

## Research Article

# Prenatal and Early Childhood Determinants of Enamel Hypoplasia in Infants

Schroth RJ<sup>1-4\*</sup>, Dhalla S<sup>4</sup>, Tate R<sup>3</sup>, Moffatt MEK<sup>2,3</sup>

<sup>1</sup>Department of Preventive Dental Science, Dr. Gerald Niznick College of Dentistry, Rady Faculty of Health Sciences, University of Manitoba, Canada

<sup>2</sup>Department of Pediatrics and Child Health, Max Rady College of Medicine, Rady Faculty of Health Sciences, University of Manitoba, Canada

<sup>3</sup>Department of Community Health Sciences, Max Rady College of Medicine, Rady Faculty of Health Sciences, University of Manitoba, Canada

<sup>4</sup>Children's Hospital Research Institute of Manitoba, Canada

**\*Corresponding Author:** Dr. Robert J Schroth, 507 - 715 McDermot Avenue, Winnipeg, Manitoba, Canada, Tel: 204-975-7764; Fax: 204-977-5691; E-mail: [robert.schroth@umanitoba.ca](mailto:robert.schroth@umanitoba.ca)

**Received:** 30 December 2021; **Accepted:** 20 January 2021; **Published:** 28 January 2021

**Citation:** Schroth RJ, Dhalla S, Tate R, Moffatt MEK. Prenatal and Early Childhood Determinants of Enamel Hypoplasia in Infants. Journal of Pediatrics, Perinatology and Child Health 5 (2021): 005-017.

### Abstract

**Objective:** Enamel hypoplasia (EH) is a recognized risk factor for dental caries. The purpose was to investigate the relationship between prenatal and early life risk factors, including prenatal nutritional status, and EH among infants.

**Methods:** Pregnant mothers from an at-risk urban population in Manitoba, Canada were recruited during the second or early third trimester into a prospective cohort study. At baseline participants completed a questionnaire and provided a serum sample to analyze

calcium and 25-hydroxyvitamin D [25(OH)D]. Dental examinations were completed at one year of age by an examiner blinded to mothers' serum nutrient levels and parents completed a questionnaire by interview. Enamel defects were determined using the DDE index. Data were analyzed using descriptive techniques and regression analysis. A p value < 0.05 was significant.

**Results:** Overall, 22% of infants had EH. Although mothers of infants with EH had lower mean concentrations of 25(OH)D, they did not statistically

differ from values associated with children without EH ( $p=0.072$ ). Infants with EH were significantly more likely to have early childhood caries (73% vs. 27%,  $p<.001$ ). Four factors were significantly and independently associated with EH: prenatal maternal calcium levels ( $p=.030$ ), not having heard of vitamin D ( $p=.033$ ), not drinking milk frequently during pregnancy ( $p=.034$ ), and not consuming margarine daily ( $p=.023$ ).

**Conclusions:** Several prenatal and early childhood determinants were identified to be associated with EH in infants and, due to the strong association with early childhood caries, the management of EH risk factors is critical to reducing oral health disparities in at-risk children.

**Keywords:** Infant; Dental enamel hypoplasia; Pregnancy; Vitamin D; Calcium

## 1. Introduction

Dental enamel is a mineralized tissue that covers the coronal surface of teeth. Developmental defects of enamel (DDE) are irreversible and permanent insults to enamel formation in both the primary and permanent dentitions that begins to form during week 18 in utero and continues to form until early infancy [1, 2]. DDE have been implicated as risk factors for early childhood caries (ECC) because the abnormal structure and morphology of the affected teeth provide suitable local environments for adhesion and colonization of cariogenic bacteria [3-6]. Although DDE are now increasingly recognized as a risk factor for caries, identification, clinical implications, preventive and restorative management of DDE are not currently well recognized by many practitioners [7, 8].

Enamel hypoplasia (EH) is one of the most frequently observed categories of DDE that is marked by reduced or altered amounts of enamel caused by insult to the

ameloblast cells [9]. The other group of DDEs are classified as opacities. Clinically, EH is visually and morphologically identified by irregularities such as pits, grooves, or missing enamel of varying size [5, 7, 10]. Over the years, several risk factors for EH have been identified, ranging from metabolic diseases and nutritional disorders/deficiencies to premature birth and low birth weight [10-18]. Nutritional deficiencies of vitamins A, C, D, and calcium are of importance due to their role in enamel development, formation, and structure. In addition to nutritional deficiencies, enamel defects may also be associated with social risk factors, including socioeconomic inequalities and demographic variables (e.g. family income, employment status, quality of life) that predispose them to insults during enamel development [4, 19-21]. Children from rural locales appear to demonstrate an increased prevalence in EH [22]. The amount of sunshine mothers received during their pregnancy is also of interest, as it alters vitamin D and calcium regulation [23]. Mothers who are young and/or have a delayed first obstetrical visit may have offspring with EH in their baby teeth [20, 24].

Because of the association between EH and ECC, risk factors and etiologies disrupting enamel formation in utero and early infancy are crucial to developing targeted prevention and restorative care, which may aid in combating the burden of ECC. The purpose of this study was to investigate the relationship between DDE and prenatal and early childhood risk factors, with particular attention to prenatal nutritional status, socioeconomic status (SES) and ethnicity, and infant birth and health status.

## 2. Materials and Methods

Participants and their offspring in this study were part of a broader prospective birth cohort investigation examining the impact of prenatal nutritional status and caries in infants [25]. Participants were recruited during

the second or early third trimester in order to later investigate the association between prenatal nutritional status and EH in a vulnerable urban population in Manitoba, Canada. A serum sample was collected from mothers during second or early third trimester, during which primary maxillary incisors begin to calcify in utero. Samples were analyzed for levels of total calcium, inorganic phosphorus, alkaline phosphatase, and 25-hydroxyvitamin D [25(OH)D]. 25(OH)D was assessed via radioimmunoassay using a DiaSorin kit (DiaSorin, Inc, Stillwater, MN). Participants also completed a baseline questionnaire conducted by interview to collect information on nutritional deficiencies, demographic characteristics, pregnancy, health conditions, nutrition, and awareness of ECC. Exposure to sunlight, family composition, finances, and employment were also assessed.

Around 12 months of age, a clinical assessment of the primary dentition was performed by RJS, who was blinded to maternal prenatal serum nutritional levels. DDE, such as EH and opacities, were also recorded. The modified DDE index was used to assess opacities and EH [26]. ECC, and Severe-ECC (S-ECC) were documented according to recognized definitions [27]. Decayed, extracted, and filled primary teeth, as well as cumulative totals of decayed primary teeth (dt score) were also recorded. A follow-up questionnaire, at the time of the infant dental examination, was completed on demographic characteristics, household finances, birth weight, prematurity, feeding practices, and infant health status. Caregivers were also asked about the age of the eruption of the first tooth, oral hygiene practices, and whether their child had visited the dentist.

Data were entered into a Microsoft Office Access database (Microsoft Corporation, Redmond, WA) and data were analyzed using NCSS2007 (Kaysville, UT) and SPSS version 17.0 (IBM SPSS Statistics, IBM

Corporation, Armonk, NY). Analysis included descriptive statistics, chi-squared analysis, *t* tests, correlation, and analysis of variance. Five separate logistic regression models for EH were performed in order to prevent over-fitting of the models due to the small sample size of maternal-infant pairs. Models were based upon the following themes: 1) prenatal serum metabolites 2) maternal awareness of calcium and vitamin D, 3) prenatal care and diet, 4) socioeconomic status (SES) and ethnicity, and 5) the infant's birth and health status. A final model was constructed, including independent variables significantly associated or approximating the threshold of significance with EH in these separate models, in addition to variables routinely reported to be associated with EH in the literature. For continuous variables, odds ratios and confidence intervals were calculated to reflect a difference of 1 SD unit of the variable. For example, the odds ratio for 25OHD reflected a 25 unit difference. A *p* value  $\leq 0.05$  was statistically significant.

### 3. Results

Overall, 207 women were recruited (mean age  $19 \pm 5$  years) and 93% resided in Winnipeg. While 71% reported taking vitamins during pregnancy, only 37% did so daily. Characteristics of participants and offspring appear in Table 1 and have been previously reported [25]. Full laboratory reports were available for 200 participants. The mean 25(OH)D level was  $48 \pm 24$  nmol/L (median 43); 65 (32.5%) participants had deficient concentrations ( $<35$  nmol/L) while only 24 (12%) had optimal levels ( $\geq 75$  nmol/L). Mean calcium, phosphate and alkaline phosphate levels were  $2.25 \pm 0.10$  mmol/L,  $1.15 \pm 0.19$  mmol/L, and  $98 \pm 52$  U/L, respectively. Despite losses to follow-up, 64% ( $n=133$ ) returned with their infant for follow-up. There were no differences in age ( $p=0.24$ ), level of education ( $p=0.74$ ), or ethnic heritage ( $p=0.24$ ) between women lost to follow-up and those remaining in the study. Further,

there was no difference in the 25(OH)D levels between these two groups ( $50 \pm 26$  nmol/L vs.  $45 \pm 20$ ,  $p=0.08$ ). Overall, 135 infants (2 sets of twins), 56% male, with a mean age of  $16 \pm 7$  months (median 13) returned.

In total, 22% had EH (29 of 134), most often as pits and missing enamel. A majority of EH in the maxillary and mandibular arch were found on the primary maxillary incisors and primary central incisors, respectively. Table 2 shows the relationship between mean 25(OH)D levels and DDE and EH. There was no significant relationship between circulating levels of maternal 25(OH)D and the presence of DDEs among infants ( $p=0.91$ ). Although mothers of infants with EH had what appeared to be lower mean concentrations of 25(OH)D, they did not statistically differ from values associated with children who did not have EH ( $p=0.072$ ). Chi-square analyses revealed no significant associations between mothers with 25(OH)D concentrations  $> 35$  nmol/L or  $\leq 35$  nmol/L and DDEs ( $p=.65$ ) and EH ( $p=0.18$ ). Likewise, no significant associations were found between whether mothers had concentrations  $\geq 50$  nmol/L or  $< 50$  nmol/L and DDEs ( $p=0.39$ ) and EH ( $p=0.18$ ). In addition, there were no apparent associations between whether mothers had concentrations  $< 75$  nmol/L or  $\geq 75$  nmol/L and DDEs ( $p=0.93$ ) and EH ( $p=0.18$ ). Mothers of infants without EH were significantly more likely to have heard of vitamin D ( $p=0.01$ ), more likely to have been recommended to take vitamins by their physician ( $p=0.012$ ), likely to have consumed milk ( $p<0.001$ ) and margarine more frequently ( $p=0.011$ ), and less likely to have had older children undergo general anesthesia for pediatric dental surgery ( $p<0.005$ ) (Table 3).

The  $\chi^2$  analysis (data not shown) revealed that infants with EH were significantly more likely to have ECC (73% vs. 27%,  $p<.001$ ). No significant age differences existed between infants with and without EH ( $14.8 \pm 5.2$  months vs.  $16.4 \pm 7.9$ ,  $p=0.18$ ). Likewise, there was no

significant association between gestational diabetes and EH ( $p=0.77$ ) or prematurity and EH ( $p=0.28$ ). Overall, no significant associations were found between the presence of EH and the remaining variables collected in the infant questionnaire. The first logistic regression model, from the series of five conducted, showed that serum calcium was the only metabolite of interest significantly associated with EH in infants ( $p=0.05$ ) (Table 4). Further, results of backwards logistic regression analysis (data not shown) also confirmed this relationship as only calcium remained in the model ( $p=0.04$ ). The second logistic regression model revealed no significant relationship with maternal awareness of calcium or vitamin D and EH (Table 4). However, it did suggest that offspring of participants who had heard of vitamin D were less likely to have EH ( $p=0.053$ ).

The logistic regression model for prenatal care and diet revealed significant associations between the presence or absence of EH and prenatal serum calcium levels of mothers ( $p=0.019$ ) and margarine use ( $p=0.022$ ). Those who did not eat margarine often, defined as once a day or more, were more than three times as likely to have an infant with EH (OR =  $1/0.31 = 3.2$ ). Results from a backwards logistic regression model involving the same variables included in this third model in Table 4 identified three variables as being significantly associated with EH: maternal prenatal calcium levels ( $p=0.01$ ), not drinking milk often ( $p=0.009$ ), and not consuming margarine often ( $p=0.017$ ). No significant association was identified between household employment and EH (Table 4). However, when a separate model was run for EH with the economic variables (data not shown) like participant's annual income during pregnancy ( $< \$18,000$  vs.  $\geq \$18,000$ ), full-time employment (not full-time vs. full-time), and whether the parent at the time of the infant's examination was receiving government assistance (yes vs. no), only those not benefiting from someone in the

home working full-time during pregnancy was significantly associated with EH (p= 0.019). In addition, no significant associations were identified between infant birth characteristics and health status and EH (Table 4).

An overall final logistic regression model for EH was constructed incorporating seven different variables, revealing that the following variables were significantly and independently associated with EH: maternal prenatal serum calcium levels (p=0.034), margarine intake (p=0.024), and maternal awareness of vitamin D (p=0.036), while not drinking milk often just failed to reach the threshold for significance (p=0.057) (Table 5). After removing the variable of serious medical problem(s) during infancy from the model, the same variables identified in Table 5 remained significant, however, not drinking milk often also became statistically significant (p=.05) (data not shown).

Overall, lower prenatal calcium concentrations were associated with EH. Infants of mothers who lived in a household where no one was employed full-time during pregnancy were at greater risk for EH, while those whose mothers ate margarine, drank milk frequently, and had heard of vitamin D were significantly less likely to have EH.

Backwards logistic regression analysis for EH using the same variables included in Table 5 revealed that the final iteration contained five of these variables, but only four were significantly and independently associated with EH (Table 5). The five remaining variables included low prenatal calcium levels (p=.030), not having heard of vitamin D (p=.033), not drinking milk frequently during pregnancy (p=.034), not using margarine daily (p=.023), and no one in the household with full-time employment during pregnancy (p=.066).

<b>Variable</b>	<b>Total Number in Cohort (% if applicable)</b>
<b>Maternal Characteristics</b>	
Mean age (years)	19 ± 5
Resided in Winnipeg	
Yes	190 (93)
No	15 (7)
Self-rated prenatal health status*	
Good	130 (64)
Average	70 (34)
Poor	5 (2)
Primigravid	
Yes	125 (61)
No	81 (39)
Drink milk	
Often (daily)	103 (50)
Sometimes (more than once a week)	68 (33)
Rarely (less than once a week)	20 (10)
Never	15 (7)
Daily vitamin use	

Yes	74 (37)
No	125 (63)
Education level	
< High school	190 (92)
≥ High school	16 (8)
Annual income	
≤ \$18,000	196 (95)
> \$18,000	10 (5)
Sun exposure (summer May - October)	
Spent time outside in sunshine	9 (7)
Did not spend time outside in sunshine	117 (93)
<b>Infant Characteristics</b>	
Sex	
Male	75 (56)
Female	60 (44)
Premature	
Yes	17 (13)
No	117 (87)
Low birth weight	
Yes	6 (5)
No	124 (95)
Mean birth weight (grams)	3490 ± 561
Breast-fed	
Yes	97 (74)
No	35 (26)
Bottle-fed	
Yes	130 (96)
No	5 (4)
Mean Age at eruption of first tooth (months)	6 ± 2
Health rating by caregiver	
Very good	75 (56)
Good	51 (38)
Fair	8 (6)

**Table 1:** Maternal and infant characteristics.

<b>Oral Health Outcomes</b>	<b>Maternal 25(OH)D</b>
-----------------------------	-------------------------

	<b>N</b>	<b>Mean ± S. D.</b>	<b>Median</b>	<b>P value</b>
Developmental Defects of Enamel				0.91
Yes	122	50.3 ± 26.5	46	
No	7	49.1 ± 25.5	45	
Enamel Hypoplasia				0.072 <sup>a</sup>
Yes	28	43.2 ± 21.1	39.5	
No	104	51.4 ± 27.4	46.5	

T test analysis <sup>a</sup>One-tailed \*2 tailed p=0.14

**Table 2:** Relationship between oral health outcomes and maternal 25(OH)D.

<b>Variable</b>	<b>No Enamel Hypoplasia (%)</b>	<b>Enamel Hypoplasia (%)</b>	<b>P value</b>
Doctor recommended vitamins			0.012
No	6 (50.0)	6 (50.0)	
Yes	99 (81.1)	23 (18.9)	
Heard of vitamin D			.01
No	34 (66.7)	17 (33.3)	
Yes	71 (85.5)	12 (14.5)	
Identified what calcium important for			.021
Correct	78 (83.9)	15 (16.1)	
Didn't Know	25 (69.4)	11 (30.6)	
Incorrect	2 (40.0)	3 (60.0)	
Weak arm/leg			.030
No	88 (82.2)	19 (17.8)	
Yes	17 (63.0)	10 (37.0)	
Drink milk			<.001
Often	58 (86.6)	9 (13.4)	
Sometimes	32 (74.4)	11 (25.6)	
Rarely	4 (33.3)	8 (66.7)	
Never	11 (91.7)	1 (8.3)	
Milk upset stomach			.016
No	87 (82.9)	18 (17.1)	
Yes	18 (62.1)	11 (37.9)	
Eat margarine			.011
Often	61 (85.9)	10 (14.1)	
Sometime	41 (71.9)	16 (28.1)	
Rarely	1 (25.0)	3 (75.0)	
Never	2 (100.0)	0	

Variable	No Enamel Hypoplasia (%)	Enamel Hypoplasia (%)	P value
Overall Milk Consumption			<.01
Often	71 (84.5)	13 (15.5)	
Sometime	26 (72.2)	10 (27.8)	
Rarely	1 (20.0)	4 (80.0)	
Never	7 (77.8)	2 (22.2)	
Heard of ECC			.044
No	25 (92.6)	2 (7.4)	
Yes	80 (74.8)	27 (25.2)	
Older children had general anaesthesia for dental surgery			<.005
No	31 (83.8)	6 (16.2)	
Yes	4 (40.0)	6 (60.0)	
Household income			.034
Full-Time	34 (91.9)	3 (8.1)	
Government Assist	51 (72.9)	19 (27.1)	
Part-Time	7 (58.3)	5 (41.7)	
Other	13 (86.7)	2 (13.3)	

**Table 3:** Prenatal variables associated with enamel hypoplasia.

Serum metabolites of alkaline phosphatase, calcium, phosphorus, and 25(OH)D (R <sup>2</sup> = 6.0%)				
Variable	Regression Coefficient (b) (SE)	Adjusted Odds Ratio	± 95% Confidence Interval	P value
Alkaline Phosphatase	-0.0057	0.74	0.42, 1.33	0.32
Calcium	-4.84	0.62	0.38, 1.00	.050
Phosphorus	-0.14	0.97	0.61, 1.54	.91
25(OH)D	-0.013	0.72	0.47, 1.14	.16
Maternal awareness and knowledge of calcium and vitamin D (R <sup>2</sup> = 6.4%)				
Heard of vitamin D (reference: no)	-0.88 (0.45)	0.42	0.17, 1.01	.053
Knew what calcium is important for (reference: no)	-0.72 (0.46)	0.48	0.20, 1.19	.11
Prenatal care and diet (R <sup>2</sup> = 17.6%)				
Calcium	-6.35 (2.70)	0.53	0.31, 0.90	.019
Gestational diabetes (reference: no)	0.004 (0.73)	1.00	0.24, 4.21	1.00
Drink milk (reference: < often)	-1.04 (0.55)	0.35	0.12, 1.05	.061
Doctor recommended vitamins (reference: no)	-0.48 (0.72)	0.62	0.15, 2.52	.50

Eat margarine (reference: < often)	-1.18 (0.52)	0.31	0.11, 0.84	.022
Received Healthy Baby benefit (reference: no)	0.41 (0.64)	1.50	0.43, 5.22	.52
Milk upsets stomach (reference: no)	0.74 (0.56)	2.10	0.70, 6.29	.19
25(OH)D	-0.0051 (0.010)	0.88	0.53, 1.45	0.62
<b>SES &amp; ethnicity (R<sup>2</sup>= 5.1%)</b>				
Not of Indigenous heritage (reference: no)	-0.86 (1.12)	0.42	0.047, 3.83	0.45
No one with full-time employment in household (reference: no)	1.21 (0.68)	3.35	0.88, 12.71	.076
<b>Infant birth characteristics and health status (R<sup>2</sup>= 4.9%)</b>				
Birth weight (grams)	0.0005 (0.00039)	1.00	0.99, 1.00	.21
Premature (reference: no)	0.99 (0.62)	2.70	0.80, 9.10	.11
Serious medical problem(s) (reference: no)	0.52 (0.45)	1.68	0.69, 4.09	.25

\*Enamel Hypoplasia reference = yes for all

**Table 4:** Logistic regression models for enamel hypoplasia.

<b>Variable</b>	<b>Regression Coefficient (b) (SE)</b>	<b>Adjusted Odds Ratio</b>	<b>± 95% Confidence Interval</b>	<b>P value</b>
<b>Final Logistic Regression Model for EH</b>				
Calcium	-5.53 (2.61)	0.57	0.35, 1.52	0.034
Drink milk (reference: < often)	-1.07 (0.56)	0.34	0.11, 1.03	.057
Eat margarine (reference: < often)	-1.14 (0.51)	0.32	0.12, 0.86	.024
No one with full-time employment in household (reference: no)	1.43 (0.83)	4.16	0.82, 21.03	.085
Heard of vitamin D (reference: no)	-1.03 (0.49)	0.36	0.14, 0.94	.036
Serious medical problem(s) (reference: no)	0.038 (0.55)	1.04	0.36, 3.03	.94
25(OH)D	-0.0017 (0.011)	0.96	0.89, 1.04	.87
<b>Final Backwards Logistic Regression Model for EH</b>				
Calcium	-5.57 (2.57)	0.57	0.35, 0.95	.030
No one with full-time employment in household (reference: no)	1.47 (0.80)	4.33	0.91, 20.69	.066
Drink milk (reference: < often)	-1.11 (0.52)	0.33	0.12, 0.92	.034
Eat margarine (reference: < often)	-1.14 (0.51)	0.32	0.12, 0.86	.023
Heard of vitamin D (reference: no)	-1.04 (0.49)	0.36	0.14, 0.92	.033

\*Enamel Hypoplasia reference = yes  $R^2= 19.9\%$

**Table 5:** Final logistic regression model for enamel hypoplasia.

#### 4. Discussion

Although several studies have reported associations between vitamin D status and caries, only two studies, to the best of our knowledge, have previously examined the relationship between prenatal vitamin D levels and the presence of EH [15, 28]. A recent pilot study reported that the presence of EH in the primary maxillary central incisors of 2-5 year old's was correlated with lower maternal serum circulating 25(OH)D concentrations during pregnancy [28]. In our current study, maternal 25(OH)D levels did not appear to be associated with the presence of EH, but was associated with ECC as reported in our past publication [25]. Variability in the maternal 25(OH)D concentrations have been previously observed between different weeks during pregnancy, suggesting a more elegant statistical method is required to examine this relationship [28].

As EH directly correlates with periods of tooth formation and maturation, we restricted our statistical analyses to those factors corresponding to the prenatal and birth periods. Some of the prenatal variables associated with EH in this study included not having heard of vitamin D, infrequent milk intake, and infrequent margarine use. Logistic regression analysis further supported these associations while controlling for additional factors such as the child's medical history and prenatal 25(OH)D concentration. The final model for EH revealed that infrequent milk consumption and infrequent margarine use were significantly associated with increased risk. Since milk intake is a significant predictor for 25(OH)D, it is possible these mothers who did not frequently consume milk and margarine had lower maternal vitamin D concentrations. This may

have resulted in disturbances of primary enamel formation during fetal development.

Additionally, awareness of vitamin D and receiving recommendations from a physician to take vitamins during pregnancy also appeared to be related to EH. Low awareness of vitamin D may be a proxy measure for dietary and supplemental intake of vitamin D. These individuals may not be consciously looking for foods rich in vitamin D when they shop for groceries and may not be taking supplements. It is plausible that women who are generally unaware of vitamin D and/or are not recommended to take vitamin supplements have lower vitamin D levels, which may have an impact on their infant's oral health.

No association was found between infant illnesses and medical conditions with EH, although several have been previously reported, ranging from metabolic diseases and nutritional disorders/deficiencies to premature birth and low birth weight [10, 13-15, 29]. When controlling for other variables in our final logistic regression model, serious childhood medical problems was not significantly associated with EH.

Results of the final logistic regression model revealed that lower maternal levels of calcium were significantly associated with EH. Although these mothers did not have extremely low or elevated calcium levels (range 2.01 - 2.57, median 2.24), results at the bivariate level revealed that mothers who had infants with EH had significantly lower calcium levels during pregnancy than those whose infants had no enamel defects ( $2.23 \pm 0.10$  mmol/L vs.  $2.27 \pm 0.10$ ,  $p=.036$ ). Calcium plays a critical role in the ordered crystalline and mineralized

structure of enamel by combining with inorganic phosphate to form hydroxyapatite and participating in ion transport during amelogenesis [30]. Disruptions in the formation of hydroxyapatite and ion exchanges result in hypomineralized enamel, which is soft and prone to caries [30]. This supports the association between low prenatal calcium and EH, and other studies have also confirmed hypocalcemia as a risk factor for EH [29, 31, 32].

An interesting finding from the bivariate analysis was the fact that participants who had a previous child undergo pediatric dental surgery in hospital to treat ECC had significantly lower vitamin D levels. These same participants were also significantly more likely to have other children with noticeable EH. While we are unaware of these participants' vitamin D status during their previous pregnancies, it is possible that their vitamin D levels were similar during both pregnancies if they had similar dietary and lifestyle choices. Low levels of vitamin D during a past pregnancy may have resulted in their older child having EH, which then placed them at increased risk for dental surgery under GA because of ECC. However, this hypothesis cannot be substantiated in this current investigation.

This study is not without limitations. We recruited a convenience sample of participants rather than randomly selecting them. Our results may not be generalizable to all populations, but our findings are generalizable to the urban Indigenous population in Winnipeg. Regardless, this investigation provides insight into the nutritional status of expectant women and the oral health of their infants.

The study was of moderate size and deliberately involved a high-risk population of mostly urban Indigenous persons, with limited education levels and incomes. The prospective design spanning the prenatal

period to infancy allowed the prevalence of EH in primary teeth of infants to be observed and permitted the study of prenatal and early childhood factors, including prenatal nutritional status. One of the strengths of cohort studies is reduced bias. Another notable strength was that dental assessments were made while being blinded to maternal prenatal levels of calcium, phosphate, alkaline phosphatase and 25(OH)D.

Overall, the study demonstrated that low calcium levels, maternal awareness of vitamin D, milk intake, and margarine use during pregnancy are associated with EH. Although there was no significant association between 25(OH)D concentration and EH, lower concentrations of 25(OH)D were observed in mothers with infants that had EH compared to those without EH. A previous publication involving this same cohort revealed that EH, along with infant age at the time of follow-up, and lower maternal prenatal 25(OH)D concentrations, were independent predictors of ECC [25]. Findings from this study have can influence early childhood oral health policies. Improving prenatal nutrition, when primary tooth formation begins may result in lower prevalence of EH and reduced risk for caries. Prevention efforts should begin during pregnancy by bolstering maternal nutrition, either through improved dietary intake or supplementation with calcium and possibly vitamin D.

#### **4. Conclusion**

Nearly one quarter of infants were identified as having EH in their primary. This study reveals that prenatal and early childhood factors, including prenatal maternal calcium levels may have an influence on the primary dentition, specifically the presence of EH. Lower prenatal calcium levels, low awareness of vitamin D, not consuming milk frequently during pregnancy, and not consuming margarine daily were independent determinants for EH.

## Acknowledgements

Grant funding for this study was provided by the Manitoba Medical Service Foundation, the Children's Hospital Research Institute of Manitoba, the Dentistry Canada Fund, and the Dr. Gerald Niznick College of Dentistry, University of Manitoba. Dr. Schroth received postdoctoral funding from the Children's Hospital Foundation of Manitoba and was a Canadian Institutes of Health Research Strategic Training Fellow in the Canadian Child Health Clinician Scientist Program. He presently holds a CIHR Embedded Clinician Researcher salary award.

## Conflict of Interest

RJS, SD, RT and MEKM declare no conflicts of interest.

## References

1. Nowak AJ. Pediatric dentistry: infancy through adolescence. 3<sup>rd</sup> Edn. Toronto: Saunders WB (1999).
2. Seow W. Oral complications of premature birth. *Aust Dent J* 31 (1989): 23-29.
3. Li Y, Navia J, Bian J. Caries experience in deciduous dentition of rural Chinese children 3-5 years old in relation to the presence or absence of enamel hypoplasia. *Caries Res* 30 (1996): 8-15.
4. Kanchanakamol U, Tuongratanaphan S, Tuongratanaphan S, et al. Prevalence of developmental enamel defects and dental caries in rural pre-school Thai children. *Community Dent Health* 13 (1996): 204-207.
5. Pascoe L, Seow W. Enamel hypoplasia and dental caries in Australian aboriginal children: prevalence and correlation between the two diseases. *Pediatr Dent* 16 (1994): 193-199.
6. Caufield PW, Li Y, Bromage TG. Hypoplasia-associated severe early childhood caries--a

proposed definition. *J Dent Res* 91 (2012): 544-550.

7. Commission on Oral Health RE. A review of the developmental defects of enamel index (DDE Index). *Int Dent J* 42 (1992): 411-426.
8. Dabiri D, Eckert GJ, Li Y, et al. Diagnosing Developmental Defects of Enamel: Pilot Study of Online Training and Accuracy. *Pediatr Dent* 40 (2018): 105-109.
9. Yadav P, Saha S, Jagannath GV, et al. Prevalence and Association of Developmental Defects of Enamel with, Dental- Caries and Nutritional Status in Pre-School Children, Lucknow. *J Clin Diagn Res* 9 (2015): 71-74.
10. Seow W. Enamel hypoplasia in the primary dentition: a review. *ASDC J Dent Child* 58 (1991): 441-452.
11. Commission on Oral Health RaE. An epidemiological index of developmental defects of dental enamel (DDE Index). *Int Dent J* 32 (1982): 159-167.
12. Organization WH. Oral health surveys: basic methods. In Ed.: Organization WH. 4<sup>th</sup> Edn. Geneva (1997).
13. Giunta J. Dental changes in hypervitaminosis D. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 85 (1998): 410-413.
14. Salanitri S, Seow W. Developmental enamel defects in the primary dentition: aetiology and clinical management. *Aust Dent J* 58 (2013): 133-140.
15. Cockburn F, Belton N, Purvis R, et al. Maternal vitamin D intake and mineral metabolism in mothers and their newborn infants. *Br Med J* 281 (1980): 11-14.
16. Seow W, Young W, Tsang A, et al. A study of primary dental enamel from preterm and full-term children using light and scanning electron microscopy. *Pediatr Dent* 27 (2006): 374-379.

17. Li Y, Navia J, Bian J. Prevalence and distribution of developmental enamel defects in primary dentition of Chinese children 3-5 years old. *Community Dent Oral Epidemiol* 23 (1995): 72-79.
18. Seow W, Perham S. Enamel hypoplasia in prematurely-born children: a scanning electron microscopic study. *J Pedod* 14 (1990): 235-239.
19. Seow W. Biological mechanisms of early childhood caries. *Community Dent Oral Epidemiol* 26 (1998): 8-27.
20. Needleman H, Allred E, Bellinger D, et al. Antecedents and correlates of hypoplastic enamel defects of primary incisors. *Pediatr Dent* 14 (1992): 158-166.
21. Sweeney E, Saffir A, De Leon R. Linear hypoplasia of deciduous incisor teeth in malnourished children. *Am J Clin Nutr* 24 (1971): 29-31.
22. Lukacs R, Walimbe S, Floyd B. Epidemiology of enamel hypoplasia in deciduous teeth: explaining variation in prevalence in western India. *Am J Hum Biol* 13 (2001): 788-807.
23. Mellander M, Noren J, Freden H, et al. Mineralization defects in deciduous teeth of low birthweight infants. *Acta Paediatr Scand* 71 (1982): 727-733.
24. Slayton R, Warren J, Kanellis M, et al. Prevalence of enamel hypoplasia and isolated opacities in the primary dentition. *Pediatr Dent* 23 (2001): 32-36.
25. Schroth RJ, Lavelle C, Tate R, et al. Prenatal Vitamin D and Dental Caries in Infants. *Pediatrics* 133 (2014): 1277-1284.
26. A review of the developmental defects of enamel index (DDE Index). Commission on Oral Health, Research & Epidemiology. Report of an FDI Working Group. *Int Dent J* 42 (1992): 411-426.
27. American Academy of Pediatric D. Policy on Early Childhood Caries (ECC): Classifications, Consequences, and Preventive Strategies. *Pediatr Dent* 39 (2017): 59-61.
28. Reed S, Voronca D, Wingate J, et al. Prenatal vitamin D and enamel hypoplasia in human primary maxillary central incisors: a pilot study *Pediatr Dent* 27 (2017): 21-28.
29. Aine L, Backstrom M, Maki R, et al. Enamel defects in primary and permanent teeth of children born prematurely. *J Oral Pathol Med* 29 (2000): 403-409.
30. Lacruz R, Habelitz S, Wright J, Paine M. Dental Enamel Formation and Implications for Oral Health and Disease. *Physiol Rev* 97 (2017): 939-993.
31. Guggenheimer J, Nowak A. Dental manifestations of the rubella syndrome. *Oral Surg Oral Med Oral Pathol* 32 (1971): 30-37.
32. Corrêa-Faria P, Martins-Júnior P, Vieira-Andrade R, et al. Perinatal factors associated with developmental defects of enamel in primary teeth: a case-control study. *Braz Oral Res* 27 (2013): 363-368.



This article is an open access article distributed under the terms and conditions of the [Creative Commons Attribution \(CC-BY\) license 4.0](https://creativecommons.org/licenses/by/4.0/)