Amoxicillin use during early childhood and fluorosis of later developing tooth zones

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Keywords
amoxicillin; dental fluorosis; enamel defects; antibiotics.

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Received: 6/8/2010; accepted: 2/5/2011.

doi: 10.1111/j.1752-7325.2011.00254.x

Abstract

Objectives: Amoxicillin use has been reported to be associated with developmental defects on enamel surfaces. This analysis assessed the association between amoxicillin use and fluorosis on late-erupting permanent teeth.
Methods: As part of the Iowa Fluoride Study, subjects were followed from birth to 32 months with questionnaires every 3-4 months to gather information on fluoride intake and amoxicillin use ($n = 357$ subjects for this analysis). Permanent tooth fluorosis on late-erupting zones was assessed by three trained dentists using the fluorosis risk index (FRI) at approximately age 13. A case was defined as fluorosis if a subject had at least two FRI classification II zone scores of 2 or 3. Chi-square tests and logistic regression were used, and relative risks (RRs) and odds ratios (ORs) were calculated.
Results: There were 113 cases and 244 controls. In bivariate analyses, amoxicillin use from 20 to 24 months significantly increased the risk of fluorosis on FRI classification II zones [44.2 percent versus 30.4 percent, [RR = 1.45, 95 percent confidence interval (CI) 1.05-2.04], but other individual time periods did not. Multivariable logistic regression confirmed the increased risk of fluorosis for amoxicillin use from 20 to 24 months (OR = 2.92, 95 percent CI = 1.34-6.40), after controlling for otitis media, breast-feeding, and fluoride intake.
Conclusions: Amoxicillin use during early childhood could be a risk factor in the etiology of fluorosis on late-erupting permanent tooth zones, but further research is needed.

Introduction

It has been suggested that amoxicillin use is associated with developmental enamel defects (1-4). These defects appear as diffuse opacities, possibly due to enamel hypomineralization. They manifest clinically similar to dental fluorosis, but are obviously different from tetracycline staining. Laboratory studies suggested that the use of antibiotics/amoxicillin is associated with the so-called molar–incisor hypomineralization among children (5-8). A more recent clinical study reported that molar–incisor hypomineralization was more common among children who had taken amoxicillin (9) during the first year of life compared with children who had not received amoxicillin [odds ratio (OR) = 2.06, 95 percent confidence interval (CI) 1.01-4.17]. A study using laboratory mice suggested that the early use of amoxicillin could alter the pattern of amelogenesis (enamel formation) and could interfere with mineralization (9). Using data collected in the Iowa Fluoride Study, we reported a weak association between primary tooth fluorosis and amoxicillin use during the first

Supported in part by NIH grants R01-DE09551, R01-DE12101, P30-DE10126 and M01-RR00059.
Study results were partially presented at the 37th Annual meeting of the American Association of Dental Research, Dallas, TX, March 21-24, 2008.

year of life (3). Also, we reported a stronger association of amoxicillin use during early life with fluorosis of early-developing permanent tooth zones (4). Amoxicillin use from 3 to 6 months significantly increased the risk of fluorosis (OR = 2.50, 95 percent CI 1.21-5.15) after adjusting for fluoride intake, breast-feeding, and otitis media. Therefore, the purpose of our study was to follow up on the children in the Iowa Fluoride Study cohort and to report associations between fluorosis of late-developing permanent tooth zones and amoxicillin use during the first 32 months of life. For the convenience of description, we use the more common term "fluorosis" for possible amoxicillin-related enamel defects in the text because they have similar clinical manifestation.

Methods

The data were collected as part of the Iowa Fluoride Study, a prospective study investigating fluoride exposures, biological and behavioral factors, and children's dental health. The details of the study have been reported elsewhere (3,4,10-14). Using institutional review board-approved informed consent procedures, 1,882 subjects were initially recruited at birth from March 1992 to February 1995, and subjects were excluded if they were too ill to participate. Participants received dental examinations of primary dentition at about age 5 ($n = 698$), mixed dentition at about age 9 ($n = 630$), and permanent dentition at about age 13 ($n = 550$). Demographic characteristics at baseline were described previously (10,11). Among those who remained in the study for age 13 examinations, 52 percent were female, 70 percent had family income of $30,000 or more at recruitment, 56 percent of mothers had completed 4 years of college at recruitment, 32 percent of children had been breast-fed for at least 6 months, 3 percent had low birth weight, and 3 percent had developmental disorders.

Questionnaires were sent to parents at 3- or 4-month intervals from birth. Antibiotic use, children's illnesses, and breast-feeding practices were assessed until 32 months of age. Data collection details have been described previously (3,10). Parents were asked to identify specific antibiotics prescribed and given to the child and to report how the antibiotics were administered, the number of episodes of illnesses for which the antibiotics were used, and the number of days the antibiotics were used. The number of days attributed to amoxicillin use was reduced when other concomitant antibiotics were reported (3,4). Topical antibiotics were excluded from the analyses. As described previously (12-15), information on fluoride intake from various sources has been collected on all questionnaires since birth. Fluoride intake in milligrams per kilogram of bodyweight (bw) per day was estimated from water, beverages and selected foods, dietary fluoride supplements, and fluoride dentifrice based on responses to a series of detailed questions. Four yearly area-under-the-curve fluoride intake estimates were computed (0-12 months, 12-24 months, 24-36 months, and 36-48 months) using the trapezoidal method. These estimates of yearly fluoride intake were categorized using tertiles of the frequency distribution based on daily combined average fluoride intake in milligrams of fluoride (F) per kilogram bw per day from drinking water, beverages and selected foods, dietary fluoride supplements, and fluoride dentifrice ingestion for each of the first 4 years of life

The term "dental fluorosis" is used to refer to the condition of diffuse opacities on tooth surfaces, although the cause might not be excessive fluoride ingestion (4). The fluorosis risk index (FRI) was used to assess this condition (16). Children were examined for dental fluorosis at about 13 years of age (mean age 13.5) by two trained and calibrated examiners. Four zones (occlusal table or incisal edge, incisal third, middle third, and cervical third) of facial surfaces of each tooth were assessed separately, with FRI scoring criteria differentiating no fluorosis, questionable fluorosis (50 percent or less of zone with white striations), definitive fluorosis (greater than 50 percent of zone with white striations), and severe fluorosis (zone displays pitting, staining, and/or deformity) (16). Fluorosis was differentiated from non-fluorosis opacities based on Russell's criteria (17) and from "white spot" carious lesions (18). FRI classification II zones are those tooth areas that form primarily during the third through sixth years of life and include the cervical third of incisors (eight zones), middle third of canines (four zones), and occlusal tables, occlusal third, and middle third of premolars and second molars (36 zones) (16). Thus, FRI classification II includes a total of 48 zones on 24 teeth. A fluorosis case was defined as having FRI definitive/severe fluorosis (score 2 or 3) on at least two FRI classification II zones ($n = 113$); controls had no definitive fluorosis (score 0 or 1) on all 48 zones ($n = 244$). Subjects were excluded if seven or more zones (out of 48) were not scorable ($n = 182$), except for four subjects with extracted first premolars whom we classified as controls. Eleven subjects with only one tooth having fluorosis on FRI classification II zones were also excluded. Therefore, the final sample size was 357 subjects (185 boys and 172 girls) for this analysis. With a high degree of certainty of diagnosis, the analysis included only individuals with definitive/severe fluorosis and those without fluorosis. Person level inter-examiner reliability was 90 percent agreement (Kappa = 0.73).

Exposure to amoxicillin was first categorized into yes or no groups, both for individual and cumulative time periods. Using midpoints of the duration intervals, the number of days of amoxicillin use were calculated as described previously (3,4). The estimated daily average fluoride intake for each year of life was categorized into tertiles (low, middle, and high fluoride intakes), based on the frequency distribution of estimated daily fluoride intake by year (milligram F per kilogram bw) (15).

The associations between fluorosis and amoxicillin use were first assessed using chi-square tests. Relative risks (RR) and 95 percent CI were calculated. Bivariate associations between fluorosis and sex, family income, mother's age at birth of child, mother's educational level, use of other antibiotics, low bw, otitis media, developmental disorders, breast-feeding, and yearly fluoride intakes were also assessed using chi-square tests. Mantel–Haenszel stratified analyses of the association between fluorosis and amoxicillin use controlled for fluoride intake and otitis media.

Variables with $P < 0.10$ in bivariate analyses were selected for inclusion in multivariable logistic regression, and the main effects and two-way interactions between them were assessed and ORs were obtained. The significance level was set at $\alpha = 0.05$. The data were analyzed with SAS statistical software for Windows version 9.1 (SAS Institute Inc., Cary, NC, USA).

Results

Based on the case and control definitions described in the methods section, there were 113 cases and 244 controls. Period-specific amoxicillin exposure was substantial among the 357 subjects, increasing from 20.1 percent (0-3 months of age) to 40.1 percent (12-16 months), before declining to 19.5 percent (28-32 months). Cumulatively, 72.3 percent had amoxicillin use by 12 months, 83.4 percent by 20 months, and 90.6 percent by 32 months.

From bivariate analyses, amoxicillin use during 20-24 months (RR = 1.45, 95 percent CI 1.05-2.04) was significantly associated with fluorosis on late-developing FRI-II zones (Table 1). In addition, analyses showed a significant dose–response relationship between the number of days of amoxicillin use during 20-24 months and fluorosis. Prevalence was 30.7 percent for children without amoxicillin, 36.8 percent for 1-10 days of amoxicillin use, and 45.6 percent for >10 days of amoxicillin use ($P = 0.035$, Cochran-Armitage trend test). For children using amoxicillin at 20-24 months, 44.2 percent (34/77) had fluorosis versus 33.3 percent (6/18) with fluorosis among children who have never used amoxicillin during all of the first 32 months of life ($P = 0.29$).

The analyses showed that fluoride intake during 24-36 months ($P = 0.004$) and 36-48 months ($P = 0.02$) were positively associated with fluorosis on the FRI II zones of these permanent teeth, while 0-12-month fluoride intake ($P = 0.14$) and 12-24-month intake ($P = 0.11$) showed positive, but non-significant, associations (Table 2). Other classes of antibiotics, including penicillin, cephalosporins, and erythromycins, were not found to be significantly associated with fluorosis (data not shown). Among all other factors (Table 2), only breast-feeding less than 6 months was moderately associated with fluorosis ($P = 0.09$) (14).

Otitis media was the predominant illness listed in conjunction with antibiotic use (59-80 percent, depending on the reporting period). Amoxicillin accounted for 70-85 percent of all antibiotics prescribed for treatment of otitis media during the first 32 months of life. Otitis media alone, both for individual time periods and cumulatively, was not significantly associated with fluorosis on FRI II zones (data not shown). However, because amoxicillin use and otitis media

Table 1 Amoxicillin Use and Prevalence of Fluorosis* on Later Developing Permanent Tooth Zones

Age	*n*†	Prevalence of fluorosis*		Relative risk (95% confidence interval)	Chi-square *P*-value
		No amoxicillin	Any amoxicillin		
Individual periods‡					
Birth to 3 months	319	32.2 (82/255)	37.5 (24/64)	1.17 (0.81-1.69)	0.42
>3-6 months	317	31.4 (69/220)	37.1 (36/97)	1.19 (0.86-1.64)	0.32
>6-9 months	317	30.5 (58/190)	36.2 (46/127)	1.19 (0.87-1.64)	0.29
>9-12 months	299	30.9 (56/181)	35.6 (42/118)	1.16 (0.83-1.62)	0.41
>12-16 months	297	30.2 (57/189)	36.1 (39/108)	1.20 (0.86-1.59)	0.30
>16-20 months	274	33.5 (65/194)	36.3 (29/80)	1.09 (0.76-1.54)	0.67
>20-24 months	284	30.4 (63/207)	44.2 (34/77)	1.45 (1.05-2.04)	0.03
>24-28 months	274	32.2 (67/208)	31.8 (21/66)	0.99 (0.66-1.49)	0.96
>28-32 months	272	33.3 (73/219)	34.0 (18/53)	1.02 (0.67-1.56)	0.93
Cumulative periods‡					
0-12 months	274	26.3 (20/76)	36.4 (72/198)	1.39 (0.91-2.12)	0.12
0-20 months	229	23.6 (9/38)	35.6 (68/191)	1.52 (0.83-2.77)	0.16
0-24 months	220	28.0 (7/25)	35.4 (69/195)	1.26 (0.66-2.43)	0.47
0-32 months	192	33.3 (6/18)	35.6 (62/174)	1.07 (0.54-2.12)	0.85

* Fluorosis is defined as having fluorosis [fluorosis risk index (FRI) score 2 or 3] on at least two FRI II zones. Subjects were excluded if seven or more zones (out of 48) were not scorable or only one tooth had fluorosis on FRI classification II zones.

† Sample sizes reflect the number of parents who returned the questionnaires for each reporting interval.

‡ Both individual and cumulative periods refer to the ages at which amoxicillin use was assessed.

Table 2 Bivariate Associations of Fluorosis* with Demographic Variables and Other Factors

	Category	Sample percentage (%)	Prevalence of fluorosis* (%)	*P*-value†
Sex	Male	51	31.0	0.54
	Female	49	34.1	
Family income at baseline	<$20,000	14	33.4	0.18
	$20,000-$39,999	37	24.5	
	$40,000 or more	49	40.0	
Mother's educational level at baseline	High school diploma (or less)	21	20.3	0.15
	Some college	23	36.8	
	4-year college degree or more	56	35.6	
Low birthweight	No (2.5 kg or more)	96	26.6	0.62
	Yes (under 2.5 kg)	4	32.8	
Illness during first year‡	No	78	32.8	0.92
	Yes	22	31.6	
Developmental disorder	No	97	32.2	0.75
	Yes	3	37.5	
Length of breast-feeding	Less than 6 months	67	34.2	0.09
	6 months or more	33	28.2	
Fluoride intake§				
0-12 months	Low level	33	29.9	0.14
	Middle level	37	29.4	
	High level	30	41.2	
12-24 months	Low level	35	23.5	0.11
	Middle level	33	35.3	
	High level	32	37.4	
24-36 months	Low level	33	22.0	0.004
	Middle level	36	27.0	
	High level	31	43.4	
36-48 months	Low level	35	20.2	0.019
	Middle level	39	30.2	
	High level	26	40.8	

* Fluorosis is defined as having fluorosis [fluorosis risk index (FRI) score 2 or 3] on at least two FRI II zones. Subjects were excluded if seven or more zones (out of 48) were not scorable or only one tooth had fluorosis on FRI classification II zones.

† *P*-value from chi-square test.

‡ A child is defined as having illness if they reported having a "serious illness" at any time before the first clinical visit (approximately age 5).

§ Fluoride intake was categorized using tertiles of the frequency distribution based on daily combined average fluoride intake in milligrams of fluoride per kilogram bodyweight per day from drinking water, beverages and selected foods, dietary fluoride supplements, and fluoride dentifrice ingestion for each of the first 4 years of life.

are clearly related in this study, association between amoxicillin and fluorosis was assessed using Mantel–Haenszel analysis, which was stratified by both fluoride intake and otitis media (Table 3). Because fluoride intake at 24-36 months and 36-48 months were both significantly associated with fluorosis, they were combined by averaging the two yearly daily fluoride intakes and then split into three levels (low, middle, and high) using tertiles. The risk of fluorosis on FRI II zones for amoxicillin use during 20-24 months (RR = 1.67, 95 percent CI 1.10-2.43) remained significant after stratification, with the risk appearing elevated at middle and high fluoride levels.

In addition to amoxicillin use (20-24 months) and concurrent otitis media, individual variables with $P < 0.10$ in the bivariate assessment were chosen for multivariable logistic regression analyses: breast-feeding during the first year of life (less than 6 months versus 6 months or more) and daily average fluoride intake during 24-48 months (low, middle, and high levels). Amoxicillin use during 20-24 months (adjusted OR = 2.92, 95 percent CI 1.34-6.40, $P = 0.01$) was still significantly associated with fluorosis after controlling for other risk factors (Table 4). Fluoride intake was also significantly related to fluorosis (high level, adjusted OR = 3.38, 95 percent CI 1.57-7.25, $P = 0.001$; middle level, adjusted OR = 2.13, 95 percent CI 1.11-4.07, $P = 0.02$), but other factors were not statistically significant. No significant two-way interactions were detected.

Discussion

The developing tooth is susceptible to various insults during enamel formation (19,20). Dental enamel develops from a

Table 3 Mantel–Haenszel Stratified Analyses for Effects of Amoxicillin Use on Fluorosis* after Jointly Controlling for Daily Average Fluoride Intake and Otitis Media (n = 269)

Age		Fluoride intake level† (24-48 months)	Prevalence of fluorosis* Amoxicillin use (20-24 months) No	Yes	Relative risk (95% CI)	Mantel–Haenszel relative risk (95% CI)
Otitis media 20-24 months‡	No	Low	23.1 (12/52)	20.0 (1/5)	0.86 (0.14-5.55)	1.67 (1.10-2.43) [P = 0.03]
		Mid	36.6 (26/71)	54.6 (6/11)	1.49 (0.81-2.77)	
		High	36.5 (19/52)	66.7 (4/6)	1.85 (0.93-3.57)	
	Yes	Low	0 (0/7)	25.0 (3/12)	NA	
		Mid	25.0 (2/8)	47.4 (9/19)	1.92 (0.52-7.14)	
		High	28.6 (2/7)	42.1 (8/19)	1.49 (0.41-5.55)	

* Fluorosis is defined as having fluorosis [fluorosis risk index (FRI) score 2 or 3] on at least two FRI II zones. Subjects were excluded if seven or more zones (out of 48) were not scorable or only one tooth had fluorosis on FRI classification II zones.

† Fluoride intake was categorized using tertiles of the frequency distribution based on daily combined average fluoride intake (area under curve) in milligrams of fluoride per kilogram bodyweight per day from drinking water, beverages and selected foods, dietary fluoride supplements, and fluoride dentifrice ingestion during 24-48 months of life.

‡ A child is defined as having otitis media during 20-24 months if the child used antibiotics for treatment of otitis media.

CI, confidence interval.

highly organic extracellular matrix (20 percent by weight) into the hardest body tissue (less than 1 percent organic material) (21-23). Because tooth enamel is not renewed, the developmental defects remain permanent. There are only a few drugs documented to disturb enamel formation (24). Some antibiotics, such as tetracyclines, clearly influence the development of the tooth, causing tooth discoloration. Our previous studies and others' (9) have indicated that the use of antibiotics/amoxicillin is associated with enamel defects, with the common feature being hypomineralization. Specifically, the hypomineralized enamel may manifest clinically as diffuse opacities (3,4) or demarcated opacities (5,7-9).

Our present study shows a link between amoxicillin use during 20-24 months and fluorosis of FRI classification II zones. These tooth areas develop at later stages of permanent dentition formation, presumably developing primarily during the third through sixth years of life (16). Therefore, exposure to amoxicillin during an earlier stage of enamel formation may also increase the risk of dental fluorosis. This result is generally consistent with our previous analysis of early-developing permanent teeth, for which amoxicillin use during 3-6 months significantly increased the risk of dental fluorosis of FRI classification I zones of early-developing permanent teeth (incisors and first molars) (4).

A recent animal study showed that amoxicillin induces earlier enamel formation and/or accelerates the enamel accretion rate, and thus, it is possible that amoxicillin interferes with ameloblast (enamel-producing cells) function and disturbs the temporal sequence of amelogenesis events (9). The same researchers' clinical investigation showed children who had amoxicillin during the first year of life were more likely to have molar/incisor demarcated opacities, with an OR of 2.06 (95 percent CI 1.01-4.17) (9). Evidence from these studies suggests that exposure to amoxicillin in early stages of enamel formation is likely among the causative factors for enamel hypomineralization. Considering the developmental

Table 4 Logistic Regression Analyses for Fluorosis* and Four Predictor Variables (n = 264)

	Risk group	Odds ratios (95% confidence interval)	P-value
Amoxicillin use (20-24 months)	Yes	2.92 (1.34-6.40)	0.007
Otitis media (20-24 months)†	Yes	1.91 (0.85-4.27)	0.12
Breast-fed 6 months or more	No	1.29 (0.69-2.39)	0.41
Average daily fluoride intake	High level	3.38 (1.57-7.25)	0.001
during 24-48 months‡	Middle level	2.13 (1.11-4.07)	0.022

* Fluorosis is defined as having fluorosis [fluorosis risk index (FRI) score 2 or 3] on at least two FRI II zones.

† Otitis media was categorized as yes or no during 20-24 months of life.

‡ Fluoride intake was categorized using tertiles of the frequency distribution based on daily combined average fluoride intake in milligrams of fluoride per kilogram bodyweigh per day from drinking water, beverages and selected foods, dietary fluoride supplements, and fluoride dentifrice ingestion.

stages of enamel formation of FRI II zones, it is possible that the critical stages for the effects of amoxicillin could be the secretory stages, which are the first stages of enamel formation.

The β-lactam antibiotics, including penicillins, amoxicillins, and cephalosporins, have been considered safe for infants and, often, are prescribed for common childhood infections such as otitis media. Our study provides additional evidence that amoxicillin use might carry a risk to the developing teeth. The findings from this study and others (3-9) are not conclusive because of various study differences and design limitations, and do not reach the level to warrant recommendations to cease use of amoxicillin early in life. Amoxicillin should remain the first pharmacological choice against otitis media and other common childhood infections. Hypomineralized enamel defects usually do not impose a significant health risk and are typically much less aesthetically objectionable than tetracycline staining. The vast majority of fluorosis cases in our study were questionable fluorosis and may not have any substantial effect on oral health quality of life (25). However, in severe cases, teeth can be prone to enamel breakdown and may require restorative treatment. When anterior teeth are involved, the aesthetic impact could be greater (26,27).

As previously reported (4), our study has limitations. These limitations include the use of a convenience sample, most children being from relatively high socioeconomic status families, and the use of self-administered questionnaires without direct verification. One concern is the fact that there were a very limited number of true non-users of amoxicillin, which made it difficult to control for the cumulative effects of amoxicillin use from previous periods. As mentioned, illnesses were reported only if they were associated with antibiotic use and thus, could underestimate their occurrence. Information on fevers and fever-reducing medication was not collected. In addition, there were incomplete (missing) questionnaire data for many individuals during some reporting periods, which is unavoidable in longitudinal studies. Some individuals left the study, so that the results could have been different if all the children had remained in the study. We excluded 182 children with seven or more zones not being scorable (mostly due to active orthodontia), and the implications of the exclusions on the study results are not known. Because no adjustment was made for testing at multiple exposure times, our results only suggest a link between amoxicillin use and fluorosis-like enamel defects. Although statistical analysis showed an independent effect of amoxicillin use, it could still be possible that the association reported in this study would be an added effect of amoxicillin on the effect of fluoride exposure. It would be necessary to use animal models to evaluate different aspects at cellular and molecular levels on the developing enamel. The findings are not conclusive and probably should not be generalized to other populations. There is a need for further research.

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