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ORIGINAL ARTICLE

Molar–incisor hypomineralization and the association with childhood illnesses and antibiotics in a group of Finnish children

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ABSTRACT

Objective: Molar–incisor hypomineralization (MIH) is a developmental enamel defect affecting 1–4 first permanent molars (FPMs) and often also incisors. The aim of this study was to assess whether childhood illnesses or medication are associated with MIH.

Material and methods: FPMs and incisors of 287 Finnish children were examined for MIH in line with the criteria of the EAPD. Health data from the first 3 years of life was collected from medical records and the associations with MIH and MIH2 (lesions in at least one FPM and incisor) were assessed using simple and multiple logistic regression analyses.

Results: The prevalence of MIH and MIH2 were 11.5% and 6.3%, respectively. During the first 3 years of life, the children with MIH had sought care for infectious illnesses more often than the children without MIH (mean number of visits (SD) 7.9(6.4) vs. 6.0(5.1), $p = 0.045$, independent samples *t*-test). After adjustment for confounding factors, children who had received penicillin or macrolides within the first year, or amoxicillin within the first 3 years had a higher risk for MIH (2.61, 4.07 and 2.58 times, adjusted OR, respectively) or MIH2 (3.16 times, aOR for penicillin and amoxicillin) compared to those who had not received that antibiotic. Of the illnesses, children with at least one episode of otitis within the first year had a higher risk for MIH (2.28 times, aOR) than those who had not suffered from otitis.

Conclusions: Acute otitis media and the use of certain antibiotics were associated with the elevated risk of MIH/MIH2.

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Introduction

Molar–incisor hypomineralization (MIH) is a developmental enamel defect where first permanent molars (FPM) exhibit enamel with reduced mineral content.[1] The number of affected FPMs can vary from one to all four of them, with frequent, but usually more mildly affected, incisors.[2] The defects are seen clinically as white or yellow demarcated opacities that vary in size and severity, sometimes leading to enamel breakdown after eruption.

The prevalence of MIH varies markedly depending on the region studied and diagnostic criteria used: between 2.9 and 44%.[3] The study of aetiology of MIH has concentrated on the factors occurring in early childhood. In FPMs, mineralization of enamel starts around birth and the crown formation is completed around the age of three years,[4,5] but the maturation continues until the tooth erupts. Many studies have shown associations between MIH and medical problems at birth or during the first years of life. Pre-term birth [6] and common childhood illnesses such as acute otitis media (AOM) and respiratory infections have been found to be associated with the defect.[7–13] The use of medication has been proposed as a putative aetiological factor.[14–16] Attention has also been paid to environmental toxicants such as dioxins [17,18] and, in a recent experimental study, bisphenol A.[19] However, the results have not been very consistent. Also, most of the clinical studies are based on parental recollection.

This retrospective cohort study is a continuum of the study of Laisi et al., where the association between MIH and the use of antibiotics during the first years of the child's life were analysed.[15] That study suggested a role for amoxicillin in MIH. We extended that study to include the episodes of illnesses and we increased the number of subjects by including children from another Finnish town, Jalasjärvi.

The aim of this study was to consistently assess whether childhood illnesses and their treatment with antimicrobial medication during the first years of life are associated with hypomineralized enamel lesions in the early-developed permanent teeth (FPMs and incisors).

Materials and methods

Subjects

The biggest comprehensive schools from two rural Finnish towns, Lammi and Jalasjärvi (population approximately 5500 and 8500, respectively [20]) were selected and the students in the 2nd to 5th grades, 396 children in total, were invited to join the study. A total of 317 children (148 children from Lammi and 169 children from Jalasjärvi) participated in the dental examination with informed parental consent. The

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percentage of participation in the examination was 80.0%. Examinations were part of the dental check-ups that are offered to every child, free of charge, at certain ages by public health care. The children were 7–12 years old.

In the Lammi region, the fluoride content in communal pipe water is <0.1 mg/L and in wells in the sparsely populated areas it is mostly <0.2 mg/L. In Jalasjärvi, the fluoride content in communal pipe water is 0.5–1 mg/L.

Ethical considerations

The Ethics Committee at South Karelia Hospital District, Lappeenranta, Finland, approved the study. Information on childhood illnesses and prescribed medications was obtained from medical records from the health centers of Lammi and Jalasjärvi with permission from the Ethics Committee (with extended ethical permission from the JIK (Jalasjärvi Ilmajoki Kurikka) municipal establishment of basic services) and from a private doctor with permission from those who had used his services.

Clinical examination

Dental examinations were performed in the dental clinic under standard dental lighting. All examinations were done by one calibrated dentist (SL) using a mirror and a periodontal probe. The examiner was blind to the information in the medical records. The teeth were examined wet for MIH-characteristic hypomineralization (demarcated opacities, posteruptive enamel loss, atypical restorations replacing the affected dental hard tissue, and extracted molars due to MIH). The judgement criteria for MIH were in line with that set by the European Academy of Paediatric Dentistry [21] except that lesions smaller than 2 mm in diameter were not recorded. Diffuse opacities indicating fluorosis were recorded but not included in the analyses. Children with hereditary defects of dental enamel were not observed. If the patient had orthodontic molar bands or partially erupted molars, only the visible teeth surfaces were examined.

The participating dentist (SL) has vast experience in the diagnosis and treatment of MIH. He was calibrated for the screening of MIH, diffuse opacities, and hypoplasia. The intra-examiner kappa coefficient for teeth with developmental defects of enamel (≥2 mm) was 0.91 and for classified defects (MIH, diffuse opacity, and hypoplasia) it was 0.90. Correspondingly, inter-examiner (SA) kappa coefficients were 0.96 and 0.81 between SL and SA.

Medical records

Medical records (documents written by doctors that include a reason for the visit, examination methods, diagnosis, treatment and prescribed medicines) from the first 3 years of participants' lives were obtained from the public health centers in Lammi and Jalasjärvi. A private doctor, whose services some of the children from Jalasjärvi ($N = 16$) had used, also provided medical records. In most of the cases when a patient had been sent to the central hospital, medical records from the hospital visit were also available for the purpose of the study.

The records were screened by authors (EW and AE) for episodes of common childhood illnesses including acute otitis media (AOM) and upper and lower respiratory tract infections, as well as the more rare infections, such as gastroenteritis, urinary tract infections, and chickenpox. To differentiate the number of episodes in a case of prolonged illness, as is common with AOM and bronchitis, a new episode was considered to begin if there was a minimum time period of 21 days between separate events, unless the patient's disease was judged as cured by a physician at an earlier date.[22] It was possible that a child got diagnosed with more than one illness during a single visit (e.g. acute otitis media and upper respiratory illness). Antibiotics were classified according to their active agent as follows: penicillin, amoxicillin, cephalosporin, sulphonamide-trimethoprim, macrolide and other agents. Other commonly used medications were also recorded, such as antitussives, antihistamines, and bronchodilators.

The total number of visits to a doctor at a health center was calculated as well as the number of visits due to the aforementioned illnesses. Visits due to trauma or allergies were not included, unless some illness was diagnosed during the same visit. Authors were not aware of MIH status of the children when screening the records.

Statistics

The data were analysed with the Statistical Package for the Social Sciences (SPSS, version 22, Chicago, IL). Diagnosis of MIH was set when at least one of the four FPMs was affected, according to EAPD criteria.[21] In addition, because MIH is defined as a demarcated enamel defect in "one or more of the four permanent first molars, as well as any associated and affected incisors",[2] an additional category of MIH was set when a child had hypomineralization defects in 1–4 FPMs and 1–8 permanent incisors (MIH2).[12]

Health data from the first year (0–1yr) and first 3 years (0–3yrs) of life was prospectively recorded by doctors and retrospectively collected (historical cohort) by the authors. The statistical differences in the mean number of visits to health centers between MIH/non-MIH and MIH2/non-MIH2 children were assessed using an independent samples *t*-test. The simple and multiple logistic regression analysis (binary logistic regression) were used to calculate the risk of MIH and MIH2 after suffering from an illness or using an antibiotic (yes/no) in the first and first 3 years (Table 2). The increase in risk of MIH or MIH2 with every extra episode of illness or course of antibiotic was calculated using the same analysis and the results are reported in Table 3. The associations between illnesses and antibiotics and MIH or MIH2 are reported as unadjusted odd ratios (uORs), adjusted odd ratios (aORs, to control for other confounders: age and location) and their 95% confidence intervals (95% CI). The association was considered statistically significant when the observed *p*-value was less than 0.05.

Results

Of the 317 children who participated in dental examinations, 27 had incomplete medical records and were excluded. Also,

one child had a low birth weight (<2000g) and two children had suffered from childhood cancer and were excluded. Thus, the final number of participants with sufficient health data from the first 3 years of life was 287 children (90.5% of the examined and 72.5% of the invited children). The mean age of the participants at the time of the examination was 10.4 years (SD 1.3).

Thirty-three children had demarcated lesions in at least one FPM, i.e. MIH (11.5%). Among them, 18 (6.3% of all children) had MIH2 (at least one FPM and one incisor affected). No FPMs had been extracted due to hypomineralization defects.

Crude associations with the definitions of MIH (MIH and MIH2) and the potential risk factors (gender, location, illnesses and medications) are seen in Table 1. MIH or MIH2 were not associated with gender ($p = 0.458$ and, $p = 0.635$, respectively). Neither age nor birth year statistically differed between children with and without MIH ($p = 0.652$ and 0.331, respectively) or MIH2 ($p = 0.356$ and 0.601, respectively).

The prevalence of MIH and MIH2 were 16.4% and 7.9% in Lammi and 6.8% and 4.8% in Jalasjärvi, respectively. The association between MIH and the locations was significant, but not between with MIH2 and locations (Table 2).

The total number of visits to a doctor by the 287 children who participated in the study was 2865 (mean 9.98 SD 8.07). Children with MIH visited a doctor in a health center more often than children without MIH, although the difference was not statistically significant (mean number of visits (SD) 12.30 (9.70) vs. 9.68 (7.81), $p = 0.079$). However, those with MIH had sought care for infectious illnesses significantly more often than children without MIH (mean (SD) 7.94 (6.40) vs. 5.99 (5.09), $p = 0.045$). There was no difference in the total number of visits between children with and without MIH2 (mean number of all visits (SD) for children with MIH2 9.94 (7.40) vs. children without MIH2 9.99 (8.13), $p = 0.984$ and the mean number of visits due to illness 6.44 (5.46) vs. 6.20 (5.28), $p = 0.848$, respectively).

Childhood illnesses

The number of children with certain illnesses and the definitions of MIH are seen in Table 1. The most commonly diagnosed illness during the first 3 years of life was upper respiratory infection with 680 episodes in total (mean 2.37, SD 2.33), followed by acute otitis media (AOM) with 617 episodes (mean 2.15, SD 2.44), lower respiratory infection with 340 episodes (mean 1.18, SD 1.83) and gastroenteritis with 89 episodes (mean 0.31, SD 0.61). Urinary tract infection was rare with 15 episodes (mean 0.05, SD 0.28). The total number of episodes of illnesses, also including more rare illnesses such as stomatitis or chicken pox, was 1763 (mean 6.14, SD 5.47).

Age- and location-adjusted associations showed that children who had suffered from at least one episode of AOM within the first year of life had a 2.28 times higher risk for MIH ($p = 0.035$) (Table 2). They also had a 2.62 times higher risk for MIH2, but this association was not statistically significant ($p = 0.056$). Every extra episode of AOM within the first year of life increased the risk of MIH by 42% ($p = 0.023$) and

Table 1. Crude associations with the definitions of MIH and potential risk factors from the first 3 years of life.

		MIH		MIH2	
	Group	No *N* (%)	Yes *N* (%)	No *N* (%)	Yes *N* (%)
Gender	Boys (159)	143 (89.9)	16 (10.1)	150 (94.3)	9 (5.7)
	Girls (128)	111 (86.7)	17 (13.3)	119 (93.0)	9 (7.0)
Location	Lammi (140)	117 (83.6)	23 (16.4)	129 (92.1)	11 (7.9)
	Jalasjärvi (147)	137 (93.2)	10 (6.8)	140 (95.2)	7 (4.8)
Acute otitis media	None (84)	79 (94.0)	5 (6.0)	82 (97.6)	2 (2.4)
	≥1 (203)	175 (86.2)	28 (13.8)	187 (92.1)	16 (7.9)
Upper resp. tract infection	None (70)	64 (91.4)	6 (8.6)	67 (95.7)	3 (4.3)
	≥1 (217)	190 (87.6)	27 (12.4)	202 (93.1)	15 (6.9)
Lower resp. tract infection	None (142)	127 (89.4)	15 (10.6)	133 (93.7)	9 (6.3)
	≥1 (145)	127 (87.6)	18 (12.4)	136 (93.8)	9 (6.2)
Gastroenteritis	None (215)	193 (98.8)	22 (10.2)	199 (92.6)	16 (7.4)
	≥1 (72)	61 (84.7)	11 (15.3)	70 (97.2)	2 (2.8)
Urinary tract infections	None (275)	244 (88.7)	31 (11.3)	257 (93.5)	18 (6.5)
	≥1 (12)	10 (83.3)	2 (16.7)	12 (100.0)	0 (0.0)
Amoxicillin	None (136)	127 (93.4)	9 (6.6)	132 (97.1)	4 (2.9)
	≥1 (151)	127 (84.1)	24 (15.9)	137 (90.7)	14 (9.3)
Penicillin	None (170)	155 (91.2)	15 (8.8)	160 (94.1)	10 (5.9)
	≥1 (117)	99 (84.5)	18 (15.4)	109 (93.5)	8 (6.8)
Cephalosporin	None (188)	170 (90.4)	18 (9.6)	175 (93.1)	13 (6.9)
	≥1 (99)	84 (84.8)	15 (15.2)	94 (94.9)	5 (5.1)
Sulfonamide and trimethoprim	None (159)	141 (88.7)	18 (11.3)	149 (93.7)	10 (6.3)
	≥1 (128)	113 (88.3)	15 (11.7)	120 (93.8)	8 (6.3)
Macrolide	None (223)	201 (90.1)	22 (9.9)	211 (94.6)	12 (5.4)
	≥1 (64)	53 (82.8)	11 (17.2)	58 (90.6)	6 (9.4)
Antitussives	None (155)	138 (89.0)	17 (11.0)	143 (92.3)	12 (7.7)
	≥1 (132)	116 (87.9)	16 (12.1)	126 (95.5)	6 (4.5)
Antihistamines	None (196)	176 (89.8)	20 (10.2)	185 (94.4)	11 (5.6)
	≥1 (91)	78 (85.7)	13 (14.3)	84 (92.3)	7 (7.7)
Bronchodilators	None (233)	204 (87.6)	29 (12.4)	215 (92.3)	18 (7.7)
	≥1 (54)	50 (92.6)	4 (7.4)	54 (100.0)	0 (0.0)
Any antibiotic	None (53)	51 (96.2)	2 (3.8)	52 (98.1)	1 (1.9)
	≥1 (234)	203 (86.8)	31 (13.2)	217 (92.7)	17 (7.3)
Any infection	None (29)	28 (96.6)	1 (3.4)	28 (96.6)	1 (3.4)
	≥1 (258)	225 (87.6)	32 (12.4)	241 (93.4)	17 (6.6)

Table 2. The unadjusted odd ratios (uOR), adjusted odds ratios (aOR), and 95% confidence intervals of the binary logistic regression model for the occurrence of illnesses and the use of medications (grouped as 0 (reference) and ≥1) and MIH definitions.

		MIH		MIH2	
		uOR	aOR	uOR	aOR
Location	Lammi(ref.)/Jalasjärvi	**0.37 (0.17–0.81)**	**0.37 (0.17–0.82)**	0.59 (0.22–1.56)	0.52 (0.19–1.41)
Acute otitis media	0–1y	1.92 (0.92–4.00)	**2.28 (1.06–4.89)**	2.24 (0.86–5.85)	2.62 (0.97–7.04)
	0–3y	2.53 (0.94–6.79)	2.63 (0.97–7.14)	3.51 (0.79–15.61)	3.38 (0.75–15.15)
Upper resp. tract infection	0–1y	0.95 (0.46–1.96)	1.06 (0.50–2.23)	1.15 (0.44–2.99)	1.28 (0.48–3.39)
	0–3y	1.52 (0.60–3.84)	1.71 (0.66–4.41)	1.66 (0.47–5.91)	1.68 (0.47–6.03)
Lower resp. tract infection	0–1y	1.24 (0.56–2.74)	1.58 (0.69–3.61)	1.07 (0.37–3.12)	1.20 (0.40–3.61)
	0–3y	1.20 (0.58–2.50)	1.58 (0.73–3.41)	0.98 (0.38–2.54)	1.04 (0.38–2.81)
Gastroenteritis	0–1y	1.53 (0.49–4.77)	1.44 (0.45–4.55)	–	–
	0–3y	1.58 (0.73–3.45)	1.61 (0.73–3.54)	0.36 (0.08–1.59)	0.36 (0.08–1.61)
Urinary tract infections	0–1y	2.62 (0.26–25.89)	3.58 (0.33–38.02)	–	–
	0–3y	1.57 (0.33–7.52)	2.14 (0.42–10.90)	–	–
Any infection	0–1y	1.49 (0.66–3.34)	1.71 (0.75–3.89)	1.94 (0.62–6.07)	2.11 (0.67–6.69)
	0–3y	3.97 (0.52–30.15)	3.76 (0.49–28.97)	1.98 (0.25–15.41)	1.74 (0.22–13.76)
Amoxicillin	0–1y	1.27 (0.54–2.99)	1.36 (0.57–3.24)	1.53 (0.52–4.48)	1.59 (0.54–4.68)
	0–3y	**2.67 (1.19–5.96)**	**2.58 (1.14–5.83)**	**3.37 (1.08–10.51)**	**3.16 (1.01–9.91)**
Penicillin	0–1y	**2.80 (1.28–6.13)**	**2.61 (1.17–5.85)**	**2.93 (1.08–7.95)**	**3.16 (1.13–8.85)**
	0–3y	1.88 (0.91–3.90)	1.69 (0.80–3.58)	1.17 (0.45–3.07)	1.18 (0.44–3.16)
Cephalosporin	0–1y	1.74 (0.70–4.33)	1.68 (0.67–4.25)	0.74 (0.16–3.33)	0.75 (0.16–3.40)
	0–3y	1.69 (0.81–3.51)	1.67 (0.80–3.52)	0.72 (0.25–2.07)	0.73 (0.25–2.11)
Sulfonamide and trimethoprim	0–1y	1.03 (0.40–2.65)	1.88 (0.65–5.49)	1.35 (0.43–4.28)	1.85 (0.50–6.84)
	0–3y	1.04 (0.50–2.16)	1.81 (0.77–4.19)	0.99 (0.38–2.59)	1.17 (0.40–3.46)
Macrolide	0–1y	**2.91 (1.07–7.97)**	**4.07 (1.39–11.92)**	2.36 (0.63–8.82)	3.04 (0.77–11.98)
	0–3y	1.90 (0.87–4.16)	**2.57 (1.11–5.91)**	1.82 (0.65–5.06)	2.18 (0.75–6.34)
Antitussives	0–1y	**2.45 (1.11–5.43)**	1.96 (0.86–4.49)	1.73 (0.59–5.07)	1.61 (0.53–4.93)
	0–3y	1.12 (0.54–2.31)	0.83 (0.38–1.78)	0.57 (0.21–1.56)	0.46 (0.16–1.32)
Antihistamines	0–1y	1.33 (0.48–3.72)	1.39 (0.49–3.93)	1.48 (0.41–5.40)	1.49 (0.40–5.49)
	0–3y	1.47 (0.70–3.10)	1.44 (6.68–3.08)	1.40 (0.53–3.74)	1.34 (0.50–3.59)
Bronchodilators	0–1y	0.26 (0.03–2.00)	0.39 (0.05–3.10)	–	–
	0–3y	0.56 (0.19–1.67)	0.83 (0.26–2.64)	–	–
Any antibiotic	0–1y	1.72 (0.83–3.58)	2.11 (0.99–4.52)	1.53 (0.59–3.99)	1.74 (0.65–4.67)
	0–3y	3.89 (0.90–16.81)	3.92 (0.90–17.09)	4.07 (0.53–31.31)	3.83 (0.50–29.58)

The model was adjusted for age and location (p-value <0.05 in bold).

the risk of MIH2 by 50% ($p = 0.023$) (Table 3). Every extra episode of AOM within the first 3 years of life increased the risk of MIH by 19% ($p = 0.009$) and MIH2 by 18% ($p = 0.039$).

Other illnesses alone were not associated with MIH or MIH2, but when considering all illnesses together, every extra episode of any illness within the first year of life increased the risk of MIH by 8% ($p = 0.025$) (Table 3).

Medications

The number of children who have received certain medicines and the definitions of MIH are seen in Table 1. The total number of antibiotics was 1019 courses (mean 3.55, SD 3.58). The most commonly prescribed antibiotic during the first 3 years of life was amoxicillin with 306 courses in total (mean 1.07, SD 1.40), followed by sulphonamide-trimethoprim with 269 courses (mean 0.94, SD 1.45), cephalosporin with 179 courses (mean 0.62, SD 1.16), penicillin with 163 courses (mean 0.57, SD 0.83), macrolides with 84 courses (mean 0.29, SD 0.63) and other antibiotics with 18 courses (mean 0.06, SD 0.33). Other commonly prescribed medicines were antitussives with 235 courses (mean 0.82, SD 1.18), antihistamines with 133 courses (mean 0.46, SD 0.84) and bronchodilators with 97 courses (mean 0.34, SD 1.0). After adjustment for age and location, children who had received at least one course of penicillin within the first year of life had a 2.61 times higher risk for MIH ($p = 0.019$) and a 3.16 times higher risk for MIH2 ($p = 0.029$) (Table 2). Every extra course of penicillin during the first year increased the risk of MIH by 92% ($p = 0.039$) and the risk of MIH2 by 120% ($p = 0.049$) (Table 3).

Children who had received at least one course of amoxicillin within the first 3 years of life had a 2.58 times higher risk for MIH ($p = 0.022$) and a 3.16 times higher risk for MIH2 ($p = 0.048$) (Table 2). Every extra course of amoxicillin during the first 3 years of life increased the risk of MIH by 35% ($p = 0.008$) and the risk of MIH2 by 32% ($p = 0.042$) (Table 3).

Children who had received at least one course of macrolides within the first year of life had a 4.07 times higher risk for MIH ($p = 0.011$) (Table 2). Every extra course of macrolide antibiotic increased the risk of MIH by 210% ($p = 0.012$) (Table 3). Children who had received at least one course of macrolides within the first 3 years of life had a 2.57 times higher risk for MIH ($p = 0.027$) (Table 2). Every extra course of macrolides increased the risk of MIH by 92% ($p = 0.011$) (Table 3). Also, the risk of MIH2 was increased, but the association was not statistically significant (Table 3).

Other antibiotics were not associated with MIH or MIH2 alone but when all antibiotics where grouped together, every extra course of any antibiotic within the first year of life increased the risk of MIH by 28% ($p = 0.024$) and within the first 3 years by 14% ($p = 0.007$) (Table 3). Similarly, statistically significant risk was not seen for MIH2.

Antitussives were associated with MIH, but after adjustment for age and location significant association was not seen. However, every extra course of antihistamines within the first 3 years of life increased the risk of MIH by 57% ($p = 0.011$) (Table 3).

Table 3. The unadjusted odd ratios (uOR), adjusted odd ratios (aOR), and 95% confidence intervals of the binary logistic regression model for the cumulative number of illnesses/medications and MIH definitions.

		MIH		MIH2	
		uOR	aOR	uOR	aOR
Acute otitis media	0–1y	1.31 (0.98–1.75)	**1.42 (1.05–1.92)**	**1.42 (1.01–1.99)**	**1.50 (1.06–2.13)**
	0–3y	**1.16 (1.03–1.32)**	**1.19 (1.04–1.35)**	**1.17 (1.00–1.37)**	**1.18 (1.01–1.38)**
Upper resp. tract infection	0–1y	0.91 (0.63–1.31)	0.97 (0.67–1.41)	0.99 (0.63–1.56)	1.03 (0.66–1.62)
	0–3y	1.07 (0.93–1.24)	1.13 (0.97–1.32)	0.99 (0.81–1.22)	1.01 (0.82–1.25)
Lower resp. tract infection	0–1y	1.05 (0.66–1.67)	1.19 (0.75–1.91)	0.88 (0.44–1.75)	0.92 (0.46–1.86)
	0–3y	0.98 (0.79–1.20)	1.06 (0.86–1.31)	0.88 (0.63–1.24)	0.90 (0.63–1.28)
Gastroenteritis	0–1y	1.41 (0.48–4.14)	1.30 (0.44–3.84)	–	–
	0–3y	1.25 (0.74–2.12)	1.26 (0.74–2.16)	0.38 (0.09–1.51)	0.38 (0.09–1.52)
Urinary tract infections	0–1y	3.23 (0.67–15.55)	4.30 (0.87– 21.34)	–	–
	0–3y	1.53 (0.56–4.17)	1.95 (0.69–5.50)	–	–
Any infection	0–1y	1.08 (0.92–1.25)	1.14 (0.97–1.34)	1.07 (0.87–1.30)	1.10 (0.98–1.35)
	0–3y	1.05 (0.99–1.11)	**1.08 (1.01–1.15)**	1.02 (0.94–1.10)	1.03 (0.94–1.12)
Amoxicillin	0–1y	1.31 (0.79–2.14)	1.42 (0.84–2.38)	1.54 (0.86–2.74)	1.60 (0.88–2.89)
	0–3y	**1.35 (1.09–1.68)**	**1.35 (1.08–1.68)**	**1.34 (1.03–1.74)**	**1.32 (1.01–1.72)**
Penicillin	0–1y	**2.11 (1.16–3.86)**	**1.92 (1.03–3.58)**	2.09 (0.99–4.44)	**2.20 (1.00–4.83)**
	0–3y	1.43 (0.97–2.09)	1.36 (0.90–2.03)	1.15 (0.68–1.97)	1.21 (0.68–2.14)
Cephalosporin	0–1y	1.18 (0.60–2.31)	1.10 (0.56–2.16)	0.69 (0.19–2.53)	0.70 (0.19–2.61)
	0–3y	1.23 (0.96–1.58)	1.25 (0.96–1.61)	0.94 (0.60–1.48)	0.96 (0.63–1.52)
Sulfonamide and trimethoprim	0–1y	0.96 (0.55–1.82)	1.40 (0.74–2.27)	0.94 (0.41–2.15)	1.06 (0.44–2.56)
	0–3y	0.93 (0.71–1.23)	1.10 (0.82–1.48)	0.94 (0.66–1.35)	0.99 (0.67–1.47)
Macrolide	0–1y	**2.42 (1.06–5.54)**	**3.10 (1.28–7.52)**	1.73 (0.56–5.34)	2.08 (0.66–6.61)
	0–3y	1.53 (0.96–2.43)	**1.92 (1.17–3.16)**	1.25 (0.65–2.38)	1.39 (0.71–2.74)
Antitussives	0–1y	**1.93 (1.16–3.21)**	1.70 (0.99–2.89)	1.13 (0.51–2.51)	1.07 (0.46–2.47)
	0–3y	1.23 (0.95–1.61)	1.11 (0.83–1.47)	0.72 (0.41–1.25)	0.63 (0.35–1.15)
Antihistamines	0–1y	1.21 (0.47–3.14)	1.30 (0.48–3.47)	1.33 (0.40–4.41)	1.38 (0.40–4.74)
	0–3y	**1.59 (1.13–2.23)**	**1.57 (1.11–2.22)**	1.21 (0.74–1.97)	1.19 (0.72–1.95)
Bronchodilators	0–1y	0.56 (0.15–2.06)	0.77 (0.22–2.70)	–	–
	0–3y	0.87 (0.53–1.41)	1.02 (0.65–1.59)	–	–
Any antibiotic	0–1y	1.21 (0.99–1.48)	**1.28 (1.03–1.58)**	1.16 (0.89–1.52)	1.22 (0.93–1.60)
	0–3y	**1.10 (1.01–1.20)**	**1.14 (1.04–1.25)**	1.05 (0.93–1.19)	1.07 (0.94–1.21)

The model was adjusted for age and location (p-value <0.05 in bold). ORs are displayed per marginal episode/course increase.

Discussion

In this study, elementary school children from two Finnish rural towns, Lammi and Jalasjärvi, were examined for demarcated hypomineralization lesions in FPMs and incisors (MIH). Their health and medication history from the first 3 years of life was screened. By locating the study into two rural towns, the health data from the subject's first 3 years of life was obtained comprehensively, since public health center services are mainly used for a number of reasons: public health centers provide a high level of medical care, are free of charge for children, and private services are rare or non-existent. All children from the 2nd to 5th grades in the biggest elementary schools who were born in these areas were invited, and 80% of them participated in the study. After excluding children with incomplete medical records and severe medical problems (cancer, low birth weight), the participation percentage was 72%. As the examinations were part of the dental check-ups that were offered to everyone regardless of the decision to participate the study, participation should not have been affected by bias.

The results of this study support the hypothesis that childhood medical problems increase the risk of MIH. The children with MIH had sought care for illnesses more often than the children without MIH. However, when different illnesses were analyzed separately, only children with at least one episode of AOM within the first year of life had an elevated risk for MIH. Other illnesses did not show any association. The association between AOM and hypomineralization lesions has been mentioned in some of the earlier studies on MIH,[7,8] as well as in an Icelandic study which found that demarcated opacities in maxillary incisors were related to a history of otitis media.[23] In this study, other common infectious illnesses were not significantly associated with MIH. Because of the small study size, it was not possible to study the more rare infections, for example chicken pox, which was rarely suffered in the present study population under the age of 3. Several questionnaire or interview based studies have found some association between MIH and respiratory illnesses.[7–11,14,24] Recently, Kuhnisch et al. conducted a prospective study on MIH and respiratory infections [12] and found that children with at least one (lower) respiratory infection during the first 4 years of life had a significantly higher risk for the development of MIH2, but not for MIH. Our results support the authors' speculation that the key factor for induction of hypomineralization could be antimicrobial therapy rather than illness itself, because in the present study, antibiotics were associated with MIH2, but respiratory illnesses were not.

Results of several studies obtained using questionnaires already suggested that antibiotics are risk factors of hypomineralization.[7,10,13,14,25] To our knowledge, no other study investigating antibiotics as etiological factors of MIH has been made using medical files. In Finland, systemic antibiotics can be received only with a prescription written by a doctor. Thus information on the use of antibiotics ought to be comprehensive except that patient compliance cannot be verified.

The results indicate that children who had received at least one course of amoxicillin or penicillin had a higher risk for both MIH and MIH2. Also, macrolides increased the risk of MIH, which is in line with the earlier study by the authors.[15] The other antibiotics included, such as sulfonamide and

trimethoprim, which were used almost as often as amoxicillin, did not increase the risk of MIH or MIH2. Fagrell et al. prospectively collected information on the use of antibiotics from diaries and interviews.[26] As opposed to the results of this study, they did not find any association with antibiotics but found that extended breastfeeding was associated with MIH. Possibly, the interview-based study setting or the grouping of antibiotics into penicillin V and others explain the difference in results compared with this study.

In experimental studies, amoxicillin has disturbed the development of enamel. Ameloblasts in mouse molar teeth cultured in a high dose of amoxicillin were poorly organized and the nascent enamel was often non-homogeneous.[15,27] Also, a dose-dependent hypomineralization was histologically observed in a study by Gottberg et al.[28] Souza et al. found that amoxicillin interfered with the initial stages of amelogenesis in the upper first molars of rats exposed pre- and postnatally. Structural changes in the ameloblasts and a reduction of the enamel matrix were observed.[29] Furthermore, amoxicillin and clavulanic acid given daily to mice for 60 days affected the function of ameloblasts in lower incisors, especially in the maturation phase. This resulted in both qualitative and quantitative defects in enamel.[30] Kuscu et al. exposed piglets to amoxicillin and did not observe any clinical signs of MIH, but reduced mineral density was seen at a microscopic level.[31] These results are not directly comparable to humans, but they give rise to the speculation that agents such as amoxicillin or penicillin, which both are beta-lactam antibiotics, cause cell-level changes in enamel development. In this study, macrolides were associated with MIH as well. In rats, a macrolide antibiotic altered the functioning of ameloblasts at the transitional stage.[32]

A study by Wogelius et al. found that inhaled β_2-agonists and inhaled corticosteroids increased the risk of more severe MIH defects.[16] In the present study, only the use of systemic (oral) asthma medication was recorded and an association with MIH or MIH2 was not found. There were a very limited number of children who had received oral bronchodilators in this study. Instead, every extra course of antihistamines within the first three years of life increased the risk of MIH. This is a new finding, which raises the question of a possible association with MIH and allergies or allergy medication which should be further studied.

Although this study was conducted retrospectively, the health and medication data were recorded prospectively by doctors at the time of the diagnosis of a health event. Still, information is missing on health events that did not require a visit to a doctor. Therefore, it is likely that some illnesses, such as common colds, i.e. upper respiratory infections, are under-reported in this study. However, the information on more severe illnesses ought to be comprehensive.

There were significantly fewer children with MIH in Jalasjärvi than in Lammi. Locations differ in water fluoride content (0.5–1.0 mg/L in Jalasjärvi vs. $<$0.2 mg/L in Lammi). As discussed in a previous study by the authors, it can be speculated that the lower prevalence of MIH in an area with higher fluoride content is due to the post-eruption mineralisation effect of fluoride from drinking water.[33] The prevalence of MIH2 was not significantly different between locations, possibly because children with MIH lesions in both molars and incisors often have moderate and severe lesions.[34,35] The more severe lesions would not be diminished by the fluoride in water after eruption. In the present study, the analyses of other risk factors were adjusted for the locality.

In conclusion, the results of this study with comprehensive medical files support the earlier finding that children with MIH have suffered from illnesses more often than their peers. When illnesses and antibiotics were classified into groups, it was seen that children who have had AOM or who have received penicillin, amoxicillin or macrolides in early childhood carry a greater risk of having demarcated hypomineralization in their permanent first molars and incisors. The occurrence of illnesses and the use of antibiotics were strongly correlated with each other, and therefore it was not possible to evaluate their independent effects. However, while experimental studies are sparse on the effects of illnesses on enamel development, there are a growing number of reports showing that certain antibiotics interfere with amelogenesis.[15,27–32] This study supports the hypothesis of illnesses and/or antibiotics as etiologic factors of MIH and can be used as a basis for later studies.

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