

#### ORIGINAL RESEARCH

# Analysis of Risk Factors Associated with Early Childhood Caries

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**Objective:** To identify the main factors associated with early childhood caries by analyzing the risk factors of early childhood caries, thus providing a reference for developing prevention programs to reduce the risk of early childhood caries.

Methods: We selected a total of 221 children aged 3-4 years from two kindergartens in Tongzhou District, Beijing for this study. We conducted oral examination and the caries activity test (Cariostat) on children and their parents / primary caregivers, and the parents / primary caregivers additionally answered a questionnaire survey. Based on the results, we comprehensively evaluated the caries status of children and statistically analyzed the caries-related factors to identify the relevant risk factors.

Results: The mean age of children in the study children was  $40.08 \pm 2.65$  months, with a caries prevalence rate of 54.97% and a mean caries value of 4.61. Early childhood caries was correlated with the intake frequency of sugary foods, intake of sugary foods before bedtime, frequency of tooth brushing, oral health knowledge of parents, caries susceptibility, and age of starting to brush teeth. Logistic regression analysis results showed that the intake frequency of sugary foods, oral health knowledge of parents, and caries susceptibility were the factors influencing early childhood caries, especially the intake frequency of sugary foods.

Conclusion: The intake frequency of sugary foods, intake of sugary foods before bedtime, frequency of brushing teeth, oral health knowledge of parents, caries susceptibility, and age of starting to brush teeth were associated with early childhood caries. Among these, the intake frequency of sugary foods, oral health knowledge of parents, and caries susceptibility, especially the intake frequency of sugary foods, were the influencing factors.

**Keywords:** correlation analysis, early childhood caries, risk factor evaluation, susceptibility

# **Background**

Childhood caries ranks first among childhood diseases in terms of prevalence, as reported in oral epidemiological data. Moreover, early childhood caries (ECC), the most common childhood oral disease with a high prevalence, has a significant impact on the health of children and imposes a considerable economic burden on the families of affected children. Young children are unable to cooperate in caries treatment to make it effective due to incomplete physical and mental development, so protective braking, sedation, or even general anesthesia may be required, posing a serious challenge for children, parents, and dentists. Therefore, it is extremely critical to strengthen the prevention and treatment of ECC. Interventions such as analyzing the factors related to caries in children, identifying the main factors related to caries in children, conducting oral education, and effective communication with parents or caregivers of children in order to control caries can help to provide appropriate caries prevention measures for children at the individual level, and at the group level, facilitate more effective use of health care resources to reduce the occurrence of ECC in a targeted way. Analysis of the factors related to childhood caries to identify the main factors related to childhood caries can facilitate targeted oral education and effective communication with parents or caregivers of children, thus effectively controlling caries. In this way, appropriate caries prevention measures can be provided for children at the individual level and health care resources can be used more effectively at the population level to reduce the occurrence of ECC in a targeted manner.<sup>2</sup>

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This study aimed to identify the main factors associated with early childhood caries by analyzing the risk factors of early childhood caries, thus providing a reference for developing prevention programs to reduce the risk of early childhood caries.

#### **Materials and Methods**

## **Experimental Materials and Tools**

In this study, we used cariostat caries susceptibility test<sup>3,4</sup> media (Beijing GangDa Medical Technology Co., Ltd., Beijing, China), constant temperature incubators at 37°C (Tianjin Zhonghuan Equipment Co., Ltd., Tianjin, China), disposable instrument boxes for oral examination (Shanghai Shengda Medical Insurance Company, Shanghai, China), oral examination forms, and oral health questionnaires.

### **Experimental Methods**

#### **Participants**

We selected 221 children aged 3–4 years from two kindergartens in Tongzhou District, Beijing. This study was conducted in accordance with the declaration of Helsinki and approved by the Ethics Committee of the School of Stomatology, Capital Medical University. Legally authorized representative and/or guardian's of all minor participants signed an informed consent form.

The inclusion criteria were as follows:

1) Kindergarten children aged 3–4 years; 2) Children without physical and psychological disease or major systemic diseases; 3) Children with parental consent to participate in the study; 4) Children who were able to cooperate with the oral examination.

#### The Caregivers of the Children Signed an Informed Consent Form

Children and their primary caregivers underwent an oral examination and the caries susceptibility test, and their primary caregivers additionally answered a questionnaire survey. We comprehensively assessed the caries status of children based on the results, developed a caries risk assessment form, and grouped the children accordingly.

#### Questionnaire Survey

Questionnaires were used to understand the extent of oral health knowledge among the caregivers of children and included the following aspects:

- 1) Oral health knowledge, including basic knowledge of oral health, oral health knowledge pertaining to young children, and caries-related knowledge.
- 2) Oral health behaviors, including daily oral hygiene habits, dental treatment-seeking behaviors, dietary habits, common oral problems, and treatment needs.
  - 3) Socio-demographic aspects, such as age, education level of parents, and family monthly income.

#### Oral Examination

An attending pediatric dentist was the examiner, and the inter-rater reliability was checked by a senior dentist (Kappa = 0.75). Another attending dentist recorded the results. The caries examinations of the children were performed using a disposable mouth mirror and CPI probe under artificial light in the kindergarten classroom. The results of the examinations were recorded in the Children's Oral Examination Form.

Examination item: Presence of caries (coronal caries).

Examination standard: World Health Organization (WHO, 1997) Diagnostic Criteria for Dental Caries.<sup>5</sup>

Dental caries: Teeth with obvious caries, enamel destruction, or detectable softened cavity at the fissure or smooth surface or caries of the cavity wall were recorded as dental caries. In addition, teeth with temporary obturation on the surface were recorded as caries. Teeth were also regarded as having caries when fissure sealants also had caries. Cariesfree tooth: a tooth with a healthy and complete crown without signs of caries and no obturation due to caries was

considered a caries-free tooth. Unformed carious cavities and similar early caries were not recorded as caries due to their unreliable diagnosis.

#### Caries Susceptibility Test

The caries susceptibility test was conducted from 9:00 am to 11:00 am. Participants were instructed to fast for more than 1 hour before the test, without brushing teeth or gargling, as well as not have any medication in the last 2 weeks.

The test was performed as per procedures, and the results were interpreted by a pediatric dentist after repeated tests (RSD = 3%).

- 1) The dentist filled in the information of participants (including name and date) on the label and affixed the label to the upper part of the culture bottle (with instructions to not block the liquid in order to facilitate the observation of the results).
- 2) The dentist wore sterile gloves and collected samples from the buccal side of the maxillary molars and the labial side of the mandibular anterior teeth by wiping them thrice with swabs.
- 3) The swabs were placed in the medium, and then the bottles were capped, shaken thoroughly, and placed in a  $37^{\circ}$ C incubator within 4 hours for  $48 \pm 6$  hours of incubation.
- 4) The results of the medium were observed and compared with the colorimetric card under natural light, and the cariostat values were recorded to determine the caries susceptibility (Table 1, Figure 1).

#### Development of the Caries Risk Assessment Form

We comprehensively analyzed the results of the questionnaire survey, oral examinations, and caries susceptibility test responses, and based on the caries risk assessment form for children aged 0–5 years in the Caries-risk Assessment Tool published by the American Academy of Pediatric Dentistry in 2010,<sup>6</sup> we developed a simplified caries risk assessment form for this study (Table 2).

#### Grouping

The participants were assessed for caries-related risk factors as shown in Table 2, and a profile was created for each child. The children were assigned to high- and low-risk groups according to the assessment results. The risk category of each child was determined by his/her highest risk factor.

# Statistical Analysis

Data were entered using SPSS 17.0 and statistically analyzed with a test level of P = 0.05. The differences in the mean value of caries and caries rates were compared with the t-test and chi-square test, respectively. The correlation between caries risk factors and ECC was evaluated using logistic regression, and the caries susceptibility results were analyzed with the rank sum test.

#### Results

# Analysis of Caries in Children

Of the 221 participants, 121 children suffered from caries, with a caries rate of 54.97% and a mean value of caries of 4.61. There was no significant difference in gender distribution as revealed by the chi-square test ( $\chi^2 = 0.003$ , P = 0.887)

Color	Cariostat Values	рН	Caries Susceptibility
Blue	0	>6.0	Safe Zone
Blackish green	0.5	5.9-5.7	Safe Zone
Dark green	1.0	5.6-5.2	Attention Zone
Green	1.5	5.1-4.9	Danger Zone
Chartreuse	2.0	4.8-4.6	Danger Zone
Light yellow	2.5	4.5-4.3	High danger zone
Yellow	3.0	<4.2	High danger zone

Table I Cariostat Values, Color, and pH

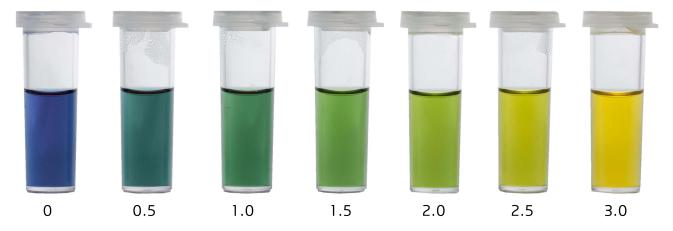


Figure I Cariostat values, color, and pH.

and mean age in months as shown by the *t*-test (T = 2.271, P = 0.307) between the high-risk and low-risk groups. Additionally, the caries rate was 50.96% in 117 boys and 58.97% in 104 girls, and the gender difference was not significant as seen from the chi-square test results ( $\chi^2 = 0.998$ , P > 0.05). The mean value of caries was 4.46 in boys and 4.74 in girls. The mean value of caries in girls was higher than that in boys, with a significant difference as seen from the *t*-test results (T = 1.218, P < 0.05) (Table 3).

# Questionnaire Analysis

The analysis of the questionnaire responses indicated that the intake frequency of sugary food, frequency of brushing, intake of sugary foods before bedtime, oral health knowledge of parents, and the age of starting to brush teeth were

Table 2 The Caries Risk Assessment Form

Factors	High-Risk	Low-Risk
Biological factors		
Mother/primary caregiver has active dental caries	Yes	
Low socioeconomic status of parents/caregivers	Yes	
Intake of sugary snacks or drinks more than 3 times a day between meals	Yes	
Intake of sugary drinks or foods before bedtime	Yes	
Protective factors		
Drinking water fluoridation or appropriate fluoride application		Yes
Brushing teeth daily with fluoride toothpaste		Yes
Regular topical fluoride treatment in an oral health institute		Yes
Regular oral examinations		Yes
Clinical examinations		
At least I decayed tooth/filled tooth/lost tooth due to caries	Yes	
Active white punctate caries or enamel caries	Yes	
Cariostat values > 1.0	Yes	
Poorly developed enamel or deep fissure	Yes	
Food impaction visible on the adjacent tooth surfaces	Yes	
Risk of childhood caries	High	Low

Table 3 The Results of Oral Examination

Gender	N	Mean Age in Months (Months)	Caries Rate	Mean Value of Caries
Male	117	38.64±2.2	50.96%	4.46
Female	104	41.52±3.1	58.97%	4.74
	0.887	0.307	0.278	<0.05

correlated with ECC. Among these, the intake frequency of sugary food, oral health knowledge of parents, and intake of sugary foods before bedtime were significantly correlated (Table 4).

### Analysis of Caries Susceptibility Test Results

The distribution of cariostat values was different between children in the high-risk and low-risk groups. Specifically, cariostat values were mainly distributed in the range of 0.5-2.0 in the high-risk group and the range of 0-1.0 in the low-risk group, with a statistically significant difference as shown by the rank sum test results (Mann–Whitney *U*-test) (Z = -6.226, P < 0.01) (Table 5). Furthermore, the rank sum test also revealed that cariostat values for the primary caregiver of children in the high-risk and low-risk groups were not statistically different (Table 6).

Table 4 Analysis of Factors Influencing Childhood Caries

Influencing Factors	High-Risk	Low-Risk	χ2	P
	Group (n)	Group (n)		
Weaning time				
≥ I year old	121	25	2.783	0.113
< I year old	55	20		
Intake frequency of snacks				
≥ 3 times/day	140	18	27.499	<0.01
< 3 times/day	36	27		
Frequency of brushing				
I times/day	68	25	10.67	0.015
≥ 2 times/day	30	12		
No brushing	78	8		
Fluoride toothpaste				
Used	49	19	3.66	0.16
Not used	72	16		
Not known	55	10		
Oral health knowledge of parents				
Do not understand	78	12	12.276	0.002
Understand	55	10		
Familiar	43	23		
Primary caregiver				
Parents	52	15	0.244	0.885
Grandparents	83	20		
Others	41	10		
Education level of parents				
Less than degree from a junior college	41	10	1.418	0.492
University degree	102	23		
Bachelor's degree or above	33	12		
Family monthly income				
Less than RMB 5000	32	5	3.008	0.214
RMB 5000-8000	48	9		
More than RMB 8000	96	31		
Intake of sugary foods before bedtime				
0 times/week	36	17	13.44	0.001
≤ 3 times/week	92	10		
> 3 times/week	48	18		
Age of starting to brush teeth				
< I year old	47	21	6.885	0.032
≥ I year old and < 2 years old	85	17		
≥ 2 years old	44	7		

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**Table 5** Comparison of Caries Susceptibility of Children in the High-Risk and Low-Risk Groups

Cariostat Values	High-Risk Group	Low-Risk Group		
0	27(15.08%)	31(68.89%)		
0.5-1.0	70(39.32%)	14(31.11%)		
1.5–2.0	47(26.40%)	0		
2.5–3.0	32(17.98%)	0		
Total	176	45		
Rank sum test	Z=-6.226	P<0.01		

**Table 6** Comparison of Caries Susceptibility of Primary Caregivers of Children in the High-Risk and Low-Risk Groups

Cariostat Values	High-Risk Group (n, %)	Low-Risk Group (n, %)		
0	65(36.93%)	21(46.67%)		
0.5-1.0	71(40.34%)	15(33.33%)		
1.5–2.0	18(10.23%)	6(13.33%)		
2.5–3.0	22(12.50%)	2 (4.44%)		
Total	176	45		
Rank sum test	Z=-0.864	P=0.387		

Spearman rank correlation analyses showed that the caries susceptibility of the primary caregiver was positively correlated with that of children ( $r_s = 0.76$ , P < 0.05). In other words, the caries susceptibility of children increased with increase in the caries susceptibility of the child's caregiver.

We compared the number of decay-missing-filled teeth (DMFT) at each cariostat value. When the cariostat value was 1, the median number of DMFT was 2 with an interquartile range of 3. When the cariostat value was 2, the median number of DMFT was 5 and the interquartile range was 7. When the cariostat value was 3, the median number of DMFT was 7 and the interquartile range was 9. These results suggested a significant difference in the number of DMFT among all cariostat values (P < 0.01). Pairwise comparisons exhibited no substantial difference in the number of DMFT between children with cariostat values of 1 and 2 (P > 0.05). The number of DMFT was higher in children with a cariostat value of 3 when compared to children with cariostat values of 1 and 2, and was markedly different between children with cariostat values of 1 and 3 (P < 0.05). The difference in the number of DMFT was significant between children with cariostat values of 2 and 3 (P < 0.05).

# Logistic Regression Analyses of Factors Influencing ECC

We used logistic regression analysis to analyze children in the two groups, and the results showed that the intake frequency of sugary foods, caries susceptibility, and oral health knowledge of parents were the factors influencing caries in children. Among these, the intake frequency of sugary foods showed the greatest influence (Table 7).

Table 7 Multi-Factor Logistic Regression Analysis of ECC

Factors	В	S.E.	Wals	df	Sig.	Exp(B)	Exp(B) 95% C.I.
Intake of sugary foods	2.020	0.533	14.366	1	0.000	7.539	2.653–21.429
Frequency of brushing	-0.122	0.352	0.120	1	0.729	0.885	2.653-21.429
Oral health knowledge of parents	-1.209	0.575	4.422	1	0.035	0.299	0.097-0.921
Caries susceptibility	0.369	0.174	4.488	1	0.034	1.447	1.028-2.036
Intake of foods before bedtime	0.063	0.173	0.132	1	0.717	0.939	0.670-1.318
Age of starting to brush teeth	-0.148	0.505	0.086	1	0.770	1.159	0.431-3.123
Constant	-1.159	0.757	2.422	I	0.147	0.172	

#### **Discussion**

We included a total of 221 children aged 3–4 years in this study, and the caries rate was 54.97%. This was higher than the caries prevalence rate among children aged 3–4 years in Beijing (47.34%) as reported by Li et al<sup>7</sup> in 2006 and children aged 1–4 years in Beijing (40.19%) as reported by Deng et al<sup>8</sup> in 2006, and lower than the prevalence of caries in 5 year old children (70.9%) in the fourth National Oral Epidemiological Survey, and similar to that in Shanghai (58.07%) in 2015. This difference may be related to sample selection. Specifically, Li et al<sup>7</sup> selected respondents from the Xicheng District of Beijing which is a relatively well developed district in Beijing and is at the forefront in Beijing in terms of education and health levels, with relatively better kindergarten conditions and families from better economic conditions, and all these factors inevitably influence the degree of attention to oral health. The kindergarten selected for this study is located in Tongzhou District, which is an urban fringe area and has a large outsider population, and these factors undoubtedly increase the caries rate of children. In addition, the difference may also be related to the small sample size in this study.

The prevalence of dental caries increases with age and is significantly elevated in children aged 3–4 years, so research pertaining to caries prevention should focus on this age group. In Beijing, the caries rate has decreased of late due to effective caries prevention and oral health care measures such as free oral examinations, use of fluoride foam, and treatment of caries, and so on, that have been implemented for kindergarten children.

The sample for this study was selected from kindergartens and consisted of young children who were in school for less than one year. Among these children, most of the children with caries already had caries before they entered kindergarten, and this indicates that we should not only take up caries prevention and health care interventions during the kindergarten period, but also offer caries prevention education to children who have not yet entered kindergarten and their parents, and even pregnant women. All these measures can effectively reduce the incidence of ECC.

In this study, the caries rate was 50.96% in 117 boys and 58.97% in 104 girls, and the gender difference was not statistically significant as seen from the chi-square test results ( $\chi^2 = 0.998$ , P > 0.05). The mean value of caries in boys was 4.46, higher than that of girls (4.74) with a marked difference as revealed by the *t*-test results (T = 1.218, P < 0.05). This difference can be explained by a possible preference among girls to eat snacks when compared with boys, or due to the sample selection and sample size, and this aspect needs to be further studied.

In this study, we found that the daily intake frequency of sugary foods or drinks and the intake of sugary foods before bedtime were significantly correlated with the caries rate of children.<sup>10</sup> Increased intake frequency of sugary foods enhances the acidity of the plaque, consolidating the dominance of *Streptococcus mutans* and accelerating enamel demineralization, as confirmed by a growing number of studies.<sup>11–13</sup> The occurrence and development of caries are not only associated with the intake amount of sugary foods but also highly associated with the frequency of intake of such foods. Therefore, for caries prevention, it is more important to control the intake frequency of sugary foods than just controlling the amount of sugar.

Intake of sugary foods before bedtime can expose the tooth tissues to an acidic environment for a long period. In particular, saliva secretion decreases after falling asleep, and this reduces the buffering effect on the acidic environment, thus predisposing the tooth to enamel demineralization and accelerating the formation of caries. With the improvement of national living standards, material life gradually diversifies and more types of sugary foods are included in the lifestyle. Therefore, it is important for parents to correct their wrong perceptions, identify the sugar content in various foods, and reduce the amount, frequency, and time of intake of sugar by children in order to prevent the occurrence of dental caries more effectively. 15

Our analysis of the questionnaire results revealed a correlation between the oral health knowledge of parents and the caries rate of children. <sup>16</sup> Better oral health knowledge of parents was associated with their greater attention to oral health, suggesting that they were more concerned about the oral health of children, thus taking steps to reduce the caries rate of children. Parents' oral health care knowledge directly affects the prevention and treatment of childhood caries. <sup>17</sup> The questionnaire responses showed that parents mainly acquired oral health care information through the media, indicating that the public has limited access to oral health care knowledge and that the extent of publicizing dentistry as a professional discipline is insufficient. Additionally, kindergartens and communities can invite parents to attend lectures

in the form of health classes to answer their doubts in face to face interactions, to strengthen the publicity of caries prevention and treatment, and to guide the attitude of parents towards prevention and treatment of dental caries. Because the attitude of parents towards oral health care directly influences the caries of deciduous teeth, doctors should not only educate parents and children about oral hygiene during caries treatment but also strengthen the education of pregnant women on oral hygiene of infants and children to lay the foundation for cultivating healthy oral hygiene habits of children at an early stage.

Brushing is recognized as the most effective mechanical method for caries prevention.<sup>14</sup> In this study, the questionnaire results indicated that the age of starting to brush teeth and the frequency of brushing were correlated with the incidence of childhood caries. Proper brushing is the most effective method to prevent caries. Therefore, cultivating favorable oral health care habits in early childhood can reduce the incidence of caries in children. As a result, parents are advised to supervise the brushing of their children while teaching them the correct way of brushing, thus ensuring the frequency and effectiveness of brushing. The occurrence of ECC is related to the frequency of daily brushing.<sup>18</sup>

Generally, infants and children have the problem of poor oral hygiene because they cannot clean their mouth on their own, cannot achieve self-cleaning through chewing, and sleep for a longer duration during which saliva secretion decreases. Therefore, it should be emphasized to children to clean the mouth immediately after teething, brush teeth once in the morning and evening, and use fluoride toothpaste as appropriate. The questionnaire survey results revealed that the usage rate of fluoride toothpaste for young children is low and that many parents have little knowledge of caries prevention with fluoride. Fluoride has the ability to disrupt bacterial metabolism and increase tooth mineralization. <sup>19</sup> In China, fluoride is not commonly used for children under 4 years, and there are several areas that are low-fluoride areas. Therefore, the routine application of a small dose of fluoride (such as fluoride toothpaste, fluoride gel, fluoride foam, fluoride mouthwash, and fluorinated water source) is one of the effective methods to prevent caries. <sup>20</sup>

Cariostat caries susceptibility test is a caries activity test to detect the acid-producing ability of caries-causing bacteria, which was developed by Shimono<sup>4</sup> in the 1970s. The principle of this test is as follows: in the kit, sucrose is used as the carbon source, protein as the nutrient source, and bromocresol as the acid-base color developer. After incubation for 48 hours at 37°C, the color of the reagent is observed and compared with the standard colorimetric plate to assess the individual's caries susceptibility.

The cariostat method has been widely used in the clinical and epidemiological study of caries since it not only reflects the current status of individual caries but also can predict the tendency for caries development, as well as reacting to many kinds of caries-causing bacteria in the oral cavity.<sup>21</sup> Kozai et al<sup>22</sup> observed a concordance of 31.4% of *Streptococcus mutans* genotypes in the oral cavity of children aged 0–11 years with their mothers in Japanese families. Zou et al<sup>23</sup> found that the genotypic similarity of *Streptococcus mutans* in the oral cavity of mothers and children in Chinese families was 35%. These findings indicate that the caries status of the caregiver of children is closely related to the occurrence of ECC, which was confirmed in the study of Van den Branden et al.<sup>24</sup> Therefore, it is necessary to control the caries condition of children's caregivers to reduce the incidence of ECC.

Our study unveiled a correlation between caries and cariostat values in children and that the distribution of cariostat values was significantly higher in the high-risk group than in the low-risk group, indicating that caries activity was substantially higher in children with caries than in children without caries, consistent with the results of a prior study. Therefore, children with cariostat value over 1.0 and caries should be actively treated with minimally invasive caries treatment and fissure sealants for deciduous molar teeth, as well as encouraged to regularly and effectively use fluoride in the oral cavity to promote tooth remineralization and undergo regular oral examination and caries susceptibility monitoring, and their parents should be educated on oral hygiene and given correct dietary advice, thus decreasing the incidence of new caries. For children with cariostat values more than 1.0 and no caries, it is necessary to strengthen training in brushing, effectively improve oral hygiene, and undergo regular oral examinations and caries susceptibility tests, in addition to topical fluoride application, to prevent the occurrence of dental caries. For children with cariostat values of 1.0 or below and no caries, the risk of caries cannot be excluded but can be prevented by improving the quality of brushing teeth or through regular oral examinations. Additionally, 17% of children had cariostat values below 1.0 and caries, which suggested that the occurrence of caries is the result of many factors, including the birth history, growth and development, diet, and oral hygiene habits of children as well as the economic status of the family and society in addition

to the main factor, bacteria. These factors should be evaluated comprehensively for effective prevention of ECC.<sup>26</sup> In addition, health policymakers should consider training primary care providers in preventive dentistry and encourage interdisciplinary collaboration between primary care providers and dental professionals to improve dental practice among pediatric primary care providers.<sup>27</sup>

#### **Conclusion**

Our results suggest that the prevention of ECC was mainly associated with the feeding habits and diet of children. Specifically, the incidence of ECC could be greatly diminished by reducing the intake frequency of sugary foods and eliminating the intake of sugary drinks or foods before bedtime for children. Additionally, the caries rate of deciduous teeth in young children can be directly influenced by increasing awareness about oral hygiene among the guardians of children. Appropriate publicity methods can be developed based on local cultural customs and the Internet can be fully utilized for disseminating oral health information. Appropriate brushing training and oral hygiene instructions can be provided for children and their families based on the results of this test, and this holds much significance in the prevention of dental caries in children as it is suitable for large-scale promotion.

### **Ethics Approval and Consent to Participate**

This study was conducted in accordance with the declaration of Helsinki and approved by the Ethics Committee of the School of Stomatology, Capital Medical University. Legally authorized representative and/or guardian's of all minor participants signed an informed consent form.

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#### **Disclosure**

The authors have no conflict of interest

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