Glucosyltransferase B, Immunoglobulin A, and Caries Experience Among a Group of Egyptian Preschool Children

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ABSTRACT

Purpose: Glucosyltransferase B enzyme (GtfB) plays an essential role in the pathogenesis of dental caries. It catalyzes the synthesis of water-insoluble glucan from sucrose, which is essential for accumulation of Streptococcus mutans in the dental biofilm. On the other hand, salivary immunoglobulin A (sIgA) plays a protective role in the same process. Therefore, the purpose of this study was to attempt to correlate glucosyltransferase B enzyme and immunoglobulin A levels in whole saliva with caries experience in preschool children.

Methods: Forty-five 3- to 6-year-old were examined clinically, and their caries experiences were recorded as decayed, missing, and filled teeth (dmft) scores. Unstimulated whole saliva was collected and analyzed for GtfB and IgA using enzyme-linked immunosorbent assay.

Results: Mixed-model analysis revealed that both GtfB and IgA are covariants regarding the effect on dmft scores (P=.008). GtfB levels displayed a simultaneous increase in caries experience (dmft) and number of carious lesions (d), while IgA levels were high in caries-free children and significantly lower values were obtained in the high caries group.

Conclusion: Salivary IgA was negatively correlated with caries experience and inversely proportional with GtfB levels in the saliva of preschool children.

(J Dent Child 2012;79(2):63-8)

Received January 26, 2011; Last Revision April 10, 2011; Revision Accepted April 14, 2011.

KEYWORDS: GTFB, IGA, DENTAL CARIES, PRESCHOOL CHILDREN

Dental caries is an infectious microbial disease with a multifactorial origin that continues to be the most common chronic disease in humans, affecting nearly the entire population.[1] *Streptococcus mutans* has been implicated as a major etiological factor for the initiation and progression of dental caries.[2] The virulence of this organism is related to many factors, including its acidogenic and aciduric properties, as well as its ability to adhere and accumulate on dental pellicle.[3,4]

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The bacterial cell surface protein antigen (**PA**) and glucosyltransferase (**Gtf**) enzymes could mediate the binding of micro-organisms to tooth surfaces. PA was found to be related to the sucrose-independent adhesion ability of *S mutans* to the tooth surface[5] and Gtf enzymes are considered essential for its sucrose-dependent adhesion.[6,7]

Gtf enzymes are essential for the expression of virulence by *S mutans* in the pathogenesis of dental caries because of their ability to synthesize glucans from sucrose.[8] *S mutans* secretes 3 types of Gtf enzymes: GtfB, GtfC, and GtfD. These enzymes are the protein products of GtfB, GtfC, and GtfD genes, respectively.[9] GtfB and GtfD have molecular weights of approximately 148 and 143 kd, respectively, and both catalyze the synthesis of water-insoluble glucan. GtfC has a molecular weight of 138 kd and forms both water-soluble and -insoluble

glucans.[10] These enzymes (GtfB, C, and D) retain their enzymatic activity when adsorbed onto the surface hydroxyapitite with the highest increase in activity observed with GtfB.[11] GtfB appears to be responsible for the formation of *S mutans* microcolonies, thus enhancing its competitiveness in the biofilm on tooth surfaces.[12] Furthermore, GtfB plays an important role in the adherence and accumulation of mutans streptococci in the dental plaque of young children.[13]

Conversely, saliva contains a number of immune and nonimmune factors, which have a protective role on oral tissues. Immunoglobulin IgA, IgG, and IgM antibodies to *S mutans* are related to caries immunity, with higher level of antibodies against *S mutans* antigen in caries-free or caries-resistant individuals.[14,15]

Kopec et al.[16] investigated the relationship between GtfB, GtfC, and immunoglobulin G (**IgG**) and found that both enzymes were inhibited by IgG; however, the relationship between immunoglobulin A and glucosyltransferase B is inadequately addressed.

Therefore, the purpose of the current study was to measure salivary levels of a proven virulent factor glucosyltransferase B enzyme and a major immune factor immunoglobulin A and correlate them to caries experience in a group of preschool children.

METHODS

This cross-sectional study was approved by the research ethics committee, Faculty of Oral and Dental Medicine, Cairo University, Cairo, Egypt. The study subjects were recruited from among outpatients of the Department of Pediatric Dentistry. Forty-five 3- to 6-year-old boys and girls with different caries experience were selected based on the following criteria: They were in the primary dentition stage, had no systemic disease or reported infections in the past month, and had received no antibiotic or anti-inflammatory drug in the past month.

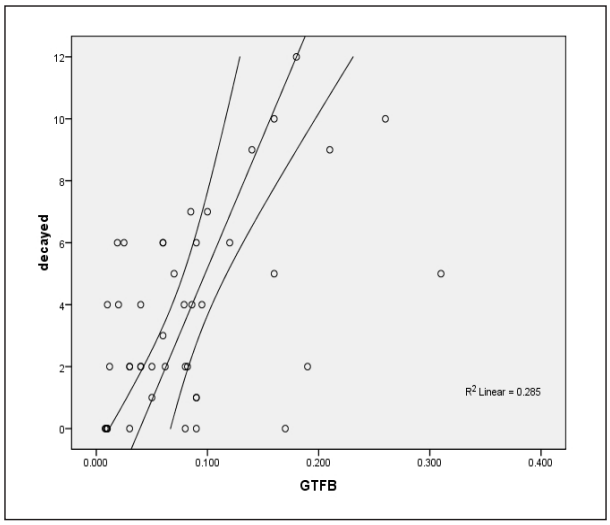

Figure 1. Glucosyltransferase B enzyme level vs decayed component.

CLINICAL EXAMINATION AND SALIVA COLLECTION

Informed consents were obtained from parents before the children's examinations and collection of their saliva samples. While the children were seated on dental chairs, clinical examinations were conducted with the aid of mouth mirrors, explorers, and dental operatory lights after the teeth had been dried with compressed air. Dental caries experience was expressed as decayed, missing, and filled teeth (**dmft**), and white spot lesions were included in caries scoring; however, they were not penetrated with an explorer. The examination was carried out by a single calibrated examiner, who was first calibrated on 5 patients who were not part of the study; duplicate examinations were conducted 1 week apart. The intra-examiner reliability for recording dental caries was 98%.

Unstimulated whole saliva (3 ml) was collected 2 hours after breakfast (from 9-11 a.m.) in sterile tubes. Children were asked to pool their saliva in the floor of the mouth and spit into a sterile tube intermittently. After collection of saliva samples, the tubes were capped tightly, ice chilled, delivered to the biochemistry lab, and assayed within 1 hour of collection. The study was conducted in a double-blind manner to ensure accuracy of measurement.

CHEMICAL ANALYSES

GTFB ASSAY

Specific GtfB present in the salivary samples was measured using an enzyme-linked immunosorbent assay (**ELISA**) by employing a special kit (Kirkegaard and Perry Laboratories, Gaithersburg, Md). Clarified saliva was mixed in a 1:1 ratio with the coating buffer supplied in the kit and was coated onto 96-well plates. After washing, antibodies to GtfB were applied. All buffers and reagents supplied in the kit were prepared according to the manufacturer's instructions, and the assays were performed according to the methods outlined in the manufacturer's information. The plate was read at 450 nm in an ELISA reader (Stat Fax 2100, Awareness Technology, Inc, Palm City, Fla., USA), and enzyme concentration was calculated from a standard curve in relation to saliva protein, which was determined via a protein assay kit (Sigma Chemical Co, St. Louis, Mo., USA) using bovine serum albumin as a standard.

*Table 1. Mean Values of Glucosyltransferase B Enzyme (GtfB) and Immunoglobulin A (IgA) Levels in Males and Females**

Sex	GtfB	IgA
Males (21)	0.09±0.07	0.72±0.35
Females (24)	0.08±0.07	0.83±0.42

** P>.05.*

ANTIBODY ANALYSIS

Salivary IgA (**sIgA**) antibodies were tested via ELISA. Antibody activity was measured by incubation with 1:8 dilutions of saliva. Plates of ELISA were incubated, followed in sequence by alkaline phosphatase coated to IgA (Bio Source, Invitrogen Corp, Carlsbad, Calif) and nitrophenylphosphate (Sigma Chemical Co). Avidin-alkaline phosphatase was used for color reaction to reveal levels of sIgA antibody to peptides. Reactivity was recorded as A_{405} in a microplate reader.

Statistical analysis was carried out using SPSS 13 software (IBM, Chicago, Ill). Descriptive statistics for data were collected in the form of means and standard deviations. A 2-tailed Pearson's correlation test was conducted to detect a possible correlation between the tested variables. A mixed model analysis was used to test the covariance effect of GtfB and IgA on caries experience. The level of significance was set at P=.05.

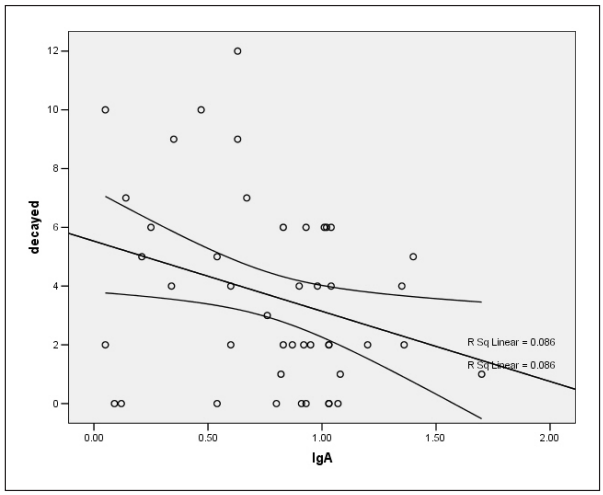

Figure 2. Immunoglobulin A level vs decayed component.

RESULTS

GtfB and IgA levels were determined in the whole saliva of 45 children and correlated to their caries experiences. The sample consisted of 21 males and 24 females. Caries active children (N=35) were ranked, according to their dmft scores, into 3 groups. Group 1 included 11 children with low caries experience and dmft scores of 1 to 3. Group 2 included 13 children with moderate caries experience and dmft scores of 4 to 6. Group 3 included 11 children who had dmft scores of at least 6 (high caries experience). The control group consisted of 10 caries-free children.

Table 1 summarizes the mean values of GtfB and IgA levels in males and females; no sex differences could be detected (P>.05). GtfB and IgA levels varied differently among the groups. In general the level of GtfB enzyme showed a significant increase with the increase of caries experience (P<.01). On the contrary, sIgA levels decreased with the increase in caries experience, except for Group 1 (low caries experience), where it was higher (1.09) than that of the caries-free control group (0.81; Table 2).

A mixed model analysis revealed that both GtfB and IgA are covariants regarding the effect on dmft (P=.008). GtfB levels increased with the increase of caries experience (dmft scores; r=0.54) and the number of decayed teeth (r=0.53), while IgA levels were inversely proportional with either dmft (r=-0.34) or the decayed component (r=-0.38; Table 3, Figures 1 and 2). Neither GtfB nor IgA values, as measured by ELISA, correlated with either the number of filled (f) or missing (m) teeth.

Higher levels of GtfB, however, were not always displayed with higher dmft scores, particularly if IgA levels were relatively low. Only one case, in which a 6-year-old had a high level of GtfB (0.31), a dmft score of 2, and an IgA level of 0.21, deviated from this rule (a statistical outlier). Other values were higher than the expected levels, but they were still statistically predictable values.

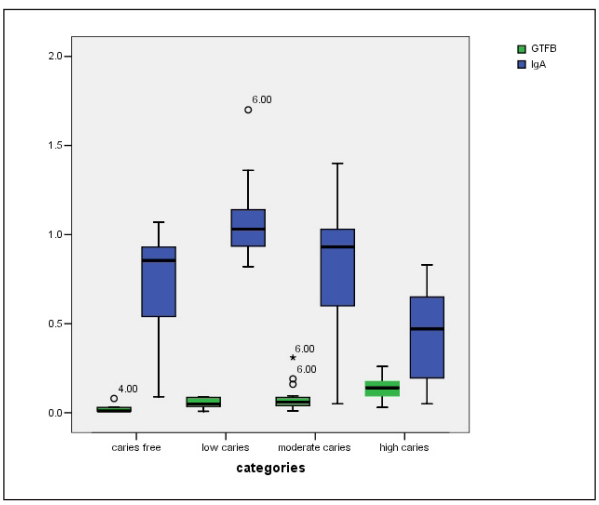

Figure 3. Glucosyltransferase B enzyme and immunoglobulin A levels in different caries categories.

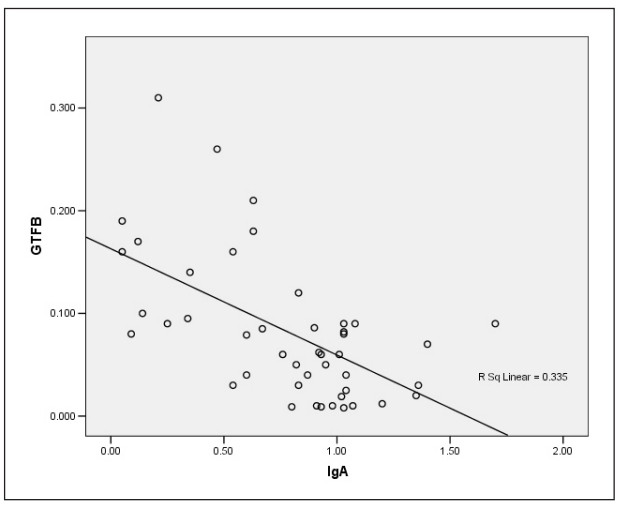

Figure 4. Correlation between immunoglobulin A and glucosyltransferase B enzyme levels in saliva.

Table 2. Glucosyltransferase B Enzyme (GtfB) and Immunoglobulin A (IgA) Levels Among Different Groups

Groups	GtfB	IgA
Caries-free	0.02±0.02*	0.81±0.38
Low caries	0.06±0.03*	1.09±0.25*
Moderate caries	0.08±0.07*	0.72±0.36
High caries	0.14±0.06*	0.45±0.29*

* P<.01.

Table 3. Correlation Between Glucosyltransferase B Enzyme (GtfB) and Immunoglobulin A (IgA) Levels and Decayed, Missing, and Filled Teeth (dmft) Components

Variable	d	m	f	dmft
GtfB	0.53*	0.14	0.14	0.54*
IgA	-0.34†	-0.22	-0.29	-0.38†

* Correlation is significant at the .01 level (2-tailed).

† Correlation is significant at the .05 level (2-tailed).

In this context, another 6-year-old with high IgA and relatively high GtfB levels (1.7 and 0.09, respectively) had a dmft score of 3 with only one decayed component. Figure 3 shows the box plot for the odds ratio values obtained for GtfB and IgA in different caries categories. The results also revealed an inverse correlation between the level of sIgA and GtfB (r=-0.58; *P*<.01). Therefore, IgA may possess an inhibitory effect on GtfB (Figure 4).

DISCUSSION

The enzyme GtfB, produced by *S mutans*, is one of the key factors in the process of dental caries.[10] The infectious nature of the disease assumes that some form of host immunity can regulate caries activity. Salivary defense factors, such as IgA, exhibit an inhibitory effect against oral pathogens, especially *S mutans.*[14] This study attempted to relate the salivary concentration of both GtfB and sIgA to caries experience in preschool children.

In Egypt, dental caries is a common problem among preschool children. Furthermore, studies in the literature assessing levels of salivary GtfB and IgA in preschool children are scarce in number. Therefore, 3- to 6-year-olds were chosen for this study. Unstimulated salivary samples were collected from these children since the concentration of salivary secretions is decreased with increased salivary flow.[17] Samples were collected 2 hours after breakfast to prevent any possible effect of circadian rhythm on the salivary concentrations.

GtfB has the highest activity among all Gtf enzymes. It is responsible for the majority of water-insoluble glucan synthesis, which is an important virulence factor in caries development, as it increases adherence and accumulation of *S mutans* in young children.[11,13] Our results support these previous reports, as the level of GtfB in the saliva of preschool children displayed a noticeable increase with higher caries experience. In addition, the correlation and logistic regression analysis revealed that GtfB levels in saliva correlated strongly with the number of carious lesions in children. Similar results were obtained by Vacca-Smith et al.,[18] who reported that GtfB activity was significantly higher in caries active than caries-free children.

Several studies assessed the sIgA levels in children and adults, and contradictory results were obtained. Some authors reported an increase in the level of sIgA with decreased caries activity,[19,20,21] which agrees with the current study's results. On the contrary, the results of other studies found that sIgA and caries activity increase simultaneously.[22] On the other hand, some studies revealed no association between caries experience and sIgA.[23,24]

The present study's results may shed light on a logical explanation for these conflicting findings. Although the sIgA level was significantly higher in caries-free (control group) children than high caries (caries active group) children, the reverse was observed in children with low caries experience, where the sIgA level in this group was significantly higher than that of the control group. As one of the immune factors, IgA may increase in response to mild insult (low caries activity) as a form of a protective mechanism of the body against caries attack. Therefore, it may be more valuable to relate IgA level to the decayed component (d) of caries index rather than total caries index (dmft) score. The present results revealed a significant correlation between the decayed component and sIgA; a similar observation has been reported by Sikorska et al.[25]

Previous studies have investigated the association between either GtfB or IgA and dental caries separately; the present study tried to find a possible relationship between both factors and dental caries; therefore, a mixed model analysis was performed. This analysis showed that both factors are covariants regarding their effect on dental caries. Furthermore, the current results imply that sIgA may possess an inhibitory effect on GtfB enzymes; when levels of GtfB increased, caries experience (dmft) increased and sIgA decreased. Thus, in high-risk children, there is a lack of protective mechanism and increased colonization of *S mutans* on tooth surfaces reflected by high levels of GtfB, which further increase the susceptibility to dental caries.

Although the dental caries experience (dmft) score was high with the increase of GtfB levels and the decrease in the IgA level, there was one case in which the highest GtfB score (0.31) was not reflected by the highest dmft score (dmft=2; a statistical outlier). This case, however,

had only decayed teeth with no missing or filled components. In the meantime, the IgA level in this case was relatively high, (0.2) suggesting a protective mechanism against caries.

The IgA level might play a role in inactivation of the GtfB enzyme, thus reducing the number of carious lesions. In addition, caries active individuals might display new carious lesions in the future, which could later correlate well with the high GtfB level; however, children in this cross sectional study were not followed. Moreover, other salivary macromolecules and local environmental factors that could influence the activity of GtfB were not assessed in the current study. Therefore, the mechanisms underlying the enhancement or inactivation of GtfB enzyme remain to be explored. Also, GtfB might be used as a predictor for future caries experience in children.

CONCLUSIONS

Based on this study's results, the following conclusions can be made:

1. The results support the protective effect of salivary immunoglobulin A in the caries process and highlights the importance of glucosyltransferase B in the pathogenesis of dental caries.
2. GtfB may have a diagnostic significance in vivo with respect to caries susceptibility and future caries experience in children.
3. Further longitudinal studies are needed to confirm the role of GtfB as a potential marker for caries activity.

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