Two-year longitudinal observations of salivary status and dental caries in children with insulin-dependent diabetes mellitus
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Birgitta Ståhl Stefan Aronson MD, PhD

Abstract
Salivary status and caries incidence were studied in 28 young, Type 1 diabetics from the onset of the disease and during a two-year period. Flow rate, buffer capacity, glucose content, total protein concentration and levels of mutans streptococci and lactobacilli were determined in stimulated whole saliva every third month. Dental caries was recorded at onset and then once a year. Forty-six per cent of the children developed caries during the observation period. Caries incidence was significantly higher (P < 0.05) during the first year of diabetes, compared with the second. Caries-active children displayed significantly higher Hb A1c levels (P < 0.001), compared with caries-inactive diabetics. The number of salivary lactobacilli dropped significantly (P < 0.05) during the first six months of the disease, while mutans streptococci levels remained unchanged during the study period. Salivary glucose concentration showed a considerable individual variation, but tended to be lower during the second year. The results suggest a possible relationship between Type I diabetes treatment and caries. (Pediatr Dent 14:184-88, 1992)

Introduction
It is well established that insulin-dependent diabetes mellitus (IDDM) is associated with an increased prevalence and severity of periodontal disease. However, the impact of diabetes on dental caries seems to be limited, though lower saliva secretion and dry mouth have been reported. The low caries frequency in diabetics has been explained mainly by the sucrose-free diet that is a part of the lifelong treatment.

In recent years, many investigators have related oral and salivary findings to the metabolic state of the disease. Thus, Harrison and Bowen found higher gingival index and increased plaque index together with decreased saliva flow rate in poorly controlled diabetic children. Salivary glucose concentration has been shown to be elevated in diabetics with poor metabolic control when they are compared with diabetics with good metabolic control.

Few studies have focused on the cariogenic microorganisms in the saliva of IDDM patients. Tenovuo and coworkers found the same amount of salivary mutans streptococci and lactobacilli in well-controlled diabetic adults as in healthy controls. Others have suggested a decreased number of salivary mutans streptococci, but not lactobacilli, in diabetic adults during a period of improved metabolic control. In a group of diabetic children, we previously found lower levels of salivary lactobacilli when compared with healthy controls; this presumably was due to their dietary regimen.

The current concept in diabetic care with blood glucose monitoring and frequent injections of short-acting insulin allows a less restricted diet. This may influence oral health rapidly, and therefore requires attention.

This study presents longitudinal data of salivary status and dental caries in a group of children with IDDM from onset and during a two-year follow-up period.

Materials and Methods
Subjects
The group studied comprised 28 children (15 boys, 13 girls) with a mean age of 11.0 years (range 3.5 – 17.0 years). They were all diagnosed and treated as Type 1 diabetics at the Department of Pediatrics, County Hospital, Halmstad, Sweden, during 1987-88. All patients volunteered for the study after receiving information and consent from their parents. Following initial diagnosis, the disease was brought under control with short- and medium-acting insulin (Actrapid Human and Protaphan Human, Novo, Bagsvaerd, Denmark) during a two-week hospitalization period. Thereafter, the children were seen at the pediatric clinic at least every third month, depending on their state of metabolic control.

Data Collection
The children underwent a thorough oral examination within three days after the initial diagnosis. Dental caries was registered according to Koch and then checked once yearly. Initial approximal lesions on molars and premolars were assessed from bite-wing radiographs. An initial lesion was defined as a radiolucent area within the enamel which could not be related to normal anatomy or hypoplasia according to grades 01 and 02 in the index of Gröndahl et al. Paraffin-stimulated whole saliva was collected and recollected every
third month. Data concerning the medical treatment were obtained from the hospital records.

**Salivary Sampling and Analysis**

Paraffin-stimulated whole saliva was collected in the mornings, for 5 min at least 1 hr after food intake. Flow rate was calculated as ml/min, and the buffer capacity was estimated using the Dentobuff test according to the manufacturer’s instructions (Orion Diagnostica, Helsinki, Finland). For quantitation of salivary *mutans* streptococci the Strip mutans (Dentocult -SM, Orion) technique as described by Jensen and Bratthall was used. During the first study year, a *Strep. mutans* prototype was used. After 1 min of paraffin-chewing, a plastic strip was rotated approximately 10 times on the tongue and then withdrawn through softly closed lips to remove excess saliva. The strips were transferred to a selective broth in glass tubes and incubated at 37°C for 48 hr. After drying, the number of colony-forming units (CFU) was counted using a stereo microscope and grouped into four scores: 15 0 = 0–10 CFU; 1 = 11–99 CFU; 2 = 100–499 CFU; 3 = 500 CFU.

The frequency of salivary lactobacilli was determined with a dip slide culture method (Dentocult -LB, Orion) described by Larmas. The slides were incubated aerobically at 37°C for four days. According to colony density, the slides were classified into five categories corresponding to 0, 10³, 10⁴, 10⁵, and 10⁶ CFU in 1 ml of saliva.

Aliquots of saliva were centrifuged at 8,700 × g for 15 min and stored frozen at −18°C for four months or less; further assays were performed later. Salivary glucose concentration was determined enzymatically with hexokinase and glucose-6-phosphate dehydrogenase (Sigma, St. Louis, MO). The total protein content was analyzed according to Lowry et al.

**Blood Sampling and Analysis**

Blood samplings were performed routinely at all visits to the clinic. HbA₁c was monitored by a liquid-chromatographic assay (Mono S, Pharmacia, Uppsala, Sweden) described by Jeppsson et al.

**Statistical Methods**

Analysis of variance (ANOVA) was used to test differences for caries data. The analysis of changes in bacterial levels was performed with the Wilcoxon two-tailed nonparametric sign test. Student’s t-test was used to compare means.

**Results**

Mean values of glycosylated hemoglobin and insulin dose at onset of diabetes and during the next two years are given in Fig 1. The remission of the disease was displayed at the three-month registration. The HbA₁c values were continuously low throughout the study period, while an increased need for insulin was apparent.

In Tables 1 and 2 (next page), the caries experience and caries incidence of the children are shown. The caries prevalence was 72% at onset. In all, 13 of the children (46%) developed caries lesions during the course of the study. Nine children developed cavities during the first study year, compared with two during the second, disclosing a statistically significant difference (P < 0.05) in caries incidence. The increment in primary teeth and the development of initial proximal lesions in permanent teeth followed the same pattern, though these were not statistically significant.

Salivary data are summarized in Figs 2 and 3 (page 187). The flow rate and buffer capacity both increased slightly during the follow-up period. The mean salivary glucose concentration was higher during the first year than during the second year, although with considerable individual variation. However, the total salivary protein concentration was stable at 0.6–0.8 g/l (SD ± 0.35) at all sampling occasions.

The percentage distribution of salivary *mutans* streptococci scores at onset of diabetes was 0.36%; 1.21%; 2.19% and 3.24%. These levels remained virtually unchanged during the two years of observation. The percentage distribution of lactobacilli at the different sampling occasions is presented in Fig 4 (page 187). A statistically significant decrease in salivary lactobacilli levels (P < 0.05) was found after three and six months, compared with the levels recorded at the onset of the disease. Thereafter, a slow recolonization by the microorganism was seen.

Some salivary data in relation to caries activity are presented in Tables 3 and 4 (page 186). Children who...
Table 1. Caries experience in 26 IDDM children with permanent teeth. The data on primary teeth are based on all children younger than 8 years old (n = 8) at the onset of diabetes.

<table>
<thead>
<tr>
<th>Index</th>
<th>Onset</th>
<th>Year 1</th>
<th>Year 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>x</td>
<td>SD</td>
<td>x</td>
</tr>
<tr>
<td>DMFS</td>
<td>3.1</td>
<td>3.8</td>
<td>4.0</td>
</tr>
<tr>
<td>Initial</td>
<td>0.7</td>
<td>1.4</td>
<td>1.0</td>
</tr>
<tr>
<td>dmfs</td>
<td>3.5</td>
<td>6.1</td>
<td>4.0</td>
</tr>
</tbody>
</table>

* Initials are approximal caries within the enamel of premolars and molars assessed from bite-wing radiographs. See Materials and Methods for definition.

Table 2. Caries incidence in 28 IDDM children during two years after onset of the disease. Number of new lesions with range in parenthesis.

<table>
<thead>
<tr>
<th>Index</th>
<th>First Year</th>
<th>Second Year</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>x</td>
<td>SD</td>
<td>x</td>
</tr>
<tr>
<td>DMFS</td>
<td>18 (1-6)</td>
<td>2* (1)</td>
<td>20 (1-6)</td>
</tr>
<tr>
<td></td>
<td>N = 9</td>
<td>N = 2</td>
<td>N = 11</td>
</tr>
<tr>
<td>Initial</td>
<td>10 (1-3)</td>
<td>6 (1-3)</td>
<td>16 (1-3)</td>
</tr>
<tr>
<td></td>
<td>N = 5</td>
<td>N = 3</td>
<td>N = 7</td>
</tr>
<tr>
<td>dmfs</td>
<td>4(4)</td>
<td>0</td>
<td>4 (4)</td>
</tr>
<tr>
<td></td>
<td>N = 1</td>
<td>--</td>
<td>N = 1</td>
</tr>
</tbody>
</table>

* Statistically different (P < 0.05) from first year.

Table 3. Salivary composition and percentage of glycosylated hemoglobin (mean ± SD) in relation to caries activity in IDDM children

<table>
<thead>
<tr>
<th>Caries Inactive</th>
<th>Caries Active</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mmol/l)</td>
<td>0.66</td>
<td>0.58&lt;br&gt; 0.95&lt;br&gt; 1.30&lt;br&gt; NS*</td>
</tr>
<tr>
<td>Protein (g/l)</td>
<td>0.65</td>
<td>0.26&lt;br&gt; 0.78&lt;br&gt; 0.33&lt;br&gt; NS</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>6.5</td>
<td>8.0&lt;br&gt; 7.8&lt;br&gt; 0.9&lt;br&gt; &lt; 0.001</td>
</tr>
</tbody>
</table>

* Student’s t-test; NS = Not significant.

Table 4. Caries promoting factors in saliva in relation to caries activity in IDDM children. Values indicate the number of children with percentage in parenthesis.

<table>
<thead>
<tr>
<th>Caries Inactive</th>
<th>Caries Active</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flowrate* (&lt; 1.0 ml/min)</td>
<td>5 (33%)&lt;br&gt; 4 (31%)</td>
</tr>
<tr>
<td>Buffer capacity (&lt; pH 5.0)</td>
<td>4 (27%)&lt;br&gt; 3 (23%)</td>
</tr>
<tr>
<td>Mutans streptococci (score 3)</td>
<td>4 (27%)&lt;br&gt; 11 (85%)</td>
</tr>
<tr>
<td>Lactobacilli (&gt; 10^8 CFU)</td>
<td>2 (13%)&lt;br&gt; 8 (62%)</td>
</tr>
</tbody>
</table>

* Stimulated whole saliva.
fluorides and antimicrobial agents could be given on an individual basis. However, further long-term investigations are needed to clarify this area.

In this study, decreased levels of salivary lactobacilli were found during the remission of the disease, probably due to the sugar-restricted diet. Low levels of salivary lactobacilli in young, type 1 diabetics have been reported previously. It can be speculated that the gradual return to onset levels reflects a somewhat normalized diet during the course of the disease. The number of *mutans* streptococci in saliva remained virtually unchanged throughout the observation period regardless to the state of metabolic control. This finding conflicts with that of an earlier report, but agrees with those of others. However, the fact that we did not find increased salivary levels of these bacteria was notable; otherwise, an increased prevalence with age could be expected.

The slightly increased mean values of salivary flow rate and buffer capacity found with time were not surprising because of the patients’ growth. All values recorded were within normal limits and subjective complaints of dry mouth stated by three children could not be verified sialometrically.

The finding that the salivary glucose concentration was higher during the first months of the disease compared with the second year, thus contrasting the levels of glycosylated hemoglobin in the blood, was notable. Increased salivary glucose output in diabetics due to elevated blood glucose levels has been reported previously. On the other hand, a nonexistent or individual relationship between serum and saliva glucose concentrations has been implied, suggesting a threshold mechanism in the salivary glands. The lower glucose concentrations during the course of the disease found here do not imply an alteration of basement membrane permeability of the glands. The individual glucose concentrations fluctuated widely and randomly. This might be explained by an individual gland leakage or by the patient’s inability to balance the short-acting insulin with eating and physical exercise, especially during the first period of treatment. Therefore, the lower values recorded during the second year probably resulted from improved management of the disease. Though brief, such elevated levels of glucose in the oral cavity may enhance the cariogenic challenge and contribute to the development of the new carious lesions and progression of existing lesions as observed during the first study year. However, while the salivary glucose concentrations seemed to be of a snapshot character, the percentage of glycosylated hemoglobin offered a long-term measure of the degree of metabolic control. Therefore, it was reasonable that the HbA1c values, but not...
glucose, were correlated to caries activity. It must, however, be emphasized that all children were judged medically to be satisfactorily controlled (HbA1c < 10%) during the follow-up period; the HbA1c levels could not be used to predict caries development on an individual basis. We intend to follow these selected children up to maturity to extend our knowledge on the relationship between type 1 diabetes mellitus and dental caries.

Dr. Twetman is associate professor in the Department of Pedodontics, Karolinska Institute, Stockholm, Sweden. Dr. Nederfors has a research fellowship, Ms. Stahl is a dental assistant, and Dr. Aronson is chairmain of the Department of Pediatrics. All are at the Medical and Dental Health Center, Länsjukhuset, Halmstad, Sweden. Reprint requests should be sent to: Dr. Svante Twetman, Department of Pedodontics, Länsjukhuset, S-301 85 Halmstad, Sweden.