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ORIGINAL ARTICLE

Protein Profiles of Individuals with Erosive Tooth Wear

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Abstract

Objective: To determine if protein profiles identified in saliva could be used to determine risk and severity of erosive tooth wear. **Material and Methods:** Three types of saliva sampling were performed to obtain saliva from 34 18-year old individuals that received regular dental check-ups, along with clinical status of the dentition and risk factor related to erosive tooth wear using the VEDE scale. 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 to test for association with erosive tooth wear status. **Results:** There were no differences in the number of detectable proteins sourced from the parotid gland, nor the unstimulated and stimulated whole saliva. Five out of the 34 individuals had no signs of erosive tooth wear despite an acidic diet and were more likely to have proteins with molecular weight smaller than 1 KDa (p=0.03). **Conclusion:** There is potential for the use of protein profiling to determine risks for erosive tooth wear.

Keywords: Dental Enamel; Dental Caries; Tooth Erosion.

Introduction

Erosive tooth wear is the loss or wear of dental hard structure caused by acids not originated from bacteria [1]. These acids may be related to vomiting or acid reflux, or acidic diets. Soft drinks such as carbonated sodas and sports drinks are the main reasons explaining erosive tooth wear. The perceived increase in erosive tooth wear and a healthier diet that can be more acidic has made us suggest the utilization of fluorides to counteract the effects of acids from the diet on the dental enamel [2]. In addition, drinking milk may be a way to minimize losses of hard tooth structure [3,4]. The mechanism underlying this effect appears to be related to an impact on bacteria adhesion to the acquired enamel pellicle when one drinks milk [5].

We have proposed that a specific genetic factor in individuals that may be more susceptible to erosive tooth wear when exposed to acidic diets may be the same operating in people more prone to dental caries under the right conditions. This common susceptibility would have to do with the formation of dental enamel and related to genetic variation in certain genes, such as amelogenin [6-8]. Studies of genetic variants are yet to readily translate to information that can be directly applied to interpretation of clinical status. Ascertaining proteins in saliva may provide a direct measure of risks that may be useful for clinical management. Saliva proteomics analyses led to the identification of more than 2,000 proteins [9]. Proteomics analyses of plasma, urine, cerebrospinal fluid, amniotic fluid, and saliva suggest that body fluids might reflect the diverse functions of the whole body rather than the characteristics of their adjacent tissues and may be useful for biomarker discovery [10].

Therefore, the presence of proteins in saliva of a group of individuals with erosive tooth wear were ascertained, with the hypothesis that specific protein profiles may be present and be potential indicators of erosive risks.

Material and Methods

Study Population

This study revisited clinical data from a previous study [11]. One trained and calibrated clinician (AM) examined a sample of 267 (response rate of 48%) 18-year-olds in Oslo, Norway during 2008, in fully equipped dental clinics using plane mouth mirrors, probes and standard lightning [11].

These individuals were receiving regular dental treatment and all 18 years old that accepted to participate in the study were examined. Calibration happened by the examiner (AM) training to detect erosive tooth wear through a series of photographs and the advice of an experienced clinician (ABT) [12].

Twenty surfaces per participant were examined: the occlusal surfaces of the first and second molars in both jaws and the labial and palatal surfaces of the upper incisors and canines. Dental erosive wear was classified by the VEDE system [12] according to the following criteria: score 0: no erosion, score 1: initial loss of enamel, no dentine exposed; score 2: pronounced loss of enamel, no dentine exposed; score 3: exposure of dentine, < ⅓ of the surface involved; score 4: ⅓ - ⅔ of the dentine exposed; score 5: > ⅔ of dentine exposed. In cases of doubt, the lower score was recorded and only lesions that were considered as obvious dental erosive wear defects were registered, including cuppings/grooves of the molar cusps. When index surfaces were either filled, bonded with a retainer, considered to have attrition and wedge-shaped defects or the tooth had been extracted, the surfaces and teeth were recorded as missing and excluded.

Caries experience, measured as DMFT (decayed, missing due to caries and filled teeth) 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 (D_{3-5}MFT). The participants were defined as having no caries experience when D0-2MFT=0, and as having caries experience when D_{3-5}MFT>0.

Saliva Collection

With the allocated resources and of convenience, the first 34 participants arriving to the oral examination provided three saliva samples in a quiet, isolated room, always at the same time (between 9 and 10 am). The participants were fully informed of the process of the saliva collection. Unstimulated and stimulated whole, and parotid saliva were collected. Table 1 shows a summary of individual's clinical status.

Subjects were told to relax in an upright sitting position for a few minutes before collecting the unstimulated whole saliva. Immediately afterwards, they performed a standardized 10-minute collection of saliva by letting the saliva drip into a graduated plastic tube. After collecting the unstimulated saliva, the subjects were given an unflavored paraffin gum to chew at a rate of their own chewing frequency for 5 minutes to collect the stimulated whole saliva. Swallowing was not permitted. The collection of the parotid saliva was performed by application of a specific collecting device (snail collector) to the emergence area of the ductus. After the collection of all tree parameters, the amount of saliva (ignoring the foam) was measured to an accuracy of 0.1 ml and flow rate (ml/min) was determined for each saliva sample. Immediately after collection, each sample was fractionated into 100 µm tubes, and frozen at -70°C until analyses.

Questionnaire

Subjects were asked about their place of residence and the national background. According to the area of residence, socio-economic status was categorized into east- and west end of the city of Oslo (east-end: low income, west-end: high income). The distinction between these areas is defined by Statistics in Norway based on the household net income [13]. National background was recorded according to mother's and participant's country of birth. Their national origin was combined into one variable and thereafter dichotomized as western origin (both mother and participant born in a western country) or non-western origin (either mother or participant born in a non-western country). Non-western origin included the individuals with birthplace from Eastern Europe, Turkey, Asia, Africa, South and Central America [14].

Ethical Considerations

The study was approved by the local Regional Committee for Medical Research Ethics and The Norwegian Social Science Data Services. Written, informed consent was obtained from all participants. The University of Pittsburgh Institutional Review Board also approved this study.

Protein Profiling

The collected saliva samples were evaluated using the Bio-Rad Experion Automated Electrophoresis System. Ten microliters of each saliva sample were loaded into a primed Experion Chip and run using the Experion Software Protein260 Assay as described previously [15]. 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 34 individuals was completed using bands located at or around certain molecular weights (Table 1).

Statistical Analysis

Chi-square or Fisher's exact tests were used in all comparisons with an alpha of 0.05.

Results

There were no differences in the number of detectable proteins sourced from the parotid gland, nor the unstimulated and stimulated whole saliva (Table 1 summarizes the clinical characteristics and total protein profiles depending of the type of saliva sample). All individuals had amylase detected as expected. Variation in the presence of certain proteins was observed, which is summarized in Table 2. Individuals without erosive tooth wear despite an acidic diet were more likely to have proteins with molecular weight smaller than 1 KDa (p=0.03).

Table 1. Clinical characteristics and total protein profiles depending of the type of saliva sample of the studied sample.

Participant	Sex	Erosive Tooth Wear	Caries Experience (DMFT score)	Number of Bands in Gel		
				Parotid Saliva	Unstimulated Saliva	Stimulated Saliva
1	Female	No Erosion	12	6	8	8
2	Male	Dentin Exposed	5	7	9	7
3	Male	No Erosion	2	6	7	7
4	Male	Dentin Exposed	2	9	7	6
5	Female	No Erosion	0	6	9	8
6	Female	No Erosion	4	6	12	7
7	Female	Dentin Exposed	7	6	8	11
8	Female	Enamel Affected Only	5	10	7	6
9	Male	Enamel Affected Only	7	8	10	6
10	Male	Dentin Exposed	2	16	10	9
11	Male	Dentin Exposed	3	10	11	7
12	Male	Dentin Exposed	2	8	16	10
13	Female	Dentin Exposed	0	6	9	7
14	Male	Dentin Exposed	0	6	13	10
15	Male	Dentin Exposed	5	8	11	12
16	Male	Dentin Exposed	1	11	10	9
17	Female	Dentin Exposed	0	9	8	12
18	Male	Dentin Exposed	6	7	4	7
19	Male	Dentin Exposed	3	9	6	7
20	Male	Dentin Exposed	5	6	10	13
21	Female	Enamel Affected Only	7	11	12	14
22	Female	Enamel Affected Only	7	9	8	7
23	Male	Dentin Exposed	3	10	6	14
24	Male	Dentin Exposed	0	8	14	11
25	Female	Enamel Affected Only	0	7	11	6
26	Male	Enamel Affected Only	3	6	6	8
27	Male	Dentin Exposed	9	7	11	6
28	Male	Dentin Exposed	3	9	11	11
29	Female	Enamel Affected Only	8	6	8	10
30	Female	No Erosion	2	7	10	7
31	Female	Dentin Exposed	4	8	11	10
32	Female	Enamel Affected Only	11	7	11	6
33	Female	Enamel Affected Only	21	7	12	10
34	Female	Enamel Affected Only	7	9	14	11

Table 2. Patterns of variation in the sample.

Proteins Patterns	Individual Samples*
Presence of proteins with molecular weight above 260 KDa	Parotid saliva of 16, 22, and 23; stimulated saliva of 23
Absent agglutinin	Parotid saliva of 21 and 28; unstimulated saliva of 10
Reduced agglutinin	Parotid saliva of 2, 8, 11, 18, and 25; unstimulated saliva of 4, 28, and 33; stimulated saliva of 4 and 10
Reduced histatin	Parotid saliva of 2, 18, 19, and 28; unstimulated saliva of 18
Presence of proteins with molecular weight smaller than 1KDa	Parotid saliva of 7, 8, 9, 12, and 14; unstimulated saliva of 1, 3, 5, 7, 8, 13, 14, and 24; stimulated saliva of 3, 6, 7, 11, and 12

*According to individual identification in Table 1.

Discussion

The main purpose of the present study was to ascertain if young individuals with erosive tooth wear show specific protein profiles. Even though no meaningful pattern was unveiled, a variation among individuals and several consistent patterns were found. In the present study the presence of amylase was detected, independently of caries experience or presence of erosive tooth wear. Another interesting finding was that out of the five individuals included who did not have clinical signs of erosive tooth wear despite an acidic diet, four presented proteins with molecular weight smaller than 1KDa in at least one type of saliva studied (Odds Ratio = 0.3, 95% confidence interval 0.14-0.69). This result points to the possibility that the presence of certain proteins in saliva protects against erosive tooth wear. These low-molecular-weight components of human saliva appear to be derived from proteolysis of the hydroxyapatite-interactive human salivary proteins, histatins, proline-rich proteins, and statherins [16].

Scientific interest in discovering new pathways of the pathology of the disease has increased in lately due to its' high prevalence. A recent review and meta-analysis estimated the prevalence among children and adolescents to be on average 30% [17]. However, estimates of the frequency of erosive tooth wear among children and adolescents show great variation (between 14% and 100%) [11]. Although most of it can be explained by different measurements used and population exposures, we believe individual susceptibility modulates a portion of the variation seen among people [7,8,18]. Individual biologic influences on erosive tooth wear apparently are similar to the ones previously detected both for dental caries and dental erosive tooth wear (i.e., variation in genes involved in enamel formation) [7,8]. It is likely to assume that heritability should show modest values for dental erosive tooth wear similar to the ones found for dental caries [6] or molar-incisor hypomineralization [19] (around 20%). However, this is probably enough to determine important differences in frequency when environmental factors apparently are similar.

Our study was modest in scope, with just 34 subjects being examined but it is remarkable to see that variation could still be detected. Studies of larger scale indeed have the potential to unveil differences that may be useful biomarkers of disease.

Conclusion

The present work suggests that protein profiling in saliva may provide a tool for determining differences in risk for erosive tooth wear. This may be of value when determining lifetime risks of loss of dental structure. To identify the risk at an early stage of the disease gives us the opportunity to implement proper individualized prevention.

Authors' Contributions

AM	0000-0002-3784-7278	Conceptualization, Methodology, Investigation and Writing – Original Draft Preparation.
ABT	————————	Conceptualization, Methodology, Resources, Writing – Original Draft Preparation, Supervision, Project Administration and Funding Acquisition.
NMV	0000-0001-5971-652X	Formal Analysis, Data Curation, Visualization and Writing – Review and Editing.
KL	0000-0003-4295-4665	Investigation, Data Curation and Writing – Review and Editing.
ARV	0000-0003-3392-6881	Conceptualization, Methodology, Formal Analysis, Resources, Data Curation, Writing – Original Draft Preparation, Writing – Review and Editing, Visualization, Supervision, Project Administration and Funding Acquisition.

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

The authors declare no conflicts of interest.

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