Effect of restorative treatment on mutans streptococci and IgA antibodies

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Abstract

Purpose: Streptococcus mutans has been implicated as the major causative agent of dental caries. Although restorative treatment for caries is thought to temporarily eliminate the carious challenge, there are few reports of alterations in salivary mutans streptococci (MS) numbers and no reports of changes in salivary IgA antibody to S. mutans following restorative treatment.

Methods: This study investigated the effects of treatment in 12 caries-active children.

Results: Numbers of MS decreased slightly from pre- to postrestoration levels in six subjects and increased in five subjects. However, there were no significant differences in pre- to postrestoration numbers of total oral streptococci, MS, the percentage of MS/total oral streptococci, salivary IgA antibody levels to S. mutans, or correlations between bacterial counts and IgA antibody levels.

Conclusions: These results indicate that successful restorative treatment does not alter mutans streptococcal numbers and suggest the need for more effective methods for reducing the cariogenic challenge. (Pediatr Dent 20:273–77, 1998)

Streptococcus mutans and other mutans streptococci have been implicated as the major etiological agents of human dental caries and have a number of important characteristics which promote their cariogenic potential. These characteristics include the ability to colonize smooth surfaces of teeth, aggregate with S. mutans and other organisms, cleave sucrose into a 1-3-linked insoluble and a 1-6-linked soluble glucans, and produce lactic acid, which demineralizes the enamel to form carious lesions. Restorative treatment for caries remains the optimal treatment and has been thought to remove cariogenic organisms from the area of the restored lesion. However, few reports have established numbers of oral bacteria before and after restorative treatment. Keene et al. reported that the prevalence of S. mutans in dental plaque was reduced following complete restorative treatment. About 1 year after treatment, several of the subjects developed additional caries at other sites that had high numbers of plaque S. mutans. They concluded that restoration was effective, but incomplete for eliminating S. mutans from plaque surfaces. Tinanoff et al. found higher numbers of salivary S. mutans in subjects after receiving restorative treatment. Wright et al. reported significant decreases in salivary numbers of MS and lactobacilli immediately following restoration, however MS levels returned to prerestoration numbers in many of their subjects. Petti et al. also reported significant decreases in salivary numbers of MS in children with restored surfaces compared to those with unrestored surfaces. As teeth are bathed in saliva and saliva may serve as a source of cariogenic organisms to susceptible sites, it is important to establish changes in numbers of salivary MS following clinically successful restorative treatment.

Secretory IgA (sIgA) bathes the surfaces of all mucosal sites and is the predominant immunoglobulin in the mouth and the bodily pool of immunoglobulins. Although other nonspecific defense factors exist at these sites, sIgA is the principal immunoglobulin in saliva, breast milk, colostrum, tears, and secretions bathing the mucosal surfaces of the gastrointestinal, respiratory, and genitourinary tracts. Peyer’s patches are specialized immune tissues located throughout the small intestine. Because MS are natural inhabitants of the oral cavity of virtually everyone and are ultimately swallowed, Peyer’s patches regularly sample and process mutans streptococcal antigens in the small intestine. Processed antigenic determinants are presented to B and T lymphocytes, which leave the Peyer’s patch and travel through the circulation and lymphatics and migrate to the mucosal sites of the body, including the lining of the gastrointestinal, respiratory, and genitourinary tracts and the salivary, mammary, and lacrimal glands. There the B lymphocytes differentiate into plasma cells and synthesize and secrete specific sIgA antibody to the foreign antigen originally encountered in the small intestine (i.e., MS cells) into the mucosal fluids. Therefore, naturally occurring sIgA antibodies to many normal flora microorganisms, as well as pathogens, are found in all secretions. The modes of action of sIgA antibodies in protection against pathogenesis include inhibition of attachment, colonization, and
penetration of antigen into mucosa; agglutination of microorganisms; neutralization of microbial enzymes, toxins and viruses; opsonization; and activation of the alternate complement pathway. 10

Naturally occurring slgA antibodies to MS are present in most individuals and first appear early in childhood as a result of swallowed mutans streptococcal antigens being processed by the common mucosal immune system. The present study examined the numbers of MS, total oral streptococci, and IgA anti-S. mutans levels in saliva of caries-active children prior to and after placement of restorations.

Methods

Clinical evaluation of subjects

Volunteers (ages 4–10 years old) with unremarkable medical histories were recruited from patients at Indiana University School of Dentistry and Riley Hospital for Children Dental Clinic, Indiana University, Indianapolis. These studies were carried out with informed consent and were approved by the Institutional Review Board of Indiana University–Purdue University at Indianapolis. Children were screened for the number of decayed, missing (extracted), and filled teeth (deft/DMFS) and were selected for inclusion if subjects had more than five unrestored carious teeth. The number of decayed, missing (extracted or exfoliated), or filled surfaces (deft/DMFS) was also recorded. Approximately 1 mL of unstimulated whole saliva was collected immediately prior to restorative treatment and another sample 1–4 weeks after restorative treatment was completed. Each saliva sample was divided into two aliquots: one was used immediately for enumeration of MS and the other was frozen at −20°C for later antibody analysis by enzyme-linked immunosorbent assay (ELISA). Restorative treatment consisted primarily of amalgam (Dispersalloy®, Dentsply International Inc., Milford, DE), composite resin (Prisma TPH®, Dentsply), or stainless-steel crown (SSC; 3M/Unitek Stainless Steel Crowns® or 3M Stainless Steel Crowns [Jon Ni-Chro]®, 3M Dental Products Division, St. Paul, MN) restorations. One patient received three restored surfaces with glass-ionomer (GI; KETAC-FIL®, Espe-Premier Sales Corp., Norristown, PA) restorative material and for the period of this study, one patient received two restored surfaces with an intermediate restorative material (IRM; Dentsply/Caulk). All restorative procedures were performed in accordance with clinically acceptable guidelines. 11 All crowns were cemented with GI cement (KETAC-CEM®, Espe-Premier). Unrestorable carious teeth were extracted.

Enumeration of total oral streptococci and MS

Streptococci were detected in fresh whole saliva samples from all volunteers by colonial morphology as described earlier. 12 Briefly, unstimulated whole saliva samples were diluted (1:10 and 1:100) in sterile saline, vortexed for 30 s and spiral plated (Spiral Systems, Inc., Cincinnati, OH) in duplicate on Mitis Salivarius agar plates (Difco Laboratories, Detroit, MI) for total oral streptococci, and Mitis Salivarius plates supplemented with bacitracin (0.2 units/mL) and 15% sucrose for MS. Streptococcal colonies were counted after incubation for 3 days at 37°C in an atmosphere of 5% CO₂ in air.

Determination of IgA anti-S. mutans antibody activity.

Saliva samples were assayed for IgA antibody activity to a clinical isolate of S. mutans (A32-2) using a previously described ELISA. 12, 13 This isolate was previously obtained from saliva of a caries-active child and the fimbrial surface appendages of A32-2 has been studied extensively in this laboratory. 14 Polystyrene microtiter plates (EIA, Linbro, Flow Laboratories, Inc., McLean, VA) were coated (100 μL/well) with A32-2 whole cells (diluted to an absorbance of 0.500 at 660 nm in carbonate/bicarbonate buffer, pH 9.6) and incubated at 37°C for 3 h. Coated plates were washed three times in Tween saline (0.9% NaCl containing 0.05% Tween 20) to remove unbound antigen. Free sites on the plates were blocked by reaction with 200 μL of a solution containing 10 μg/mL of globulin-free human serum albumin (Sigma) for 1 h at 25°C. Saliva (diluted 1:4 in Tween saline) samples, in triplicate, were added to the wells (100 μL/well) and incubated for 2 h at 37°C. The plates were washed three times with Tween saline and incubated for 3 h at 37°C with 100 μL of horseradish peroxidase-labeled anti-human IgA heavy chain-specific reagent (Sigma; 1:1000).

After washing three times with Tween saline, orthophenylenediamine dihydrochloride in citrate buffer containing H₂O₂ was added (100 μL/well). Color development was monitored between 10 and 30 min, and the reaction was stopped using 2 N H₂SO₄. The amount of color that developed was measured at 490 nm in the microtiter plate with a Titertek Multispec spectrophotometer (Flow). The data were reduced by computing the means and standard errors of the mean of the absorbances of triplicate determinations per sample.

Statistical analysis

The bacterial and antibody results were analyzed by a paired Student’s t test, and significant differences were defined as P < 0.05. Correlations of salivary bacterial numbers with IgA antibody levels were determined by Pearson’s product-moment correlation coefficient.

Results

Subject demographics

The mean age of the subjects was 7.0 ± 1.9 years old (ranging from 4 to 10 years old). The subjects were seven males and five females—four were Afro-American and eight were Caucasian. Each
Numerous studies have examined the numbers of plaque S. mutans, however, few studies have addressed this important aspect. Keene et al. examined the numbers of plaque S. mutans in carious sites of five US Navy personnel and found that 96% of the sites contained S. mutans. After restorative treatment, the prevalence was significantly lower, but S. mutans remained present. Approximately 12 months after treatment, four of the five subjects developed new lesions at sites containing high levels of S. mutans. They concluded that standard restorative treatment was not effective for eliminating S. mutans from tooth surfaces or for arresting caries activity. Tinanoff et al. reported that subjects receiving restorations had higher levels of total salivary bacteria, Streptococcus sanguis, and S. mutans after treatment than before. Wright et al. examined 52 postpartum females with high pre-existing levels of salivary MS. Following restorative treatment of a mean of 16.4 surfaces, a significant reduction in MS and lactobacilli was observed. However, the numbers of MS in many of the subjects returned to high levels within an average of 3 months.

Similar conclusions were reached by Petti et al. in that of 809 6- to 7-year-old children, those with sound numbers of oral streptococci

Whole saliva samples were collected prior to restorative treatment and 1–4 weeks after restoration. Numbers of MS decreased slightly from pre- to postrestoration samples in six subjects and increased in five subjects (Table 1). One subject (#12) had no detectable MS following restorative treatment. However, there were no significant overall differences in numbers of total oral streptococci (1.70 x 10^7 and 1.56 x 10^7; P = 0.68), MS (9.81 x 10^5 and 5.69 x 10^5; P = 0.42), or the percentage of MS to total oral streptococci (5.97 and 8.82%; P = 0.65) from the pre- to postrestoration saliva samples.

**Table 1. Numbers of Mutans Streptococci and Total Oral Streptococci/mL of Pre- and Postrestoration Saliva Samples**

<table>
<thead>
<tr>
<th>Patient #</th>
<th>Pre-rest.</th>
<th>Post-rest.</th>
<th>Pre-rest.</th>
<th>Post-rest.</th>
<th>% MS/Oral Streptococci</th>
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<tr>
<td>1</td>
<td>6.86 x 10^6</td>
<td>2.48 x 10^7</td>
<td>2.64 x 10^5</td>
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<td>2</td>
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<td>6.68 x 10^6</td>
<td>3.22 x 10^5</td>
<td>NA</td>
<td>2.21 x 10^5</td>
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<tr>
<td>3</td>
<td>1.93 x 10^7</td>
<td>2.69 x 10^7</td>
<td>6.28 x 10^5</td>
<td>9.84 x 10^5</td>
<td>32.54 x 10^5</td>
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<tr>
<td>4</td>
<td>2.25 x 10^7</td>
<td>1.31 x 10^7</td>
<td>3.25 x 10^5</td>
<td>1.63 x 10^5</td>
<td>1.44 x 10^5</td>
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<tr>
<td>5</td>
<td>1.79 x 10^7</td>
<td>1.77 x 10^7</td>
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<td>1.01 x 10^5</td>
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<tr>
<td>6</td>
<td>7.99 x 10^6</td>
<td>4.64 x 10^6</td>
<td>4.59 x 10^5</td>
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<tr>
<td>7</td>
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<td>2.44 x 10^7</td>
<td>1.41 x 10^5</td>
<td>3.33 x 10^5</td>
<td>1.27 x 10^5</td>
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<td>8</td>
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<td>3.47 x 10^7</td>
<td>5.30 x 10^5</td>
<td>1.01 x 10^5</td>
<td>0.00 x 10^5</td>
</tr>
<tr>
<td>Mean ±</td>
<td>1.70 x 10^7</td>
<td>1.56 x 10^7</td>
<td>9.81 x 10^5</td>
<td>5.69 x 10^5</td>
<td>5.97 x 10^5</td>
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<td>SEM</td>
<td>8.35 x 10^6</td>
<td>1.02 x 10^6</td>
<td>1.66 x 10^6</td>
<td>6.14 x 10^5</td>
<td>8.75 x 10^5</td>
</tr>
</tbody>
</table>

*Number of mutants streptococci (MS)/total oral streptococci x 100. Restoration. Not available.

1 Standard error of the mean.

Patient had at least five decayed teeth and the group had a mean combined deft DMFT score of 9.50 ± 3.80 ranging from 5 to 15. The group's mean combined defs/DMFS score was 18.17 ± 10.44, range 6 to 33. Treatment of all carious teeth resulted in 64 restored primary teeth (137 restored surfaces), 12 restored permanent teeth (19 restored surfaces), 20 extracted primary teeth, and two primary teeth with smooth surface-incipient lesions confined to enamel were treated with topical fluoride alone.

**Discussion**

The ability of MS to cause carious lesions is well documented. However, effective treatment to remove MS from either lesions or the entire oral cavity is not extremely advanced. Restorative treatment is used to repair damage to tooth surfaces and has been thought to reduce the cariogenic load in the mouth, however, few studies have addressed this important aspect. Keene et al. examined the numbers of plaque S. mutans in carious sites of five US Navy personnel and found that 96% of the sites contained S. mutans. After restorative treatment, the prevalence was significantly lower, but S. mutans remained present. Approximately 12 months after treatment, four of the five subjects developed new lesions at sites containing high levels of S. mutans. They concluded that standard restorative treatment was not effective for eliminating S. mutans from tooth surfaces or for arresting caries activity.
numbers of MS, but that was not the focus of this study. Alternatively, as numbers of MS vary over time, it is possible that additional samples may reflect altered bacterial numbers following restorative treatment. However, there was no correlation between either MS or total oral streptococci and IgA antibody to *S. mutans*, which should have been observed if there were varying numbers of oral bacteria.

Camling and Kohler reported that slgA antibody to *S. mutans* appears between the ages of 1 and 5 years. Smith et al., reported in a longitudinal study that the IgA antibody level to *S. mutans* continues to increase from 5 months to 3 years of age and Gahnberg et al. confirmed these findings in the age group from 2 to 48 months of age. Changes in numbers of salivary *S. mutans* should alter the immunological load affecting induction of the mucosal immune response. Because immunization with *S. mutans* cells increases specific slgA antibody, it may be inferred that increases of indigenous salivary MS should result in higher levels of slgA anti-*S. mutans* antibodies, whereas lower numbers of MS should contribute to stable or lower levels of specific antibodies. However, our data did not support this hypothesis as there was no significant correlation between slgA anti-*S. mutans* and the numbers of MS or total oral streptococci. It is possible that changes in total oral streptococci may mask changes in antibody to *S. mutans* due to cross-reactive antigens, however, there were no correlations between numbers of total oral streptococci and levels of IgA antibody to *S. mutans*.

Caries-free adults and children have been reported to have significantly higher levels of naturally occurring salivary IgA and serum antibodies to *S. mutans* and lower numbers of salivary *S. mutans* than caries-active subjects. Specific salivary antibodies to *S. mutans* inhibit adherence and acid production and other enzyme activities of *S. mutans*. There is speculation that although there are other factors such as susceptible enamel surfaces, oral hygiene, diet, and oral microflora, caries-active subjects are not capable of inducing protective salivary antibodies, while caries-free subjects produce effective antibody responses. While both caries-free and caries-active subjects usually have at least some minimum level of slgA antibody to *S. mutans*, differences exist in the antigens recognized. Studies in this laboratory suggest that caries-active subjects do not produce antibody to the *S. mutans*-protective antigens and that this may contribute to the caries-active state of these patients.

The conventional restorative treatment used in this study is important to return the tooth structure to proper form and function. But the lack of reduction in mutans streptococcal numbers suggest much additional work remains to be done to accomplish truly long-term preventative antimicrobial therapy. The use of fluorides, chlorhexidine and other antimicrobials, appropriate nutrition, diet modification, vaccines, and dental materials that have antimicrobial properties are methods that need to be addressed in identifying more permanent and effective therapies to manage this infectious disease.

**Conclusions**

Based upon the results of this study, we conclude:

1. There were no significant differences in pre- to postrestoration numbers of total oral streptococci, mutans streptococci, or the percentage of mutans streptococci/total oral streptococci.

2. There was no significant difference in slgA antibody levels to *S. mutans* between pre- and postrestoration samples by ELISA.

3. There were no significant correlations between bacterial counts and IgA antibody levels.

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References