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Original Article

Correlation between salivary mutans streptococci, lactobacilli and the severity of early childhood caries

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Abstract *Background/Purpose:* Mutans streptococci (MS) and lactobacilli (LB) are associated with dental caries, but the acquisition of these organisms in young children with early childhood caries (ECC) and severe early childhood caries (S-ECC) has only been partly described. The purpose of this study was to evaluate the salivary MS and LB levels of preschool children with ECC and S-ECC, and the correlation of MS, LB levels with caries severity.
Materials and methods: The study population was comprised of children from 3 to 6 years of age who visited the Pediatric Dental clinic of TCVGH. Oral examinations were performed, and whole unstimulated salivary samples were collected for buffer capacity, MS and LB counts by using commercially available diagnostic kits (CRT bacteria kit). The participants were divided into mild (dmft < 6), moderate (dmft:6–9) and severe caries (dmft > 9) groups; the salivary microbiological tests and buffer capacity were compared.
Results: A total of 72 children with a mean age of 4.5 years participated in this study. The salivary test showed that the higher the salivary MS level the more severe the caries status in children ($P = 0.001$). There was also a tendency toward a higher salivary LB level in children with more severe caries, but the difference was not significant ($P = 0.088$). There was no significant correlation between buffer capacity and caries status.
Conclusion: The salivary MS level in preschool children was significantly correlated with the severity of early childhood caries.

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Introduction

Early childhood caries (ECC) is a chronic, transmissible, infectious disease whose etiology is complex and multifactorial. ECC exhibits a distinct caries pattern that is often associated with prolonged, frequent, and inappropriate consumption of cariogenic foodstuffs. Mutans streptococci colonization is regarded as one of the principal factors involved in ECC.[1–3] Early childhood caries results in a considerable direct burden of pain and suffering as well as poorer general health than that of caries free children.[3]

Despite efforts in restorative therapy, children who experience ECC continue to be at a higher risk for new lesions in both the primary and the permanent dentition.[4] A retrospective study showed that despite engagement in preventive dental caries, children with too numerous counts MS were six times more likely to experience cavity increments after 5 years than preschoolers with no recoverable MS at first visit.[5] Interventions that disrupt the pathobiology of caries are needed to prevent and treat this aggressive infectious disease.

In previous studies, conventional culturing methods have been used to show that well-known bacteria species, such as Streptococcus mutans and Lactobacilli (LB), are associated with dental caries.[1,2] A delay in mutans streptococci colonization is likely to be beneficial as early detection was found to be coupled with a higher caries experience in children.[6,7] The combined "mutans streptococci + lactobacilli + past caries" model is useful for identifying at-risk children reported in a one year prospective study.[8]

According to the definition of American Academy of Pediatric Dentistry, early childhood caries (ECC) is the caries occurring in children below 71 months of age, and severe early childhood caries (S-ECC) is the severe form of early childhood caries.[9] The overall prevalence of ECC in 5 years old children of Taiwan is estimated to be 89% or more.[10] This creates very serious oral health problems in Taiwan. Therefore understanding the role of specific bacterial species is important for creating a complete model of caries etiology and caries prevention.

The purpose of this study was to evaluate the salivary MS and LB levels of preschool children with ECC and S-ECC and the correlation of MS, LB levels with caries severity.

Materials and methods

Participant selection

Children who visited the pediatric dentistry at Taichung Veterans General Hospital, Taiwan, from July 2009 to Dec 2009, participated in this study. Children suffering from systemic disease or taking antibiotics were excluded from the study. Written informed consent was obtained from all parents who agreed to allow their children to participate in this study. This study protocol was approved by the Institutional Review Board of the Taichung Veterans General Hospital, Taiwan.

Oral examination

A complete dental examination was performed in the dental operating room of the pediatric dental clinic by two examiners. The examiners were trained regarding diagnosis criteria and clinical examination procedures. The subjects were examined in a supine position in a dental chair. An overhead light, a plane mouth mirror and an explorer were used. The caries status was first assessed by visual inspection and then confirmed by tactile inspection. The caries status of each tooth surface was evaluated and coded as "s" (sound), "d" (decayed), "m" (missed), or "f" (filled) surface. After oral examination, the participants were divided into three groups according to the dmft score. Mild caries is dmft smaller than 6 (dmft < 6), moderate caries is dmft between 6 and 9 (dmft: 6—9) and severe caries is dmft greater than 9 (dmft > 9).

Saliva tests

Unstimulated saliva samples were collected for buffer capacity, mutans streptococci and lactobacilli counts. The subjects were instructed to spit a total of 3 ml of saliva into a vial. The whole saliva samples were collected for buffer capacity and bacterial counts immediately. CRT® Bacteria kit (Ivoclar Vivadent AG products) was used for evaluating buffer capacity and bacterial cultures. The salivary cultures were incubated at 37 °C for 48 h. Then, the bacteria counts were measured according to the standard chart of CRT® Bacteria kit. The counts of colony forming units per milliliter (CFU/ml) saliva were categorized into four levels by two calibrated examiners who were blinded to the subject's caries status. CRT scores 0 to 3 denote a MS level of "less than 10^4,"

"10^4—10^5," "10^5—10^6," and "greater than 10^6," respectively, and CRT scores 1 to 4 denote a LB level of "less than 10^3," "equal to or greater than 10^4," "equal to or greater than 10^5," and "greater than 10^6," respectively. CRT scores 1 to 3 denotes a buffer capacity of "high", "medium" and "low" respectively.

Statistical analysis

Data were analyzed using SPSS 24.0 soft-ware (SPSS Inc, Chicago, Ill., USA). The Pearson correlation coefficient was used to identify the correlation between age and dmft score. The correlation between bacteria counts and caries status were also analyzed by using Pearson correlation coefficient test. Differences in bacterial counts between the three caries severity groups were evaluated by using analysis of variance (ANOVA) and Tukey's test at a p value of 0.05. Chi-square test and Bonferroni test were used to analyze the correlation of caries severity in different bacteria concentration at a p value of 0.05.

Results

A total of 72 children (45 boys, 27 girls) with a mean age of 4.5 years (range 3.5—6.0years) participated in this study.

Caries status

The mean dmft was 8.0 for boys and 7.9 for girls. The mean dmfs was 17.6 for boys and 16.7 for girls. There were no

significant differences between girls and boys in dmf scores. There was a tendency that older children had higher dmft score (Fig. 1), but the difference was not significant (Pearson correlation coefficient, $P = 0.114$, $R = 0.188$).

Bacteria counts

The salivary test showed that the higher the salivary MS level the higher the dmft score (Fig. 2). There were significant differences with p value <0.001 (Pearson correlation coefficient, $R = 0.428$). The participants were divided into three groups: mild, moderate and severe caries. A positive correlation was found between the severity of caries and MS level, the higher the MS score (CRT score) the more severe the caries status (Table 1). There was a significant difference in the salivary MS level between these three groups of children (ANOVA test, $P = 0.001$). Tukey's test showed that there was a significant difference ($p < 0.05$) between severe caries and mild caries, moderate caries and mild caries in MS counts (Table 1).

In severe caries group, 48% of children had a MS level higher than 10^6, whereas in mild caries group, only 8.3% of children had a MS level higher than 10^6 (Table 2). In the mild caries group, 54.2% of children had a MS level less than 10^4, but in severe caries group, only 24% of children showed a MS level less than 10^4 (Table 2). The salivary MS concentration was significant different between these three groups of children (Chi-square Test, $P = 0.002$). There was a significant difference ($p < 0.05$) between severe and mild caries group in MS concentration greater than 10^6.

There was also a tendency toward a higher salivary LB level in children with more severe caries, but the difference was not significant (Table 1, ANOVA test, $P = 0.088$). Table 3 showed that in different salivary LB concentrations, there was also no significant difference between these three groups of children (Chi-square Test, $P = 0.279$). Although it revealed that in high LB concentration ($LB = 10^5$–10^6, $LB > 10^6$), only 12.5% mild caries children had LB greater than 10^5, however 26.1% moderate caries and 32% severe caries children had LB level greater than 10^5. In low LB concentration ($LB < 10^4$), it also revealed less severe caries children (24%) than mild caries (50%) and moderate caries (52%) children (Table 3).

Buffer capacity

The salivary buffer capacity was not associated with caries severity. There were no significant differences of buffer capacity between mild, moderate and severe caries groups (Table 1, ANOVA test, $P = 0.898$).

Discussion

Since salivary micro flora reflects the gross composition of the microbial deposits on the various oral surfaces, salivary counts of cariogenic bacteria can be used as an indicator of dental plaque cariogenicity.[11] Theoretically plaque is more appropriate for estimating MS infection,[12] and several studies have shown that MS levels in stimulated saliva correctly reflect those in plaque.[13] In this study

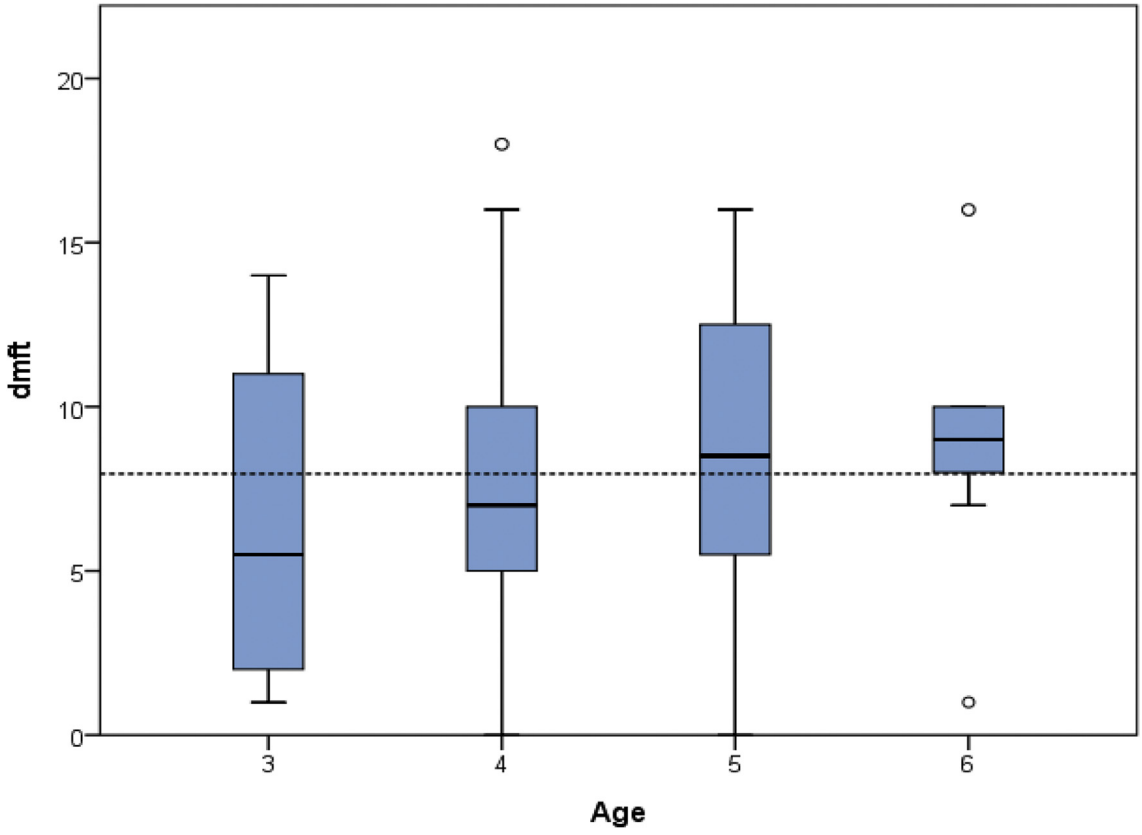

Figure 1 Correlation of age and dmft (P-value* = 0.114). *Pearson Correlation Coefficient (R = 0.188).

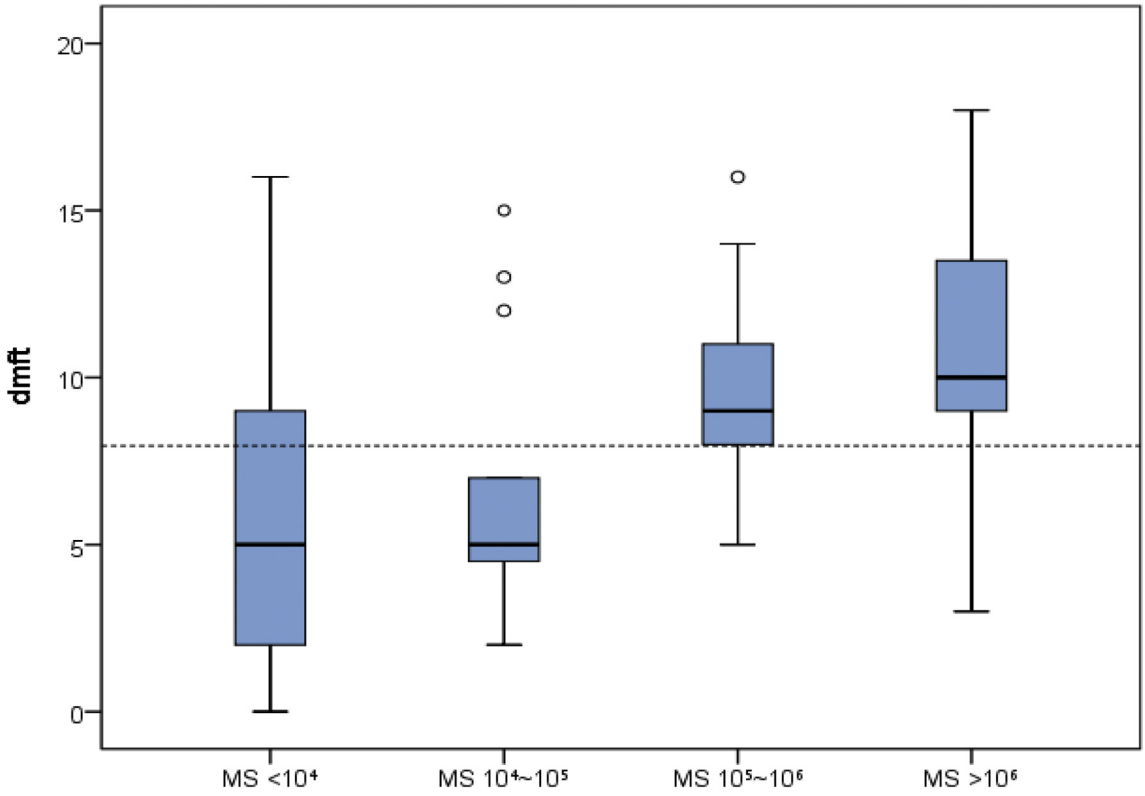

Figure 2 The correlation of salivary *mutans streptococci* (MS) and the caries status. (P-value* <0.001). *Pearson Correlation Coefficient(R = 0.428).

Table 1 Comparison of buffer capacity, MS and LB level in three caries severity groups with CRT score.

Characteristic	Mild caries (n = 24) Mean ± SD	Moderate caries (n = 23) Mean ± SD	Severe caries (n = 25) Mean ± SD	P-value[a]
Buffer (CRT score)	1.54 ± 0.72	1.61 ± 0.72	1.64 ± 0.81	0.898
MS (CRT score)[b]	0.67 ± 0.92	1.52 ± 1.12	1.88 ± 1.27	0.001
LB (CRT score)	1.63 ± 0.71	1.78 ± 0.95	2.16 ± 0.90	0.088

[a] One-way ANOVA.
[b] Tukey's Test for MS (P-value <0.05): Mild vs Moderate, Mild vs Severe.

Table 2 The salivary MS level in mild, moderate and severe caries groups.

MS	Mild caries (n = 24) n (%)	Moderate caries (n = 23) n (%)	Severe caries (n = 25) n (%)	P-value[a]
$<10^4$	13 (54.2)	6 (26.1)	6 (24.0)	0.002
10^4—10^5	8 (33.3)	4 (17.4)	3 (12.0)	
10^5—10^6[b]	1 (4.2)	8 (34.8)	4 (16.0)	
$>10^6$[c]	2 (8.3)	5 (21.7)	12 (48.0)	

[a] Chi-square Test.
[b] Bonferroni Test for severity (P-value <0.05): Mild vs Moderate.
[c] Bonferroni Test for severity (P-value <0.05): Mild vs Severe.

unstimulated saliva was used because stimulated saliva sampling by paraffin is often impossible in preschool children. According to the study done by Seki et al., the unstimulated saliva could replace stimulated saliva for testing of preschool children.[14]

Previous studies have shown that MS and LB are associated with dental caries.[1,15] These bacteria species have been reported as potential contributors to caries onset and development.[1,15] Therefore in this study, the salivary MS and LB levels in preschool children were evaluated in an attempt to determine the correlation between MS, LB level and the severity of early childhood caries.

A study done by Ramos-Gomez and associates showed that salivary MS levels among young children with ECC were higher than those in dentally healthy children.[16] In other studies,

Table 3 The salivary LB level in mild, moderate and severe caries groups.

LB	Mild caries (n = 24) n (%)	Moderate caries (n = 23) n (%)	Severe caries (n = 25) n (%)	P-value[a]
$<10^4$	12 (50.0)	12 (52.2)	6 (24.0)	0.279
10^4–10^5	9 (37.5)	5 (21.7)	11 (44.0)	
10^5–10^6	3 (12.5)	5 (21.7)	6 (24.0)	
$>10^6$	0 (0.0)	1 (4.4)	2 (8.0)	

[a] Chi-square Test.

which compared children with S-ECC and caries free children, the S-ECC group had higher salivary MS levels than the caries free group.[17,18] A systematic review article showed that MS level is a strong risk indicator for ECC.[19] In present study, a positive correlation was found between severity of ECC (measured by the dmft index) and high levels of MS counts, indicating that as number of MS colonies increased, the number of teeth affected by caries also increased. Therefore, not only caries occurrence but also caries severity was significantly associated with salivary MS counts in these children. Therefore preventing early colonization of MS in young children may play an important role in reducing the risk of early childhood caries.

On the other hand, the association between lactobacilli and caries remain equivocal.[20–22] In present study, there was also a tendency toward a higher salivary LB level in children with more severe caries, but the difference was not significant.

Our study population comprised of patients who visited Pediatric Dentistry of Taichung Veterans General Hospital, Taiwan. Most of them were referral patients with a high caries rate; it was not easy to find caries free children in this population. In our study population, approximately one third of children had early childhood caries, and two thirds of them were severe early childhood caries. Therefore we divided participants into mild, moderate and severe caries groups, and significant difference between the salivary MS levels and the severity of early childhood caries was found in the present study.

A systemic review in 2015 revealed that salivary markers have generally proved unhelpful in the formal assessment of caries risk in 0–5 year old age group.[23] The present study confirmed that buffer capacity was not correlated with the severity of early childhood caries in preschool children.

ECC is a chronic, transmissible, infectious disease whose etiology is complex and multifactorial. Therefore, control of other risk factors is also import in preventing early childhood caries. In conclusion, the salivary MS levels in preschool children were significantly correlated with the caries severity. The higher the salivary MS level was, the more severe the caries was in preschool children. The levels of MS in the oral cavity play an important role in caries development of preschool children. There was also a tendency toward a higher salivary LB level in children with more sever caries. The salivary buffer capacity was not associated with caries severity.

Conflicts of interest

The authors have no conflicts of interest relevant to this article.

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References

1. Tanzer JM, Livingston J, Thompson AM. The microbiology of primary dental caries in humans. *J Dent Educ* 2001;65:1028–37.
2. Loesche WJ. Role of Streptococcus mutans in human dental decay. *Microbiol Rev* 1986;50:353–80.
3. Low W, Tan S, Schwartz S. The effect of severe caries on the quality of life in young children. *Pediatr Dent* 1999;21:325–6.
4. Tinanoff N, O'Sullivan DM. Early childhood caries: overview and recent findings. *Pediatr Dent* 1997;19:12–6.
5. Edelstein BL, Ureles SD, Smaldone A. Very high salivary streptococcus mutans predicts caries progression in young children. *Pediatr Dent* 2016;38:325–30.
6. Alaluusua S, Renkonen OV. Streptococcus mutans establishment and dental caries experience in children from 2 to 4 years old. *Scand J Dent Res* 1983;91:453–7.
7. Kőhler B, Andréen I, Jonsson B. The earlier the colonization by mutans streptococci, the higher the caries prevalence at 4 years of age. *Oral Microbiol Immunol* 1988;3:14–7.
8. Gao X, Hsu CYS, Loh T, Hwarng B, Koh D. Role of microbiological factors in predicting early childhood caries. *Pediatr Dent* 2014;36:348–54.
9. American Academy of Pediatric Dentistry. Policy on early childhood caries(ECC):classifications, consequences, and preventive strategies. *Pediatr Dent* 2018;40:60–2. Reference Manual 2018–2019.
10. Tsai AI, Chen CY, Li LA, Hsiang CL, Hsu KH. Risk indicators for early childhood caries in Taiwan. *Community Dent Oral Epidemiol* 2006;34:437–45.
11. Axelsson P. *Diagnosis and risk prediction of dental caries*, 2nd ed. Chicago: Quintessence, 2000:249–80.
12. Gibbons RJ, Van Houte J. Dental caries. *Annu Rev Med* 1975;26:121–35.
13. Togelius J, Kristoffersson K, Anderson H, et al. Streptococcus mutans in saliva: intra-individual variations and relation to the number of colonized sites. *Acta Odontol Scand* 1984;42:157–63.
14. Seki M, Karakama F, Terajima T, et al. Evaluation of mutans streptococci in plaque and saliva: correlation with caries development in preschool children. *J Dent* 2003;31:283–90.
15. Van Houte J. Bacterial specificity in the etiology of dental caries. *Int Dent J* 1980;30:305–26.
16. Ramos-Gomez FJ, Weintraub JA, Gansky SA, Hoover CI, Featherstone JD. Bacterial, behavioral and environmental factors associated with early childhood caries. *J Clin Pediatr Dent* 2002;26:165–73.
17. Priyadarshini HR, Hiremath SS, Fernandes B, Rohini M, Nagarathnamma T. Relationship between severe-early childhood caries, salivary mutans streptococci, and lactobacilli in preschool children of low socioeconomic status in Bengaluru city. *J Indian Soc Pedod Prev Dent* 2014;32:44–7.
18. Ge Y, Caufield PW, Fisch GS, Li Y. Streptococcus mutans and Streptococcus sanguinis colonization correlated with caries experience in children. *Caries Res* 2008;42:444–8.

19. Parisotto TM, Steiner-Oliveira C, Silva CM, Rodrigues LK, Nobre-dos-Santos M. Early childhood caries and mutans streptococci: a systematic review. *Oral Health Prev Dent* 2010;8:59—70.
20. Barsamian-Wunsch P, Park JH, Watson MR, Tinanoff N, Minah GE. Microbiological screening for cariogenic bacteria in children 9 to 36 months of age. *Pediatr Dent* 2004;26:231—9.
21. Slayton RL, Cooper ME, Marazita ML. Tuftelin, mutans streptococci, and dental caries susceptibility. *J Dent Res* 2005;84:711—4.
22. Gudkina J, Brinkmane A. The impact of salivary mutans streptococci and sugar consumption on caries experience in 6-year-olds and 12-year-olds in Riga. *Stomatologia* 2010;12:56—9.
23. Fontana M. The clinical, environmental, and behavioral factors that foster early childhood caries: evidence for caries risk assessment. *Pediatr Dent* 2015;37:217—25.