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Article

Explore Ways for Children to Maintain a Caries-Free State While Receiving the Benefits of Breast Milk for as Long as Possible: A Longitudinal Analysis

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Abstract

Background/Objectives: The relationship between prolonged breastfeeding and ECC remains inconclusive and equivocal. However, dentists must manage children's oral health while respecting the mother's chosen feeding habits and provide information on maintaining a caries-free state while receiving the benefits of breast milk for as long as possible. This longitudinal study aimed to investigate the occurrence of dental caries in children who were breastfed for at least 18 months and to explore ways for children to remain caries-free while receiving the benefits of prolonged breastfeeding. **Methods:** Of 6746 children aged 42 months who underwent physical and oral examinations at public healthcare centers in Toyonaka City, Japan, 1210 who had continued breastfeeding for at least the first 18 months were enrolled in the study. Oral examinations and anthropometric height and weight measurements were performed at 18 and 42 months of age. Additionally, a structured self-administered interview sheet was provided directly to the guardians when the child was 18 months old. Caries risk factors were evaluated using a logistic regression model, with caries occurrence at 42 months of age as the dependent variable. **Results:** Among the enrolled children, 24.3% had experienced dental caries. Logistic regression analysis results revealed that the child factors of birth order ($P<0.001$), snacking ($P<0.05$), and colorimetric caries-risk test results ($P<0.001$) were significantly associated with dental caries occurrence at 42 months. The child factor of wake-up time ($P<0.05$) and the guardian factors of parental exhaustion ($P<0.05$) and postnatal oral health class participation ($P<0.01$) were significant preventive factors. **Conclusions:** These results suggest that establishing early waking habits in early childhood and providing psychological support and postnatal oral health instruction for mothers help their children to remain caries-free while receiving the benefits of prolonged breastfeeding.

Keywords: breastfeeding; dental caries; postnatal class; mental health; longitudinal analysis

1. Introduction

Breastfeeding is one of the most effective ways to ensure a child's health and survival [1] and might permanently shape an individual's life course [2]. Breast milk is the ideal food for infants, and it benefits both the infants and the mothers [1]. Breastfed children perform better on intelligence tests, are less likely to be overweight or obese, or to experience inflammatory bowel disease, allergic rhinitis, asthma, or diabetes later in life. [1,3,4]. Breastfeeding women also have a reduced risk of breast and ovarian cancers, and metabolic and cardiovascular disease [1,5,6]. Therefore, the World Health Organization (WHO) recommends breastfeeding up to 2 years of age or beyond [7].

However, prolonged breastfeeding can be a risk factor for early childhood caries (ECC) [8-10]. Some recent systematic reviews have shown that breastfeeding beyond 12 months of age is associated with severe ECC [8,9] and another concluded that breastfeeding beyond 24 months could increase the risk of ECC [10]. The International Association of Paediatric Dentistry has recommended against breastfeeding and bottle use beyond 12 months to prevent ECC [11]. Conversely, a recent cohort study found that exclusive breastfeeding for 6–17 months provides a protective effect against ECC [12]. Thus, the relationship between prolonged breastfeeding and ECC remains inconclusive and equivocal. However, dentists must manage children's oral health while respecting the mother's chosen feeding habits and provide information on maintaining a caries-free state while receiving the benefits of breast milk for as long as possible, especially for mothers who wish to breastfeed. Therefore, research focusing on the characteristics of long-term breastfed children is needed.

In this longitudinal study, the aims were to investigate caries occurrence in children who were breastfed for at least 18 months, to clarify the factors preventing the development of caries, and to explore ways for children to remain caries-free while receiving the benefits of prolonged breastfeeding.

2. Materials and Methods

2.1. Study Participants

The study participants were selected from 6746 children who received free medical and dental health examinations at 42 months of age between April 2018 and March 2020 in Toyonaka City, a core city of Osaka prefecture, Japan. The inclusion criteria were any child who had received these services in Toyonaka City at 18 months of age, had a complete set of records of their dental and clinical examination, and whose examination data and the interview sheet provided by the mothers were complete. The exclusion criterion was any child who had weaned by 18 months of age. Finally, 1210 children aged 42 months who had continued breastfeeding for at least the first 18 months were included. This study was conducted with full adherence to the Declaration of Helsinki. The study protocol was approved by the Ethics Committee of Osaka University Graduate School of Dentistry (approval number R2-E25). Informed consent was obtained from all the participants and their mothers in the form of an opt-out on the website.

2.2. Clinical Examination

The physical examinations included anthropometric measurements of height and weight at 18 months of age. In the oral examinations, the number of erupted teeth (at 18 months of age), the number of decayed teeth (at 18 months of age), and decayed, missing, and filled primary teeth (dmft) per child (at 18 and 42 months of age) were recorded for each child by visual inspection with a dental mirror under lighting according to criteria established by the WHO [13]. The number of erupted teeth at 18 months was divided into three categories based on the eruption order of primary teeth in Japanese children (≤ 12 , 13–16, $17 \leq$) [14]. The oral examinations were performed by dentists from the Toyonaka Dental Association.

2.3. Microbiological Assessment

The microbiological status was assessed at 18 months of age using Cariostat® (Dentsply Sirona, Tokyo, Japan), which is a caries-risk test measuring the presence of acidogenic microorganisms in a colorimetric manner [15]. Plaque was collected from the buccal surfaces of all maxillary teeth using a sterilized cotton swab, which was soaked in a test medium in a Cariostat® test tube and incubated at 37°C for 48 hours. After incubation, the color of the test medium turns from blue to yellow via green, determined by the sampled plaque acidity. The color was graded according to the color chart and classified into four categories: (-), (+), (++) and (+++) [15].

2.4. Interview Sheet

The structured self-administered interview sheet developed by the public healthcare centers was given to the mothers and collected at the 18-month examinations. The interview sheet was completed at the 18-month examinations, which included the following information about the children: sex, birth order (first, second, third or later), parenting environment (commuting to nursery or not), bottle feeding (weaned or ongoing), snacking habits per day (never, once or twice, 3 times or more), eating before bed (never, weekly, daily), wake-up time (before 7:00, 7:00–8:00, after 8:00, unsettled), bedtime (before 21:00, 21:00–22:00, after 22:00, unsettled), topical fluoride application experience (yes or no), and use of fluoride-containing toothpaste (yes or no). Information collected about the mothers included: exhausted with parenting (yes or no), enjoying parenting (yes or no), anxiety (yes or no), physical condition (healthy or unhealthy), participation in prenatal classes (yes or no), and participation in postnatal oral health classes (yes or no).

2.5. Statistical Analysis

Descriptive analyses included calculation of relative frequencies, absolute frequencies, means, and standard deviations with the univariate analysis. The number of erupted teeth, dmft, Cariostat score, and body mass index (BMI) in the caries-free group and the caries experience group were compared using Student’s *t*-test. Dental caries prevalence was defined as the frequency of children with at least one carious tooth. We performed logistic regression with dental caries occurrence at 42 months of age as the dependent variable. The odds ratio (ORs) and their 95% confidence intervals (CI) were estimated as risk factors for dental caries occurrence at 42 months of age. The fit of the data to the model was tested using the Hosmer-Lemeshow test, and multicollinearity was assessed by using the variance inflation factor (VIF). All data were analyzed using IBM SPSS Statistics version 28.0.1.0® (IBM Japan, Tokyo, Japan), and the level of statistical significance was set at *P*<0.05.

3. Results

3.1. Characteristics of the Participants

The characteristics of the participants are shown in Table 1. Of 1210 children, 294 (24.3%) had experienced dental caries at 42 months of age. In the caries experience group, dmft at 18 months of age was 0.36±1.3, and dmft at 42 months of age was 3.3±2.6. There were no significant differences in the number of erupted teeth or BMI at 18 months of age between the caries-free group and the caries experience group. However, the caries experience group had a significantly higher Cariostat score at 18 months of age than the caries-free group (*P*<0.001).

Table 1. Descriptive statistics.

	Caries-free N=916	Caries experience N=294	<i>P</i>
Number of erupted teeth at 18 months of age	14.5±2.3	14.4±2.5	0.545
dmft at 18 months of age	0	0.36±1.3	<0.001
BMI at 18 months of age	15.9±1.8	16.0±1.8	0.874
Cariostat score at 18 months of age	1.1±0.5	1.3±0.6	<0.001
dmft at 42 months of age	0	3.3±2.6	<0.001

Mean ± SD. Student’s *t*-test was used to compare two groups.

3.2. Univariate Analysis

Table 2 shows the results of the univariate analysis of dental caries distribution among factors in children. The following factors were significantly associated with dental caries experience at 42 months of age: Cariostat score at 18 months of age (+: *P*<0.001; ++: *P*<0.001), birth order (second: *P*<0.001; third or later: *P*<0.001), snacking at 18 months of age (once or twice: *P*<0.001; ≥3 times:

$P<0.05$), wake-up time at 18 months of age (before 7:00: $P<0.001$), bedtime at 18 months of age (before 21:00: $P<0.01$), and fluoride-containing toothpaste at 18 months of age ($P<0.05$). For factors relating to the mother, exhausted with parenting when the child was 18 months old ($P<0.05$), anxiety when the child was 18 months old ($P<0.05$), and participation in postnatal oral health classes ($P<0.001$) were significantly associated with dental caries experience at 42 months of age (Table 3).

Table 2. Dental caries distribution among factors in children.

Variables	Caries Prevalence		<i>P</i>
	N	(%)	
Bottle-feeding (at 18 months of age)			
Weaned	275/1143	24.1	0.426
Ongoing	19/67	28.4	
Snacking (at 18 months of age)			
No	193/899	21.5	<0.001
Once or twice	57/170	33.5	
≥3 times	44/141	31.2	
Eating before bed (at 18 months of age)			
No	166/726	22.9	0.185
Weekly	45/162	27.8	
Daily	83/322	25.8	
Wake-up time (at 18 months of age)			
Before 7:00	63/368	17.1	<0.001
7:00–8:00	152/547	27.8	
After 8:00	51/189	27.0	0.831
Unsettled	28/106	26.4	
Bedtime (at 18 months of age)			
Before 21:00	45/262	17.2	0.008
21:00–22:00	173/683	25.3	
After 22:00	53/186	28.5	0.383
Unsettled	23/78	29.5	
Topical Fluoride Application (at 18 months of age)			
No	268/1067	25.1	0.071
Yes	26/143	18.2	
Fluoride-containing toothpaste (at 18 months of age)			
No	172/643	26.7	0.034
Yes	122/567	21.5	

Univariate analysis was used to compare each item.

Table 3. Dental caries distribution among factors in mothers.

Variables	Caries Prevalence		<i>P</i>
	N	(%)	
Exhausting with parenting (at 18 months of age)			
No	231/1000	23.1	0.035
Yes	63/210	30.0	

Enjoying parenting (at 18 months of age)				
No	2/12	16.7		
Yes	292/1198	24.4	0.539	
Anxiety (at 18 months of age)				
No	251/986	25.5		
Yes	43/224	19.2	0.049	
Physical condition (at 18 months of age)				
Healthy	252/1073	23.5		
Unhealthy	42/137	30.7	0.066	
Participation in prenatal class				
No	276/1111	24.8		
Yes	18/99	18.2	0.141	
Participation in postnatal oral health class				
No	254/938	27.1		
Yes	40/272	14.7	<0.001	

Univariate analysis was used to compare each item.

3.3. Logistic Regression Analyses

The results of the logistic regression analyses for dental caries experience at 42 months of age are shown in Table 4. Adjusted for potential confounders, the following factors were significantly associated with dental caries experience at 42 months of age: Cariostat score (++) (OR=1.93; 95% CI: 1.32–2.82, $P<0.001$), Cariostat score (+++) (OR=5.09; 95% CI: 2.41–10.75, $P<0.001$), second child (OR=1.80; 95% CI: 1.29–2.53, $P<0.001$), third or later child (OR=2.29; 95% CI: 1.51–3.45, $P<0.001$), snacking once or twice per day (OR=1.52; 95% CI: 1.02–2.25, $P<0.05$), wake-up time before 7:00 (OR=0.63; 95% CI: 0.43–0.91, $P<0.05$), exhausted with parenting (OR=1.51; 95% CI: 1.02–2.25, $P<0.05$), and participation in postnatal oral health classes (OR=0.56; 95% CI: 0.37–0.84, $P<0.01$). In the regression model, the Hosmer-Lemeshow test showed a good fit ($P=0.733$), and the VIF showed the absence of multicollinearity for each independent variable (<4.0).

Table 4. Logistic regression analysis with occurrence of dental caries as the dependent variable (only significant variables are listed).

Variables	Adjusted odds ratio (95% CI)	P	VIF
Cariostat score (at 18 months of age)			1.013
+	Ref.		
–	0.68 (0.29–1.58)	0.373	
++	1.93 (1.32–2.82)	<0.001	
+++	5.09 (2.41–10.75)	<0.001	
Birth order			1.115
First	Ref.		
Second	1.80 (1.29–2.53)	<0.001	
Third or later	2.29 (1.51–3.45)	<0.001	
Snacking (at 18 months of age)			1.089
No	Ref.		
Once or twice	1.52 (1.02–2.25)	0.038	
≥3 times	1.27 (0.83–1.96)	0.273	
Wake-up time (at 18 months of age)			1.052

7:00–8:00	Ref.	
Before 7:00	0.63 (0.43–0.91)	0.015
After 8:00	0.98 (0.65–1.49)	0.930
Unsettled	0.95 (0.57–1.59)	0.850
Exhausting with parenting (at 18 months of age)		1.238
No	Ref.	
Yes	1.51 (1.02–2.25)	0.041
Participation in postnatal oral health class		1.135
No	Ref.	
Yes	0.56 (0.37–0.84)	0.005

Adjusted for sex, birth order, eating before bed, snacking, bottle-deeding (at 18 months of age), bedtime (at 18 months of age), wake-up time (at 18 months of age), exhausted with parenting (at 18 months of age), enjoying parenting (at 18 months of age), anxiety (at 18 months of age), physical condition (at 18 months of age), nursery (at 18 months of age), topical fluoride application (at 18 months of age), fluoride-containing toothpaste (at 18 months of age), pregnancy class, oral health class, number of erupted teeth (at 18 months of age), and cariostat score (at 18 months of age).

4. Discussion

The relationship between prolonged breastfeeding and ECC remains inconclusive and equivocal. Dentists must manage children’s oral health and provide information on maintaining a caries-free state while receiving the benefits of breast milk over the long term for mothers who wish to breastfeed. However, few studies have focused on the characteristics of long-term breastfed children. The present longitudinal study clarified the importance of comprehensive social support for mothers to achieve both children’s caries-free state at 42 months of age and the benefits of breastfeeding for at least the first 18 months.

Of the children enrolled in this study, 24.3% had experienced dental caries at 42 months of age. According to some data from the last few years in Japan, the dental caries prevalence rate at 3 years of age was 14.6%–20.0%, which is lower than the present results [16–18]. Previous research findings have indicated that prolonged breastfed children are more likely to experience dental caries [8-10]. However, a recent systematic review concluded that breastfeeding for less than 24 months is not associated with an increased risk of ECC [10], and a longitudinal study showed that a higher intake of free sugars in breastfed children, not breastfeeding itself per se, is associated with an increased risk of ECC [19]. This result is plausible based on the results of a recent cohort study that found that exclusive breastfeeding for 6–17 months provides a protective effect against ECC [12]. Additionally, a systematic review showed that nocturnal breastfeeding is one of the strongest risk factors for ECC [8]. In the present study, these two important caries risk factors, intake of free sugars and nocturnal breastfeeding, couldn’t be evaluated in breastfed children; thus, further research focusing on the amount and frequency of intake of free sugars and the duration and frequency of nocturnal breastfeeding in breastfed children is warranted.

In long-term breastfed children, birth order was significantly associated with dental caries experience at 42 months of age in the present study. Later-born children tend to be influenced by first-born children and exposure to free-sugar-containing foods in earlier childhood [20]. As a result, they can easily acquire snacking habits and have higher acidity in dental plaque in the oral cavity. Therefore, birth order, snacking and the Cariostat score, high-risk factors for ECC in the present study as well as a previous study [14], may be interconnected through dietary issues. Future cluster analyses would be useful to clarify the interconnection among risk factors for ECC. In contrast, snacking three or more times per day at 18 months of age was not a significant risk factor for dental caries experience at 42 months of age. Taking this result into account, snacking habits after 18 months

of age rather than at 18 months of age may have a greater impact on caries development at 42 months of age.

Waking up before 7:00 was a longitudinal ECC preventive factor for long-term breastfed children in the present logistic regression analysis result. Additionally, going to bed before 21:00 also prevented caries development longitudinally in our univariate analysis. Some population studies and systematic reviews concluded that irregular or late bedtime and fewer sleeping hours may be risk factors for ECC [21,22]. The present results suggest that establishing a rhythm of life and sleeping habits by 18 months of age is important for maintaining oral health and may assist in preventing ECC in long-term breastfed children. However, the relationship between the establishment of circadian rhythm and nocturnal breastfeeding remains inconclusive and equivocal [23–2], and nocturnal breastfeeding does not necessarily lead to an irregular rhythm of life, resulting in ECC.

The mother's exhaustion with parenting when her child was 18 months of age was significantly associated with dental caries experience at 42 months of age in long-term breastfed children in the present study. Few studies have clarified the associations between chronic maternal stress and children's dental caries experience [26]. Although the exact mechanisms are unclear, multiple mechanisms could explain the relationship. One is the quality of breast milk from mothers. It has been reported that maternal stress may significantly reduce the amount of immunoglobulin A in breast milk [27]. Additionally, maternal psychosocial stress may affect mammary epithelial cells and secretory mechanisms, which indirectly affect the production and secretion of lactoferrin and lysozyme [28]. A decrease in these antibacterial components of breast milk may contribute to ECC in long-term breastfed children. Another is that parental exhaustion may lead to a tendency to neglect toothbrushing and diet management, except for breastfeeding. Parenting stress may lead to less proper care for children [29]. Therefore, psychological and social support for mothers is warranted for preventing ECC [30].

The key strength of the present study is that it demonstrated, through a large-scale longitudinal study, that participation in postnatal oral health classes can be a preventive measure for ECC for long-term breastfed children. Previous studies have noted that prenatal and/or postnatal oral supports by dental health professionals or non-professionals may offer benefits for maternal and infant oral health [31,32]. In the city in which this study took place, a postnatal oral health class is performed for children aged 8–10 months and their mothers to provide dietary and toothbrushing instruction and dental consultations for the child. These postnatal oral health classes could contribute to ECC prevention by increasing mothers' interest, awareness, and motivation in caring for their child. However, the class is only held on weekdays, and the participants tend to be highly interested in caring for their child and also have sufficient time to attend the classes. Therefore, both the positive effects of the classes and the participants' backgrounds may have had an impact on the present result. Postnatal oral health instruction for mothers as a public health measure is important to maintain children's oral health while benefiting from breastfeeding as much as possible.

The present study had several limitations. First, although we focused on children who continued breastfeeding for at least the first 18 months, the definition of prolonged breastfeeding remains inconclusive and equivocal [7–12]. Nevertheless, we believe that our results have established a basis for further research to evaluate the actual conditions of long-term breastfed children. Second, nocturnal feeding and the frequency of breastfeeding were not investigated in the present study. Third, the reliability and validity of the interview sheet used in the present study have not been evaluated, and the self-reported data may be subject to recall bias. Further research addressing these limitations may contribute to the eradication of ECC in the future.

5. Conclusions

Providing psychological support and postnatal oral health instruction for mothers helps their children to remain caries-free while receiving the benefits of prolonged breastfeeding. Additionally, establishing a rhythm of life and sleeping habits by the age of 18 months is important for maintaining oral health, and may assist in preventing ECC in long-term breastfed children.

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Institutional Review Board Statement: This study was conducted with full adherence to the Declaration of Helsinki. The study protocol was approved by the Ethics Committee of Osaka University Graduate School of Dentistry (approval number R2-E25).

Informed Consent Statement: Informed consent was obtained from all the participants and their mothers in the form of an opt-out on the website.

Data Availability Statement: All data generated or analyzed during this study are included in this published article.

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Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

BMI	body mass index
CI	confidence interval
ECC	early childhood caries
OR	odds ratio
VIF	variance inflation factor
WHO	World Health Organization

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