

**Effect of Toothbrushing Frequency on Incidence and Increment of Dental Caries: A Systematic Review and Meta-Analysis**

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## Effect of Toothbrushing Frequency on Incidence and Increment of Dental Caries: A Systematic Review and Meta-Analysis

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Abstract:	<p>Toothbrushing with fluoridated toothpaste has long been the foundation for preventing dental caries and maintaining periodontal health: brushing twice a day has become a social norm but the evidence base for this frequency is weak. This systematic review and meta-analysis aims to assess the effect of toothbrushing frequency on incidence and increment of carious lesions. Medline, Embase, Cinahl and Cochrane databases were searched. Screening and quality assessment was performed by two independent reviewers. Three different meta-analyses were conducted: two based on the caries outcome reported in the studies (incidence and increment) with subgroup analyses of categories of toothbrushing frequency; another included all studies irrespective of the caries outcome reported with the type of dentition as subgroups. Meta-regression was conducted to assess the influence of sample size, follow-up period, diagnosis level for carious lesions, and methodological quality of the articles on the effect estimate. Searches retrieved 5494 titles: after removing duplicates 4305 remained. Of these, 74 were reviewed in full but only 33 were eligible for inclusion. Self-reported infrequent brushers demonstrated higher incidence (odds ratio [OR], 95% confidence interval [CI] = 1.50, 1.34-1.69) and increment (standardised mean difference [SMD]: 0.28; 95% CI: 0.13-0.44) of carious lesions than frequent brushers. The odds of having carious lesions differed little when subgroup analysis was conducted to compare the incidence between <math>\geq 2</math> times/day Vs <math>&lt; 2</math> times (OR: 1.45, 95%CI: 1.21-1.74) and <math>\geq 1</math> time /day Vs <math>&lt; 1</math> time/day brushers (OR: 1.56, 95%CI: 1.37-1.78). When meta-analysis was conducted with the type of dentition as subgroups, the effect of infrequent brushing on incidence and increment of carious lesions was higher in deciduous (OR: 1.75, 95%CI: 1.49-2.06) than the permanent dentition (OR: 1.39, 95%CI: 1.29-1.49). Findings from meta-regression indicated that none of the included variables influenced the effect estimate.</p>

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3 **Title:** Effect of Toothbrushing Frequency on Incidence and Increment of Dental Caries: A  
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5 Systematic Review and Meta-Analysis  
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3 **Title:** Effect of Toothbrushing Frequency on Incidence and Increment of Dental Caries: A  
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5 Systematic Review and Meta-Analysis  
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8 **Abstract**  
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10  
11 Toothbrushing with fluoridated toothpaste has long been the foundation for preventing dental  
12 caries and maintaining periodontal health: brushing twice a day has become a social norm but  
13 the evidence base for this frequency is weak. This systematic review and meta-analysis aims  
14 to assess the effect of toothbrushing frequency on incidence and increment of carious lesions.  
15  
16 Medline, Embase, Cinahl and Cochrane databases were searched. Screening and quality  
17 assessment was performed by two independent reviewers. Three different meta-analyses were  
18 conducted: two based on the caries outcome reported in the studies (incidence and increment)  
19 with subgroup analyses of categories of toothbrushing frequency; another included all studies  
20 irrespective of the caries outcome reported with the type of dentition as subgroups. Meta-  
21 regression was conducted to assess the influence of sample size, follow-up period, diagnosis  
22 level for carious lesions, and methodological quality of the articles on the effect estimate.  
23  
24 Searches retrieved 5494 titles: after removing duplicates 4305 remained. Of these, 74 were  
25 reviewed in full but only 33 were eligible for inclusion. Self-reported infrequent brushers  
26 demonstrated higher incidence (odds ratio [OR], 95% confidence interval [CI] = 1.50, 1.34-  
27 1.69) and increment (standardised mean difference [SMD]: 0.28; 95% CI: 0.13-0.44) of  
28 carious lesions than frequent brushers. The odds of having carious lesions differed little when  
29 subgroup analysis was conducted to compare the incidence between  $\geq 2$  times/day Vs  $< 2$   
30 times (OR: 1.45, 95%CI: 1.21-1.74) and  $\geq 1$  time /day Vs  $< 1$  time/day brushers (OR: 1.56,  
31 95%CI: 1.37-1.78). When meta-analysis was conducted with the type of dentition as  
32 subgroups, the effect of infrequent brushing on incidence and increment of carious lesions  
33 was higher in deciduous (OR: 1.75, 95%CI: 1.49-2.06) than the permanent dentition (OR:  
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3 1.39, 95%CI: 1.29-1.49). Findings from meta-regression indicated that none of the included  
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5 variables influenced the effect estimate.  
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## 8 **Introduction**

9  
10  
11 Toothbrushing is considered fundamental self-care behaviour for maintenance of oral health  
12  
13 (Poklepovic et al. 2013) and brushing twice a day is a social norm. It is common practice for  
14  
15 dentists and professional organisations to advise this: e.g. Centers for Disease Control  
16  
17 recommends brushing twice a day specifically for preventing dental caries (CDC 2014).  
18  
19 Nevertheless, the effect of toothbrushing frequency on prevention of dental caries is unclear:  
20  
21 the evidence is inconsistent and conflicting. In 1986, based on conclusions from several  
22  
23 Workshops on oral hygiene, Addy stated that other than the delivery of fluoride ions from the  
24  
25 toothpaste, brushing frequency by itself has no additional benefit in preventing dental caries  
26  
27 (Addy 1986). Many studies have found an association between cumulative levels of dental  
28  
29 caries and reported toothbrushing frequency but only one published experimental trial could  
30  
31 be found that also evaluated the effect of toothbrushing frequency on caries increment: this  
32  
33 observed a strong inverse correlation (Chestnutt et al. 1998). A Cochrane review also  
34  
35 concludes that brushing twice daily increases the effectiveness of fluoridated toothpaste in  
36  
37 decreasing caries increment (Marinho et al. 2003).  
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42 Several systematic reviews and meta-analyses have reported associations between  
43  
44 toothbrushing frequency and gingival recession (Rajapakse et al. 2007), head and neck cancer  
45  
46 (Zeng et al. 2015) and periodontitis (Zimmermann et al. 2015). However, the evidence for a  
47  
48 clear association between toothbrushing frequency *per se* and dental caries remains  
49  
50 ambiguous and no systematic review could be found which specifically explored this matter.  
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54 The present systematic review and meta-analysis aims to assess the effect of toothbrushing  
55  
56 frequency on incidence and increment of carious lesions.  
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## 58 **Methods**

### *Eligibility criteria*

This systematic review conforms to Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines (Moher et al. 2009). Case-control, prospective cohort, retrospective cohort and experimental trials which evaluated the effect of toothbrushing frequency on incidence or increment of new carious lesions were considered for inclusion. When similar data from the same study population was reported in subsequent published papers, all except the latest record that provided the required data were excluded. Studies reported prior to 1980 and not published in English were excluded. There was no restriction with respect to the characteristics of the study population. Studies with participants of any and all ages were included. As we aimed to observe the effect of the frequency of toothbrushing on the development of dental caries, those studies that analysed the effect of other caries-related factors such as diet, but not toothbrushing frequency, were excluded. The exposure/intervention variable was self-reported toothbrushing frequency, the reported categories of which varied considerably between studies. The outcomes of interest were incidence (proportion of individuals developing new carious lesions) and increment (mean of new carious lesions or caries experience). The increment was reported in any of the following ways: mean of new decayed teeth or surfaces; mean of new decayed and filled teeth and mean of new decayed, missing and filled surfaces. Studies which had tooth loss, tooth pain or self-reported dental decay as outcome measures were excluded.

### *Information sources and search strategy*

A systematic search for literature was performed in January 2016 in four electronic databases; Medline via PubMed, Embase, Cinahl and Cochrane (for trials and economic assessments). Search filters were used to restrict retrieval to studies in humans, published in English between the January 1980 and December 2015, and to journal articles. There were very few longitudinal studies published prior to 1980 on this topic and it proved difficult to



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3 retrieve full texts of these articles and even abstracts in many instances. Books, letters to the  
4 editor and personal opinions were not considered. The search strategy used in PubMed is  
5 provided in Appendix table 1.  
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#### 8 9 *Study selection and data extraction*

10 Screening of titles and abstracts was performed by two independent reviewers (SK, JT).  
11 Abstracts found relevant were scheduled for full-text review, including those which  
12 apparently focussed on oral hygiene behaviour or oral health-related behaviour. There was no  
13 discrepancy between the reviewers in study selection. Data extraction from the full texts of  
14 the articles was independently performed by two reviewers (SK & JT). Pre-piloted forms  
15 were used for this purpose and extracted data were re-checked for accuracy by the senior  
16 author (NWJ). Data on study setting, study design, sample size, follow-up period, dental  
17 caries outcome and diagnostic criteria, categories used to record the frequency of  
18 toothbrushing, absolute values necessary for meta-analysis, findings, and information on  
19 other sources of fluoride were collected. The original corresponding authors were contacted  
20 when the data required for meta-analysis were missing: reminders were sent by e-mail twice  
21 at one-week intervals when a response was not obtained.  
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#### 38 *Quality assessment*

39 Studies were assessed for methodological quality by two reviewers (SK and JT)  
40 independently. The quality assessment tool for quantitative studies developed by the  
41 Effective Public Health Practice Project was used for this purpose (EPHPP 2010). The level  
42 at which a diagnosis of a carious lesion was made was also recorded for every study (i.e.,  
43 whether at pre-cavity or cavity level). The EPHPP tool has six components (selection bias,  
44 study design, confounders, blinding, data collection method and withdrawals & dropouts)  
45 with a rating of 'strong', 'moderate' or 'weak' provided for each component, utilising the  
46 criteria described in the EPHPP dictionary itself. A final global rating of strong is given to a  
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3 study if it does not have weak ratings in any of the six components. A study is rated moderate  
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5 if it has one weak rating and weak if it has two or more weak ratings.  
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### 7 *Data synthesis*

8  
9 Revman 5.3 (The Cochrane Collaboration, Copenhagen) was used for conducting the meta-  
10  
11 analysis. The odds ratio was the summary estimate reported in most of the studies (16  
12  
13 articles). Seven studies reported continuous data as ‘mean increment’ in carious lesions,  
14  
15 along with standard deviations and sample sizes for each toothbrushing category, allowing  
16  
17 computing of standardised mean differences and standard errors. Effect Estimate of Odds  
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19 Ratio =1 was imputed for two studies (Fure 2004; Takano et al. 2003) which did not report  
20  
21 any values, but stated that the effect of toothbrushing frequency was statistically insignificant,  
22  
23 the standard error was imputed as the mean of the reported values in that comparison  
24  
25 (Higgins and Green 2011; Schwendicke et al. 2015). Sensitivity analysis excluding these  
26  
27 studies was performed using a random effects model. Unadjusted effect estimates were used  
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29 in the meta-analysis as the confounding variables which might have been utilised for  
30  
31 statistical adjustment varied between studies. For one study (Mattila et al. 2001), unadjusted  
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33 data were not available and could not be retrieved by contacting the authors, so adjusted  
34  
35 estimates were used.  
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40 The categorization of exposure variable (toothbrushing frequency) differed between studies  
41  
42 and some studies had more than two categories. In the latter situation, a single effect estimate  
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44 was generated by comparing the caries increment or incidence in the highest brushing  
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46 frequency category with the pooled data from the other categories. In 15 studies, frequent  
47  
48 brushers were those brushing  $\geq 2$ /day while in 7 and 1 studies respectively they were those  
49  
50 brushing  $\geq 1$ /day and  $> 2$ /day respectively.  
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52 Heterogeneity was examined using ‘I<sup>2</sup>’ statistic. An I<sup>2</sup> value of less than 40% is considered  
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54 ‘not important’, 30% to 60% is ‘moderate heterogeneity’ while a value between 75% and  
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3 100% represents 'considerable heterogeneity' (Higgins and Green 2011). Subgroup and  
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5 meta-regression analyses were conducted to determine the sources of heterogeneity. Two  
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7 different meta-analyses were conducted based on the caries outcome reported, (viz: incidence  
8  
9 and increment) with subgroup analyses based on the categories of toothbrushing frequency  
10  
11 reported. To report the pooled effect of toothbrushing frequency on incidence or increment of  
12  
13 carious lesions, the exposure variable has been categorized as frequent (subjects in highest  
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15 brushing category in each study) and infrequent brushers (other brushing categories of each  
16  
17 study). A third meta-analysis was conducted by pooling the data from all the studies  
18  
19 irrespective of the caries outcome reported with the type of dentition as subgroups. For the  
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21 latter, standardised mean differences were re-expressed as Log odds ratios using the formula  
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23 suggested in Cochrane handbook (Higgins and Green 2011). A random effect model was  
24  
25 used because study characteristics varied so widely. A general inverse variance method was  
26  
27 used for meta-analysis as many studies only provided overall effect estimate rather than  
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29 summary data for each exposure group. When the caries assessment in a study was restricted  
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31 to specific teeth or surfaces, this was included along with the author's name in the Forest  
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33 plots for ease of understanding.

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Meta-regression analysis was conducted using Comprehensive Meta-Analysis 3.3.070  
(Biostat, Englewood, NJ) to explore the effect of confounding variables that were not  
considered in subgroup analyses on the effect size. Variables considered were sample size,  
follow-up period, diagnosis level for the presence of a carious lesion, and methodological  
quality of the articles. For assessing publication bias, visual inspection of Funnel plots was  
conducted and Egger's regression intercept test was also conducted. For meta-regression,  
data on each confounding variable were obtained from all the 25 studies included in the meta-  
analysis. A single funnel plot was constructed to demonstrate publication bias as the number

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3 of studies was not sufficient to conduct analyses for caries incidence and caries increment  
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5 separately (Higgins and Green 2011).  
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## 7 **Results**

### 8 *Study selection*

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10 A flowchart describing the selection of records identified, included and excluded, with  
11 reasons, is presented in Figure 1. Searches in Medline, Embase, Cinahl and Cochrane  
12 databases retrieved 3796, 533, 814 and 346 results respectively. After removing duplicates,  
13 4305 remained. Five of these articles were identified by manually searching the references of  
14 the included articles and from recently published literature that has not yet been indexed in  
15 Medline by reviewing the recent issues of dental epidemiology, public health and hygiene  
16 journals. A total of 74 articles were reviewed in full, of which 33 were considered eligible for  
17 inclusion (Appendix table 2). For quantitative synthesis, data could only be extracted and  
18 imputed from 25 articles.  
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### 31 *Study characteristics*

32 Appendix table 3 presents the characteristics and findings of the included studies. Most were  
33 conducted in high-income countries except four from Brazil (Lawrence and Sheiham 1997;  
34 Rodrigues and Sheiham 2000; Rossete Melo et al. 2013; Tagliaferro et al. 2006) and one  
35 from China (Zhou et al. 2012). Almost half (16) were conducted on European populations  
36 with six and five studies each from Finland and Sweden respectively. There were seven  
37 studies from the USA. Follow-up for the incidence or increment of carious lesions in the  
38 studies ranged from 11 months (Stecksen-Blicks and Gustafsson 1986) to 15 years (Bjertness  
39 et al. 1992). Except eight, all studies were on infant or child populations. Eleven of the  
40 included studies had caries in the deciduous dentition as outcome. In three articles  
41 (Chankanka et al. 2011; Maserejian et al. 2009; Stecksen-Blicks and Gustafsson 1986),  
42 cumulative caries in deciduous and permanent dentitions together was the outcome reported  
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3 but only one of these (Maserejian et al. 2009) could be included in the meta-analysis. Sample  
4 sizes at follow-up in three and seven studies were less than 100 and greater than 1000  
5 individuals respectively.  
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#### 9 10 *Quality of studies*

11 Most studies were of strong (13 studies) or moderate quality (14 studies) (Appendix table 4).  
12 Six studies could be rated 'weak'. Most of the studies diagnosed a carious lesion only when it  
13 was cavitated.  
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#### 17 18 *Effect of toothbrushing frequency on incidence and increment of carious lesions*

19  
20 Compared with frequent brushers, infrequent brushers demonstrated a higher  
21 incidence of carious lesions (OR: 1.50, 95% CI: 1.34-1.69). The odds of having carious  
22 lesions differed little when subgroup analysis was conducted to compare the incidence  
23 between  $\geq 2$  times/day Vs  $< 2$  times (OR: 1.45, 95% CI: 1.21-1.74) and  $\geq 1$  time /day Vs  $< 1$   
24 time/day brushers (OR: 1.56, 95% CI: 1.37-1.78). Only one study utilised exposure variable  
25 categorised as  $> 2$  times/day and  $\leq 2$  times/day. No heterogeneity ( $I^2=0$ ) was observed between  
26 the subgroups (Figure 2).  
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36 Figure 3 demonstrates that brushing  $< 2$  times /day significantly caused an increment  
37 of carious lesions compared with  $\geq 2$ /day brushing (SMD: 0.34; 95% CI: 0.18-0.49). There  
38 were no differences between  $> 2$ /day and  $\leq 2$ /day brushers for an increment of carious lesions  
39 (SMD: -0.12; 95% CI:-0.38-0.15,  $p=0.39$ ). Overall, infrequent brushing was associated with  
40 an increment of carious lesions (SMD: 0.19; 95% CI: 0.04-0.34). 'Considerable  
41 heterogeneity' was observed between the subgroups of studies with increment as an outcome.  
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49 When meta-analysis was conducted with the type of dentition as subgroups, there was  
50 an increased chance of incidence or increment of carious lesions among infrequent brushers  
51 than those brushing frequently in both the dentitions (Figure 4). However, the strength of this  
52 association was greater in the deciduous dentition (OR: 1.75, 95% CI: 1.49-2.06) than that  
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3 found in the permanent dentition (OR: 1.39, 95% CI: 1.29-1.49). Heterogeneity among the  
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5 studies describing the deciduous ( $I^2 = 0$ ) and permanent dentitions ( $I^2 = 54\%$ ) was not  
6  
7 'considerable'.  
8

#### 9 10 *Sensitivity analysis, meta-regression and publication bias*

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12 A sensitivity analysis was performed by excluding two studies whose data were imputed; the  
13  
14 pooled estimate thus obtained was only minutely different (OR: 1.41, 95% CI: 1.31-1.51)  
15  
16 from the estimate obtained by including them in the analysis (OR: 1.39, 95% CI: 1.29-1.49).  
17  
18 Results of the meta-regression analysis (Appendix table 5) indicate that none of the included  
19  
20 variables influenced the effect estimate. There was no evidence of publication bias among the  
21  
22 included studies ( $t=1.40$ , 95% CI:-0.52-2.71,  $p=0.174$ ): visual inspection of the funnel plot in  
23  
24 Figure 5 also demonstrates that no significant asymmetry existed.  
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#### 27 **Discussion**

28  
29 In this meta-analysis, we aimed to quantify the effect of toothbrushing frequency on  
30  
31 incidence and increment of carious lesions. We have considered only longitudinal studies as  
32  
33 we aimed to find if tooth brushing frequency is predictive of the development of carious  
34  
35 lesions. To our knowledge, this is the first systematic review and meta-analysis on this topic.  
36  
37 Most of the included studies recorded toothbrushing frequency at baseline and the increment  
38  
39 of carious lesions at follow-up. Eight articles could not be included in the data synthesis as  
40  
41 the data provided were insufficient.  
42  
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44  
45 Although most studies were of moderate or even strong quality, they differed in nature of  
46  
47 population, study setting, follow-up period, a method for diagnosis of a carious lesion and  
48  
49 caries outcome used. In most of the studies, a lesion was diagnosed as carious only when it  
50  
51 was cavitated, although a few studies diagnosed non-cavitated lesions also as carious this  
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53 would have caused under and over estimation of dental caries in these studies respectively.  
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3 However, results from meta-regression analysis indicated that none of the potential  
4  
5 confounding variables had an influence on effect estimate.  
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8 Irrespective of the brushing frequency category used in the studies, those brushing  
9  
10 less frequently were at greater risk for incidence and increment of carious lesions than those  
11  
12 brushing frequently. However, the risk for an increment of carious lesions in those brushing  
13  
14 >2 times/ day did not differ significantly from those brushing  $\leq 2$  times/ day, but this  
15  
16 estimate comes from only one study and should be considered with caution. Toothbrushing  
17  
18 frequency was self-reported and in the case of children it was parent/caregiver reported, so  
19  
20 the accuracy of information cannot be assumed. There is a likely tendency for subjects to  
21  
22 inflate their answers for this type of socially acceptable behaviour. This kind of reporting  
23  
24 would have caused smaller effect estimates. Toothbrushing frequency was more effective in  
25  
26 controlling incidence or increment in the deciduous dentition than the permanent dentition,  
27  
28 possibly because the former has greater susceptibility to dental caries (Lynch 2013).  
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32  
33 It is widely believed that effective removal of dental biofilm by toothbrushing can  
34  
35 reduce the development of new carious lesions but the evidence base is weak – especially  
36  
37 when it comes to frequency of brushing. It is recognised that most of the population cannot  
38  
39 achieve optimal control of biofilm with toothbrushing alone, and fluoride in the toothpaste is  
40  
41 considered of major importance in caries prevention (Choo et al. 2001). In this meta-analysis,  
42  
43 we could not separate the contribution of fluoride in toothpaste as none of the studies  
44  
45 provided data to make this possible. We have established, however, that frequent brushers are  
46  
47 at less risk for incidence of carious lesions independent of fluoride in toothpaste based on the  
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49 findings from independent studies. Three studies (Grindefjord et al. 1995; Leroy et al. 2005;  
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51 Wong et al. 2012) considered toothbrushing frequency and fluoride in tooth paste as separate  
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53 variables and found that the effect of the type of toothpaste was insignificant while infrequent  
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55 toothbrushing frequency was associated with the incidence of carious lesions. Two studies  
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3 (Wendt et al. 1994; Wong et al. 2012) found both frequent brushing and the presence of  
4 fluoride in toothpaste to be associated with decreased incidence of carious lesions.  
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7 This study has several limitations. Toothbrushing per se is associated with many factors like  
8 nature and design of the brush and bristles, duration of brushing, brushing method and the  
9 type of dentifrice. These effects cannot be separated in observational studies without  
10 diligently collecting comprehensive information on all of these, and applying statistical  
11 adjustments. None of the studies we found have attempted this. There was also a marked  
12 variation between studies in the way toothbrushing frequency was reported. This required us  
13 to perform several subgroup analyses based on the categories given. Another limitation of  
14 this meta-analysis is that none of the studies had the primary aim of assessing the influence of  
15 toothbrushing frequency on dental caries incidence or increment. Different caries diagnosis  
16 criteria and methods might have introduced heterogeneity between the studies. Further, we  
17 restricted our search to only studies published in English that were published prior to 1980,  
18 comparing the findings of older studies with no fluoride in toothpaste with newer studies  
19 that could have allowed interpreting the relevance of brushing versus fluoridated toothpaste.  
20 Lastly, exposure to fluoride dentifrice was not statistically adjusted in any of the included  
21 studies. A majority of studies were from developed countries. More longitudinal studies from  
22 developing and low-income countries might be helpful in assessing the independent effect of  
23 toothbrushing frequency on dental caries as it is easier to identify populations not using  
24 fluoridated products in some of these countries. Further, it would be helpful for future  
25 research if studies can use a uniform protocol for reporting toothbrushing frequency which  
26 could be one of the constituents of a core outcome set for toothbrushing studies. With the  
27 likelihood of toothbrushing frequency being considered as an indicator of oral health literacy  
28 (Parker and Jamieson, 2010) and social status (Levin and Currie, 2009), using a uniform  
29 protocol has wider implications on population oral health research.  
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## Conclusions

Individuals who state that they brush their teeth infrequently are at greater risk for incidence or increment of new carious lesions than those brushing more frequently. The effect is more pronounced in the deciduous than in the permanent dentition. A few studies indicate that this effect is independent of the presence of fluoride in toothpaste. It is also possible that other factors in those claiming a higher frequency of brushing, such as greater health awareness and motivation, higher socioeconomic status and a healthier diet are responsible for the observed effects.

## Acknowledgments

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## Conflict of Interest

Authors declare no conflicts of interest.

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For Peer Review

## Figure Legends

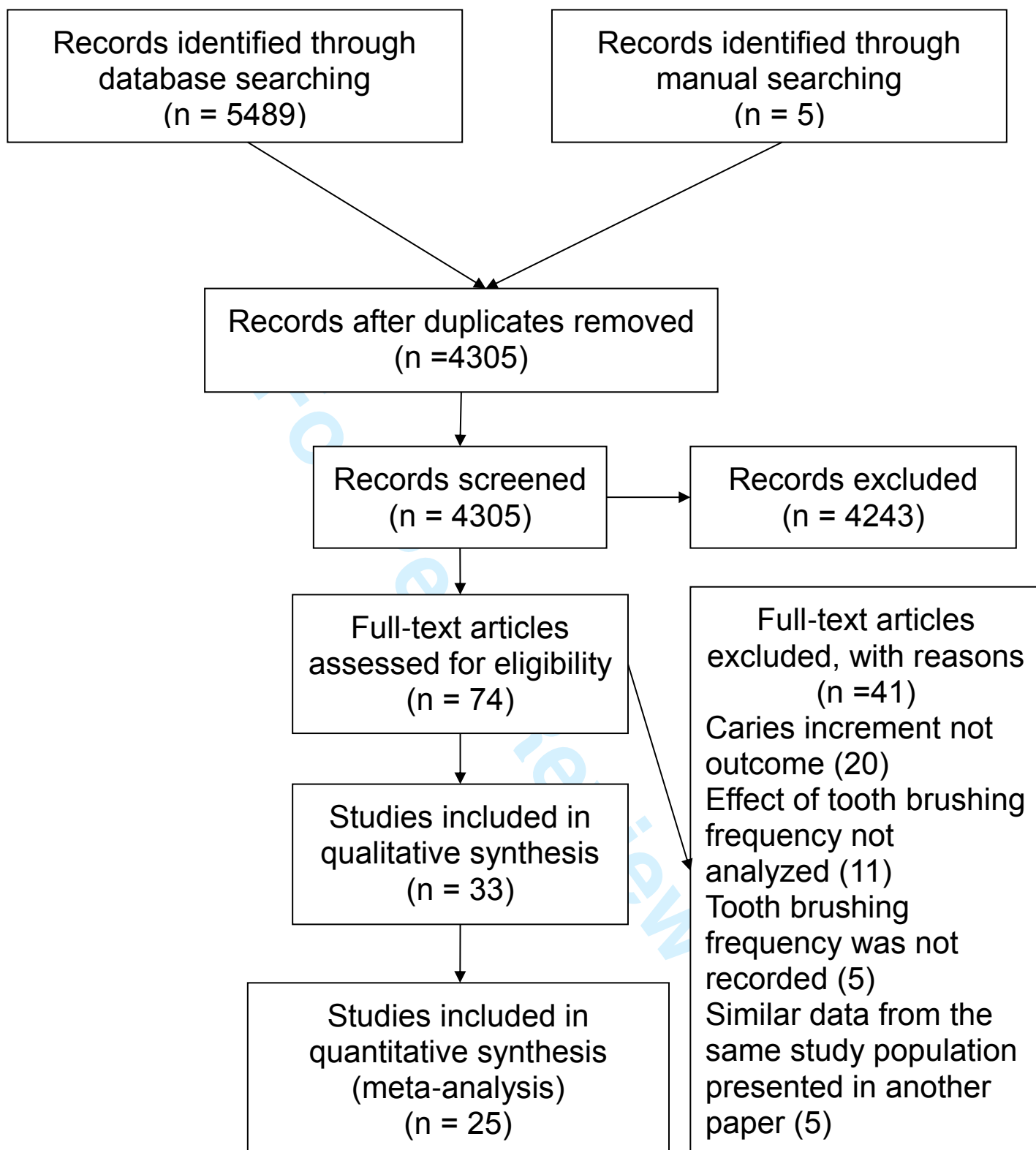
**Figure 1:** PRISMA flowchart depicting the studies identified, included and excluded with reasons

**Figure 2:** Effect of frequent toothbrushing compared with infrequent brushing on the incidence of dental caries.

**Figure 3:** Effect of frequent toothbrushing compared with infrequent brushing on the increment of dental caries

**Figure 4:** Effect of frequent toothbrushing compared with infrequent brushing: incidence or increment of dental caries is the outcome

**Figure 5:** Funnel plot to detect publication bias from all the studies included in the meta-analysis



Comparison: Frequent toothbrushing versus infrequent toothbrushing  
 Outcome: Incidence of dental caries

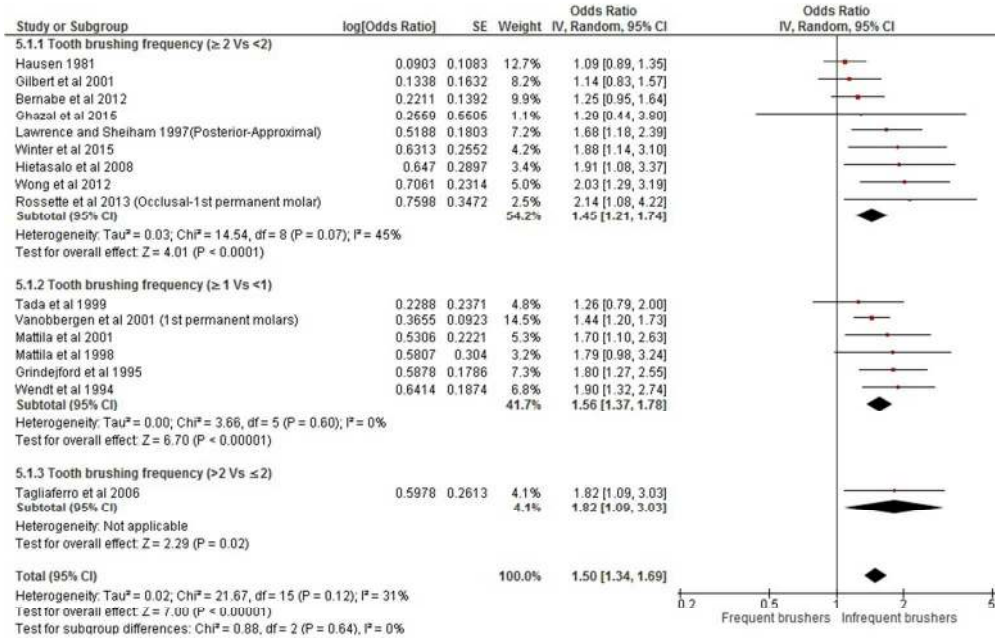


Figure 2: Effect of frequent toothbrushing compared with infrequent brushing on the incidence of dental caries  
 247x218mm (96 x 96 DPI)

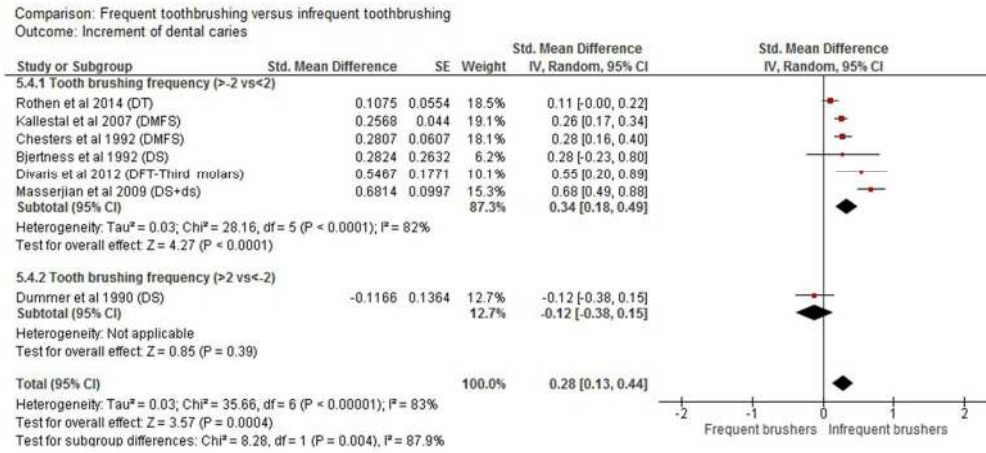


Figure 3: Effect of frequent toothbrushing compared with infrequent brushing on the increment of dental caries  
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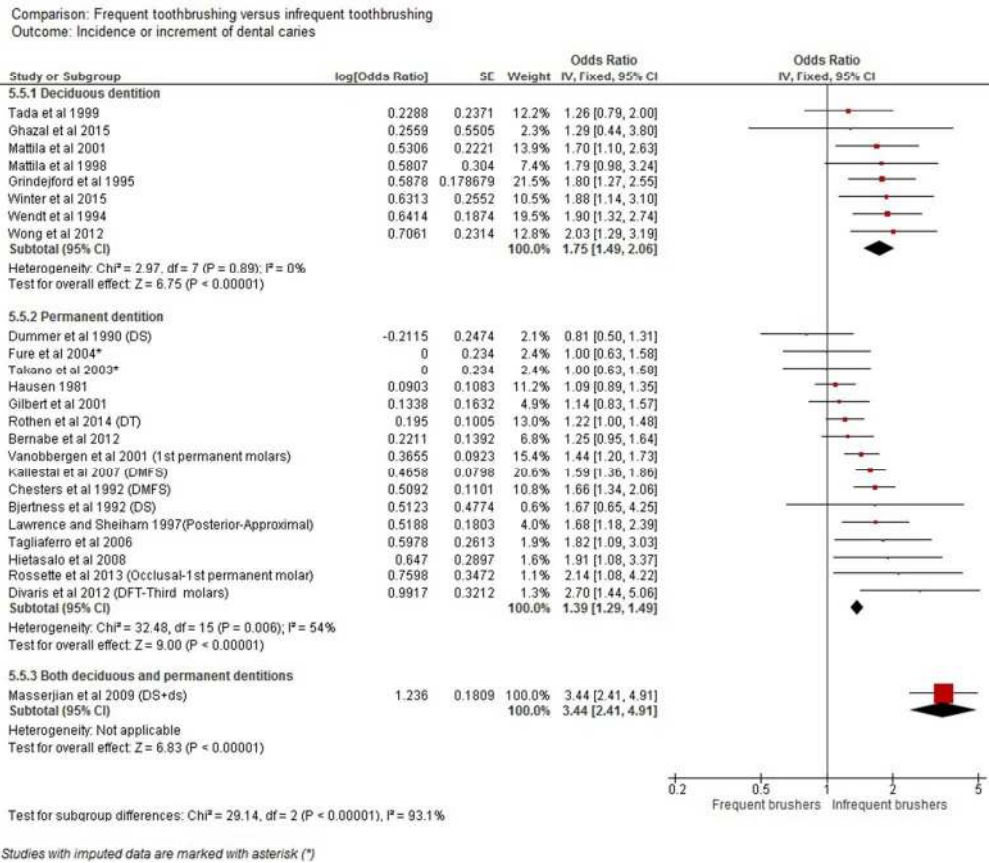
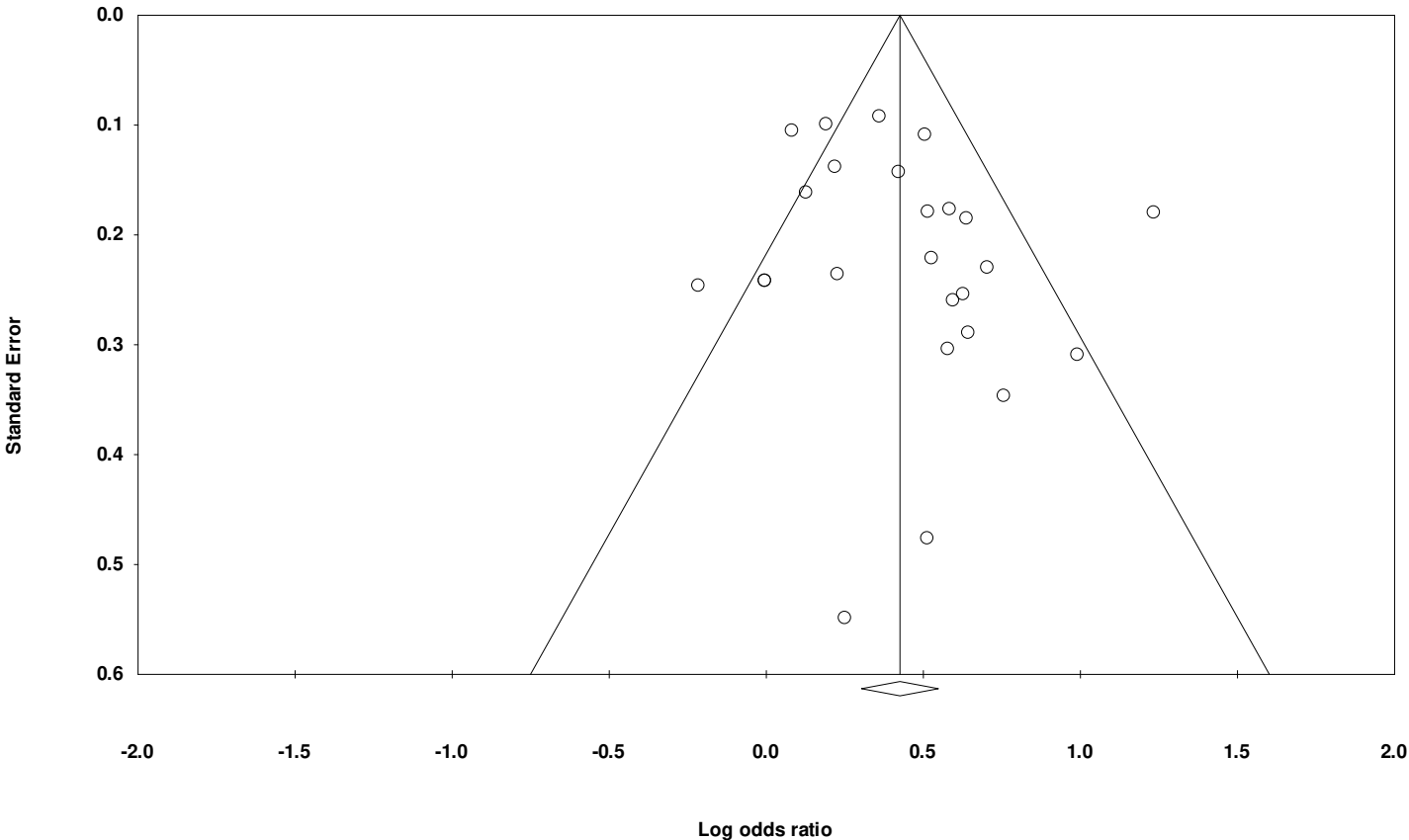


Figure 4: Effect of frequent toothbrushing compared with infrequent brushing: incidence or increment of dental caries is the outcome  
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**Appendix Table 1:** Search strategy used in Pubmed

#1 dental caries [MeSH Terms]

#2 dental [All Fields] AND caries [All Fields]

#3 dental caries [All Fields]

#4 tooth [All Fields] AND decay [All Fields]

#5 tooth decay [All Fields]

#6 (#1 or #2 or #3 or #4 or #5)

#7 toothbrushing [MeSH Terms]

#8 toothbrushing [All Fields]

#9 tooth [All Fields] AND brushing [All Fields]

#10 oral hygiene [MeSH Terms]

#11 oral [All Fields] AND hygiene [All Fields]

#12 oral hygiene [All Fields]

#13 (#7 or #8 or #9 or #10 or #11 or #12)

#14 #6 AND #13

Search limited to Journal Article[ptyp] AND ("1980/01/01"[PDAT] : "2015/12/31"[PDAT])  
AND "humans"[MeSH Terms] AND English[lang])

**Appendix Table 2:** Articles included and excluded with reasons

ARTICLES INCLUDED IN SYSTEMATIC REVIEW	
1	Winter J, Glaser M, Heinzl-Gutenbrunner M, Pieper K. Association of caries increment in preschool children with nutritional and preventive variables. <i>Clin Oral Investig</i> 2015; 19(8): 1913-9.
2	Ghazal T, Levy SM, Childers NK, et al. Factors associated with early childhood caries incidence among high caries-risk children. <i>Community Dentistry &amp; Oral Epidemiology</i> 2015; 43(4): 366-74 9p.
3	Rothen M, Cunha-Cruz J, Lingmei Z, Mancl L, Jones JS, Berg J. Oral hygiene behaviors and caries experience in Northwest PRECEDENT patients. <i>Community Dentistry &amp; Oral Epidemiology</i> 2014; 42(6): 526-35 10p.
4	Rossete Melo R, Rezende JS, Gomes VE, Ferreira EFE, Oliveira AC. Sociodemographic, biological and behavioural risk factors associated with incidence of dental caries in schoolchildren's first permanent molars: a 3-year follow-up study. <i>European journal of paediatric dentistry : official journal of European Academy of Paediatric Dentistry</i> 2013; 14(1): 8-12.
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8	Bernabe E, Newton JT, Uutela A, Aromaa A, Suominen AL. Sense of coherence and four-year caries incidence in Finnish adults. <i>Caries Res</i> 2012; 46(6): 523-9.
9	Chankanka O, Cavanaugh JE, Levy SM, et al. Longitudinal associations between children's dental caries and risk factors. <i>J Public Health Dent</i> 2011; 71(4): 289-300.
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11	Ismail AI, Sohn W, Lim S, Willem JM. Predictors of dental caries progression in primary teeth. <i>J Dent Res</i> 2009; 88(3): 270-5.
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ARTICLES INCLUDED IN THE META-ANALYSIS

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7 toothbrushing and sugar exposure in fluoridated and nonfluoridated areas. *Community*  
8 *Dent Oral Epidemiol* 1981; 9(3): 103-7.  
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ARTICLES EXCLUDED WITH REASONS

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11 **Caries incidence/increment not the outcome**  
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**Appendix Table 3:** Background characteristics and findings from the included studies

Study population	Age of the study population at baseline	Sample size	Follow-up period	Exposure variable Tooth brushing frequency (TBF)	Caries increment outcome	Association between TBF and caries	Findings from Univariate or bivariate analysis	Findings from Multivariate analysis	Information on Fluoride toothpaste usage	Effect of other fluoride sources	Reference
Children attending municipal dental health centres of Kuopio, Finland	7-16	2024	1 year	TBF/day: ≥1 <1 Reported by children or families	% developing new caries lesions (permanent dentition)	No association. An association was observed when fluoride and sugar exposure were considered	Caries risk increased with more frequent tooth brushing among children using fluoridated water & consuming sugars frequently; caries decreased among children receiving local decay preventives containing fluoride	Caries risk increased with more frequent tooth brushing among children using fluoridated water & consuming sugars frequently; caries decreased among children receiving local decay preventives containing fluoride	Fluoride dentifrice use was recorded but its effect on dental caries not analysed	Data on exposure to Fluoridated water supply and topical fluorides recorded	Hausen et al., 1981
Children of Umea, North Sweden	8 & 13 years old	At baseline: 88 (8yrs) 97 (13 yrs) At follow up: 83 (8yrs) 88 (13yrs)	11-13 months	TBF/day at baseline: <1 times ≥1 times Reported by children	Mean brushing frequency in 8 and 13 year olds with 1. 0-2 decayed surfaces increment 2. ≥3 decayed surfaces increment (both deciduous and permanent dentition)	No association	No association	Not conducted	Fluoridated toothpaste was used by 84% and 91% of 8 and 13 yr old respectively, it was not associated	Effect of mouth rinse and varnish was also insignificant	Stecksen-Blicks et al, 1986

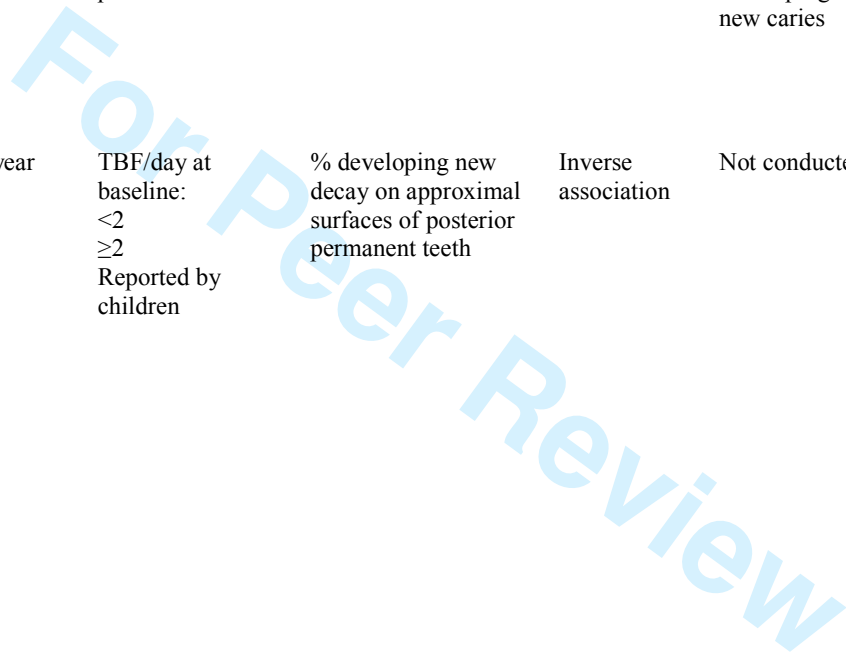
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										with caries increment		
Children from schools in Cardiff, Barry and Penarth, South wales, UK	11-12 years	At baseline: 1015 At follow-up: 798	4 years	TBF/ week at baseline: 0-6 times 7-13 times 14 times >14 times Reported by children	Mean increment of decayed surfaces in 1. Pit and fissure surfaces of posterior teeth 2. Approximal surfaces of posterior teeth 3. Approximal surfaces of incisor and canine teeth 4. Buccal & lingual surfaces all teeth (permanent dentition)	Inverse association	TBF associated with caries in approximal surfaces of posterior teeth & approximal surfaces of incisor & canine teeth.	TBF associated Approximal surfaces of anterior teeth	-	-	Dummer et al., 1990	
Citizens of Oslo, Norway	35	At baseline: 116 At follow-up: 81	15 years	TBF/day: 1 >1	Decayed surfaces increment (permanent dentition)	No association	No association	TBF was not entered into multivariate analysis as it was not significant at bivariate level	Use of fluoride recorded but was not explained if this meant fluoridated tooth paste	Effect of fluoride usage was insignificant	Bjertness et al., 1992	
Adults living independently in two metropolitan communities in Ontario, Canada	>50 years	At baseline: 699 At follow-up: 493	3 years	TBF/day at baseline: ≥1/day <1/day	- % developing new caries - Decayed Surfaces (DS) increment (root)  (permanent dentition)	Inverse association with DS increment only	Mean decayed surfaces increment more in <1/day brushers than ≥1/day	Insignificant		Exposure to Fluoride in water recorded. Water fluoridation was not significant	Locker. 1996	
Schoolchildren of Lanarkshire	11-12	At baseline: 4294 At follow-up:	3 years	TBF/day in consistent brushers on all	Decayed, Missing and Filled Surfaces increment	Inverse association	- ≥2/day brushers had lower	Not conducted	All the subjects used	-	Chesters et al., 1992	

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5	, Scotland		2317		three follow-ups: (permanent dentition)							
6					≤1							
7					≥2							
8					TBF/day based on							
9					responses on three							
10					occasions:							
11					G1: <1/day in at							
12					least 2 of 3							
13					occasions							
14					G2: 1/day at least							
15					2 of the 3							
16					instances							
17					G3: >1/day at							
18					least 2 of the 3							
19					instances							
20					G4: All others							
21					Reported by							
22					children							
23	Preschool	1 year	At baseline:	2 years	TBF/day at 1& 2	% developing new	Inverse	More children	Not conducted	Fluoridate	Use of	Wendt et
24	children of		632		years of age:	caries lesions	association	in with no		d	Fluoride	al, 1994
25	Jonkoping,		At follow-up:		<1	(deciduous dentition)		caries at 1 and		toothpaste	tablets also	
26	Sweden		593		≥1			3 years		was used	recorded	
27					Reported by			brushed their		by 87%	whose	
28					parents			teeth at age 1		participants	effect was	
29								more often		s at 2 yrs.	insignifica	
30								(≥1/day) than		More	nt.	
31								those who had		children		
32								caries at 3		who were		
33								years; More		caries free		
34								children with		at all ages		
35								no caries at		used F-		
36								ages 1, 2 and 3		toothpaste		
37								brushed more		than those		
38								often than		with caries		
39								children who		at 3 years		
40								had caries at 3		but not at 1		
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Children living in suburbs of Stockholm, Sweden	2.5 years	At baseline: 832 At follow-up: 692	1 year	TBF/day at baseline: <1 ≥1 Reported by parents	% developing new caries lesions (deciduous dentition)	Inverse association	Children brushing less than once/day were at greater risk of developing new caries	Not conducted	Fluoride toothpaste usage recorded and had no significant effect on caries increment	Use of fluoride tablets recorded. Children not using F tablets were at greater risk for caries increment.	Grindejford et al., 1995
School children of Rio de Janeiro state, Brazil	12-16	At baseline: 420 At follow-up: 290	1 year	TBF/day at baseline: <2 ≥2 Reported by children	% developing new decay on approximal surfaces of posterior permanent teeth	Inverse association	Not conducted	Subjects brushing <2/day were at 1.68 times greater risk than those brushing ≥2	All the subjects used fluoride toothpaste	Information on professionally applied fluorides, F mouth rinses, fluoride supplements & water fluoridation obtained. Only effect of water was fluoridation analysed (greater caries increment in those living in F deficit areas)	Lawrence and Sheiham, 1997
Children born at	3 years	At baseline: 1059	2 years	TBF/day at baseline:	- % developing new decay	Inverse association	Among Daily	Not significant	-	-	Mattila et al., 1998



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5	Maternity		At follow-up:		- $\geq 1$	- Mean dmfs		brushers, 21.8					
6	Health		828		- <1	increment		percent					
7	Centers in					(Deciduous dentition)		had new					
8	province of				Reported by			caries, while					
9	Turku &				mothers			34 % of					
10	Poori,							occasional					
11	south-							brushers					
12	western							developed					
13	Finland							new caries.					
14	Infants	18 months	At baseline: 392	18 months	Tbf/day with	% developing new	No	No association	Not conducted	-	-	Tada et al.,	
15	attending				guardians help:	carious lesions	association					1999	
16	mass				<1	(Deciduous dentition)							
17	check-ups				1								
18	in Chiba				$\geq 2$								
19	city, Japan				Reported by								
20					parent/guardian								
21	Persons	$\geq 45$ years	At baseline: 873	2 years	Tbf/day at	% developing new	Inverse	In those	Insignificant	-	-	Gilbert et	
22	$\geq 45$ years		At follow-up:		baseline:	decay on root	association	brushing				al., 2001	
23	(black and		723		$\leq 1$	surfaces		$\leq 1$ /day, 62%					
24	rural				$> 1$	(permanent		or filling while					
25	residents)					dentition)		in those					
26	of four							brushing					
27	counties of							$> 1$ /day, 65%					
28	North							had no decay					
29	Florida, US							or filling					
30	Nursery	36-47	At baseline:	1 year	TBF/day at	% developing new	Inverse	Stated as	Children	Has been	Fluoride	Rodrigues	
31	school	months	650		baseline:	caries lesions	association	significant in	brushing	reported	gel usage	and	
32	children of		At Follow-up:		<1	(Deciduous dentition)		the results but	<1/day 1.77	that effect	was	Sheiham,	
33	metropolita		510		$\geq 1$			no values	times more	of	considered.	2000	
34	n area of							provided	likely at risk	Fluoridate	Children		
35	Recife,				Reported by				of caries than	d	who have		
36	Pernambuc				mothers				those brushing	toothpaste	not		
37	o state,								at least	has been	received		
38	Brazil								once/day	studied but	Fluoride		
39										no findings	gel were		
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714	Children born at Maternity Health Centers in province of Turku & Pori, south-western Finland	7 years	At baseline: 1070 At Follow-up: 1074	3 years	Tbf/day at age 3 and 5: ≥1 <1  Reported by mothers	% developing new caries (deciduous dentition, permanent dentition and either of the dentitions)	Inverse association in deciduous dentition only.	Child's TBF at 5 associated with caries incidence in deciduous dentition. No values provided for association of TBF with caries incidence with other dentitions. Not conducted	Children brushing occasionally at 5 yrs of age were 1.7 more likely for caries incidence in deciduous dentition	-	for caries increment -	Mattila et al., 2001
205	Cohort of schoolchildren of Flanders, Belgium born in 1989	7 years	At baseline: 3,303 At follow-up: 2691	3 years	Tbf/day at each year from baseline: ≥1 <1  Reported by parents	% developing new caries in the 1 <sup>st</sup> permanent molars	Inverse association	Not conducted	Children brushing less than once a day were at 1.44 and 2.24 times more risk for dental caries incidence in at least one and two or more first permanent molar surfaces respectively than those brushing ≤1/day	99% of the study subjects used fluoridated toothpaste	Use of systemic fluorides recorded but its effect on increment of Decayed surfaces of 1 <sup>st</sup> permanent molars was insignificant	Vanobbergen et al., 2001
16	70 year old dentate individuals living in	70 years	At baseline: 544 At follow-up: 379	2 years	TBF/day at baseline: <2 ≥2	Root caries incidence at 1 or 2 years follow-up: ≥1 surfaces Vs no	No association	Not significant with chi square (No values)	TBF was not entered into multivariate analysis as it	Use of fluoridated toothpaste recorded		Takano et al., 2003

For Peer Review

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5	Niigata												
6	City, Japan												
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13	Residents	55, 65 and	At baseline: 208	10 years	TBF (no	- Increment in	No	Not conducted	Not significant	Almost, all	Fluoride	Fure et al.,	
14	of	75 years old	At follow-up:		categories	decayed and	association			the subjects	rinse,	2004	
15	Municipalit	at baseline	102		provided)	filled coronal				used	tablets or		
16	y of					surfaces				Fluoride	chewing		
17	Gotherburg,					surfaces				toothpaste	gums		
18	Sweden					and filled root					usage		
19						surfaces					registered		
20						Increment in					but their		
21						decayed and					effect on		
22						filled surfaces					dental		
23						with coronal and					caries		
24						root surface					increment		
25						counted as one					was		
26						surface					insignifica		
27											nt		
28	School	7 years	At baseline:	5 years	TBF/day at	% developing new	Inverse	In those who	Teeth in	Fluoridate	Use of	Leroy et	
29	children of		4351		baseline:	decay in first	association	didn't brush	frequent	d toothpaste	systemic	al., 2005	
30	Flanders,		At follow -up:		≥1	permanent molars		daily caries	brushers	usage	Fluoride		
31	Belgium		3291		<1			occurrence	had the best	recorded	was		
32					Reported by			was	survival	and	recorded		
33					parents			accelerated	estimates	its effect	and its		
34										was	effect was		
35										insignifica	only in		
36										nt (only	univariate		
37										6% did not	analysis		
38										use			
39										fluoridated			
40	Elderly of	67, 72 and	At baseline:	5 years	TBF at baseline:	- Coronal DMFT	Inverse	TBF	Not significant	Fluoridate		Siukosaari	
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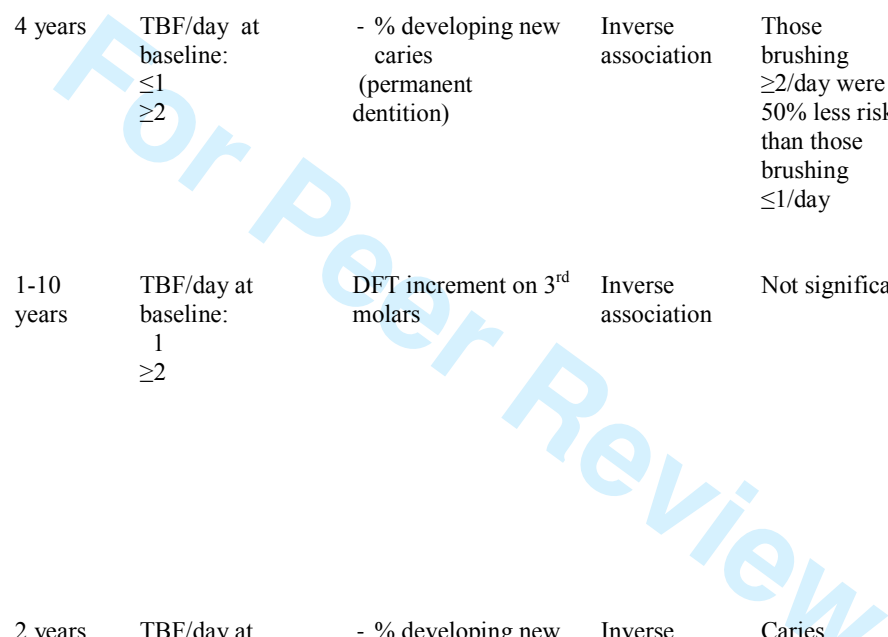


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Helsinki, Finland	77 age cohorts	364 At follow-up: 209		≥2/day 1/day Few times/week Occasionally	increment - Root caries index increment	association with coronal caries	significantly related to DMFT increment (no values provided)		d tooth paste usage recorded but its effect was not evaluated		et al., 2005
School children of Piracicaba, Brazil	6 – 8 years	At baseline: 480 At follow-up: 206	7 years	TBF/day at baseline: ≤2 >2  Reported by parents	% developing new decay (permanent dentition)	Inverse association	Caries incidence in ≤2/day brushers 52.4% and in those brushing >2 was 38.7%	Not significant	Fluoridated toothpaste usage recorded but its effect on DMFS increment not assessed	Use of other topical fluorides recorded. Type of Fluoride use had no effect on DMFS increment	Tagliaferro et al., 2008
Children attending public dental health clinics of Sweden	12 years	At baseline: 3373 At follow-up: 2848	4 years	TBF/day based on information provided at each year intervals: At 3 examinations, ≥2 At 2 examinations, ≥2 At 1 examination, ≥2 <2 all examinations Reported by children	Increment of DMFS (caries at dentinal level on all surfaces) – included in meta-analysis Increment of DeMFS (caries at dentinal level on all surfaces and at enamel level in proximal surfaces)	Inverse association with both outcomes	Children brushing, ≥2 times at 3 occasions had lower caries increment than those brushing less frequently	Inverse association but the strength of association was very weak	All subjects used fluoridated toothpaste	Water Fluoride level recorded & was significantly associated with both outcomes	Kallestal et al., 2007
All fifth and six grade children with at least once active caries in	11-12 years	At baseline and follow-up: 497 Effect of TBF on dental caries increment was assessed only in	4 years	TBF/day with Fluoridated toothpaste at baseline: <1 1 2	% developing new decay - ≤0vs≥1surfaces (used in meta-analysis) - ≤2vs≥3surfaces - ≤4vs≥5 surfaces	Inverse association	Those brushing at least twice a day did not develop new caries compared to	Not conducted	TBF with Fluoridated toothpaste was the exposure variable	-	Hietasalo et al., 2008

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5	Pori town,		250 subjects		Reported by				those brushing				
6	Finland				children				less often				
7									daily only				
8									when the				
9									outcome was				
10									$\leq 0$ vs $\geq 1$				
11	African –	0-5 years	At baseline: 1021	2 years	TBF during the	Increment in	No		Not conducted	Not significant	-	-	Ismail et
12	American		At follow-up: 788		preceding week	- d3-6 (cavitated	association						al., 2009
13	children				$\leq 7$	- d1-6 (cavitated &							
14	from low				$\geq 7$	non-							
15	income				Reported by	cavitated)mfs							
16	families of				parents	(deciduous dentition)							
17	Detroit,												
18	Michigan,												
19	USA												
20	Children	6-10 years	At baseline: 534	5 years	TBF/day at	Increment of decayed	Inverse		Not conducted	Children who	-	-	Masserjian
21	attending 5		At follow-up: 429		baseline:	surfaces	association			brushed their			et al., 2009
22	community				$< 1$	(deciduous and				teeth $< 1$ /day			
23	health				1	permanent dentition				were at greater			
24	dental				$\geq 2$	together)				risk of			
25	clinics in				Reported by					decayed teeth			
26	Boston and				children's					& surfaces			
27	1 in				guardians					than $\geq 2$			
28	Farmington									brushers			
29	, USA with												
30	2 or more												
31	posterior												
32	teeth with												
33	caries on												
34	occlusal												
35	surfaces												
36	Newborns	1.5 months	156	Three	Information on	- New Cavitated	Inverse	TBF	TBF	Almost, all	Composite	Chankanka	
37	at 8 IOWA			follow-	TBF collected at	decayed surfaces at	association	associated	associated	the	water	et al., 2011	
38	hospital			ups:	frequent intervals	all three		with new non-	with new non-	subjects	fluoride		
39	postpartum			5 &	from 6 weeks to	examinations		cavitated	cavitated	used	levels		
40	units			13 years	13 years and	- New Non-cavitated		caries	caries	fluoridated	recorded		
41				13 years	average TBF was	decayed surfaces at		( $p=0.03$ ). With	( $p=0.044$ ). With	toothpaste	and was		
42					generated (no	all three		increase in tbf	increase	not	associated		
43					categories	examinations		of 1/day, the	in tbf of 1/day,				
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provided)	(deciduous and	proportion of	the proportion	with
Reported by	permanent dentition	new non-	of new non-	cavitated
parents	together)	cavitated	cavitated	or non-
		caries	caries	cavitated
		decreased by	decreased by	caries
		33%	33%	

Randomly selected from those who attended Finnish Health 2000 survey	Subjects aged ≥30 years	At baseline: 1248 At follow-up: 944	4 years	TBF/day at baseline: ≤1 ≥2	- % developing new caries (permanent dentition)	Inverse association	Those brushing ≥2/day were at 50% less risk than those brushing ≤1/day	Those brushing ≥2/day were at 36% less risk than those brushing ≤1/day	-	-	Bernabe et al., 2012
Patients attending clinical centers at University of Kentucky and University of North Carolina, USA	14 – 45 years old with at least one 3 <sup>rd</sup> molar erupted at the occlusal plane	At baseline: 389 At follow-up: 215	1-10 years	TBF/day at baseline: 1 ≥2	DFT increment on 3 <sup>rd</sup> molars	Inverse association	Not significant	With unit increase in TBF, caries incidence on 3 <sup>rd</sup> molars increase by 30%	-	-	Divaris et al., 2012
Children from randomly selected kindergartens of Hong Kong	3-4 years	At baseline: 465 At Follow-up: 358	2 years	TBF/day at baseline: ≤1 ≥2 Reported by parents	- % developing new caries - dft increment (deciduous dentition)	Inverse association	Caries incidence and mean caries increment more in ≤1/day brushers than ≥2/day	Not significant	Fluoride toothpaste usage recorded and its effect was insignificant	-	Wong et al., 2012
Children attending a hospital in	8 months	At baseline: 225 At follow-up:	2 years	TBF/day at baseline: ≥1	Decayed surfaces increment (Deciduous dentition)	No association	Not conducted	Not significant	-	-	Zhou et al., 2012

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town of Xinhua, China	155	<1	Reported by parents										
Children from two public schools in Belo Horizonte, Brazil	6-8 years	At baseline: 272 At follow-up: 224	3 years	TBF/day at baseline: <2 ≥2	Reported by parents/guardians	% developing new caries on occlusal surfaces of first permanent molars	Inverse association	Those brushing <2/day at 1.56 times greater risk for dental caries than those brushing >2/day	Not significant	-	Fluoride use recorded as yes or no (this was insignificant)	Rossette Melo et al., 2013	
Patient attending 63 dental practices in 5 states of the US	Four different age groups 9-17 18-64 ≥65	At baseline: 1763 At follow-up: 1400	2 years	TBF/day with fluoridated toothpaste at baseline: <1 1 ≥2		Increment in decayed teeth during the past 24 months (Permanent dentition)	Inverse association	Those brushing ≥2/day had lesser caries increment than those brushing <2/day	Those brushing ≥2/day had 30% lesser new caries in past 24 months than those brushing <2/day	TBF with fluoridated toothpaste was the exposure variable	Frequency of usage of other fluoride products was recorded but its effect was insignificant	Rothen et al., 2014	
Children from high caries risk lower SES, single parent African American households in Alabama, USA	3-22 months (approximately 1 year old)	At baseline: 86 At follow-up: 81	3 years	TBF/day at age 1 (used as continuous variable-categorical data was obtained by request from author) Reported by parents/caregivers		- % developing new caries at 4 years (Used in meta-analysis) - dmfs increment from 1 to 4 years old - dmfs increment from 2- 3 years old - % developing new caries at 3 years (deciduous dentition)	Inverse association	Not significant	TBF at age 1 was associated with incidence of ECC from 2-3 years (OR-0.34 (age adjustment))	-	-	Ghazal et al., 2014	
Children attending kindergartens in districts of	1-4 years	At baseline and follow-up: 566	3 years	TBF/day just before follow-up examination: >1 ≤1		- % developing new caries – used in meta-analysis - dmft increment (deciduous dentition)	Inverse association	Greater risk of caries incidence and mean increment of	Not significant	Fluoridated toothpaste use recorded and its	Information on professional applied topical	Winter et al., 2015	

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Reported by  
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effect was  
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For Peer Review

**Appendix Table 4:** Quality rating of the included studies according to Effective Public Health Practice Project's Qualitative Assessment Tool for Quantitative Studies

	<b>Selection bias</b>	<b>Study design</b>	<b>Confounders</b>	<b>Blinding</b>	<b>Data collection</b>	<b>Withdrawal &amp; dropouts</b>	<b>Overall Quality rating</b>	<b>Caries diagnosis</b>	
1	Strong	Moderate	Strong	Moderate	Strong	Strong	Strong	Not described	Hausen et al., 1981
2	Weak	Moderate	Weak	Moderate	Strong	Strong	Weak	Cavitated	Steckesen-Blicks.1986
3	Moderate	Moderate	Weak	Moderate	Strong	Moderate	Moderate	Non-cavitated	Dummer et al., 1990
4	Moderate	Moderate	Weak	Moderate	Strong	Strong	Moderate	Cavitated	Bjertness.,1992
5	Weak	Moderate	Strong	Moderate	Strong	Moderate	Moderate	Cavitated	Locker, 1996
6	Moderate	Moderate	Strong	Moderate	Strong	Moderate	Strong	Cavitated	Chesters et al., 1992
7	Moderate	Moderate	Weak	Moderate	Strong	Strong	Moderate	Cavitated	Chest nut et al., 1998
8	Strong	Moderate	Weak	Moderate	Moderate	Strong	Moderate	Non-cavitated	Wendt et al., 1994
9	Strong	Moderate	Strong	Moderate	Strong	Strong	Strong	Non-cavitated	Grindejford et al., 1995
10	Strong	Moderate	Weak	Moderate	Strong	Moderate	Moderate	Non-cavitated	Lawrence and Sheiham, 1997
11	Weak	Moderate	Weak	Moderate	Strong	Strong	Weak	Cavitated	Mattila et al., 1998
12	Moderate	Moderate	Strong	Moderate	Strong	Strong	Strong	Cavitated	Tada et al., 1999
13	Moderate	Moderate	Strong	Moderate	Strong	Moderate	Strong	Non-cavitated	Gilbert et al.,2000
14	Strong	Moderate	Moderate	Moderate	Strong	Strong	Strong	Cavitated	Gilbert et al., 2001
15	Strong	Moderate	Weak	Moderate	Strong	Strong	Moderate	Non-cavitated	Rodrigues & Sheiham, 2000
16	Strong	Moderate	Weak	Moderate	Strong	Weak	Weak	Cavitated	Mattila et al., 2001
17	Moderate	Moderate	Weak	Mode	Strong	Weak	Weak	Coronal caries - Cavitated & non-	Vanobbergen et al., 2001
									Takano et al., 2003
									Fure et al., 2004

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									cavitated Root caries- only cavitated	
18	Strong	Moderate	Strong	Moderate	Strong	Moderate	Strong	Strong	Non-cavitated	Leroy et al., 2005
19	Strong	Moderate	Weak	Moderate	Strong	Weak	Weak	Weak	Cavitated	Siukosaari et al., 2005
20	Moderate	Moderate	Strong	Moderate	Strong	Weak	Moderate	Moderate	Cavitated	Tagliaferro et al., 2006
21	Moderate	Moderate	Weak	Moderate	Strong	Strong	Moderate	Moderate	Non-cavitated	Kallestal et al., 2005
22	Strong	Moderate	Weak	Moderate	Strong	Strong	Moderate	Moderate	Cavitated	Hietasalo et al., 2008
23	Moderate	Moderate	Strong	Moderate	Strong	Moderate	Strong	Strong	Non-cavitated	Ismail et al., 2009
24	Strong	Moderate	Strong	Moderate	Strong	Strong	Strong	Strong	Cavitated	Masserjian et al., 2009
25	Moderate	Moderate	Strong	Moderate	Strong	Weak	Moderate	Moderate	Cavitated and non- cavitated	Chankanka et al., 2011
26	Strong	Moderate	Strong	Moderate	Strong	Moderate	Strong	Strong	Cavitated	Bernabe et al., 2012
27	Weak	Moderate	Strong	Moderate	Strong	Weak	Weak	Weak	Cavitated	Divaris et al
28	Strong	Moderate	Strong	Moderate	Strong	Moderate	Strong	Strong	Cavitated	Wong et al., 2012
29	Moderate	Moderate	Strong	Moderate	Strong	Moderate	Strong	Strong	Cavitated	Zhou et al., 2012
30	Weak	Moderate	Strong	Moderate	Strong	Strong	Moderate	Moderate	Cavitated	Rossetto et al., 2013
31	Moderate	Moderate	Strong	Moderate	Strong	Moderate	Strong	Strong	Cavitated	Rothen et al., 2014
32	Weak	Moderate	Strong	Moderate	Strong	Strong	Moderate	Moderate	Cavitated	Ghazal et al., 2015
33	Moderate	Moderate	Strong	Moderate	Strong	Weak	Moderate	Moderate	Cavitated	Winter et al., 2015

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**Appendix Table 5:** Meta-regression analysis of the influence of potential confounding variables on the effect estimate for the association of tooth brushing frequency and either caries incidence or caries increment

	<b>Regression coefficient</b>	<b>SE</b>	<b>95% CI</b>	<b>P</b>
<b>Sample size</b>	-0.00001	0.0001	-00003-0.0001	0.2903
<b>Follow-up period</b>	0.0008	0.0024	-0.0039-0.0056	0.7268
<b>Caries diagnosis level</b>				
Cavitated	Reference			
Non-cavitated	-0.1243	0.1569	-0.4318-0.1831	0.4279
<b>Methodological Quality</b>				
Strong	Reference			
Moderate	0.0052	0.1555	-0.2995-0.3099	0.9733
Weak	-0.2533	0.2368	-0.7174-0.2108	0.2847

$R^2 = 0.00$ ,  $p=0.43$ . Data on each confounding variable were obtained from all the 25 studies included in the meta-analysis