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Protein content of human mixed saliva of subjects with different caries risk

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Authors: Dr. Thorsten Henning, Prof. Dr. Roswitha Heinrich-Weltzien, Dr. Bernd Röhrig, Dr. Uwe Kehrer, Prof. Dr. Lutz Stößer
University of Jena, Department of Preventive Dentistry

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Introduction

Saliva proteins after adsorption on teeth as pellicle facilitate bacteria adhesion and as a consequence the formation of plaque. Therefore, the protein composition of saliva can have a certain impact on the caries risk. To proof this hypothesis the investigations as describe as follows were accomplished.

Aims

The proteins from saliva by using reversed phase high performance chromatography (RPLC) were to separate. By using cluster analysis it was to proof, if the children could be separated into distinct groups according to similar protein content of whole saliva. Then, a possible correlation of protein content and caries incidence was to examine.

Material and Methods

Saliva collection:
Unstimulated mixed saliva from school children (n=111) was collected three times every six month (E3 to E5) on ice (4°C). After centrifugation (4000 x g, 10 min.) and filtration (0.2 µm RC 25 Sartorius) the samples were investigated by reversed phase liquid chromatography (RPLC).

Protein separation:
RPLC was done by a HP ChemStation LC1100 (Agilent Technologies) with a Zorbax 300 SB-C8 (Agilent Technologies) column and a gradient of water/acetonitrile (ACN) (0 min 5%, 15 min 40%, 20 min 95% ACN) with 0,05% trifluoroacidic acid (TFA)). The results of separation (Fig. 1) were elucidated by UV detection (lambda = 215 nm). The protein content of a particular fraction (n=9) and the total protein was calculated using bovine serum albumin (BSA) as internal standard.

Statistics:
The computer programs SAS and SPSS were employed to determine the mean values and the standard deviations. Friedman tests estimated the significance of results. Cluster analysis (Ward method) based on the absolute protein content [µg/ml] of nine RPLC fractions was accomplished.

Determination of caries risk:
Caries was determined according to the WHO standard (1997). As initial lesions both white and dark spots were recognised.

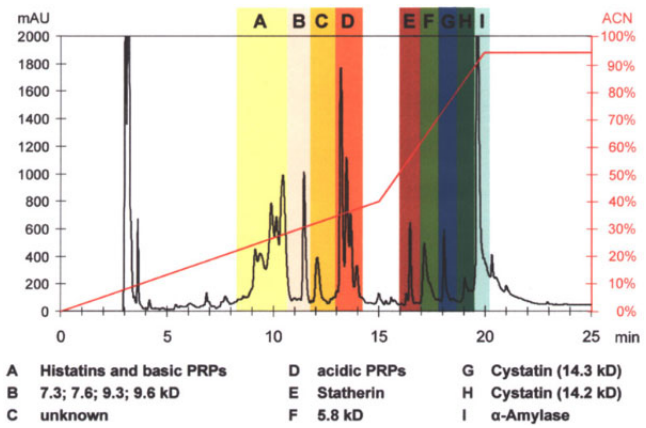


Fig. 1: Standardised chromatographic profile of proteins of human mixed saliva separated RPLC

Results

The protein content was subject to change during this study (Tab. 1). Cluster analysis separated constantly two distinct groups of children with either high or low protein content for each RPLC fraction during this study. The number of children in the second cluster was getting smaller during this study while the ratio between both clusters was rising for some protein fractions (Tab. 2). A relation between protein content and caries incidence was not found by Spearman correlation.

Examination (E)	**3**	**4**	**5**	**p Value** (Friedman-Test)
n	**111**	**111**	**111**	**111**
Total protein	**1254** ± 478	**1120** ± 452	**1361** ± 898	**0.002**
Fraction				
A	371	417	559	<0.001
B	78	57	84	<0.001
C	30	26	49	<0.001
D	297	192	208	<0.001
E	52	36	28	<0.001
F	73	61	62	<0.001
G	43	38	71	<0.001
H	140	138	133	0.302
I	169	156	167	0.053

Tab. 1: Protein content [µg/ml] and RPLC fraction A to I from saliva in children with increasing age of children

Examination (E)	**3**			**4**			**5**		
Cluster (C)	**C1**	**C2**	**C2/C1**	**C1**	**C2**	**C2/C1**	**C1**	**C2**	**C2/C1**
n	**39**	**72**	**111**	**101**	**10**	**111**	**106**	**5**	**111**
Total protein	**815** ± 148	**1492** ± 422	**1.8**	**1011** ± 291	**2218** ± 298	**2.2**	**1223** ± 620	**4303** ± 881	**3.5**
Fraction									
A	239	443	1.9	379	808	2.1	493	1966	4.0
B	54	90	1.7	50	125	2.5	71	369	5.2
C	16	38	2.4	23	62	2.7	38	274	7.2
D	177	363	2.0	164	472	2.9	183	740	4.0
E	37	60	1.6	32	73	2.3	26	67	2.6
F	43	89	2.1	55	118	2.1	57	173	3.0
G	33	48	1.4	35	67	1.9	66	178	2.7
H	109	158	1.4	133	193	1.5	130	198	1.5
I	105	204	1.9	141	301	2.1	159	339	2.1

Tab. 2: Saliva samples of children separated by cluster analysis (Ward) based on the protein content of the nine RPLC fractions With a few exceptions the subject groups found by cluster analysis are statistically significant (U test, $p < 0.05$)

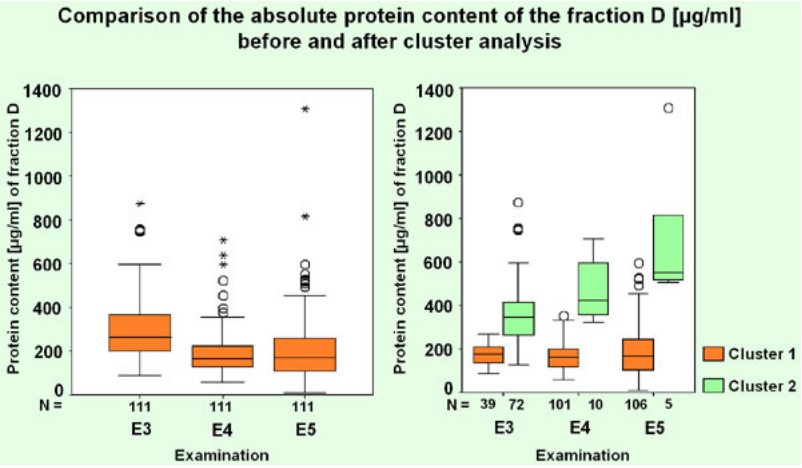


Fig. 2: Comparison of the absolute protein content of the fraction D [µg/ml] before and after cluster analysis

Examination (E)	**3**		**4**		**5**	
Cluster (C)	**C1**[1]	**C2**[2]	**C1**	**C2**	**C1**	**C2**
Clinical Parameter						
n	**39**	**72**	**101**	**10**	**106**	**5**
DMF/S	0.23	0.36	0.52	0.30	0.62	1.40
delta DMF/S	-	-	0.19	0.20	0.32	0.38
Initial lesions	0.64	0.74	0.50	0.60	1.00	1.60
delta Initial lesions	-	-	0.50	0.80	1.19	2.00
API	55.77	53.31	52.98	53.70	57.20	79.60

Tab. 3: Caries data of 111 subjects separated by cluster analysis into two groups using the protein content of nine saliva fractions
(1) Cluster 1 (2) Cluster 2

Conclusions

Quantitative studies relating concentrations of the predominant salivary protein components have been already conducted using RPLC (Dodds et al., 1997; Kehrer et al., 1999). The reproducibilty of protein composition in human salivary proteins was tested (Kehrer et al., 2000).
The clusters formed using the content of nine saliva protein fractions presented here showed differences in terms of protein content but were not related to the clinical data. Whether the protein concentration is changed during increasing age of children and subsequently influences the pellicle formation is subject to further work.

Literature

- Dodds MWJ, Johnson DA, Mobley CC, Hattaway K.M.: Parotid saliva protein profiles in caries-free and caries-active adults. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1997;83:244-251.
- Kehrer U, Fischer T, Kneist S, Stoesser L: Reversed phase liquid chromatography of human salivary proteins. Caries Res 1999;33:309-310.
- Kehrer U, Fischer T, , Stoesser L: Reproducibility of protein composition in human salivary proteins. Caries Res 2000;34:366-377.
- World Health Organisation (WHO) Application of the international classification of diseases to dentistry and stomatology (ICD-DA) 4th ed. WHO, Geneva, 1997.

This Poster was submitted by Dr. Thorsten Henning.

Correspondence address:
Dr. Thorsten Henning
University of Jena
Department of Preventive Dentistry
Nordhäuser Str. 78
D-99089 Erfurt
Germany

Poster Faksimile:

T 10 Protein content of human mixed saliva of subjects with different caries risk

Th. Henning*, B. Röhrig, U. Kehrer, L. Stößer

Department of Preventive Dentistry, University of Jena, Germany

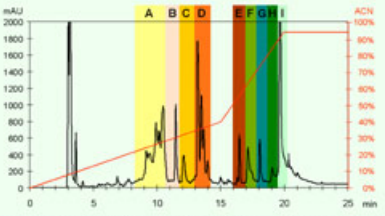
AIMS

- Separation of proteins from saliva by using reversed phase high performance chromatography (RPLC)
- Grouping of subjects according to the similar protein content of their mixed saliva
- Correlation of the protein content and the clinical data of school children aged eight to ten years taking part in a caries prevention study

MATERIAL AND METHODS

- Saliva collection
  - Unstimulated mixed saliva from school children (n=111)
  - Collected three times every six month (E3 to E5) on ice (4°C)
  - 4°C, centrifuged (4000 x g, 10 min.) and filtered (0.2 µm RC 25 Sartorius)
- Protein separation
  - RPLC: HP ChemStation LC1100 (Agilent Technologies)
  - Column: Zorbax 300 SB-C8 (Agilent Technologies)
  - Gradient: water/acetonitrile (ACN) (0 min 5%, 15 min 40%, 20 min 95% ACN) with 0,05% trifluoroacidic acid (TFA))
  - Detection: UV detection (λ = 215 nm)
  - Calculation of the protein content of a particular fraction (n=9) and the total protein using bovine serum albumin (BSA) as internal standard
- Statistics
  - Mean values and the standard deviations were determined by the computer programs SAS and SPSS. Friedman tests estimated the significance of results.
  - Cluster analysis (Ward method) based on the absolute protein content [µg/ml] of nine RPLC fractions was accomplished.

Standardised chromatographic profile of proteins of human mixed saliva separated RPLC



A	Histatins and basic PRPs	D	acidic PRPs	G	Cystatin (14.3 kD)
B	7.3; 7.6; 9.3; 9.6 kD	E	Statherin	H	Cystatin (14.2 kD)
C	unknown	F	5.8 kD	I	α-Amylase

RESULTS

Protein content [µg/ml] and RPLC fraction A to I from saliva in children with increasing age of children

Examination (E)	3	4	5	p value (Friedman-Test)
n	111	111	111	111
Total protein	1254 ± 476	1120 ± 462	1361 ± 866	0.002
Fraction				
A	371	417	559	< 0.001
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Saliva samples of children separated by cluster analysis (Ward) based on the protein content of the nine RPLC fractions

Examination (E)	3			4			5		
Cluster (C)	C1	C2	C2/C1	C1	C2	C2/C1	C1	C2	C2/C1
n	39	72	111	101	10	111	106	5	111
Total protein	815 ± 146	1492 ± 422	1.8	1011 ± 201	2218 ± 298	2.2	1223 ± 430	4303 ± 991	3.5
Fraction									
A	239	443	1.9	379	808	2.1	493	1966	4.0
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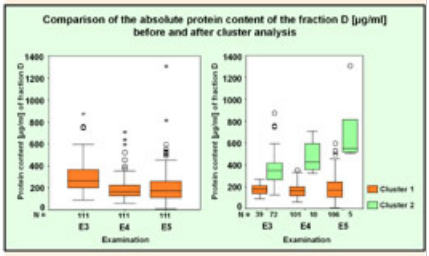
With a few exceptions the subject groups found by cluster analysis are statistically significant (U test, $p < 0.05$)

Comparison of the absolute protein content of the fraction D [µg/ml] before and after cluster analysis



Caries data of 111 subjects separated by cluster analysis into two groups using the protein content of nine saliva fractions

Examination (E)	3		4		5	
Cluster (C)	C1[1]	C2[2]	C1	C2	C1	C2
Clinical Parameter						
n	39	72	101	10	106	5
DMF/S	0.23	0.36	0.52	0.30	0.62	1.40
Δ DMF/S	-	-	0.19	0.20	0.32	0.38
Initial lesions	0.64	0.74	0.50	0.60	1.00	1.60
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[1] Cluster 1 [2] Cluster 2

CONCLUSIONS

- Quantitative changes in salivary protein content were observed during this study.
- The clusters formed using the content of nine saliva protein fractions showed differences in terms of protein content.
- These clusters were not related to the clinical data.
- Whether the protein concentration is changed during increasing age of children and subsequently influences the pellicle formation is subject to further work.

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