Evaluation of Salivary Secretory Immunoglobulin A Levels in Diabetic Patients and Association with Oral and Dental Manifestations

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ABSTRACT: Objectives: Oral and dental manifestations in diabetic patients can arise due to numerous factors, including elevated salivary secretory immunoglobulin A (s-IgA) levels. This study aimed to evaluate s-IgA concentrations in patients with type 2 diabetes mellitus (T2DM) and to investigate the association between s-IgA levels and oral and dental manifestations of T2DM. Methods: This cross-sectional descriptive study was carried out between October 2011 and September 2012 in Kerman, Iran, and included 260 subjects (128 patients with T2DM and 132 healthy controls). Unstimulated salivary samples were collected from all subjects and s-IgA levels were determined using the immunoturbidimetric method. The oral cavities and teeth of T2DM patients were evaluated for oral and dental manifestations. Results: Both diabetic and control subjects with higher concentrations of s-IgA had significantly higher numbers of decayed, missing or filled teeth (DMFT) and periodontal index (PDI) scores (P <0.050). s-IgA levels were significantly higher in subjects with oral candidiasis (P <0.050). Among diabetic patients, significantly higher s-IgA levels were concomitant with xerostomia and denture stomatitis (P ≤0.050). There were no significant differences between s-IgA concentrations and other oral or dental manifestations in either group. Conclusion: Individuals with a greater number of DMFT, a higher PDI score and oral candidiasis had significantly higher s-IgA levels. s-IgA levels were not significantly higher among diabetic patients in comparison to the control group. However, significantly higher s-IgA levels occurred with xerostomia and denture stomatitis in diabetic patients. In addition, s-IgA was significantly higher in patients with uncontrolled diabetes compared to those with controlled diabetes.

Keywords: Diabetes Mellitus; Saliva; Secretory Immunoglobulin A; Oral Manifestations; Iran.
Type 2 diabetes mellitus (T2DM) is one of the most prevalent metabolic disorders worldwide; by 2025, approximately 320 million people will have the disease. The disease has a wide range of signs and symptoms and manifests differently in various organs. In the oral cavity, manifestations of diabetes can include a dry or burning mouth, periodontal diseases, bone loss, dental abscesses, fungal and bacterial infections, oral lichen planus and delayed wound healing.

Immunoglobulin A (IgA) and immunoglobulin G affect oral cavity microorganisms in the saliva, gingival sulcular fluid and plasma. These antibodies prevent bacterial metabolism and adhesion of microorganisms to the oral tissue. Although there are many non-specific defensive elements in the saliva, such as lactoferrin and lysozymes, salivary secretory IgA (s-IgA) is the foremost protective mechanism against bacterial colonisation of the oral mucous membranes. As s-IgA plays an important role in protecting against these pathogens, the antibody might also protect against periodontal diseases. Changes in salivary IgA concentrations in diabetic patients could have an effect on their oral health. Several studies have sought to determine salivary flow rates and components that can affect the progression, symptoms and varieties of oral changes in diabetic patients. Overall, determining the salivary components of diabetic patients can be useful in detecting and managing their oral manifestations.

Previous research has assessed s-IgA levels and oral conditions among diabetic patients. A Brazilian study reported that diabetic patients with lower s-IgA levels had more severe and frequent periodontal disease. However, two Iranian studies yielded different results: Mohiti-Ardekani et al. recorded significantly higher s-IgA levels in diabetic patients while Bakianian Vaziri et al. found no significant differences between diabetic and non-diabetic groups. Other studies have also reported conflicting results with regards to s-IgA levels and their relationship to various diseases. The current study aimed to compare s-IgA levels between healthy subjects and T2DM patients and determine the association between s-IgA levels and various oral and dental manifestations in diabetic patients. This relationship could help determine the prognosis of oral diseases in diabetic patients and serve as a guide for their dental care.

**Methods**

This cross-sectional descriptive study was carried out between October 2011 and September 2012 in Kerman, Iran, and included 260 subjects divided into two groups. The first group consisted of 128 patients with T2DM who routinely attended follow-up appointments at the Diabetes Center of Shahid Bahonar Hospital in Kerman. The non-diabetic control group was composed of 132 healthy individuals with no history of DM who attended annual check-ups at either the Razi Laboratory or the Besat Clinical Laboratory in Kerman. Information regarding the subjects’ gender, age, diabetes type and medical results (including assessments of glycated haemoglobin [HbA1C] and fasting blood sugar [FBS] levels) was compiled by a trained dental student. Smokers and subjects <20 years old were excluded from the study. Control subjects were included only if they had FBS levels of <100 mg/dL, no systemic disorders and did not take medications that affected either IgA secretion or its levels in the saliva. A diagnosis of T2DM was made with a HbA1C level of ≥6.5%, fasting plasma glucose (PG) level of ≥126 mg/dL and a two-hour PG level of ≥200 mg/dL. For patients exhibiting classic symptoms of hyperglycaemic crises, a random PG measurement of ≥200 mg/dL met the criteria for a diagnosis of T2DM. Known diabetic patients who were already taking medicine for lowering blood glucose and who had a HbA1C level of ≥6.5% were determined to have uncontrolled T2DM.

The oral mucosa of all subjects was checked for abnormalities, including manifestations of oral candidiasis (erythematous candidiasis, thrush, angular cheilitis, median rhomboid glossitis and denture stomatitis), lichenoid reactions and frequent abscesses. The characteristics of any noted lesions and their locations were recorded. In addition, a tongue blade test was performed on all participants to detect xerostomia. The guidelines of the World Health Organization for assessing dental cavities were used to calculate the number of decayed, missing or filled teeth (DMFT). The periodontal disease index (PDI) were used to evaluate the effect of any periodontal disease on the supporting tissues of the oral mucosa. Xerostomia was assessed using Fox et al’s standardised questionnaire.
Table 1: Correlation between salivary secretory immunoglobulin A concentrations and other variables among control and diabetic subjects in Kerman, Iran (N = 260)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Correlation with s-IgA concentration (r)</th>
<th>Control group (n = 132)</th>
<th>Diabetic group (n = 128)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.05</td>
<td>(0.520)</td>
<td>0.15 (0.070)</td>
<td>0.10  (0.080)</td>
</tr>
<tr>
<td>HbA1C</td>
<td>-</td>
<td>(0.30 (0.009)*</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>FBS</td>
<td>-0.07</td>
<td>(0.370)</td>
<td>-0.06 (0.460)</td>
<td>-0.01 (0.830)</td>
</tr>
<tr>
<td>DMFT</td>
<td>0.06</td>
<td>(0.510)</td>
<td>0.22 (0.040)*</td>
<td>0.14  (0.04)*</td>
</tr>
<tr>
<td>PDI</td>
<td>0.40</td>
<td>(0.001)*</td>
<td>0.50 (0.004)*</td>
<td>0.12  (0.001)*</td>
</tr>
</tbody>
</table>

s-IgA = salivary secretory immunoglobulin A; HbA1C = glycated haemoglobin; FBS = fasting blood sugar; DMFT = decayed, missing or filled teeth; PDI = periodontal index. *Highly statistically significant at P < 0.001. †Statistically significant at P < 0.05.

Table 2: Comparison between salivary secretory immunoglobulin A levels and oral and dental manifestations among control and diabetic subjects in Kerman, Iran (N = 260)

<table>
<thead>
<tr>
<th>Oral/dental manifestation</th>
<th>Control group</th>
<th>Diabetic group</th>
<th>P value</th>
<th>Control group</th>
<th>Diabetic group</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral candidiasis</td>
<td>79.5 ± 16.5</td>
<td>67.5 ± 10.6</td>
<td>0.006†</td>
<td>74.0 ± 16.8</td>
<td>0.160</td>
<td></td>
</tr>
<tr>
<td>Erythematous candidiasis</td>
<td>-</td>
<td>-</td>
<td></td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Thrush</td>
<td>-</td>
<td>-</td>
<td></td>
<td>49.0 ± 11.7</td>
<td>0.630</td>
<td></td>
</tr>
<tr>
<td>Median rhomboid glossitis</td>
<td>-</td>
<td>-</td>
<td></td>
<td>35.7 ± 15.9</td>
<td>0.810</td>
<td></td>
</tr>
<tr>
<td>Denture stomatitis</td>
<td>64.1 ± 22.9</td>
<td>71.4 ± 14.7</td>
<td>0.002</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Angular cheilitis</td>
<td>95.0 ± 25.0</td>
<td>89.2 ± 27.9</td>
<td>0.110</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Lichenoid reactions</td>
<td>34.6 ± 6.6</td>
<td>126.5 ± 76.5</td>
<td>0.460</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Frequent abscesses</td>
<td>-</td>
<td>-</td>
<td></td>
<td>35.0 ± 6.5</td>
<td>0.650</td>
<td></td>
</tr>
<tr>
<td>Xerostomia</td>
<td>35.0 ± 15.0</td>
<td>51.8 ± 7.5</td>
<td>0.050†</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

IgA = immunoglobulin A; SE = standard error. *Statistically significant at P ≤ 0.05.

Unstimulated salivary samples were collected from all subjects in the morning (between 7:30 and 9:30 a.m.) after fasting for eight hours and having cleaned their mouths and teeth 90 minutes beforehand. Participants were requested to keep their mouths closed for a few moments in order for saliva to pool and then hold their heads over a container and release, resulting in a sample of 1–2 mL of saliva. Samples were then frozen to -20°C and sent to a laboratory for testing. Concentrations of s-IgA were determined using the immunoturbidimetric method with measurements obtained from a commercial kit (Pars Azmoon Co., Tehran, Iran) and an automated continuous flow analyser (AutoAnalyzer, TechniCon Systems Inc., Oakland, California, USA).

Data were analysed using the Statistical Package for the Social Sciences (SPSS, Version 17 (IBM Corp., Chicago, Illinois, USA). Pearson's correlation coefficient test was used to determine the relationship between s-IgA levels and age, HbA1C and FBS. The independent t-test was used to compare DMFT and PDI indices between the groups.

This study was approved by the Ethics Committee of Kerman University of Medical Sciences (#K/91/05). All subjects gave informed consent before participating in the study.

Results

A total of 128 T2DM patients and 132 healthy adults were included in the study. The mean age of the subjects was 47.96 years (range: 20–83 years old). Mean s-IgA levels in the diabetic and control groups were 45.40 ± 6.87 mg/dL and 41.17 ± 7.66 mg/dL, respectively; this difference between the two groups was not significant (P > 0.050). There was no significant difference in s-IgA levels between genders or with age. However, there was a significant increase in s-IgA levels among patients with uncontrolled T2DM compared to those with controlled disease (P < 0.050). Both the DMFT and PDI indices showed significant increases among the diabetic patients in comparison with the control group (P < 0.050). Between the two groups, s-IgA levels were significantly higher among subjects with a higher PDI index. Correlations between s-IgA levels and other variables are shown in Table 1.

Table 2 shows the distribution of various oral and dental manifestations among the two groups. s-IgA levels were significantly higher in subjects with oral candidiasis in both the diabetic and control groups. Tongue blade signs were positive for two subjects in the control group and 38 patients in the diabetic group. s-IgA levels were significantly higher for diabetic patients with xerostomia in comparison to the control subjects (P = 0.050). Diabetic patients also suffered from denture stomatitis more frequently than control subjects with significantly higher s-IgA levels (P ≤ 0.050).

Discussion

As patients with systemic diseases such as DM become better educated regarding self-management...
and beneficial lifestyle choices, they are increasingly seeking to address their oral health issues.15 While various research has focused on diabetic subjects, few studies have evaluated salivary immunoglobulin levels among this patient population. Unlike other body fluids, saliva is easily accessible and therefore no need for aggressive sampling techniques in order to make a diagnosis or determine the prognosis of a disease. As such, many researchers prefer to use saliva instead of blood for sampling purposes.16,17 Saliva is a complex biological fluid and conditions which affect saliva production—such as xerostomia and saliva excretion dysfunction—decrease its buffering and cleansing capacity. In addition, neuropathic disturbances in diabetic patients can influence the development of dental caries and periodontal diseases.18

Within the oral cavity, s-IgA can act as the first line of defence against pathogens that affect mucous membranes by preventing the aggregation and adhesion of bacteria to the mucosa and neutralising enzymes, toxins and viruses.19 The concentrations of various salivary elements, such as s-IgA, have been investigated in a number of systemic diseases as well as many oral conditions, including periodontitis, xerostomia, lichen planus and smoking-induced oral diseases.14,17–20 Immune suppression or disturbances have been observed in diabetic patients; it is possible that T2DM affects the secretion of IgA in the saliva and, consequently, the defence reaction of the mucosa.

In the present study, s-IgA levels in diabetic patients were not significantly higher in comparison with non-diabetic individuals. This result is similar to those seen in previous studies.5,7,9,22 In contrast, Mohiti-Ardekani et al. demonstrated that s-IgA levels in diabetic patients were higher than in non-diabetics.1 Other studies have also found higher s-IgA levels in diabetic patients.2,21 Discrepancies between these results could be attributed to differences in study designs and sampling and measuring techniques (e.g. the use of stimulated versus unstimulated saliva or immunoturbidimetric versus immunonephelometric methods).5 s-IgA levels were considerably higher in patients with uncontrolled T2DM in the current study. Harrison et al. and Malicka et al. found similarly high s-IgA levels in patients with poorly controlled diabetes.10,24

In the current research, there was a significant positive relationship between s-IgA and PDI and DMFT indices in both groups, suggesting that the innate immune system tries to synthesise high levels of IgA to reduce periodontal tissue infections.19 In addition, Kakoei et al. also noted a positive association between high salivary glucose levels and DMFT and PDI indices in diabetic patients.20 This is compatible with other findings showing that s-IgA levels were significantly higher in diabetic patients with periodontitis.19,21,28 Using the gingival and modified sulcus bleeding indices, Malicka et al. reported that the periodontal status of diabetic patients was significantly worse than that of healthy subjects.12 Ranadheer et al. showed that s-IgA levels were significantly higher among individuals with more than three DMFT.15 In studies by Bakianian Vaziri et al. and Lopez et al., diabetic patients had significantly increased numbers of DMFT in comparison with healthy subjects.1,27

In the current study, the prevalence of oral candidiasis was significantly higher in the subjects with higher s-IgA levels in both groups; this is consistent with the results observed by Jaganathan et al.29 However, no significant relationship between s-IgA and oral lichen planus was found. This correlates with previous research by Divya et al., who did not report any significant association between s-IgA levels and pre-cancerous lesions like lichen planus.20 In contrast, Ghelayani et al. found higher s-IgA levels in patients with oral lichen planus in comparison with normal individuals.21 In the present study, a significant increase in s-IgA levels was found in diabetic patients with denture stomatitis. A study by Papova et al. also showed that s-IgA levels in patients with denture stomatitis were significantly higher in comparison to a control group.28 The results of the current study differed from those observed in a study by Wilson et al., who found that that s-IgA levels were significantly lower in denture wearers with denture stomatitis in comparison to healthy subjects.30

Changes in the function and components of saliva as a result of different conditions among diabetic patients (e.g. dental caries, periodontitis, burning mouth and sensory problems) are not yet fully understood. Previous investigations have shown the efficacy of the buffering capacity and cleansing effect of saliva in negating some of these changes.16 However, the differences in s-IgA levels in different studies could be attributed to different sampling techniques or detection methods. One of the limitations of the current study was an inability to assess oral hygiene among the subjects. Further investigations are recommended to detect salivary immunoglobulins among diabetic patients in order to determine a precise and more reliable technique to predict and manage oral manifestations of the disease.

**Conclusion**

The s-IgA levels of the diabetic patients were not significantly higher than those of the control group among the studied population. In both groups, subjects with a higher number of DMFT, greater PDI scores and oral candidiasis were found to have significantly...
higher s-IgA levels. Among diabetic patients, significantly higher s-IgA levels were concomitant with xerostomia and denture stomatitis. Furthermore, s-IgA levels were found to be significantly elevated in patients with uncontrolled T2DM compared to those with controlled T2DM. Further research is needed to investigate s-IgA concentrations among diabetic patients in order to aid in the prediction and management of oral manifestations.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

References