Association between toothbrushing frequency and dental caries and tooth loss in adolescents: a cohort study

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Abstract: This cohort study assessed the association between toothbrushing frequency and the increment of dental caries and tooth loss in a population-based sample of southern Brazilian adolescents, to investigate whether there is any additional benefit in performing a third daily brushing. At baseline, 1,528 12-year-old schoolchildren attending 42 schools were examined for gingivitis and dental caries, and answered a questionnaire. After a mean period of 2.5 years, 801 schoolchildren were re-examined. Dental caries and tooth loss increment were outcomes of the study. The main predictor variable was toothbrushing frequency (≥3 times/day vs. twice/day or ≤1 time/day). Poisson regression models were used to estimate the risk for caries and tooth loss increment. Incidence risk ratios (IRR) and 95% confidence intervals (CI) were estimated. The final model adjusted for sociodemographic, behavioral, and clinical variables showed that brushing twice/day afforded 40% greater risk (IRR = 1.40; 95%CI: 1.02–1.92) for caries increment than ≥3 times/day. Regarding the tooth loss increment, adolescents who brushed their teeth twice/day had a fourfold greater risk (IRR = 3.92; 95%CI: 1.23–12.49) than those who brushed ≥ 3 times/day. Sex, school type, and gingivitis were found to act as effect modifiers, inasmuch as a third daily brushing presented advantages against tooth loss only for girls, public school attendees, and those with ≥ 50% of bleeding sites. This study suggests that adolescents benefit from a third daily toothbrushing. Increasing brushing frequency to 3 times/day may be a suitable strategy to control dental caries and tooth loss among high-risk adolescents.

Keywords: Toothbrushing; Dental Caries; Tooth Loss; Adolescent; Cohort Studies; Risk Assessment.

Introduction

Brushing one's teeth twice a day has been considered a social norm in many countries.[1] The American Dental Association recommends: "For a healthy mouth and smile, the ADA recommends you brush your teeth twice/day with a soft-bristled brush." The Centers for Disease Control and Prevention (CDC) recommend brushing teeth twice a day specifically for preventing dental caries. Comparative international studies on oral hygiene practices in 22 European countries showed that most children

(73%-83%) are used to brushing their teeth twice/day in Sweden, Denmark, Germany, Austria, and Norway. Toothbrushing more than once a day was especially uncommon (26-33%) among boys in Finland, Lithuania, Russia, Estonia, and Latvia.[2]

Most studies in children/adolescents have considered the toothbrushing frequency of twice/day as the cut-off, and found a higher caries risk in individuals who brush their teeth less than twice/day.[3,4] In a systematic review with meta-analysis, Kumar et al.[1] studied the effect of toothbrushing frequency on dental caries incidence and increment. Infrequent brushers demonstrated higher incidence and increment of caries lesions than frequent brushers. Brushing one's teeth <twice/day resulted in a significantly higher increment of caries lesions than ≥twice/day (standardized mean difference = 0.34; 95% confidence interval [95%CI] = 0.18–0.49). Only two studies evaluated a brushing frequency >twice/day, and no difference in caries incidence[5] or increment[6] was found between >twice/day and ≤twice/day.

Tooth cleaning can be a highly effective method of controlling caries development and progression, especially when a fluoride toothpaste is used. Consensus between researchers and local public health authorities consider toothbrushing with fluoride toothpaste as the method of choice to prevent dental caries. The beneficial effect of frequent toothbrushing is related not only to the effect of frequent cleaning (mechanical removal of biofilm), but also to that of frequent application of small amounts of fluoride in the oral environment to increase fluoride effectiveness by maintaining high levels throughout the day. In a systematic review investigating the effect of fluoride toothpaste in preventing dental caries, Marinho et al.[4] included 74 studies, almost all reporting a frequency of once or twice daily. They reported an increase in the prevented fraction of 14% (95%CI:6–22) when changing from once to twice/day. No comparison was performed between 2 and 3 times/day.

A toothbrushing frequency of 3 times/day is the most prevalent in Brazil,[7] where there is a culture of associating toothbrushing with the three main meals of the day. A brushing frequency ≥ 3 times/

day is recommended by the Brazilian Ministry of Health, which advises "brush your teeth after each meal and before bedtime." Although an *in situ* study showed that the use of fluoride toothpaste 3 times/day enhanced demineralization protection compared with twice/day,[8] there is a lack of evidence supporting the Brazilian recommendation. In this respect, it is important to assess whether a third daily toothbrushing offers an additional benefit, since it increases the cost and time spent on oral hygiene, not to mention the demotivating aspect of making the daily task a greater hindrance. Therefore, the aim of this cohort study was to assess the association between toothbrushing frequency and dental caries and tooth loss increment in a population-based sample of southern Brazilian adolescents, over a 2.5-year period. We hypothesized that toothbrushing 3 times/day afforded higher protection against caries and tooth loss increment than twice/day.

Methodology

This cohort study was conducted in southern Brazil. Detailed information on sample size calculation, sampling strategy, and eligibility criteria adopted at baseline can be found elsewhere.[9] In brief, at baseline (2009–2010), a representative sample of the population of 12-year-old schoolchildren from Porto Alegre was drawn using a multistage probability sampling strategy. The primary sampling unit consisted of five geographical areas organized according to the municipal water fluoridation system. The schools within each area were selected randomly, proportional to the number of private/public schools. Schoolchildren born in 1997 or 1998 and regularly attending school were considered eligible for the study. Those presenting special needs or using fixed orthodontic appliances were excluded. A total of 1,528 schoolchildren attending 42 schools (33 public and 9 private) were examined (response rate of 83.2%), far more than the minimum sample size of 1,331 required for the study.

At baseline, a structured questionnaire containing questions on sociodemographic information and behavioral aspects was sent to the parents/legal guardians of the selected students. The clinical

examination was conducted at the schools, with the students in a supine position, under artificial light, using a flat mirror, a periodontal probe, and portable equipment (air compressor and suction). First, a trained examiner recorded the gingival bleeding index[10] at four sites per tooth (buccal, lingual, mesial, and distal). The examiner cleaned and dried the teeth, and then recorded the presence of caries lesions (non-cavitated and cavitated, active and inactive), and both missing and filled surfaces. Clinical examinations comprised all erupted permanent teeth.

Every effort was made at the follow-up (2012–2013) to contact the participants (their parents/legal guardians) examined at baseline, by telephone calls and visits to the schools. A total of 801 schoolchildren were re-examined after a mean period of 2.5 years (standard deviation = 0.3), representing 52.4% of the initially examined sample. Clinical follow-up examinations were performed according to the same protocol. The flowchart of the study, including the reasons for non-participation and the comparison between individuals followed-up and those lost to follow-up, can be found elsewhere.[9]

Reliability

Caries examination was performed at baseline by a single calibrated examiner (LSA). Training sessions included a theoretical explanation on the caries index using clinical photographs and clinical examination of patients. The sessions were performed under the supervision of a benchmark examiner before the study began. The examiner's calibration was checked before the study began, and was monitored over the data collection period by repeated examinations conducted on 5% of the sample after a minimal time interval of 2 days. The overall unweighted Cohen's kappa value was 0.84. Gingivitis was recorded by another examiner, who was trained for the gingival bleeding index, but was not calibrated for this index because of the temporary nature of the condition.

Clinical examination was conducted at follow-up by another examiner (CDB), who was trained and calibrated by the first examiner (LSA), as previously described. The interexaminer unweighted Cohen's kappa value was 0.78. During the survey, calibration

was also monitored by repeated examinations of 5% of the sample (10 double examinations were conducted at every 200 schoolchildren included in the sample). The lowest intraexaminer unweighted Cohen's kappa value was 0.81.

Data analysis

Caries and tooth loss increment were the outcomes of this study, and were modeled as count variables. The caries increment was defined as the difference between follow-up DMFS and baseline DMFS. The DMFS index was calculated as the sum of decayed, missing, or filled surfaces according to the WHO criteria (cavity level). The tooth loss increment was defined as the difference between the number of missing teeth at follow-up and at baseline, and included extracted teeth as well as those indicated for extraction (residual roots).

The main predictor variable was toothbrushing frequency (≥ 3 times/day vs. twice/day or $\leq$ once/day). Other predictors included in the study comprised sociodemographic, behavioral, and clinical variables. Sociodemographic variables were sex (girls vs. boys), socioeconomic status (classified according to the cut-offs proposed by the standard Brazilian economic classification[11] as high, mid-high, mid-low or low), and school type (private vs. public). The other behavioral variable assessed in addition to toothbrushing frequency was soft drink consumption (daily vs. non-daily). This variable was considered an indicator of healthy/unhealthy dietary pattern. The clinical variable was gingivitis (< 50% of bleeding sites *vs.* $\geq 50\%$ to < 75% or $\geq 75\%$), as an indicator of tooth cleaning.[10] All of these adjusting variables were selected based on the previous literature.

Data analysis was performed using STATA (Stata 14.2; Stata Corp., College Station, USA). A weight variable was used in the statistical analysis, considering the inverse probability of participation at follow-up according to sex and socioeconomic status. Preliminary analysis comparing the mean increment of dental caries and tooth loss by categories of predictors was performed using the Wald test. Poisson regression models (unadjusted and adjusted) were used to estimate the risk for caries and tooth loss increment over the study period. Incidence risk ratios

(IRR) and their respective 95%CI were estimated. Two different adjusted models were presented. The first was adjusted for sociodemographic and behavioral variables (sex, socioeconomic status, type of school, and soft drink consumption), and the second included the clinical variable (gingivitis). These variables were included and maintained in the adjusted models, irrespective of their p-values.

The mean increment of dental caries and tooth loss, and the risk assessment analysis comparing the toothbrushing frequencies of ≥3 times/day versus twice/day were calculated after stratifying the sample by predictor variables to investigate whether any of the variables acted as an effect modifier of the association between toothbrushing frequency and study outcomes.

Ethical aspects

The study protocol was approved by the Federal University of Rio Grande do Sul Research Ethics Committee (299/08), and by the Porto Alegre Municipal Health Department Ethics Committee (registration no. 001.049155.08.3/register no. 288 and registration no. 001.028618.12.2/register no. 807). The research was conducted ethically, in accordance with the World Medical Association Declaration of Helsinki. All the participants and their parents/legal guardians provided written informed consent. Participants received a report of their oral health status, and were referred for dental treatment as needed.

Results

The comparison between the followed-up individuals and those lost to follow-up showed a higher proportion of public-school attendees and a lower caries experience among study participants than those lost to follow-up (p < 0.05).[9] Among the 801 followed-up adolescents, 370 were classified as caries-free (DMFT = 0) at baseline, 91 of whom developed caries, thus yielding a caries incidence of 24.5% (95%CI: 20.1–29.0). Regarding tooth loss, an incidence of 3% was detected (95%CI: 1.8–4.2), representing 23 out of 761 adolescents who had no missing teeth at baseline.

The sample distribution and the dental caries and tooth loss increments over 2.5 years by predictor variables are described in Table 1. This followed-up population had a mean DMFT of 1.35 (95%CI: 1.23–1.47) and a mean DMFS of 2.06 (95%CI: 1.83–2.29) at baseline. After a 2.5-year period, an overall average caries increment of 0.97 (95%CI: 0.84–1.11) surfaces was observed. Adolescents who brushed their teeth ≤once/day had a mean caries increment of 1.45 (95%CI: 1.08–1.82) surfaces, which was significantly higher than those who reported a brushing frequency of twice/day (0.95, 95%CI: 0.76-1.15) or ≥3 times/day (0.70, 95%CI: 0.53–0.88). The comparison between these two last categories showed a borderline p-value of 0.054. Regarding tooth loss, an overall average increment of 0.05 (95%CI: 0.03–0.07) tooth was found. The tooth loss increment was significantly lower among adolescents who brushed their teeth ≥ 3 times/day, compared with the other two categories.

Table 2 describes the association between predictor variables and dental caries increment. Toothbrushing twice/day afforded 40% greater risk for caries increment, while toothbrushing ≤ once/day afforded about a twofold greater risk for caries increment than ≥ 3 times/day, in both adjusted models. The inclusion of gingivitis in the second adjusted model had no effect on this association.

The association between predictor variables and tooth loss increment is shown in Table 3. Adolescents who reported brushing their teeth twice/day had a fourfold greater risk for tooth loss increment than those who reported a frequency of ≥ 3 times/day. The lowest toothbrushing frequency of ≤once/day resulted in a 7-fold greater risk for tooth loss increment, compared with the highest frequency of ≥ 3 times/day, even after the inclusion of gingivitis in the second adjusted model.

Table 4 presents the tooth loss increment and its association with toothbrushing frequency (twice/day vs. ≥ 3 times/day), stratified by sex, school type, and gingivitis, for the purpose of assessing effect modification. Sex, school type, and gingivitis were found to modify the effect of toothbrushing frequency on tooth loss estimates. A significantly greater risk for tooth loss increment was found

Table 1. Sample distribution, and dental caries and tooth loss increments over 2.5 years among southern Brazilian adolescents. Mean (95% confidence interval).

Variable	n (%)	Dental caries	Tooth loss
Sociodemographic			
Sex			
Girls	387 (48.3)	1.05 (0.85–1.25)[a]	0.06 (0.03–0.09)[a]
Boys	414 (51.7)	0.90 (0.73–1.07)[a]	0.04 (0.02–0.07)[a]
Socioeconomic status			
High/mid-high	258 (32.2)	0.71 (0.51–0.90)[a]	0.03 (0.01–0.05)[a]
Mid-low	461 (57.6)	1.06 (0.88–1.24)[b]	0.06 (0.03–0.08)[a]
Low	82 (10.2)	1.36 (0.88–1.84)[b]	0.10 (0.02–0.18)[a]
School type			
Private	117 (14.6)	0.51 (0.31–0.71)[a]	0.01 (–0.01–0.02)[a]
Public	684 (85.4)	1.05 (0.90–1.20)[b]	0.06 (0.04–0.08)[b]
Behavioral			
Toothbrushing frequency			
≥ 3 times/day	275 (34.3)	0.70 (0.53–0.88)[a]	0.01 (0.01–0.03)[a]
Twice/day	349 (43.6)	0.95 (0.76–1.15)[a]	0.05 (0.02–0.08)[b]
≤ Once/day	177 (22.1)	1.45 (1.08–1.82)[b]	0.11 (0.05–0.16)[b]
Soft drink consumption			
Daily	227 (28.3)	0.94 (0.71–1.17)[a]	0.03 (0.01–0.06)[a]
Non-daily	574 (71.7)	0.99 (0.83–1.15)[a]	0.06 (0.03–0.08)[a]
Clinical			
Gingivitis*			
<50%	341 (42.7)	0.80 (0.63–0.97)[a]	0.03 (0.01–0.05)[a]
≥ 50% and < 75%	388 (48.6)	1.05 (0.85–1.25)[ab]	0.06 (0.03–0.09)[a]
≥ 75%	69 (8.7)	1.47 (0.90–2.04)[b]	0.09 (0.01–0.16)[a]
Total	801 (100)	0.97 (0.84–1.11)	0.05 (0.03–0.07)

*Figures do not total 801 due to missing data. Different letters indicate statistically significant differences between categories ($p < 0.05$, adjusted Wald test).

among girls and adolescents with ≥ 50% of bleeding sites, who brushed their teeth twice/day than those who brushed ≥3 times/day, whereas no association was found among boys or adolescents with < 50% of bleeding sites. Regarding school type, a significantly higher risk for tooth loss increment was found among public school attendees who brushed their teeth twice/day than those who brushed ≥3 times/ day. No case of tooth loss was found among private school attendees who reported a toothbrushing frequency of ≥twice/day, a datum that enabled this comparison to be made. No effect modification was detected for caries increment.

The study power was calculated *a posteriori* for both outcomes comparing exposed (toothbrushing twice/day) and non-exposed (toothbrushing ≥ 3 times/day) individuals, using 95%CI. Estimates of 48% and 100% study power were observed for caries and tooth loss increments, respectively.

Discussion

This study was carried out to investigate whether toothbrushing a third time daily would provide benefits for dental caries and tooth loss in a population-based sample of adolescents from southern Brazil,

Table 2. Association between predictor variables and dental caries increment over 2.5 years among southern Brazilian adolescents.

Variable	Unadjusted		Adjusted[a]		Adjusted[b]	
	IRR (95%CI)	p-value	IRR (95%CI)	p-value	IRR (95%CI)	p-value
Sociodemographic						
Sex						
Girls	1.00		1.00		1.00	
Boys	0.86 (0.66–1.13)	0.27	0.78 (0.60–1.01)	0.06	0.76 (0.61–1.04)	0.09
Socioeconomic status						
High/mid-high	1.00		1.00		1.00	
Mid-low	1.49 (1.08–2.06)	0.01	1.19 (0.84–1.67)	0.32	1.16 (0.82–1.64)	0.39
Low	1.92 (1.22–3.01)	0.005	1.45 (0.92–2.28)	0.11	1.42 (0.90–2.24)	0.13
School type						
Private	1.00		1.00		1.00	
Public	2.06 (1.36–3.12)	0.001	1.65 (1.04–2.60)	0.03	1.59 (1.01–2.53)	0.049
Behavioral						
Toothbrushing frequency						
≥ 3 times/day	1.00		1.00		1.00	
Twice/day	1.36 (0.99–1.87)	0.06	1.41 (1.03–1.93)	0.03	1.40 (1.02–1.92)	0.04
≤ Once/day	2.07 (1.45–2.94)	< 0.001	2.01 (1.41–2.86)	< 0.001	1.96 (1.38–2.77)	< 0.001
Soft drink consumption						
Daily	1.00		1.00		1.00	
Non-daily	1.05 (0.78–1.40)	0.74	1.05 (0.79–1.39)	0.75	1.05 (0.79–1.38)	0.76
Clinical						
Gingivitis						
< 50%	1.00		–	–	1.00	
≥ 50% and < 75%	1.31 (0.98–1.74)	0.07	–	–	1.12 (0.84–1.49)	0.43
≥ 75%	1.83 (1.18–2.85)	0.007	–	–	1.49 (0.96–2.30)	0.08

IRR: Incidence risk ratio obtained with Poisson regression models; CI: Confidence interval. [a] Estimates are adjusted for sociodemographic and behavioral variables; [b] Estimates are adjusted for sociodemographic, behavioral and clinical variables.

over a period of 2.5 years. Our hypothesis failed to be rejected, since adolescents who brushed their teeth at least 3 times/day were less likely to present an increment in dental caries or tooth loss than those used to brushing their teeth twice/day. To the best our knowledge, no previous cohort study has investigated the effect of toothbrushing 3 times/day on tooth loss increment among adolescents.

The main finding of the present study was that toothbrushing twice/day afforded greater risk for caries and tooth loss increment than ≥3 times/day. This is in disagreement with the systematic review on toothbrushing frequency and dental

caries by Kumar et al., who found no difference between caries increment estimates of >twice/day and ≤twice/day by toothbrushers.[1] Nevertheless, the authors stressed that this estimate came from only one study (Dummer et al.[6]), and should be considered with caution. Their study included a similar sample in terms of size and age group (798 adolescents followed from 11–12 to 15–16 years); however, the toothbrushing frequency was categorized on a weekly basis, and the category of > 14 times/week was considered a >twice/day frequency. This frequency can explain, at least in part, why there was no difference between the

Table 3. Association between predictor variables and tooth loss increment over 2.5 years among southern Brazilian adolescents.

Variable	Unadjusted		Adjusted[a]		Adjusted[b]	
	IRR (95%CI)	p-value	IRR (95%CI)	p-value	IRR (95%CI)	p-value
Sociodemographic						
Sex						
Girls	1.00		1.00		1.00	
Boys	0.74 (0.35–1.55)	0.43	0.58 (0.28–1.19)	0.14	0.60 (0.29–1.25)	0.17
Socioeconomic status						
High/mid–high	1.00		1.00		1.00	
Mid–low	2.06 (0.85–4.99)	0.11	1.15 (0.48–2.78)	0.76	1.12 (0.45–2.80)	0.80
Low	3.52 (1.17–10.60)	0.03	1.74 (0.60–5.02)	0.30	1.69 (0.58–4.94)	0.34
School type						
Private	1.00		1.00		1.00	
Public	7.11 (0.97–52.02)	0.06	4.71 (0.62–35.72)	0.13	4.44 (0.58–34.24)	0.15
Behavioral						
Toothbrushing frequency						
≥ 3 times/day	1.00		1.00		1.00	
Twice/day	3.64 (1.17–11.38)	0.03	3.96 (1.24–12.67)	0.02	3.92 (1.23–12.49)	0.02
≤ Once/day	7.53 (2.48–11.89)	< 0.001	7.53 (2.40–23.66)	0.001	7.09 (2.24–22.49)	0.001
Soft drink consumption						
Daily	1.00		1.00		1.00	
Non–daily	1.61 (0.72–3.62)	0.25	1.63 (0.75–3.54)	0.22	1.61 (0.73–3.52)	0.23
Clinical						
Gingivitis						
< 50%	1.00				1.00	
≥ 50% and < 75%	1.91 (0.82–4.47)	0.13			1.36 (0.54–3.41)	0.51
≥ 75%	2.72 (0.88–8.37)	0.08			1.79 (0.58–5.56)	0.31

IRR = Incidence risk ratio obtained with Poisson regression models; CI: Confidence interval. [a] Estimates are adjusted for sociodemographic and behavioral variables; [b] Estimates are adjusted for sociodemographic, behavioral and clinical variables.

groups (standardized mean difference = -0.12; 95%CI: -0.38 to 0.15; p = 0.39).[6] In the present study, the brushing frequency was categorized as ≥3 times/day, which would result in a frequency of > 21 times/week, clearly indicating a greater daily frequency of fluoride application than that observed by Dummer et al.[6] The other study addressing a > 2 times/day brushing frequency was conducted by Tagliaferro et al.[5] Although toothbrushing frequency was associated significantly with a high caries level at baseline, no association was found with the study outcome (high caries increment over a 7-year follow-up period, defined as a DMFS increment ≥

4). The variables that remained in the final model were only baseline dmfs and mother's educational level. The definition of a binary instead of count outcome, the cut-off used to define the outcome (≥ 4 surfaces), and the reduced sample size (n=206) may have compromised the statistical power of the study, hence explaining the disagreement between their findings and ours.

Reducing the toothbrushing frequency from 3 to 2 times/day increased the risk of caries and tooth loss increment in this sample of Brazilian adolescents by 40% and fourfold, respectively. Notwithstanding, an interesting finding of our study was that the

Table 4. Tooth loss increment and its association with toothbrushing frequency stratified by sex, school type, and gingivitis.

Variable	Increment	Unadjusted		Adjusted[a]		Adjusted[b]	
	Mean (95%CI)	IRR (95%CI)	p-value	IRR (95%CI)	p-value	IRR (95%CI)	p-value
Sex							
Female							
≥ 3 times/day	0.02 (−0.01–0.04)[a]	1.00		1.00		1.00	
Twice/day	0.07 (0.02–0.13)[a]	4.23 (1.08–16.6)	0.04	4.34 (1.09–17.29)	0.04	4.38 (1.10–17.3)	0.04
Male							
≥ 3 times/day	0.01 (−0.01–0.03)[a]	1.00		1.00		1.00	
Twice /day	0.03 (0.01–0.06)[a]	3.51 (0.43–28.89)	0.24	3.52 (0.43–28.66)	0.24	3.26 (0.41–26.04)	0.27
School type							
Private							
≥ 3 times/day	0						
Twice /day	0						
Public							
≥ 3 times/day	0.02 (0.01–0.03)[a]	1.00		1.00		1.00	
Twice /day	0.06 (0.03–0.10)[b]	3.65 (1.17–11.37)	0.03	3.99 (1.24–12.78)	0.02	3.97 (1.24–12.67)	0.02
Gingivitis							
< 50% bleeding sites							
≥ 3 times/day	0.01 (−0.01–0.3)[a]	1.00		1.00			
Twice /day	0.02 (−0.01–0.04)[a]	1.38 (0.23–8.15)	0.72	1.53 (0.26–8.99)	0.64		
≥ 50% bleeding sites							
≥ 3 times/day	0.01 (−0.01–0.03)[a]	1.00		1.00			
Twice /day	0.08 (0.02–0.13)[b]	5.46 (1.18–25.25)	0.03	6.00 (1.22–29.48)	0.03		

IRR: Incidence risk ratio obtained with Poisson regression models; CI: Confidence interval. Different letters indicate statistically significant differences between categories (p < 0.05, adjusted Wald test).[a] Estimates are adjusted for sociodemographic and behavioral variables; [b]Estimates are adjusted for sociodemographic, behavioral and clinical variables.

benefit provided by a third daily brushing was not observed in the whole sample. Sex, school type, and gingivitis modified the effect of toothbrushing on the tooth loss increment. A greater risk of tooth loss increment among adolescents who brushed their teeth twice/day was observed only in girls, public school attendees, and those with ≥50% of bleeding sites. All of these findings are conceivable, since these subgroups may be considered at a greater risk for caries than their counterparts. The relationship between sex and caries/tooth loss during childhood and adolescence may be explained by the difference in tooth eruption time, inasmuch as girls have earlier tooth eruption than boys,[12] resulting in a longer time at risk for caries development and progression. In addition to this biological aspect,

there is some evidence in the literature that women have a higher DMFT index than men, because they visit the dentist more often,[13,14] but the effect of this aspect on the present study is uncertain due to the age group under investigation.

The type of school has been consistently used as a proxy indicator of socioeconomic status,[15,16] in which public school attendees are more likely to have caries than their private school attendee counterparts. Maltz & Silva have previously shown that parents of Brazilian public school attendees tend to have lower socioeconomic and educational levels than parents of private school attendees.[17] The authors argued that the most likely composition of parental education and income variables in Brazilian public and private education networks,

associated with other variables, such as lifestyle, may make this variable more sensitive to capture the effect of socioeconomic condition on oral health outcomes than the variable "socioeconomic status" itself.[17] The feasibility of using type of school as an alternative indicator for socioeconomic status was also demonstrated by Piovesan et al.[18] Lastly, gingivitis was used as an indicator of an oral hygiene pattern targeting biofilm control. The association between a greater proportion of bleeding sites and greater risk of caries and tooth loss has been previously reported in the literature.[13] In general, these results may support a recommendation of higher toothbrushing frequency for patients considered as having a "high caries risk."

The benefit of a third daily brushing observed in the present study does not seem to be related to mechanical cleaning or the presence of biofilm, since the adjustment for gingivitis had no effect on the models. This corroborates the literature review by Bellini et al., conducted in the early 80's, prior to the widespread use of fluoride toothpaste, by way of which the effect of brushing frequency could be studied without the interference of fluoride.[19] The authors found no relationship between brushing frequency and dental caries. There is no evidence that an increase in brushing frequency is associated with an improvement in the quality of biofilm control. In fact, brushing once a day should be sufficient for caries control, since dental biofilm must be 2 days old to produce sufficient acid levels to cause enamel demineralization.[20] However, cleaning effectiveness with a toothbrush is not high, and dental caries is a multifactorial disease, with numerous determinants that influence the disease, such as sugar consumption, salivary flow rate, saliva buffer capacity, and others. Oral hygiene performed with fluoride toothpaste (FT) combines mechanical cleaning and topical fluoride application. Twice-a-day brushing has been suggested as a measure to increase the frequency of fluoride availability in the oral environment. In an *in situ* study, Dijkman et al. evaluated two oral hygiene protocols, i.e. twice-daily brushing with FT (1250 ppm F) and twice-daily brushing with non-FT, compared with a non-brushing control group.[21] The authors demonstrated that brushing twice/day

with non-FT resulted in a 50% reduction in mineral loss, compared to the non-brushing control group (cleaning effect only), inasmuch as this protection increased to 90% when a FT was used (combination of two factors, a cleaning effect and a fluoride effect). In another *in situ* study, Souza et al. observed that the use of FT 3 times/day enhanced demineralization protection, compared to twice/day. This result showed the added benefit of a third daily fluoride application.[8] The decrease in the caries and tooth loss increment observed in our study with a brushing frequency of ≥3 times/day, compared with twice/day, is likely due to a higher frequency of topical fluoride application.

Among the strengths of this study, we can highlight its longitudinal design with a 2.5-year follow-up period, and high intra- and interexaminer reproducibility. In addition, its pioneering aspect must also be acknowledged, since this was the first cohort study to assess the tooth loss increment among adolescents, and also the first to investigate the toothbrushing frequency of 3 times/day. Of the original sample of 1,528 adolescents, 801 (52.4%) were re-examined after 2.5 years, at which time the main reason for losses was moving to another school, which is a common finding at an age group that commonly transitions from elementary to high school. Although a weight variable was used in an attempt to mitigate the impact of a non-response in the study findings, the possibility that we lost the representativeness of the original sample cannot be ruled out, and must be addressed as a possible limitation of the study. It should also be recognized that the present sample did not attain sufficient statistical power to investigate the association between toothbrushing 2 or ≥3 times/day and the caries increment. However, even though our study was considered underpowered for this outcome, it did find a significantly greater risk for caries increment among twice/day than ≥3 times/day toothbrushers in the adjusted models, despite the weak magnitude of the association (about 40%).

Conclusion

The results of this population-based cohort study suggest that Brazilian adolescents benefit from a

third daily toothbrushing in regard to dental caries and tooth loss. Those considered as having "a high caries risk" (girls, public school attendees, and those with ≥ 50% of bleeding sites) were more likely to benefit from a third daily brushing in regard to tooth loss. Therefore, increasing the toothbrushing frequency to 3 times/day may be a suitable strategy to control dental caries and tooth loss among high-risk adolescents.

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