Self-perceived oral health and salivary proteins in children with type 1 diabetes

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SUMMARY The aim was to validate self-perceived oral health with salivary IgG as an inflammatory parameter in children with type 1 diabetes. Unstimulated whole saliva samples were collected from 36 children with well controlled and 12 with poorly controlled type 1 diabetes and 40 non-diabetic children (Controls). Salivary flow rate, random blood glucose level, salivary protein concentration and immunoglobulin A and G levels were recorded using standard techniques. Data concerning oral health and diabetes status were collected. Self-perceived gingival bleeding (bleeding gums), bad breath and dry mouth were higher in diabetic children when compared with those in controls ($P < 0.05$). Gingival bleeding was frequently perceived by children with poorly controlled compared to well-controlled type 1 diabetes ($P < 0.05$) and controls ($P < 0.001$). Bad breath was common perceived by children with poorly controlled compared to well-controlled type 1 diabetes ($P < 0.05$) and controls ($P < 0.0001$). Salivary flow rate was lower in the diabetic children compared to controls ($P < 0.01$) with no difference between children with poorly controlled and well-controlled type 1 diabetes. Salivary IgG per mg protein concentration was higher in the diabetics when compared with the control group ($P < 0.0001$). IgG per mg protein levels were also higher in children with poorly controlled when compared with well-controlled type 1 diabetes ($P < 0.05$). There was no difference in IgA per mg protein and total protein concentrations between children with poorly controlled and well-controlled type 1 diabetes. Self-perceived gingival bleeding and salivary IgG per mg protein concentration were increased in children with type 1 diabetes compared with controls. These variables were also increased in children with poorly controlled compared with well-controlled type 1 diabetes.

KEYWORDS: self-perceived, gingival bleeding, saliva, IgG, type 1 diabetes

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Introduction

Self-perceived health may be defined as an individual’s evaluation of his or her general health status (1). However, the manner in which the questions are administered and education of subjects may influence the validity of the questionnaire. Therefore, the reliability of data may remain questionable (2). Clinical examinations are commonly performed to verify self-perceived oral health (2, 3).

A recent clinical study showed that gingival bleeding (GB) is significantly increased in children with poorly controlled type 1 diabetes (T1D) compared with non-diabetic controls (4). In addition to the conventional clinical parameters, laboratory assays have also been used to validate self-reported health (5). Laboratory-based techniques have shown an association between self-perceived health and serum immunological markers that reflect disease (6). It
has been shown that poor self-perceived health, presenting with features such as pain and discomfort, is mainly triggered by circulating inflammatory markers such as cytokines (6). Saliva may also be considered as essential for diagnostic laboratory analysis (7). Certain immunological parameters in saliva may be related with perceived oral health. Salivary immunoglobulins, especially immunoglobulin (Ig) A, play a significant role in protecting oral mucosal and teeth surfaces from microbes (8). Several studies have been performed to determine the Ig levels in saliva (8–10). Concentration of IgG in saliva is low; however, in periodontal inflammation, high levels of IgG have been reported (10). As GB is increased in subjects with T1D and is reflected by raised levels of salivary IgG, therefore, such parameters might also be helpful in verifying self-perceived oral health.

The aim of the present study was to validate self-perceived oral health with salivary inflammatory parameter, IgG in children with T1D.

Materials and methods

Information and consent

An information sheet printed in simple English and Urdu (native language of Pakistan) was provided. It described the purpose of the research and clearly stated that participation is absolutely voluntary. Parents who allowed their children to participate in the study were requested to sign a consent form.

Inclusion and exclusion criteria

Type 1 diabetic and non-diabetic children aged 10–19 years with similar socio-economic statuses were included. The children whose parents did not disclose the medical and family history of diabetes of the child, served as ‘Controls’.

The exclusion criteria were based on smoking habit, infection with Hepatitis B or Hepatitis C, acquired immune deficiency syndrome (AIDS), human immunodeficiency virus (HIV) and usage of narcotic drugs. All type 1 diabetic (n = 1) as well as non-diabetic (n = 9) smokers were males. There were no children infected with Hepatitis B, Hepatitis C, AIDS and/or HIV. The diabetic and control children participating were not using any narcotic drugs.

Recruitment of type 1 diabetic and control subjects

Diabetic children were recruited from the diabetic care unit of a local hospital in Karachi, Pakistan. Their medical records were evaluated to identify the duration and treatment modalities for T1D. ‘Controls’ were also residents of Karachi, Pakistan visiting an oral healthcare centre.

Questionnaire

The children filled the oral health questionnaire together with the investigator. The children were attended to individually and were not permitted to consult each other and/or their parents/guardians. Questions regarding self-perceived GB, bad breath, dry mouth and burning sensation in mouth were also included. Xerostomia is the subjective feeling of dry mouth and hence can be assessed through questioning (11). A single-item approach may be used to measure dry mouth (12). Data regarding dry mouth and bad breath were elicited by the following questions: ‘Does your mouth usually feel dry, especially during meals’? and ‘Does your mouth have a bad smell almost all day’?

Socio-demographic characteristics of children with T1D and non-diabetic controls

Parents of the diabetic and control children were inquired about their monthly salary. The salary was recorded in Pakistani currency (Rupees, Rs.) and presented in US Dollars ($) for international readers. One US$ was equivalent to Rs. 60.7. The socio-demographic characteristics of type 1 diabetic and control children are presented in Table 1.

Family history of diabetes mellitus

For children with T1D, information concerning the duration, family history of diabetes and medications for T1D were obtained from hospital records. Parents of the control children were inquired about the presence of diabetes in their families (Table 1).

Glycosylated haemoglobin and random blood glucose level

Fasting blood samples were analysed for HbA1c in children with T1D. HbA1c was determined by high precision liquid chromatography (13). The HbA1c cut-off level for the diagnosis of diabetes has been reported
to be 6.5% (14). Therefore, in the present study, pre-
diagnosed type 1 diabetic children with HbA1c levels <
and ≥6.5% were categorized as having ‘well-controlled’
and ‘poorly controlled’ T1D, respectively (15). The
HbA1c levels were measured between 7 and 30 days
before the current study. All children with T1D were on
insulin therapy.

Random blood glucose level (RBGL) was measured
using ACCU CHEK Advantage system⁄Sensor comfort
strips (Roche Diagnostics, Mannheim, Germany). Sub-
jects were instructed not to eat or drink for at least 2 h
before their RBGL was recorded.

Collection of unstimulated whole saliva samples and
determination of salivary IgG, IgA and total protein
concentration

Unstimulated whole saliva samples were collected and
salivary flow rates (SFRs) were determined as described
earlier (9). Levels of total salivary IgG and IgA were
determined by direct enzyme-linked immunosorbent assay
as described earlier (16). Microtitre plates (Costar; Corning
Inc., Corning, NY, USA) were coated with 100 lL per well
of anti-human IgG and anti-human IgA (DAKO A⁄S,
Glostrup, Denmark) in coating buffer (0.05 M carbonate-
bicarbonate buffer, pH 9⁄6) and incubated at room
temperature for 24 h. After washing, 100 lL of appro-
priately diluted IgG (Human serum protein calibrator;
DAKO A⁄S, Denmark) and IgA (human colostrum)
standards, positive control (saliva from a healthy subject),
negative control (saliva from IgA deficient adult subject)
and saliva samples were added to the respective micro-
plate wells. After incubation at room temperature, the
microplates were washed to remove unbound proteins.

Purified alkaline phosphatase-conjugated anti-human IgG
and IgA (IgA⁄AP; DAKO A⁄S) were added (100 lL per
well) and the microplates were incubated for 3 h at room
temperature. After washing, 100 lL per well of substrate
(p-nitrophenyl phosphate) in 10 M diethanolamine,
0.5 mM MgCl2, pH 9.8, (S-0942; Sigma-Aldrich, St Louis,
MO, USA) was added. The absorbance was read at
405 nm in a microtitre plate photometer (Molecular
Devices Corp., Menlo Park, CA, USA).
The bicinchoninic acid (BCA TM) Protein Assay
Reagent Kit (Product No. 23227)* was used to deter-
mine the total protein concentration in the saliva
supernatants. Using albumin as standard, aliquots of
saliva (200 lL per well) were placed in microtitre
plates. The protein assay reagent was added and the
plates were incubated at 37 °C for 30 min. Optical
densities were read at 550 nm in a microtitre plate
photometer (Vmax; Molecular devices).

In total, 48 children (27 males and 21 females) with
T1D and 40 non-diabetic controls (19 males and 21
females) participated in this study. The study was
approved by the regional ethical review board in
Stockholm, Sweden and ethical committee of Altamash
Institute of Dental Medicine, Karachi, Pakistan.

Statistical analysis

All statistical analyses were performed using STATISTICA
7-1 (1984–2005).† Multiple logistic regression was used

Table 1. Socio-demographic vari-
ables and family history of diabetes
among children with type 1 diabetes
(T1D) and non-diabetic controls

<table>
<thead>
<tr>
<th>Children</th>
<th>Children</th>
</tr>
</thead>
<tbody>
<tr>
<td>with T1D</td>
<td>with poorly controlled T1D</td>
</tr>
<tr>
<td>Number (n)</td>
<td>48</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>15</td>
</tr>
<tr>
<td>Range</td>
<td>10–19</td>
</tr>
<tr>
<td>Duration of T1D (years)</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>4.6</td>
</tr>
<tr>
<td>Range</td>
<td>2–12</td>
</tr>
<tr>
<td>Family history of diabetes (%)</td>
<td>81.3%</td>
</tr>
<tr>
<td>Parental SES ($)</td>
<td>$90.8</td>
</tr>
<tr>
<td>Mean</td>
<td>$84.2–$93.6</td>
</tr>
<tr>
<td>Range</td>
<td></td>
</tr>
</tbody>
</table>

SES, socio-economic status.

*Pierce Chemical Co., Rockford, IL, USA.
†Statsoft Inc., Tulsa, OK, USA.

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to determine whether the dependent variables (salivary IgG per mg protein, IgA per mg protein, total protein concentration and self-perceived oral health) were statistically significant with the independent variables. The independent variables were transformed into dichotomous variables, for example, children with T1D, 1; and control children, 0. For multiple comparisons, Bonferroni adjustment post hoc test was performed. P-values <0.05 were considered as statistically significant.

**Results**

**HbA1c and RBGL**

The mean HbA1c levels for children with poorly controlled and well-controlled T1D were 12.2% (11.5–15.2%) and 5.5% (5.1–6.2%), respectively. The mean RBGL in children with poorly controlled and well-controlled T1D were 13.5 (11.7–14 mmol L\(^{-1}\)) and 5.8 mmol L\(^{-1}\) (5.4–6 mmol L\(^{-1}\)), respectively. In controls, the mean RBGL was 5.3 ± 0.8 mmol L\(^{-1}\) ranging between 5.2 and 5.9 mmol L\(^{-1}\).

**Self-perceived oral health among the type 1 diabetic and control children**

Self-perceived GB, bad breath and dry mouth were reported to be higher in diabetic children when compared with those in controls (\(P < 0.05\)). Bleeding gums were more often reported by children with poorly controlled T1D compared to well-controlled T1D (\(P < 0.05\)) and non-diabetic controls (\(P < 0.001\)). There was no difference in self-rated GB between the controls and children with well-controlled T1D. Bad breath was commonly perceived by subjects with poorly controlled T1D compared to well-controlled T1D (\(P < 0.005\)) and non-diabetic children (\(P < 0.0001\)). There was no difference in self-rated burning sensation in mouth between the type 1 diabetic and control subjects. Children with poorly controlled and well-controlled T1D showed no significant difference in relation to self-perceived dryness in oral cavity. These results are shown in Fig. 1.

**Salivary flow rate**

There was a significant difference in SFR between the diabetic (mean: 0.2 mL min\(^{-1}\), range: 0.1–0.4 mL min\(^{-1}\)) and control (mean: 0.5 mL min\(^{-1}\), range: 0.3–0.7 mL min\(^{-1}\)) (\(P < 0.01\)) groups. There was no difference in SFR between children with poorly controlled (mean: 0.1 mL min\(^{-1}\), range: 0.1–0.3 mL min\(^{-1}\)) and well-controlled T1D (mean: 0.2 mL min\(^{-1}\), range: 0.1–0.4 mL min\(^{-1}\)).

**Salivary IgG (\(\mu\)g) per mg protein, IgA (\(\mu\)g) per mg protein and total protein concentration**

Levels of IgG per mg protein were higher in the diabetics when compared with those in control children (\(P < 0.05\)). There was no difference in IgA per mg protein levels in diabetic and control groups and is shown in Table 2. IgG per mg protein levels were raised in children with poorly controlled T1D compared to well-controlled T1D (\(P < 0.05\)). There was no difference in IgA per mg protein and total protein concentration between the diabetic groups, which is shown in Table 3.

**Discussion**

Differences in oral health in individuals with and without T1D have been extensively studied. In this study, self-perceived oral health was assessed by means of questionnaires, which have been shown to be reliable (17). However, unawareness of individuals
Salivary IgG and IgA concentrations have been reported to be higher in diabetic patients compared with non-diabetic individuals (21). However; it is notable that a low SFR in diabetic individuals raises the concentration of salivary immunoglobulins thereby expressing raised concentrations. Therefore, in the current study, IgG and IgA were presented as ‘Ig per milligram of the salivary total protein concentration’, that is, IgG per mg protein and IgA per mg protein to normalize against volume. The current results showed no difference in SFR between children with poorly controlled and well-controlled T1D, as reported earlier (22). This study investigated salivary IgG per mg protein levels in type 1 diabetic and control (non-diabetic) children and also among children with poorly controlled and well-controlled T1D. Self-reported GB as well as experimentally determined salivary IgG per mg protein levels were elevated in children with T1D compared to controls.

The increased perception of gingival inflammation and raised levels of salivary IgG per mg protein in diabetic children may be associated with the formation of irreversibly glycated proteins called ‘advanced glycation end products’ (AGEs). Hyperglycaemia has been correlated with AGEs (23). Advanced glycation end products have been associated with inflammation and dysfunction in several tissues including the periodontium (23). This may be an explanation for the raised IgG per mg protein levels and self-perceived GB in children with T1D compared to controls. There was no significant difference in salivary IgA per mg protein concentration between the diabetic and non-diabetic subjects. Most of the salivary IgA originates from glandular secretion.

It has been reported that self-perceived bad breath is associated with GB (24). The present study supports these results. The current results are in accordance with another study which showed that perception of bad breath is increased in subjects with a poorer metabolic control of TID (25).

In conclusion, self-perceived GB as well as salivary IgG per mg protein concentration was higher in children with T1D compared with non-diabetic controls.

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