BBA Clinical 3 (2015) 135–140

Contents lists available at ScienceDirect

BBA Clinical

journal homepage: http://www.journals.elsevier.com/bba-clinical/

Secretion of salivary statherin is compromised in uncontrolled diabetic patients

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ARTICLE INFO

Article history:
Received 27 October 2014
Received in revised form 6 January 2015
Accepted 8 January 2015
Available online 15 January 2015

Keywords:
Statherin
Diabetes
Saliva
Oral health

ABSTRACT

Background: Statherin is an important salivary protein for maintaining oral health. The purpose of the current study was to determine if differences in statherin levels exist between diabetic and healthy subjects.
Methods: A total of 48 diabetic and healthy controls were randomly selected from a community-based database. Diabetic subjects ($n = 24$) had fasting glucose levels >180 mg/dL, while controls ($n = 24$) had levels <110 mg/dL. Parotid saliva (PS) and sublingual/submandibular saliva (SS) were collected and salivary flow rates determined. Salivary statherin levels were determined by densitometry of Western blots. Blood hemoglobin A1c (HbA1c) and total protein in saliva were also obtained.
Results: SS, but not PS, salivary flow rate and total protein in diabetics were significantly less than those in healthy controls ($p = 0.021$ & $p < 0.001$ respectively). Correlation analysis revealed the existence of a negative correlation between PS statherin levels and HbA1c ($p = 0.012$) and fasting glucose ($p = 0.021$) levels, while no such correlation was found for SS statherin levels. When statherin levels were normalized to total salivary protein, the proportion of PS statherin, but not SS statherin, in diabetics was significantly less than that in controls ($p = 0.032$). In contrast, the amount of statherin secretion in SS, but not PS, was significantly decreased in diabetics compared to controls ($p = 0.016$).
Conclusions and general significance: The results show that synthesis and secretion of statherin is reduced in diabetics and this reduction is salivary gland specific. As compromised salivary statherin secretion leads to increased oral health risk, this study indicates that routine oral health assessment of these patients is warranted.

1. Introduction

Diabetes mellitus is a growing global epidemic with more than 439 million projected to be affected by 2030 [3]. Oral diseases, such as periodontitis, dental caries, fungal infection, xerostomia, and salivary gland dysfunction, are all major complications associated with diabetes [6,17]. Saliva contains a number of components, such as electrolytes, multiple buffering systems, digestive enzymes, lubricant glycoproteins, and antimicrobial proteins, to maintain oral homeostasis and preserve the health of the teeth and oral mucosa and prevent infection. Therefore, salivary dysfunction may account for a number of the oral diseases associated with diabetes and may be a risk factor for these patients. Indeed, decreased salivary flow rates have been documented in clinical cohort studies and animal models of diabetes [17,23,31]. Further, a limited number of studies have reported changes in saliva composition, such as amylase, total protein and antimicrobial proteins, in diabetic patients [6,14,23,27].

Human salivary statherin is a low molecular weight phosphoprotein, containing 43 amino acids, that functions to inhibit spontaneous precipitation of calcium and phosphate salts (primary precipitation) from saliva and the growth of hydroxyapatite crystals (secondary precipitation) on the surface of the teeth [18,20]. In addition, statherin is a major component of the "acquired" dental pellicle and functions to regulate mineralization at the surface through binding of its hydrophilic N-terminal domain to hydroxyapatite and selection of oral microorganisms that bind to the

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http://dx.doi.org/10.1016/j.bbacli.2015.01.002

pellicle through its C-terminal domain [7,18,20]. Further, the level of statherin in whole saliva has been reported to be higher in caries-free patients than in caries-susceptible patients and those with elevated decayed, missing, and filled teeth (DMFT) indices [29]. Based on these observations, it is generally believed that statherin plays a critical role in protecting oral tissues from a number of common dental disorders (e.g., periodontal diseases, dental caries and oral mucosa infections) [11,25].

Previous studies have shown that oral health in patients with type 2 diabetes is compromised and that many of the problems these patients have are attributable to reduced salivary production or secretion and alterations in the composition of their saliva [17,23,31]. In other studies, statherin expression in labial, submandibular, and parotid gland tissue, obtained from control and diabetic (type 2) patients undergoing head and neck tumor resection, was examined using immunogold labeling and transmission electron microscopy (TEM). Statherin immunoreactivity was detected in small vesicles, diffusely localized throughout the cytoplasm of labial serous cells, and in secretory granules of serous acinar cells in both submandibular and parotid glands [11–13]. Detailed statistical analyses revealed that the number of stained particles was significantly lower in tissues of diabetic subjects than non-diabetic controls. Recent proteome and peptidome analyses of saliva from children with type 1 diabetes also suggest a relatively lower production of statherin than those in controls [1].

Since the earlier studies, mentioned above, demonstrated decreased production of statherin in salivary gland tissue (type 2 diabetics) and saliva (type 1 diabetics), the current study aimed to determine whether there are measurable differences in salivary statherin from sublingual/submandibular and parotid glands from normal ($n = 24$) and type 2 diabetic ($n = 24$) middle aged subjects. Changes in statherin production were also evaluated for correlation with fasting blood glucose and HbA1c levels and DMFT indices. The overall goal was to evaluate whether salivary statherin had potential as a marker of salivary dysfunction, oral health, and overall disease activity in type 2 diabetes.

2. Materials and methods

2.1. Subjects

A total of 48 subjects, 24 diabetic and 24 healthy age- and gender-matched controls, were randomly selected from 1322 subjects in the Oral Health: San Antonio Longitudinal Study of Aging (OH: SALSA) database [6] which included both Mexican American and European American ethnic groups. The number of subjects to be included in the study was estimated using a power analysis (effect size = 0.85,αerror = 0.05, power = 0.80) and based on a preliminary study of PS. The study protocol was approved by the Institutional Review Board of the University of Texas Health Science Center at San Antonio and informed consent was obtained from each participant. Diabetic subjects were identified based on fasting plasma glucose levels >180 mg/dL. Control subjects (non-diabetic), with comparable age and gender to the diabetic cohort, were identified by having no major health problems and fasting plasma glucose levels <110 mg/dL. Some of the control subjects, however, were taking anti-cholesterolemic or anti-hypertensive medications. Body mass index (BMI), HbA1c, and oral health status were also obtained for both groups, but not used as selection criteria.

2.2. Saliva collection

Parotid saliva (PS) and sublingual/submandibular saliva (SS) production were stimulated and collected as previously described [6]. In brief, PS was collected using a modified Carlson–Crittenden cup, while SS was collected using a micropipette connected to a mini-vacuum pump. Stimulation of saliva production was achieved by swabbing the lateral surfaces of the tongue with 0.1 M (≈2%, w/v) citric acid every 30 s. PS was collected for 5 min and SS for 3 min. Salivary flow rate was calculated by dividing the total volume of saliva obtained by the length of time taken to collect it and expressing the result as mL/min. Saliva samples were divided into 100 μL aliquots and stored at −80 °C until analyzed.

2.3. Immunoblot analysis

Salivary statherin levels were determined using a previously described method with some modification [6,30]. Saliva (PS and SS) samples were thawed and ethylenediamine tetraacetic acid (EDTA) was added to a final concentration of 0.5 mM to minimize aggregate formation. Following a series of pilot experiments, the volume of saliva required to obtain an optimal statherin signal in the immunoblot analysis was found to be 0.125 μL for both control and diabetic samples. Total protein content of the saliva was determined by measuring absorbance at 215 nm using a DU® spectrophotometer (Beckman Coulter, CA, USA) [26]. Bovine serum albumin was used as a standard.

Saliva samples were separated on precast 4–20% SDS-PAGE gels (Mini-PROTEAN® TGX™, Bio-Rad Laboratories, Inc., CA, USA) and then transferred to polyvinylidene difluoride membranes (Millipore Corp, Bedford, MA). The membranes were immunoblotted with a primary antibody against human statherin (1:2000 dilution; STATH Polyclonal Antibody [catalog #19724-1-AP], Proteintech, IL, USA) at 4 °C overnight, followed by incubation with horseradish peroxidase-labeled secondary antibody (1:10,000) (GE Healthcare Life Sciences, PA, USA). The statherin immunoreactive band was visualized using an ECL detection kit (ECL Advance™ Western Blotting Detection Kit, GE Healthcare, Buckinghamshire, UK) and band images were acquired and analyzed with an AutoChemi system (UVP, Inc., CA, USA). Gray-scale values for statherin-positive bands were quantified using Scion Image software (Scion Corporation, MD, USA).

2.4. Quantification of statherin

To compensate for variations in immunoblotting, one PS sample was selected from the normal controls that produced an average level of band intensity after SDS-PAGE and immunoblotting. The integrated gray value of this standard (0.125 μL saliva) was assigned an arbitrary value of 100. Statherin levels in all samples were calculated relative to this PS standard and are reported as the percent (%) statherin level for a particular PS or SS sample.

To normalize the data, the % statherin level (calculated above) was divided by the protein content in the saliva sample (% statherin level divided by μg protein/μL saliva). To obtain an estimate of the rate of statherin secretion in PS and SS, the % statherin level was multiplied by the measured salivary flow rate (mL/min).

2.5. Statistical analysis

A power analysis was performed using the statistical software, G*Power (version 3.1.9.2) [8,9] to determine the appropriate number of subjects to include in the study. The Mann–Whitney U-test was used for comparing data from control and diabetic subjects. Spearman's rank correlation coefficient was used for the test of correlation between statherin level and HbA1c and blood glucose levels. These statistical analyses were carried out using PASW Statistics 17.0 software (SPSS, Inc., IL, USA). A p-value less than 0.05 was considered statistically significant.

3. Results

The demographics of the control and diabetic subjects are shown in Table 1. The average age of the subjects in both groups was nearly identical and the male/female ratio was identical in each. In diabetic subjects, median body mass index (BMI) and HbA1c level were statistically higher when compared with controls ($p = 0.049$ and $p < 0.001$,