The Association of Early Childhood Caries with Salivary Antimicrobial Peptide LL37 and Mutans Streptococci

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Purpose: *This study aims to determine the relation of salivary LL37 level and mutans streptococci levels in early childhood caries (ECC).* ***Study design:*** *A case-control study was performed in children ≤71 months old. Unstimulated whole saliva was collected and the level of salivary LL37 was measured using an enzyme-linked immunosorbent assay kit. The mutans streptococci oral bacteria were isolated from saliva and identified using a modified SB-20 culture medium (SB-20M). Data were analyzed using descriptive statistics, bivariate, and Spearman's rank correlation analysis.* ***Results:*** *The was a variability of salivary LL37 level among the children and the level was significantly associated with age and races. The median (IQR) value of salivary LL37 in caries-free (CF) children was significantly higher 393.50 (580.55) ng/mL compared to 172.50 (234.65) ng/mL in the ECC group. The ECC children exhibited a significantly higher count of S. mutans and S. sobrinus compared to the CF children. An inverse weak correlation between salivary LL37 and dmft was also observed.* ***Conclusions:*** *The low salivary LL37 level and higher S. mutans and S. sobrinus count in ECC supported the protective role of salivary LL37 against dental caries. Further studies are required to explore the definite relation between salivary LL37 levels and dental caries.*

Keywords*: Salivary LL37, Early childhood caries, Mutans streptococci, Children.*

INTRODUCTION

Dental caries is a common public health problem in children. It is a multifactorial disease influenced by several factors including social, host, and bacterial infection.[1] The parents educational level and employment status are significant risk factors for dental caries in children.[1] Additionally, susceptibility to dental caries is influenced by the salivary antimicrobial peptides of the host. [2]

Microbiological studies suggest that the development and progression of ECC started with the accumulation of cariogenic bacteria on the tooth surface. [3] The most common mutans streptococci, mainly *S. mutans* and *S. sobrinus* are the major causative bacteria responsible for initiating dental caries in children. [4,5] In the presence of sucrose, fructose, and glucose, extracellular polysaccharides are formed by these bacteria. In addition, these bacteria bind strongly to the teeth and are able to survive the acidic environment. [6]

Human antimicrobial peptides (AMPs) are one of the essential parts of human innate immunity. These are natural antibiotics possess bactericidal activities. AMPs have a wide antimicrobial activity against Gram positive and Gram negative bacteria besides providing primary protection against different types of microorganisms and infections. [2] In human, there are different types of AMPs [7] but human cathelicidin (LL37) is considered as the major AMPs being produced. [8]

Invasion by viruses, fungi, and bacteria, and the active form of vitamin D ($1,25(OH)_2D_3$) [10] activated the formation of LL37 by the

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epithelial cells membrane and natural killer cells such as neutrophils and monocytes. [9]

In the oral cavity, the main sources of LL37 are saliva, gingival crevicular fluid, and epithelial cells. [8] LL37 provides a natural defense mechanism against many types of bacteria residing in the oral cavity. The peptide also helps to keep the oral tissue, mucosa, and dental structure healthy by preventing the formation of dental biofilm and kills pathogenic bacteria causing oral diseases and dental caries. [2,8]

The salivary LL37 peptide is considered as a biological factor that contributes to caries resistance and susceptibility. [8] Lower level of LL37 in the saliva may contribute to caries susceptibility [8,11] hence, suggesting the peptide as an essential innate immunity factor in the mouth protecting against dental caries. [12] This is proven by the antibacterial role of salivary LL37 against different species of oral bacteria including mutans Streptococci. [7,8]

Several studies have explored the association between salivary LL37 and dental caries in children, [2,11–13] however, the results are still a controversial debate in the field. A study in 149 children age between 11-15 years old in the US reported a non-statistically significant higher level of salivary LL37 in CF children compared to caries active children. [2] In contrast to the previous study, a statistically significant outcome of LL37 was observed in CF children compared to caries active in a study of 49 Greek children between 2-18 years old. [11] Few other studies showed no association between salivary LL37 and dental caries in children. [12,13] There are limited studies investigating the association of LL37 concentration and ECC. [12] Additionally, no data is available on the association between Asian children with different races. Therefore, this study aims to determine the association between salivary LL37 level and mutans streptococci levels and early childhood caries in a multiracial society.

MATERIALS AND METHOD

Ethical approval

This case-control study was conducted as part of a larger study evaluating the level of vitamin D and its association with ECC among Malaysian young children. This study was approved by the Human Ethics Committee of Universiti Teknologi MARA [600-RMI (5/1/6)] and Medical Research and Ethics Committee [(MREC) NMRR-15-1857-25950 (IIR)]. Written informed consent was obtained from the parents prior to the study.

Study design and sampling

A total of 120 healthy Malaysian children in the age of ≤71 months old were recruited from the pediatric dental clinic at faculty of dentistry Universiti Teknologi MARA and Tengku Ampuan Rahimah Klang hospital, Selangor, Malaysia. The children were divided into two groups namely ECC group consisting of children undergoing comprehensive dental care under GA and children with no dental caries experience (dmft=0). Data were collected from November 2016 until August 2019.

Clinical and dental examination

The heights and weights of the children were measured after recording the child's information on the morning of the data collection day. The children were requested to wear minimal clothes with no shoes on to record their heights and weights. The measurement was done twice and the mean reading was recorded. The BMI expressed as weight/height^2 (kg/m^2) was calculated using an online child and teen BMI calculator. [14]

Dental examination of each child was performed using a dental mirror and caries experience was diagnosed according to the WHO dental caries diagnostic criteria based on *the number of decayed, missing, and filled primary teeth (dmft) scores.* [15]

Saliva samples collection

Unstimulated saliva was collected in a sterile disposable container and the salivary LL37 peptides, *S. mutans*, and *S. sobrinus* were determined using an expectorating method. [2] All saliva samples were obtained between 8.00 and 11.00 am to avoid the diurnal effect on the salivary content. The samples were kept on dry ice and transported in a cooler box to the research laboratory. Approximately, 0.5-1 mL of the saliva was transferred to a sterile cryovial and stored at–80°C for salivary LL37 analysis [13] and microbiological analysis was performed immediately.

Microbiological analysis

A saliva volume of 100 µl of each sample was transferred into a sterile Eppendorf tube (2mL) containing 900 µl Phosphate Buffer Saline (PBS) and serially diluted to establish dilutions of 1:100, 1:1000, and 1:10,000. For each dilution factor, 100 µL volume was plated in triplicate onto a modified SB-20 sucrose-bacitracin culture medium (SB-20M) (a selective medium that only grows *S. mutans* and *S. sobrinus* to reveal the morphological differences). [16] The plates were then incubated aerobically at 37°C for 72 hours. After incubation, *S. mutans* and *S. sobrinus* colonies were counted using a colony counter and the number of colony forming unit (CFU) was multiplied by the number of times the original mL of sample was diluted (the dilution factor of the plate counted) and expressed as CFU/mL of saliva. The cariogenic activity of mutans streptococci bacteria was classified as very low risk (<10,000), low risk (10,000–100,000), average risk (>100,000–500,000), and high risk (>500,000) CFU/ml of saliva. [17]

Salivary LL37 analysis

The saliva samples were thawed, cleared by centrifugation at 15,000 rpm for 5 minutes at 4°C and the supernatant was collected. [13] The salivary LL37 level was determined using an enzyme-linked immunosorbent assay kit (ELISA) (HyCult Biotechnology, Uden, The Netherlands) in duplicate following the manufacturer's instructions.

Statistical analysis

The data obtained were coded and analyzed using the Statistical Package for Social Science (SPSS) version 25.0. SPSS (SPSS Inc, Chicago, IL, USA). Nonparametric statistical methods were used since the data failed the normality test. Descriptive statistical analysis including (median ± IQR, 25th quartile; 75th quartile) was reported. Chi-Square and Fisher's exact tests were used for categorical variables. The statistical comparison of continues variables between groups of children was performed using Mann–Whitney tests and Kruskal-Wallis test. The correlation test was evaluated using Spearman's rank test. Binary regression analysis was performed to evaluate the influence of salivary LL37 and oral bacteria on dental caries. A p-value ≤ 0.05 was considered statistically significant.

RESULTS

Out of a total of 120 participants, only 106 saliva samples were available for salivary LL37 and microbiological analysis. The other 14 children were devoid of saliva samples. There were 26 CF children and 80 ECC children. Among these children, 55 (51.9%) participants were females and 51 were males (48.1%). The mean age for these children was 55.10 ± 11.04 months ranging from 31 to 71 months old. The majority of the children were Malay with 94 children (88.7%), 10 (9.4%) children were Indian, and only 2 children were Chinese (1.9%). No significant differences were found in relation to age, gender, race, and birthweight between CF and ECC children. However, Fisher's exact test result indicated that children with premature birth (<37 weeks) were more likely to experience ECC (p=0.023). In addition, the education level of the parents appeared to be significantly associated with caries experience among their children. The Chi square test results indicated that both mothers and fathers with secondary education and below were more likely to have children affected with ECC (p<0.001, mother; p=0.002, father). The employment status of the father is inversely correlated with the caries status whereby employed fathers were more likely to have children affected with ECC (p=0.014). Children that live in the urban areas were more likely to be affected with ECC compared to children living in rural areas (p=0.021).

Salivary LL37 and demographic characteristics of the children

Salivary LL37 was detected in the children with high variability. The overall median (IQR) of LL37 was 183.55 (282.15) ng/mL (25th quartile; 75th quartile) were (93.25, 375.40). Table 1 shows no significant difference in salivary LL37 levels between gender. However, the levels are significantly associated with children's age and race. Children with the age <60 months old showed a significantly higher level of LL37 in the saliva (p=0.012). Additionally, the highest level of median salivary LL37 was seen in the Chinese children and the lowest median level of salivary LL37 was identified in the Indian children (p=0.009).

Table 1. The level of salivary LL37 of children by their demographic charactersitic

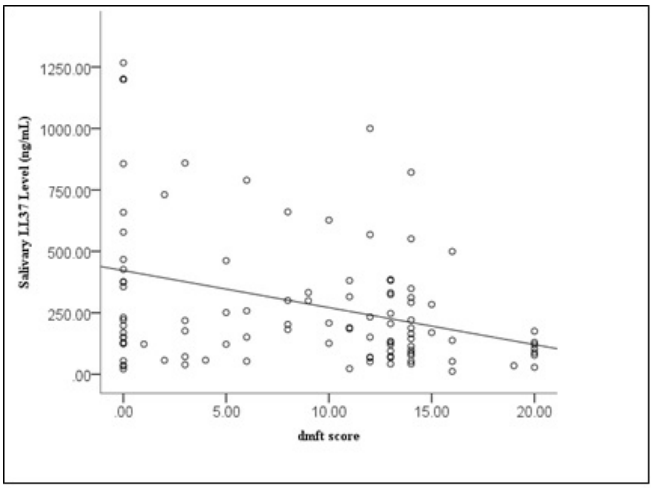
Variables	Salivary LL37 ng/mL n Median (IQR)	Z statistic[a]	X^2 statistic (df)[b]	*P*-value
Age (months)				
<60	58 232.05 (311.50)	-2.520		0.012[a]
≥60	48 130.60 (208.95)			
Gender				
Male	51 218.60 (300.50)	-1.717		0.086[a]
Female	55 164.40 (242.53)			
Race				
Malay	94 200.60 (261.90)		9.516	**0.009[b]**
Chinese	2 310.05 (189.65)		(2)	
Indian	10 70.05 (79.26)			
Caries status		-2.11		0.034[a]
CF	26 293.50 (580.55)			
ECC	80 172.50 (234.65)			

[a]Mann-Whitney test, [b]Kruskal-Wallis test, significant at p=≤0.05, CF; Caries-free, ECC; Early Childhood Caries

Association of salivary LL37 and caries experience

The median (IQR) salivary LL37 of CF children was 393.50 (580.55) ng/mL (25th quartile; 75th quartile) (127.62, 708.17) compared to 172.50 (234.65) ng/mL (79.72, 314.57) in the ECC group. Mann-Whitney test revealed that children with caries showed a significantly lower level of salivary LL37 compared to the CF children (p=0.034) as shown in Table 1. This is strengthened with a significant inverse weak correlation between salivary LL37 and dmft score ((ρ (rho) =–0.275; p =0.004) examined using Spearman's rho correlation coefficient as shown in Figure 1.

Figure 1. The association between salivary LL37 levels and dmft score



Oral bacteria and demographic characteristics of the children

Regarding the oral bacteria detection, 54.7% of *S. mutans* and 87.7% of *S. sobrinus* were found in the children. *S. mutans* colonization was significantly correlated with the children's age. Chi-square test indicated that children at the age of ≥60 months old have a high proportion of *S. mutans* in the saliva compared to <60 months old children, although no statistical significance was achieved (p=0.063). A statistically significant was seen for *S. mutans* in the older children (p=0.047) using a t-test. Furthermore, children born prematurely (<37 weeks) showed a significantly higher percentage of *S. mutans* (p=0.001) and *S. sobrinus* (p=0.040) as shown in Table 2.

Association of oral bacteria and caries experience

In this study, a strong association was seen between the presence of oral bacteria and caries experience. The frequency of *S. mutans* in the ECC children was 91.4% while only 8.6% was recorded in the CF children and the difference was statistically significant (p<0.001). In addition, the frequency of *S. sobrinus* in the ECC children was 76.3% compared to 23.7% in the CF children. No statistically significant difference (p=0.577) was observed as shown in Table 3.

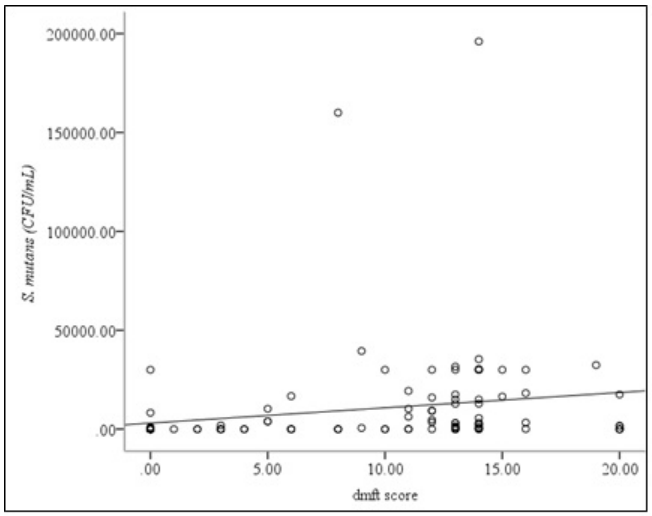
Nonetheless, a significantly higher percentage of *S. mutans* and *S. sobrinus* CFU/mL was found in the ECC group (p=0.005; p=0.029), respectively (Table 3) compared to the CF group. Comparing the frequency of both mutans streptococci, this study revealed that a higher proportion of ECC children (92.2%) comprises both *S.*

Table 2. Mutans streptococci bacteria of children by their demographic characteristic

Variables	*S. mutans*	n (%) / Mean (SD)	P-value	*S. sobrinus*	n (%) / Mean (SD)	*P*-value
Age (months)						
<60	*S. mutans*- *S. mutans*+	31 (53.4) 27 (46.6)	0.063	*S. sobrinus*- *S. sobrinus*+	8 (13.8) 50 (86.2)	0.598
≥60	*S. mutans*- *S. mutans*+	17 (35.4) 31 (64.6)		*S. sobrinus*- *S. sobrinus*+	5 (10.4) 43 (89.6)	
Age (months)						
	S. mutans- *S. mutans*+	48 54.12 (10.77) 58 58.91 (8.85)	**0.047**[*]	*S. sobrinus*- *S. sobrinus*+	13 53.15 (9.78) 93 57.19 (10.06)	0.184
Gender						
Male	*S. mutans*- *S. mutans*+	27 (52.9) 24 (47.1)	0.127	*S. sobrinus*- *S. sobrinus*+	9 (17.6) 42 (82.4)	0.104
Female	*S. mutans*- *S. mutans*+	34 (38.2) 34 (61.8)		*S. sobrinus*- *S. sobrinus*+	4 (7.3) 51 (92.7)	
Race						
Malay	*S. mutans*- *S. mutans*+	45 (47.9) 49 (52.1)		*S. sobrinus*- *S. sobrinus*+	11 (11.7) 83 (88.3)	0.700
Chinese	*S. mutans*- *S. mutans*+	1 (50.0) 1 (50.0)	0.175	*S. sobrinus*- *S. sobrinus*+	0 (0.0) 2 (100.0)	
Indian	*S. mutans*- *S. mutans*+	2 (20.0) 8 (80.0)		*S. sobrinus*- *S. sobrinus*+	2 (20.0) 8 (80.0)	
Low birth weight						
	S. mutans- *S. mutans*+	10 (55.6) 8 (44.4)	0.317	*S. sobrinus*- *S. sobrinus*+	4 (22.2) 14 (77.8)	0.316
Premature birth (Yes)						
	S. mutans- *S. mutans*+	3 (37.5) 5 (62.5)	**0.001**[b]	*S. sobrinus*- *S. sobrinus*+	3 (37.5) 5 (62.5)	**0.040**[b]

[a]Chi-square test, [b]Fisher's exact test , [*]t-test, statistically significant at p≤0.05,

Figure 2. The association of *S. mutans* (CFU/mL) and dmft score



mutans and *S. sobrinus* (p<0.001) as shown in Table 3. Spearman's rho indicates a weak positive correlation between the colony counts (CFU/mL) of *S. mutans* and dmft score ((ρ (rho) = 0.483 p<0.001) as shown in Figure 2. However, no correlation was identified between dmft score and *S. sobrinus* (ρ (rho) = 0.027 p=0.786).

Association of salivary LL37 and oral bacteria

In this section, any association between salivary LL37 level and oral bacteria (*S. mutans* and *S. sobrinus*) was investigated. Children that harbor both *S. mutans* and *S. sobrinus* bacteria have a lower salivary LL37 level compared to children with no bacteria. The median salivary LL37 level reported in children with detection of *S. mutans* was 172.50 ng/mL compared to 193.15 ng/mL in children with no detection (p=0.195). Furthermore, the median salivary LL37 level in children with detected *S. sobrinus* was 182.10 ng/mL compared to 198.20 ng/mL in children with no detection of *S. sobrinus* (p=0.473).

Table 4 shows the results of binary regression analysis revealing that salivary LL37 and oral bacteria have a significant influence on dental caries in children. Two units reduction in salivary LL37 (ng/mL) will increase caries experience by 20%. Furthermore, children that have both *S. mutans* and *S. sobrinus* bacteria were 10-fold at risk of dental caries compared to children without any presence of bacteria.

Table 3. The association of caries status of children and their mutans streptococci bacteria levels

Bacteria	Caries status		*P*-value
	CF n (%)	ECC n (%)	
S. mutans^{+}	5 (8.6)	53 (91.4)	
S. mutans^{-}	21 (43.8)	27 (56.2)	<0.001[a]
Total	26 (24.5)	80 (75.5)	
S. sobrinus^{+}	22 (23.7)	71 (76.3)	
S. sobrinus^{-}	4 (30.8)	9 (69.2)	0.577
Total	26 (24.5)	80 (75.5)	
S. mutans^{-}/*S. sobrinus*$^{-}$	3 (50.0)	3 (50.0)	
S. mutans^{+}/*S. sobrinus*$^{-}$	1 (14.3)	6 (85.7)	<0.001[a]
S. mutans^{+}/*S. sobrinus*$^{+}$	4 (7.8)	47 (92.2)	
S. mutans^{-}/*S. sobrinus*$^{+}$	18 (42.9)	24 (57.1)	
Total	26 (24.5)	80 (75.5)	
*S. mutans (*CFU/mL)			
<10,000	25 (32.1)	53 (67.9)	**0.005**[b]
10,000-100,000	1 (3.8)	25 (96.2)	
>100,000	0 (0.0)	2 (100.0)	
Total	26 (24.5)	80 (75.5)	
*S. sobrinus (*CFU/mL)			
<10,000	17 (20.0)	68 (80.0)	**0.029**[b]
10,000-100,000	9 (42.9)	12 (57.1)	
>100,000	—	—	
Total	26 (24.5)	80 (75.5)	

[a]Chi-Square test, [b]Fisher's exact test statistically significant at ≤0.05, CF; Caries-free , ECC; Early Childhood Caries

DISCUSSION

It is well documented that dental caries is a multifactorial disease influenced by several factors including social, host, dietary, and bacterial infection.[1] This study examined the socioeconomic factors, salivary LL37, and the presence of cariogenic bacteria that have a significant influence on dental caries.

In this study, there is no significant difference was observed between the ECC and CF groups on age, gender, and race, which is aligned with a previous study. [18] This study also reported a high proportion of premature birth children in the ECC group. This is in line with a previous study that showed a significant association between premature birth and dental caries in 3 to 5 years-old US children. [19] In addition, there is no relationship was indicated between low birth weight and dental caries, which is in accordance with other studies. [19,20]

The finding in this study suggested that parents with higher education level have a positive influence on caries incidence in their children. This is in line with the results from previous studies. [21,22] The parents with low educational level may have a lack of attention to the essential dental care measures, hence leading to caries incidence in their children. [23] Working parents are able to provide the basic needs for their children, however, this situation causes the parents to seek help from day-care or extended family members in managing their children. [24] The less time spent with their children cause the parents to overlook their children's oral health care. [24] The association between parental employment status and dental caries is still controversial. Few studies reported lower caries prevalence in the high occupation levels of either one of the parents or both. [25,26] Nonetheless, there are several studies suggested an inverse association between the parent's employment status and dental caries prevalence in their children. [24,27]

Another finding in this study is that working fathers are prone to have children with dental caries. This could be due to the no engagement with the spouse in providing care to their young children. [28] However, a crude measurement of employment status, employed, and unemployed were examined in this study to establish the association between parental employment status and dental caries. A detailed employment classification might give another insight into this finding.

In addition, children from rural areas have better dental health than the children in urban areas. This is in accordance with a finding reported in a previous study among 5 years-old Scotch children. [29] This result could be explained by the difference lifestyle by the children living in the rural areas including diet and eating habits. The children in the rural areas may have less caries prevalence due to the fact that these children consume healthy homemade meals, less fast food, and limited choice of snacks and ready-made meals that are mainly high in sugar. [29]

Greater attention was given to the saliva as it offers unique advantages compared to a serum since it can be collected non-invasively, no special equipment is required for the collection, and is inexpensive.[30] Saliva is an important body fluid contains various host factors that can be used to diagnose dental caries and monitor its progress. [31] Therefore, saliva is a useful specimen to be used to develop caries risk tools to easily and accurately identify patients at high caries risk for preventative measures and better treatment options, especially among young children. Unstimulated saliva

Table 4. Logistic regression of dental caries, salivary LL37 and oral bacteria

Variables	Regression coefficient (b)	Standard error	Wald statistics	*P*-value	Adjusted odds ratio (95% CI)
Salivary LL37	-0.002	0.001	6.150	**0.013**	0.998 (0.997- 1.000)
Oral bacteria Reference= *S. mutans*-/*S. sobrinus*-			12.527	**0.005**	
S. mutans^{+}/*S. sobrinus*-	2.289	1.561	2.151	0.142	9.863 (0.463- 110.088)
S. mutans^{+}/*S. sobrinus*$^{+}$	2.282	1.004	5.166	**0.022**	10.008 (1.399- 71.587)
S. mutans-/*S. sobrinus*$^{+}$	0.278	0.914	0.092	0.761	1.320 (0.220- 7.921)

Statistically significant at ≤0.05

samples are easily collected compared to stimulated saliva since the children were unable to follow the instructions and have limited ability to chew the tools that are used for saliva collection. [32] The unstimulated whole saliva is, therefore, collected in a less invasive method. It represents the systemic clinical conditions since some materials that were used to stimulate flow may change the saliva composition. [33] A study conducted by Seki et al., (2003) [34] comparing stimulated and unstimulated saliva in evaluating oral bacteria reported a significant correlation. Thus, suggesting that evaluation of oral bacteria in preschool children have to be on the unstimulated saliva.

LL37 level in the unstimulated saliva of the children showed a great variation, [8,11] which could be influenced by genetic. [8] The finding in this study showed a significant difference in the level of salivary LL37 between different races with Chinese children exhibit higher level compared to Malay and Indian ethnic groups. However, this result is not a real representative in the population as there were only 2 Chinese and 10 Indian children in the sample size. Larger sample size is required to represent different ethnic groups.

A review paper indicated that the concentration of LL37 in the saliva ranging from 0.14-3µg/mL.[35] Other studies reported a range of 0.22 ng/mL to 12 µg/mL. [2,11] The concentration of LL37 observed in this study is higher in younger children, which contradicts with a report proving a significant positive correlation between salivary LL37 and age. [11] The salivary LL37 level also has been shown to be higher in children with mixed or permanent dentition and the level diminished in individuals with high caries activity. [11] This study only examined LL37 level in preschool children and only children with primary teeth were enrolled. Furthermore, the results also showed that older children are significantly detected with *S. mutans* than younger children. Although non-statistically significant, older children tend to have caries experience, which may have led to a decrease in salivary LL37 concentration in older children.

Salivary LL37 is an essential innate immunity component and the finding in this study exhibits that CF children tend to have a higher level of salivary LL37 compared to ECC. This is in line with findings from other studies. [2,11] Nonetheless, several studies reported contradicting findings. [12,13] This suggests that LL37 level could be an important parameter for caries risk assessment in children and as a tool for prognosis of dental caries or as a therapeutic agent.

Streptococcus mutans plays an important role in dental caries pathogenesis. The majority of studies support a positive correlation between salivary levels of mutans streptococci and ECC. Mutans streptococci could serve as a marker for early childhood caries onset. [36] This study revealed that the frequency of *S. mutans* and *S. sobrinus* was significantly higher in ECC children (91.4%, 76.3%) compared to CF children (8.6%, 23.7%), respectively. Similar findings of higher frequency of *S. mutans* and *S. sobrinus* in ECC compared to CF children was also reported. [37, 38]

Another significant finding in this study is the presence of both *S. mutans* and *S. sobrinus* with 92.2% in the ECC group compared to 7.8% in the CF children. This showed a 12-fold higher than the CF children and a similar finding was reported in other studies. [38, 39] Children harboring both bacterial species were at higher risk of dental caries. [4,5] Therefore, the presence of both *S. mutans* and *S. sobrinus* is considered an important pathogenic factor in the development of carious lesions and may increase the risk of ECC.

Gestational age and birth weight are a useful indicator for the nutritional status of the young children. [40] A higher proportion of *S. mutans* and *S. sobrinus* is present in the premature children in this study and this could support the association between dental caries in children and premature birth. Premature birth child acquired more oral bacteria at a very early in life hence increases the risk of dental caries. However, no significant association was found relating to birth weight. This is supported by a previous study showing a significant association between *S. mutans* and birth weight, but not *S. sobrinus.* [41]

Moreover, an investigation on the association between salivary LL37 and the presence of oral bacteria was also performed. Although non-statistically significant was achieved, children harboring both *S. mutans* and *S. sobrinus* bacteria have lower salivary LL37 levels compared to children with no bacteria detection. This could be due to the sample size in this study. However, the influence of both salivary LL37 and mutans streptococci on dental caries may support the main aim of this study that ECC may be influenced by both variables. Caries experience was significantly influenced by a lower level of salivary LL37 and the presence of both *S. mutans* and *S. sobrinus* bacteria. This strengthens the notion that salivary LL37 has a protective role against dental caries.

The main limitation of this study is the small sample size especially the limited number of CF children as majority of the recruited children present with ECC. As reported by a previous study, the prevalence of ECC in Malaysia is 71.3%. [42] This could explain the percentage of CF to ECC in this study. Biased recall may exist during data collection mainly in questions related to the child's early life. However, despite these limitations, the findings on the role of LL37 in ECC in this study are useful.

CONCLUSION

This study indicated the importance of social and biological factors in the development of dental caries in preschool children. CF children have higher levels of salivary LL37 and low loads of *S. mutans* and *S. sobrinus*. This study supported that elevated salivary LL37 level might have a role in the inhibition of oral bacteria, hence reducing caries incidence. These valuable findings could lead to the development of a new screening tool used to determine caries susceptibility in children. Further studies are recommended to investigate other antimicrobial peptides and cariogenic bacteria in relation to the incidence and the severity of dental caries.

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