Clinical Epidemiology

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ORIGINAL RESEARCH

Factors Affecting Future Caries Occurrence Among Preschoolers in Northern Guangdong: A Longitudinal Study

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Purpose: This study aimed to investigate the new development of caries among preschoolers in northern Guangdong and to assess caries-related factors to distinguish groups with different caries risk levels.

Methods: Baseline data were recorded for participants from September to November 2019, and participants were reexamined from September to November 2020. A longitudinal observation of 11,973 preschoolers was conducted. The simplified debris index (DI-S) and decayed-missing-filled tooth (dmft) index values were obtained for each participant.

Results: Factors associated with whether caries would occur in the future and one-year increase in dmft (Δdmft) included baseline dmft, baseline DI-S, and baseline age. The risk ratio (RR) of caries occurrence and the number of teeth with new-onset caries were 4.482 (95% confidence interval, 4.056–4.957) and 2.945 (2.742–3.165) in the participants with baseline dmft ≥3, which were higher than those with baseline dmft =1 or 2. In the baseline caries-free group, whether caries would occur in the future was related to the baseline DI-S (95% confidence interval, 0.022–0.062). The caries incidence of maxillary central incisors (27.9%) was the highest among teeth of preschoolers without caries at baseline, whereas the caries incidence of mandibular first deciduous molars (42.7%) was the highest among teeth of preschoolers with caries at baseline.

Conclusion: Baseline dmft is a good predictor of future caries. Children with baseline caries-free status, baseline dmft >0, and baseline dmft ≥3 should be treated with preventive interventions of different intensities and frequencies. The occurrence of future caries in baseline caries-free participants is related to oral hygiene status. Measures to prevent caries on smooth surfaces, such as topical fluoridation, should be applied to all preschoolers. Preschoolers with caries at baseline may be given priority for pit and fissure sealing.

Keywords: preschool children, caries risk, previous caries experience, simplified debris index, oral epidemiology

Introduction

Caries is the most common oral disease in children, and it seriously affects children's oral health and even their whole-body health; furthermore, it causes harm to children's mental health. The high incidence of caries has not only caused a serious burden to individuals and families but has also become one of the health burdens of society as a whole.

A study showed that in individuals in the United States, 75% of caries occurred in 8.1% of the population under 5 years of age.[1] A survey of caries prevalence in children aged 3–5 years in Guangdong Province found that 84.8%, 79.8% and

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Received: 21 March 2021
Accepted: 6 May 2021
Published: 25 May 2021

72.3% of caries cases were centralized in one-third of children aged 3, 4 and 5 years, respectively.[2] The caries incidence in children is not average, but there are children with a higher risk of caries. In recent years, there have been numerous studies on the risk of caries;[3–6] these studies were conducted with the hope of identifying groups at high risk of caries so that public health resources for caries prevention can be appropriately allotted to the high-risk groups and to improve the efficiency of caries prevention. Exact caries risk assessment at the population level and "precision dentistry" at the individual level are both advisable and viable but must be based on high-quality longitudinal data and strict methodologies.[7] Evans et al have shown that previous caries experience increases the likelihood of caries occurrence in the future.[8] Sonoda et al found that previous caries experience affected the incidence of caries.[9] Isaksson et al and Corrêa-Faria et al also showed that previous caries experience has good predictability for future caries development.[10,11] Previous caries experience is regarded as the best predictor of new caries.[7] Oral hygiene status has also been confirmed by some studies to predict the occurrence of caries.[12,13]

However, studies on caries-related factors carried out in different regions showed great differences in the caries incidence rate and caries severity of the selected participants, as well as in the detection criteria of the variables included in the analysis. Most of the previous studies were cross-sectional or descriptive studies, and there is a lack of long-term longitudinal studies with large samples.

In the past decade, with the rapid economic development of Guangdong Province, widely recognized prevention techniques, such as topical application of fluoride and pit and fissure sealing, have been promoted among preschoolers in the province. A large amount of manpower and material resources have been invested, but the prevention effect on young children is still unsatisfactory. The caries-free rate (21.6%) is much lower than the 50% caries-free rate for 5-year-old children set by the World Health Organization. Therefore, the benefits of these prevention resources are worth further study and consideration.

In this study, children aged 3–4 years at baseline were observed longitudinally within one year. This study evaluated the incidence, severity, and caries-related factors among preschoolers attending kindergarten in northern Guangdong, China. The purpose of this study was to identify participants with different caries risk levels by investigating caries-related factors, including baseline decayed-missing-filled tooth (dmft), age, sex, and oral hygiene (DI-S). The identification of these factors will affect the formulation of prevention policies related to caries, and the implementation of different intensities of preventive interventions for children with different risk levels will improve the effectiveness of caries prevention.

Materials and Methods

Study Population

The baseline values of participants were recorded in September to November 2019, and participants were reexamined from September to November 2020. Data for this longitudinal study were collected from the annual oral health examination among kindergartens in Guangdong Province. Before entering the kindergarten for health examination, the inclusion criteria were established: the baseline age of 3–4 years old, and participants' willingness to cooperate with the researchers and maintain good compliance. The selection criteria for legal guardians included the ability to understand the research and the willingness to sign relevant informed consent letter. Prior to the examination, we excluded children who did not meet the inclusion requirements. Exclusion criteria entailed legal guardians who were unaware of the study, a child with an existing oral disease that required medical treatment or even emergency treatment or a child with a major systemic disease that affects the safety and data integrity of children, and children who will leave the current kindergarten within one year. Figure 1 is a flowchart illustrating the entire recruitment process. The participants of this study were preschoolers in urban kindergartens in five cities of northern Guangdong. The valid sample size was 11,973 children. The sample size of the group of 3-year-old and 4-year-old children was essentially the same, and the ratio of females to males in each age group was close to the ratio of females to males in that age group under local natural conditions.

Ethical Consideration

The study passed ethical review by the Ethics Committee of the Stomatological Hospital, Southern Medical University (Approval No. 2019–01). This study was conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from legal guardians of all children included in the study.

Quality Control

Eight experienced dentists and 8 recorders were trained and standardized before the formal examination. We

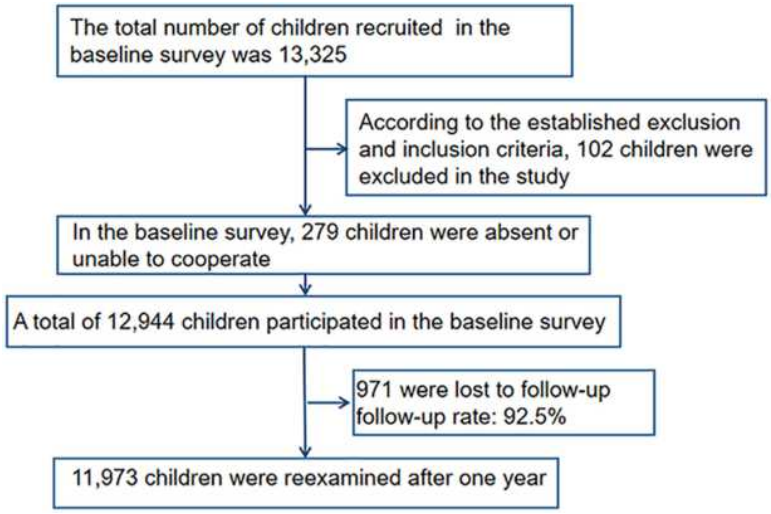

Figure 1 Flowchart illustrating the entire recruitment process of the study population.

recruited a group of volunteers, and then the dentists were trained under the guidance of a standard examiner until the kappa values for dmft of inter-examiner reproducibility were >0.85. Through the reexamination of 5% of children, the final inter-examiner reproducibility was obtained.

Method of Data Collection

The participants were examined while seated in a portable dental chair with a light. We used the simplified debris index[14] to record oral hygiene, with 6 index teeth (55, 51, 65, 71, 75 and 85). Then, the teeth were cleaned and dried using cotton swabs. According to the dmft index of the WHO criteria,[15] the presence of dental caries was determined. A general dental examination was performed on all children with a CPI probe and a plane mirror under lamplight. The ball end of the CPI probe was used to diagnose dental caries at the cavitation level. The recorders recorded the caries status of each tooth of every participant.

Follow-Up of Caries Status

The caries examination was repeated after one year with the same criteria and procedures. The one-year increase in dmft (Δdmft) was calculated. 971 were lost to follow-up, with a loss to follow-up rate of 7.5%. The valid sample size was 11,973 children. The reason for the loss to follow-up was that at the time of the second examination, some children asked for leave or were transferred to another kindergarten. There were no significant differences in census register type, sex ratio and caries between children who were followed up and those who were not.

Statistical Analysis

First, a descriptive analysis was performed on the characteristics of the study population, and the new progress of caries within one year. Caries-related factors included in the analysis included baseline dmft, baseline DI-S, baseline age, and sex. For each caries-related factor, univariate logistic regression was used to analyze the correlation between the factors and whether caries occurred, and negative binomial regression was used to analyze the correlation between the factors and Δdmft. Multivariate logistic regression and multivariate negative binomial regression were used to analyze caries onset and Δdmft relative to caries-related factors with $P < 0.2$ in the bivariate analyses. Risk ratios (RRs) with 95% confidence intervals (CIs) were reported. If the 95% CI did not include 1, the estimated value differed significantly from the reference value. The American Academy of Pediatric Dentistry (AAPD) uses a decayed, missing, and filled surfaces (dmfs) value >1 as a crucial factor in determining the overall caries risk in a child aged 0–5 years.[5] Gao et al found that a community-screening model requiring only a questionnaire could identify a quarter of the children with high caries burden (baseline dmft >2) well.[6] The participants of the study initiated by Gao et al were preschoolers in Asian, which was similar to our participants. We divided participants into four groups with baseline

dmft =0, 1, 2, ≥3 for a preliminary exploration to try to find out whether the incidence and severity of future caries were different among the four groups. The number of teeth with new-onset caries and caries occurrence in different groups were compared by using negative binomial regression analysis and logistic regression analysis. Among participants without caries at baseline, the difference of the DI-S between participants with new-onset caries and those without new-onset caries was compared by *t*-test. All statistical analyses were conducted using SPSS 27.0 software.

The participants' baseline dmft scores ranged from 0 to 20. New caries lesions at the time of follow-up were defined as Δdmft and recorded as a dichotomous outcome. Caries incidence was defined as the percentage of participants with Δdmft >0 within one year among all participants in this research. Δdmft is the dmft value 1 year later minus the baseline dmft value. New caries of a tooth was defined as one of the four conditions in which a tooth could not be initially categorized as crown caries, filled with caries, filled without caries, or lost due to caries at baseline, and one year later developed one of the four conditions. DI-S of 6 index teeth were examined, which represented the overall oral hygiene situation. The personal DI-S score was the sum of the scores of each tooth divided by the number of teeth examined (excluding the teeth that could not be examined).

Results

A total of 6000 children aged 3 years at baseline and 5973 children aged 4 years at baseline were included in this study. The caries incidence was 39.2% and 44.8% in children aged 3 years and 4 years at baseline, respectively. The mean Δdmft values were 1.293 and 1.419 in children aged 3 years and 4 years at baseline, respectively. The number of participants, caries incidence, and mean Δdmft values in males and females are shown in Table 1. Table 2 illustrates the risk factors related to the occurrence of caries and Δdmft, including baseline dmft, baseline DI-S, and baseline age.

As shown in Figure 2, among children aged 3 years at baseline, caries incidence of maxillary central incisors was the highest, followed by caries incidence of mandibular deciduous molars and then caries incidence of maxillary deciduous molars. However, in children aged 4 years at baseline, caries incidence of the two maxillary central incisors decreased, caries incidence of the maxillary deciduous molars increased significantly, and caries incidence of the mandibular deciduous molars remained high; however, the increment of the latter was not obvious. The caries incidence of a tooth is related to the length of time since the tooth erupted into the mouth.

Interestingly, as shown in Figure 3, although the caries incidence in most of the teeth in the baseline with caries group was higher than that in the baseline caries-free group, caries incidence in the maxillary central incisors was nearly the same in the two groups. In the baseline caries-free participants, the proportion of two maxillary central incisors with new-onset caries was 27.9% within one year, which was the tooth type most prone to caries among all the teeth. In the participants with baseline caries, the mandibular first deciduous molars had the highest caries incidence (42.7%), and the caries incidence in the other teeth was also high.

The frequency distribution of Δdmft between individuals with and without baseline caries is shown in Figure 4. Most of the children without baseline caries did not develop caries in the future, but among participants without caries at baseline with new-onset caries, it was exceedingly likely that two teeth with new caries would occur within one year. However, among participants with caries at baseline that developed new-onset caries, it was most common that one tooth with new caries would occur within one year.

Figure 5 shows the caries incidence and the number of teeth with new-onset caries per capita in the participants with baseline dmft values of 0, 1, 2 and ≥3. With the increase in baseline dmft, the caries incidence and the number of teeth

Table 1 The Number, Caries Incidence (%), and Δdmft (Mean) of Participants at Different Baseline Ages and Grouped by Sex

Baseline Age	**No. of Participants**		
	Total	**Male**	**Female**
3 y 4 y	6000 5973	3226 3205	2774 2768
Baseline Age	Caries Incidence (%)		
	Total	Male	Female
3 y 4 y	39.15 44.77	39.24 44.77	39.04 44.76
Baseline Age	Δdmft (Mean, SD)		
	Total	Male	Female
3 y 4 y	1.293, 2.382 1.419, 2.416	1.292, 2.376 1.419, 2.446	1.293, 2.389 1.419, 2.382

Abbreviation: SD, standard deviation.

Table 2 Factors Related to a One-Year Increase in Dmft (Δdmft) and to Caries Occurrence Within One Year

Factors	RR (95% CI)[a]	Z[a]	RR (95% CI)[b]	Z[b]
Baseline dmft	1.120 (1.105, 1.135)	19.518	1.176 (1.158, 1.195)	20.179
Baseline DI-S[c]	1.232 (1.147, 1.324)	5.743	1.336 (1.227, 1.457)	6.617
Baseline age	1.066 (1.004, 1.132)	2.088	1.124 (1.048, 1.205)	3.265

Notes: [a]Factors related to a one-year increase in dmft (Δdmft) by multivariate negative binomial regression analysis. [b]Factors related to caries occurrence within one year by multivariate logistic regression analysis. [c]Participants whose DI of six teeth was not all "unrecorded" were included in the analysis.
Abbreviations: RR, risk ratio; CI, confidence interval.

with new-onset caries per capita in the participants also showed an increasing trend.

Figure 6 shows that children with caries at baseline occupied 50.9% of the total participants with new-onset caries. When the number of teeth with new-onset caries was assessed, children with caries at baseline occupied 57.3% of the total. Among children with caries at baseline, the number of teeth with new-onset caries (63.2%) and the number of participants with new-onset caries (59.2%) were concentrated in the participants with baseline dmft ≥3. The risk ratio (RR) and 95% CI of caries occurrence and the number of teeth with new-onset caries were 4.482 (95% CI, 4.056–4.957) and 2.945 (95% CI, 2.742–3.165) in the participants with baseline dmft ≥3, which were higher than those with baseline dmft =1 or 2. The 95% CI of participants with baseline dmft =1 overlapped with

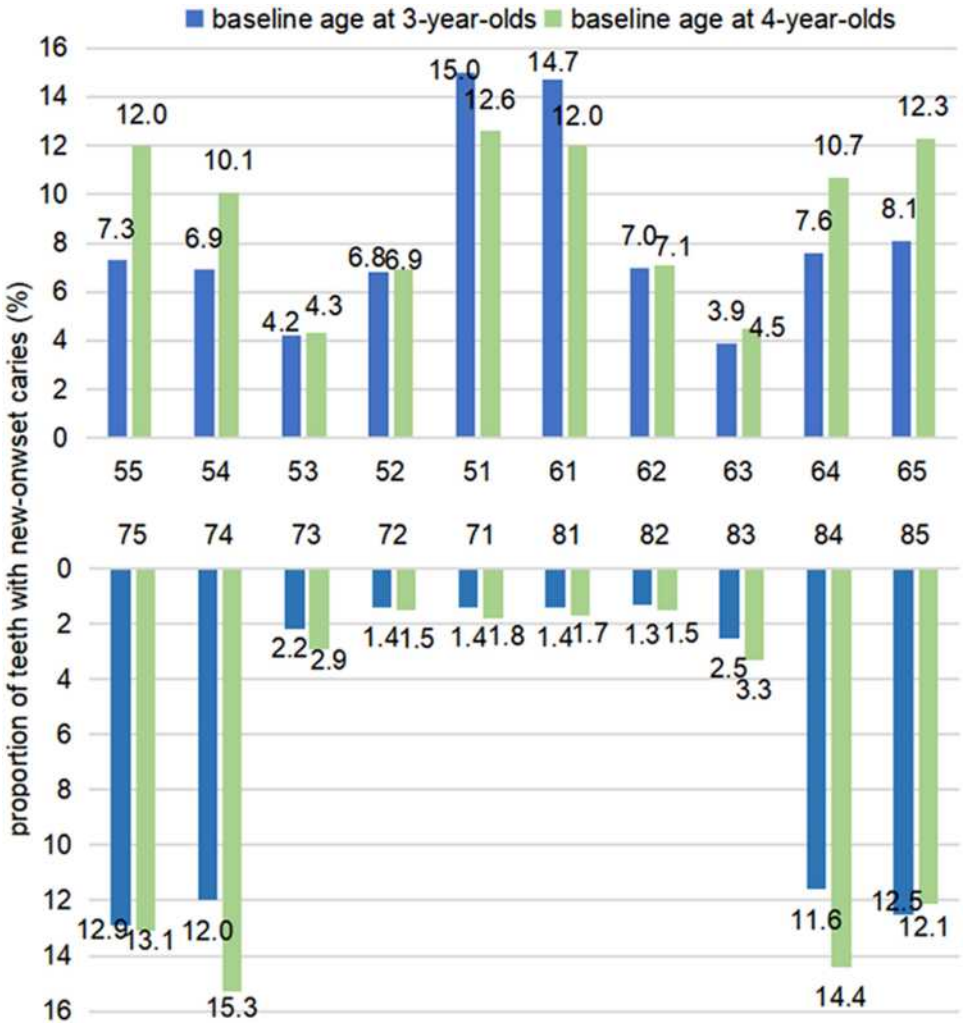

Figure 2 The distribution of teeth with new-onset caries by tooth position for children in different baseline age groups.

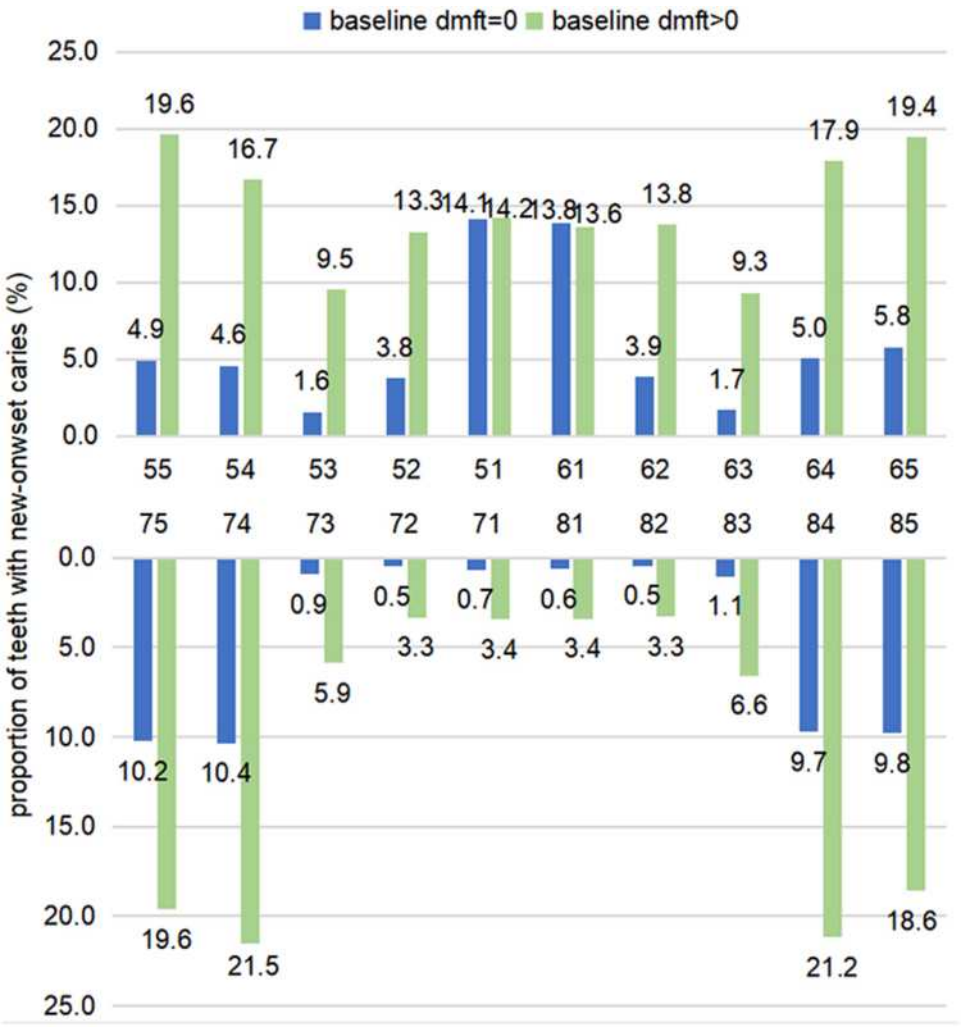

Figure 3 The distribution of teeth with new-onset caries by tooth position for children in different baseline dmft score groups.

the 95% CI of participants with baseline dmft =2, but the 95% CI of participants with baseline dmft ≥3 and participants with baseline dmft =2 did not overlap (in Table 3). Notably, the group with baseline dmft >0 was the high-risk group of future caries; moreover, the most resources should be allocated to the group with baseline dmft ≥3. Using baseline dmft, we could simply and efficiently identify groups at different risk for caries.

This study preliminarily found that among participants without caries at baseline, the mean and standard deviation (SD) of the DI-S for participants with new-onset caries and those without new-onset caries were 0.188 (SD: 0.417) and 0.146 (SD: 0.364), respectively. The *t*-test statistic (Z) was −4.161 (95% CI, 0.022–0.062). Therefore, the maintenance of favorable oral hygiene status is of positive significance for caries prevention among the relatively low-risk population. It is difficult to predict future caries among baseline caries-free participants, and more risk predictors are needed for a comprehensive risk assessment model.

Discussion

The caries incidence of this large longitudinal study in children with baseline ages of 3 years and 4 years were 39.2% and 44.8%, respectively, and the Δdmft values were 1.293 and 1.419, respectively, indicating that the onset of deciduous caries was early and that the progression was fast; these findings are similar to the results of Lim et al.[16] Age is a risk factor for the incidence and severity of deciduous caries because caries develops due to the effect of multiple factors, and time plays a major role in the development of caries. The more time that has passed since deciduous teeth erupted into the mouth, the greater the possibility of caries. As a child's jaw grows and

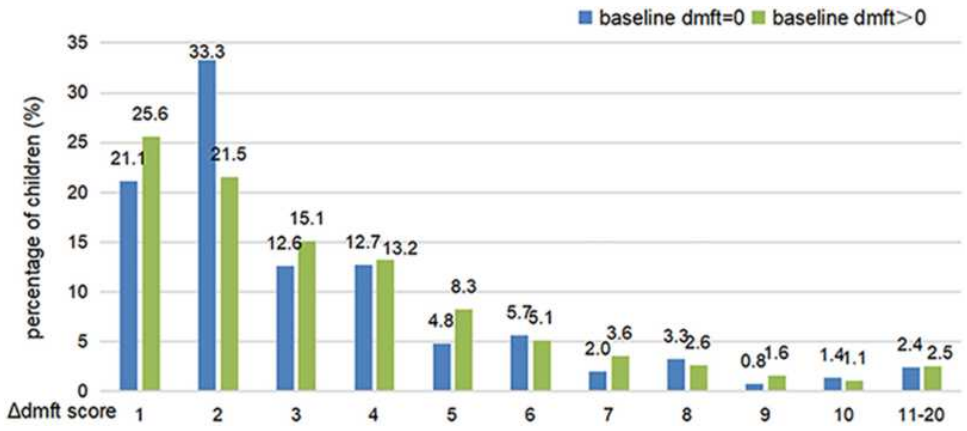

Figure 4 The frequency distribution of Δdmft.

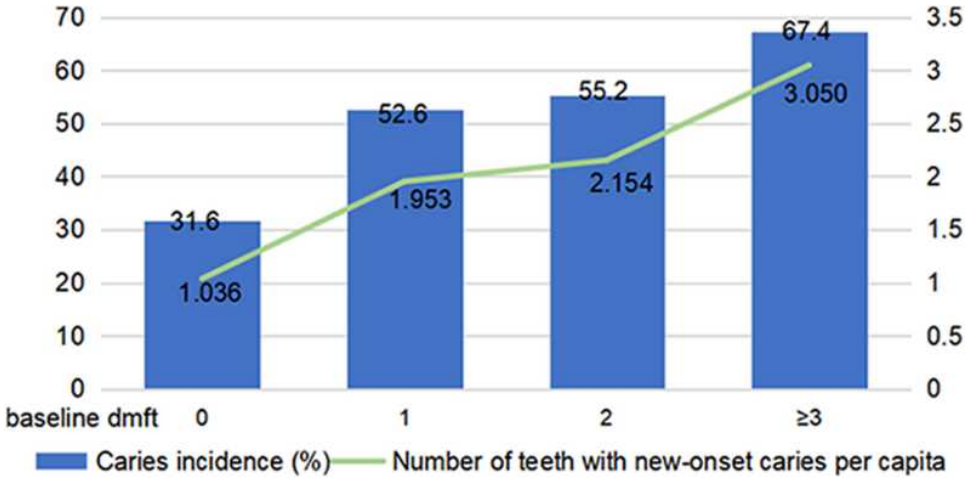

Figure 5 Caries incidence (%) and number of new-onset caries per capita.

develops, the developmental space between deciduous teeth provides opportunities for food impaction, causing interproximal caries to be more likely to develop. This suggests that the prevention of deciduous teeth caries should be timely screening and early intervention and even advocates beginning a prevention strategy during the mother's pregnancy.[17]

The occurrence and severity of new-onset caries are significantly correlated with dmft and DI-S at baseline. The occurrence likelihood and severity of new-onset caries in children with baseline caries are significantly higher than those without baseline caries; this pattern of occurrence has been confirmed by a large number of previous studies.[7,10,11,18] The American Academy of Pediatric Dentistry (AAPD) uses a decayed, missing, and filled surfaces (dmfs) value >1 as a crucial factor in determining the overall caries risk in a child aged 0–5 years.[5] The California Dental Association proposed the Caries Management by Risk Assessment (CAMBRA).[3] The four disease indicators are all related to previous caries experience and are all considered as high-risk factors to evaluate caries risk. Cariogram (a multifactorial risk assessment model) designates previous caries experience as an important risk factor.[4] Previous caries experience seems to be a single factor index, but it is actually a comprehensive reflection of the sensitivity of tooth tissue to caries, plaque microecology, diet, hygiene habits, fluoride use, dental treatment and other caries-related factors; these factors play an important role in predicting

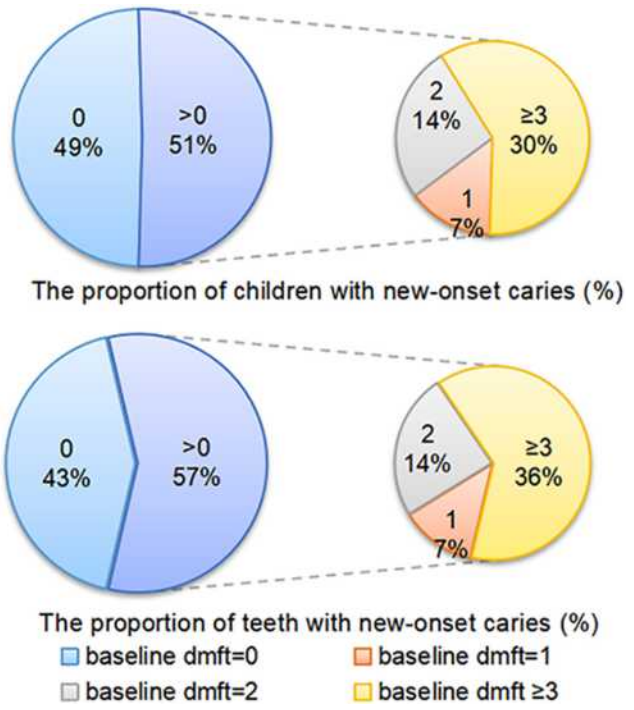

Figure 6 The proportion of children and teeth with new-onset caries.

future caries. In other words, those who have had more teeth with caries in the past will have a higher risk of future caries, and this risk increases exponentially.[9] In this study, children with caries at baseline comprised 50.9% and 57.3% of the total participants with new-onset caries and the total number of teeth with new-onset caries, respectively. Among the participants with caries at baseline, the number of teeth with new-onset caries (63.2%) and the number of participants with new-onset caries (59.2%) were concentrated in the participants with baseline dmft ≥3. Moreover, the RRs of caries occurrence and the number of teeth with new-onset caries in the participants with baseline dmft ≥3 were higher than those with baseline dmft =1 or 2. The dmft of 3 was used as a cut-off point to distinguish the extremely high-risk population. This finding has a certain correlation with the conclusion that in the caries risk model of children aged 3–6 years established by Gao et al, new-onset caries could be well predicted by a six-question questionnaire in children with baseline dmft >2.[6] Therefore, children with baseline caries-free status, baseline dmft >0, and baseline dmft ≥3 represent different caries risk levels to some extent, and they should be treated with preventive interventions of different intensities and frequencies. According to caries management protocol for 3 to 5 years old children put forward by American Academy of Pediatric Dentistry (AAPD),[5] duration between visits, dietary intervention, frequency and dose of fluoride, restoration treatments are different between different caries risk groups. Based on different risk groups of caries, it is of great significance to establish a caries management protocol for preschoolers in northern Guangdong.

Interestingly, in this study, the caries incidence of maxillary central incisors within one year was similar between the caries-free and caries-present participants at baseline. Maxillary central incisors had the highest caries incidence among all the teeth in the caries-free participants at baseline, which also explains the results shown in Figure 3 that those without baseline caries were most likely to have two teeth decayed simultaneously once new caries occurred. It has been suggested that focusing on the prevention of caries of maxillary central incisors in baseline caries-free children may improve the caries-free rate of preschoolers[2,19] and improve the efficiency of caries prevention. Caries of the central incisors are caries that affect smooth surfaces. Caries in deciduous teeth, unlike those in permanent teeth, usually affect smooth surfaces, followed by pits and fissures.[2] Caries of the maxillary central incisors are often nursing caries.[20] However, children with caries at baseline are a high-risk group, and the caries incidence of mandibular first deciduous molars was obviously the highest, and the caries incidence in the other teeth was not low either. Each tooth needs early prevention and intervention. It is suggested that the

Table 3 Association Between Baseline Dmft Scores and Number of Teeth with New-Onset Caries and Association Between Baseline Dmft Scores and Caries Occurrence

Baseline dmft	No. of Participants	RR (95% CI)[a]	Z[a]	RR (95% CI)[b]	Z[b]
0	7806	Ref		Ref	
1	701	1.886 (1.674, 2.129)	10.349	2.407 (2.060, 2.813)	11.052
2	1219	2.080 (1.896, 2.285)	15.355	2.669 (2.362, 3.018)	15.700
≥3	2247	2.945 (2.742, 3.165)	29.513	4.482 (4.056, 4.957)	29.311

Notes: [a]Association between baseline dmft scores and number of teeth with new-onset caries by negative binomial regression analysis. [b]Association between baseline dmft scores and caries occurrence by logistic regression analysis.
Abbreviations: RR, risk ratio; CI, confidence interval.

prevention measures of caries in all preschoolers should be carried out by applying fluoride, changing feeding habits and popularizing dental floss use to prevent smooth surface caries. The operation of pit and fissure sealing is relatively complex and the cost is high. In order to improve efficiency and save costs, for children with baseline caries, in addition to the above measures, they should be given priority for pit and fissure sealing of deciduous molars.

However, individuals without previous caries experience may be at risk for new-onset caries, and baseline dmft may not be an effective predictor of these individuals.[21] In addition, the trajectory of disease in caries patients may not always be linear or static, especially after effective behavioral and clinical interventions.[22] Although the risk of new-onset caries is higher in individuals with more previous caries experience,[16,23] the risk is not zero in individuals without previous caries experience.[24] In this study, among children without baseline dmft, there was a statistically significant difference in DI-S between those with new-onset caries and those without new-onset caries. It has been suggested that oral hygiene status is useful to predict new-onset caries in individuals without previous caries experience. Of course, it is not enough to predict caries risk only by oral hygiene status, and a more comprehensive caries risk prediction model needs to be established. This study is a preliminary exploration to establish a caries risk model for preschoolers in northern Guangdong. Compared with the prediction methods using single or several risk factors, this caries risk assessment system can more accurately predict the occurrence of caries, with an accuracy of 60%-90%.[25]

The advantages of this study include that the sample of preschoolers was large and representative; the loss of follow-up rate was relatively low. This prospective longitudinal study could better explain the causal relationship between caries occurrence and related factors. The results of this study offer a credible estimate of the level of dental caries development in preschoolers in northern Guangdong. However, our study also has some limitations. An examination standard based on the WHO criteria was adopted in this study; however, these criteria do not have the advantages of the International Caries Detection and Assessment System (ICDAS) in the detection of early caries. In addition, the dmfs index is more sensitive than the dmft index. Moreover, there was a lack of proper questionnaire surveys to explore children's caries-related knowledge, attitudes, behavior, and socioeconomic status. Furthermore, dental plaque microecology, saliva, caries-related genes,[26] fluoride use have not been assessed.[27–29] A meta-analysis indicated that the strongest risk factors associated with early childhood caries (ECC) are enamel defects, dentine caries and high levels of Streptococcus mutans.[30,31] In the following research, we will investigate more caries associated factors. Because observational data were used in this study, the possibility that the association between risk factors and dental caries may be biased by unmeasured confounders cannot be excluded.

Rose pointed out that one of the most basic principles of preventive medicine is that "a large number of low-risk populations may produce more cases than a few high-risk populations",[32] so focusing only on high-risk populations will cause the majority of new cases of disease to be overlooked. A longitudinal study by Kusama et al confirmed this principle by showing that the majority (59.7%) of new-onset caries in primary school students occurred in children without caries at baseline, and the study findings support the importance of a "health for all" strategy.[33] Some studies have questioned the prevention of high-risk populations and have indicated that prevention strategies targeting individuals "at risk" would not be able to cope with the majority of new lesions. The main focus of prevention strategies should be on a population approach.[34,35] In our study, the baseline prevalence of caries was 34.8%. However, the baseline prevalence of caries in these previous studies was lower than that in our study, which led to different conclusions being drawn from the data evaluated in our study. Whether the children with new-onset caries and the teeth with new-onset caries were most often in the high-risk population with caries at baseline was related to the baseline caries prevalence. Pertinently, the formulation of prevention strategies must be based on local conditions.

Conclusion

In conclusion, the onset of deciduous caries occurs early and progresses quickly. Baseline dmft is a good predictor of future caries. Children with baseline caries-free status, baseline dmft >0, and baseline dmft ≥3 represent different caries risk levels to some extent, and they should be treated with preventive interventions of different intensities and frequencies. Among children without baseline caries, the maintenance of favorable oral hygiene status is of positive significance for the prevention of future caries. Measures to prevent caries on smooth surfaces, such as topical fluoridation, changing feeding habits and popularizing dental floss use, should be applied to all preschoolers whether they are caries-free at baseline or not.

Preschoolers with caries at baseline may be given priority for pit and fissure sealing. Appropriate prevention strategies should be made according to baseline caries prevalence, preventive resources and economic development of the study area. This study was a preliminary exploration to establish a caries risk model for preschoolers in northern Guangdong.

Abbreviations

DI-S, simplified debris index; dmft, decayed, missing, filled tooth; Δdmft, one-year increase in dmft; CPI, community periodontal index; CI(s), confidence interval(s); RR(s), risk ratio(s); SD, standard deviation; dmfs, decayed, missing, filled tooth surface; ECC, early childhood caries.

Ethical Approval and Consent to Participate

The study passed ethical review by the Ethics Committee of the Stomatological Hospital, Southern Medical University (Approval No. 2019-01). This study was conducted in accordance with the Declaration of Helsinki. The legal guardians of all children signed informed consent documents before the beginning of the study.

Acknowledgments

The authors would like to thank all of the children and their parents/grandparents, and all the staff who participated in the survey.

Author Contributions

All authors made significant contributions to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, substantially revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agreed to take responsibility and be accountable for all aspects of the work.

Funding

This work was funded by Guangzhou Science and Technology Bureau Project- "Caries Prevention Platform and Network Construction Based on Internet + Health Care" (201903010038). The Clinical Research Startup Program of Southern Medical University by High-level University Construction Funding of Guangdong Provincial Department of Education (LC2017PY001) also provided support for this study.

Disclosure

The authors declare no conflicts of interest in this work.

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