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Cancer and Potentially Malignant Disorders

Investigating the Association Between Oral Hygiene and Oral Cancer: A Systematic Review and Meta-Analysis

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ABSTRACT

Objective: We aim to investigate the association between oral hygiene habits, including frequency of toothbrushing and regularity of dental check-ups, and oral cancer risk.

Materials and Methods: A systematic review and meta-analysis was developed following the PRISMA and the MOOSE guidelines. We searched electronic databases until 4th December 2024 (CRD42021242709).

Results: A total of 15 studies were included. Overall, the results indicated that poor oral hygiene habits are potential risk factors for oral cancer. Compared to brushing once/day or more, toothbrushing < 1/day or never significantly increased the risk for oral cancer, with an OR of 1.85 (95% CI, 1.06–3.22). Toothbrushing < 2/day compared with ≥ 2/day also showed a positive association with oral cancer risk, with an OR of 1.32 (95% CI, 1.17–1.48). Irregular dental check-ups, defined as less than once a year or never, also significantly increased oral cancer risk (OR, 1.58; 95% CI, 1.29–1.92).

Conclusion: Infrequent toothbrushing and irregular dental check-ups are risk factors for oral cancer. Toothbrushing twice or more daily and visiting a dentist once a year could be recommended to reduce oral cancer risk. The study reinforces the importance of good oral health as a preventive measure in the control of oral cancer.

1 | Introduction

Oral cancer is a significant public health problem worldwide. In 2022, lip and oral cavity cancer combined amounted to an estimated incidence of 389,485 new cases and 188,230 deaths (Bray et al. 2024). The major risk factors are tobacco smoking, smokeless tobacco use, alcohol consumption, and areca nut chewing (Hashibe 2020; Bouvard et al. 2022). Nevertheless, poor oral hygiene has often been observed in oral cancer patients, and the question arises whether it may contribute to oral carcinogenesis (Mathur et al. 2019). The oral cavity is inhabited by the oral microbiome, and the presence of dental plaque, which consists of a bacterial biofilm that covers the surface of the teeth and gums, may promote tissue inflammatory processes (Cekici et al. 2014). Poor oral hygiene leads to the formation of dental plaque and thus may contribute to an inflammatory process in the oral cavity, consequently leading to cancer development (Hashim et al. 2016).

A few epidemiological studies have investigated the possible role of poor oral hygiene and of low oral microbiome diversity (leading to dysbiosis) on susceptibility to oral cancer. Indicators of poor oral hygiene such as low frequency of toothbrushing and irregular dental check-ups have been linked to oral cancer, independently of tobacco use and alcohol consumption (Guha et al. 2007). Results from the International Head and Neck Cancer Epidemiology (INHANCE) consortium showed that good oral hygiene, as characterized by few missing teeth, annual dental check-ups, and daily

toothbrushing, may modestly reduce the risk of head and neck cancer (Hashim et al. 2016). Several meta-analyses of individual studies have been conducted, which have assessed the association between toothbrushing and/or dental check-ups and risk for head and neck cancer (Zeng et al. 2015), esophageal carcinoma (Chen et al. 2015), and upper aerodigestive tract (UADT) cancer and subsites of the UADT (Gupta et al. 2019; Wu et al. 2021).

The aim of this systematic review and meta-analysis is to evaluate quantitatively the association between oral hygiene habits, including frequency of toothbrushing and regularity of dental check-ups, and oral cancer risk.

2 | Methods

2.1 | Protocol and Registration

This systematic review and meta-analysis protocol was developed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) checklist (Page et al. 2021) and also complied with the MOOSE reporting guidelines (Stroup et al. 2000). The protocol for this study was registered prospectively in the International Prospective Register of Systematic Reviews (PROSPERO), with the registration number CRD42021242709.

2.2 | Eligibility Criteria

The focused question of this systematic review and meta-analysis was formulated using the PECOS framework, with P (Population): individuals of any age, sex or geographic origin; E (Exposition): individuals with poor oral hygiene habits, that is, individuals who do not brush their teeth or do it infrequently, and individuals who do not have regular check-ups with a dentist or oral hygienist; C (Comparison): individuals with good oral hygiene habits, that is, individuals who brush their teeth frequently and individuals who have regular dental check-ups; O (Outcomes): pathologically confirmed oral cancer; S (Studies): clinical trials, cohort studies, case–control studies or cross-sectional studies. No restrictions in regard to the time of publication or language were applied. Based on this framework, the research question was: Are poor oral hygiene habits, defined here as infrequent toothbrushing and irregular dental check-ups, associated with the development of oral cancer?

The following exclusion criteria were applied: (1) letters, reviews, case series, case reports; (2) studies that did not report separate data for the oral cavity from other head and neck sites; (3) studies that did not report effect estimates or had insufficient information for analysis; (4) molecular studies, animal models and in vitro studies; and (5) studies with missing information or reporting on outcomes other than toothbrushing or dental check-ups.

2.3 | Information Sources and Search Strategy

The search strategy was performed using the electronic databases PubMed, Scopus, and Cochrane Library until 4th December 2024. Additionally, a search was carried out into the gray literature databases of Open Gray and Proquest for dissertations (Table S1). Furthermore, an extensive hand-search was carried out on the list of references of included studies.

2.4 | Study Selection Process and Data Extraction

Following removal of duplicates, the reviewers (LCM and LM) screened the articles by title and abstract. Then, the full text of the included articles was assessed by the same authors. Disagreements were discussed and resolved in a consensus with a third author (SW). We calculated the percentage of agreement between the reviewers and Cohen's kappa coefficient using the IBM Statistics 29 program (SPSS, Chicago, Illinois, USA). All records were managed with the EndNote X9 software (Thomson Reuters, New York, USA).

Two authors (LCM and LM) independently extracted the data. Data extracted consisted of study characteristics (first author, year of publication, country, and study design), population characteristics (sample size, age, sex), exposure variables (frequency of toothbrushing and regularity of dental check-ups), and results of the analyses (crude and adjusted risk estimates). Any disagreements were discussed and resolved with a third author (SW). Exposure variables were extracted as described in the original articles, and values were subsequently standardized into clinically meaningful categories, namely: (1) toothbrushing frequency of < 1/day or never compared to ≥ 1/day (reference group); (2) toothbrushing frequency of < 2/day compared to ≥ 2/day (reference group). Dental check-ups were classified as "not regular or never" compared to "regular" (reference group). We indicate in Table S2 how the original exposure variables were assigned to the categories for the quantitative analyses.

2.5 | Risk of Bias Assessment

For each included study, the risk of bias was assessed independently by two reviewers (LCM and LM) using the Newcastle-Ottawa Scale (NOS) (Stang 2010). This scale assesses study quality based on three domains: selection (assesses the quality of the selection process for study groups), comparability (evaluates the comparability of the groups in the study), and exposure (looks at how outcomes are assessed and the adequacy of follow-up). The NOS has a maximum score of 9 points: studies that score less than four points were regarded as low quality, four to six points as moderate quality, and seven or more points as high quality (Stang 2010).

2.6 | Data Analysis and Synthesis

A quantitative analysis of the results was performed using the software program Review Manager (RevMan) version 5.3. (Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2012). Adjusted effect estimates were favored to minimize confounding. Statistical heterogeneity was assessed by performing Q test and I^2 tests. A significant heterogeneity was defined as Cochrane $Q < 0.10$ and/or $I^2 > 50\%$. A random-effect model was used when there was significant between-study

heterogeneity ($I^2 > 50\%$); otherwise, a fixed-effect model was conducted following the Cochrane Collaboration guidelines (Deeks et al. 2019). Sensitivity analyses were carried out by excluding studies that did not adjust for important potential confounders. When data in a study were reported separately for different categories or subgroups of the study population, such as sex or age groups, a fixed-effect meta-analysis was conducted to combine these data and calculate an overall OR.

3 | Results

3.1 | Study Selection

A total of 1537 records were initially identified. The duplicates (1046 records) were then removed. Following title-abstract selection, 44 records were assessed for eligibility through full-text evaluation, after which 29 records were excluded, with an absolute agreement between the two reviewers of 93.8% and a kappa coefficient of 0.81 (95% CI, 0.61–0.95). Thus, 15 eligible studies were identified after full-text evaluation. A detailed flowchart of the selection process is shown in Figure S1. The reasons for exclusion of the studies are presented in Table S3.

3.2 | Characteristics of the Included Studies

The main characteristics of the included studies are listed in Tables S4 and S5. The studies were carried out from 1989 to 2020 in populations from Asia (6), Europe (2), Latin America (5), North America (1), and Oceania (1). A total of 6 studies (Chang et al. 2013; Chen et al. 2016; Franco et al. 1989; Gupta et al. 2017; Hashim et al. 2016; Zheng et al. 1990) used "oral cancer" as the classification of type of cancer, 8 studies (Balaram et al. 2002; Bundgaard et al. 1995; Divaris et al. 2010; Guha et al. 2007; Marques et al. 2008; Pereira et al. 2020; Sato et al. 2011; Velly et al. 1998) used "oral cavity" as the classification of type of cancer, and one used "oral squamous cell carcinoma" (Yan et al. 2017). Based on the ICD codes reported in the original articles (listed in Tables S4 and S5), the subsites have been standardized; as a result, all "oral cancer" could be identified as "oral cavity" cancers (Tables S4 and S5).

Outcomes for toothbrushing frequency were reported in 13 studies (Balaram et al. 2002; Chang et al. 2013; Chen et al. 2016; Franco et al. 1989; Guha et al. 2007; Gupta et al. 2017; Hashim et al. 2016; Marques et al. 2008; Pereira et al. 2020; Sato et al. 2011; Velly et al. 1998; Yan et al. 2017; Zheng et al. 1990) and for regularity of dental check-ups in 10 studies (Balaram et al. 2002; Bundgaard et al. 1995; Chang et al. 2013; Chen et al. 2016; Divaris et al. 2010; Guha et al. 2007; Gupta et al. 2017; Marques et al. 2008; Pereira et al. 2020; Yan et al. 2017).

3.3 | Quality Assessment of the Included Studies

All studies included were of moderate quality (NOS scale ranging from 4 to 6), with most studies having an NOS scale of 5 (Tables S4 and S5). Only three studies selected controls from community sources, while the remainder relied on hospital-based controls. Additionally, most studies reported that interviews were conducted without blinding to case/control status. Furthermore, none of the studies provided information on response rates. All but one study (Pereira et al. 2020) adjusted for tobacco smoking and alcohol consumption, and most studies also adjusted for additional variables likely to be associated with oral cancer (Tables S4 and S5).

3.4 | Meta-Analysis

3.4.1 | Association of Toothbrushing Frequency With Oral Cancer

The MA assessing the association between toothbrushing frequency and oral cancer risk encompassed 13 studies (Balaram et al. 2002; Chang et al. 2013; Chen et al. 2016; Franco et al. 1989; Guha et al. 2007; Gupta et al. 2017; Hashim et al. 2016; Marques et al. 2008; Pereira et al. 2020; Sato et al. 2011; Velly et al. 1998; Yan et al. 2017; Zheng et al. 1990). The combined effect estimates (meta-OR) using a random-effects model revealed a significant association between infrequency of toothbrushing and risk for oral cancer, with a meta-OR for <1/day or never compared to ≥ 1/day of 1.85 (95% CI, 1.06–3.22), with high heterogeneity ($I^2 = 87\%$) (Figure 1).

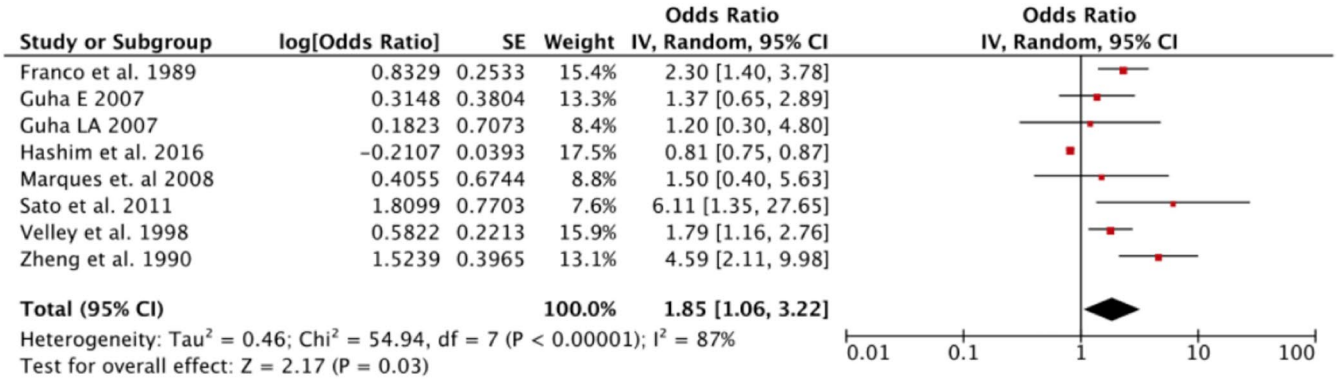

FIGURE 1 | Forest plot of studies assessing toothbrushing frequency in relation to oral cancer risk, using <1/day or never compared to the reference group of ≥ 1/day (random-effect model). Guha E, data for Europe; Guha LA, data for Latin America; For Zheng et al. 1990, a fixed-effect MA was first conducted combining data for men and women to calculate a unique OR. For other specific indications on the odds ratios included in the forest plot analysis, please see Table S4.

Based on those studies where data were available, we then estimated the risk for oral cancer using the exposure categories of toothbrushing of <2/day compared to ≥2/day (Figure 2). The meta-OR using a fixed-effects model revealed a significant association, with an OR of 1.32 (95% CI, 1.17–1.48) and a heterogeneity of 20% (Figure 2A). Even after excluding the study by Pereira et al. (Pereira et al. 2020) because of the lack of adjustment for smoking status and alcohol consumption, the results remained significant (Figure 2B). Using the raw data of events and controls (Table S2), we also calculated crude risk estimates with other cut-offs of toothbrushing frequency. We investigated the association between oral cancer risk and toothbrushing frequency of <1/day compared to 1/day (but not more), as well as the lowest frequency score compared to the highest frequency score (Figures S2 and S3). These comparisons showed meta-ORs of 2.06 (95% CI, 1.54–2.76; $I^2 = 56\%$) and 2.14 (95% CI, 1.81–2.52; $I^2 = 62\%$), respectively.

3.4.2 | Association Between Regularity of Dental Check-Up and Oral Cancer Risk

We first carried out a MA including studies that compared exposure categories of irregular dental check-ups (i.e., less than once a year, including never) with regular dental check-ups, defined as a dental check at least once a year, as the reference group (Table S5). We found a significant positive association, with a meta-OR of 1.58 (95% CI, 1.29–1.92; $I^2 = 54\%$) (Figure 3A). Additionally, we conducted a MA including only studies that assessed "never" compared to regular dental check-ups, which

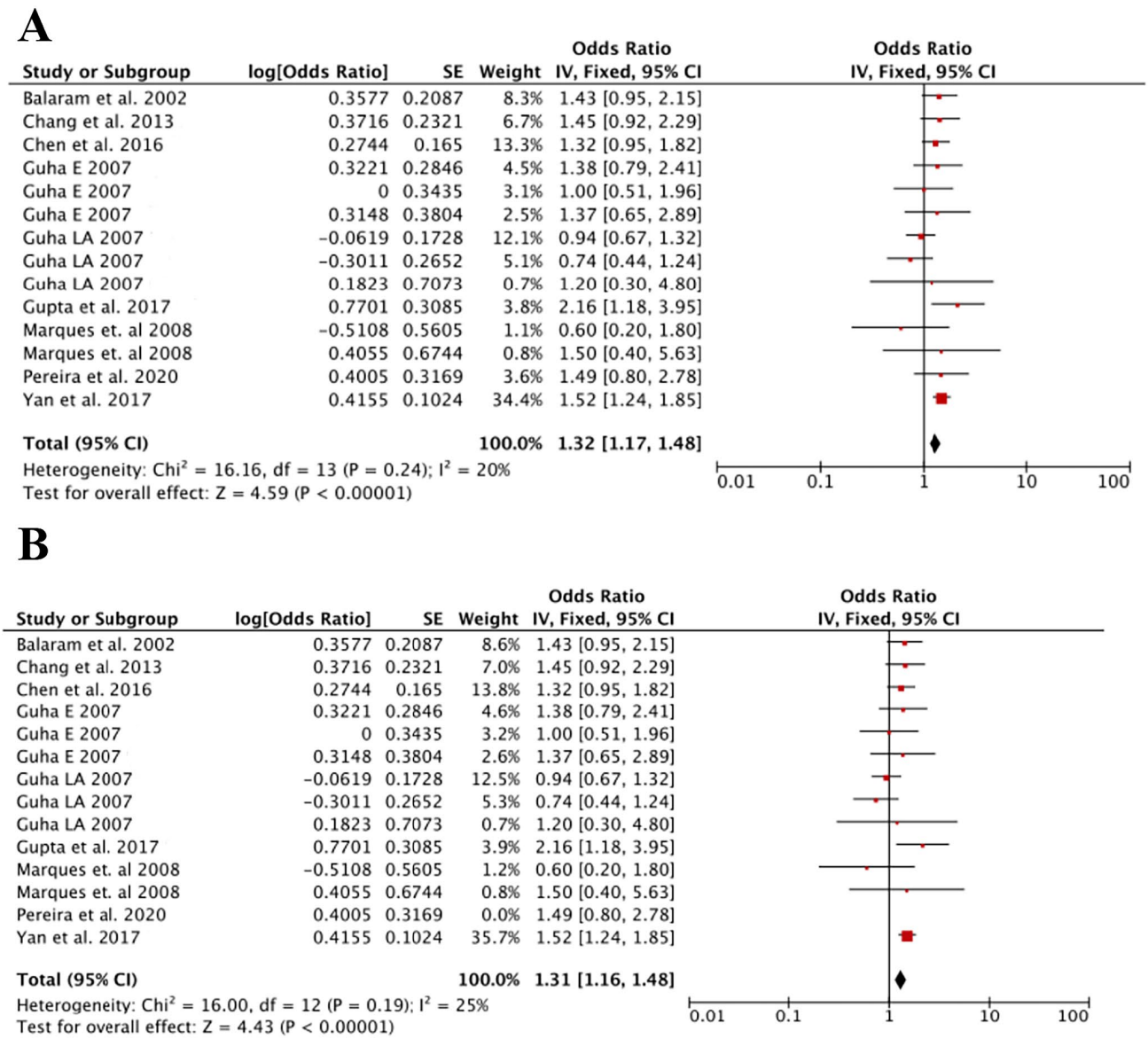

FIGURE 2 | Forest plot of studies assessing tooth brushing frequency in relation to oral cancer risk, using <2/day compared to the reference group of ≥2/day (fixed-effect model). A – All studies; B – Excluding Pereira et al. (2020) due to the lack of adjustment for smoking and alcohol consumption. Notes: Guha E, data for Europe; Guha LA, data for Latin America. For Guha et al. (2007; E and LA), the forest plot includes ORs for the reference group versus "once a day", "<1/day", and "never" groups; for Marques et al. (2008), the forest plot includes OR for the reference group versus "<1/day" and "never" groups. For other specific indications on the odds ratios included in the forest plot analysis, please see Table S4.

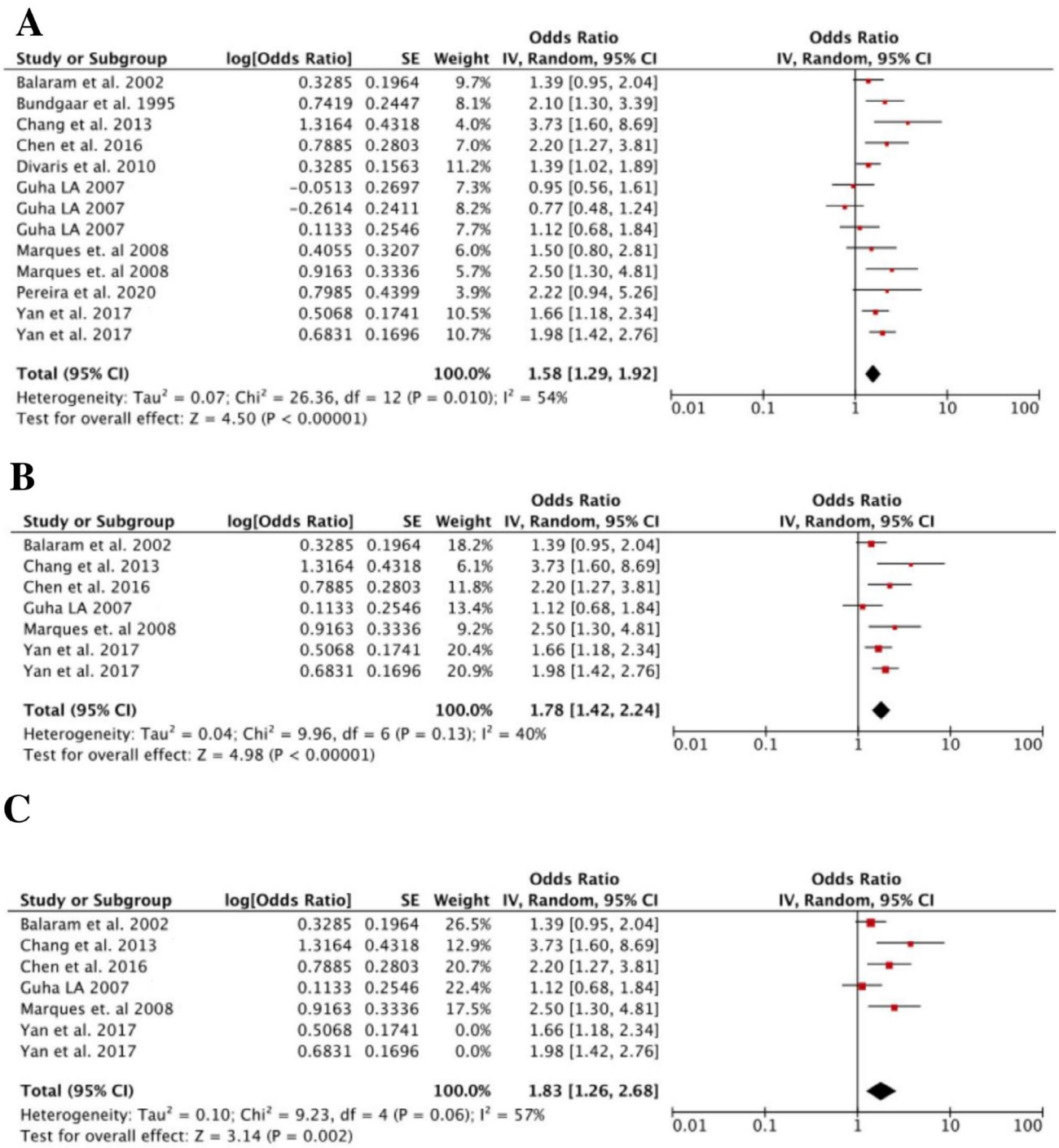

FIGURE 3 | Forest plot of studies assessing regularity of dental check-up in relation to oral cancer risk by comparing "not regularly or never" to the reference group of "regularly" (random-effect model). (A) All studies; (B) Excluding studies that only compared "not regularly" to the reference group of "regularly"; (C) Excluding Yan et al. (2017), which presented exposure groups with a broader time range. In figure A, for Guha et al. (2007), the forest plot includes OR for the reference group versus "dental check-up every 2-5 years"; "dental check-up less than every 5 years"; and "never" groups; for Marques et al. (2008), the forest plots include OR for the reference group versus "occasionally" and "never" groups; for Yan et al. (2017), the forest plots include OR for the reference group "> 5 years/time" versus "never" group and "≤ 5 years/time" versus "never" group; in figures B and C, for Yan et al. (2017), the forest plots include OR for the reference group "> 5 years/time" versus "never" and reference group "≤ 5 years/time" versus "never." For other specific indications on the odds ratios included in the forest plot analysis, please see Table S5.

showed a meta-OR of 1.78 (95% CI, 1.42–2.24), with moderate heterogeneity ($I^2 = 40\%$) (Figure 3B), and excluding Yan et al. (2017), which presented exposure groups with a broader time range (Figure 3C).

4 | Discussion

Few systematic reviews have evaluated the association between toothbrushing frequency and/or dental check-up regularity and

oral cancer risk (Gupta et al. 2019; Wu et al. 2021). The earlier review (Gupta et al. 2019) estimated the association between regularity of dental check-ups and incidence of head and neck cancers, including a sub-analysis on oral cancers; based on 13 original studies, the meta-OR of dental check-ups categorized as "past never/irregular/not frequent" was significantly increased in oral cancer cases (1.93; 95% CI, 1.47–2.52) compared with controls (which included both hospital-based or population-based controls). In a meta-analysis of studies assessing the association between toothbrushing frequency and gastric and upper aerodigestive tract cancer risk, Wu et al. (2021) also evaluated as a secondary outcome the risk for oral cavity cancer (including 14 articles) and observed that a high frequency of toothbrushing, defined by a score from 0 to 4 times/day, was associated with a significantly reduced risk of oral cavity cancer (OR = 0.52, 95% CI, 0.40–0.64), with moderate heterogeneity ($I^2 = 68.3\%$). More recently, another systematic review (Mahuli et al. 2023) reported poor oral hygiene as a risk factor for oral cancer (OR = 1.29; 95% CI, 1.04–1.54), but the review is lacking definitions on poor oral hygiene and doesn't provide an evaluation of toothbrushing or dental visit variables for comparison to our results.

In our study, we investigate quantitatively the association between oral hygiene habits, that is, toothbrushing frequency and dental check-ups regularity, and oral cancer risk using clear definitions of the exposure categories and focusing on cancer of the oral cavity. We calculated meta-ORs for toothbrushing frequency of <1/day or never compared to ≥ 1/day, of <2/day compared to ≥ 2/day, of 1/day (but not more) compared to <1/day, as well as for highest frequency score compared to lowest frequency score. All analyses showed an increased risk for oral cancer, and the increase was highest when comparing the lowest to the highest toothbrushing frequency for each study, although with an increase in heterogeneity. We also observed a significant association between irregular dental check-ups (i.e., less than once a year, including never) and oral cancer risk (OR, 1.58; 95% CI, 1.29–1.92), which was stronger when using solely "never." Our results are in line with other systematic reviews and give a more precise estimate of the risk, focusing on several exposure categories and oral cavity cancer as an outcome. Based on this evidence, we recommend that future studies examining risk factors for oral cancer also adjust for oral hygiene habits in addition to known risk factors such as smoking and alcohol consumption.

We observed a lack of clear definitions of the exposure categories in some articles, particularly for the number of visits to a dentist in a defined period (e.g., per year), or with subjective information such as "regular" or "not," which constitutes a limitation for the evaluation of these articles. This highlights the need for quantitative objective information on frequency/time/type of these variables in future research to enable the assessment of continuous variables.

All but one study (Pereira et al. 2020) accounted for the key risk factors relevant to our research, that is, alcohol and tobacco consumption. Noteworthy, excluding this study did not modify the association. Nevertheless, smoking is a strong predictor of oral cavity cancer and there could be some residual confounding by smoking.

The oral health status of an individual is associated with socioeconomic status and is influenced by access to education and prevention programs in oral health (Mathur et al. 2019); furthermore, low socioeconomic status has been reported as an independent risk factor for oral cancer (Conway et al. 2008; Warnakulasuriya 2009). None of the studies adjusted for socioeconomic status directly, but most adjusted for education (Balaram et al. 2002; Bundgaard et al. 1995; Chang et al. 2013; Chen et al. 2016; Divaris et al. 2010; Franco et al. 1989; Guha et al. 2007; Gupta et al. 2017; Hashim et al. 2016; Marques et al. 2008; Sato et al. 2011; Velly et al. 1998; Yan et al. 2017; Zheng et al. 1990), which can be used as an indirect measure of socioeconomic status (Hashim et al. 2016), thus leaving only a residual confounding. Nevertheless, this lack of adjustments for socioeconomic status constitutes a limitation of the analysis and underscores the need for future studies to incorporate direct measures of socioeconomic status.

All studies were of moderate study quality, which can originate from several factors. First, reliance on hospital-based controls may introduce biases and affect the generalizability of the findings. Additionally, conducting interviews without blinding to status introduces a potential for information bias. Furthermore, the lack of information on response rates leaves the possibility of response bias unaddressed.

We found moderate statistical heterogeneity among the included studies, mostly around 55%–60%. Such heterogeneity may arise from differences in study design, geographical location, sample sizes, and methodological approaches. The included studies (all with a case–control design) were conducted in many different countries and settings around the world, where toothbrushing may be practiced in a variety of ways. Also, the reporting of toothbrushing frequency varied widely among the included studies. Furthermore, we only assessed toothbrushing frequency and not the utensil used for brushing. In some regions in the world where the population does not have access to a toothbrush, notably in rural populations in Asia and Africa, individuals brush their teeth with their index finger or other materials such as the neem tree (*Azadirachta indica*), miswak (*Salvadora persica*), or twigs (Chatterjee et al. 2011; Gupta and Shetty 2018; Kumar et al. 2022; Sultan et al. 2024). Such practices may contribute to the observed heterogeneity and affect the strength of the observed associations. Although heterogeneity reduces the precision of pooled estimates, the direction of the association between poor oral hygiene and oral cancer remained consistent.

Another potential limitation of our study is the magnitude and clinical relevance of the effect estimates. Although the meta-ORs demonstrated statistical significance, interpretation according to Cohen's *d* thresholds (Chen et al. 2010) indicates that the effect sizes were generally small (OR 1.32–2.06, corresponding to $d \approx 0.15$–0.30). Thus, while poor oral hygiene and irregular dental check-ups are consistently associated with oral cancer, the strength of these associations is modest, and their clinical significance should be interpreted with caution, particularly considering the observed heterogeneity.

The mechanisms involved in the carcinogenesis associated with poor oral hygiene are not fully understood. Pathogenic

microbes found in the oral biofilm and associated with periodontal disease (D'Aiuto et al. 2004; Gopinath et al. 2020; Javed and Warnakulasuriya 2016; Karin et al. 2006; Nwizu et al. 2020) may contribute to carcinogenesis by inducing chronic inflammation, promoting cell proliferation, triggering cellular invasion, and deploying immune evasion strategies (D'Aiuto et al. 2004; Karin et al. 2006; Nwizu et al. 2020). Abundant oral flora may also contribute to cancer development through the metabolism of procarcinogens—e.g., conversion of ethanol to acetaldehyde—by *Candida*, *Neisseria*, and streptococci. IARC has classified acetaldehyde as a Group 1 carcinogen (IARC 2012), and accumulation of acetaldehyde in biopsied tissues of oral leukoplakia and oral cancer has been demonstrated in histochemical studies (Warnakulasuriya et al. 2008). Using multi-omics approaches to explore complex host-microbiota interactions, significant enrichment in the relative abundance of seven bacteria species (*Fusobacterium nucleatum*, *Treponema medium*, *Peptostreptococcus stomatis*, *Gemella morbillorum*, *Catonella morbi*, *Peptoanaerobacter yurli*, and *Peptococcus simiae*) was observed in the tumor microenvironment of oral cancers (Cai et al. 2024). Nonetheless, susceptibility to these exposures may be modified by other lifestyles (IARC 2012).

The *IARC Monograph* program has not evaluated poor oral hygiene as a risk factor for cancer in humans. The IARC Advisory Group that convened in 2019 (IARC 2019) recommended an evaluation of poor oral hygiene with high priority. In the more recent Advisory Group meeting in 2024 (de Berrington González et al. 2024), the most appropriate agents identified in relation to oral hygiene for consideration by the *IARC Monographs* programme were the pathogenic bacterial species recognized as causes of chronic periodontitis and related conditions, with *Fusobacterium nucleatum* being the best-studied specific agent in this regard. Based on our current results, we recommend that the *IARC Monographs* Programme consider epidemiological evidence on poor oral hygiene in addition to the evidence on these specific bacteria.

Recently, a Task Team appointed by the World Dental Federation (FDI) to assess the benefits of oral health developed evidence-based recommendations for toothbrushing through professional consensus. The Task Team recommended that toothbrushing should be undertaken at least twice a day, with 96% agreement among its members (Glenny et al. 2024). Our results are in full agreement with this report.

5 | Conclusion

This systematic review and meta-analysis further strengthens the evidence that poor oral hygiene is a risk factor for oral cancer. Toothbrushing at least twice daily and regular dental check-ups contribute to good oral hygiene and may be protective of oral cancer. Frequent toothbrushing effectively removes dental plaque, thus preventing gingival bleeding and periodontal disease, and in turn may contribute to reducing the risk of oral cancer. In addition, regular dental check-up provides an opportunity to reduce inflammatory conditions associated with periodontal disease. These measures should be recommended for oral cancer control. In view of the body of evidence presented here it is timely that the *IARC Monographs Program* takes up this topic for a future evaluation.

Author Contributions

Lorena C. Mariano: methodology, software, data curation, investigation, formal analysis, writing – original draft, writing – review and editing. **Saman Warnakulasuriya:** conceptualization, methodology, validation, supervision, writing – review and editing, writing – original draft. **Beatrice Lauby-Secretan:** validation, conceptualization, methodology, writing – review and editing, supervision. **Luis Monteiro:** conceptualization, methodology, investigation, writing – original draft, writing – review and editing.

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The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Data S1:** odi70201-sup-0001-Supinfo.docx.