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Meta-analysis on the association between the frequency of tooth brushing and diabetes mellitus risk

Running title: tooth brushing and diabetes

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Abstract

***Background and Objective*:** Epidemiological studies suggested that the frequency of tooth brushing might be associated with the risk of diabetes mellitus (DM), but the results were inconsistent and no systematic review was conducted to focus on this topic. In this meta-analysis, we synthesized available observational epidemiological evidences to identify the association between tooth brushing and DM risk and investigate the potential dose-response relationship of them.

***Methods*:** We searched PubMed and Embase from their inception through December 2017 to identify observational studies examining the association between tooth brushing and the risk of DM. Reference lists from retrieved articles were also reviewed. We quantitatively combined results of the included studies using a random-effects model. Dose-response meta-analysis was conducted to further examine the effect of tooth brushing frequency on DM risk.

Results: We identified 20 relevant studies (one cohort study, 14 case-control studies, and five cross-sectional studies) involving161,189 participants and 10,884 patients with DM. Compared with the highest tooth brushing frequency, the lowest level was significantly associated with an increased risk of DM (OR 1.32, 95% CI: 1.19 to 1.47), and there was no significant heterogeneity across the

included studies (P = 0.119, I^2 = 28.1%).

Exclusion of any single study did not materially alter the combined risk estimate. The dose–response analysis indicated that the summary odds of DM for an increment of one time of tooth brushing per day was 1.20 (95% CI: 1.16–1.24).

Conclusions: Integrated epidemiological evidence supports the hypothesis that low frequency of tooth brushing may be a risk factor of DM, and lower frequencies of tooth brushing were significantly associated with higher risk of DM.

Keywords: tooth brushing, diabetes, meta-analysis

Introduction

Diabetes mellitus (DM) is one of major public health threats in both developed and developing countries [1]. Between 2012 and 2013, 1.5-5.1 million people died from diabetes per year, making it the 8th leading cause of death around the world. It is predicted that about 592 million people would die from diabetes by the year of 2035 [2]. Studies have shown that reducing modifiable risk factors might contribute to the prevention and control of DM [3-4]. Therefore, identifying possible risk factors of DM is an important task.

Tooth brushing is considered as a fundamental self-care behavior for the maintenance of oral health. Some studies have suggested that tooth brushing was associated with many health problems, including gingivitis, dental caries, tooth decay [5], periodontal disease [6], head and neck cancer [7], dyslipidemia, [8] and endothelial dysfunction [9].

A growing number of epidemiological studies have investigated the association of tooth brushing and DM, although these studies had modest sample sizes and reported inconclusive results. Meta-analysis, as a statistical tool that can be used to integrate results of multiple independent studies, is considered to be ‘combinable’ for a more precise estimation [10-11]. To our knowledge, tooth brushing and DM risk has not yet been evaluated using a meta-analytic method. Therefore, we conducted a meta-analysis to investigate the association between tooth brushing and the risk of DM, and quantify the dose–response relationship between them. Given the heavy economic and health burden of DM, the results of our study may provide additional practical and valuable clues for the prevention of DM.

Materials and Methods

This systematic review was performed according to the Meta-analysis of Observational Studies in Epidemiology（MOOSE）statements[12] . Ethical approval is not required for this systematic review.

Eligibility criteria

Studies meeting the following criteria were included in the meta-analysis: (1) the study design was observational; (2) tooth brushing was an exposure variable and the outcome was DM; and (3) the study reported risk estimates (ORs/RRs) with 95% confidence intervals (CIs) of DM related to tooth brushing or provided sufficient data to calculate them. Animal studies, clinical trials, reviews, letters and commentaries were excluded. Additionally, in this meta-analysis, DM is defined as follows:（1）a glycated hemoglobin (HbA_{1c}) concentration of ≥ 6.5% (as per National Glycohemoglobin Standardization Program) and/or the subjects who received medication for their diseases；（2）a fasting plasma glucose level ≥126 mg/dl or a non-fasting plasma glucose level ≥200 mg/dl and/or receiving treatment for diabetes. We only included the one with the most detailed information for both tooth brushing and the incidence of DM if there were more than one report in the same study.

Literature search strategy

We conducted a literature search of PubMed and Embase through April 2017. The following words were used to collect relevant citations: ‘tooth brushing’ or ‘oral hygiene’ or ‘oral health’ combination with ‘diabetes’. The language was restricted to English. Only human studies were considered. Additionally, we also reviewed the reference lists of retrieved original articles and relevant reviews.

Data extraction and quality assessment

Two authors (WF and SC) independently extracted the following information from included studies: first author, publication year, country (state), study design, sex, age, diabetes type, number of

participants, adjusted OR/RR with 95% CI, and adjusted factors. Differences in data extraction were resolved by discussion with a third author (ZL).

The Newcastle-Ottawa Scale was used to evaluate the qualities of cohort studies and case-control studies [13], which is a nine-point scale allocating points based on the selection of participants, comparability of groups, and exposure/outcome. The total score of the Newcastle-Ottawa Scale was nine, and included studies were classified as having a high quality (scores of 7-9), moderate quality (4-6), or low quality (0-3), respectively. For cross-sectional studies, assessment involving 11 items recommended by the Agency for Healthcare Research and Quality was applied [14]. An item was scored "1" if it was clearly considered, and "0" otherwise. Each study was rated independently by two authors (WF and SC). Discrepancies were resolved by consultation with a third investigator (ZL).

Statistical analyses

OR was used as the common measure of the association between the frequency of tooth brushing and DM risk. The reported RR was considered approximately as OR. The highest vs. lowest frequencies of tooth brushing were used to assess the association of tooth brushing and DM risk [15]. We calculated an overall pooled OR using a random effects model for the main analysis. In addition, if the articles included at least three quantitative categories of tooth brushing, they were included in the dose-response meta-analysis. For the dose–response analysis, we used the method described by Greenland and Longnecker [16] to calculate the trend from the correlated estimates for log relative risks across categories of tooth brushing. The frequency of tooth brushing, the distributions of cases and person years, and ORs with 95% CIs were extracted according to the method. A dose value for each tooth brushing exposure group was assigned as suggested by [17]: The median or mean level of tooth brushing within each category was used as the corresponding dose value. When the median or mean

frequency of tooth brushing was not available, the midpoint of the upper and lower boundaries was considered as the dose of each category. If the highest category was open-ended, the midpoint of the category was set at 1.5 times that of the lower category. Additionally， we assessed a potential non-linear dose-response relation between tooth brushing and DM using restricted cubic splines with three knots at 10%, 50%, and 90% centiles of the distribution, which were combined using multivariate meta-analysis[18-19]. The 95% confidence intervals were derived from the standard errors of the differences in linear predictors between each given point on the dose-response curve and a stated reference value, computed from the covariate values and the covariance matrix of the estimated coefficients [20]. A likelihood ratio test was used to assess the difference between the non-linear and linear models to test for non-linearity [21]. The estimation method of random-effects in our meta-analysis was inverse-variance-weighted.

Q statistic (with a significance level at $P<0.10$) and I^2 statistic were used to test the heterogeneity. The I^2 statistic measures the percentage of total variation across studies due to heterogeneity rather than chance. It was calculated according to the formula by Higgins [22]. The substantial heterogeneity was defined as I^2 value of ≥50%.

Subgroup analyses were performed to determine the possible influence of some factors, such as study design, diabetes type, and state. We conducted a sensitivity analysis to explore potential sources of heterogeneity and to investigate the influences of various exclusion criteria on the pooled risk estimate. Using the Begg's rank correlation and the Egger's linear regression tests to assess the potential publication bias [23-24]. Duval and Tweedie's nonparametric trim-and-fill method were used to adjust potential publication bias [25-26]. All analyses were performed using STATA statistical software (version 12.0; College Station, TX, USA) and all tests were two-sided with a significance level of less

than 0.05.

Results

Literature search

Figure 1 shows the literature research and study selection process. After excluding 721 duplicates, we identified 19,721 potentially relevant studies from electronic database. Of these, we excluded 19,542 papers because they were experimental, biomechanics, reviews or irrelevant studies. After retrieving full-text of the remaining 179 articles, 32 articles were excluded because of insufficient data to calculate the risk estimates, and 127 were excluded as tooth brushing was not a risk factor. Eventually, we included 20 studies [27-46] in this meta-analysis.

Characteristics of the included studies

Table 1 shows the abstracted characteristics of the 20 studies included. These studies were published between 1971 and 2016, and involved a total of 161,189 participants. Among them, six studies were from Europe, seven studies were from Asia, five studies were from America, one each from Africa and Australia. One study was published before 2000 and the remaining were published after 2000. All of the included studies have moderate or high qualities. The quality score for the five cross-sectional studies was on average 7, and ranged from 6 to 9. The quality evaluation score for the cohort study was 9 points. There was a total of 14 case-control studies, and the quality scores ranged from 6 to 10, with a mean score of 7.5 points.

Results of meta-analysis

Association between tooth brushing and the risk of DM

Figure 2 shows the results of the random-effects meta-analysis. Compared with the highest tooth brushing frequency, the lowest level of tooth brushing frequency was significantly associated with an

increased risk of DM (OR = 1.32, 95% CI: 1.18-1.47), and there was no significant heterogeneity across the included studies (P = 0.119, I^2 = 28.1%).

Dose-response analysis

Thirteen of the included studies reported the dose-response analysis of frequency of tooth brushing and the risk of DM. Pooling these studies, the risk of DM decreased by 20% for an increment of one time of tooth brushing per day (OR: 1.20; 95% CI: 1.16, 1.24), and there was no significant heterogeneity (I^2 = 67.2%) (Figure. 3). In the cubic spline model that including all studies, we did not find evidence suggesting any nonlinear association between tooth brushing and the risk of DM (Figure. 4; P for nonlinearity = 0.42).

Results of subgroup analyses and sensitivity analyses

Table 2 shows the results of subgroup analyses by study design, diabetes type, and state or country. In general, there was no statistically significant differences between subgroups.

Excluding any single study from meta-analysis did not change the pooled OR materially, with a range from 1.26 (95% CI: 1.17-1.38) to 1.42 (95% CI: 1.23-1.64).

Publication bias

The funnel plot visually showed substantial asymmetry (Figure 5). The Egger and Begg test suggested evidence of publication bias (Egger, p=0.000 and Begg, p=0.004). The Trim-and-Fill method was used to evaluate the impact of any potential publication bias, indicating that seven potentially missing studies would be needed to make the funnel plot symmetric (Figure 6). By using the trim-and-Fill method, the corrected OR was 1.26 (95% CI: 1.10 to 1.43; random-effects model, $p<0.001$). Therefore, the pooled OR was not substantially changed by the correction for potential publication bias.

Discussion

Tooth brushing is a widely existing oral care around the world and a number of previous studies have suggested that tooth brushing may affect health. In our meta-analysis, we found that low frequency of tooth brushing might be a risk factor of DM, and lower frequencies of tooth brushing were significantly associated with higher risk of DM, which was in line with findings from mechanism studies [47-50]. Previous evidences found that tooth brushing was an effective method for removing dental plaque [51] and indicated that tooth brushing might reduce the risk of periodontal disease [52]. Iacopino and colleagues found that periodontal bags of harmful bacteria could enter the blood, causing the body's inflammatory response and activation of certain cytokines. If this situation exists for a long time, immune inflammation can damage Islet beta cells and then cause DM [53]. Other studies shown that low frequency of tooth brushing activity facilitated the proliferation of P. gingivalis, and these breeding bacteria could increase insulin resistance and systemic inflammation by producing a worse enteric environment [54-55].

The interest on the association between tooth brushing and the risk of DM has been increased in the general public recently. Though the potential mechanisms of tooth brushing raising DM risk have not been studied thoroughly, our meta-analysis of 20 studies involving 10,884 patients with DM in 161,189 participants showed an inverse association between tooth brushing and the risk of DM. Evidence from these studies suggested that individuals who brushed teeth with the lowest frequency had an increased risk of DM by 32%, compared with those who brushed teeth with the highest frequency. We also found that there was no substantial heterogeneity among the included studies on the association between frequency of tooth brushing and the risk of DM.

In the dose–response analysis, we found that lower frequencies of tooth brushing were significantly associated with higher risk of DM, and the risk of DM increased by 20% for a decrement of one time of tooth brushing per day. From our analysis, we failed to suggest an appropriate tooth brushing frequency. Given the tooth brushing frequency of the reference groups in most of the included studies were two or more times per day, it should not be beneficial for the prevention of DM that the tooth brushing frequency per day was less than two.

Considering the different ways of designing those studies, subgroup analysis by study design was conducted, and no statistically significant differences across subgroups were found. Only one cohort study with a small sample size was examined in our review, and more prospective cohort studies were needed to explore the relationships of the frequency of tooth brushing and DM. Due to the difference of the pathogenesis, onset age, symptoms, complications, and treatment in type 1 and type 2 DMs, we conducted a subgroup analysis about the DM type but found insignificant difference between the pooled result of type 1 and type 2 DM studies. Given the different diets, lifestyles and the prevalence of DM in different regions, we also conducted subgroup analysis by region.

We observed that brushing teeth was a protective measure for diabetes. Based on the habits of individuals, the frequencies of toothbrushes are different. Oral medical and dental workers suggest that brushing teeth two times per day, especially brushing before going to sleep, has a significant effect on maintaining a good oral health status [52-53]. Food debris and bacteria accumulated during the day harm the oral health and often lead to periodontal diseases followed by the consequent series of adverse reactions. Brushing before going to bed is more important, because it can remove the food residue dirt from daily meals. Moreover, oral saliva secreted at night can reduce the ability to resist

bacteria and that is more likely to lead to tooth decay and periodontal diseases. Besides, the mouth is closed at night and the food will be processed with glycolysis under hypoxia conditions, which provides a good breeding condition for anaerobic bacteria. Dental healthcare professionals should educate their patients on the importance of tooth brushing every morning and evening. Teachers, especially those working in rural kindergartens and primary schools, should also educate young students in order to cultivate the good habits of regular tooth brushing.

Our meta-analysis has several strengths. Firstly, this is the first meta-analysis to systematically quantify the strength of association between tooth brushing and DM. Secondly, when several ORs were reported separately in terms of the different frequencies of tooth brushing in the same study, we combined the results of subgroups and calculated a common OR by a fixed-effects model. Therefore, we could pool the outcomes of the DM risk with highest tooth brushing frequency compared with those who brush-their teeth once a day or less. In addition, sensitivity analysis and consistent results in various subgroup analyses indicated that our findings were reliable and robust, although heterogeneity existed among the included studies.

Some limitations in the present meta-analysis should be of concern. First of all, the methodological weakness of the four cross-sectional studies might weak the validity of our results. Secondly, due to the various reported frequency of tooth brushing, we were unable to select uniform standards. Thus, the use of the highest and lowest levels of frequency to assess the association between tooth brushing and DM risk can be a source of heterogeneity and some information might be lost. Thirdly, considering the small sizes in most included studies, the risk of incomparability and selection bias cannot be ruled out, the researches based on larger sample sizes are needed in the future. Lastly, tooth brushing frequency was self-reported, and in the case of children, it was reported by their

parent/caregiver, so the accuracy of information cannot be assumed. There is a likely tendency for subjects to inflate their answers for this type of socially acceptable behavior. This kind of reporting would have caused smaller effect estimates.

Conclusion

In conclusion, our meta-analysis suggested that low frequency of tooth brushing is significantly associated with an increased risk of DM. A positive and statistically significant dose-response relationship was found between the frequency of tooth brushing and the DM risk. Given the heavy economic burden of DM, the results of our study provide additional valuable clues for the prevention of DM. For future studies, more prospective and interventional studies are needed to explore the underlying mechanisms of the relationships between tooth brushing and DM.

Abbreviations

DM: Diabetes mellitus

CI: confidence interval

MOOSE: the meta-analysis of observational studies in epidemiology

OR: odds ratio

RR: relative risk

WF: Wenning Fu

SC: Shiyi Cao

ZL: Zuxun Lu

Ethics approval and consent to participate

Ethical approval is not required for this systematic review.

Consent for publication

Not applicable.

Availability of data and materials

Data sharing is not applicable to this article as no datasets were generated or analysed during the current study.

Competing interests

The authors declare that they have no competing interests.

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Author Contributions

Zuxun Lu, Shiyi Cao had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Wenning Fu and Shiyi Cao independently extracted the related data information.

Check the related data information again: Chuanzhu Lv，Li Zou，Fujian Song，Xiantao Zeng，Chao Wang, Shijiao Yan, Yong Gan and Fan Chen.

Data analysis and writing articles: Wenning Fu and Shiyi Cao

Analysis, or interpretation of data: All authors.

Critical revision of the manuscript for important intellectual content: All authors.

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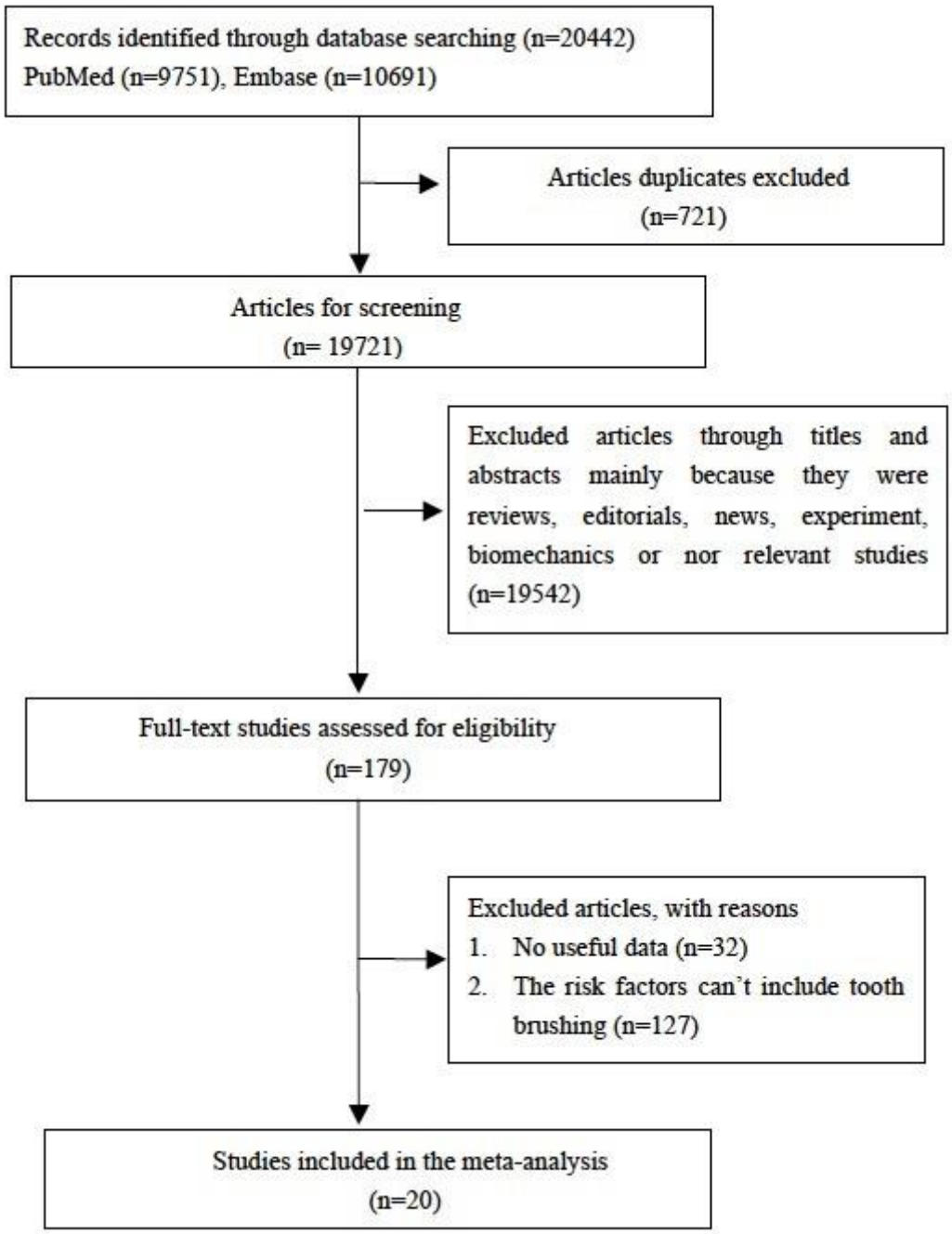

Figure 1 Flow diagram of identification of relevant observational studies of tooth brushing in relation to the risk of diabetes mellitus

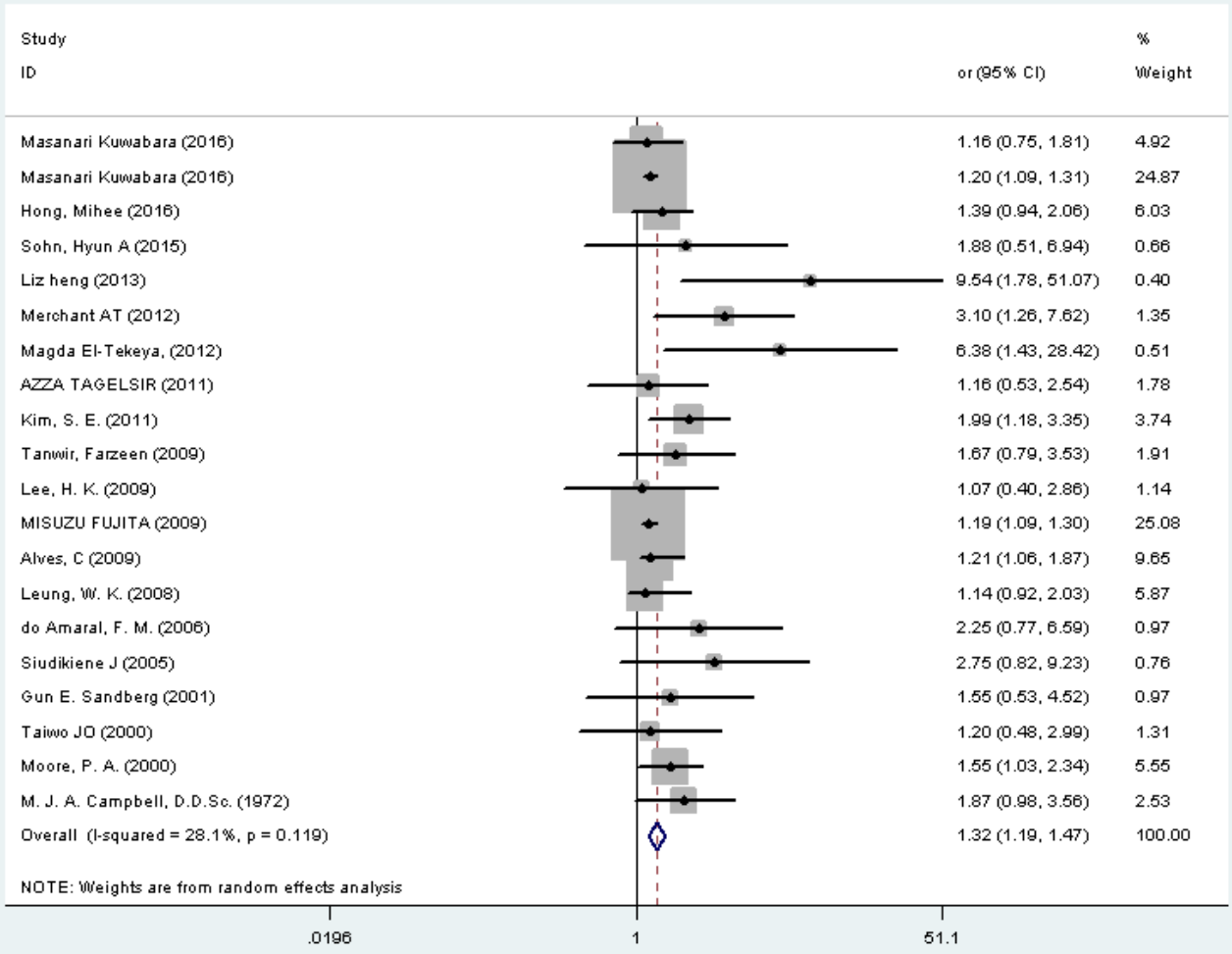

Figure 2 Association between tooth brushing and the risk of diabetes mellitus in a meta-analysis of observational studies

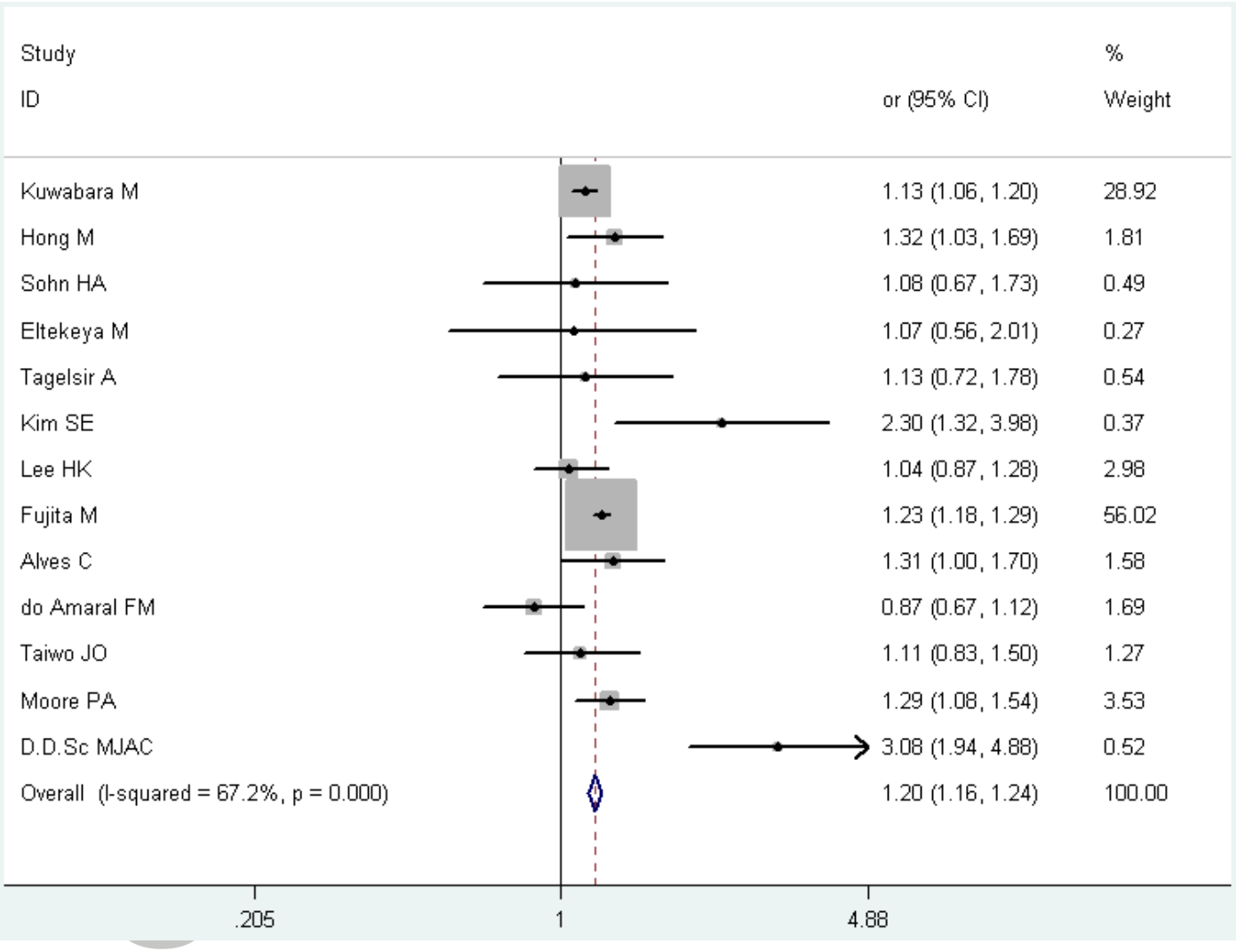

Figure 3 Dose-response relationships for the association between tooth brushing and risk of diabetes mellitus. CI = confidence Interval

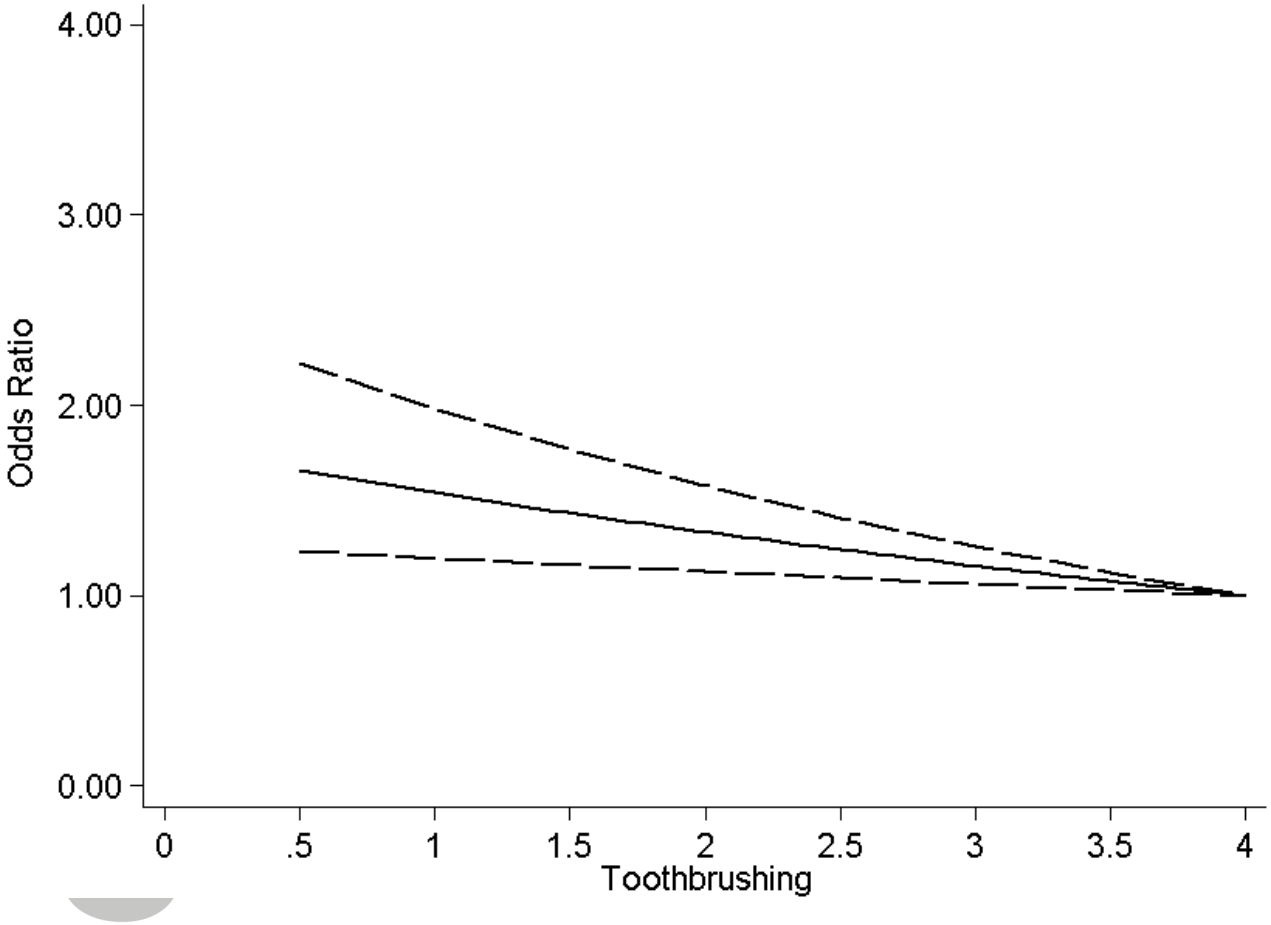

Figure 4 Forest plot of the summary odds ratio of diabetes mellitus for a decrement of one time of tooth brushing per day. CI = confidence interval

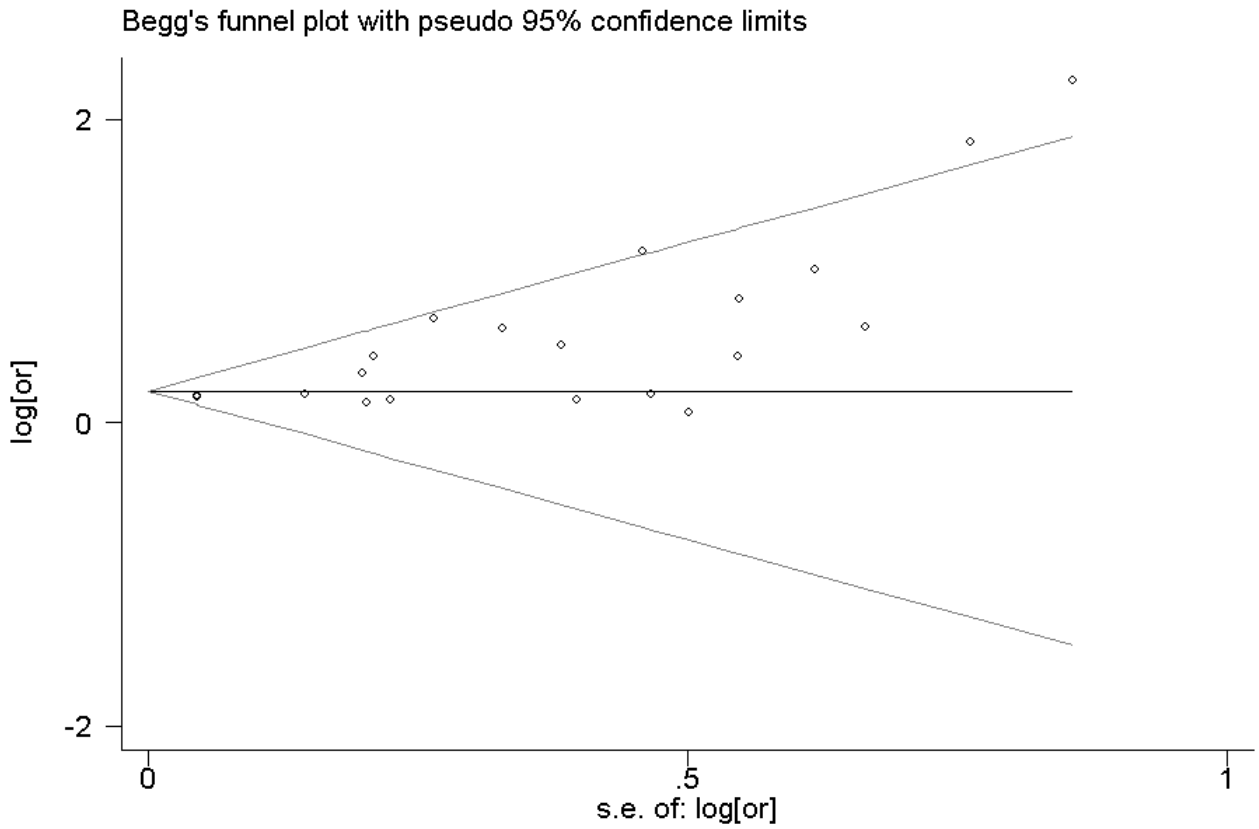

Figure 5 Funnel plot with 95% confidence limits

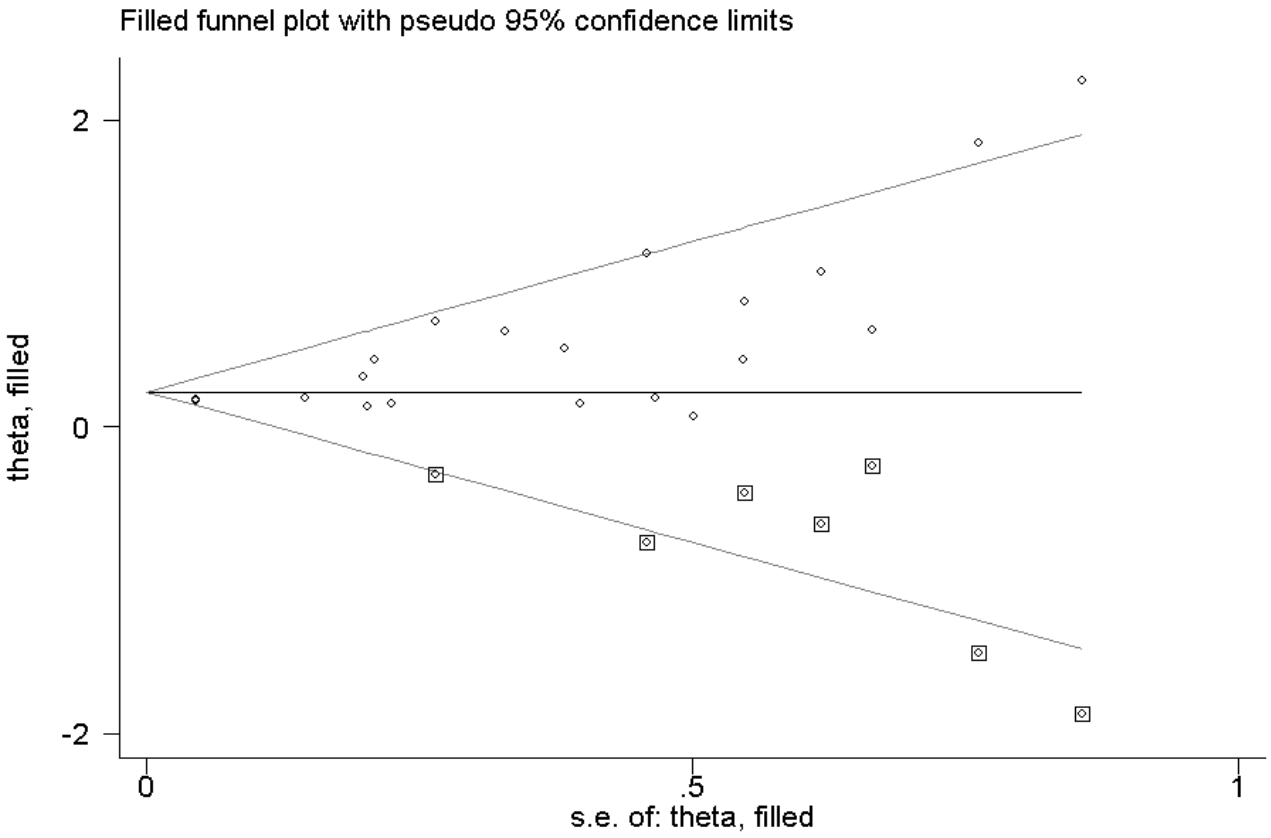

Figure 6 Filled funnel plot of OR from studies that investigated the association between tooth brushing and the risk of diabetes mellitus

Table 1 Main characteristics of the included studies involving tooth brushing and the risk of diabetes mellitus

Author	Year	Country(state)	Age	Sample size	Number of cases	Adjustment
Masanari Kuwabara	2016	Japan（Asia）	M&F.30-85	2	575	13070
Masanari Kuwabara	2016	Japan（Asia）	M&F.30–84	2	3698	85861
Hong, Mihee	2016	Korea（Asia）	M&F.≥30	2	617	4,477
Sohn, Hyun A	2015	California(America)	M&F.6-13	1	46	92
Li Z	2013	china（Asia）	M&F.62.7	2	83	83
Merchant AT	2012	United States（North America）	M&F.≥18＜18	1	126	155
Eltekeya, M	2012	America	M&F.6-9	1	50	100
Tagelsir, A	2011	Belgium（Europe）	M&F.3-16	1	52	102
Kim, S. E.	2011	Michigan（America）	M&F.18-72	2	77	448
Tanwir, Farzeen	2009	Sweden（Europe）	M&F.	2	88	168
Lee, H. K.	2009	Daegu （Asia）	M&F.＜45，≥45	2	40	75
MISUZU FUJITA	2009	Japan（Asia）	M&F.40-79	2	4250	54551
Alves, C	2009	Moscoso（Europe）	M&F.11.3±3.7	1	55	110
Leung, W. K.	2008	Hong Kong（Asia）	M&F.41-85	2	364	525
do Amaral, F. M.	2006	（Europe）	M&F.17-28	1	30	124

Siudikiene, J	2005	Lithuani（Europe）	M&F.10-15	1	70	140
Gun E. Sandberg	2009	Stockholm（Europe）	M&F.≤75	2	102	204
Taiwo JO	2000	Nigeria(Africa)	22-83	2	101	137
PAUL A. MOORE	2000	Pittsburgh（North America）	M&F.13-52	1	390	592
D.D.Sc MJAC	1972	Australia	17-39	2	70	172

Table 2 Results of subgroup analyses about tooth brushing and the risk of diabetes mellitus

Subgroup	Number of studies	OR/RR	95% confidence intervals	P for heterogeneity	I-square (%)
Study design					
Cohort	1	1.163	0.747-1.810	—	—
Cross-sectional	5	1.021	1.128-1.278	0.037	60.80
Case-control	14	1.511	1.273-1.794	0.690	0.00
Diabetes type					
type 1	8	1.473	1.200-1.809	0.171	32.20
type 2	12	1.213	1.142-1.288	0.298	14.90
State					
Asia	7	1.196	1.125-1.272	0.358	9.30
America	5	1.919	1.436-2.255	0.328	13.50
Europe	6	1.337	1.058-1.691	0.659	0.00
Africa	1	1.200	0.481-2.993	—	—
Australia	1	1.869	0.981-3.561	—	—