Blood levels of lead and dental caries in permanent teeth

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Abstract

Objectives: The purpose of this study was to determine if there is an association between lead exposure within the ages of one to four years and dental caries in the permanent dentition between ages 9-17 among Mexican youth.

Methods: Data were collected for the Early Life Exposures in Mexico to Environmental Toxicants (ELEMENT) cohort from a group of 490 children born and reared in Mexico City. Among ages one to four years, blood lead levels were measured in micrograms of lead per deciliter of blood (μg/dL) and the presence of caries in adolescence was determined using the International Caries and Detection and Assessment System (ICDAS). The relationship between blood levels of lead and decayed, missing, or filled surfaces (DMFS) was examined using negative binomial regression. Covariates were selected based on previous studies and included age, gender, socioeconomic status, oral hygiene, BMI, and diet. The nonlinear relationship between lead and DMFS was examined using smoothing splines.

Results: The mean overall blood lead level (BLL) was 4.83 μ g/dL (S.D of 2.2). The mean overall caries level (DMFS) was 4.1. No statistically significant association was found between early childhood blood lead levels and dental caries in adolescence.

Conclusion: This study shows a lack of association between exposure to lead between the ages of one to four years of age and dental caries in permanent dentition later in life. Other covariates, such as age and sugar consumption, appeared to play a more prominent role in caries development.

Keywords:

*Dental caries, blood lead levels, risk factors

Introduction

It has been well established that high levels of lead exposure can have an impact on overall health. There are several sources of lead exposure, including *in utero* from lead in maternal blood.¹ Many household and everyday items are known to contain lead, as well as many aspects of a person's environment, such as food and jewelry. Within Mexican populations, lead-glazed pottery is a major source of lead exposure.² There are three main sources of lead contamination seen today: gasoline in certain countries, solder used to seal food and beverage cans, and household paint.³ However, the most common way lead enters the body is from non-nutritive hand-to-mouth activity during childhood.³

Because of the varying effects lead can produce, it is important to measure the amount of lead in a person's body, particularly within communities where lead exposure is more common. The standard for measuring the health effects that lead can cause was defined by The Centers for Disease Control and Prevention (CDC). The CDC has estimated 5 μ g/dL as the reference value "based on 97.5th percentile of population blood lead level (BLL) in children aged 1-5 years to identify children living or staying for long periods in environments that expose them to lead hazards." However, no safe level of lead exposure has been identified by the CDC. 3.5

There is conflicting evidence reporting the effects lead may have on one's teeth. In 1994, Gil et al. conducted a study using 220 extracted teeth from a population in Spain which determined no direct correlation with dental caries.⁶ This study was expanded in 1996, and researchers found a positive relationship with lead and dental caries, dental plaque, *Salivalis*

lactobacilli levels, and Streptococcus mutans levels.⁷ In 2016, Shishniashvili et al. saw a negative association between dental caries and communities with environmentally favorable conditions without lead.⁸ A positive correlation between lead exposure and dental caries was also found in 2002 by Gemmel et al.⁹ In 2015, Wiener et al. conducted a secondary data analysis of de-identified data collected from children aged 24-72 months old and a strong link was found between high blood lead levels and caries.⁴ In addition, Moss et al. performed a cross-sectional study in which he found a significant positive correlation with blood lead levels and the DMF caries rate in deciduous and permanent teeth.¹⁰ However, in 2004, Gomes et al. also found no link between lead concentrations and DMF count of 329 preschool children.¹¹ Both Youravong et al. in 2006 and Cenić-Milosević et al. in 2013 found positive associations with lead exposure and high DMF counts.^{12,13}

There are several hypotheses as to how lead may affect dental caries. One proposed mechanism for the correlation between lead and caries is the replacement of calcium by lead in dental hydroxyapatite during tooth formation. Another suggested reason for the relationship was an interference in the process of amelogenesis by lead. Wiener's study also discussed lead's role in decreasing the bioavailability of fluoride, which in turn would decrease fluoride's remineralization's ability, after tooth eruption. It has also been suggested lead exposure disrupts odontoblast development and function. Enamel and dentin may serve as reservoirs for heavy metal ions within the body, while bone does not store heavy metal ions. This is believed to be due to the decreased amount of metabolism of enamel and dentin when

compared to bone. A study conducted by Costa de Almeida et al. in 2007 showed a strong correlation between high levels of lead exposure and accumulation of lead in enamel. Cleymaet et al. performed a study in which they discussed the use of surface enamel as an indicator of lead exposure. Youravong et al. conducted a study involving two groups of children: one with low blood levels of lead and the other with high blood levels of lead. Using secondary ion mass spectrometry, they found children with high blood levels of lead had evidence of lead within dentin. One with light blood levels of lead had evidence of lead within dentin.

The purpose of this study was to determine if there is an association between exposure to blood lead levels early in life when permanent teeth are developing (between the ages of one and four years), and the development of dental caries in the permanent dentition within a population of Mexican children ages nine to seventeen years.

Methods

This was a longitudinal birth cohort study of measurements of lead taken in the blood of children every six months from ages 1-4 and the association with measures of dental caries, as categorized by the International Caries Detection and Assessment System (ICDAS), in permanent dentition in participants 9-17 years old. The collection of the primary data was approved by the IRB's of the National Institute of Public Health-Mexico and the Schools of Public Health at Harvard University, the University of Michigan, and the University of Toronto. Secondary approval was obtained by the Indiana University Institutional Review Board (#1706774130) for this analysis. Data collected for the Early Life Exposures in Mexico to

Environmental Toxicants (ELEMENT) cohort from a group of 490 children in Mexico City was statistically analyzed to determine any association between dental caries, exposure to lead, and many co-variates. Blood measurements obtained from complete years (12 months, 24 months, etc.) were venous blood, whereas blood measurements obtained from the halves of years (18 months, 30 months, etc.) were capillary blood. Analysis has been conducted regarding this population's prenatal exposure to lead and will be reported in another publication. ¹¹ ICDAS development and approach to caries detection and assessment has been explained in detail elsewhere. ²¹ Each carious lesion was classified based on depth of the lesion within the tooth. There are seven ICDAS codes, which correlate to the extent of decay, ranging from sound dentition to large cavitated lesions (more than 50% of surface) with visible dentin. ICDAS includes codes for both cavitated and non-cavitated lesions. Once all of the variables are accounted for, an overall score can be provided for the carious lesion, leading to a caries risk assessment. ²²

The covariates measured included age, sex, BMI (Body Mass Index) category, SES level, sugar intake (g/day), water intake (ml/day), amount of beverages with sugar per day (ml/day), amount of toothpaste child used regularly from birth to 2 years of age, amount of toothpaste child used regularly from 2-4 years of age, amount of toothpaste child used regularly from 4-6 years of age, and amount of toothpaste child currently uses regularly. Socioeconomic status (SES) was calculated using metrics from the Mexican Association of Marketing Intelligence. This model divides the

Mexican population in seven groups based on six determinants: human capital, infrastructure, connectivity, sanitation, planning, and housing. BMI was categorized as underweight (< 18.5), normal weight (18.5-24.9), overweight (25.0-29.9), and obese (≥30.0). The decayed, missing, or filled surfaces (DMFS) scores were calculated from the ICDAS data with several cutoffs for defining decay. Blood lead levels are categorized as greater than 5 and less than 5 and the number visits with blood lead levels greater than 5 was included. Mean and maximum lead levels across all visits were also calculated. The relationship of blood levels of lead with DMFS was examined using negative binomial regression, with other caries risk factors included as covariates. Two additional types of analyses were explored but not presented in the results. Zero inflated negative binomial models were examined but found to have similar results to the standard negative binomial, so those results are not shown. Smoothing splines were used to explore possible nonlinear relationships between lead and DMFS, but nonlinearity was not found.

Results

A total of 490 children from Mexico City, 50% which were male, participated in this study. Of this population, 56% were considered to have normal weight, or a body mass index (BMI) of 18.5-24.99. The overall mean BLL was found to be 4.83 µg/dL (S.D of 2.2) and the geometric mean was found to be 4.36 (S.D. 2.01) µg/dL. The overall DMFS mean was found to

be 4.1 (S.D. 5.1, maximum 7.46 and minimum 2.07) Please refer to Table 1 for full descriptive results.

Mothers or caregivers reported oral hygiene habits of their children using a survey.

Analysis of oral hygiene habits within this population revealed 37% of participants reported receiving professional fluoride. Caregivers reported they began to brush their children's teeth before the age of two 73% of the time and 77% of the caregivers stated teeth were brushed two or more times a day. Please refer to Table 2 to view the complete list of oral hygiene variables.

When considering the mean and maximum blood lead level, no statistically significant association was found with DMFS (Tables 3 and 4, respectively). Also, no association was found between the number of visits with BLL > $5\mu g/dL$ and DMFS (data not shown). Statistically significant positive associations were found between DMFS and increasing age, as well as grams of sugar consumed per day. Similar results were found when using the maximum BLL, however D2MFS was significantly correlated with maximum lead levels (as this was the only significant association, it may be a spurious result). When considering models using mean BLL, an inverse relationship with the amount of water intake (ml/day) and D2MFS was found to be statistically significant. A statistically significant inverse correlation was also determined between the amount of water intake (ml/day) and D1MFS and D2MFS in the models using mean BLL. In models using either the mean and maximum BLL, subjects with normal BMI had significantly lower D3MFS than underweight subjects, but no associations were found for any other BMI range for any other DMFS definition.

Discussion

The purpose of this study was to analyze data provided from a longitudinal cohort study of children born and living in Mexico City to determine if levels of lead exposure during the ages of dental development have any consequence on the caries susceptibility of permanent dentition. No statistically significant association was found to relate early lead exposure to dental caries susceptibility, however other sociodemographic and lifestyle factors were associated with caries in this cohort. These results suggest that lead may not be a major factor in dental caries in the multifactorial disease of dental caries.

Our results can be compared to those studies that have assessed lead exposure and dental caries using the DMFS [decayed (D), missing (M), and filled (F) surfaces (S)] count to evaluate level of caries. Results from researchers who used DMFS can be compared to results from researchers who used the ICDAS system because the data categories in the ICDAS can be combined to DMFS categories. In our study, there was no statistically significant association found between the mean or maximum blood lead level of participants between the ages of 1-4 years and dental caries of permanent dentition between the ages of 9-17 years. These results are consistent with existing literature providing lack of evidence of a relationship between dental caries and lead exposure, including a retrospective cohort study conducted in 2000 by Campbell et al. who found no relationship between lead exposure of toddlers and future DMF counts.²³

According to McDonald and Avery's *Dentistry for the Child and Adolescent*, calcification of teeth is the "precipitation of inorganic calcium salts within the deposited matrix"

of the developing dental hard tissues.²⁴ Casamassimo et al. stated hard tissue calcification of the permanent teeth begins at birth with the first molar. Calcification of the remaining dentition begins prior to the age of three and the development of enamel of permanent teeth (excluding third molars) is not complete until about the age of eight.²⁵ Our knowledge of the timing of developing permanent dentition and the results from this analysis can be used to help determine that lead exposure of our participants from ages 1-4 did not appear to have a detrimental effect on the calcification of the developing permanent teeth causing increased caries susceptibility.

The positive significant association found between age and dental caries is consistent with existing literature. According to the NIH statement made in 2001, the "most consistent predictor of caries risk in children is past caries experience." Both Hall-Scullin et al. and Li et al. conducted studies which verify the increased risk of caries in permanent dentition of a child who had caries in primary dentition. This population also shows a correlation between increasing age and dental caries. The consistency between this study and existing literature demonstrates the validity of the data analyzed and the determined results.

It is well known sugar plays an integral part in the development of dental caries. One of the most well-known studies to exemplify this relationship was the Vipeholm study conducted from 1945-1952 which showed the importance of sugar in the development of caries in a population of institutionalized adults in Sweden. ²⁹ Caries develops when cariogenic bacteria break down simple sugars to create an acidic environment, which promotes demineralization of the dental enamel. Within this study, dental caries was found to be significantly associated with

the number of grams of sugar each participant consumed per day. Just as with the association of age with dental caries, the significant not only shows sugar consumption played a part in the development of caries within this population, but also validates the results obtained.

Another aspect of diet which can contribute to differing caries rates are beverages. No statistically significant relationship was found between dental caries and beverages with or without sugar. However, a statistically significant inverse relationship was found between the amount of water intake as milliliters per day and D1MFS and D2MFS. Water plays a very important role in reducing the amount of caries production, as it can act to rinse away simple sugars and food debris which may accumulate in the mouth and contribute to caries development. Water is not fluoridated in Mexico, but according to Martinez-Mier et al., tap water and community water in Mexico City contain varying amounts of natural fluoride. The results from our study are consistent with existing literature showing drinking water may have a caries reducing effect.

Limitations regarding this study include the lack of a control group with zero exposure to lead. Lead exposure is very common in Mexico City and therefore the participants within the population selected all had variations of lead within their blood. Therefore, this analysis aimed to determine if differing levels of lead aided in caries development. A follow-up study from this would be to utilize the population provided and compare to a population with no history of lead exposure. Another follow-up study to be considered would be to observe the effect continuous and present exposure to lead has on caries susceptibility.

This study provided no evidence of association between exposure to lead during ages of dental development and caries in the permanent dentition. Other confounding variables, such as increasing age and sugar consumption, were found to be more statistically associated with the development of caries in the permanent dentition of this population.

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Table 1: Descriptive Statistics. n (%) or Mean (SD)

Variable	Mean / Frequency
Sex, n (%)	
Male	245 (50%)
Female	245 (50%)
BMI, n (%)	
Underweight (< 18.5)	127 (26%)
Normal (18.5-24.9)	276 (56%)
Overweight (25.0-29.9)	73 (15%)
Obese (≥ 30.0)	18 (4%)
Age, years	14.3 (2.1)
Age, years, N (%)	
10	11 (2%)
11	77 (16%)
12	72 (15%)
13	79 (16%)
14	44 (9%)
15	55 (11%)

D5MFS	2.07 (3.7)

^{*:} D#MFS represents DMFS counts using all ICDAS scores ≥ # as Decay.

^{*} BLL: Blood Lead Level * BMI: Body Mass Index * SES: Socioeconomic Status

Table 2: Oral Hygiene Variables:

VARIABLE	n (%)
Did the child receive any fluoride from a dentist before the age of 6?	
Yes, frequently (4 or more times)	38 (10%)
Yes, occasionally (1-3 times)	63 (16%)
No	282 (72%)
Missing	161
What age did you start to brush your child's teeth?	
< 2yrs	403 (73%)
2-4 yrs	127 (23%)
4-6 yrs	13 (2%)
How often do you usually brush your child's teeth after age 2?	
Twice a day or more	422 (77%)
Once a day	122 (22%)
From birth to 2 years, the amount of paste your child used	
regularly.	
Thick band that covers the brush	-

Thin band that covers the brush	-
Covers ¾ of the brush	7 (1.28%)
Covers ½ of the brush	28 (5.10%)
Covers ¼ of the brush	24 (4.37%)
Covers less than ¼ of the brush	51 (9.29%)
Very small amount of place on the brush	52 (9.47%)
Didn't use toothpaste	387 (70.49%)
From 2-4 years, the amount of paste your child used regularly	
Thick band that covers the brush	-
Thin band that covers the brush	12 (2.18%)
Covers ¾ of the brush	50 (9.09%)
Covers ½ of the brush	129 (23.45%)
Covers ¼ of the brush	103 (18.73%)
Covers less than ¼ of the brush	92 (16.73%)
Very small amount of place on the brush	52 (9.45%)
Didn't use toothpaste	111 (20.18%)
From 4-6 years, the amount of paste your child used regularly	
Thick band that covers the brush	12 (2.18%)
Thin band that covers the brush	78 (14.18%)
Covers ¾ of the brush	155 (28.18%)
Covers ½ of the brush	153 (27.82%)

Covers ¼ of the brush	81 (14.73%)
Covers less than ¼ of the brush	57 (10.36%)
Very small amount of place on the brush	12 (2.18%)
Currently, the amount of paste your child uses regularly	
Thick band that covers the brush	134 (24.36%)
Thin band that covers the brush	278 (50.55%)
Covers ¾ of the brush	102 (18.55%)
Covers ½ of the brush	24 (4.36%)
Covers ¼ of the brush	11 (2%)
Covers less than ¼ of the brush	1 (0.18%)

Table 3: Negative Binomial Regression for association of Mean BLL with DMFS.*

Parameter estimate (95% CI) [p-value].

Parameter	D1MFS [@] (95%	D2MFS	D3MFS	D4MFS	D5MFS
	CI) [p-value]				
Mean BLL	0.0295 (0318,	0.0151 (0520,	0096 (0957,	0508 (1507,	0550 (1572,
	0.0908)	0.0821)	0.0764)	0.0490)	0.0473)
	[0.3455]	[0.6593]	[0.8265]	[0.3181]	[0.2920]
Age			0.1182	0.1192	
	0.0743 (0.0118,	0.0913 (0.0223,	(0.0313,	(0.0164,	0.1270 (0.0205,
	0.1367)	0.1603)	0.2050)	0.2219)	0.2335)
	[0.0198]	[0.0095]	[0.0076]	[0.0230]	[0.0194]
BMI-	2409 (5315,	2749 (5976,	5052 (9182,	4254 (9226,	3748 (9003,
normal	0.0497)	0.0478)	0923)	0.0718)	0.1506)
	[0.1042]	[0.0949]	[0.0165]	[0.0935]	[0.1621]

Parameter	D1MFS [@] (95%	D2MFS	D3MFS	D4MFS	D5MFS
	CI) [p-value]				
BMI-	0.0912 (3108,	0.1982 (2493,	0.0300 (-	0.2779 (-	0.3395 (3787,
overweight	0.4931)	0.6456)	.5425, 0.6025)	.4104, 0.9662)	1.0577)
	[0.6566]	[0.3853]	[0.9182]	[0.4287]	[0.3542]
BMI-obese	0381 (6726,	1180 (8307,	5679 (-1.485,	9266 (-2.056,	9017 (-2.085,
	0.5964)	0.5947)	0.3493)	0.2026)	0.2814)
	[0.9063]	[0.7456]	[0.2249]	[0.1078]	[0.1352]
Sugar			0.0048	0.0064	
intake	0.0014 (0011,	0.0022 (0006,	(0.0014,	(0.0024,	0.0067 (0.0024,
(g/day)	0.0039)	0.0050)	0.0082)	0.0105)	0.0111)
	[0.2792]	[0.1190]	[0.0056]	[0.0020]	[0.0023]
Water	0002 (0004,	0002 (0005,	0001 (0004,	0002 (0006,	0002 (0006,
intake	0.0000)	0.0000)	0.0002)	0.0001)	0.0002)
(ml/day)	[0.0224]	[0.0463]	[0.3855]	[0.1931]	[0.3569]

^{*:} Only covariates with a statistically significant association with DMFS were included in the table. The complete list of covariates included: age, sex, BMI category, SES level, sugar intake (g/day), water intake (ml/day), amount of beverages with sugar per day, amount of beverages

without sugar per day, amount of toothpaste child used regularly from birth to 2 years of age, amount of toothpaste child used regularly from 2-4 years of age, amount of toothpaste child used regularly from 4-6 years of age, amount of toothpaste child currently uses regularly.

(a): D#MFS represents DMFS counts using all ICDAS scores $\geq \#$ as Decay.

BMI: Body Mass Index BLL: Blood Lead Level

Table 4: Negative Binomial Regression for association of Maximum BLL with DMFS.*

Parameter estimate (95% CI) [p-value].

Parameter	D1MFS [@]	D2MFS	D3MFS	D4MFS	D5MFS
	(95% CI) [p-				
	value]				
Max BLL	0.0131 (0146,	0.0073 (0232,	0050 (0455,	0148 (0652,	0160 (0683,
	0.0408)	0.0379)	0.0355)	0.0356)	0.0363)
	[0.3546]	[0.6370]	[0.8097]	[0.5649]	[0.5492]
Age	0.0743			0.1133	
	(0.0117,	0.0908 (0.0216,	0.1185 (0.0316,	(0.0099,	0.1207 (0.0133,
	0.1368)	0.1601)	0.2055)	0.2168)	0.2280)
	[0.0199]	[0.0102]	[0.0075]	[0.0317]	[0.0276]
BMI-	2433 (5349,	2757 (5987,	5054 (9173,	4439 (9397,	3990 (9224,
normal	0.0482)	0.0472)	0935)	0.0520)	0.1244)
	[0.1018]	[0.0942]	[0.0162]	[0.0794]	[0.1351]

Parameter	D1MFS [@]	D2MFS	D3MFS	D4MFS	D5MFS
	(95% CI) [p-				
	value]				
BMI-	0.0922 (3099,	0.1993 (2473,	0.0280 (5401,	0.2341 (-	0.2878 (4230,
overweight	0.4944)	0.6459)	0.5961)	.4474, 0.9156)	0.9985)
	[0.6531]	[0.3817]	[0.9231]	[0.5008]	[0.4275]
BMI-obese	0438 (6798,	1210 (8346,	5659 (-1.484,	9434 (-2.075,	9213 (-2.106,
	0.5921)	0.5925)	0.3520)	0.1878)	0.2634)
	[0.8925]	[0.7396]	[0.2269]	[0.1021]	[0.1275]
Sugar				0.0065	
intake	0.0014 (0011,	0.0022 (0006,	0.0048 (0.0014,	(0.0024,	0.0068 (0.0024,
(g/day)	0.0039)	0.0050)	0.0082)	0.0105)	0.0111)
	[0.2763]	[0.1186]	[0.0056]	[0.0020]	[0.0023]
Water	0002 (0004,	0002 (0005,	0001 (0004,	0002 (0006,	0002 (0005,
intake	0.0000)	0.0000)	0.0002)	0.0001)	0.0002)
(ml/day)	[0.0193]	[0.0432]	[0.3886]	[0.2244]	[0.4075]

^{*:} Only covariates with a statistically significant association with DMFS were included in the table. The complete list of covariates included: age, sex, BMI category, SES level, sugar intake (g/day), water intake (ml/day), amount of beverages with sugar per day, amount of beverages

without sugar per day, amount of toothpaste child used regularly from birth to 2 years of age, amount of toothpaste child used regularly from 2-4 years of age, amount of toothpaste child used regularly from 4-6 years of age, amount of toothpaste child currently uses regularly.

@: D#MFS represents DMFS counts using all ICDAS scores \geq # as Decay.

* BMI: Body Mass Index BLL: Blood Lead Level

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Table 1: Descriptive Statistics. n (%) or Mean (SD)

Variable	Mean / Frequency
Sex, n (%)	
Male	245 (50%)
Female	245 (50%)
BMI, n (%)	
Underweight (< 18.5)	127 (26%)
Normal (18.5-24.9)	276 (56%)
Overweight (25.0-29.9)	73 (15%)
Obese (≥ 30.0)	18 (4%)
Age, years	14.3 (2.1)
Age, years, N (%)	
10	11 (2%)
11	77 (16%)
12	72 (15%)
13	79 (16%)
14	44 (9%)
15	55 (11%)
16	102 (21%)
17	52 (11%)
SES Level	8.6 (3.2)

Sugar Intake, g/day	41.9 (45.8)
Water Intake, ml/day	667 (564)
Beverages with Sugar, ml/day	650 (504)
Beverages without Sugar,	51 (123)
ml/day	
Number of visits with BLL > 5	2.73 (2.2)
μg/dL	
Number of visits with BLL ≤ 5	4.81 (2.3)
μg/dL	
Mean BLL, μg/dL	4.83 (2.2)
Maximum BLL among all visits,	8.26 (4.7)
μg/dL	
Mean D#MFS	4.1 (5.1)
D1MFS*	7.46 (6.9)
D2MFS	5.76 (6.9)
D3MFS	2.98 (4.3)
D4MFS	2.22 (3.9)
D5MFS	2.07 (3.7)

^{*:} D#MFS represents DMFS counts using all ICDAS scores ≥ # as Decay.

^{*} BLL: Blood Lead Level * BMI: Body Mass Index * SES: Socioeconomic Status

Table 2: Oral Hygiene Variables:

n (%)	
38 (10%)	
63 (16%)	
282 (72%)	
161	
403 (73%)	
127 (23%)	
13 (2%)	
422 (77%)	
122 (22%)	
-	
-	

Covers ¾ of the brush	7 (1.28%)
Covers ½ of the brush	28 (5.10%)
Covers ¼ of the brush	24 (4.37%)
Covers less than ¼ of the brush	51 (9.29%)
Very small amount of place on the brush	52 (9.47%)
Didn't use toothpaste	387 (70.49%)
From 2-4 years, the amount of paste your child used regularly	
Thick band that covers the brush	-
Thin band that covers the brush	12 (2.18%)
Covers ¾ of the brush	50 (9.09%)
Covers ½ of the brush	129 (23.45%)
Covers ¼ of the brush	103 (18.73%)
Covers less than ¼ of the brush	92 (16.73%)
Very small amount of place on the brush	52 (9.45%)
Didn't use toothpaste	111 (20.18%)
From 4-6 years, the amount of paste your child used regularly	
Thick band that covers the brush	12 (2.18%)
Thin band that covers the brush	78 (14.18%)
Covers ¾ of the brush	155 (28.18%)
Covers ½ of the brush	153 (27.82%)
Covers ¼ of the brush	81 (14.73%)
Covers less than ¼ of the brush	57 (10.36%)
Very small amount of place on the brush	12 (2.18%)
Currently, the amount of paste your child uses regularly	
Thick band that covers the brush	134 (24.36%)

Thin band that covers the brush	278 (50.55%)
Covers ¾ of the brush	102 (18.55%)
Covers ½ of the brush	24 (4.36%)
Covers ¼ of the brush	11 (2%)
Covers less than ¼ of the brush	1 (0.18%)

Table 3: Negative Binomial Regression for association of Mean BLL with DMFS.*

Parameter estimate (95% CI) [p-value].

Parameter	D1MFS [@] (95%	D2MFS	D3MFS	D4MFS	D5MFS
	CI) [p-value]				
Mean BLL	0.0295 (0318,	0.0151 (0520,	0096 (0957,	0508 (1507,	0550 (1572,
	0.0908)	0.0821)	0.0764)	0.0490)	0.0473)
	[0.3455]	[0.6593]	[0.8265]	[0.3181]	[0.2920]
Age			0.1182	0.1192	
	0.0743 (0.0118,	0.0913 (0.0223,	(0.0313,	(0.0164,	0.1270 (0.0205,
	0.1367)	0.1603)	0.2050)	0.2219)	0.2335)
	[0.0198]	[0.0095]	[0.0076]	[0.0230]	[0.0194]
BMI-	2409 (5315,	2749 (5976,	5052 (9182,	4254 (9226,	3748 (9003,
normal	0.0497)	0.0478)	0923)	0.0718)	0.1506)
	[0.1042]	[0.0949]	[0.0165]	[0.0935]	[0.1621]

Parameter	D1MFS [@] (95%	D2MFS	D3MFS	D4MFS	D5MFS
	CI) [p-value]				
BMI-	0.0912 (3108,	0.1982 (2493,	0.0300 (-	0.2779 (-	0.3395 (3787,
overweight	0.4931)	0.6456)	.5425, 0.6025)	.4104, 0.9662)	1.0577)
	[0.6566]	[0.3853]	[0.9182]	[0.4287]	[0.3542]
BMI-obese	0381 (6726,	1180 (8307,	5679 (-1.485,	9266 (-2.056,	9017 (-2.085,
	0.5964)	0.5947)	0.3493)	0.2026)	0.2814)
	[0.9063]	[0.7456]	[0.2249]	[0.1078]	[0.1352]
Sugar			0.0048	0.0064	
intake	0.0014 (0011,	0.0022 (0006,	(0.0014,	(0.0024,	0.0067 (0.0024,
(g/day)	0.0039)	0.0050)	0.0082)	0.0105)	0.0111)
	[0.2792]	[0.1190]	[0.0056]	[0.0020]	[0.0023]
Water	0002 (0004,	0002 (0005,	0001 (0004,	0002 (0006,	0002 (0006,
intake	0.0000)	0.0000)	0.0002)	0.0001)	0.0002)
(ml/day)	[0.0224]	[0.0463]	[0.3855]	[0.1931]	[0.3569]

*: Only covariates with a statistically significant association with DMFS were included in the table. The complete list of covariates included: age, sex, BMI category, SES level, sugar intake (g/day), water intake (ml/day), amount of beverages with sugar per day, amount of beverages without sugar per day, amount of toothpaste child used regularly from birth to 2 years of age, amount of toothpaste child used regularly from 2-4 years of age, amount of toothpaste child used regularly from 4-6 years of age, amount of toothpaste child currently uses regularly.

@: D#MFS represents DMFS counts using all ICDAS scores $\geq \#$ as Decay.

BMI: Body Mass Index BLL: Blood Lead Level

Table 4: Negative Binomial Regression for association of Maximum BLL with DMFS.*

Parameter estimate (95% CI) [p-value].

Parameter	D1MFS@	D2MFS	D3MFS	D4MFS	D5MFS
	(95% CI) [p-				
	value]				
Max BLL	0.0131 (0146,	0.0073 (0232,	0050 (0455,	0148 (0652,	0160 (0683,
	0.0408)	0.0379)	0.0355)	0.0356)	0.0363)
	[0.3546]	[0.6370]	[0.8097]	[0.5649]	[0.5492]
Age	0.0743			0.1133	
	(0.0117,	0.0908 (0.0216,	0.1185 (0.0316,	(0.0099,	0.1207 (0.0133,
	0.1368)	0.1601)	0.2055)	0.2168)	0.2280)
	[0.0199]	[0.0102]	[0.0075]	[0.0317]	[0.0276]
BMI-	2433 (5349,	2757 (5987,	5054 (9173,	4439 (9397,	3990 (9224,
normal	0.0482)	0.0472)	0935)	0.0520)	0.1244)
	[0.1018]	[0.0942]	[0.0162]	[0.0794]	[0.1351]

Parameter	D1MFS [@]	D2MFS	D3MFS	D4MFS	D5MFS
	(95% CI) [p-				
	value]				
BMI-	0.0922 (3099,	0.1993 (2473,	0.0280 (5401,	0.2341 (-	0.2878 (4230,
overweight	0.4944)	0.6459)	0.5961)	.4474, 0.9156)	0.9985)
	[0.6531]	[0.3817]	[0.9231]	[0.5008]	[0.4275]
BMI-obese	0438 (6798,	1210 (8346,	5659 (-1.484,	9434 (-2.075,	9213 (-2.106,
	0.5921)	0.5925)	0.3520)	0.1878)	0.2634)
	[0.8925]	[0.7396]	[0.2269]	[0.1021]	[0.1275]
Sugar				0.0065	
intake	0.0014 (0011,	0.0022 (0006,	0.0048 (0.0014,	(0.0024,	0.0068 (0.0024,
(g/day)	0.0039)	0.0050)	0.0082)	0.0105)	0.0111)
	[0.2763]	[0.1186]	[0.0056]	[0.0020]	[0.0023]
Water	0002 (0004,	0002 (0005,	0001 (0004,	0002 (0006,	0002 (0005,
intake	0.0000)	0.0000)	0.0002)	0.0001)	0.0002)
(ml/day)	[0.0193]	[0.0432]	[0.3886]	[0.2244]	[0.4075]

*: Only covariates with a statistically significant association with DMFS were included in the table. The complete list of covariates included: age, sex, BMI category, SES level, sugar intake (g/day), water intake (ml/day), amount of beverages with sugar per day, amount of beverages without sugar per day, amount of toothpaste child used regularly from birth to 2 years of age, amount of toothpaste child used regularly from 2-4 years of age, amount of toothpaste child used regularly from 4-6 years of age, amount of toothpaste child currently uses regularly.

@: D#MFS represents DMFS counts using all ICDAS scores $\geq \#$ as Decay.

* BMI: Body Mass Index BLL: Blood Lead Level

Blood levels of lead and dental caries

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