Impact of Cord Blood Vitamin D Level on Early Childhood Caries in Infancy: A Pilot Study

Sila Korun,1,2 Serap Cetiner1

1DDS, Department of Pediatric Dentistry, Faculty of Dentistry, Near East University, Turkey
2MD, Prof., Department of Pediatrics, Faculty of Medicine, Near East University, Turkey

Abstract

Background: Early childhood caries (ECC) is one of the most frequent chronic diseases among infants and preschool-aged children.

Objectives: Our objective was to determine an association between umbilical cord blood 25(OH)D level and early childhood caries.

Methods: This prospective cohort study was conducted at Near East University (NEU) Medical hospital in Cyprus during 2013-2016. All pregnant women followed by NEU gynecology and obstetrics clinic were invited to participate in the study. Cord blood serum samples were collected after birth and analyzed for 25(OH)D levels. Infants underwent dental examinations, and questionnaire information was gathered from parents of infants between 1 and 2 years of age.

Results: Among 120 deliveries, 90 mothers accepted to participate and supply cord blood. 40 of them were excluded; therefore, 50 toddlers were recruited to participate in the study. Cord blood serum samples were collected after birth and analyzed for 25(OH)D levels. Infants underwent dental examinations, and questionnaire information was gathered from parents of infants between 1 and 2 years of age.

Results: Among 120 deliveries, 90 mothers accepted to participate and supply cord blood. 40 of them were excluded; therefore, 50 toddlers were recruited to participate in the study. Cord blood serum samples were collected after birth and analyzed for 25(OH)D levels. Infants underwent dental examinations, and questionnaire information was gathered from parents of infants between 1 and 2 years of age.

Discussion: This study demonstrated the impact of low neonatal 25(OH)D level on the development of ECC. Based on these results, it can be suggested that measuring 25(OH)D levels of mothers during pregnancy can help maintain an optimal maternal and offspring vitamin D level, thereby protecting the infant’s dental health.

Keywords: Dental Caries, Vitamin D, Chronic Disease, Oral Health

1. Background

Early childhood caries (ECC) is one of the most frequent chronic diseases among infants, toddlers, and preschoolers. The American academy of pediatric dentistry (AAPD) defines ECC as “the presence of one or more decayed (non-cavitated or cavitated lesions), missing (due to caries), or filled tooth surfaces [dmfs] in any primary tooth in a child under 72 months of age.” Signs of any smooth-surface caries in children younger than 3 years of age indicate severe early childhood caries (5-ECC) (1).

ECC is the major cause of tooth pain and loss, and it is associated with impaired growth and development, decreased weight gain, detrimental effects on linguistic development, and reduced self-confidence, as well as, lower quality of life and decreased school performance (2). The etiology of ECC is multi-factorial that includes biomedial factors, such as microorganisms, diet, tooth resistance, and social determinants (3). Major risk factors for ECC are high levels of cariogenic bacteria, frequent consumption of sugar, and refined carbohydrates, inappropriate bottle-feeding habits, low secretion of saliva, developmental enamel defects, history of caries experience, poor oral hygiene habits of the family and child, drinking water with low fluoride content, lack of knowledge regarding risk factors and low socio-economic status (2). In addition, lack of enamel maturation or the presence of developmental enamel defects increases the risk of ECC by enhancing the retention of plaque, thereby increasing the colonization of streptococcus mutans (4).

Vitamin D is an essential pro-hormone that regulates calcium levels, playing a key role in bone health and craniofacial development. Vitamin D deficiency may result in impaired bone mineralization and ossification, leading to bone softening. In addition, as ameloblasts and odontoblasts are among the target cells for 1,25 hydroxy-vitamin D, vitamin D has a critical role in enamel, dentin, and oral bone formation, thereby affecting tooth mineralization. Furthermore, there is literature supporting the role of vitamin D in immune response to pathogens. Vitamin D may reduce the risk of developing caries by the production of antimicrobial peptides, such as cathelicidin and defensins, which attack cariogenic bacteria (5, 6).

Although mineralization of primary teeth starts in uterine life and continues after birth, there are limited studies evaluating maternal contribution to development of teeth. Cord blood reflects the materno-fetal interaction and the constituents of the newborn in early postnatal life. Although there are number of studies on the relation-
ship between serum vitamin D level and ECC in children (3, 5, 7), the impact of cord blood 25(OH)D level on ECC has not been studied yet.

Maternal deficiency of vitamin D during gestation has previously been demonstrated to have a detrimental effect on the development of primary teeth of the offspring. What is more, vitamin D deficiency during early childhood also creates similar effects on the development of permanent teeth (8).

Therefore, we hypothesized that low cord blood 25 hydroxy vitamin D (25(OH)D) levels increase the risk of development of ECC and enamel hypoplasia during infancy.

2. Methods

This was a prospective cohort study evaluating the association between cord blood 25(OH)D level and ECC during infancy (between the age of 1 and 2 years). The study was approved by the ethics committee of Near East University (NEU) Medical Faculty (code: YDU/2015/33-235, date: 05.11.2015), Nicosia/Cyprus, which evaluates studies according to the renewed version of declaration of Helsinki (2008).

2.1. Sample Size

Before the start of the study, we applied power analysis to calculate the required sample size to achieve 80% statistical power with a confidence interval of 95% and 5% level of significance. Results showed that 42 patients would be sufficient to reach the goal. Considering the possible exclusions and problems that might decrease the power of the study, we decided to recruit at least 50 patients (20% higher than the initial sample size) in the research.

2.2. Sample Collection and Study Flow

All pregnant women followed by the gynecology and obstetrics clinic of NEU Hospital between January 2013 and January 2014 were invited to participate in this study. Written informed consents to collect cord blood samples from expecting mothers were obtained. Those who accepted to participate were enrolled in the study. After the enrollment, the newborns’ umbilical cord blood was collected following labour in the delivery room. Exclusion criteria included congenital anomalies (cardiac, pulmonary, gastrointestinal, and cranial), metabolic diseases, TORCH infections, prematurity (< 34 weeks), and low weight for gestational age (< 2500 g) identified immediately after birth or loss to/incomplete follow-up (Figure 1). All newborns’ cord blood serum samples were kept at -80°C until analysis for 25(OH)D levels.

The toddlers were invited for their first dental examination between the age of 12 and 24 months. Those who were routinely followed at the department of pediatrics at NEU Hospital were approached by their pediatricians, while those who were followed by an external pediatrician were contacted over the phone. The dental examinations were conducted by the same dentist who was blinded to cord blood 25(OH)D levels from NEU, department of pediatric dentistry.

Parents completed a questionnaire, which collected information regarding the child and parents. Parents were queried about their demographic (nationality: local/foreign, age), financial (monthly income: ≤ 900 €/ > 900 €), educational (education level of parents: primary/high school/university), and employment (employed/unemployed parents) characteristics. Mothers were asked about the duration and frequency of sun exposure (15 minutes per day/less than 15 minutes per day), nutritional status (frequency of dairy product consumption during pregnancy), and medications used during pregnancy. The questionnaire collected information regarding the child’s duration and frequency of sun exposure (15 minutes per day/less than 15 minutes per day), diseases (systemic disease: yes/no), vitamins (use of vitamins: yes/no), and medications used (yes/no), duration of mother’s milk consumption, time of onset of solid food, daily consumption of sweet products (frequency), use of pacifier (yes/no), and bottle feeding habits (yes/no). In addition, data on child and parental oral hygiene habits, knowledge of early childhood caries, and preventive treatments for the infant were gathered.
2.3. Dental Examination Protocol

Each infant was evaluated for enamel hypoplasia and ECC. Enamel hypoplasia and ECC were categorized as binary variables (yes/no). Early childhood and severe early childhood caries were assessed based on the recommendations of the workshop held by experts of the National Institute of dental and craniofacial research group (9). White spot lesions, which are seen as chalky white and opaque patches, enamel caries especially in pits and fissures, which are presented as light to dark discolored areas no larger than the size of the pit or fissure, and dentine caries are defined as ECC (8). ECC are diagnosed by visual exam only. No radiographic examination and tactile explorations were included. The knee-to-knee technique was utilized for examination. The dmft index was calculated based on the cumulative score of decayed, missing, and filled primary teeth.

2.4. Measurements

25(OH)D levels were studied in serum samples obtained from umbilical cord blood after delivery. All samples were analyzed by a single investigator from the NEU Medical Hospital, Department of Biochemistry, who was blinded to the caries status of the child. 25(OH)D levels were analyzed using the electrochemiluminescence immunoassay and a Roche Cobas e601 machine (Roche Diagnostics GmbH, Mannheim, Germany). 25(OH)D levels < 10 ng/mL were categorized as seriously deficient, ≥ 10 - 20 ng/mL as low, 21 - 29 ng/mL as deficient, and ≥ 30 ng/mL as normal (10).

2.5. Statistical Analysis

All data were entered into a Microsoft Office 2010 Database program and analyzed by means of statistical package for the social sciences (SPSS) version 18.0. Descriptive statistics (frequencies, mean ± SD values), Chi square test, Student’s t test, and Pearson Correlation were used for analyses. Although the size of the study group was not enough for an efficient logistic regression analysis, a model was constructed including independent variables (25(OH)D levels, child’s tooth brushing habits, mother’s tooth brushing habits, and father’s dental status) significantly associated with ECC.

3. Results

120 newborns delivered in NEU Hospital between January 2013 and 2014 were invited to participate. Of those, 90 mothers accepted to participate and cord blood samples were obtained during labour. All serum samples were kept at -80°C until final analysis. 40 of them were excluded due to loss to/incomplete follow-up. The remaining 50 participants were invited for a dental examination and questionnaire evaluation after their first birthday in consecutive order. All of those 50 toddlers participated in the final evaluation (Figure 1).

Of the 50 toddlers, 23 were male (46%) and 27 were female (54%). The mean age was 20.2 ± 3.1 months (range of 14-24 months). The demographic characteristics obtained from the questionnaires are presented in Table 1. The majority of the newborns were born by C-section (90%) to local families (63%) (Table 1). The majority of the pregnancies were not complicated by infectious diseases (82%) or medication use (86%) and the mothers tended to get daily sun exposure of 15 minutes (76%). Toddlers were generally healthy (88%), did not need medication (88%), and received 15 minutes of daily sun exposure (78%).

Cord blood 25(OH)D level (mean 25.6 ± 11.6 ng/mL) was found to be seriously low (< 10 ng/mL) in 12%, low (< 20 ng/mL) in 20%, deficient (21 - 29 ng/mL) in 28%, and optimal (≥ 30) in 40% of the study participants. The study group was divided into 2 categories, based on cord blood 25(OH)D (< 30 vs. ≥ 30 ng/mL). Comparison of those 2 groups based on maternal health status, use of vitamin D supplements, sun exposure or consumption of milk and dairy products during pregnancy demonstrated no statistically significant difference.

Enamel hypoplasia was detected in 8 (16%) of the toddlers. The toddlers with and without enamel hypoplasia were compared based on maternal characteristics (use of medication, experience of any infectious disease, use of vitamin D supplements, exposure to sun, and consumption of dairy products) in addition to cord blood 25(OH)D levels. The results revealed that the umbilical cord blood level of 25(OH)D < 30 was significantly associated with enamel hypoplasia of the toddlers detected between 12 and 24 months of age (P = 0.018). Furthermore, toddlers who had enamel hypoplasia demonstrated significantly more ECC (P = 0.001) (Table 2).

Dental examination of the 50 infants demonstrated ECC in 34%. Children with and without ECC were compared based on cord blood 25(OH)D levels, oral hygiene habits, use of pacifier, bottle feeding habits, night time feeding habits, systemic disorders, parents’ oral hygiene habits, nationality, maternal employment, monthly income of family, use of medications, and consumption of sweet food products. Low cord blood level of 25(OH)D and enamel hypoplasia (P = 0.002 and P = 0.001, respectively), mother’s tooth brushing frequency (P = 0.012), father’s oral hygiene (P = 0.047), and inappropriate oral hygiene habits (P = 0.039) were the parameters which were significantly related to the development of ECC (Table 2). Furthermore, no significant difference was found between ECC and dental
examination age (P = 0.108).

The mean value of calculated dmft (decay, missing, filling, total) was 0.82 ± 1.35 with a range of 0 - 4. The score of dmft was significantly in negative correlation with the level of cord blood 25(OH)D (r = -0.45, P = 0.01) (Figure 2).

Figure 2. Cord Blood 25(OH)D Levels and dmft Values

The score of dmft was significantly in negative correlation with the level of cord blood 25(OH)D (r = -0.45, P = 0.01).

Regression analysis was performed to assess the relationship between independent variables and the dependent outcomes of ECC. Logistic regression model for ECC constructed 5 different variables which were significantly associated with ECC. Variables except low vitamin D value (P = 0.039) were insignificant from the results of the logistic regression analysis. The limited sample size was considered as the reason. As the 25(OH)D level increases one unit, there is a 0.910 less chance for the child to have ECC. $R^2$ value of the model was 0.407. Confidence interval was between 0.832 and 0.995 (Table 3).

Table 3. Logistic Regression Analysis of Risk Factors for Development of ECC Between 1 and 2 Years of Age

| Risk Factors                      | P Value | Confidence Interval |
|----------------------------------|---------|---------------------|
|                                  |         | Lower   | Upper   |
| Mother tooth brushing            | 0.176   | 0.035   | 1.847   |
| Infant tooth brushing            | 0.999   | 0.000   |         |
| Father’s bad dental status       | 0.115   | 0.721   | 19.907  |
| Enamel hypoplasia                | 0.438   | 0.022   | 5.212   |
| Low 25(OH)D vitamin D level      | 0.039   | 0.832   | 0.995   |
| Constant                         | 0.999   |         |         |

4. Discussion

In this study, 25(OH)D levels were found to be seriously low, low, or deficient in 60% of cord blood samples in our study population, although 72% of the mothers received vitamin D supplementation during pregnancy.

Among the 50 toddlers studied, 16% demonstrated enamel hypoplasia, while 34% presented ECC. The only risk factor for the development of enamel hypoplasia was demonstrated to be decreased cord blood 25(OH)D level among all the other risk factors evaluated. Meanwhile, in infants who developed ECC within 1 - 2 years of age, the frequency of 25(OH)D level below 30 ng/ml, the inadequacy of tooth brushing habits of the infant and mother, the level of the father’s bad dental status, and the amount of enamel hypoplasia were significantly higher compared to those without ECC. In addition, there was a significant negative correlation between dmft score and 25(OH)D levels.

Although there are a number of studies which evaluated the association between serum vitamin D level and ECC in children (3, 5, 7), there is no published study to date investigating the impact of cord blood 25(OH)D level on ECC in a newborn cohort.

It is well-known that mineralization of primary teeth starts in uterine life and continues after birth. In spite of this fact, there are limited studies evaluating maternal impact on tooth development. Cord blood reflects the maternal effect on the fetus as well as in the first months of postnatal life (11). Therefore, based on the effect of vitamin D on tooth mineralization of the primary teeth, hereby, by measuring the cord blood level, we detected the maternal effect on postnatal tooth mineralization.

The other factors that have an impact on vitamin D synthesis are sun exposure, diet, and vitamin D supplementation of the expectant mother and infant (12). Although all those factors were evaluated in this study, none of them were shown to have an impact on the cord blood levels of vitamin D.

In the study of Schroth et al. (3), serum 25(OH)D measurements were studied during pregnancy (between second-third trimester) and the association between maternal vitamin D levels and infants’ ECC were evaluated. Low maternal 25(OH)D levels were associated with the development of ECC in early childhood. Moreover, there was a significant relationship between enamel hypoplasia and ECC, and a negative correlation between dmft and levels of 25(OH)D in this study. Our results are in accordance with this study findings (3).

In another study conducted on children with ECC, those who had 25(OH)D levels below the optimal level demonstrated more severe S-ECC. This study demonstrated the impact of vitamin D level on the severity of ECC (5).
The positive relationship between enamel hypoplasia and childhood ECC was shown in another study, as well (4).

A recent meta-analysis revealed that supplementation with vitamin D in children decreases the risk of dental caries by 47% although statistical significance was insufficient. The authors suggested that further investigations are warranted to evaluate the long-term effect of vitamin D supplementation in infancy on the development of dental caries (7). In another study, T. Dudding et al. evaluated the association between 25(OH)D level and the presence and severity of caries with little evidence. However, the authors declared that larger and long-term studies are required (6).

In a recently published survey, performed on 5-12 year-old children, no significant association was demonstrated between dental caries and 25(OH)D vitamin levels. Based on the results of this survey, it can be suggested that cross-sectionally measured 25(OH)D levels may not have an association with current dental caries; but prenatal or early childhood vitamin D status is more predictive for future mineralization, thereby dental caries and hypoplasia (13).

Although one of the limitations of our study was the low sample size, power analysis was performed to calculate the required sample size for sufficient statistical power. On the other hand, the current study evaluated the impact of low cord 25(OH)D level on development of ECC for the first time. ECC is the most frequently observed chronic disease among children. Therefore, our results bring new approaches into mind towards the prevention of ECC.

In conclusion, this study demonstrated that among our study group, one of the most important factors in development of ECC and enamel hypoplasia is cord blood 25(OH)D level. Besides, the importance of familial dental care habits is confirmed. Based on our results, it can be suggested to measure 25(OH)D levels during pregnancy in order to maintain an optimal maternal and offspring vitamin D level by vitamin D supplementation and thereby protect the child’s dental health. In addition, oral hygiene education should be given to pregnant women as well as to the child postnatally.

Acknowledgments

The authors thank the staff of the Near East University hospital, department of pediatrics and department of pediatric dentistry.

Footnote

Financial Disclosure: There are no financial interests related to the materials in the manuscript. Also, there is no conflict of interest in this study.

References

1. Pediatrics AAoPatAAo . Policy on early childhood caries (ecc): classifications, consequences, and preventive strategies 2014. Available from: http://www.aapd.org/media/policies_guidelines/p_eccclassifications.pdf.
2. Chou R, Cantor A, Zakher B, Mitchell JP, Pappas M. Preventing dental caries in children 5-12 years: systematic review updating USPSTF recommendation. Pediatrics. 2013;132(2):332-50. doi: 10.1542/peds.2013-1469. [PubMed: 23854849].
3. Schroth RJ, Lavelle C, Tate R, Bruce S, Billings RJ, Moffatt ME. Prenatal vitamin D and dental caries in infants. Pediatrics. 2014;133(5):e1277-84. doi: 10.1542/peds.2013-2215. [PubMed: 24753535].
4. Tinanoff N, Reisine S. Update on early childhood caries since the Surgeon General’s Report. Acad Pediatr. 2009;9(8):396-403. doi: 10.1016/j.acap.2009.08.006. [PubMed: 19945074].
5. Schroth RJ, Levi JA, Sellers EA, Friel J, Kleweer I, Moffatt ME. Vitamin D status of children with severe early childhood caries: a case-control study. BMC Pediatr. 2013;13:174. doi: 10.1186/1471-2431-13-174. [PubMed: 24160554].
6. Dudding T, Thomas SJ, Duncan K, Lawlor DA, Timpson NJ. Re-examining the Association between Vitamin D and Childhood Caries. PLoS One. 2015;10(12):e0143769. doi: 10.1371/journal.pone.0143769. [PubMed: 26692015].
7. Hujoel PP. Vitamin D and dental caries in controlled clinical trials: systematic review and meta-analysis. Nut Rev. 2013;71(2):86-97. doi: 10.1111/njr.12044. [PubMed: 23356936].
8. Davit-Beal T, Gabay J, Antoniolli P, Masle-Farquhar J, Wolikow M. Dental complications of rickets in early childhood: case report on 2 young girls. Pediatrics. 2014;133(4):e1077-81. doi: 10.1542/peds.2013-0733. [PubMed: 24616355].
9. Drury TF, Horowitz AM, Ismail AI, Maertens MP, Rozier RG, Selwitz RH. Diagnosing and reporting early childhood caries for research purposes. A report of a workshop sponsored by the National Institute of Dental and Craniofacial Research, the Health Resources and Services Administration, and the Health Care Financing Administration. J Public Health Dent. 1999;59(3):192-7. [PubMed: 10649591].
10. Holick MF. Vitamin D status: measurement, interpretation, and clinical application. Ann Epidemiol. 2009;19(2):73-8. doi: 10.1016/j.annepidem.2007.12.001. [PubMed: 18329892].
11. Pinkham JRCP, Fields HW, McTigue DJ, Nowak A. Pediatric Dentistry. Elsevier Saunders; 2005.
12. Anderson PGW, Halderson H, Sommerfeldt C, Agnew G. The influence of vitamin D in the prevention of dental caries. J Am Dent Assoc. 1934;21(8):1149-66.
13. Herzog K, Scott JM, Hujoel P, Seminario AL. Association of vitamin D and dental caries in children: Findings from the National Health and Nutrition Examination Survey, 2005-2006. J Am Dent Assoc. 2016;147(6):433-20. doi: 10.1016/j.adaj.2015.12.013. [PubMed: 26827077].
Table 1. Characteristics of the Study Group

| Characteristics (n = 50) | No. (%) |
|-------------------------|---------|
| **Demographic**         |         |
| Birth                   |         |
| Normal                  | 5 (10)  |
| Caesareansection        | 45 (90) |
| Nationality             |         |
| Local                   | 32 (63) |
| Foreign                 | 19 (37) |
| Monthly income, €       |         |
| ≤ 900                   | 24 (48) |
| > 900                   | 26 (52) |
| **Pregnancy**           |         |
| Infectious disease      |         |
| Yes                     | 9 (18)  |
| No                      | 41 (82) |
| Medication              |         |
| Yes                     | 7 (14)  |
| No                      | 43 (86) |
| Exposure to sun         |         |
| Daily 15 min            | 38 (76) |
| Sometimes               | 12 (24) |
| **Infancy**             |         |
| Systemic disorder       |         |
| Yes                     | 6 (12)  |
| No                      | 44 (88) |
| Medication              |         |
| Yes                     | 68 (12) |
| No                      | 44 (88) |
| Exposure to sun         |         |
| Daily 15 min            | 39 (78) |
| Sometimes               | 11 (22) |
| Pacifier                |         |
| Yes                     | 16 (32) |
| No                      | 34 (68) |
| Bottle-feeding          |         |
| Yes                     | 26 (52) |
| No                      | 24 (48) |
| Night-time snacking     |         |
| Yes                     | 36 (72) |
| No                      | 14 (28) |
| Activity                                | Yes  | No  |
|-----------------------------------------|------|-----|
| Adding sugar to milk                    | 10 (20) | 40 (80) |
| Using sugar for bottle feeding          | 3 (6) | 47 (94) |
| Consumption of sweet food               | 19 (38) | 31 (62) |
| Brushing teeth                          | 8 (16) | 42 (84) |
| 25(OH)D level                           | 19 (38) | 31 (62) |
| Knowledge on ECC                        | 5 (10) | 45 (90) |
| Dental visit                            | 0 (0) | 50 (100) |
| Knowledge on preventive applications    | 1 (2) | 49 (98) |
| Performed preventive applications       | 0 (0) | 50 (100) |
| Use of flouride tablets                 | 0 (0) | 50 (100) |
Table 2. Comparison of Infants with and Without ECC

| Toddler Characteristics          | ECC % of Toddlers | No ECC % of Toddlers | P Value |
|----------------------------------|-------------------|----------------------|---------|
| 250 HD level                     |                   |                      | 0.002^a |
| Optimum                          | 6                 | 32                   |         |
| Deficient                        | 28                | 34                   |         |
| Brushing teeth                   |                   |                      | 0.039^b |
| Yes                              | 0                 | 16                   |         |
| No                               | 34                | 50                   |         |
| Systemic disorder                |                   |                      | 0.396^b |
| Yes                              | 6                 | 6                    |         |
| No                               | 28                | 60                   |         |
| Using pacifier                   |                   |                      | 0.318^b |
| Yes                              | 14                | 18                   |         |
| No                               | 20                | 48                   |         |
| Use of medication                |                   |                      | 0.650^b |
| Yes                              | 2                 | 10                   |         |
| No                               | 52                | 56                   |         |
| Consumption of sweet food        |                   |                      | 0.369^b |
| Yes                              | 24                | 38                   |         |
| No                               | 10                | 28                   |         |
| Night time feeding               |                   |                      | 0.187^b |
| Yes                              | 20                | 52                   |         |
| No                               | 14                | 14                   |         |
| Adding sweet products to milk    |                   |                      | 0.707^b |
| Yes                              | 8                 | 12                   |         |
| No                               | 26                | 54                   |         |
| Adding sweet products to bottle  |                   |                      | 1.0^h   |
| Yes                              | 2                 | 4                    |         |
| No                               | 32                | 62                   |         |
| Mother brushing her own teeth    |                   |                      | 0.012^b |
| Sometimes once a day             | 16                | 8                    |         |
| Twice a day or more              | 18                | 58                   |         |
| Nationality                      |                   |                      | 0.275^b |
| Local                            | 18                | 44                   |         |
| Foreign immigrant                | 16                | 20                   |         |
| Monthly income                   |                   |                      | 0.272^b |
| ≤ 2 subsistence level            | 20                | 28                   |         |
| > 2 subsistence level            | 14                | 38                   |         |
| Mother employed                  |                   |                      | 0.442^b |
| No                               | 14                | 20                   |         |
| Yes                              | 20                | 46                   |         |
|                          |         |     |
|--------------------------|---------|-----|
| **Mother's dental status** |         |     |
| Good                     | 20      | 40  |
| Bad                      | 14      | 26  |
| **Father's dental status** |         |     |
| Good                     | 18      | 54  |
| Bad                      | 16      | 12  |
| **Enamel hypoplasia**     |         |     |
| Yes                      | 14      | 2   |
| No                       | 20      | 64  |

Abbreviation: ECC, early childhood caries.

*Student's t tests were used.

*Chi square tests were used.