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

<table>
<thead>
<tr>
<th>Journal:</th>
<th><em>Journal of Dental Research</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Manuscript ID:</td>
<td>JDR-15-1270.R4</td>
</tr>
<tr>
<td>Manuscript Type:</td>
<td>Clinical Review</td>
</tr>
<tr>
<td>Date Submitted by the Author:</td>
<td>n/a</td>
</tr>
<tr>
<td>Complete List of Authors:</td>
<td>Tadakamadla, Santhosh Kumar TADAKAMADLA, JYOTHI Johnson, N.W.; Griffith University, Griffith Health Institute;</td>
</tr>
<tr>
<td>Keywords:</td>
<td>Caries, Systematic reviews and evidence-based medicine, Oral hygiene, Meta-analysis, Fluoride(s), Preventive Dentistry</td>
</tr>
</tbody>
</table>

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

Authors

Kumar S¹, Tadakamadla J¹, Johnson NW²

¹Population and Social Health Research Programme, Menzies Health Institute Queensland and School of Dentistry and Oral Health, Griffith University, Queensland, Australia

²Population and Social Health Research Programme, Menzies Health Institute Queensland, Griffith University, Queensland, Australia

Corresponding author

Santhosh Kumar

Population and Social Health Research Programme Menzies Health Institute Queensland and School of Dentistry and Oral Health, Griffith University, Queensland, Australia

E-mail: santoshkumar.tadakamadla@griffithuni.edu.au

Mobile: +61415060506

Short title: Effect of Toothbrushing frequency on dental caries

Keywords: Dental caries; Meta-analysis; Systematic review; Toothbrushing frequency

Number of words in Abstract: 290

Number of words in the abstract and the text (excluding tables, figure legends, acknowledgments, and cited references): 3406

Number of tables: 0
Number of figures: 5

Number of cited references: 32
Title: Effect of Toothbrushing Frequency on Incidence and Increment of Dental Caries: A Systematic Review and Meta-Analysis

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.

**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**

http://mc.manuscriptcentral.com/jdr
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
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 focused 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)
individually. 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 ‘I²’ statistic. An I² 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 (I²=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 ($t=1.40, 95\% \text{ CI: }-0.52\text{-}2.71, p=0.174$): 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 differ 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 (Grindfjord et al. 1995; Leroy et al. 2005; Wong et al. 2012) considered toothbrushing frequency and fluoride in toothpaste 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

We wish to thank Dr Martin Downes for statistical advice. This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of Interest

Authors declare no conflicts of interest.

References


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)

Studies included in qualitative synthesis (n = 33)

Studies included in quantitative synthesis (meta-analysis) (n = 25)

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)

http://mc.manuscriptcentral.com/jdr
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)
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

<table>
<thead>
<tr>
<th>ARTICLES INCLUDED IN SYSTEMATIC REVIEW</th>
</tr>
</thead>
</table>

http://mc.manuscriptcentral.com/jdr


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


ARTICLES INCLUDED IN THE META-ANALYSIS


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


24 Dummer PM, Oliver SJ, Hicks R, Kindon A, Addy M, Shaw WC. Factors influencing


25 ARTICLES EXCLUDED WITH REASONS

Caries incidence/increment not the outcome


Effect of tooth brushing frequency not analysed


8 Bernabe E, Stansfeld SA, Marcenes W. Roles of different sources of social support on caries experience and caries increment in adolescents of East London. Caries Res.
Ferreira de Camargo MA, Frias AC, Antunes JL. The incidence of dental caries in children and adolescents who have cerebral palsy and are participating in a dental program in Brazil. Special care in dentistry: official publication of the American Association of Hospital Dentists, the Academy of Dentistry for the Handicapped, and the American Society for Geriatric Dentistry. 2011;31(6):210-5.


Similar data from the same study population presented in another paper


Tooth brushing frequency was not recorded


### Appendix Table 3: Background characteristics and findings from the included studies

<table>
<thead>
<tr>
<th>Study population</th>
<th>Age of the study population at baseline</th>
<th>Sample size</th>
<th>Follow-up period</th>
<th>Exposure variable</th>
<th>Caries increment outcome</th>
<th>Association between TBF and caries</th>
<th>Findings from Univariate or bivariate analysis</th>
<th>Findings from Multivariate analysis</th>
<th>Information Fluoride toothpaste usage</th>
<th>Effect of other fluoride sources</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children attending municipal dental health centres of Kuopio, Finland</td>
<td>7-16</td>
<td>2024</td>
<td>1 year</td>
<td>TBF/day:</td>
<td>% developing new caries lesions (permanent dentition)</td>
<td>No association. An association was observed when fluoride and sugar exposure were considered</td>
<td>Caries risk increased with more frequent tooth brushing among children using fluoridated water &amp; consuming sugars frequently; caries decreased among children receiving local decay preventives containing fluoride</td>
<td>No association</td>
<td>Fluoride dentifrice use was recorded but its effect on dental caries not analysed</td>
<td>Data on exposure to Fluoridated water supply and topical fluorides recorded</td>
<td>Hausen et al., 1981</td>
</tr>
<tr>
<td>Children of Umea, North Sweden</td>
<td>8 &amp; 13 years old (8yrs)</td>
<td>88 (8yrs)</td>
<td>11-13 months</td>
<td>TBF/day at baseline:</td>
<td>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)</td>
<td>No association</td>
<td>Fluoridate toothpaste was used by 84% and 91% of 8 and 13 yr old respectively, it was not associated</td>
<td>Fluoridate toothpaste was used by 84% and 91% of 8 and 13 yr old respectively, it was not associated</td>
<td>Stecksen-Blicks et al, 1986</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

http://mc.manuscriptcentral.com/jdr
<table>
<thead>
<tr>
<th>Study</th>
<th>Age Range</th>
<th>Baseline Data</th>
<th>Follow-up Data</th>
<th>TBF Frequency</th>
<th>Caries Impact</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citizens of Oslo, Norway</td>
<td>35 years</td>
<td>At baseline: 116, At follow-up: 81</td>
<td>15 years</td>
<td>Decayed surfaces increment (permanent dentition)</td>
<td>No association</td>
</tr>
<tr>
<td>Adults living independently in two metropolitain communities in Ontario, Canada</td>
<td>&gt;50 years</td>
<td>At baseline: 699, At follow-up: 493</td>
<td>3 years</td>
<td>% developing new caries - Decayed Surfaces (DS) increment (root) (permanent dentition)</td>
<td>Inverse association Mean decayed surfaces increment more in &lt;1/day brushers than ≥1/day</td>
</tr>
<tr>
<td>Schoolchildren of Lanarkshire</td>
<td>11-12 years</td>
<td>At baseline: 4294, At follow-up:</td>
<td>3 years</td>
<td>TBF/day in consistent brushers on all Decayed, Missing and Filled Surfaces increment</td>
<td>Inverse association - ≥2/day brushers had lower</td>
</tr>
</tbody>
</table>

Dummer et al., 1990
Bjertness et al., 1992
Locker. 1996
Chesters et al., 1992
Three follow-ups: (permanent dentition)

- \( \leq 1 \)
- \( \geq 2 \)

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

DMFS increments than \( \leq 1/day \) brushers

- G3 - lowest DMFS increment & G1 – highest DMFS increment

Preschool children of Jonkoping, Sweden

At baseline: 632

At follow-up: 593

TBF/day at 1 & 2 years of age:

- \(<1\>
- \(\geq 1\)

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 (\(\geq 1/day\)) 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.

http://mc.manuscriptcentral.com/jdr
<table>
<thead>
<tr>
<th>Study</th>
<th>Population</th>
<th>Age</th>
<th>At baseline</th>
<th>TBF/day at baseline</th>
<th>% developing new caries lesions</th>
<th>Inverse association</th>
<th>Caries risk factors</th>
<th>Fluoride use</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>Children living in suburbs of Stockholm, Sweden</td>
<td>2.5 years</td>
<td>832</td>
<td>&lt;1</td>
<td>Children brushing less than once/day were at greater risk of developing new caries</td>
<td>Not conducted</td>
<td>Fluoride toothpaste usage recorded and had no significant effect on caries increment</td>
<td>Use of fluoride tablets recorded. Children not using F tablets were at greater risk for caries increment.</td>
<td>Grindejford et al., 1995</td>
</tr>
<tr>
<td>9</td>
<td>School children of Rio de Janerio state, Brazil</td>
<td>12-16</td>
<td>420</td>
<td>&lt;2</td>
<td>Subjects brushing &lt;2/day were at 1.68 times greater risk than those brushing ≥2</td>
<td>Not conducted</td>
<td>All the subjects used fluoride toothpaste</td>
<td>Information on professionally applied fluorides, F mouth rinses, fluoride supplements &amp; water fluoridation obtained. Only effect of water was fluoridation analysed (greater caries increment in those living in F deficit areas)</td>
<td>Lawrence and Sheiham, 1997</td>
</tr>
<tr>
<td>10</td>
<td>Children born at 3 years</td>
<td>2 years</td>
<td>1059</td>
<td>-</td>
<td>Among Daily</td>
<td>Not significant</td>
<td>-</td>
<td>Mattila et al., 1998</td>
<td></td>
</tr>
<tr>
<td>Study Description</td>
<td>Maternity Health Centers in province of Turku &amp; Poori, southwestern Finland</td>
<td>Infants attending mass check-ups in Chiba city, Japan</td>
<td>Persons ≥45 years (black and rural residents) of four counties of North Florida, US</td>
<td>Nursery school children of metropolitan area of Recife, Pernambuco state, Brazil</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------------------------------------------------------------------------</td>
<td>-------------------------------------------------------------------------------</td>
<td>------------------------------------------------------</td>
<td>-----------------------------------------------------------------------------------</td>
<td>----------------------------------------------------------------------------------</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At follow-up: 828</td>
<td>- ≥1 Mean dmfs increment (Deciduous dentition)</td>
<td>At baseline: 392 18 months Tbf/day with guardians help: &lt;1 1 ≥2 % developing new carious lesions (Deciduous dentition)</td>
<td>No association In those brushing ≤1/day, 62% had no decay or filling while in those brushing &gt;1/day, 65% had no decay or filling</td>
<td>Stated as significant in the results but no values provided</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>- &lt;1</td>
<td>18 months Reported by parent/guardian Tbf/day at baseline: ≤1 &gt;1 % developing new decay on root surfaces (permanent dentition)</td>
<td>Inverse association Insignificant - -</td>
<td>Inverse association</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Reported by mothers</td>
<td>723</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>650</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>510</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 year TBF/day at baseline: &lt;1 ≥1 % developing new caries lesions (Deciduous dentition)</td>
<td>Inverse association</td>
<td>Stated as significant in the results but no values provided</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Children brushing &lt;1/day 1.77 times more likely at risk of caries than those brushing at least once/day</td>
<td>Has been reported that effect of Fluoridated toothpaste has been studied but no findings to be seen</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fluoride gel usage was considered. Children who have not received Fluoride gel were 2.6 times more likely</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Tada et al., 1999
Gilbert et al., 2001
Rodrigues and Sheiham, 2000
<table>
<thead>
<tr>
<th>Study</th>
<th>Participants</th>
<th>Baseline</th>
<th>Follow-up</th>
<th>TBF/day</th>
<th>% Developing New Caries</th>
<th>Inverse Association</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mattila et al., 2001</td>
<td>Children born at Maternity Health Centers in province of Turku &amp; Poori, southwestern Finland</td>
<td>7 years</td>
<td>7 years</td>
<td>3 years</td>
<td>≥1 or &lt;1</td>
<td>Inverse association</td>
<td>Child’s TBF at 5 yrs of age was 1.7 more likely for caries incidence in deciduous dentition. No values provided for association of TBF with caries incidence with other dentitions.</td>
</tr>
<tr>
<td>Vanobbergen et al., 2001</td>
<td>Children brushing occasionally at 5 yrs of age were 1.7 more likely for caries incidence in deciduous dentition. No values provided for association of TBF with caries incidence with other dentitions.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Takano et al., 2003</td>
<td>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.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-</td>
</tr>
</tbody>
</table>

**Cohort of Schoolchildren of Flanders, Belgium born in 1989**

At baseline: 3,303
At follow-up: 2,691

<table>
<thead>
<tr>
<th>TBF/day at each year from baseline:</th>
<th>% Developing New Caries in the 1st Permanent Molars</th>
<th>Inverse Association</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Reported by parents

**70 Year Old Dentate Individuals Living in**

At baseline: 544
At follow-up: 379

<table>
<thead>
<tr>
<th>TBF/day at baseline:</th>
<th>Root Caries Incidence at 1 or 2 Years</th>
<th>No Association</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥2</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Root caries incidence at 1 or 2 years follow-up: ≥1 surfaces vs no

Not significant with chi square (No values)

Use of fluoridated toothpaste recorded

Use of systemic fluorides recorded but its effect on increment of decayed surfaces of 1st permanent molars was insignificant
<table>
<thead>
<tr>
<th>Location</th>
<th>Age</th>
<th>Baseline</th>
<th>Follow-up</th>
<th>TBF/day</th>
<th>DMFT</th>
<th>Decayed and Filled Surfaces</th>
<th>Fluoride Usage</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Niigata City, Japan</td>
<td>55-75 years</td>
<td>208</td>
<td>102</td>
<td></td>
<td></td>
<td>Increment in decayed and filled surfaces</td>
<td></td>
<td>Not significant</td>
</tr>
<tr>
<td>Residents of Municipality of Gothenburg, Sweden</td>
<td>55, 65, and 75 years</td>
<td>208</td>
<td>102</td>
<td>TBF (no categories provided)</td>
<td></td>
<td>No association</td>
<td>Oral hygiene</td>
<td>Not conducted</td>
</tr>
<tr>
<td>School children of Flanders, Belgium</td>
<td>7 years</td>
<td>4351</td>
<td>3291</td>
<td>TBF/day at baseline</td>
<td></td>
<td>% developing decay in first permanent molars</td>
<td>Fluoride variance</td>
<td>Inverse association</td>
</tr>
<tr>
<td>Elderly of 67, 72 and 75 years</td>
<td>5 years</td>
<td>4351</td>
<td>3291</td>
<td>TBF at baseline</td>
<td></td>
<td>Coronal DMFT</td>
<td>Use of systemic Fluoride</td>
<td>Inverse TBF</td>
</tr>
</tbody>
</table>

Fure et al., 2004
Leroy et al., 2005
Siukosaari
<table>
<thead>
<tr>
<th>Location</th>
<th>Age</th>
<th>Baseline</th>
<th>Follow-up</th>
<th>TBF/day</th>
<th>% Developing New Decay</th>
<th>Inverse Association</th>
<th>Effect</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Helsinki, Finland</td>
<td>77 age cohorts</td>
<td>364</td>
<td>209</td>
<td>≥2/day</td>
<td>- Root caries index increment</td>
<td>significantly related to DMFT increment (no values provided)</td>
<td>Not significant</td>
<td>et al., 2005</td>
</tr>
<tr>
<td>School children of Piracicaba, Brazil</td>
<td>6 – 8 years</td>
<td>480</td>
<td>206</td>
<td>TBF/day at baseline:</td>
<td>% developing new decay (permanent dentition)</td>
<td>Inverse association</td>
<td>Caries incidence in ≤2/day brushers</td>
<td>Not significant</td>
</tr>
<tr>
<td>Children attending public dental health clinics of Sweden</td>
<td>12 years</td>
<td>3373</td>
<td>2848</td>
<td>TBF/day based on information provided at each year intervals:</td>
<td>Increment of DMFS (caries at dentinal level on all surfaces) – included in meta-analysis</td>
<td>Inverse association with both outcomes</td>
<td>Children brushing ≥2 times at 3 occasions had lower caries increment than those brushing less frequently</td>
<td>Inverse association but the strength of association was very weak</td>
</tr>
<tr>
<td>All fifth and sixth grade children with at least once active caries in</td>
<td>11-12 years</td>
<td>497</td>
<td>Effect of TBF on dental caries increment was assessed only in</td>
<td>TBF/day with Fluoridated toothpaste at baseline:</td>
<td>% developing new decay</td>
<td>Inverse association</td>
<td>Those brushing at least twice a day did not develop new caries compared to</td>
<td>Not conducted</td>
</tr>
</tbody>
</table>

http://mc.manuscriptcentral.com/jdr
<table>
<thead>
<tr>
<th>Study</th>
<th>Country</th>
<th>Age</th>
<th>Sample Size</th>
<th>Outcome Measure</th>
<th>Frequency</th>
<th>Outcome Type</th>
<th>Association</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pori town, Finland</td>
<td>250 subjects reported by children</td>
<td>0-5 years</td>
<td>At baseline: 1021; follow-up: 788</td>
<td>TBF during the preceding week &lt; 7 vs ≥ 7</td>
<td>2 years</td>
<td>Increment in d3-6 (cavitated decay) mfs; d1-6 (cavitated &amp; non-cavitated) mfs (deciduous dentition)</td>
<td>No association</td>
<td>those brushing less often daily only when the outcome was ≤0 vs ≥1</td>
</tr>
<tr>
<td>African–American children from low income families of Detroit, Michigan, USA</td>
<td>0-5 years; 6-10 years</td>
<td>0-5 years</td>
<td>At baseline: 1021; follow-up: 788</td>
<td>TBF during the preceding week &lt; 7 vs ≥ 7</td>
<td>2 years</td>
<td>Increment in d3-6 (cavitated decay) mfs; d1-6 (cavitated &amp; non-cavitated) mfs (deciduous dentition)</td>
<td>No association</td>
<td>African–American children from low income families of Detroit, Michigan, USA</td>
</tr>
<tr>
<td>Children attending 5 community health clinics in Boston and 1 in Farmington, USA with 2 or more posterior teeth with caries on occlusal surfaces</td>
<td>6-10 years; 156</td>
<td>5 years</td>
<td>At baseline: 534; follow-up: 429</td>
<td>TBF/day at baseline: &lt;1 vs ≥1</td>
<td>5 years</td>
<td>Increment of decayed surfaces (deciduous and permanent dentition together)</td>
<td>Inverse association</td>
<td>Children who brushed their teeth &lt;1/day were at greater risk of decayed teeth &amp; surfaces than ≥2 brushes</td>
</tr>
<tr>
<td>Newborns at 8 IOWA hospital postpartum units</td>
<td>1.5 months; 156</td>
<td>1.5 months</td>
<td>At baseline: 156; follow-up: 156</td>
<td>Information on TBF collected at frequent intervals from 6 weeks to 13 years and average TBF was generated (no categories)</td>
<td>13 years</td>
<td>- New Cavitated decayed surfaces at all three examinations; - New Non-Cavitated decayed surfaces at all three examinations</td>
<td>Inverse association</td>
<td>Composite water fluoride levels recorded and was not associated</td>
</tr>
</tbody>
</table>

Ismail et al., 2009
Masserjian et al., 2009
Chankanka et al., 2011
<table>
<thead>
<tr>
<th>Study</th>
<th>Participants</th>
<th>Study Design</th>
<th>Baseline</th>
<th>Follow-up</th>
<th>TBF/day</th>
<th>Main Findings</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bernabe et al., 2012</td>
<td>Randomly selected subjects aged ≥30 years from those who attended Finnish Health 2000 survey</td>
<td>At baseline: 1248 At follow-up: 944</td>
<td>4 years</td>
<td>TBF/day at baseline: ≤1 ≥2</td>
<td>% developing new caries (deciduous and permanent dentition together)</td>
<td>Inverse association</td>
<td>Those brushing ≥2/day were at 50% less risk than those brushing ≤1/day</td>
</tr>
<tr>
<td>Divaris et al., 2012</td>
<td>Patients attending clinical centers at University of Kentucky and University of North Carolina, USA</td>
<td>At baseline: 389 At follow-up: 215</td>
<td>1-10 years</td>
<td>TBF/day at baseline: 1 ≥2</td>
<td>DFT increment on 3rd molars</td>
<td>Inverse association</td>
<td>Not significant With unit increase in TBF, caries incidence on 3rd molars increase by 30%</td>
</tr>
<tr>
<td>Wong et al., 2012</td>
<td>Children attending a hospital in Hong Kong</td>
<td>At baseline: 225 At follow-up:</td>
<td>8 months</td>
<td>TBF/day at baseline: ≥1</td>
<td>Decayed surfaces increment (Deciduous dentition)</td>
<td>No association</td>
<td>Not significant Fluoride toothpaste usage recorded and its effect was insignificant</td>
</tr>
<tr>
<td>Zhou et al., 2012</td>
<td>Patients attending clinical centers at University of Kentucky and University of North Carolina, USA</td>
<td>At baseline: 465 At follow-up: 358</td>
<td>3-4 years</td>
<td>TBF/day at baseline: ≤1 ≥2</td>
<td>% developing new caries (deciduous dentition)</td>
<td>Inverse association</td>
<td>Caries incidence and mean caries increment more in ≤1/day brushers than ≥2/day Not conducted</td>
</tr>
</tbody>
</table>

*Deciduous and permanent dentition together*
<table>
<thead>
<tr>
<th>Study</th>
<th>Location</th>
<th>Population Details</th>
<th>Sample Size</th>
<th>Follow-Up Duration</th>
<th>TBF/day Information</th>
<th>Caries Incidence</th>
<th>Summary of Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study 1</td>
<td>China</td>
<td>155 children from two public schools in the town of Xinhua, China</td>
<td>155</td>
<td>3 years</td>
<td>At baseline: 272, At follow-up: 224</td>
<td>those brushing 2/day at baseline: ≥2</td>
<td>Inverse association</td>
</tr>
<tr>
<td>Study 2</td>
<td>Brazil</td>
<td>30 children from two public schools in Belo Horizonte, Brazil</td>
<td>6</td>
<td>3 years</td>
<td>At baseline: 91, At follow-up: 33</td>
<td>those brushing 2/day had 30% lesser new caries in past 24 months than those brushing &lt;2/day</td>
<td>Inverse association</td>
</tr>
<tr>
<td>Study 3</td>
<td>US</td>
<td>Patient attending 63 dental practices in 5 states of the US</td>
<td>1763</td>
<td>2 years</td>
<td>At baseline: 1763, At follow-up: 1400</td>
<td>Those brushing ≥2/day had lesser caries increment than those brushing &lt;2/day</td>
<td>Inverse association</td>
</tr>
<tr>
<td>Study 4</td>
<td>US</td>
<td>3-22 months (approximately 1 year old)</td>
<td>86</td>
<td>3 years</td>
<td>At baseline: 86, At follow-up: 81</td>
<td>% developing new caries at 4 years (used in meta-analysis)</td>
<td>Inverse association</td>
</tr>
<tr>
<td>Study 5</td>
<td>US</td>
<td>1-4 years</td>
<td>566</td>
<td>3 years</td>
<td>At baseline and follow-up: 566</td>
<td>TBF/day just before follow-up examination: ≤1</td>
<td>Greater risk of caries incidence and mean increment of</td>
</tr>
<tr>
<td>Study 6</td>
<td>US</td>
<td>31 children attending kindergartens in districts of</td>
<td>31</td>
<td>3 years</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Rossette Melo et al., 2013

Rothen et al., 2014

Ghazal et al., 2014

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

<table>
<thead>
<tr>
<th>Study Design</th>
<th>Confounders</th>
<th>Blinding</th>
<th>Data Collection</th>
<th>Withdrawal &amp; Dropouts</th>
<th>Overall Quality Rating</th>
<th>Caries Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Strong</td>
<td>Moderate</td>
<td>Strong</td>
<td>Moderate</td>
<td>Strong</td>
<td>Strong</td>
<td>Not described</td>
</tr>
<tr>
<td>2 Weak</td>
<td>Moderate</td>
<td>Weak</td>
<td>Moderate</td>
<td>Strong</td>
<td>Strong</td>
<td>Not described</td>
</tr>
<tr>
<td>3 Moderate</td>
<td>Moderate</td>
<td>Weak</td>
<td>Moderate</td>
<td>Strong</td>
<td>Moderate</td>
<td>Non-cavitated</td>
</tr>
<tr>
<td>4 Moderate</td>
<td>Weak</td>
<td>Moderate</td>
<td>Strong</td>
<td>Strong</td>
<td>Moderate</td>
<td>Cavitated</td>
</tr>
<tr>
<td>5 Weak</td>
<td>Moderate</td>
<td>Strong</td>
<td>Moderate</td>
<td>Strong</td>
<td>Moderate</td>
<td>Cavitated</td>
</tr>
<tr>
<td>6 Moderate</td>
<td>Moderate</td>
<td>Strong</td>
<td>Moderate</td>
<td>Strong</td>
<td>Moderate</td>
<td>Cavitated</td>
</tr>
<tr>
<td>7 Moderate</td>
<td>Moderate</td>
<td>Weak</td>
<td>Moderate</td>
<td>Strong</td>
<td>Moderate</td>
<td>Cavitated</td>
</tr>
<tr>
<td>8 Strong</td>
<td>Moderate</td>
<td>Weak</td>
<td>Moderate</td>
<td>Strong</td>
<td>Moderate</td>
<td>Non-cavitated</td>
</tr>
<tr>
<td>9 Strong</td>
<td>Moderate</td>
<td>Strong</td>
<td>Moderate</td>
<td>Strong</td>
<td>Strong</td>
<td>Non-cavitated</td>
</tr>
<tr>
<td>10 Strong</td>
<td>Moderate</td>
<td>Weak</td>
<td>Moderate</td>
<td>Strong</td>
<td>Moderate</td>
<td>Non-cavitated</td>
</tr>
<tr>
<td>11 Weak</td>
<td>Moderate</td>
<td>Strong</td>
<td>Strong</td>
<td>Strong</td>
<td>Weak</td>
<td>Cavitated</td>
</tr>
<tr>
<td>12 Moderate</td>
<td>Moderate</td>
<td>Strong</td>
<td>Strong</td>
<td>Strong</td>
<td>Strong</td>
<td>Cavitated</td>
</tr>
<tr>
<td>13 Moderate</td>
<td>Moderate</td>
<td>Strong</td>
<td>Moderate</td>
<td>Strong</td>
<td>Strong</td>
<td>Non-cavitated</td>
</tr>
<tr>
<td>14 Strong</td>
<td>Moderate</td>
<td>Moderate</td>
<td>Strong</td>
<td>Strong</td>
<td>Strong</td>
<td>Cavitated</td>
</tr>
<tr>
<td>15 Strong</td>
<td>Moderate</td>
<td>Weak</td>
<td>Moderate</td>
<td>Strong</td>
<td>Moderate</td>
<td>Non-cavitated</td>
</tr>
<tr>
<td>16 Strong</td>
<td>Moderate</td>
<td>Weak</td>
<td>Moderate</td>
<td>Strong</td>
<td>Weak</td>
<td>Cavitated</td>
</tr>
<tr>
<td>17 Moderate</td>
<td>Moderate</td>
<td>Weak</td>
<td>Strong</td>
<td>Weak</td>
<td>Weak</td>
<td>Coronal caries -</td>
</tr>
</tbody>
</table>

Hausen et al., 1981
Steckesen-Blicks, 1986
Dummer et al., 1990
Bjertness, 1992
Chesters et al., 1992
Chestnut et al., 1998
Wendt et al., 1994
Grindeland ef al., 1995
Lawrence and Sheiham, 1997
Mattila et al., 1998
Tada et al., 1999
Gilbert et al., 2000
Gilbert et al., 2001
Rodrigues & Sheiham, 2000
Mattila et al., 2001
Vanobbergen et al., 2001
Takano et al., 2003
Fure et al., 2004
<table>
<thead>
<tr>
<th></th>
<th>Strength</th>
<th>Moderate</th>
<th>Strong</th>
<th>Moderate</th>
<th>Strong</th>
<th>Moderate</th>
<th>Strong</th>
<th>Non-cavitated</th>
<th>Cavitated</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>Strong</td>
<td>Moderate</td>
<td>Strong</td>
<td>Moderate</td>
<td>Strong</td>
<td>Moderate</td>
<td>Strong</td>
<td>Non-cavitated</td>
<td>Cavitated</td>
<td>Leroy et al., 2005</td>
</tr>
<tr>
<td>19</td>
<td>Strong</td>
<td>Moderate</td>
<td>Weak</td>
<td>Moderate</td>
<td>Strong</td>
<td>Weak</td>
<td>Strong</td>
<td>Non-cavitated</td>
<td>Cavitated</td>
<td>Siukosaari et al., 2005</td>
</tr>
<tr>
<td>20</td>
<td>Moderate</td>
<td>Moderate</td>
<td>Strong</td>
<td>Moderate</td>
<td>Strong</td>
<td>Weak</td>
<td>Moderate</td>
<td>Non-cavitated</td>
<td>Cavitated</td>
<td>Tagliaferro et al., 2006</td>
</tr>
<tr>
<td>21</td>
<td>Moderate</td>
<td>Moderate</td>
<td>Weak</td>
<td>Moderate</td>
<td>Strong</td>
<td>Strong</td>
<td>Moderate</td>
<td>Non-cavitated</td>
<td>Cavitated</td>
<td>Kallestal et al., 2005</td>
</tr>
<tr>
<td>22</td>
<td>Strong</td>
<td>Moderate</td>
<td>Weak</td>
<td>Strong</td>
<td>Moderate</td>
<td>Strong</td>
<td>Moderate</td>
<td>Non-cavitated</td>
<td>Cavitated</td>
<td>Hietasalo et al., 2008</td>
</tr>
<tr>
<td>23</td>
<td>Moderate</td>
<td>Strong</td>
<td>Moderate</td>
<td>Strong</td>
<td>Strong</td>
<td>Moderate</td>
<td>Strong</td>
<td>Cavitated</td>
<td></td>
<td>Ismail et al., 2009</td>
</tr>
<tr>
<td>24</td>
<td>Strong</td>
<td>Moderate</td>
<td>Strong</td>
<td>Moderate</td>
<td>Strong</td>
<td>Strong</td>
<td>Strong</td>
<td>Cavitated</td>
<td></td>
<td>Masserjian et al., 2009</td>
</tr>
<tr>
<td>25</td>
<td>Moderate</td>
<td>Moderate</td>
<td>Strong</td>
<td>Moderate</td>
<td>Strong</td>
<td>Weak</td>
<td>Moderate</td>
<td>Cavitated and non-cavitated</td>
<td></td>
<td>Chankanka et al., 2011</td>
</tr>
<tr>
<td>26</td>
<td>Strong</td>
<td>Moderate</td>
<td>Strong</td>
<td>Moderate</td>
<td>Strong</td>
<td>Moderate</td>
<td>Strong</td>
<td>Cavitated</td>
<td></td>
<td>Bernabe et al., 2012</td>
</tr>
<tr>
<td>27</td>
<td>Weak</td>
<td>Moderate</td>
<td>Strong</td>
<td>Moderate</td>
<td>Strong</td>
<td>Weak</td>
<td>Weak</td>
<td>Cavitated</td>
<td></td>
<td>Divaris et al</td>
</tr>
<tr>
<td>28</td>
<td>Strong</td>
<td>Moderate</td>
<td>Strong</td>
<td>Moderate</td>
<td>Strong</td>
<td>Moderate</td>
<td>Strong</td>
<td>Cavitated</td>
<td></td>
<td>Wong et al., 2012</td>
</tr>
<tr>
<td>29</td>
<td>Moderate</td>
<td>Moderate</td>
<td>Strong</td>
<td>Moderate</td>
<td>Strong</td>
<td>Moderate</td>
<td>Strong</td>
<td>Cavitated</td>
<td></td>
<td>Zhou et al., 2012</td>
</tr>
<tr>
<td>30</td>
<td>Weak</td>
<td>Moderate</td>
<td>Strong</td>
<td>Moderate</td>
<td>Strong</td>
<td>Strong</td>
<td>Moderate</td>
<td>Cavitated</td>
<td></td>
<td>Rossetto et al., 2013</td>
</tr>
<tr>
<td>31</td>
<td>Moderate</td>
<td>Moderate</td>
<td>Strong</td>
<td>Moderate</td>
<td>Strong</td>
<td>Moderate</td>
<td>Strong</td>
<td>Cavitated</td>
<td></td>
<td>Rothen et al., 2014</td>
</tr>
<tr>
<td>32</td>
<td>Weak</td>
<td>Moderate</td>
<td>Strong</td>
<td>Moderate</td>
<td>Strong</td>
<td>Strong</td>
<td>Moderate</td>
<td>Cavitated</td>
<td></td>
<td>Ghazal et al., 2015</td>
</tr>
<tr>
<td>33</td>
<td>Moderate</td>
<td>Moderate</td>
<td>Strong</td>
<td>Moderate</td>
<td>Strong</td>
<td>Weak</td>
<td>Moderate</td>
<td>Cavitated</td>
<td></td>
<td>Winter et al., 2015</td>
</tr>
</tbody>
</table>
### 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

<table>
<thead>
<tr>
<th></th>
<th>Regression coefficient</th>
<th>SE</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size</td>
<td>-0.00001</td>
<td>0.0001</td>
<td>-0.0003-0.0001</td>
<td>0.2903</td>
</tr>
<tr>
<td>Follow-up period</td>
<td>0.0008</td>
<td>0.0024</td>
<td>-0.0039-0.0056</td>
<td>0.7268</td>
</tr>
<tr>
<td>Caries diagnosis level</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cavitated</td>
<td>Reference</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-cavitated</td>
<td>-0.1243</td>
<td>0.1569</td>
<td>-0.4318-0.1831</td>
<td>0.4279</td>
</tr>
<tr>
<td>Methodological Quality</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strong</td>
<td>Reference</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>0.0052</td>
<td>0.1555</td>
<td>-0.2995-0.3099</td>
<td>0.9733</td>
</tr>
<tr>
<td>Weak</td>
<td>-0.2533</td>
<td>0.2368</td>
<td>-0.7174-0.2108</td>
<td>0.2847</td>
</tr>
</tbody>
</table>

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