Systematic Review
Oral Pathology

Effects of tobacco on the DNA of smokers and non-smokers affected by OSCC: systematic review and meta-analysis

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Abstract: Scientific evidence about genetic and molecular changes in oral squamous cell carcinoma (OSCC) among smokers and non-smokers is inconclusive. This systematic review and meta-analysis assessed the effects of tobacco on the DNA of individuals with OSCC based on protein mutations. Electronic searches were conducted on PubMed, Ovid, Web of Science, and Scopus to identify observational studies published up to January/2022. The Joanna Briggs Institute tool was used for the critical appraisal of studies. The certainty of the evidence was evaluated. Twenty-three studies assessing 4,060 individuals (2,967 smokers vs. 1,093 non-smokers) were included in this review. Fifteen groups of proteins/genes were investigated. Analysis of the quality of articles revealed low risk of bias in most studies. The certainty of the evidence was very low. The meta-analysis confirmed no significant difference between smokers and non-smokers with respect to damage to GSTM1 (OR: 0.60; 95%CI: 0.30–1.18), GSTT1 (OR: 1.18; 95%CI:0.49–2.83), hydrolase proteins (Ku70 and Ku80) (OR: 0.74; 95%CI: 0.18–3.05), and transferase proteins (GSTM1, GSTT1, GSTM3) (OR: 0.74; 95%CI: 0.47–1.18). Most of the studies included showed that smokers are more likely to exhibit genetic instability. However, the meta-analysis revealed that smokers do not necessarily have more genetic alterations in the DNA than non-smokers.

Keywords: DNA Damage; Head and Neck Neoplasms; Meta-Analysis; Mouth Neoplasms; Systematic Review.

Introduction

Oral squamous cell carcinoma (OSCC) remains one of the deadliest types of cancers of the head and neck worldwide and is the sixth most prevalent type of cancer.[1] Oral cancer is a global health issue with an annual incidence of 300,000 and approximately half of affected individuals succumb to the disease.[2–4] OSCC manifests as an outcome of several biochemical, cellular, and clinical changes in the epithelium of the affected oral mucosa.[5]

The etiology of OSCC is multifactorial and the main risk factors are tobacco, alcohol, genetic predisposition, biological agents, systemic status,

and diet.[2,3,6,7] Nearly 80% of affected individuals report a history of smoking. Indeed, smokers are five to nine times more likely to develop oral cancer than non-smokers.[8] Some smokers may be inherently more susceptible to the development of OSCC due to patterns of tobacco use, innate metabolism of carcinogens, and altered excretion or variation in DNA damage and repair.[9] The occurrence of OSCC, excluding cases affecting the oropharynx, is increasing among non-smokers and little is known about the process of carcinogenesis and the clinical outcomes of this cancer in these individuals.[10,11]

Carcinogens may induce various types of DNA damage, including DNA adducts and single- and double-strand breaks.[12,13] DNA damage is a generic term for many different DNA modifications that activate apoptosis.[14] Moreover, the various DNA repair pathways provide a first line of defense for maintaining genome stability, which protects against carcinogenesis. Individuals with suboptimal DNA repair capacity are at increased risk of smoking-related cancers.[14] Smoking may also induce oxidative damage to human genome.[15]

Although the factors involved in smokers with OSCC have been widely discussed in the literature, the determinants of the development of a malignant lesion among non-smokers remain uncertain.[11,16] Therefore, the purpose of the present systematic review and meta-analysis was to synthetize the effects of tobacco on the DNA of individuals with OSCC. The specific aim of the study was to describe the proteins/genes investigated and the molecular changes observed among individuals with OSCC, comparing smokers and non-smokers.

Methodology

Study design

This systematic review and meta-analysis was conducted using the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) checklist as a reporting guide.[17]

Protocol and registration

The study was registered with the International Prospective Register of Systematic Reviews in Health and Social Care (Prospero, National Institute for Health Research, UK, CRD42018112409).

The research question was: Does DNA damage in OSCC patients who are smokers differ from that of non-smokers OSCC patients? Thus, the following acronym PECOS was used to support the research question:

(P) Population: individuals with OSCC;

(E) Exposure: tobacco;

(C) Comparator: non-tobacco;

(O) Outcomes: effect on DNA (difference in pattern expression in smokers and non-smokers);

(S) Study design: observational studies.

Eligibility criteria

Inclusion criteria were cross-sectional, case-control, or longitudinal studies assessing the effects of tobacco on the DNA of individuals with OSCC by comparing the expression pattern of genes and protein of smokers and non-smokers. Reviews, letters, personal or expert opinions, meeting abstracts, case reports, case series, *in vitro* or *ex vivo* studies, and animal studies were excluded.

Search strategy

Computerized searches without restrictions of publication date, geographic region, or publication language were undertaken in March/2020 in the following electronic databases: PubMed (National Library of Medicine), Ovid (Wolters Kluwer), Web of Science (Clarivate Analytics), and Scopus (Elsevier). An update took place in January/2022. A manual search screening of the reference lists of the selected articles was also performed to retrieve studies that may have been missed in the electronic searches. In addition, the grey literature was accessed by Google Scholar and Open Grey by reading the first 100 results of each website.

Keywords included the following medical subject headings (MeSH) and free terms: "DNA damage" OR "DNA injury" OR "Genotoxic Stress" OR "DNA" OR "genotoxic" OR "cytotoxic" OR "DNA damage response" OR "cell cycle" OR "cytotoxicity" OR "genotoxicity" AND "oral squamous cell cancer" OR "mouth neoplasm" OR "oral neoplasm" OR "mouth cancer" OR "oral cancer" OR "epidermoid

carcinoma" OR "oral tumor" OR "mouth tumor" OR "oral tumour" OR "mouth tumour" AND smoking OR smoke OR smoker OR tabagism OR tobacco OR nicotine.

Study selection

The reference were managed using the EndNote X7.4 software (Clarivate Analytics, Toronto, Canada). Duplicates were removed upon identification. After duplicate removal, the titles/abstracts of the retrieved references were assessed by two independent reviewers (L.F.S. and K.S.S.V.). Percent inter-observer agreement was calculated.[18] The references whose title/abstract seemed to meet the eligibility criteria were selected for full-text reading. Full text evaluation was also performed by the two reviewers independently. After assessment of the full texts, those that met the eligibility criteria were included in this systematic review and meta-analysis. Disagreements between reviewers were resolved by a third examiner (V.F.B.).

Data extraction

Data were extracted by one author (KSV), and double-checked by a second author (LFS). Disagreements were resolved by discussions, and if needed, another author (VFB) was consulted. The following items were extracted from the articles included in the study: name of author(s), year of publication, country where the study was conducted, study design, overall sample size, participants' sex, number of individuals who were smokers and non-smokers, gene/protein analyzed, method for gene/protein assessment, and main findings. If necessary, contact with authors was made to obtain additional information.

Appraisal of the methodological quality of the included studies

The Critical Appraisal Checklist for cross-sectional studies recommended by the Joanna Briggs Institute of the University of Adelaide was employed.[19] The included articles were evaluated according to specific parameters. Two reviewers (L.F.S. and K.S.S.V.) independently evaluated the included studies. For each parameter, the included articles were rated as "low risk of bias", "high risk of bias", "unclear risk of bias", or "not applicable". Any discrepancy between reviewers was resolved by discussion. If necessary, a third examiner (V.F.B.) was consulted.

Synthesis of the results

A meta-analysis was conducted on the included studies that showed methodological homogeneity. The Review Manager 5.3 software (Review Manager (RevMan) [Computer program], version 5.3; Copenhagen: The Nordic Cochrane Center, The Cochrane Collaboration, 2014) was used. Statistical heterogeneity was assessed using the I^2 statistic. The fixed model was deployed.[20] To illustrate the conducted meta-analysis, a forest plot was provided.

Additional analyses

Four subgroup analyses were conducted: by protein, by gene, by group of proteins/genes assessed, and by method of assessment. In the analyses, dichotomous data (number of smokers with DNA damage among the total number of smokers evaluated and number of non-smokers with DNA damage among the total number of non-smokers evaluated) were used. Comparisons between smokers and non-smokers were carried out. The results are reported as odds ratio (OR) and confidence intervals (CI). Two p values were also reported, one from the chi-square test related to heterogeneity and one from the Z test related to the summary effect, all with the significance level set at $p<0.05$.

Assessment of the certainty of evidence

The Grading of Recommendations, Assessment, Development and Evaluations (Grade) was used as a tool for evaluation of the certainty of evidence. The Grade has two sections: the first is the certainty assessment with which publication bias, imprecision, indirectness, inconsistency, risk of bias, studies' design, and number of studies were evaluated. The second is the summary of findings with which the number of participants was evaluated. According to the assessment, the certainty of evidence could be rated high, moderate, low, or very low. [21] The GRADEpro GDT was used.[22]

Results

Study selection

The computerized searches yielded 1,581 references. After the removal of 629 duplicates, inclusion and exclusion criteria were applied to 952 references. The agreement between observers was 94.0%. A total of 110 articles were selected for full-text assessment. Twenty-three articles fulfilled the eligibility criteria and were included in this systematic review and meta-analysis.[8,13,23-42] A flowchart of the process of study selection is outlined in Figure 1.

Study characteristics

The included articles, all of them in English, were published between 1998 and 2020. All articles were cross-sectional studies with control groups. They were conducted in Taiwan,[13,23-28] India,[8,29-34] Japan,[35,36] Thailand,[37,38], Brazil,[39] England,[40] Germany,[41] and the United States[42].

The included studies showed wide variation in sample size (ranging from 27 to 680 individuals). The total number of individuals evaluated in the 23 included studies was 4,060. Of these, 2,967 (73.07%) were smokers and 1,093 (26.92%) were non-smokers.

Regarding the protein used for the identification of DNA damage, a high heterogeneity was observed among studies. Different methods of protein evaluation (PCR, IHC, and ELISA) were also employed. Table 1 shows the characteristics (including protein used and method of protein evaluation) and the results of the included studies.

Appraisal of the methodological quality of the included studies

Overall, the 23 included studies showed a low risk of bias for inclusion criteria of the sample, detailed description of sample characteristics and study setting, measurement of exposure in

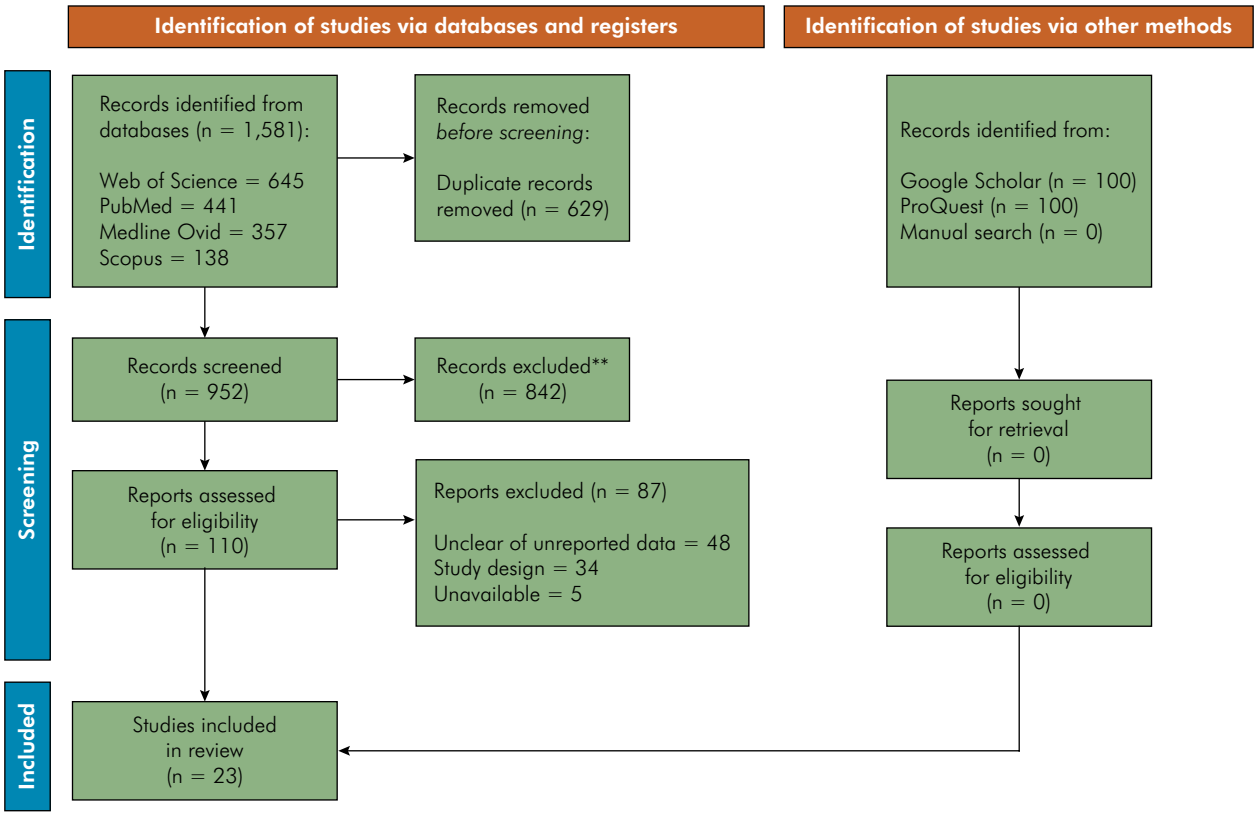

Figure 1. Flowchart showing the results of the search process.

Table 1. Articles included in this systematic review.

Author	Sample	Smoker		Gene/Protein	Method	Results
		Yes	No			
Katoh et al.[33]	62	39	23	NAT1 e 2	PCR	Individuals with NAT1*10 alleles are at higher risk for oral squamous cell carcinoma, but smoking history may not play a role in this genetic relationship. The role of NAT1 appears to be independent of smoking behavior.
Tanimoto et al.[34]	100	58	42	CYP1A1, GSTM1	PCR	The ORs of the individual genotypes increases as cigarette dose increases; however, the genetic difference in susceptibility was largest with ORs of 7.0 for genotype m2/m2 at the lower smoking level. These results indicate that the patients with m2/m2 contracted oral squamous cell carcinoma from a significantly lower cigarette dose than those with other genotypes. The fact that there is less difference in susceptibility among the genotypes at high cigarette dose levels may be ascribable to a saturation of the metabolic response. On the other hand, the amount of life-time alcohol consumption did not show a significant difference in the distribution of genotypes of either CYP1A1 or GSTM1, when estimated by drinking index (DI=the amount of alcohol converted into ethanol per day x the number of years of drinking).
Park et al.[40]	164	147	17	GSTM1 e 3	PCR	For African-American subjects who smoked more than 24 PY, risk for oral cancer was significantly associated with the GSTM1 null polymorphism (OR: 5.4, 95%CI: 1.2 ± 24). No association was observed in African-Americans who were light-smokers (*i.e.* 24 PY). A test for interaction between smoking and the GSTM1 genotype was not significant when the smoking-GSTM1 genotype interaction variable was introduced into the multiple logistic regression model for oral cancer risk. Significant associations were not observed between the GSTM3 genotype and oral cancer risk in African-Americans after stratification by smoking dose, although a trend was observed between the GSTM3 (B/B) genotype and oral cancer risk in the light-smoking African-American group (OR: 0.19, 95%CI: 0.03 ± 1.3).
Hsieh et al.[20]	187	182	5	p53	PCR	A specific pattern of mutation was observed in exons 5–9 of the p53 gene in OSCCs from smokers, alcohol users and BQ chewers. G:C to A:T transitions were the predominant mutations observed and associated with BQ and tobacco use. Seventeen of the 18 (94.44%) frameshift mutations including deletions and insertions occurred in smokers. Among them, 14 patients (82.35%) were also BQ chewers. In addition, most (20/22, 90.91%) G:C to T:A transversions occurred in smokers. All A:T to T:A and G:C mutations (n = 11) occurred in BQ chewers. All G:C to C:G transversions occurred in either smokers or BQ chewers. All of the mutations identified in patients with OSCCs in this series were somatic and not germ-line in origin, as DNA from normal tissue adjacent to p53-mutated tumor was negative for p53 mutations by both PCR–SSCP and DNA sequencing analysis.
Kietthubthew et al.[35]	53	50	3	GSTM1, GSTT1	PCR	The GSTM1 null genotype had a significant effect on oral cancer risk (OR: 3.0, 95%CI: 1.4–6.7), whereas the GSTT1 revealed no association (OR: 0.6, 95%CI: 0.3–1.3). The effect of the GST-susceptible genotypes on oral cancer risk was not increased with the combined deletion of GSTM1 and GSTT1 (OR: 2.0, 95%CI: 0.5–7.8). The GSTM1 wild type and GSTM1 null genotypes had no influence on oral cancer among nonsmokers and occasional smokers. However, frequent smokers with the GSTM1 null had a significantly increased risk for oral cancer (OR: 4.1; 95%CI: 1.5–11.3). With respect to the betel-chewing habit, the GSTM1 null increased the risk among frequent chewers (OR: 4.0; 95%CI: 1.3–12.9). Interestingly, for individuals who chew betel without smokeless tobacco, the risk was raised to 22-fold (95%CI: 2.2–222.0).

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Chaves et al.[37]	71	66	5	p53	PCR	The presence of TP53 mutation was independent of tobacco consumption. There was a predominance of A-G (45%) substitutions in current smokers and drinkers, followed by A-C transversions in 20% of the cases. The G-T mutation characteristic of tobacco smoke was found in only 1 tumor. Non-smokers and non-alcohol users were found to have 42.8% of G-A mutations, 2 of them were located in CpG sites and 1 in non-CpG sites.
Kietthubthew et al.[36]	106	72	34	XRCC1 e 3, XPC, XPD	PCR-RFLP	The homozygous variant genotype of XRCC1 399GIn reduced the OSCC risk (OR: 30, 95%CI: 0.1-0.88, p=0.03). The heterozygous genotypes for XRCC1 194Trp and XRCC3 241Met significantly increased the risk (OR: 2.26, 95%CI: 1:20-4.28, p = 0.01; OR: 2.31, 95%CI: 1.09-4.91, p = 0.03, respectively). There was a marginally higher risk for the heterozygous XPD exon 6 (OR: 1.74, 95% CI =0.94-3.22, p =0.08).
Korabiowska et al.[39]	40	28	12	Ku70, Ku80	IHC	In carcinomas from smokers, Ku70-positive cells were found in 82.9% of tumors and Ku80 positivity was observed in 85.7% of carcinomas. The maximum values of Ku70 and Ku80 expressions in carcinomas from smokers reached 60% and 50%, respectively. In tumors from non-smokers, Ku70 positivity was observed in 87.5% of cases and Ku80 positivity in 93.8% of tumors. Ku70 and Ku80 expression values reached maxima of 40%. The comparison of Ku70 and Ku80 expressions in tumors from smokers and non-smokers demonstrated a highly significant result for Ku70 ($p = 0.008$). Significant correlations between Ku70 and Ku80 expression were found in carcinomas from non-smokers ($p < 0.05$). In tumors from smokers, these significant relationships were not preserved ($p > 0.05$).
Prior et al.[38]	27	21	6	ND2	PCR	For ND2 gene, nucleotide 4917 was a significant mutation hotspot (P 1/4 0.027) and thus a potential smoking-associated biomarker in oral SCC. All patients having a mutation were males and classed as smokers with the exception of patient 5 whose smoking status was not known. Seven different types of mutation were discovered in the region of the D-Loop between nt 8 and 429. Base substitutions were observed in 16 (53.3%) different patients, 15 of whom had a classified smoking status. Of these, the 10 male patients with mutations were all self-classified smokers whereas, conversely, 4 of the 5 females with mutations were self-classified as non-smokers. This association of sex (males) and smoking status was statistically significant ($p = 0.003$) for patients with mutations.
Sharma et al.[27]	40	18	22	GSTM1, GSTT1	PCR	The prevalence of the GSTM1 null genotype in cancer cases was 52.5% (21/40). A total of 42.5% (17/40) of oral cancers had homozygous deletion of GSTT1 genotype as compared to 14.9% (13/87) of the controls. Only 14 individuals with cancer were heavy smokers (> 40 pack years) and 7 were alcohol consumers. Four individuals were occasional smokers and the rest were non-smokers. Three individuals were pan/tobacco chewers. The GSTM1 null genotype prevalence was 35.71% (5/14) in smokers. In the case of GSTT1, the differences between oral cancer cases and control smokers were significant ($p = 0.04$) (OR: 6.33; 95%CI: 1.0-44.1).
Anantharaman et al.[28]	458	391	67	CYP1A1, GSTM1, GSTT1	PCR	Among mixed habits of tobacco (chewers and smokers), *CYP1A1* MspI homozygous variant genotype (m2/m2) contributed a 3.2-fold increased risk to OSCC [95% CI, 1.10–10.28; p=0.05]. *GSTM1* null genotype shows a 1.29 times greater risk for OSCC (95% CI, 1.04– 1.65; P=0.05). *GSTT1* null genotype offered protection to OSCC. Individuals carrying this genotype were at 0.5 times reduced risk for cancer conditions (95%C: 0.39–0.83; p =0.004). Among tobacco chewers, *GSTT1* null genotype offered protection by decreasing the risk for OSCC by 0.27-fold (95%CI: 0.14–0.53; p = 0.0001).

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Bau et al.[13]	154	137	17	XPA A-23G, XPD Lys751Gin	PCR	The crude OR of the stratification with either harboring variant XPA genotype (A/G or G/G, "variant") or with smoking habit was 3.52 (95%CI: 1.26-9.84), and the crude OR of those with both harboring variant XPA genotype and smoking habit was increased to 47.7 (95%CI: 15.48-147.01). By the same analyses strategy, the same trend was observed and the joint effect of XPD genotype and smoking habit on oral cancer were also significant. The "common" group with putative low-risk XPD A/A genotype and without smoking habit was used as reference. The crude OR of the stratification with either harboring variant XPD genotype (A/C or C/C, "variant") or with smoking habit was 28.48 (95%CI: 13.93-58.23), and the crude OR of those with both harboring variant XPD genotype and smoking habit was 26.33 (95%CI: 7.87-88.04). The crude ORs of the stratification with one of the three factors, variant XPA (A/G or G/ G), variant XPD (A/C or C/C) genotype, or smoking habit, was 3.59 (95%CI: 1.27-10.19), and the crude ORs of the stratification with two or all of the three factors were significantly increased to 24.05 (95%CI: 8.38-68.95). The 7-fold synergistic increase from 3.59 to 24.05 suggested that genetic factors (XPA and XPD), modified by the environmental factor (smoking), may also contribute to oral cancer risk.
Chen et al.[21]	78	61	17	hTERT	IHC	OSCC patients with areca quid chewing (p=0.029), cigarette smoking (p=0.027), or alcohol drinking habits (p = 0.025) were prone to have a higher mean cytoplasmic hTERT labeling score in OSCC samples than OSCC patients without these oral habits. OSCC patients with all 3 oral habits (p = 0.005) or with at least one oral habit (p = 0.007) also had a significantly higher cytoplasmic hTERT LS than OSCC patients without any oral habit.
Tsai et al.[22]	680	512	168	Exo1	PCR	The Exo1 K589E was associated with higher susceptibility of oral cancer. The allele frequency distributions of the Exo1 K589E showed that A allele of Exo1 K589E was associated with higher susceptibility for oral cancer, while others were not. Genotype distribution of various genetic polymorphisms of exo1 K589E was significantly different between oral cancer and control groups with smoking habit (p = 2.41E-11). Distributions of Exo1 K589E A homozygote/heterozygote and G homozygote in controls and oral cancer patients with no smoking habit were 76/116 and 59/109, respectively (p = 0.344, OR: 0.795, 95%CI: 0.519-1.218).
Anantharaman et al.[29]	665	325	340	GSTM1null, GSTT1null	PCR	Increased risk of oral cancer was associated with rs4646903 (OR: 1.66; 95%CI: 1.16-2.43), GSTM1 null (OR: 1.50; 95%CI: 1.20-1.87) and GSTT1 null genotype (OR: 0.47; 95%CI: 0.32-0.69). Additionally, rs2031920 and rs3813867 (OR: 0.39; 95%CI: 0.18-0.86) and rs1381 (OR: 0.75; 95%CI: 0.63-0.89) significantly reduced the risk of oral cancer. rs2031920 and rs3813867 significantly reduced oral cancer risk among exclusive tobacco chewers, (OR = 0.13; 95%CI = 0.03–0.59) and a positive dose-response relationship was observed. Similar results were observed for rs13181 (OR = 0.76; 95% CI = 0.59–0.97).
Tsai et al.[23]	213	159	54	CCND1	PCR	The genotype distribution of the polymorphisms of *CNND1* A870G was significantly different between oral cancer patients and controls with a smoking habit (p = 0.0006). The GG genotype frequency was still significantly lower (12.9%) in cancer patients with a smoking habit than in smoking controls (16.6%).
Mallick et al.[30]	39	31	8	p53, BCL-XL	IHC	In patients with tobacco habits (chewers + smokers), increased p53 intensity (p = 0.063) was observed compared to those with no habits, although it did not reach statistical significance. The probability of treatment failure (hazard ratio) was 3.2 times higher in the unfavorable responders compared to that of favorable response group. Bcl-xL protein was significantly upregulated (p=0.048) in the unfavorable responders compared to the favorable responders.

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Tsai et al.[24]	230	168	62	hOGG1	PCR	The genotype distribution of hOGG1 codon 326 polymorphism was significantly different between oral cancer and control groups who had a smoking habit (p = 0.0198), but was not significant (p = 0.8357) in non-smokers. Consistent with the findings, the C allele frequency was still significantly higher in patients with cancer with a smoking habit than in smoking controls. There was no such difference between the non-smoking groups
Zavras et al.[25]	239	194	45	ERCC5	PCR	The use of areca nut products, a prevalent habit in southern and eastern Asia that has been linked with higher frequency of epithelial tumor, was significantly higher among subjects with cancer (77.8%) compared with control subjects (15%).
Chuang et al.[26]	158	101	57	CYP1A1, GSTM1	IHC, ELISA	In smoking groups, the DNA adduct levels were 93.18 ± 81.67 adducts/108 nucleotides, which were significantly higher than in the non-smoking group (0.04 ± 0.33 adducts/108 nucleotides; p < 0.0001). Cigarette smoking may be the major cause of DNA adduct formation in oral tissue
Mondal et al.[31]	124	89	35	GSTM1, GSTT1	PCR	The risk of OSCC is 2.2-fold higher (95%CI: 1.31–3.68; p = 0.002) in tobacco-betel quid chewers, which is one of the main factors for oral cancer and is a common practice in Northeast India. The association between mtDNA copy number and OSCC risk was evident among tobacco-betel quid chewers rather than tobacco-betel quid non-chewers; the interaction between mtDNA copy number and tobacco-betel quid was significant (P = 0.0005). Similar results were observed when cases and controls were classified as tobacco- betel quid chewers and non-chewers based on low and high mtDNA copy number: the tobacco-betel quid chewers with the low mtDNA copy number had a 3.54-fold increased risk of OSCC (95%CI: 1.59–7.87).
Anil et al.[8]	100	72	28	PARP-1 variants	PCR	A strong association was observed with PARP1 SNP rs1136410 homozygous genotype "C/C" polymorphism in OSCC patients with smoking habit. OSCC patients with smoking habit showed nearly 12-fold higher risk compared to healthy individuals with "CC" genotype of SNP rs1136410 (p = 0.01923; OR: 12.615; 95%CI: 0.682-233.37). Similarly, variant allele C imposed 2.547-fold increased risk of OSCC in patients with smoking habit (p = 0.01617; OR: 0.547; 95%CI: 1.165–5.568). PARP1 SNP rs3219090, which didn't show any association with OSCC cancer in overall study, showed no association with OSCC in patients with smoking habit. No significant association was observed in case of interaction of smoking status with any genotypes in PARP1 SNP rs3219090 and rs1136410 for OSCC risk in non-smokers.
Nigam et al.[32]	72	46	26	XPC	PCR	Frequency of smokers among cases was significantly higher than in healthy controls. However, compared to oral cancer subjects, the proportion of nonsmokers was significantly higher among the healthy control group (p = 0.001). The result shows that smoking increases the risk of oral cancer by five times (OR 5.03; 95%CI: 2.91–8.69).

a valid and reliable way, use of standard criteria for measurement of the condition, adequate identification of confounding factors, statement of strategies used to deal with confounding, and use of appropriate statistical analysis. All studies showed unclear risk of bias for measurement of outcome in a valid and reliable way, as illustrated in Figure 2.

Results of the individual studies

The role for NAT1 appears to be independent of smoking behavior.[32] GSTM1 was found to have a protective role against OSCC, since a higher risk for OSCC was associated with GSTM1 null polymorphisms.[28,29,31,33,36,37,42] Also, a protective factor was detected for GSTT1,[29-31,33,37] as well as for CYP1A1,[28,30,36] whose null polymorphisms were related

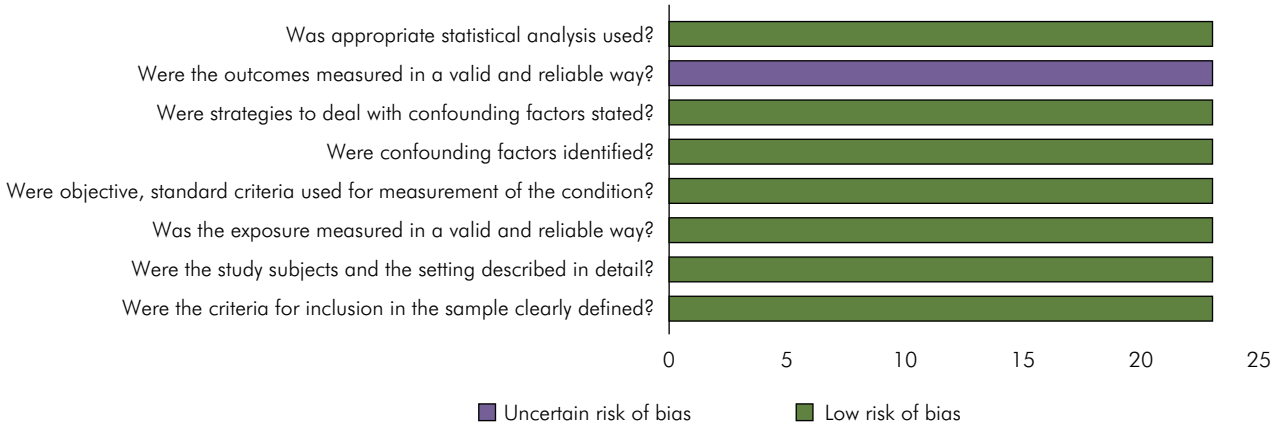

Figure 2. Risk of bias summary.

to a higher risk of OSCC. Park et al.[42] also described a protective factor for GSTM3, with no significant associations between GSTM3 genotype and oral cancer risk among African-Americans after stratification by smoking dose. Likewise, authors reported that the presence of p53 mutations was independent of tobacco consumption[39] and the difference between smokers and non-smokers was not statistically significant.[23,32] Mallick et al.[32] reported that the Bcl-xL protein was significantly increased in patients who responded to unfavorable situations, compared to patients who responded to factors favorable to OSCC treated with radiotherapy. The XPA and XPD genetic factors were modified by smoking.[13] XPC genes were also modified by smoking and had heterozygous genotypes associated with elevated risk for OSCC.[34,38] PARP-1 variants and ERCC5, hOGG1, and hTERT mutations were significantly higher in patients with OSCC who were smokers.[8,24,26,27] Ku70, Ku80, Exo1, and CCND1 were significantly lower in OSCC patients who were non-smokers.[15,41] For the ND2 protein, all patients with mutation were classified as smokers.[40]

Synthesis of results and additional analysis

Three studies reporting dichotomous data regarding GSTM1 were incorporated into one subgroup for analysis.[29,37,42] No significant difference was observed between smokers and non-smokers with respect to damage to GSTM1 (OR: 0.60; 95%CI: 0.30–1.18; I^2: 0%). Two studies[29,37] reporting dichotomous data regarding glutathione S-transferase theta 1 (GSTT1) were incorporated into the second subgroup. No significant difference was observed between smokers and non-smokers with respect to damage to GSTT1 (OR: 1.18; 95%CI: 0.49–2.83; I^2: 0%). Dichotomous data regarding hydrolase proteins (Ku70 and Ku80) were incorporated into the third subgroup.[41] No significant difference was observed between smokers and non-smokers with respect to damage to hydrolase proteins (OR:0.74; 95%CI: 0.18–3.05; I^2: 0%). Dichotomous data with respect to transferase proteins (GSTM1, GSTT1, and GSTM3) were incorporated into the last subgroup.[37,38] No significant difference was observed between smokers and non-smokers with respect to damage to transferase proteins (OR: 0.70; 95%CI: 0.42–1.12; I^2: 0%). Figure 3 shows the subgroup analyses.

Assessment of the certainty of evidence

The certainty of evidence was very low. Table 2 shows the complete information on evaluation of certainty of evidence.

Discussion

Summary

OSCC follows a multifactorial and dynamic course, with numerous changes contributing to the development of the disease. This systematic review and meta-analysis investigated the effects of tobacco on DNA of individuals with OSCC, comparing smokers and non-smokers. To our knowledge, this

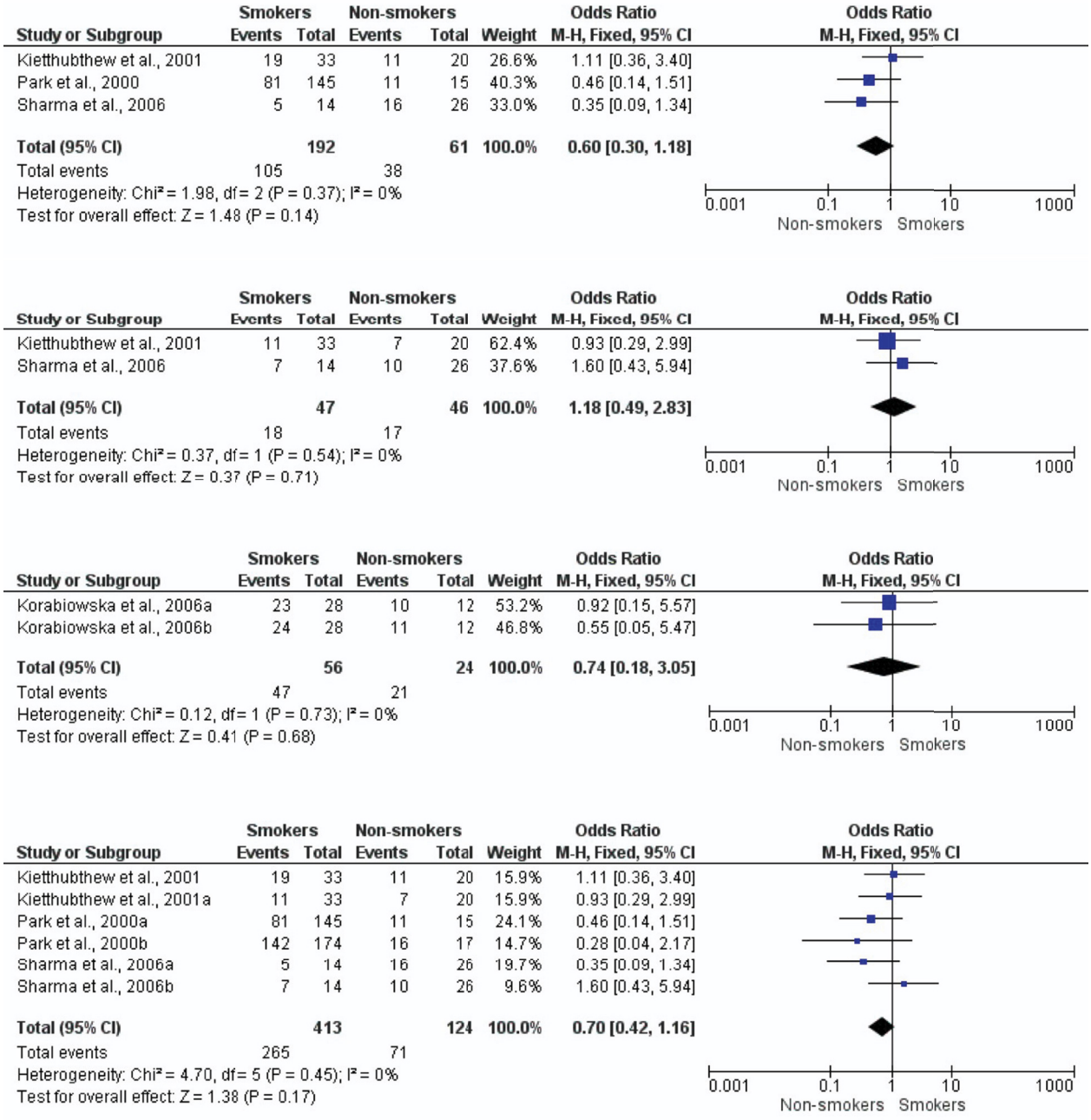

Figure 3. Forest plot of meta-analysis for the studies including (A) glutathione S-transferase mu 1 (GSTM1), (B) glutathione S-transferase theta 1 (GSTT1), (C) hydrolase proteins (Ku70 and Ku80), and (D) transferase proteins (NAT1*4, NAT1*10, GSTM1, GSTT1, and GSTM).

is the first comprehensive analysis about the effect of tobacco on DNA of smokers and non-smokers with OSCC. DNA damage response is a complex signaling network involving cell cycle checkpoints as well as DNA damage and repair pathways.[43] Herein, 14 molecular changes in gene/protein groups and altered genes/proteins, such as tumor suppressor, antiapoptotic, cyclin, monooxigenase, glycosidase, enzyme binding, transferase, DNA binding, hydrolase, helicase, ribonucleoprotein, exonuclease, endonuclease, and translocase were examined. Meta-analysis for GSTM1 and GSTT1, as well as for transferase and

Table 2. Assessment of the certainty of the evidence.

Variable		Certainty assessment						Number of individuals		Certainty
Outcome	Studies	Design	Bias	Inconsistency	Indirectness	Imprecision	Publication	Smokers	Non-smokers	
glutathione S-transferase mu 1 (GSTM1)	3	observational studies	not serious	not serious	serious[a]	serious[b]	publication bias strongly suspected[c]	105/192 (54.7%)	38/61 (62.3%)	⊕◯◯◯ Very low
glutathione S-transferase theta 1 (GSTT1)	2	observational studies	not serious	not serious	serious[a]	serious[b]	publication bias strongly suspected[c]	18/47 (38.3%)	17/46 (37.0%)	⊕◯◯◯ Very low
hydrolase proteins (Ku70 and Ku80)	2	observational studies	not serious	not serious	serious[a]	serious[b]	publication bias strongly suspected[c]	47/56 (83.9%)	21/24 (87.5%)	⊕◯◯◯ Very low
transferase proteins (NAT1*4, NAT1*10, GSTM1, GSTT1, and GSTM)	6	observational studies	not serious	not serious	serious[a]	serious[b]	publication bias strongly suspected[c]	265/413 (64.2%)	71/124 (57.3%)	⊕◯◯◯ Very low

[a]The certainty of the evidence has been downgraded by one level. The studies did not take the characteristics of smokers and non-smokers into account in the analyses; [b]The certainty of the evidence has been downgraded by one level. The number of individuals is lower than the optimal information size. [c]Studies incorporated with non-significant results.

hydrolase groups, showed no significant difference between smokers and non-smokers regarding the damage/polymorphism of these proteins. Although meta-analysis was impossible for tumor suppressor and anti-apoptotic genes, changes in these two groups were increased in OSCC smokers, as reported by Hsieh et al.[23] and Mallick et al.[32], respectively. As to the other protein groups, the number of studies was insufficient to allow solid conclusions.

Transferase proteins

Transferase proteins belong to a class of enzymes that transfer a specific functional group from the donor molecule and catalyze numerous biological reactions of critical importance for a living system. In this study, four proteins from this group were analyzed, i.e., GSTM1, GSTM3, GSTT1, and NAT1. Glutathione S-transferases (GSTs) are an important group of these enzymes, which detoxify both endogenous compounds and foreign chemicals such as pharmaceuticals and environmental pollutants.[44] The presence of GSTT1 and GSTM1 is essential for carcinogenic detoxification.[33] Some authors have shown that null GSTM1 and GSTT1 genotypes were likely to be associated with a higher risk of different types of cancers such as hepatocellular and thyroid malignancies.[45,48] The increased risk factor of null GSTM1 in OSCC is higher than that of null GSTT1, as revealed by the findings presented herein. In this regard, the GSTM1 enzyme possibly plays an important role inside the mitochondrial matrix as an mtDNA-protecting factor for damage caused by reactive oxygen species.[33] GSTM1 and GSTT1 polymorphisms, as well as detoxification enzymes have been identified in individuals with OSCC, but are not believed to be risk factors.[47] For instance, Kietthubthew et al.[37] reported that the frequencies of null GSTM1 and GSTT1 in their non-cancer sample were 30.2% and 47.2%, respectively. On the other hand, the results showed that individuals with a susceptible version of the GSTM1 genotype (null genotype) had a 2.6 times higher risk of OSCC, regardless of exposure to environmental hazards such as tobacco. However, Minina et al.[48] suggested that the GSTM1 null genotype increased the frequency of chromosomal damage in smoking patients with lung cancer. In addition, Park et al.[42] showed that the risk of oral cancer was significantly associated with GSTM1 null polymorphism among African American individuals who had smoked heavily for more than 24 years.

Polymorphisms of N-acetyltransferase-1 and -2 (NAT-1/2), another type of transferase responsible for the metabolism of tobacco carcinogens, have been investigated for a potential role in oral carcinogenesis. Nevertheless, no correlation was found indicating that

they do not themselves contribute to the carcinogenic process.[49] Unfortunately, there are discrepancies among studies associating these polymorphisms with OSCC, possibly related to demographics, as observed for GSTM1 associated with oral cancer in Asians, but not in Caucasians.[50] Based on a hypothesized role for NAT1 in modulating the effects of carcinogens present in tobacco smoke, Katoh et al.[35] investigated a combined role for smoking and the NAT1 genotype. The authors suggested that individuals with NAT1*10 alleles were at higher risk for OSCC, but that smoking history did not play a role in this genetic relationship. Smoking behavior in cases or controls (either smoker or non-smoker index) was not associated with any NAT genotype. The role of NAT1 appears to be independent of smoking behavior.

Hydrolase proteins

Hydrolase proteins are an enzyme system that catalyzes hydrolysis reactions. In the present study, Ku represented the protein associated with this group. It is now well established that, while not essential for individual life in the short term, Ku function is critical for the maintenance of genomic integrity and for proper cellular and organismal development.[51] Ku70 and Ku80 regulate subunits of the DNA-dependent protein kinase, a crucial enzyme involved in the repair of double-strand breaks in DNA. Along this line, Korabiowska et al.[41] investigated the role of the Ku70 and Ku80 genes in the progression of OSCC. Among their findings, Ku70 expression correlated very strongly with smoking habits. The authors demonstrated that dysregulation of the Ku70 and Ku80 axis may be influenced by tobacco.[41]

Tumor suppressor and antiapoptotic proteins

Two groups deserve recognition in this study, even though no meta-analysis was possible. Tumor suppressor and antiapoptotic proteins have also been highlighted in the literature when cancer is involved.[52] The inactivation of tumor suppressor genes result in a phenotype only if both copies of the gene are lost. In the carcinogenic process, inactivation of one copy of a tumor suppressor gene must usually be followed by loss of the remaining copy of the gene and by the emergence of the tumor phenotype.[53] The importance of the p53 tumor suppressor gene in the process of carcinogenesis has been well established in the current literature.[23,54] Mutation of P53 has been reported in over 80% of all cancers,[55] with a higher incidence in tobacco-related cancers. Mallick et al.[32] reported an increased intensity of p53 among patients with tobacco habits compared to non-smokers. Notably, tobacco carcinogens played an important role in p53 mutations in Taiwanese patients with OSCCs.[23]

Antiapoptotic proteins were represented by BCL-2. The BCL-2 family may be understood as a tripartite apoptosis control system comprising one set of anti-apoptotic proteins and two sets of pro-apoptotic proteins, which interact to determine whether cells will live or die in many pathophysiological states.[56] Overexpression of BCL-2 was originally described in leukemia and in B-cell non-Hodgkin's lymphoma. BCL-2 overexpression is, in most cases, the consequence of a *t(14,18)* translocation that has its break-point close to the BCL-2 gene. However, BCL-2 overexpression is, by itself, insufficient for malignant transformation, but may provide a predisposition to the development of B-cell lymphomas.[57]

Limitations

The inherent limitations of a systematic review and meta-analysis should be considered here. First, due to the heterogeneity of genes/proteins, the comparison of a significant number of studies on the same molecule was unfeasible. Moreover, some studies did not show a relationship between smoking and gene/protein changes in their results and were thus unfit for inclusion in this systematic review and meta-analysis and for the analysis of some protein groups.

Conclusions

In summary, the articles included in the present systematic review and meta-analysis diverged in relation to the role of tobacco in genetic changes that predispose to OSCC. While in some studies smoking history has not been shown to play a differential role in carcinogenesis,[35,37,39] the vast

majority confirm that smokers are more likely to have DNA alteration – mainly associated with genetic polymorphisms.[13,24,25,29,40,42] Therefore, our study demonstrates that major changes in genes or proteins do not necessarily occur in smoking patients. Indeed, the role of tobacco in carcinogenesis is well known. As far as we know, there are nearly 60 carcinogenic compounds in tobacco smoke. However, great genetic changes in non-smoking patients were a common finding in some studies,[35] while other studies found similar patterns of genetic alterations between smokers and non-smokers,[41] suggesting that the genetic alteration evaluated was not related to smoking habit. It is possible that the referred genes do not play a relevant role in tobacco-related carcinogenesis, but are relevant to the carcinogenesis process as a whole. Thus, further studies are needed to understand OSCC pathways in smokers and non-smokers.

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Effects of tobacco on the DNA of smokers and non-smokers affected by OSCC: systematic review and meta-analysis. Braz Oral Res. 2023;37:e008.

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Where is read: José Alcides Almeida de ARRUDA

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Legend

Where is read: *Schuch LF, Viana KSS, Arruda JAA, Abreu LG, Aguiar MCF, Bernardes VF*

Should read: *Schuch LF, Viana KSS, De Arruda JAA, Abreu LG, Aguiar MCF, Bernardes VF*

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