Prediction Baby Bottle Tooth Decay based on *Streptococcus Mutans* Glucosyltransferase Polymorphisms and Salivary Mucin MG2

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Abstract

Baby bottle tooth decay syndrome (BBTDS) can compromise the development of the child. The prevention of BBTDS requires an analysis of microbiological aspects. Factors known to play a role in BBTDS include glucosyltransferase (Gtf) polymorphisms of *S.mutans* and salivary level of mucin MG2. Factors involved in BBTDS caused by *S. mutans* serotypes c and f are unknown. The present study aimed to detect the risk of caries associated with *gtfB* and *gtfC* polymorphisms in *S. mutans* serotypes c and f, in addition to mucin MG2 levels, in children aged 3–5 years with BBTDS. **Methods**: This was a cross-sectional study of prospective data on 41 children in a caries-free group and 41 in a caries group. Salivary and plaque samples were collected from for clinical examinations and laboratory analyses. Polymorphisms band detected by PCR analyses and Mucin levels measured in Elisa reader. **Results**: For the *gtfB* polymorphism, bands at 700, 800, 1500,and 2000 were an indicator of caries, whereas bands at 500 and 2000 were an indicator of caries in the presence of the *gtfC* polymorphism. An increase of 0.02 inmucin levels increased the tooth deft score by ±1. **Conclusion**: *S.mutans* serotype c and a combination of *gtfB* and *gtfC* polymorphisms were associated with the greatest increase in mucin levels. The mucin MG2 level exhibited a significant association with the incidence of caries, *S.mutans* serotype, and *gtf* polymorphism.

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Introduction

Dental caries involves an infectious pathological process in which local destruction of hard tissue is caused by microorganisms due to the interaction of several factors in the oral cavity.[1-3] In children, caries is a primary dental health problem, which can compromise a child's growth and development. The most common types of caries in 1children are rampant caries and baby bottle tooth decay syndrome (BBTDS), which is also known as nursing bottle caries syndrome, labial caries, and maxillary anterior caries, and is found in children under the age of 5 years.[4,5]

Dental caries associated with BBTDS usually affect the upper primary incisors and lower primary molars in accordance with the eruption sequence of the teeth. The lower incisors are usually not affected because these teeth are covered by the tongue during bottle nursing. The duration of bottle nursing during sleep affects the incidence of BBTDS.[6,7]

According to a report by the WHO in 2003, the prevalence of caries in children was 60–90%.[4,8,9] The prevalence of caries in children in Indonesia was reported to be approximately 70–90.05%, with BBTDS accounting for the highest incidence.[5,10,11] In a study conducted in 2009, Fahlevi reported that the proportion of children with BBTDS was 72.6%.[12] Therefore, it can be concluded that the prevalence of caries in children remains high.

Streptococcus mutans is the main caries-causing bacterium, and early colonization by *S. mutans* acts as a trigger for caries.[8,13-15] A study in Jakarta of the distribution of *S.mutans* serotypes in children aged 3–5 years with caries of varying degrees of severity demonstrated that

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serotype f bacteria were the most common (91%), followed by serotype c (79%) and serotype e (29%).[16] The ability of *S. mutans* to adhere to the tooth surface, which is aided by the availability of sucrose, is an important process in the development of dental caries. Glucosyl transferase (Gtf) enzymes play a role in the adherence of *S. mutans* to the tooth surface and cariogenic characteristics of this bacterium.[17-20]

The Gtf enzyme, which is encoded by *gtfB* and *gtfC* genes, can transform sucrose to insoluble glucan. Glucan plays a role in plaque formation by facilitating bacterial adherence and colonization on the tooth surface.[20]

Previous research showed that Gtf B, GtfC, and GtfD enzymes played an active role in the production of glucan and adherence of *S. mutans* to the pellicle. Thus, the present study focused on Gtf polymorphisms of c and f serotypes of *S. mutans*.[20]

Research has also shown that saliva helps *S.mutans* adhere to the tooth surface.[21] Mucin MG2 plays a vital role in bacterial adherence to mucosal tissue in the oral cavity. In a previous study of 60 saliva samples, the concentration of mucin MG2 was 13.3 ± 11.6 mg% according to an ELISA and Western blot analysis, and it constituted 16% of total salivary protein.A study conducted by Fahlevi in 2008 showed that a higher incidence of caries was correlated with an increased level of mucin MG2 in saliva.[16]

Both host-related (level of mucin MG2) and agent-related (*S. mutans* serotype and type of *gtf* polymorphism) factors are involved in the incidence of caries. The present study adopted a microbiology perspective and focused on the role of *gtf* polymorphisms of serotypes c and f of *S. mutans* in the incidence of caries in a population of Indonesian children.The results of this study will hopefully aid caries prevention.

The level of mucin MG2 (host factor) was analyzed using a monoclonal antibody. This methodology has most likely never been used in an attempt to develop prediction indicators of the incidence of caries in bottle-fed children aged 3–5 years.

Methods

This study was approved by the Ethics Committee of the Faculty of Dentistry of the University of Indonesia, and informed consent was obtained from the parents of all the children. The study was divided into two stages: Stage I and State II, as described below.

Stage I

Children aged 3–5 years underwent screening, and all the parents completed a questionnaire. The deft index of the children who met the inclusion criteria was determined, and saliva and plaque samples were collected for analysis of the serotype (c and f) and genotype of *S. mutans*. The polymerase chain reaction (PCR) amplified DNA and restriction fragment length polymorphism (RFLP) method was used to detect *gtfB* and *gtfC* genes and polymorphisms.

Stage II

The mucin MG2 levels in saliva were determined using a mucin MG2 monoclonal antibody and an ELISA test. The optical density value from each saliva sample was recorded.

Results and Discussion

This was a cross-sectional study of 200 children. The children were assigned to a caries-free group (n = 41) or caries group (n = 41). The results revealed the presence of *S. mutans* serotype c, *S. mutans* serotype f, or a combination of both serotypes. Distribution studies of the serotype, type of *gtf* polymorphism, and mucin MG2 level were performed. The analysis of the *S. mutans* serotype c and f combination in the caries and caries-free groups showed that the highest proportion of *S. mutans* serotype c and f combination in the caries group was associated with a combination of the *gtfB* and *gtfC* polymorphisms. In contrast, the caries-free group was characterized by a high proportion of *S. mutans* serotype and non-*gtf B* or non-*gtfC* polymorphisms.

Thus, it can be concluded that a combination of *gtfB* and *gtfC* polymorphisms might be used as an indicator of caries versus caries-free groups (Table 1).

Table 1. Variations in *gtfB* and *gtfC* polymorphisms of *S.mutans* serotypes c and f

	Non Polymorph *gtf* B & non Polymorph *gtf* C		Polymorph *gtf* C		Polymorph *gtf* B		Polymorph *gtf* B & *gtf* C		Total	
	N	%	N	%	n	%	N	%	N	%
Free caries*										
Sf	15	88,2	0	0,0	2	11,8	0	0,0	17	41,5
Sc	3	20,0	7	46,7	2	13,3	3	20,0	15	36,6
Sf and Sf	6	66,7	1	11,1	0	0,0	2	22,2	9	22,0
Total	24	58,5	8	19,5	4	9,8	5	12,2	41	100,0
Caries**										
Sf	7	100,0	0	0,0	0	0,0	0	0,0	7	17,1
Sc	4	30,8	7	53,8	1	7,7	1	7,7	13	31,7
Sc and Sf	6	28,6	6	28,6	2	9,5	7	33,3	21	51,2
Total	17	41,5	13	31,7	3	7,3	8	19,5	41	100,0

*X^2=25,876; df=6; p<0,001

** X^2=18,424; df=6; p=0,005

The findings of the present study are in accordance with those of previous research, which showed that combinations of *S. mutans* serotypes were commonly found in patients with caries as compared to those without caries.Previous studies also demonstrated that the deft index of children with combinations of *S. mutans* serotypes was higher than that of children with a single *S. mutans* serotype.[15,22]

In addition, the *gtfB/gtfC* combination was more common in children with caries than in those without caries. This result is in accordance with findings reported in previous studies, which highlighted the importance of *gtfB* and *gtfC* in the binding of bacteria to the tooth surface.[23-26]

The present study used the PCR amplified DNA RFLP method to detect *gtf* polymorphisms. This method, which cuts DNA at a certain locus sequence, is frequently used in clinical molecular diagnostics. An understanding of DNA and its replication is vital in molecular diagnostics. As reported elsewhere, DNA polymorphisms are useful for the determination of gene loci and for finding factors that affect the incidence of a disease.[27]

In this study, the following *gtf* bands were most commonly observed in the caries-free group: *gtfB* 700, 800, and 1500 bands (*n* = 2); *gtfB* 500, 700, 1000, and 1500 (*n* = 2); and *gtfB* 150, 300, and 600 (*n* = 2). In contrast, *gtfB* 700, 800, 1500, and 2000 bands were more common in the caries group (*n* = 3) (Table 2).

Table 2. Band distribution profiles of the *gtfB* polymorphism.

Gtf B Band Combination	Free Caries		Caries		Total	
	N	%	N	%	N	%
non *gtf* B band	32	51.6	30	48.4	62	75.6
800, 1500, 3000	0	0	1	100	1	1.2
700, 1500	0	0	2	100	2	2.4
700, 800, 1500	2	100	0	0	2	2.4
700, 800, 1500, 2000	1	33.3	2	66.7	3	3.7
500, 700, 1000, 1500	2	100	0	0	2	2.4
500, 600, 700, 1000, 1400	0	0	1	100	1	1.2
450, 500, 600, 700, 850, 1000, 1250	0	0	1	100	1	1.2
400, 500, 700, 1000, 1300	1	100	0	0	1	1.2
400, 500, 700, 800, 1300	1	100	0	0	1	1.2
400, 500, 600, 1000, 1300	0	0	1	100	1	1.2
400, 500, 600, 700, 1000, 1300	0	0	1	100	1	1.2
300, 400, 600, 1500	0	0	1	100	1	1.2
300, 350, 400, 500, 700, 900	0	0	1	100	1	1.2
150, 300, 600	2	100	0	0	2	2.4
Total	41	50	41	50	82	100

With regard to the polymorphic patterns associated with the incidence of caries, the most frequent polymorphic combinations in the caries group were *gtfB* 700, 800, 1500, and 2000 bands and *gtfC* 1500 and 2000 bands (Fig. 1).

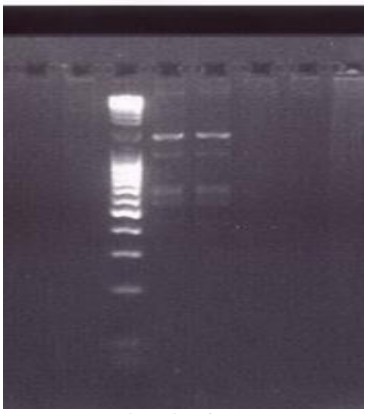

Figure 1. Polymorphic pattern of *gtfB* (700, 800, 1500, and 2000).

A previous study reported that the most common *S. mutans gtf* polymorphic patterns were a combination of *gtfB* 600, 700, and 800 bands and *gtfC* 600, 700, and 1300 bands. The findings of the present study are in accordance with those in the literature (Table 3).

Table 3. Band distribution profiles of the *gtfC* polymorphism.

	Free Caries		Caries		Total	
***Gtf* C Band Combination**	**n**	**%**	**N**	**%**	**N**	**%**
Non *gtf* C band	28	57.1	21	42.9	49	59.8
1500, 2000	0	0	4	100	4	4.9
600, 1300, 1500	3	60	2	40	5	6.1
600, 1250, 2500	0	0	1	100	1	1.2
600, 1000, 1400, 1500	0	0	1	100	1	1.2
600, 700	1	50	1	50	2	2.4
500, 650, 700, 1400, 1500	0	0	2	100	2	2.4
500, 600, 1000, 1300, 1500	0	0	1	100	1	1.2
500, 600, 700, 2800	0	0	1	100	1	1.2
450, 600, 700, 1200, 1300	1	100	0	0	1	1.2
400, 600, 1000, 2400	0	0	1	100	1	1.2
250, 600, 1000, 1300, 1500	1	33.3	2	66.7	3	3.7
250, 600, 700	1	100	0	0	1	1.2
250, 550, 600, 700	1	100	0	0	1	1.2
250, 550, 600, 700, 2000	0	0	1	100	1	1.2
250, 500, 600, 700	1	50	1	50	2	2.4
200, 250, 300, 2000	0	0	1	100	1	1.2
200, 250, 300, 600, 700	3	75	1	25	4	4.9
200, 250, 300, 500, 600, 700	1	100	0	0	1	1.2
Total	41	50	41	50	82	100

The results of this study demonstrated that the main target of the caries-causing Gtf enzyme was GtfB, encoded by the *gtfB* gene, with band polymorphisms of 700, 800, 1500, and 2000, and GtfC, encoded by the *gtfC* gene, with band polymorphisms of 1500 and 2000 (Fig. 2).

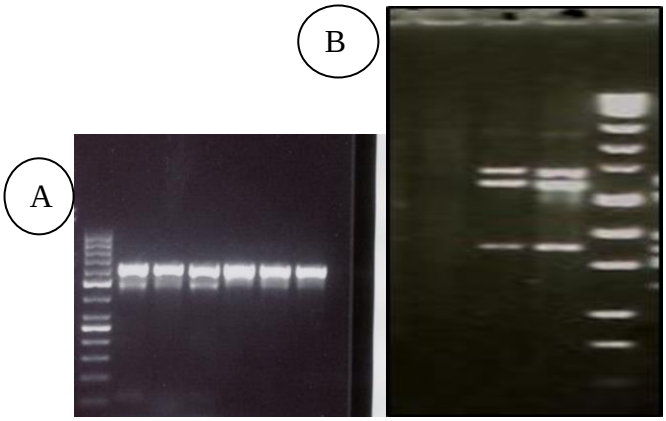

Figure 2. Polymorphic pattern of *gtfC.*

A. Polymorphic pattern of *gtfC* (1500 and 2000)
B. Polymorphic pattern of *gtfC* (600, 1300, and 1500)

Correlation between *S. mutans* serotypes c and f, *gtfB* and *C* polymorphisms, and mucin MG2 level in the caries group. Based on the results of the regression shown in Table 4, the correlation between *S.mutans* serotypes c and f, *gtfB* and *C* polymorphisms, and mucin MG2 level in the caries group was significant ($p< 0.05$). This finding exhibits a linear correlation that can explain 29.2% of the mucin MG2 level. In the caries group, the mucin MG2 level showed a significant regression coefficient ($p< 0.05$) with the *gtfB* polymorphism and *S. mutans* serotype c and f combination. This finding was in accordance with that of a study by Vacca Smith et al., who stated that the salivary level of the GtfB enzyme showed a strong relationship with the incidence of caries in children.[28] However, the analysis of the positive or negative direction of the regression showed that the *gtf* polymorphism and *S. mutans* serotype decreased the mucin MG2 level the most.

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Table 4. Correlation of *S. mutans* serotypes c and f with *gtfB* and *gtfC* polymorphisms and mucin MG2 levels in the caries group

Regression of mucin MG2* levels	B	SE	*P*	95CI β	
Constant	0.238	0.034	0.000	0.170	.307
Caries	0.122	0.027	0.000	0.067	.176
gtfC□ polymorphism	-0.030	0.037	0.424	-0.105	.045
gtfB□ polymorphism	-0.131	0.049	0.008	-0.228	-.035
gtfB and *C*□ polymorphisms	-0.009	0.041	0.833	-.0091	.074
Serotypes c and f©	-0.083	0.033	0.014	-0.149	-.018
Serotype f©	-0.054	0.039	0.171	-0.133	.024

*F = 5, 155; df1 = 6 and df2 = 75; p = <0.001; R = 0.054 R^2= 29.2%
Comparison of non-*gtfB* and non-*gtfC* polymorphismsin the caries group
© serotype c comparison

As shown in Table 4, the *S. mutans* serotype c and f combination appeared to play an important role in increasing the incidence of caries as compared with that of other serotypes (Table 4).

$$MG2\ mucin\ level = 0.238 + 0.122 * caries - 0.030 * gtfC\ polymorphism - 0.131 * gtfB\ polymorphism - 0.009 * gtfB\ and\ C\ polymorphisms - 0.083 * serotype\ c\ and\ f - 0.054 * serotype\ f + \varepsilon. \quad (1)$$

The mucin MG2 level was 0.122-fold higher in the caries group as compared with the caries-free group with the same *gtf* poly morphisms and *S. mutans* serotype. This result is in accordance with that found in previous studies, which stated that the presence of mucin MG2 in saliva triggered bacterial adhesion to oral tissue. The binding of mucin MG2 and *S. mutans* α-enolase allowed *S. mutans* to colonize and thus increased the incidence of caries. A higher caries incidence was also correlated with a higher level of mucin MG2 in saliva.[10,12,18]

The MG2 mucin level was 0.131-fold lower in the presence of the *gtfB* polymorphism as compared to the presence of the non-*gtf*B polymorphism and non-*gtfC* polymorphism. This finding indicated that both types of polymorphisms (*gtfB* and *gtfC*) were associated with elevated mucin MG2 levels. In addition, the mucin MG2 level was 0.083-fold lower in the presence of the S. *mutans* serotype c and f combination as compared with *S. mutans* serotype c only. This result suggested that mucin MG2 levels were higher in the presence of *S. mutans* serotype c than in the presence of the serotype c and f combination. These results are in accordance with the findings of previous studies, which found a high prevalence of *S. mutans* serotype c in children.[29,30]

Previous studies reported that *S. mutans* serotype c, which expressed a surface protein called antigen I/II, played a role in the pathogenesis of dental caries and that it was effective as a vaccine for the prevention of dental caries.[17,30] However, other studies failed to confirm these findings. For example, in an analysis of loci required for determination of serotype antigenicity in *S. mutans*, Shibata et al. found *S. mutans* serotypes other than serotype c were more common in a caries group than a caries-free group.[22] Fahlevi (2009) stated that the risk increased 1.13 fold in bottle-fed children with the *S.Mutans* serotype c and f combination as compared to children without this serotype combination.[11]

In the present study, the presence of both *gtfB* and *gtf*C polymorphisms was associated with the highest increase in mucin MG2 levels. This finding is in accordance with that of previous research, which demonstrated that these two polymorphisms played an important role and that they were a strong indicator of the presence or absence of caries. As mentioned previously, both *gtfB* and *gtfC* aid the accumulation of *S. mutans* on the tooth surface, which facilitates the occurrence of caries[23,26]. Therefore, *gtf* polymorphic variations and *S.mutans* serotypes can be used to predict the level of mucin MG2 in saliva and the incidence of caries. To examine the correlation between caries and mucin MG2 levels, we examined mucin levels and the incidence of caries in children with BBTDS.

Association of mucin MG2 levels with def-t scores

Picture 1 shows the distribution of mucin MG2 levels as a function of def-t scores. As can be seen, mucin MG2 levels exhibited a positive linear relationship, with an increase in levels associated with a rise in the caries incidence. The correlation coefficient was 0.664, which indicated that the linear correlation between the mucin MG2 level and def-t score was sufficiently strong. Furthermore, increases in mucin MG2 levels as a function of def-t scores followed a linear regression, as shown in Table 5.

Table 5. Regression of mucin MG2 level as a function of def-t scores

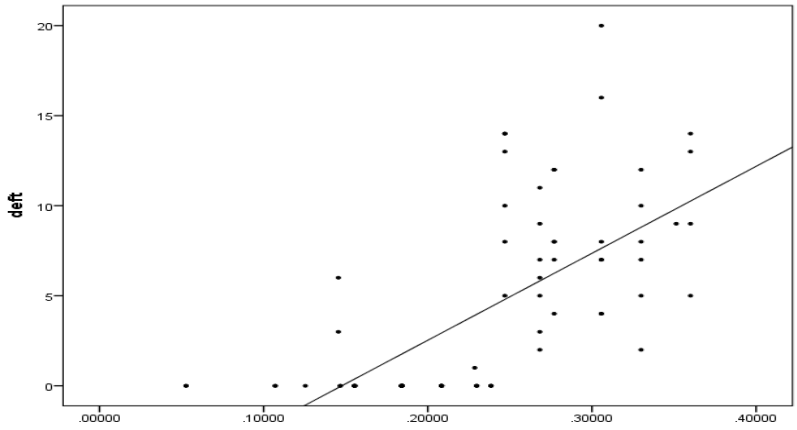
Regression in def-t*	B	SE	*P*	95 CI β	
Constant	-6.839	1.431	<0.001	-9.687	-3.991
Prediction of mucin MG2 level	46.693	5.88	<0.001	34.991	58.395

*ANOVA, F = 63,06; df1 = 1; df2=80; p< 0.001; R = 0.664; R^2 = 44.1%

Based on the results of the regression analysis (Table 5), the mucin MG2 level significantly influenced the incidence of caries, explaining up to 44.1% of caries (p<0.05). For every 0.02-step increase in the mucin MG2 level, the caries incidence increased by ±1, as shown below:

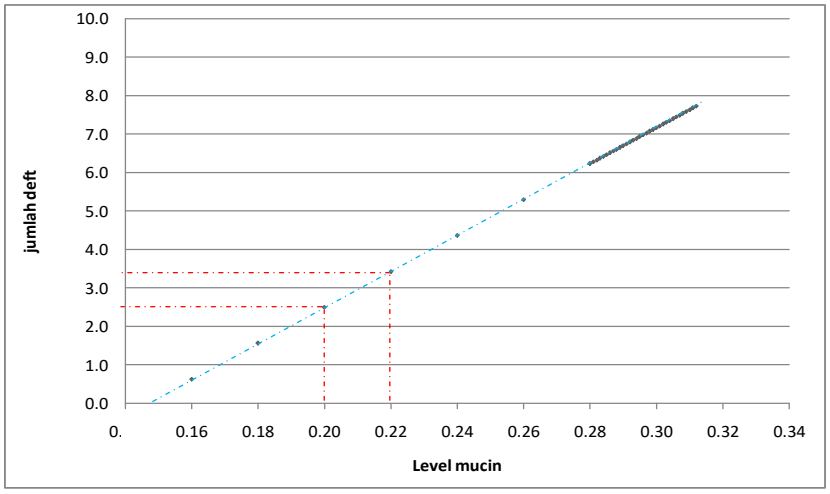
$$def - t = -6.839 + 46.693 * mucin\ level + \varepsilon. \quad (2)$$

Based on the distribution of def-t scores according to linear regression equations (1) and (2) and mucin MG2 levels, we developed a prediction model for the incidence of dental caries. In this model, the caries, *S. mutans* serotype, and *gtf* polymorphism variables were controlled (Picture1 and Picture 2)



$$y = a\alpha + b$$

Picture1. Predictive value of MG2 mucin level according to def-t scores



Picture 2. Predictive value of the distribution of the MG2 mucin level according to def-t scores

As shown by the results of the regression analysis of the two linear equation models (eq. 1 and 2) in Table 6, the incidence of dental caries increased in accordance with a rise in the mucin MG2 level and differences in the *gtf* polymorphic pattern for the same *S.mutans* serotype, and vice versa. Of the three *gtf* polymorphisms, the prevalence of caries was highest with *S.mutans* serotype c, followed by serotype f and the serotype c and f combination. The incidence of dental caries was highest in the presence of both *gtfB* and *gtf*C polymorphisms of *S.mutans* serotype c.

Table 6. Results of the regression analysis of two linear equation prediction models of dental caries, depending on the mucin MG2 level, serotype, and type of *gtf* polymorphism.

Mucin MG2 levels and def-t scores based on serotype and type of *gtf* polymorphism	Serotype c and f		Serotype f		Serotype c	
	MG2 mucin	def-t	MG2 mucin	def-t	MG2 mucin	def-t
gtfB polymorphism	0.146	0	0.175	1	0.229	4
gtfC polymorphism	0.247	5	0.276	6	0.330	9
Combination of *gtfB* and *gtfC* polymorphisms	0.268	6	0.297	7	0.351	10
Combination of non-*gtfB* and non-*gtfC* polymorphisms	0.277	6	0.306	7	0.360	10

The results of the analysis of mucin MG2 levels in saliva samples obtained from the children with a nursing bottle habit revealed a linear relationship with the total caries incidence in each child. This finding showed that an increase in the mucin MG2 level was associated with a rise in the incidence of dental caries in children.

Equations formulated from data are a simple way of predicting the probability of the caries incidence in children. In the present study, each increase of 0.02 in the mucin level was associated with an increase in the caries incidence by 1 point. Based on these equations and the information in Table 6, it is important to screen for caries in children younger than 5 years. The salivary mucin levels can be compared to the values obtained in this study to calculate the caries incidence in children.

Based on the findings of the present study, the deft score and mucin MG2 level in children with caries were highest in the presence of S*. mutans* serotype c, followed by serotype f and the serotype c and f combination. This finding is contradictory to that of a previous study, which stated that the serotype c and f combination was more common than serotype c in children with caries. The discord in the findings of the two studies might be because the previous study analyzed only caries and caries-free groups, whereas this study, in addition to caries and caries-free groups, analyzed the total of number of caries (def-t index) and mucin MG2 levels.

The dominant presence of *S. mutans* serotype c in the present study is in accordance with the finding of a previous study, which found that *S. mutans* serotype c was present in as many as 68%-70% of humans.[30] The same study found only a low incidence of serotype f.[30]

As shown by the polymorphic patterns, the caries incidence and mucin MG2 level were highest in the presence of a combination of*gtfB* and *gtfC* polymorphisms, followed by a combination of non-*gtfB* and *gtfC* polymorphisms, *gtfB* polymorphism, and *gtfC* polymorphism. The highest number of caries was found in the presence of both *gtfB* and *gtfC* polymorphisms of *S.mutans* serotype c. This finding is in accordance with that of previous studies and highlights the importance of these two *gtf* genes.[23-26]

A previous study reported that the activity of *S. mutans* was associated with three Gtf enzymes (GtfB, GtfC, and GtfD), which are the protein products of the *gtf* gene. The *gtfB* and *gtfC* genes can transform sucrose to insoluble glucan. Another study showed that GtfB, GtfC, and GtfD were involved in the production of glucan, which facilitated bacterial adherence to the tooth surface and biofilm formation.[23] Thus, *gtfB* and *gtfC* play important roles in the caries process.

The findings of the present study are in accordance with those of research conducted by McCabe and Smith, who reported that *gtf* bound to glucan during glucan synthesis under sucrose growth conditions. In addition, *gtfB* produced water-insoluble glucan through additional α(1,3) bonds, whereas *gtfC* produced water-soluble glucan (dextran) with α(1,6) bonds. As reported earlier, all the enzymes produced by *gtfB* and the *gtfC* genes are closely related to bacterial adherence to the tooth surface. Other studies

showed that this enzyme was the main target in the prevention of biofilm formation during the formation of caries.[23,26,28] According to one hypothesis, the dominant *gtf* gene in *S. mutans* most likely occurred through the remodeling of chromosomes, which involved different but partially homologous genes. Research confirmed that the sequence of the *gtfC* gene was similar to that of the *gtfB* gene and concluded that it was most likely the result of recombination and mutation of *gtfB*.[25-27] Thus, these two genes have similar roles.

Conclusions

In conclusion, as shown by the results of the present study, the *S. mutans* serotype c and f combination of *gtf* B and *gtfC* polymorphisms in bottle-fed children aged 3–5 years showed a significant association with the caries incidence as compared with other serotypes or *gtf* polymorphisms. The mucin MG2 level exhibited a significant association with the caries incidence, serotype, and *gtf* polymorphic pattern. In the presence of a constant *gtf* polymorphic pattern, serotype c exerted the greatest effect on the mucin level as compared with serotype f and the serotype c and f combination.

The prediction of the caries incidence using the deft score can be determined by the level of mucin MG2 in saliva. A combination of *S. mutans gtfB* and *gtfC* polymorphisms (i.e., GtfB enzyme encoded by the *gtfB* gene, with 700, 800, 1500, and 2000 bands and GtfC enzyme encoded by the *gtfC* gene, with 1500 and 2000 bands) can be used as an indicator of the presence of caries.

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