoking is a safe addiction.[40,44,47,48]

Conclusions

Waterpipe use has genotoxic and cytotoxic effects on human oral cells, 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 that of cigarette smoke.

AUTHORS' CONTRIBUTIONS

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

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