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Cord-blood levels of heavy metals, vitamin D and calcium and the occurrence of development defects of enamel in primary incisors: A birth cohort study

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ABSTRACT

This study investigated the influence of vitamin D, calcium and heavy metals on the frequency of development defects of enamel (DDE) in primary incisors of infants. Serum levels of vitamin D, calcium, arsenic, cadmium, lead, and mercury in cord blood, as well as prenatal and perinatal data, were collected during pregnancy, at birth, and in follow-ups with newborns and infants ≤ 24 months. This was done using laboratory exams, interviews, and newborns' and infants' records. An examiner performed dental exams using the Modified DDE. DDE type, color, location, and severity were evaluated, and logistic multiple regression models were analyzed ($p < 0.05$). Of the infants (n = 306), 52.3 % were boys; 14.4 % were premature; 93.0 % were born to mothers taking medication, 89.9 % to mothers taking vitamin supplements, 38.1 % to mothers who used alcohol, and 11.4 % to mothers who used tobacco. Most newborns had normal weight (88.9 %), adequate size for gestational age (88.1 %), 1-minute APGAR score ≥ 7 (88.6 %), and were exclusively breastfed at discharge from maternity (90.6 %). Arsenic ≥ 0.23 μg/L (53.4 %), cadmium < 0.20 μg/L (52.6 %), lead ≥ 0.8 μg/dL (60.5 %) and mercury ≥ 0.8 μg/L (55.2 %) were found in this population. Most had normal vitamin D (52.6 %) and calcium (76.3 %) levels. The incidence of DDE in infants was 27.1 % (83/306). The number of DDE per child ranged from 1 to 8 with demarcated opacity being the most common defect, while upper central incisors and the incisal third were the teeth and location most affected, respectively. Lead was associated with DDE in primary incisors, except in cases of pregnant women taking vitamin supplements and when the newborn, at the time of discharge, was being breastfed. In the present population, high cord blood levels of lead were associated with the occurrence of DDE in primary incisors.

1. Introduction

The mineralization of enamel during amelogenesis of the primary incisors begins between the 13th and 16th intrauterine weeks, and most of the dental crowns have already been mineralized at birth [43], except the cervical portions which have enamel formation completed by the 3rd month of life.

Enamel development occurs through a sequence of biochemical, molecular, and cellular phenomena, and any change in this phase results in definitive records, which are developmental defects of enamel (DDE) [68]. The prevalence of DDE in the primary dentition ranges from 3.9 % to 81.3 % worldwide [34,72]. This broad rate possibly occurs due to the different indices used for classification and the types of defects and teeth studied. The location of DDE will depend on the time in which the incident occurred and have been related to possible complications during the prenatal, perinatal, and postnatal periods, varying according to the group of primary teeth studied.

Among these complications, factors such as maternal alcohol

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consumption [21] or smoking [70] during pregnancy, the mother's age being under 24 years [15], infection/illness during pregnancy [13,67], gestational diabetes mellitus [48,51], intrauterine/perinatal nutritional deficiency [13,26,61], gene polymorphism [30], low household income [52,73], cesarean birth [2], prematurity [5,16,36,41,42,76,83], low birth weight [21,36,46,59,61,83], low APGAR score [53], jaundice at birth [1], neonatal hypoxia [48], orotracheal intubation [16,2], hospitalization [76], lack of breastfeeding [1,15,42,52], deficiency of vitamin D [48,70], low calcium levels [48], and exposure to heavy metals [44] have been investigated.

It is known that the environment harbors chemical components, including heavy metals, which can originate from both natural sources and human activities. As a result, the population is constantly exposed to these substances in their daily lives, through soil, air, water, and food. Some trace metals are essential to humans in very small amounts, while others are harmful to the environment and health [79] and can be carcinogenic or toxic, affecting the central nervous system, kidneys, liver, skin, bones, and teeth [78].

Heavy metals such as lead, arsenic, cadmium, and mercury have been listed among the 10 chemicals of major public health concern [78,80]. Susceptibility to health problems caused by the exposure to these metals varies between men, women, and children, with the latter being particularly more vulnerable [79]. It is widely recognized that dental tissue can be affected by environmental pollutants—such as heavy metals—which are deposited and bio accumulated. Thus, primary teeth can be used as biomarkers of past exposure [50,6]. However, although many studies address primary teeth as biomarkers [27,28,3,9], few of them investigate whether exposure to metals can cause enamel defects [19,32,55,82].

Vitamin D is a very important micronutrient for health and its low levels in blood have been identified as a silent epidemic [45]. The consequences from its severely low levels on bone health are clearly known; however, the impact of slightly lower levels, particularly during pregnancy and the neonatal period, is less clearly understood [4]. Serum 25(OH)D levels are currently the main indicator of vitamin D status and are reported in nanomoles per liter (nmol/L) or nanograms per milliliter (ng/mL) [35]. Levels $\geq$ 50 nmol/L (20 ng/mL) are sufficient for most people, as well as in the umbilical cord. Vitamin D participates in the absorption of calcium in the intestine and maintains adequate serum concentrations of calcium and phosphate, to allow normal bone mineralization [35,39,49] and tooth development [23]. Thus, it is plausible that the calcium deficit is related to DDE.

The investigation of DDE in primary dentition has great clinical relevance, as they are responsible for esthetic issues, hypersensitivity [68] and mainly, a greater risk of developing carious lesions [12,17,34,66,65,69,72], which leads to high treatment costs. Therefore, identifying the incidence and etiological factors for DDE is important, since affected patients require differentiated and early management [37]. However, there is a lack of well-designed studies that investigate the associations between exposure of heavy metals, and to low levels of vitamin D and calcium, with the occurrence of DDE.

Therefore, the objective of this study was to investigate the influence of serum levels of vitamin D, calcium, and heavy metals (lead, mercury, arsenic and cadmium) from cord blood on the occurrence of DDE in primary incisors of infants from the Rio de Janeiro Birth Cohort Study on Environmental Exposure and Childhood Development (PIPA Project). In addition, possible prenatal and perinatal effect modifying factors were considered. The hypothesis was that low levels of vitamin D and calcium, and high levels of heavy metals at birth, are related to DDE in primary incisors.

2. Materials and methods

2.1. Study design and setting

This cohort study nested in the PIPA project birth cohort followed the STROBE guideline [75]. It was approved by the Research Ethics Committee of the Maternity School of the Federal University of Rio de Janeiro (MS-UFRJ) with protocol number 4.676.124. The recruitment period went from June 2021 to July 2022, at the MS-UFRJ.

Serum levels of micronutrients (vitamin D and calcium) and heavy metals (arsenic, cadmium, lead, and mercury) in cord blood, such as data relating to pregnancy / birth and sociodemographic information were investigated in relation to the occurrence of DDE in primary incisors of the infants. Data collection took place in the third trimester of pregnancy, at birth and during follow-up appointments of children at 3, 6, 12, and 24 months of life (from September 2021 to April 2024). The follow-up took place at MS-UFRJ.

2.2. Participants and eligibility criteria

The sample comprised mother-infant pairs who attended follow-up in the PIPA birth cohort until April 5, 2024, and agreed to participate in this study, and signed an informed consent form. Thus, pregnant women in the 3rd trimester, over 16 years old, residents in Rio de Janeiro city, who were monitored or who arrived to give birth at MS-UFRJ during the study period were included. Their infants who were born at MS-UFRJ, without gestational age limit, including twins, were also included.

In the event of fetal death, miscarriage, or neonatal death, participants were excluded. Also, infants diagnosed with genetic malformation, with conditions such as amelogenesis imperfecta, the second twin in both identical and fraternal twins (to avoid bias in relation to genetic similarity, opting for the second because he or she usually suffers the most complications) [60,40], and those who could not undergo dental examination, were excluded. Infants who presented their eight incisors affected by caries, restorations, or pigmentations, as well as those who did not have the eight incisors erupted in the mouth, were excluded from the analysis but continued to be followed up in the cohort.

2.3. Sample size

To estimate the minimum sample size to obtain the prevalence of DDE, the following parameters were considered: a finite sample of 1608 infants born alive at MS-UFRJ, during the year prior (2020) to the recruitment period (https://www.me.ufrj.br/index.php/atencao-a-saude/relatorio-assistencial/187-indicadores-geral-2020.html), an error of 4 %, a confidence interval of 95 %, and a DDE prevalence of 13 % [53]. Therefore, 233 infants were needed to estimate the prevalence outcome, which was measured using the University of São Paulo Bauru calculator (http://estatistica.bauru.usp.br/calculoamostral/calculos.php).

2.4. Variables

The exposure variables investigated comprised the following data: serum levels of vitamin D, calcium, arsenic, cadmium, lead, and mercury from cord blood.

The following effect modifying variables were also collected: health problems during pregnancy; use of medication, vitamins, alcohol, tobacco, and drugs during pregnancy; breastfeeding upon discharge from maternity; size categorization according to the gestational age (SG); color/race of pregnant women [8]; primiparous or multiparous; complete prenatal care; education level and per capita income; diabetes, hypertension, and infections during pregnancy; body mass index (BMI); delivery type; mother's age; reduced intrauterine growth (IUGR); APGAR index in the 1st and 5th minutes; newborn resuscitation in the delivery room; stay in the Neonatal Intensive Care Unit (NICU); clinical changes in the newborn; twin; sex and color/race of the infant [8]; newborn hospitalization at birth; newborn intubation at birth; and breastfeeding an the time of mother-infant discharge from the maternity ward.

Prematurity (gestational age < 37 weeks) and low birth weight (< 2500 g) were considered predictor variables, and the presence of DDE in primary incisors were the outcome studied. Data on the number and type of DDE per incisor and per child, color and location of DDE, and DDE severity were also investigated.

2.5. Data sources and measurement

A trained and calibrated examiner (inter-examiner kappa = 0.915) on the Modified DDE Index [22], blinded to the results of vitamin D, calcium, and heavy metal levels, carried out examinations of the infant's primary incisor buccal surfaces. With the help of the caregiver, the infant exam was performed in the knee-to-knee position, under artificial light, after brushing teeth without dentifrice, and drying with gauze. During the examination, the examiner made movements within his field of vision to facilitate the visualization of the DDE.

The Modified index [22] comprises the following classifications: normal (0), demarcated opacity (1), diffuse opacity (2), hypoplasia (3), other defects (4), combinations of demarcated and diffuse opacities (5); demarcated opacity and hypoplasia (6); diffuse opacity and hypoplasia (7); and all the three conditions together (demarcated opacity, diffuse opacity and hypoplasia) (8). Teeth that had not erupted in the mouth, those exfoliated, lost, or affected by caries, restorations, or pigmentations, that could mask the possible DDE limits (9). Those classified as (9) were not included in the evaluation [22].

Development defects of enamel were assessed for color (white, cream, yellow, or brown) in case of opacities, and for location, considering the thirds of the tooth's surface (incisal, middle, cervical). Due to the young age of the children, to make the examination less time consuming, the recommendations of the index were followed, which indicates that not all teeth/faces should be used for this purpose when necessary. Therefore, only the vestibular surfaces were included [22].

To assess the severity of DDE, the location of the defect was used instead of extension [14,22]. Mild DDE was reported when the defect presented was in only one third of the tooth; a severe DDE was reported when it was present in more than one third of the tooth. Children who had at least one tooth with DDE were classified as having DDE [18].

The serum levels of the exposure variables were assessed from samples of cord blood collected at birth. Serum samples for heavy metals were analyses at Diagnostics of Brazil laboratory (DB, Brazil). Detection of arsenic, cadmium, lead, and mercury levels was performed using an inductively coupled plasma mass spectrometer (ICP-MS 7800, Agilent Technologies, California, USA). Internal quality control was carried out using a commercial kit (ClinChek Serum – RECIPE Chemicals, Germany) through multiple Westgard rules, individual evaluation, and critical analysis [11]. The proficiency test was carried out by the national control program for quality control (PNCQ) for arsenic (Orthomolecular Medicine) and PNCQ/Controllab (Toxicology III) for the other heavy metals (Occupational Medicine/Toxicology). The detection limit for all metals was 0.1 μg/L, while for lead it was 0.1 μg/dL.

The median values of each detected heavy metal's serum levels in the study population were considered the cut-off points of these metals, which were categorized into < the median, and ≥ the median. The unit of measurement for lead was μ/dL, while for the others it was μ/L.

The analyses of vitamin D were carried out at the Clinical Analysis Laboratory of the Faculty of Pharmacy at UFRJ (LACFar/UFRJ). The 25-hydroxy vitamin D (nmol/L) was evaluated through the electrochemiluminescence method [54] using the commercial kit Elecsys® Vitamin D total III (Roche Diagnostics Corporation, Indianápolis, Indiana, EUA); and calcium (mg/dL) by the arsenazo III method [38] using commercial kit (Labtest / Labmax Plenno Equipment, Buenos Aires, Argentina), both from aliquots of 750 μL of blood (plasma).

Vitamin D levels were considered insufficient (<50 nmol/L), normal (≥50 nmol/L), or above normal (>250 nmol/L) [25]. Moreover, the levels of vitamin D were also analysed considering the value of 75 nmol/L as a cutoff point (<75nmol/L or ≥ 75 nmol/L), since the Endocrine Society stated that, for clinical practice, a serum 25(OH)D concentration of more than 75 nmol/L is necessary to maximize the effect of vitamin D on calcium and bone [33]. The calcium levels were categorized as low (< 8.2 mg/dL), normal (8.2–11.2 mg/dL), or high (> 11.2 mg/dL) [10,71]. The detection limits for vitamin D and calcium were 7.5 nmol/L (3.0 ng/mL) and 0.083 mg/dL, respectively.

Face-to-face interviews were carried out to collect prenatal information, such as mother's age; color/race (white, brown, black, yellow, indigenous); education level (<12 / ≥12 years of study); monthly income (≤1 / >1 Brazilian minimum wage); weight; and height. Dichotomous variables (yes/no) were also obtained after the delivery, such as primiparous; use of medication, vitamin supplements, alcohol, tobacco, or drugs; and suffered from diabetes, hypertension, or infections during pregnancy. The information about prenatal care was collected from the records. The BMI was calculated based on the weight and height information, and the following parameters were considered: underweight (degrees I, II, and III), adequate weight, overweight, and obese (degrees I, II, and III). The mother's age was categorized by the median in ≤29 / > 29 years old.

Sex; color/race of the infant (white, brown, black, other - yellow/indigenous); SG (adequate — appropriate size for the gestational age (AGA) / inadequate — small for the gestational age (SGA) or large for the gestational age (LGA)); and the following dichotomous variables (yes / no) were investigated: twin; prematurity; newborn intubation and hospitalization at birth; IUGR; delivery room resuscitation; a stay in NICU; newborn clinical alterations; and breastfeeding at the time of the mother/infant discharge. The APGAR index scores in the 1st and 5th minutes were collected, and for both it was considered inadequate when the score was lower than 7. Also, the delivery type (vaginal, cesarian, or forceps), the gestational age, and the birth weight (≥2500 g / < 2500 g) were registered. All these variables were obtained from the newborn's medical records at MS-UFRJ, except the color/race that was reported by the main caregiver.

The interviews and data extraction were performed by trained examiners to avoid information bias.

2.6. Statistical analyses

Data were analysed using the IBM SPSS Statistics version 24.0 software (SPSS Inc., Chicago, USA). Categorical variables were represented by absolute and percentual values. The Kolmogorov-Smirnov test was applied to verify the normality of distribution of continuous variables. Thus, the Mann-Whitney test was used to observe differences in the micronutrients and heavy metals serum levels values between the infants with and without DDE.

The frequency of all categorical variables was tested in regard to the presence or absence of DDE by chi-square or Fisher's exact tests, and a Poisson regression model was used to estimate the crude and adjusted relative risks (RR) and their 95 % confidence intervals. The statistical significance of the RR was obtained using a robust variance estimator covariance matrix, assessed by the Wald chi-square test. Variables with significance lower than 0.25 and with a minimum number of 10 cases per category were the parameters used to remain as adjustment variables in the multivariate models that tested the influence of the exposure factors on the presence of DDE (dependent variable). Each multivariate model was constructed separately, meaning that each exposure variable (lead, mercury, arsenic, cadmium, vitamin D and calcium) was analyzed isolatedly with respect to the outcome, and the adjustment factors were also considered one at a time. Thus, models with multiple exposures simultaneously were not performed to avoid collinearity among variables and to preserve the interpretability of the results. Furthermore, predictor variables with theoretical value (prematurity and low birth weight) were also used for adjustment.

The statistical significance was 5 %.

3. Results

Of the 1513 eligible pregnant women who attended MS-UFRJ for prenatal care during the recruitment period, 901 were enrolled. Eleven lived outside Rio de Janeiro and 46 gave birth to their infants in other hospitals. Thus, 844 pregnant women participated in the study. Thirteen infants died and 23 moved out of Rio de Janeiro, resulting in 808 infants born at MS-UFRJ that could be followed-up with. Of these, 33 withdrew from participating in the study. Therefore, 775 remained in the cohort and 703 attended follow-up appointments until April 2024. A total of 647 infants were examined at least once by a calibrated dentist. From this sample, 341 were excluded due to the following reasons: congenital malformation (n = 8); being the second twin (n = 10); impossible to evaluate DDE (n = 1); have no teeth (n = 99); and have less than eight incisors erupted until completion of the study (n = 223). No child was excluded because all eight incisors had cavities. Thus, 306 mother-infant pairs were included. The participants' flow chart is shown in Fig. 1.

The mean age of the pregnant woman was 29.94 (±7) years and most of them were brown (162/306; 52.9 %), with 12 years or more of education (202/306; 66.0 %), who earned up to one Brazilian minimum wage (194/240; 80.8 %), and completed the prenatal care (255/299; 85.3 %). Most were multiparous women (183/305; 60 %), with some degree of obesity (149/285; 52.3 %), that used medication (280/301; 93.0 %) and vitamin supplements (268/298; 89.9 %). Few used alcohol (115/302; 38.1 %), illicit drugs (10/300; 3.3 %), and tobacco (34/298; 11.4 %). Regarding health problems during pregnancy, the most common were diabetes (84/301; 27.9 %), hypertension (59/301;19.6 %), or infection (44/301; 14.6 %) (Table 1).

The mean gestational age at delivery was 38.02 ± 2.28 weeks and ranged from 22 (1/306) to 41 (23/306) weeks. Among those with gestational age < 37 weeks, most (29/44; 65.9 %) delivered at 35 or 36 weeks. Among the infants, 51.8 % (158/305) were born by cesarean section, 52.3 % (160/306) were male, 46.2 % (139/301) were brown, 14.8 % (44/306) were premature, and 3.2 % (9/281) presented with IUGR. The majority had normal weight (272/306; 88.9 %), adequate gestational size (258/293; 88.1 %), and were exclusively breastfeeding when discharged from the maternity (222/245; 90.6 %). Regarding the health condition at birth, most of the infants had an APGAR score ≥ 7 in

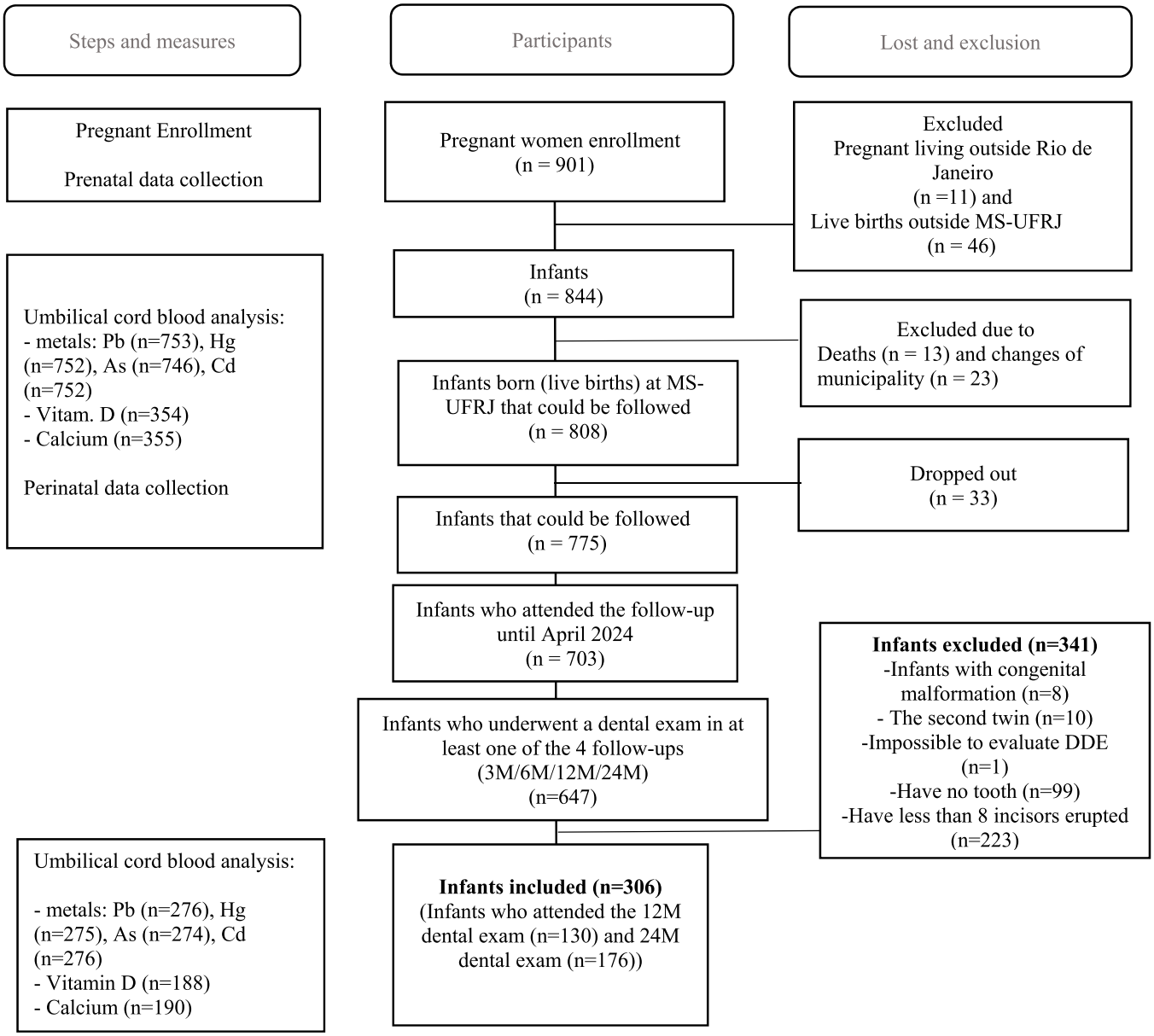

Fig. 1. Flow chart of the birth cohort study. M=months.

Table 1
The prenatal variables and their association with the presence or absence of DDE, demonstrated by the crude relative risks (RR), their respective 95 % CI, and P values.

		Presence of DDE			Poisson Regression		
	N	No	Yes		Crude Model		
		N (%)	N (%)	P[1]	RR	IC95 %	P[2]
Mother's age (median)							
≤ 29	150	113 (75.3)	37(24.7)	0.412	1		
> 29	156	110 (70.5)	46(29.5)		1.195	[0.826;1.731]	0.345
Color/race							
White	74	54 (73)	20 (27)	0.085	1		
Brown	162	115 (71)	47 (29)		1.073	[0.688;1.675]	0.755
Black	66	53 (80.3)	13 (19.7)		0.729	[0.394;1.347]	0.313
Yellow	2	1 (50)	1 (50)		1.850	[0.440;7.774]	0.401
Indigenous	2	0 (0)	2 (100)		3.700	[2.545;5.380]	< 0.001
Education level (years of study)							
< 12	104	72 (69.2)	32 (30.8)	0.342	1		
≥ 12	202	151 (74.8)	51 (25.2)		0.821	[0.565;1.192]	0.299
Income (Brazilian minimum wage)							
≤ 1	194	142 (73.2)	52 (26.8)	> 0.999	1		
> 1	46	34 (73.9)	12 (26.1)		0.973	[0.568;1.669]	0.921
Primiparous							
No	183	131 (71.6)	52 (28.4)	0.655	1		
Yes	122	91 (74.6)	31 (25.4)		0.894	[0.611;1.309]	0.565
BMI							
Underweight	-	-	-	-	-	-	
Adequate weight	43	32 (74.4)	11 (25.6)	0.689	1		
Overweight	93	70 (75.3)	23 (24.7)		0.967	[0.520;1.799]	0.915
Obesity	149	105 (70.5)	44 (29.5)		1.154	[0.655;2.035]	0.620
Use of vitamin supplements							
No	30	19 (63.3)	11 (36.7)	0.288	1		
Yes	268	199 (74.3)	69 (25.7)		0.702	[0.421;1.172]	0.176
Use of medication							
No	21	18 (85.7)	3 (14.30	0.272	1		
Yes	280	202 (72.1)	78 (27.9)		1.950	[0.673;5.654]	0.219
Use of alcohol							
No	187	140 (74.9)	47 (25.1)	0.477	1		
Yes	115	81 (70.4)	34 (29.5)		1.176	[0.808;1.712]	0.396
Use of drugs							
No	290	212 (73.1)	78 (26.9)	0.733	1		
Yes	10	7 (70)	3 (30)		1.115	[0.425;2.929]	0.825
Use of tobacco							
No	264	197 (74.62)	67 (25.38)	0.507	1		
Yes	34	23 (67.6)	11 (32.35)		1.275	[0.752;2.162]	0.368
Complete prenatal care							
No	44	32 (72.7)	12 (27.3)	0.994	1		
Yes	255	189 (74.1)	66 (25.9)		0.949	[0.561;1.605]	0.845
Diabetes during pregnancy							
No	217	162 (74.7)	55 (25.3)	0.402	1		
Yes	84	58 (69)	26 (31)		1.221	[0.825;1.808]	0.318
Hypertension during pregnancy							
No	242	175 (72.3)	67 (27.7)	0.652	1		
Yes	59	45 (76.3)	14 (23.7)		0.857	[0.519;1.414]	0.546
Infections during pregnancy							
No	257	189 (73.5)	68 (26.5)	0.808	1		
Yes	44	31 (70.5)	13 (29.5)		1.117	[0.677;1.841]	0.665

Note: 1. Chi-Square test; 2. Poisson Regression; DDE = Dental defects of enamel; BMI = Body mass index

the 1st (271/306; 88.6 %) and 5th minutes (299/305; 98.0 %). They did not require resuscitation in the delivery room (257/292; 88.0 %), hospitalization (249/301; 82.7 %), NICU (257/304; 84.5 %), or intubation (290/301; 96.3 %). In addition, they did not present clinical changes (211/306; 69 %) (Table 2).

As for metals, the majority of the population presented levels above the median for lead (60.5 %) and mercury (52.4 %), while the opposite was true for arsenic (66.1 %) and cadmium (93.5 %), where the majority of the population presented levels below the median. When looking at micronutrient levels, vitamin D was evenly distributed across the population (52.7 % with normal levels), while for the calcium, the majority of the population presented normal levels (76.3 %). The results of heavy metals and micronutrients in the umbilical cord are presented in Tables 3 and 4.

There were 187 (7.6 %) incisors affected by DDEs in 83 (27.1 %) infants. The number of teeth affected by DDE per child ranged from 1 to 8, and most presented 1 (33/83; 39.7 %) or 2 defects (30/83; 36.1 %). Eleven children had combinations of defects in the same tooth. The most common type of DDE was demarcated opacity (104/187; 55.6 %) followed by diffuse opacity (38/187; 20.3 %), hypoplasia (20/187; 10.7 %), and the combination of demarcated opacities and hypoplasia (12/187; 6.4 %). The distribution of DDE types in the eight incisors is shown in Table 5. The most affected teeth were upper central incisors (95/187; 50.8 %), followed by the upper laterals (47/187; 25.1 %), the lower central incisors (29/187; 15.5 %), and the lower laterals (16/187; 8.6 %). Considering the children with DDE, 26 (26/83; 31.3 %) presented defects in more than one third of the tooth surface, 3 (3/83; 3.6 %) had yellow or brown DDE, and 26 (26/83; 31.3 %) showed severe DDE. Regarding the location of DDE, the majority were in the incisal third (107/187; 57.2 %), followed by the incisal and middle thirds together (31/187; 16.6 %), and incisal, middle and cervical thirds simultaneously (30/187; 16 %). One tooth showed DDE involving only the cervical surface, however, this participant had other teeth with DDE that affected the incisal and middle thirds.

Table 2
The perinatal variables and their association with the presence or not of DDE, demonstrated by the crude relative risks (RR), their respective 95 % CI, and P values.

		Presence of DDE			Poisson Regression		
	N	No	Yes		Crude Model		
		N (%)	N (%)	P[1]	RR	IC95 %	P[2]
Gender							
Male	160	118 (73.8)	42 (26.3)	0.817	1		
Female	146	105 (71.9)	41 (28.1)		1.070	[0.741;1.544]	0.719
Color/race							
White	118	79 (66.9)	39 (33.1)	0.24	1		
Brown	139	107 (77)	32 (23)		0.697	[0.468;1.037]	0.075
Black	41	31 (75.6)	10 (24.4)		0.738	[0.406;1.341]	0.318
Yellow/Indigenous	3	3 (100)	0 (0)		0.000		
Prematurity							
No	262	189 (72.1)	73 (27.9)	0.599	1		
Yes	44	34 (77.3)	10 (22.7)		0.816	[0.457;1.455]	0.490
Twin							
Yes	4	1 (25)	3 (75)	0.062	1		
No	302	222 (73.5)	80 (26.5)		0.353	[0.195;0.641]	0.001
Size categorization according to GA							
Adequate (AGA)	258	194 (75.2)	64 (24.8)	0.320	1		
Inadequate (SGA+LGA)	35	23 (65.7)	12 (34.3)		1.382	[0.834;2.291]	0.210
Birth weight							
$\geq$ 2500 g	272	198 (72.8)	74 (27.2)	$>$ 0.999	1		
$<$ 2500 g	34	25 (73.5)	9 (26.5)		0.973	[0.538;1.760]	0.928
Hospitalization at birth							
No	249	186 (74.7)	63 (25.3)	0.228	1		
Yes	52	34 (65.4)	18 (34.6)		1.368	[0.890;2.104]	0.153
Intubation at birth							
No	290	215 (74.1)	75 (25.9)	0.075	1		
Yes	11	5 (45.5)	6 (54.5)		2.109	[1.188;3.743]	0.011
Breastfeeding at the time of discharge							
No	23	13 (56.5)	10 (43.5)	0.127	1		
Yes	222	164 (73.9)	58 (26.1)		0.601	[0.359;1.007]	0.053
Reduced intrauterine baby's growth (IUGR)							
No	272	198 (72.8)	74 (27.2)	0.269	1		
Yes	9	5 (55.6)	4 (44.4)		1.634	[0.767;3.479]	0.203
Delivery type							
Vaginal	146	108 (74)	38 (26)	0.784	1		
Forceps	1	1 (100)	0 (0)		-	-	-
Cesarian	158	113 (71.5)	45 (28.5)		1.094	[0.757;1.582]	0.632
APGAR in the 1st minute $\geq$ 7							
No	35	22 (62.9)	13 (37.1)	0.225	1		
Yes	271	201 (74.2)	70 (25.8)		0.695	[0.432;1.119]	0.135
APGAR in the 5th minute $\geq$ 7							
No	6	2 (33.3)	4 (66.7)	0.049	1		
Yes	299	220 (73.6)	79 (26.4)		0.396	[0.218;0.720]	0.002
Delivery room resuscitation							
No	257	189 (73.5)	68 (26.5)	0.676	1		
Yes	35	24 (68.6)	11 (31.4)		1.188	[0.699;2.018]	0.525
Neonatal Intensive Care Unit (NICU)							
No	257	191 (74.3)	66 (25.7)	0.192	1		
Yes	47	30 (63.8)	17 (36.2)		1.408	[0.913;2.172]	0.121
Newborn clinical alterations							
No	211	155 (73.5)	56 (26.5)	0.839	1		
Yes	95	68 (71.6)	27 (28.4)		1.071	[0.725;1.582]	0.731

Notes: 1. Chi-Square test; 2. Poisson Regression; DDE = Dental defect of enamel; GA = Gestational age; AGA = Adequate for gestational age; SGA = Small for gestational age; LGA = Large for gestational age.

In the crude Poisson regression, the following variables presented p value less than 0.25 (cut off point) and at least 10 cases in each category: 1-minute APGAR index, exclusively breastfeeding at the mother-infant discharge, the use of vitamin, SG, newborn hospitalization, and NICU (Tables 1 and 2). Thus, they were included in the final models of the multiple regression (Table 6).

Table 6 presents the regression models adjusted for various factors: "vitamin supplements" (Model 1), "APGAR score at 1 min" (Model 2), "size categorization according to gestational age (SG)" (Model 3), "hospitalization at birth" (Model 4), "Neonatal Intensive Care Unit (NICU) admission" (Model 5), "exclusive breastfeeding at discharge" (Model 6), "prematurity" (Model 7), and "birth weight" (Model 8). The Model 0, referred to as the crude model, analysed the individual association between each exposure variable (levels of vitamin D, calcium, lead, mercury, arsenic, and cadmium) and the presence of DDE in primary incisors, without controlling for potential confounding factors. Among these models, only Model 1 did not demonstrate an association between exposure factors with the presence of DDE in the primary incisors. Lead was consistently associated with DDE across all adjusted models, except those adjusted for vitamin supplementation during pregnancy (Model 1) and exclusive breastfeeding at discharge (Model 6). Additionally, mercury was associated with DDE in the model adjusted for exclusive breastfeeding at discharge. No significant associations were observed between DDE and the other metals (arsenic and cadmium), nor with vitamin D and calcium levels in the regression analyses.

4. Discussion

The hypothesis of the present study was partially refuted, since low

Table 3
Levels of vitamin D, calcium and heavy metals in the umbilical cord in association with DDE.

	Total	Presence of DDE		
		No	Yes	P
	N (%)	N (%)	N (%)	
Vitamin D (25-Hydroxy vitamin D)				
≥ 75nmol/L	27 (14.4)	19 (70.4)	8 (29.6)	0.648
< 75nmol/L	161 (85.6)	120 (74.5)	41 (25.5)	
Vitamin D (25-Hydroxy vitamin D)				
Normal (≥ 50nmol/L)	99 (52.7)	71 (71.7)	28 (28.3)	0.572
Insufficient (< 50nmol/L)	89 (47.3)	68 (76.4)	21 (23.6)	
Calcium				
Low (< 8.2 mg/dL)	12 (6.3)	11 (91.7)	1 (8.3)	0.343
Normal (8.2–11.2 mg/dL)	145 (76.3)	105 (72.4)	40 (27.6)	
High (> 11.2 mg/dL)	33 (17.4)	24 (72.7)	9 (27.3)	
Lead				
< 0.800 μg/dL	109 (39.5)	88 (80.7)	21 (19.3)	0.041
≥ 0.800 μg/dL	167 (60.5)	115 (68.9)	52 (31.1)	
Mercury				
< 0.800 μg/L	131 (47.6)	101 (77.1)	30 (22.9)	0.242
≥ 0.800 μg/L	144 (52.4)	101 (70.1)	43 (29.9)	
Arsenic				
< 0.230 μg/L	181 (66.1)	135 (74.6)	46 (25.4)	0.384
≥ 0.230 μg/L	93 (33.9)	64 (68.8)	29 (31.2)	
Cadmium				
< 0.200 μg/L	258 (93.5)	192 (74.4)	66 (25.6)	0.267
≥ 0.200 μg/L	18 (6.5)	11 (61.1)	7 (38.9)	

Note: Chi-Square test; DDE = Dental defects of enamel.

levels of vitamin D and calcium were not associated to DDE. In fact, lead levels in the umbilical cord at birth were associated with DDE in primary incisors, in the crude regression and in almost all adjusted models. The exceptions happened when there was an adjustment for the use of vitamin supplementation during pregnancy and the mother was exclusively breastfeeding at discharge. The literature has highlighted that lack of breastfeeding [1,15,42,52] and serum micronutrient levels in the pre and perinatal periods [20,64] are possible factors associated with DDE. In the present study, these factors do not seem to be associated with DDE. However, the use of vitamin supplements during pregnancy and exclusively breastfeeding at discharge acted as effect modifier variables. This means that the presence of higher levels of lead in the cord no longer had an association with DDE, when pregnant women used vitaminic supplementation and infants were breastfed. The present authors suggest that both vitamins and breast milk, rich in calcium, act as protective factors against lead. According to Gomes et al. [31], calcium supplementation may help to reduce adverse health effects induced by lead exposure.

However, it was also observed that even during breastfeeding, when lead lost its effect on the occurrence of DDE, mercury stood out in the model. Mercury only showed influence on DDE in this model—where mothers breastfed until being discharged—where lead no longer had influence on the presence of DDE. In the literature, it is stated that the consumption of selenium, zinc, and magnesium helps to strengthen the body against mercury [24,77], but calcium does not play this role. Thus, even when lead did not have an effect on DDE due to vitamin supplementation by the pregnant women, mercury did not exert influence on the presence of DDE, since vitamin supplements are rich in selenium, magnesium, and zinc, as well as calcium. Therefore, when adjusting the model only for breast milk consumption, the effect of lead was nullified, highlighting the effect of mercury. Thus, the authors suggest that lead was the metal that had the greatest influence on the development of primary enamel defects.

Table 4
Levels of vitamin D, calcium and heavy metals in the umbilical cord in association with DDE.

	TOTAL	Presence of DDE		
		No	Yes	P
	n	n	n	
25(OH)D (vitamin D) levels in cord blood				
n	188	139	49	0.322
Mean (SD)	52.9 (20.7)	52.4 (21.4)	53.9 (18.7)	
P50 [P25; P75]	50.9 [37.2; 67.1]	50.2 [36.4; 66.1]	53.9 [38.2; 67.4]	
min - max	15.2; 126.3	15.2; 126.3	20.5; 94.8	
Calcium levels in cord blood				
n	190	140	50	0.195
Mean (SD)	10.02 (1.94)	9.94 (2.02)	10.39 (1.70)	
P50 [P25; P75]	10.21 [9.62; 10.83]	10.10 [9.58; 10.80]	10.39 [9.82; 10.89]	
min - max	0.02; 13.66	0.02; 13.66	0.02; 12.98	
Lead levels in cord blood				
n	276	203	73	**0.004**
Mean (SD)	1.13 (1.14)	1.10 (1.25)	0.70 (1.10)	
P50 [P25; P75]	0.90 [0.60; 1.35]	0.80 [0.60; 1.20]	1.10 [0.70; 1.50]	
min - max	0.00; 13.70	0.00; 13.70	0.40; 3.50	
Mercury levels in cord blood				
n	275	202	73	0.638
Mean (SD)	1.20 (1.18)	1.22 (1.24)	0.99 (0.90)	
P50 [P25; P75]	0.80 [0.40; 1.70]	0.75 [0.40; 1.70]	0.90 [0.50; 1.40]	
min - max	0.00; 6.90	0.00; 6.90	0.00; 4.50	
Arsenic levels in cord blood				
n	274	199	75	0.110
Mean (SD)	0.39 (1.10)	0.34 (0.91)	1.47 (0.18)	
P50 [P25; P75]	0.15 [0.00; 0.32]	0.14 [0.00; 0.29]	0.18 [0.00; 0.54]	
min - max	0.00; 11.32	0.00; 10.11	0.00; 11.32	
Cadmiun levels in cord blood				
n	276	203	73	0.400
Mean (SD)	0.02 (0.06)	0.02 (0.06)	0.07 (0.00)	
P50 [P25; P75]	0.00 [0.00; 0.00]	0.00 [0.00; 0.00]	0.00 [0.00; 0.00]	
min - max	0.00; 0.30	0.00; 0.30	0.00; 0.30	

Note: Mann-Whitney test; DDE = dental defects of enamel; SD = standard deviation; P = percentile

Few studies have evaluated the results of heavy metals exposure to teeth [19,32,55]. In the present study, the heavy metals investigated are among the ten listed by the World Health Organization as of major public health concern [78,80], and children are most vulnerable to their harmful effects [79]. A previous study [32] showed that the association between lead concentrations and enamel defects was weak. However, the amount of calcium from teeth collected in areas with high levels of lead and cadmium seems to be much smaller, and an increase in heavy metal quantities was detected in more-deteriorated teeth [19] or those with higher topography roughness [55]. Although the methodological design of these studies did not investigate any exposure factors during the period of tooth development, they brought us important notes about tooth surface [55] and the state of deterioration of teeth [19] which presented heavy metals in their structure. In the present study, we investigated the association of heavy metals with DDE, not the effects of accumulated metals in the tooth structure due to past exposure. Moreover, aother difference was that we also considered possible modifying factors present at the time of mineralization of the evaluated teeth. The authors suggest that the association of lead with DDE happened because lead follows the calcium metabolic pathway—accumulating in the teeth, as well as in the bones, and possibly inhibiting the activity of enamel

Table 5
The distribution of DDE types in the eight primary incisors.

	Demarcated opacity	Diffuse opacity	Hypoplasia	Demarcated opacity and Diffuse opacity	Demarcated opacity and Hypoplasia	Diffuse opacity and Hypoplasia	Demarcated opacity and Diffuse opacity and Hypoplasia	Total
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Maxillary								
Right central incisors (51)	28 (15.0)	3 (1.6)	4 (2.1)	1 (0.5)	4 (2.1)	3 (1.6)	1 (0.5)	44 (23.5)
Left central incisors (61)	28 (15.0)	8 (4.3)	8 (4.3)	0 (0.0)	3 (1.6)	3 (1.6)	1 (0.5)	51 (27.3)
Right lateral incisors (52)	17 (9.1)	5 (2.7)	0 (0.0)	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	23 (12.3)
Left lateral incisors (62)	13 (7.0)	9 (4.8)	2 (1.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	24 (12.8)
Mandibular								
Right central incisors (71)	8 (4.3)	4 (2.1)	3 (1.6)	0 (0.0)	1 (0.5)	1 (0.5)	0 (0.0)	17 (9.1)
Left central incisors (81)	4 (2.1)	4 (2.1)	1 (0.5)	0 (0.0)	2 (1.1)	1 (0.5)	0 (0.0)	10 (5.3)
Right lateral incisors (72)	3 (1.6)	3 (1.6)	2 (1.1)	0 (0.0)	1 (0.5)	1 (0.5)	0 (0.0)	12 (6.4)
Left lateral incisors (82)	3 (1.6)	2 (1.1)	0 (0.0)	0 (0.0)	1 (0.5)	0 (0.0)	0 (0.0)	6 (3.2)
Total	104 (55.6)	38 (20.3)	20 (10.7)	2 (1.1)	12 (6.4)	9 (4.8)	2 (1.1)	187 (100.0)

Note: A total of 306 infants and 2448 teeth were evaluated. Of these, 83 infants and 187 teeth presented DDE. DDE = dental defects of enamel.

matrix proteinases, even at low concentrations, thus interfering with amelogenesis, as demonstrated in rats. Gerlach et al., [29]

Unlike other authors [48], we did not find an association between low levels of calcium and DDE. Although we cannot compare our calcium levels with those of Neto et. al (2020), our results were probably different because the majority (76.3 %) who had calcium tests presented normal levels. Thus, further investigation into this issue is suggested. No association was found between low levels of vitamin D and the presence of DDE, corroborating some reports in the literature [4,47,57,58,65,7,74]. However, Silva et al. [70] reported an association between second primary molar hypomineralization and deficiency of vitamin D serum levels at birth. Once more, it is difficult to compare our results with this finding, since we did not evaluate molars. Moreover, the mean vitamin D reading of the whole sample of the present study was 21.2 (8.3) nmol/L, which probably influenced the results as it means that many patients with insufficiency presented vitamin D serum levels close to normal.

Although prematurity and low weight were considered predictive variables, as they have been reported as factors associated to DDE [36,5,81], we did not find an association between these variables and DDE in the present population, corroborating to the results of Ruschel et al. [63]. This could be because most premature infants were categorized as having adequate size and weight according to the gestational age, with most being born at 35–36 weeks, which is considered moderate prematurity. A recent study [56] suggested that a child's longer gestational age, especially beyond 36 weeks, was associated with the occurrence of opacities. Thus, further studies are necessary to elucidate this issue.

This study addresses several important points: it explores the association of heavy metals, vitamin D, and calcium levels in umbilical cord blood with DDE in a specific group of primary teeth (incisors); the study population consisted solely of children with all eight incisors erupted; the analysis included only predictors and effect modifier variables to which the pregnant women or infants were exposed during the mineralization period of the incisors' enamel; and the assessment was conducted early (between 6 and 30 months of age), thereby reducing the risk of underestimating DDE due to wear, fractures, or carious lesions, which are more likely to occur when examinations are conducted at an older age. The prevalence of DDE in primary incisors in the present study was 27.1 %, a higher prevalence than reported by Ruschel et al. [62], that evaluated children of an older age (from 2 to 5 years old). The authors decided to analyze data exclusively from children with eight incisors. This approach allowed the study to specifically assess the prevalence of DDE within this group of teeth and to effectively compare those affected by DDE with those who were not.

Although the study had an adequate sample size, the number of cases between the categories of some variables was small. Mother's skin color/race being indigenous presented significance for the occurrence of DDE in the crude Poisson regression when compared to mother's skin color/race being white; however, as in this population only two pregnant women declared themselves to be indiginous, we were unable to draw conclusions about this. This points to the need for more studies involving indigenous people. The same happened with the variables 'use of medication', 'single newborn', 'intubation at birth', '5-minute APGAR score', 'IUGR', and 'color/race of infant'.

There could have been information bias related to the use of alcohol, drugs, and tobacco, since these are delicate questions to answer. However, interviewers were trained to let participants feel comfortable to provide such information. In addition, the majority of the study population had cadmium levels below the median, and many had undetectable levels. Since smoking is the main source of cadmium exposure, results seem consistent.

Higher levels of lead in the umbilical cord were associated with developmental defects of enamel (DDE) in the primary incisors of infants born from women participating in the PIPA-UFRJ birth cohort. Moreover, we can point out that higher levels of mercury in cord blood, the

Table 6
Multivariable regression models.

	Model 0					Model 1					Model 2				
	Total	n	RR	[IC95 %]	P	Total	n	RR	[IC95 %]	P	Total	n	RR	[IC95 %]	P
Vitamin D < 75 nmol/L	188	161	0.959	[0.797; 1.154]	0.659	180	153	0.968	[0.804; 1.165]	0.730	188	161	0.955	[0.792; 1.152]	0.628
Vitamin D < 50 nmol/L	188	89	0.834	[0.512;1.359]	0.467	180	84	0.949	[0.834; 1.079]	0.422	188	89	0.819	[0.502; 1.337]	0.425
Calcium < 8.2 mg/dL	190	12	0.302	[0.045; 2.010]	0.216	184	12	0.314	[0.047; 2.100]	0.232	190	12	0.298	[0.044; 2.008]	0.214
Calcium > 11.2 mg/dL		33	0.989	[0.534; 1.831]	0.971		33	1.024	0.551; 1.902]	0.941		33	0.961	[0.501; 1.846]	0.906
Lead (Pb) ≥ 0.800 μg/dL	276	167	1.616	[1.035;2.524]	**0.035**	268	162	1.469	[0.936; 2.307]	0.095	276	167	1.590	[1.019; 2.484]	**0.041**
Mercury (Hg) ≥ 0.800 μg/L	275	144	1.304	[0.873;1.949]	0.195	267	138	1.235	[0.823; 1.853]	0.309	275	144	1.315	[0.880; 1.966]	0.181
Arsenic (As) ≥ 0.230 μg/L	274	93	1.227	[0.829;1.815]	0.306	266	90	1.271	[0.854; 1.893]	0.237	274	93	1.223	[0.828; 1.808]	0.312
Cadmium (Cd) ≥ 0.200 μg/L	276	18	1.520	[0.822;2.813]	0.182	268	17	1.369	[0.723; 2.594]	0.335	276	18	1.515	[0.840; 2.733]	0.167
	Model 3					Model 4					Model 5				
	Total	n	RR	[IC95 %]	P	Total	n	RR	[IC95 %]	P	Total	n	RR	[IC95 %]	P
Vitamin D < 75 nmol/L	183	158	1.010	[0.844; 1.208]	0.914	185	159	0.980	[0.812; 1.181]	0.829	187	161	0.951	[0.785; 1.151]	0.603
Vitamin D < 50 nmol/L	183	86	0.813	[0.490; 1.347]	0.421	185	89	0.871	[0.530; 1.433]	0.587	187	89	0.826	[0.508; 1.346]	0.443
Calcium < 8.2 mg/dL	185	12	0.298	[0.043; 2.061]	0.220	187	12	0.312	[0.047; 2.074]	0.228	189	12	0.297	[0.044; 2.002]	0.213
Calcium > 11.2 mg/dL		32	1.020	[0.550; 1.890]	0.950		33	1.026	[0.552; 1.909]	0.935		33	0.981	[0.529; 1.819]	0.952
Lead (Pb) ≥ 0.800 μg/dL	264	157	1.59	[1.002; 2.523]	**0.049**	271	164	1.64	[1.033; 2.604]	**0.036**	274	166	1.655	[1.054; 2.599]	**0.029**
Mercury (Hg) ≥ 0.800 μg/L	263	139	1.306	[0.855; 1.994]	0.216	270	140	1.254	[0.836; 1.882]	0.274	273	143	1.300	[0.871; 1.939]	0.199
Arsenic (As) ≥ 0.230 μg/L	262	91	1.209	[0.802; 1.822]	0.365	269	91	1.214	[0.814; 1.810]	0.341	272	92	1.228	[0.830; 1.817]	0.305
Cadmium (Cd) ≥ 0.200 μg/L	264	18	1.642	[0.874; 3.086]	0.123	271	17	1.411	[0.719; 2.767]	0.317	274	18	1.577	[0.859; 2.896]	0.142
	Model 6					Model 7					Model 8				
	Total	n	RR	[IC95 %]	P	Total	n	RR	[IC95 %]	P	Total	n	RR	[IC95 %]	P
Vitamin D < 75 nmol/L	154	132	0.936	[0.759; 1.155]	0.538	188	161	0.907	[0.744; 1.106]	0.335	188	161	0.933	[0.769; 1.131]	0.481
Vitamin D < 50 nmol/L	154	75	0.75	[0.439; 1.280]	0.291	188	89	0.829	[0.511; 1.346]	0.448	188	89	0.849	[0.521; 1.385]	0.513
Calcium < 8.2 mg/dL	156	11	0.314	[0.047; 2.082]	0.230	190	12	0.327	[0.049; 2.171]	0.247	190	12	0.325	[0.048; 2.189]	0.248
Calcium > 11.2 mg/dL		25	0.827	[0.390; 1.753]	0.620		33	1.006	[0.544; 1.859]	0.985		33	0.983	[0.529; 1.825]	0.956
Lead (Pb) ≥ 0.800 μg/dL	219	132	1.508	[0.938; 2.424]	0.090	276	167	1.603	[1.028; 2.501]	**0.037**	276	167	1.616	[1.035; 2.523]	**0.035**
Mercury (Hg) ≥ 0.800 μg/L	219	117	1.588	[1.019; 2.475]	**0.041**	275	144	1.320	[0.882; 1.975]	0.177	275	144	1.304	[0.873; 1.948]	0.195
Arsenic (As) ≥ 0.230 μg/L	217	76	1.261	[0.826; 1.926]	0.283	274	93	1.239	[0.838; 1.832]	0.284	274	93	1.227	[0.828; 1.817]	0.307
Cadmium (Cd) ≥ 0.200 μg/L	219	17	1.279	[0.649; 2.520]	0.478	276	18	1.502	[0.821; 2.750]	0.187	276	18	1.522	[0.819; 2.830]	0.184

Note: **Model 0**: Crude. Reference Category: Vitamin D ≥ 75nmol/L. Vitamin D ≥ 50nmol/L. Calcium 8.2–11.2 mg/dL. Lead < 0.800 μg/dL. Mercury < 0.800 μg/L. Arsenic < 0.230 μg/L. Cadmium < 0.200 μg/L; **Model 1**: adjusted for "Vitamin supplements". Reference Category: took vitamin supplements during pregnancy; **Model 2**: adjusted for "APGAR score at 1 min ≥ 7". Reference Category: APGAR at 1 min ≥ 7; **Model 3**: adjusted for "Size categorization according to GA (SG)". Reference Category: Adequate (AGA); **Model 4**: adjusted for "Hospitalization at birth". Reference Category: newborn was not hospitalized; **Model 5**: adjusted for "Neonatal Intensive Care Unit (NICU)". Reference Category: newborn was not at NICU; **Model 6**: adjusted for "Exclusively breastfeeding at the time of discharge". Reference Category: newborn was exclusively breastfed; **Model 7**: adjusted for "Prematurity". Reference Category: baby was not premature; **Model 8**: adjusted for "Birth weight". Reference Category: ≥ 2500 g.

lack of multivitamins during pregnancy, and lack of breastfeeding at discharge could be seen as risk factors for DDE in the present population.

Author statement

We, the authors of the article entitled " Cord-blood levels of vitamin D, calcium and heavy metals, and the occurrence of development defects of enamel in primary incisors: a birth cohort study," hereby declare that we have made significant contributions to the conception, design, execution, and interpretation of the research presented in this manuscript.

We affirm that this work is original, has not been published elsewhere, and is not under consideration by any other journal. We have adhered to ethical standards in conducting this research, including obtaining informed consent from participants and ensuring the confidentiality of their data.

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By submitting this manuscript, we express our commitment to the integrity of the scientific process and to the advancement of knowledge in the field of trace elements in medicine and biology.

CRediT authorship contribution statement

Vollú A.L.: Writing – original draft, Methodology, Investigation, Formal analysis. **Nunes A.F.:** Investigation. **Oliveira I.M.C.:** Investigation. **Barboza R.R.:** Investigation. **Lanna M.F.N.:** Investigation. **de Oliveira T.B.:** Investigation. **de Figueiredo N.D.:** Writing – review & editing, Project administration, Methodology. **Froes-Asmus C.I.R.:** Writing – review & editing, Project administration, Methodology. **Fonseca-Gonçalves A.:** Writing – review & editing, Supervision, Methodology, Formal analysis, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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