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

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Abstract:	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 ≥2 times/day Vs <2 times (OR: 1.45, 95%CI: 1.21-1.74) and ≥1 time /day Vs <1 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.

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1.39, 95%CI: 1.29-1.49). Findings from meta-regression indicated that none of the included variables influenced the effect estimate.

Introduction

Toothbrushing is considered fundamental self-care behaviour for maintenance of oral health (Poklepovic et al. 2013) and brushing twice a day is a social norm. It is common practice for dentists and professional organisations to advise this: e.g. Centers for Disease Control recommends brushing twice a day specifically for preventing dental caries (CDC 2014). Nevertheless, the effect of toothbrushing frequency on prevention of dental caries is unclear: the evidence is inconsistent and conflicting. In 1986, based on conclusions from several Workshops on oral hygiene, Addy stated that other than the delivery of fluoride ions from the toothpaste, brushing frequency by itself has no additional benefit in preventing dental caries (Addy 1986). Many studies have found an association between cumulative levels of dental caries and reported toothbrushing frequency but only one published experimental trial could be found that also evaluated the effect of toothbrushing frequency on caries increment: this observed a strong inverse correlation (Chestnutt et al. 1998). A Cochrane review also concludes that brushing twice daily increases the effectiveness of fluoridated toothpaste in decreasing caries increment (Marinho et al. 2003).

Several systematic reviews and meta-analyses have reported associations between toothbrushing frequency and gingival recession (Rajapakse et al. 2007), head and neck cancer (Zeng et al. 2015) and periodontitis (Zimmermann et al. 2015). However, the evidence for a clear association between toothbrushing frequency *per se* and dental caries remains ambiguous and no systematic review could be found which specifically explored this matter.

The present systematic review and meta-analysis aims to assess the effect of toothbrushing frequency on incidence and increment of carious lesions.

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 retrieve full texts of these articles and even abstracts in many instances. Books, letters to the editor and personal opinions were not considered. The search strategy used in PubMed is provided in Appendix table 1.

Study selection and data extraction

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

Quality assessment

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

Data synthesis

Revman 5.3 (The Cochrane Collaboration, Copenhagen) was used for conducting the meta-analysis. The odds ratio was the summary estimate reported in most of the studies (16 articles). Seven studies reported continuous data as 'mean increment' in carious lesions, along with standard deviations and sample sizes for each toothbrushing category, allowing computing of standardised mean differences and standard errors. Effect Estimate of Odds Ratio =1 was imputed for two studies (Fure 2004; Takano et al. 2003) which did not report any values, but stated that the effect of toothbrushing frequency was statistically insignificant, the standard error was imputed as the mean of the reported values in that comparison (Higgins and Green 2011; Schwendicke et al. 2015). Sensitivity analysis excluding these studies was performed using a random effects model. Unadjusted effect estimates were used in the meta-analysis as the confounding variables which might have been utilised for statistical adjustment varied between studies. For one study (Mattila et al. 2001), unadjusted data were not available and could not be retrieved by contacting the authors, so adjusted estimates were used.

The categorization of exposure variable (toothbrushing frequency) differed between studies and some studies had more than two categories. In the latter situation, a single effect estimate was generated by comparing the caries increment or incidence in the highest brushing frequency category with the pooled data from the other categories. In 15 studies, frequent brushers were those brushing ≥2/day while in 7 and 1 studies respectively they were those brushing ≥1/day and >2/day respectively.

Heterogeneity was examined using 'I^2' statistic. An I^2 value of less than 40% is considered 'not important', 30% to 60% is 'moderate heterogeneity' while a value between 75% and

100% represents ‘considerable heterogeneity’ (Higgins and Green 2011). Subgroup and meta-regression analyses were conducted to determine the sources of heterogeneity. Two different meta-analyses were conducted based on the caries outcome reported, (viz: incidence and increment) with subgroup analyses based on the categories of toothbrushing frequency reported. To report the pooled effect of toothbrushing frequency on incidence or increment of carious lesions, the exposure variable has been categorized as frequent (subjects in highest brushing category in each study) and infrequent brushers (other brushing categories of each study). A third meta-analysis was conducted by pooling the data from all the studies irrespective of the caries outcome reported with the type of dentition as subgroups. For the latter, standardised mean differences were re-expressed as Log odds ratios using the formula suggested in Cochrane handbook (Higgins and Green 2011). A random effect model was used because study characteristics varied so widely. A general inverse variance method was used for meta-analysis as many studies only provided overall effect estimate rather than summary data for each exposure group. When the caries assessment in a study was restricted to specific teeth or surfaces, this was included along with the author’s name in the Forest plots for ease of understanding.

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 of studies was not sufficient to conduct analyses for caries incidence and caries increment separately (Higgins and Green 2011).

Results

Study selection

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

Study characteristics

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

Quality of studies

Most studies were of strong (13 studies) or moderate quality (14 studies) (Appendix table 4). Six studies could be rated ‘weak’. Most of the studies diagnosed a carious lesion only when it was cavitated.

Effect of toothbrushing frequency on incidence and increment of carious lesions

Compared with frequent brushers, infrequent brushers demonstrated a higher incidence of carious lesions (OR: 1.50, 95% CI: 1.34-1.69)**.** The odds of having carious lesions differed little when subgroup analysis was conducted to compare the incidence between ≥2 times/day Vs <2 times (OR: 1.45, 95% CI: 1.21-1.74) and ≥1 time /day Vs <1 time/day brushers (OR: 1.56, 95% CI: 1.37-1.78). Only one study utilised exposure variable categorised as >2 times/day and ≤2 times/day. No heterogeneity (I^2=0) was observed between the subgroups (Figure 2).

Figure 3 demonstrates that brushing <2 times /day significantly caused an increment of carious lesions compared with ≥2/day brushing (SMD: 0.34; 95% CI: 0.18-0.49). There were no differences between >2/day and ≤2/day brushers for an increment of carious lesions (SMD: -0.12; 95% CI:-0.38-0.15, p=0.39). Overall, infrequent brushing was associated with an increment of carious lesions (SMD: 0.19; 95% CI: 0.04-0.34). ‘Considerable heterogeneity’ was observed between the subgroups of studies with increment as an outcome.

When meta-analysis was conducted with the type of dentition as subgroups, there was an increased chance of incidence or increment of carious lesions among infrequent brushers than those brushing frequently in both the dentitions (Figure 4). However, the strength of this association was greater in the deciduous dentition (OR: 1.75, 95% CI: 1.49-2.06) than that found in the permanent dentition (OR: 1.39, 95% CI: 1.29-1.49). Heterogeneity among the studies describing the deciduous (I^2 = 0) and permanent dentitions (I^2 = 54%) was not 'considerable'.

Sensitivity analysis, meta-regression and publication bias

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

Discussion

In this meta-analysis, we aimed to quantify the effect of toothbrushing frequency on incidence and increment of carious lesions. We have considered only longitudinal studies as we aimed to find if tooth brushing frequency is predictive of the development of carious lesions. To our knowledge, this is the first systematic review and meta-analysis on this topic.

Most of the included studies recorded toothbrushing frequency at baseline and the increment of carious lesions at follow-up. Eight articles could not be included in the data synthesis as the data provided were insufficient.

Although most studies were of moderate or even strong quality, they differed in nature of population, study setting, follow-up period, a method for diagnosis of a carious lesion and caries outcome used. In most of the studies, a lesion was diagnosed as carious only when it was cavitated, although a few studies diagnosed non-cavitated lesions also as carious this would have caused under and over estimation of dental caries in these studies respectively.

However, results from meta-regression analysis indicated that none of the potential confounding variables had an influence on effect estimate.

Irrespective of the brushing frequency category used in the studies, those brushing less frequently were at greater risk for incidence and increment of carious lesions than those brushing frequently. However, the risk for an increment of carious lesions in those brushing >2 times/ day did not different significantly from those brushing ≤2 times/ day, but this estimate comes from only one study and should be considered with caution. Toothbrushing frequency was self-reported and in the case of children it was parent/caregiver reported, 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 behaviour. This kind of reporting would have caused smaller effect estimates. Toothbrushing frequency was more effective in controlling incidence or increment in the deciduous dentition than the permanent dentition, possibly because the former has greater susceptibility to dental caries (Lynch 2013).

It is widely believed that effective removal of dental biofilm by toothbrushing can reduce the development of new carious lesions but the evidence base is weak – especially when it comes to frequency of brushing. It is recognised that most of the population cannot achieve optimal control of biofilm with toothbrushing alone, and fluoride in the toothpaste is considered of major importance in caries prevention (Choo et al. 2001). In this meta-analysis, we could not separate the contribution of fluoride in toothpaste as none of the studies provided data to make this possible. We have established, however, that frequent brushers are at less risk for incidence of carious lesions independent of fluoride in toothpaste based on the findings from independent studies. Three studies (Grindefjord et al. 1995; Leroy et al. 2005; Wong et al. 2012) considered toothbrushing frequency and fluoride in tooth paste as separate variables and found that the effect of the type of toothpaste was insignificant while infrequent toothbrushing frequency was associated with the incidence of carious lesions. Two studies

(Wendt et al. 1994; Wong et al. 2012) found both frequent brushing and the presence of fluoride in toothpaste to be associated with decreased incidence of carious lesions.

This study has several limitations. Toothbrushing per se is associated with many factors like nature and design of the brush and bristles, duration of brushing, brushing method and the type of dentifrice. These effects cannot be separated in observational studies without diligently collecting comprehensive information on all of these, and applying statistical adjustments. None of the studies we found have attempted this. There was also a marked variation between studies in the way toothbrushing frequency was reported. This required us to perform several subgroup analyses based on the categories given. Another limitation of this meta-analysis is that none of the studies had the primary aim of assessing the influence of toothbrushing frequency on dental caries incidence or increment. Different caries diagnosis criteria and methods might have introduced heterogeneity between the studies. Further, we restricted our search to only studies published in English that were published prior to 1980, comparing the findings of older studies with no fluoride in toothpaste with newer studies could have allowed interpreting the relevance of brushing versus fluoridated toothpaste. Lastly, exposure to fluoride dentifrice was not statistically adjusted in any of the included studies. A majority of studies were from developed countries. More longitudinal studies from developing and low-income countries might be helpful in assessing the independent effect of toothbrushing frequency on dental caries as it is easier to identify populations not using fluoridated products in some of these countries. Further, it would be helpful for future research if studies can use a uniform protocol for reporting toothbrushing frequency which could be one of the constituents of a core outcome set for toothbrushing studies. With the likelihood of toothbrushing frequency being considered as an indicator of oral health literacy (Parker and Jamieson, 2010) and social status (Levin and Currie, 2009), using a uniform protocol has wider implications on population oral health research.

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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Figure Legends

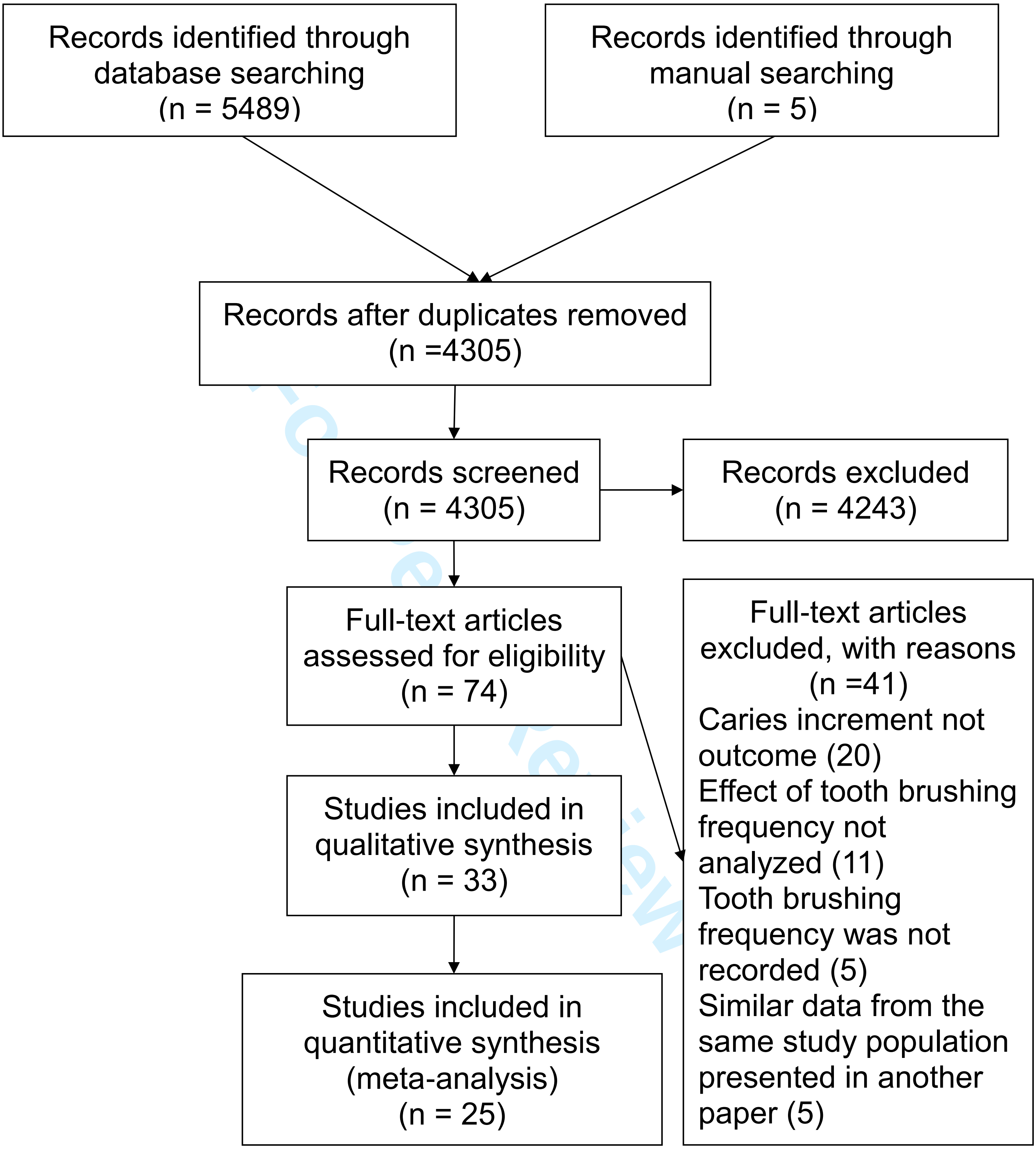
Figure 1: PRISMA flowchart depicting the studies identified, included and excluded with reasons

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


Records identified through database searching (n = 5489)
Records identified through manual searching (n = 5)
Records after duplicates removed (n =4305)
Records screened (n = 4305)
Records excluded (n = 4243)
Full-text articles assessed for eligibility (n = 74)
Full-text articles excluded, with reasons (n =41)
Caries increment not outcome (20)
Effect of tooth brushing frequency not analyzed (11)
Tooth brushing frequency was not recorded (5)
Similar data from the same study population presented in another paper (5)
Studies included in qualitative synthesis (n = 33)
Studies included in quantitative synthesis (meta-analysis) (n = 25)

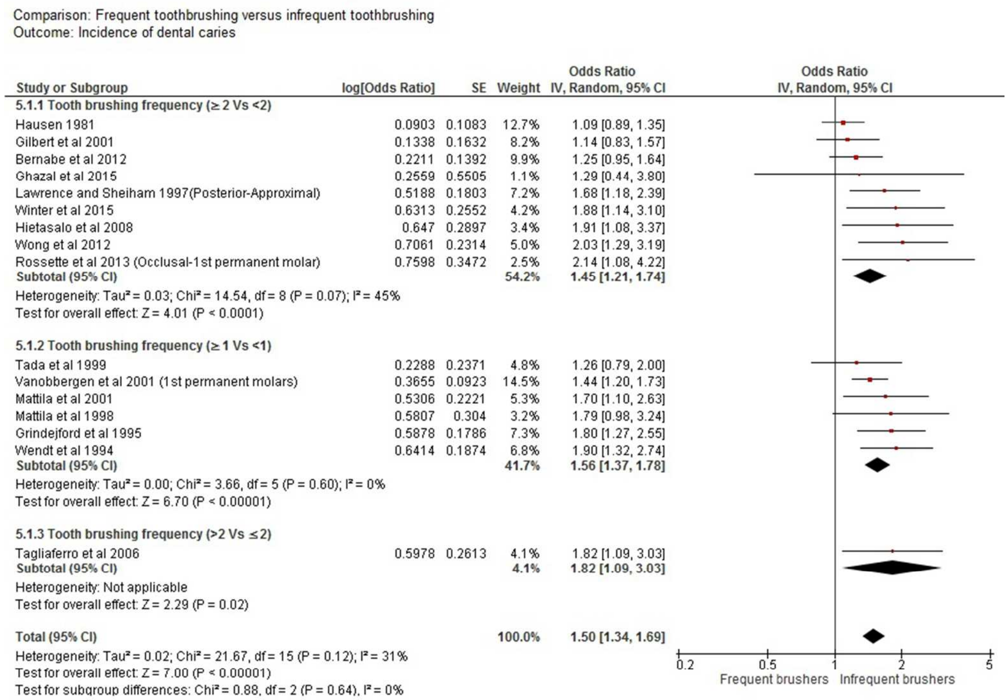


Figure 2: Effect of frequent toothbrushing compared with infrequent brushing on the incidence of dental caries
247x218mm (96 x 96 DPI)

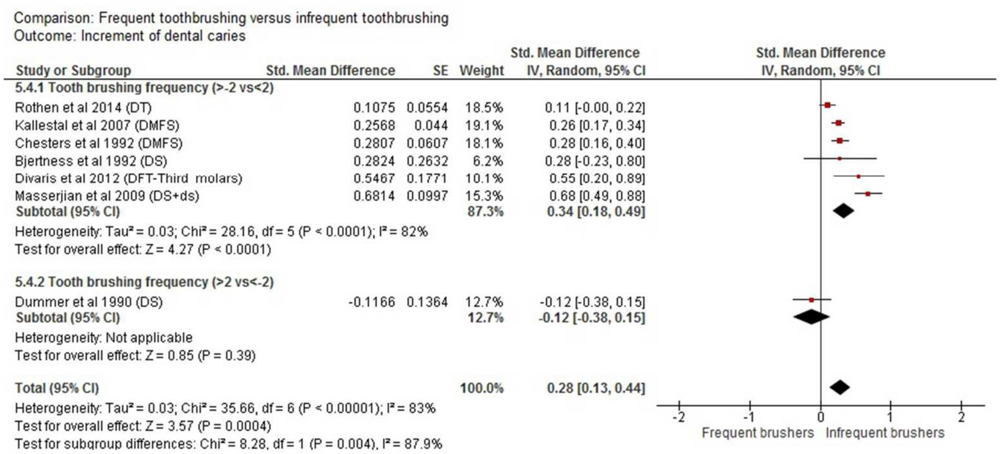


Figure 3: Effect of frequent toothbrushing compared with infrequent brushing on the increment of dental caries

239x113mm (96 x 96 DPI)

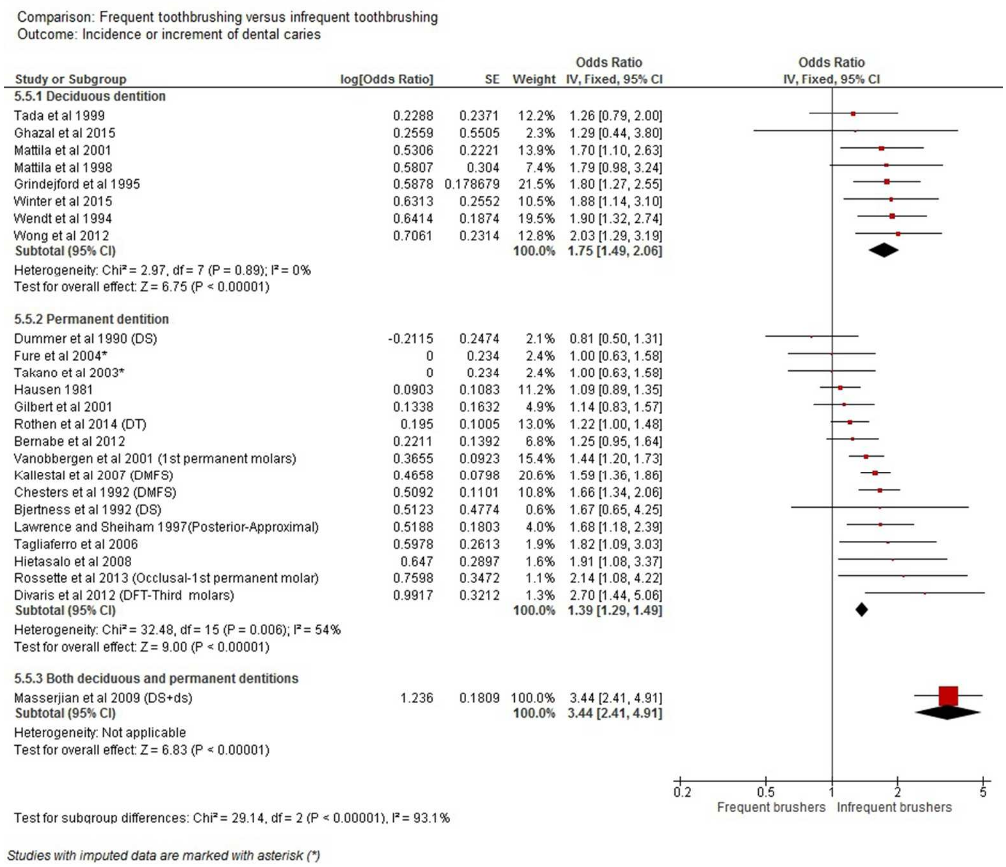
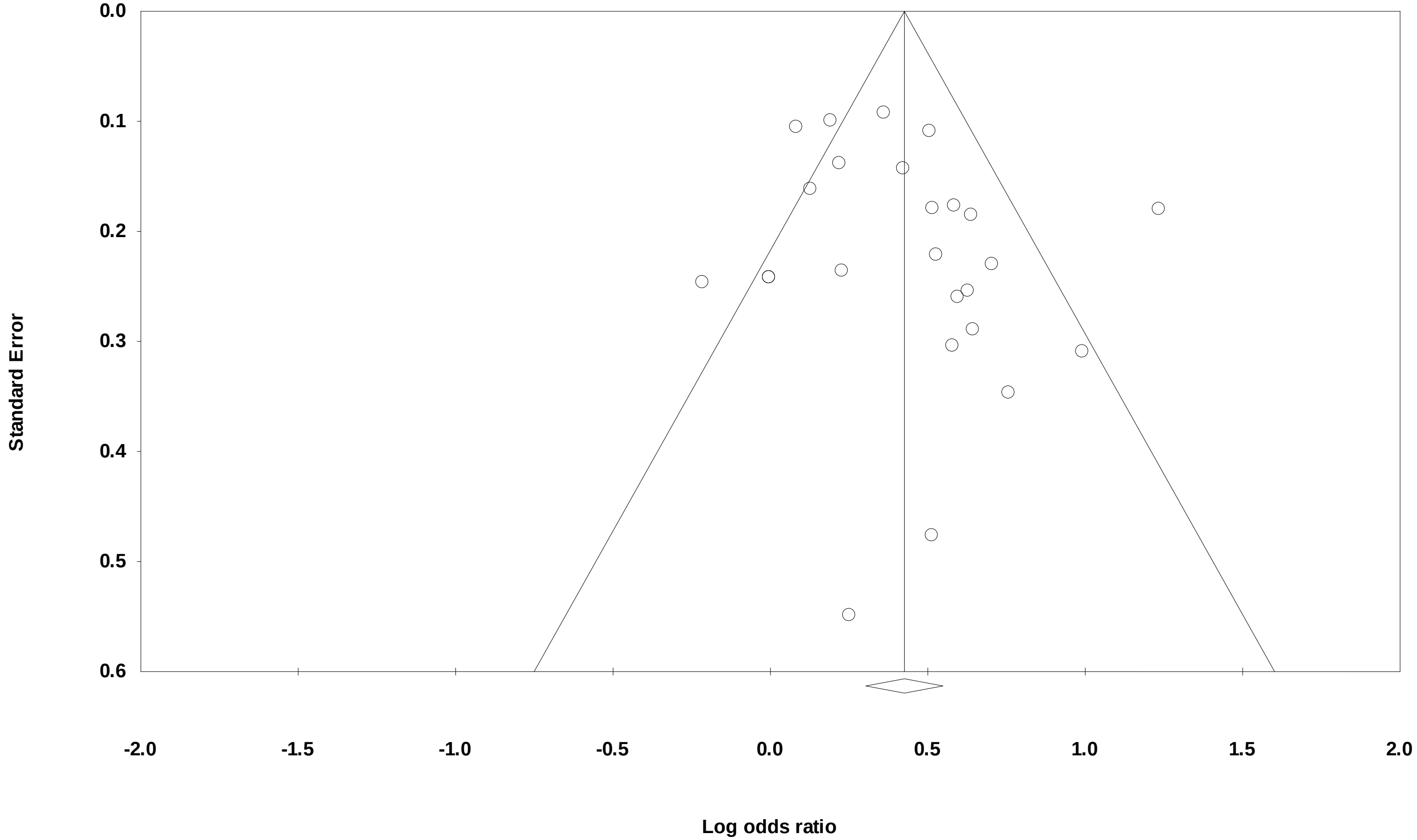


Figure 4: Effect of frequent toothbrushing compared with infrequent brushing: incidence or increment of dental caries is the outcome
246x218mm (96 x 96 DPI)


0.0
0.1
0.2
0.3
0.4
0.5
0.6
Standard Error
-2.0
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5
2.0
Log odds ratio

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, Heinzel-Gutenbrunner M, Pieper K. Association of caries increment in preschool children with nutritional and preventive variables. Clin Oral Investig 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. Community Dentistry & Oral Epidemiology 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. Community Dentistry & Oral Epidemiology 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. European journal of paediatric dentistry : official journal of European Academy of Paediatric Dentistry 2013; 14(1): 8-12.
5	Zhou Y, Yang JY, Lo EC, Lin HC. The contribution of life course determinants to early childhood caries: a 2-year cohort study. Caries Res 2012; 46(2): 87-94.
6	Wong MC, Lu HX, Lo EC. Caries increment over 2 years in preschool children: a life course approach. Int J Paediatr Dent 2012; 22(2): 77-84.
7	Divaris K, Fisher EL, Shugars DA, White RP, Jr. Risk factors for third molar occlusal caries: a longitudinal clinical investigation. Journal of oral and maxillofacial surgery : official journal of the American Association of Oral and Maxillofacial Surgeons 2012; 70(8): 1771-80.
8	Bernabe E, Newton JT, Uutela A, Aromaa A, Suominen AL. Sense of coherence and four-year caries incidence in Finnish adults. Caries Res 2012; 46(6): 523-9.
9	Chankanka O, Cavanaugh JE, Levy SM, et al. Longitudinal associations between children's dental caries and risk factors. J Public Health Dent 2011; 71(4): 289-300.
10	Maserejian NN, Tavares MA, Hayes C, Soncini JA, Trachtenberg FL. Prospective study of 5-year caries increment among children receiving comprehensive dental care in the New England children's amalgam trial. Community Dent Oral Epidemiol. 2009 Feb;37(1):9-18.
11	Ismail AI, Sohn W, Lim S, Willem JM. Predictors of dental caries progression in primary teeth. J Dent Res 2009; 88(3): 270-5.
12	Hietasalo P, Tolvanen M, Seppa L, et al. Oral health-related behaviors predictive of failures in caries control among 11-12-yr-old Finnish schoolchildren. Eur J Oral Sci 2008; 116(3): 267-71.
13	Kallestal C, Fjelddahl A. A four-year cohort study of caries and its risk factors in adolescents with high and low risk at baseline. Swed Dent J. 2007;31(1):11-25.
14	Tagliaferro EP, Pereira AC, Meneghim Mde C, Ambrosano GM. Assessment of dental caries predictors in a seven-year longitudinal study. J Public Health Dent 2006; 66(3):

169-73.

15 Siukosaari P, Ainamo A, Narhi TO. Level of education and incidence of caries in the elderly: a 5-year follow-up study. Gerodontology 2005; 22(3): 130-6.

16 Leroy R, Bogaerts K, Lesaffre E, Declerck D. Multivariate survival analysis for the identification of factors associated with cavity formation in permanent first molars. Eur J Oral Sci 2005; 113(2): 145-52.

17 Fure S. Ten-year cross-sectional and incidence study of coronal and root caries and some related factors in elderly Swedish individuals. Gerodontology 2004; 21(3): 130-40.

18 Takano N, Ando Y, Yoshihara A, Miyazaki H. Factors associated with root caries incidence in an elderly population. Community Dent Health 2003; 20(4): 217-22.

19 Vanobbergen J, Martens L, Lesaffre E, Bogaerts K, Declerck D. The value of a baseline caries risk assessment model in the primary dentition for the prediction of caries incidence in the permanent dentition. Caries Res 2001; 35(6): 442-50.

20 Mattila ML, Rautava P, Paunio P, et al. Caries experience and caries increments at 10 years of age. Caries Res 2001; 35(6): 435-41.

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22 Gilbert GH, Foerster U, Dolan TA, Duncan RP, Ringelberg ML. Twenty-four month coronal caries incidence: the role of dental care and race. Caries Res 2000; 34(5): 367-79.

23 Tada A, Ando Y, Hanada N. Caries risk factors among three-year old children in Chiba, Japan. Asia Pac J Public Health. 1999;11(2):109-12

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28 Wendt LK, Hallonsten AL, Koch G, Birkhed D. Oral hygiene in relation to caries development and immigrant status in infants and toddlers. Scand J Dent Res. 1994 Oct;102(5):269-73.

29 Chesters RK, Huntington E, Burchell CK, Stephen KW. Effect of oral care habits on

caries in adolescents. Caries Res. 1992;26(4):299-304.

30 Bjertness E, Eriksen HM, Hansen BF. Factors of importance for changes in dental caries among adults. A follow-up study of Oslo citizens from the age of 35 to 50 years. Acta Odontol Scand 1992; 50(4): 193-200.

31 Dummer PM, Oliver SJ, Hicks R, Kindon A, Addy M, Shaw WC. Factors influencing the initiation of carious lesions in specific tooth surfaces over a 4-year period in children between the ages of 11-12 years and 15-16 years. J Dent 1990; 18(4): 190-7.

32 Stecksen-Blicks C, Gustafsson L. Impact of oral hygiene and use of fluorides on caries increment in children during one year. Community Dent Oral Epidemiol 1986; 14(4): 185-9.

33 Hausen H, Heinonen OP, Paunio I. Modification of occurrence of caries in children by toothbrushing and sugar exposure in fluoridated and nonfluoridated areas. Community Dent Oral Epidemiol 1981; 9(3): 103-7.

ARTICLES INCLUDED IN THE META-ANALYSIS

1 Winter J, Glaser M, Heinzel-Gutenbrunner M, Pieper K. Association of caries increment in preschool children with nutritional and preventive variables. Clin Oral Investig 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. Community Dentistry & Oral Epidemiology 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. Community Dentistry & Oral Epidemiology 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. European journal of paediatric dentistry : official journal of European Academy of Paediatric Dentistry 2013; 14(1): 8-12.

5 Wong MC, Lu HX, Lo EC. Caries increment over 2 years in preschool children: a life course approach. Int J Paediatr Dent 2012; 22(2): 77-84.

6 Divaris K, Fisher EL, Shugars DA, White RP, Jr. Risk factors for third molar occlusal caries: a longitudinal clinical investigation. Journal of oral and maxillofacial surgery : official journal of the American Association of Oral and Maxillofacial Surgeons 2012; 70(8): 1771-80.

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8 Maserejian NN, Tavares MA, Hayes C, Soncini JA, Trachtenberg FL. Prospective study of 5-year caries increment among children receiving comprehensive dental care in the New England children's amalgam trial. Community Dent Oral Epidemiol. 2009 Feb;37(1):9-18.

9 Hietasalo P, Tolvanen M, Seppa L, et al. Oral health-related behaviors predictive of

failures in caries control among 11-12-yr-old Finnish schoolchildren. Eur J Oral Sci 2008; 116(3): 267-71.

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14 Vanobbergen J, Martens L, Lesaffre E, Bogaerts K, Declerck D. The value of a baseline caries risk assessment model in the primary dentition for the prediction of caries incidence in the permanent dentition. Caries Res 2001; 35(6): 442-50.

15 Mattila ML, Rautava P, Paunio P, et al. Caries experience and caries increments at 10 years of age. Caries Res 2001; 35(6): 435-41.

16 Gilbert GH, Foerster U, Dolan TA, Duncan RP, Ringelberg ML. Twenty-four month coronal caries incidence: the role of dental care and race. Caries Res 2000; 34(5): 367-79.

17 Tada A, Ando Y, Hanada N. Caries risk factors among three-year old children in Chiba, Japan. Asia Pac J Public Health. 1999;11(2):109-12

18 Mattila ML, Paunio P, Rautava P, Ojanlatva A, Sillanpaa M. Changes in dental health and dental health habits from 3 to 5 years of age. J Public Health Dent 1998; 58(4): 270-4.

19 Lawrence HP, Sheiham A. Caries progression in 12- to 16-year-old schoolchildren in fluoridated and fluoride-deficient areas in Brazil. Community Dent Oral Epidemiol 1997; 25(6): 402-11.

20 Grindefjord M, Dahllof G, Modeer T. Caries development in children from 2.5 to 3.5 years of age: a longitudinal study. Caries Res 1995; 29(6): 449-54.

21 Wendt LK, Hallonsten AL, Koch G, Birkhed D. Oral hygiene in relation to caries development and immigrant status in infants and toddlers. Scand J Dent Res. 1994 Oct;102(5):269-73.

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the initiation of carious lesions in specific tooth surfaces over a 4-year period in children between the ages of 11-12 years and 15-16 years. J Dent 1990; 18(4): 190-7.

25 Hausen H, Heinonen OP, Paunio I. Modification of occurrence of caries in children by toothbrushing and sugar exposure in fluoridated and nonfluoridated areas. Community Dent Oral Epidemiol 1981; 9(3): 103-7.

ARTICLES EXCLUDED WITH REASONS

Caries incidence/increment not the outcome

1 Honkala E, Nyyssonen V, Kolmakow S, Lammi S. Factors predicting caries risk in children. Scandinavian journal of dental research. 1984;92(2):134-40.

2 Grytten J, Rossow I, Holst D, Steele L. Longitudinal study of dental health behaviors and other caries predictors in early childhood. Community dentistry and oral epidemiology. 1988;16(6):356-9.

3 Ainamo J, Parvianinen K. Influence of increased toothbrushing frequency on dental health in low, optimal, and high fluoride areas in Finland. Community dentistry and oral epidemiology. 1989;17(6):296-9.

4 Reisine S, Litt M, Tinanoff N. A biopsychosocial model to predict caries in preschool children. Pediatr Dent. 1994;16(6):413-8.

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10 Law V, Seow WK. A longitudinal controlled study of factors associated with mutans streptococci infection and caries lesion initiation in children 21 to 72 months old. Pediatr Dent. 2006;28(1):58-65.

11 Ollila P, Larmas M. A seven-year survival analysis of caries onset in primary second molars and permanent first molars in different caries risk groups determined at age two years. Acta Odontol Scand. 2007;65(1):29-35.

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2008;17(2):267-86.

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15 Jamieson LM, Roberts-Thomson KF, Sayers SM. Dental caries risk indicators among Australian Aboriginal young adults. Community dentistry and oral epidemiology. 2010;38(3):213-21.

16 Niji R, Arita K, Abe Y, Lucas ME, Nishino M, Mitome M. Maternal age at birth and other risk factors in early childhood caries. Pediatr Dent. 2010;32(7):493-8.

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20 Heima M, Lee W, Milgrom P, Nelson S. Caregiver's education level and child's dental caries in African Americans: a path analytic study. Caries Research. 2015;49(2):177-83.

Effect of tooth brushing frequency not analysed

1 Rugg-Gunn AJ, Hackett AF, Appleton DR, Jenkins GN, Eastoe JE. Relationship between dietary habits and caries increment assessed over two years in 405 English adolescent school children. Archives of oral biology. 1984;29(12):983-92

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2011;45(4):400-7.
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Similar data from the same study population presented in another paper

1 Chestnutt IG, Schafer F, Jacobson AP, Stephen KW. The influence of toothbrushing frequency and post-brushing rinsing on caries experience in a caries clinical trial. Community dentistry and oral epidemiology. 1998;26(6):406-11.

2 Tagliaferro EP, Ambrosano GM, Meneghim Mde C, Pereira AC. Risk indicators and risk predictors of dental caries in schoolchildren. Journal of applied oral science : revista FOB. 2008;16(6):408-13.

3 Chankanka O, Marshall TA, Levy SM, Cavanaugh JE, Warren JJ, Broffitt B, et al. Mixed dentition cavitated caries incidence and dietary intake frequencies. Pediatr Dent. 2011;33(3):233-40
4 Broffitt B, Levy SM, Warren J, Cavanaugh JE. Factors associated with surface-level caries incidence in children aged 9 to 13: the Iowa Fluoride Study. J Public Health Dent. 2013;73(4):304-10.

5 Sabbah W, Suominen AL, Vehkalahti MM, Aromaa A, Bernabé E. The role of behaviour in inequality in increments of dental caries among Finnish adults. Caries Research. 2015;49(1):34-40.

Tooth brushing frequency was not recorded

1 Meurman PK, Pienihakkinen K. Factors associated with caries increment: a longitudinal study from 18 months to 5 years of age. Caries Res. 2010;44(6):519-24.
2 Nelson S, Albert JM, Lombardi G, Wishnek S, Asaad G, Kirchner HL, et al. Dental caries and enamel defects in very low birth weight adolescents. Caries Res. 2010;44(6):509-18.

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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	Fluoridate 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

										with caries increment		
3	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
4	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 insignifica nt	Bjertness et al., 1992
5	Adults living independent ly in two metropolita n communitie s 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 fluoridatio n was not significant	Locker. 1996
6	Schoolchild ren 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

, Scotland		2317		three follow-ups: ≤1 ≥2 TBF/day based on responses on three occasions: G1: <1/day in at least 2 of 3 occasions G2: 1/day at least 2 of the 3 instances G3: >1/day at least 2 of the 3 instances G4: All others Reported by children	(permanent dentition)		DMFS increments than ≤1/day brushers - G3 - lowest DMFS increment & G1 – highest DMFS increment		fluoridated toothpaste		Chestnut et al.,1998
Preschool children of Jonkoping, Sweden	1 year	At baseline: 632 At follow-up: 593	2 years	TBF/day at 1& 2 years of age: <1 ≥1 Reported by parents	% developing new caries lesions (deciduous dentition)	Inverse association	More children in with no caries at 1 and 3 years brushed their teeth at age 1 more often (≥1/day) than those who had caries at 3 years; More children with no caries at ages 1, 2 and 3 brushed more often than children who had caries at 3 yrs but not at 1 & 2 yrs	Not conducted	Fluoridated toothpaste was used by 87% participants at 2 yrs. More children who were caries free at all ages used F-toothpaste than those with caries at 3 years but not at 1 &2 yrs	Use of Fluoride tablets also recorded whose effect was insignificant.	Wendt et al, 1994

8	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
9	School children of Rio dejanerio 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
10	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

	Maternity Health Centers in province of Turku & Poori, south-western Finland		At follow-up: 828		- ≥1 - <1 Reported by mothers	- Mean dmfs increment (Deciduous dentition)		brushers, 21.8 percent had new caries, while 34 % of occasional brushers developed new caries.				
11	Infants attending mass check-ups in Chiba city, Japan	18 months	At baseline: 392	18 months	Tbf/day with guardians help: <1 1 ≥2 Reported by parent/guardian	% developing new carious lesions (Deciduous dentition)	No association	No association	Not conducted	-	-	Tada et al., 1999
12	Persons ≥45 years (black and rural residents) of four counties of North Florida, US	≥45 years	At baseline: 873 At follow-up: 723	2 years	Tbf/day at baseline: ≤1 >1	% developing new decay on root surfaces (permanent dentition)	Inverse association	In those brushing ≤1/day, 62% had no decay or filling while in those brushing >1/day, 65% had no decay or filling	Insignificant	-	-	Gilbert et al., 2001
13	Nursery school children of metropolitan area of Recife, Pernambuco state, Brazil	36-47 months	At baseline: 650 At Follow-up: 510	1 year	TBF/day at baseline: <1 ≥1 Reported by mothers	% developing new caries lesions (Deciduous dentition)	Inverse association	Stated as significant in the results but no values provided	Children brushing <1/day 1.77 times more likely at risk of caries than those brushing at least once/day	Has been reported that effect of Fluoridated toothpaste has been studied but no findings to be seen	Fluoride gel usage was considered. Children who have not received Fluoride gel were 2.6 times more likely	Rodrigues and Sheiham, 2000

											for caries increment	
14	Children born at Maternity Health Centers in province of Turku & Poori, 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.	Children brushing occasionally at 5 yrs of age were 1.7 more likely for caries incidence in deciduous dentition	-	-	Mattila et al., 2001
15	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 1st 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 1st 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

	Niigata City, Japan					new ≥2 surfaces Vs no new ≥3 surfaces vs no new		provided)	was not significant at bivariate level	but even it was not associated with incidence of root surface caries		
17	Residents of Municipality of Gotherburg, Sweden	55, 65 and 75 years old at baseline	At baseline: 208 At follow-up: 102	10 years	TBF (no categories provided)	- Increment in decayed and filled coronal surfaces - Increment in decayed and filled root surfaces - Increment in decayed and filled surfaces with coronal and root surface counted as one surface	No association	Not conducted	Not significant	Almost, all the subjects used Fluoride toothpaste	Fluoride rinse, tablets or chewing gums usage registered but their effect on dental caries increment was insignificant	Fure et al., 2004
18	School children of Flanders, Belgium	7 years	At baseline: 4351 At follow -up: 3291	5 years	TBF/day at baseline: ≥1 <1 Reported by parents	% developing new decay in first permanent molars	Inverse association	In those who didn't brush daily caries occurrence was accelerated	Teeth in frequent brushers had the best survival estimates	Fluoridated toothpaste usage recorded and its effect was insignificant (only 6% did not use fluoridated dentifrice)	Use of systemic Fluoride was recorded and its effect was significant only in univariate analysis	Leroy et al., 2005
19	Elderly of	67, 72 and	At baseline:	5 years	TBF at baseline:	- Coronal DMFT	Inverse	TBF	Not significant	Fluoridate		Siukosaari

	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
20	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
21	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 bushing ,≥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
22	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

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

					provided) Reported by parents	(deciduous and permanent dentition together)		proportion of new non-cavitated caries decreased by 33%	the proportion of new non-cavitated caries decreased by 33%		with cavitated or non-cavitated caries	
26	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
27	Patients attending clinical centers at University of Kentucky and University of North Carolina, USA	14 – 45 years old with at least one 3[rd] 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[rd] molars	Inverse association	Not significant	With unit increase in TBF, caries incidence on 3[rd] molars increase by 30%	-	-	Divaris et al., 2012
28	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
29	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

	town of Xinhua, China		155		<1 Reported by parents							
30	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
31	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
32	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
33	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 tooth paste use recorded and its	Information on professional applied topical	Winter et al., 2015

Waldeck-Frankenberg and Marburg-Biedenkopf, Germany	Reported by parents	dmft in children brushing ≤1/day than those brushing >1/day	effect was significant only in bivariate analysis	fluoride recorded & those who received Fluoride application had greater caries increment in bivariate analysis

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

	Selection bias	Study design	Confounders	Blinding	Data collection	Withdrawal & dropouts	Overall Quality rating	Caries diagnosis	
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 etal., 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 Chest nut et al., 1998
7	Moderate	Moderate	Weak	Moderate	Strong	Strong	Moderate	Cavitated	Wendt et al., 1994
8	Strong	Moderate	Weak	Moderate	Moderate	Strong	Moderate	Non-cavitated	Grindejford et al., 1995
9	Strong	Moderate	Strong	Moderate	Strong	Strong	Strong	Non-cavitated	Lawrence and Sheiham, 1997
10	Strong	Moderate	Weak	Moderate	Strong	Moderate	Moderate	Non-cavitated	Mattila et al., 1998
11	Weak	Moderate	Weak	Moderate	Strong	Strong	Weak	Cavitated	Tada et al., 1999
12	Moderate	Moderate	Strong	Moderate	Strong	Strong	Strong	Cavitated	Gilbert et al.,2000 Gilbert et al., 2001
13	Moderate	Moderate	Strong	Moderate	Strong	Moderate	Strong	Non-cavitated	Rodrigues & Sheiham, 2000
14	Strong	Moderate	Moderate	Moderate	Strong	Strong	Strong	Cavitated	Mattila et al., 2001
15	Strong	Moderate	Weak	Moderate	Strong	Strong	Moderate	Non-cavitated	Vanobbergen etal., 2001
16	Strong	Moderate	Weak	Moderate	Strong	Weak	Weak	Cavitated	Takano et al., 2003
17	Moderate	Moderate	Weak	Mode	Strong	Weak	Weak	Coronal caries - Cavitated & non-	Fure et al., 2004

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

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

	Regression coefficient	SE	95% CI	P
Sample size	-0.00001	0.0001	-00003-0.0001	0.2903
Follow-up period	0.0008	0.0024	-0.0039-0.0056	0.7268
Caries diagnosis level				
Cavitated	Reference			
Non-cavitated	-0.1243	0.1569	-0.4318-0.1831	0.4279
Methodological Quality				
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