

Differences in Proteomic Profiles Between Caries Free and Caries Affected Children

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Abstract

Objective: To determine if there are differences in protein profiles in saliva depending if children of caries-free versus caries affected. **Material and Methods:** A cohort of 91 children with ages between 6 and 19 years, along clinical status of caries experience. Protein profiles in saliva were determined using electrophoresis and the calculation of the percentage of a specific band at a specific molecular weight in relationship to the total protein in that sample (% of total) using molecular weight standards. This quantification was repeated for each protein band across a range of molecular weights for each sample. Chi-square, Fisher's exact, and Student t-tests were used to compare the distributions between caries-free and caries affected children ($\alpha=0.05$). **Results:** Histatin was more likely to be non-detectable or reduced in caries-free children (OR=7.56; 95% CI 1.62-35.13) and these children had on average one less gel band detected by the assay we used. **Conclusion:** We have found differences in proteins between caries affected and caries-free children, suggesting that this line of investigation holds the promise of providing new tools for caries management.

Keywords: Dental Caries; Proteome; Proteomics; Saliva.

Introduction

Blood has been the substance of choice for diagnostics. However, obtaining saliva has the advantage of being non-invasive in nature and saliva has been explored as a potential source for discriminating, detecting, and monitoring biomarkers. Published evidence suggests the identification of peptides in saliva may become a tool for determining disease risk and activity. However, except for Sjögren's syndrome, most studies of the same disease are conflicting in regard to the suggested saliva biomarkers [1].

For dental caries, levels of statherin (a protein that prevents precipitation of calcium phosphate) and a truncated cystatin S (a proteinase inhibitor) missing the first eight N-terminal amino acids showed inversed correlation with dental caries experience [2]. Dental caries biofilm composition and protein relative abundance in the presence and absence of sucrose also showed promising results related to changes in protein relative abundance as indicators of dysbiosis [3].

In cases of severe early childhood caries, two segments of the histatin-1 (which exhibits antibacterial and antifungal activities) peptide can be detected in saliva by magnetic bead (MB)-based matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) [4]. Tandem mass spectrometry of saliva samples from individuals with dental caries showed overexpression of markers of the complement system and inflammation [5].

Further work suggests differences in the complexity of the proteins identified depending on whether samples come from individuals resistant or affected by dental caries [6]. This evidence suggests that there are differences in proteomic profiles of individuals affected or not by dental caries. Therefore, we aimed to test the hypothesis that differences in salivary peptides that can be useful for future research or clinical management of patients can be identified when unstimulated whole saliva is used.

Material and Methods

Study population

A cohort of 91 children with ages between 6 and 19 years were consecutively recruited for these analyses using the University of Pittsburgh, School of Dental Medicine, Dental Registry and DNA Repository project (University of Pittsburgh Institutional Review Board (IRB) approval # 0606091).

This project was started in September 2006 and all the patients treated at the Dental Clinics of the University are invited to be part of the registry. All subjects provided written informed consent authorizing the extraction of dental and medical information from their records and provided a saliva sample for future studies.

Saliva samples were obtained prior to the subject's dental appointment and were frozen until analysis. Age, sex, ethnicity, caries experience in the primary dentition and caries experience in the permanent dentition were considered. Caries experience, measured as DMFT and DMFS (decayed, missing due to caries and filled teeth or surfaces) at the time of examination, was based on oral inspections and bite-wing radiographs and were collected from each participant's dental record, based on already existing values. Surfaces were defined as decayed if the carious lesions extended into dentine or were circumscribed to the dental enamel and appeared active (white spot lesions). Twenty children were caries-free and were compared to the remaining children with past caries experience.

Protein Profiling

The collected saliva samples were evaluated using the Bio-Rad Experion Automated Electrophoresis System (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Ten microliters of each saliva sample were loaded into a primed Experion Chip and run using the Experion Software Protein 260 Assay (Bio-Rad Laboratories, Inc., Hercules, CA, USA), as described previously [7]. The protein banding profile from each sample was used to generate a composite image similar to a Coomassie stain. Experion image analysis software calculated the percentage of a specific band at a specific molecular weight in relationship to the total protein in that sample (% of total) using molecular weight standards. This quantification was repeated for each protein band across a range of molecular weights for each sample. Analysis of the 91 individuals was completed using bands located at or around certain molecular weights.

Data Analysis

Chi-square, Fisher's exact, and Student *t*-tests were used to compare the distributions between caries-free and caries affected children ($\alpha=0.05$).

Results

Children with caries experience were on average, four years older than children that comprised the caries-free comparison group, but their distribution of sex and ethnic background was not different. More children with caries-free permanent dentition had substantial caries experience (seven affected surfaces or more) in the primary dentition. Histatin was more likely to be non-detectable or reduced in caries-free children (OR = 7.56; 95% CI 1.62-35.13) and these children had, on average, one less gel band detected by the assay we used. All detected proteins are listed in Table 1.

Table 1. Demographics, caries prevalence, and proteins identified in the study subjects.

Variables	Caries Affected (N = 71)	Caries Free (N = 20)	p-value
Age (Mean Years and Range)	13.53 (6-19)	9.15 (6-14)	<0.00001*
Sex			
Females	33	10	0.78#
Males	38	10	
Ethnicity			
Blacks	16	4	0.92#
Whites	40	11	
Others	15	5	
DMFT (Mean and Range)	4.82 (1-17)	0	
DMFS (Mean and Range)	8.9 (1-41)	0	
High Caries Experience in the Primary Dentition with dmfs 7 or Higher (N)	13	11	0.001#
Amylase not Detected or Reduced (N)	3	0	0.21&
Agglutinin not Detected or Reduced (N)	20	3	0.23&
Histatin not Detected or Reduced (N)	3	5	0.01&
Presence of Proteins with Molecular Weight Smaller Than 1KDa (N)	48	11	0.3#
Number of Bands in Gel (Mean and Range)	8.93 (3-14)	7.85 (4-12)	0.03*

**t*-test; #Chi-square; &Fisher's exact.

Discussion

Dental caries continues to affect a large segment of the population, but its epidemiology has changed from affected essentially everyone to pockets of people who are caries-free and individuals at higher risk that

experience most of the disease in the population [8]. Although preventive measures such as school-based sealant applications, fluoride exposure of the population through toothpastes or fluoridated drinking water, and facilitation of access to dental care played an important role in reducing caries experience globally, a segment of the population remains experiencing dental caries, which justifies research taking advantage of the molecular knowledge gained in the last decades. Genomic approaches have been considered as potential tools in helping determining dental caries risks [9,10], as well as evaluations that take advantage of longitudinal assessments of the disease to determine disease trajectories [11].

Proteomic approaches are emerging in oral health and the combination of the easiness of obtaining saliva and investigating a disease highly prevalent such as dental caries provide an opportunity to perform proteomic studies in cariology. Since the disease affected the hard structures of the mouth, which are washed by saliva, it is reasonable to believe that saliva will reflect dental caries activity. We have shown that differences between individuals affected by dental caries and caries-free people exist, which agrees to previously published work [1,2,4-6].

Our sample is a little more robust than previous studies but has some interesting aspects such as a relevant frequency of more severe caries experience in the primary dentition in particular in the children that was included in the caries-free group. It is difficult to speculate if this would necessarily impact our results. The technology we used is simpler than other studies. This brings up the possibility that this kind of approach can be one day translated to clinical practice and used as a tool for decision-making regarding clinical care.

Conclusion

We have found differences in proteins between caries affected and caries-free children, suggesting that this line of investigation holds the promise of providing new tools for caries management.

Authors' Contributions

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All authors declare that they contributed to critical review of intellectual content and approval of the final version to be published.

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Conflict of Interest

All authors declare that there are no conflicts of interests related to the present article.

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