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A preliminary study on the relationship between stimulated saliva and periodontal conditions in community-dwelling elderly people.

Abstract

Objectives: The purpose of this study was to explore the relationship between flow rate and spinnbarkeit of stimulated whole saliva and periodontal conditions in healthy elderly people. Methods: 355 dentate subjects aged 76 years were included. The pocket probing depth (PD), attachment level (AL), and bleeding on probing (BOP) were measured. Stimulated whole saliva was collected and the salivary flow rate (SFR) was calculated. Then, salivary spinnbarkeit (SS) was immediately measured. Results: The mean SFR and SS were 1.44 ml/min and 1.91 mm, respectively. SFR was not significantly related to each periodontal parameter. On the other hand, subjects with SS $\leq$2.00 mm had a significantly lower mean AL (p<0.05). When subjects were divided into four groups according to a combination of SFR and SS, subjects with SFR <0.7 ml/min and SS >2.00 mm exhibited a significantly higher mean PD (p<0.05), % of sites with PD $\geq$4 mm (p<0.05), mean AL (p<0.01), % of site with AL$\geq$4 mm (p<0.05) and % of sites with AL$\geq$6 mm (p<0.01) than subjects in the other 3 groups (one-way ANOVA). In the logistic regression analysis, the factors significantly associated with the highest quintile of PD $\geq$4mm were: the low salivary flow and the high salivary spinnbarkeit (OR 3.84), current smokers (OR 5.08), cleaning interdental spaces rarely/never (OR 2.12), and frequent BOP (OR 5.20). Conclusion: These findings suggest that high salivary spinnbarkeit in addition to a low salivary flow rate might be a high risk for periodontal disease in elderly people.

Table 1. The mean values on salivary parameters by gender, number of prescription medications, and number of systemic diseases

subject characteristics		n	salivary flow rate (ml/min)		spinnbarkeit of saliva (mm)	
gender						
	male	188	1.62±0.93	***	1.95±0.42	NS
	female	167	1.23±0.65		1.87±0.31	
number of medications						
	0	152	1.50±0.83	NS	1.92±0.35	NS
	1-4	129	1.40±0.80		1.90±0.37	
	5≤	74	1.38±0.87		1.91±0.42	
number of diseases[1]						
	0	78	1.54±0.84	NS	1.92±0.39	NS
	1-2	246	1.41±0.84		1.92±0.38	
	3-5	30	1.43±0.76		1.86±0.27	

Mean values ± SD are given.
***p<0.001, NS: not significant. P values between genders were obtaind from student t-test and in other variables from one-way analysis of variances.
[1] Data missing for 1 subjects.

Table 2. The mean values on periodontal parameters by gender, number of teeth present, smoking status, and oral hygiene

subject characteristics	n	mean PD (mm)	% sites with PD ≥4 mm	% sites with PD ≥6 mm	mean AL (mm)	% sites with AL ≥4 mm	% sites with AL ≥6 mm	% sites with BOP
gender								
male	188	2.2±0.5 ***	12.3±12.9 ***	2.1±4.4 **	3.6±1.1 ***	44.0±29.5 ***	11.1±16.2 ***	11.5±12.4 NS
female	167	2.1±0.4	7.9± 9.4	1.0±2.5	3.0±0.8	28.9±24.2	4.0± 7.7	12.4±13.9
number of teeth present								
1-9	73	2.3±0.6 *	14.1±15.9 **	3.0±6.2 ***	4.1±1.3 ***	58.8±29.1 ***	17.7±20.9 ***	18.5±17.4 ***
10-19	97	2.2±0.5	11.1±11.0	1.5±2.8	3.4±0.9	40.6±27.3	8.5±12.3	11.7±12.8
20-32	185	2.1±0.4	8.2± 9.2	1.0±2.4	2.9±0.7	26.3±22.1	3.4± 6.1	9.4±10.0
smoking status[1]								
current	45	2.4±0.5 ***	15.8±12.8 ***	1.4±2.0 *	3.8±1.1 ***	49.2±29.1 ***	13.7±19.5 ***	9.2± 9.5 NS
former	121	2.2±0.5	11.8±13.5	2.2±4.9	3.5±1.1	42.5±29.4	10.2±15.5	13.1±13.9
never	187	2.1±0.4	7.8± 9.1	1.1±3.0	3.0±0.8	29.9±24.8	4.7± 8.6	11.7±13.2
interdental cleaning[2]								
daily/frequently	183	2.0± 0.4 ***	7.1± 8.2 ***	1.0± 2.3 **	3.0± 0.9 ***	29.9±26.2 ***	5.4± 9.6 ***	7.7± 9.8 ***
rarely/never	169	2.3± 0.5	13.5±13.6	2.2± 4.7	3.6± 1.1	43.7±28.1	10.2±16.3	16.5±14.6
last dental visit[2]								
within 1 year	248	2.1± 0.5 NS	9.6 ±11.2 NS	1.5± 3.3 NS	3.2± 0.9 NS	34.8±27.4 *	7.1±12.3 NS	10.6±12.6 **
more than 1 year	104	2.2± 0.5	11.8±12.3	1.8± 4.5	3.5± 1.1	41.3±28.9	9.2±15.8	15.1±13.6

Mean values ± SD are given.
***p<0.001, **p<0.01, *p<0.05, NS: not significant. P values in variables with two alternatives were obtaind from student t-test and in variables with three alternatives from one-way analysis of variances.
[1] Data missing for 2 subjects.
[2] Data missing for 3 subjects.

Table 3. The mean values on periodontal parameters by salivary flow rate (SFR) ↑

periodontal parameters	SFR <0.7 (n=73)	SFR ≥0.7 (n=282)	
mean PD (mm)	2.3± 0.5	2.1± 0.5	NS
% sites with PD ≥4 mm	12.5±13.6	9.6±10.9	NS
% sites with PD ≥6 mm	2.0± 4.9	1.4± 3.3	NS
mean AL (mm)	3.3± 1.2	3.3± 1.0	NS
% sites with AL ≥4 mm	36.7±27.8	36.9±28.2	NS
% sites with AL ≥6 mm	8.7±15.4	7.5±12.9	NS
% sites with BOP	12.1±11.5	11.9±13.5	NS

Mean values ± SD are given.
NS: not significant. P values were obtaind from student t-test.

Table 4. The mean values on periodontal parameters by salivary spinnbarkeit (SS) ↑

periodontal parameters	SS ≤2.00 (n=275)	SS >2.00 (n=80)	
mean PD (mm)	2.1± 0.5	2.2± 0.5	NS
% sites with PD ≥4 mm	9.6±10.9	12.4±13.6	NS
% sites with PD ≥6 mm	1.5± 3.5	1.6± 4.4	NS
mean AL (mm)	3.2± 1.0	3.5± 1.1	*
% sites with AL ≥4 mm	35.5±28.0	41.6±28.2	NS
% sites with AL ≥6 mm	7.4±12.8	9.0±15.3	NS
% sites with BOP	11.3±12.5	14.0±14.7	NS

Mean values ± SD are given.
*$p<0.05$, NS: not significant. P values were obtaind from student t-test.

Table 5. The mean values on periodontal parameters by salivary flow rate (SFR) and salivary spinnbarkeit (SS) ↑

periodontal parameters	SS ≤2.00		SS >2.00		
	SFR <0.7 (n=56)	SFR ≥0.7 (n=219)	SFR <0.7 (n=17)	SFR ≥0.7 (n=63)	
mean PD (mm)	2.2± 0.5	2.1± 0.5	2.5± 0.6	2.2± 0.5	*
% sites with PD ≥4 mm	10.6±11.3	9.3±10.8	18.7±18.3	10.6±11.6	*
% sites with PD ≥6 mm	1.5± 3.2	1.5± 3.5	3.7± 8.1	1.1± 2.4	NS
mean AL (mm)	3.1± 0.8	3.3± 1.0	4.2± 1.7	3.3± 0.8	**
% sites with AL ≥4 mm	31.3±24.6	36.6±28.7	54.3±31.3	38.2±26.5	*
% sites with AL ≥6 mm	5.4± 7.6	7.9±13.8	19.8±26.4	6.1± 8.7	**
% sites with BOP	10.5± 9.6	11.5±13.1	17.5±15.3	13.1±14.5	NS

Mean values ± SD are given.
**p<0.01, *p<0.05, NS: not significant. P values were obtaind from one-way analysis of variances.

Table 6. Multivariate logistic regression analysis to explore factors for subjects with PD ≥4 mm in the highest 20th percentile ↑

independent variables	odds ratio	*p*	95% CI
subjects with			
SFR ≥0.7 and SS ≤2.00 (ref.)	1.00		
SFR <0.7 and SS ≤2.00	1.74	0.197	0.75-4.01
SFR ≥0.7 and SS >2.00	1.56	0.263	0.72-3.37
SFR <0.7 and SS >2.00	3.84	0.028	1.15-12.77
gender (0: female, 1: male)	0.88	0.811	0.31-2.50
number of teeth present			
1-9	1.37	0.426	0.63-2.94
10-19	1.16	0.701	0.55-2.42
20-32 (ref.)	1.00		
smoking status			
current	5.08	0.007	1.57-16.44
former	2.16	0.155	0.75-6.26
never (ref.)	1.00		
number of medications			
0 (ref.)	1.00		
1-4	0.84	0.641	0.40-1.75
5≤	0.60	0.278	0.24-1.51
number of diseases			
0 (ref.)	1.00		
1-2	1.70	0.215	0.74-3.92
3-5	0.48	0.403	0.09-2.68
interdental cleaning (0: daily/frequently, 1: rarely/never)	2.12	0.028	1.08-4.16
last dental visit (0: within 1 year, 1: more than 1 year)	1.16	0.661	0.59-2.28
% sites with BOP (0: ≤19.4%, 1: >19.4%)	5.20	0.000	2.58-10.46

dependent variable: subjects with PD ≥4 mm in the highest 20th percentile
N=350 ; p <0.001, Pseudo R^2=0.19

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「地域住民の口腔保健と全身的な健康状態の関係についての総合研究」

C．研究協力課題名　：　「高齢者における *Streptococcus mutans* 歯表面付着阻害抗体と歯周疾患および血液検査値との関係」

研究要約：近年、*Streptococcus mutans* の歯表面付着を阻害する PAc(361-386)抗体が明らかになり、その抗体とバイオフィルム形成との関係が注目されるようになった。歯周疾患もバイオフィルムの形成と密接な関係があることからも、この抗体が歯周疾患にもなんらかの関係がある可能性が考えられる。そこで、新潟市で行われている厚労省科学研究の対象者である77歳の高齢者281名を無作為に抽出し調査対象とし、唾液 PAc(361-386)抗体と歯周検査値｛付着歯肉の遊離量(AL)、歯周ポケットの深さ(PD)、プロービング時の出血(BOP)の有無｝および様々な血液検査値との関係について検討を行った。その結果、抗体を有することとALの減少とに有意な関係が認められ、女性においてはALに加えてBOPやPDの減少とも有意な関係が認められた。また、抗体と好酸球および好塩基球との間に逆相関関係も認められ、このことから、唾液 PAc(361-386)抗体の産生は、歯周疾患の進行および全身状態に何らかの関係があることが明らかとなった。

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目的：口腔バイオフィルムは、口腔微生物が歯面および口腔内表層に付着し一部の細菌が菌体外に多糖体を産生し、その内側で増殖コロニーを形成した後組織表面をフイルム状に被覆したものである。このバイオフィルムの形成に深く関与しているのがミュータンスレンサ球菌(MS菌)や嫌気性菌などの口腔細菌である。我々は、MS菌の中で特に *Streptococcus mutans* の歯表面への付着に深く関わる菌体表層蛋白質抗原（PAc）に着目して、付着領域や抗原性を有する領域の解析を行った。その結果、PAc のアミノ酸残基番号 361-386 領域は、*S. mutans* の歯表面への付着部分であるのと同時にヒト HLA-DR 分子に結合するマルチなモチーフを含んでいること明らかにした。PAc (361－386) ペプチドに対する唾液 IgA 抗体価を測定すると、抗体価の高いグループは低いグループに比べ、唾液中の *S. mutans* が少なくなっていることを明らかにした（参考文献１）。そこで、本研究ではこの抗体と全身状態と歯周検査における指標とがどのように関係するか解析をおこない、歯周疾患におけるこの抗体検査の意義について全身

疾患との関係も併せて検討することを目的とした。

対象：
新潟市で行われている厚労省科学研究の対象者である77歳の高齢者281名（女性118名、男性163名）を無作為に抽出し、調査対象とした。

方法：
抗体価の測定
それぞれの被験者から3分間パラフィンガムを咬ませ唾液を採取し、PAc(361－386)ペプチドに対する唾液IgA抗体価をELISA法にて測定した。抗体価は、OD405で0.1を越える2^n乗最小希釈値で表した。

歯周検査
歯周検査は、1歯につき6点、全歯についてプロービング時の出血(BOP)の有無、歯石の付着部位の割合（CA)、歯周ポケットの深さ（PD)、付着歯肉の遊離量（AL，セメントエナメルジャンクションからポケット底部までの距離）の測定をmm単位で行った。

血液検査
被験者の静脈血10mlをヘパリンを入れたガラス管に採取し、検査会社である新潟臨床にて白血球数、赤血球数、ヘモグロビン値、ヘマトクリット、血小板、MCV、MCHC，総IgA、白血球の中の好酸球の割合（%)、好塩基球(%)、リンパ球(%)、単級(%)、好中球（%）を算定した。

結果：抗体価の高いグループ（$>2^2$）と低いグループ（$<2^2$）に分け、1人あたりの歯石付着部位、BOP、最大PD 4，6mm以上、最大AL 4，6mm以上を有する部位のそれぞれの割合において、グループ間の比較を行った。その結果、AL値（6mm以上：低；14.7±22.9%，高；8.7±14.1%)において、5%危険率でグループ間の有意差が認められた。女性においては、平均AL値（低；4.1±3.4，高；3.4±1.0)、AL値（6mm以上：低：22.1±32.6%，高；9.0±13.8%)、BOP値（低；16.8±17.2%，高；8.9±11.1%)、平均PD値（低；2.5±0.7%，高；2.2±0.5%）において5%危険率で有意差が認められた。また、好酸球率（低；4.0±2.0%，高；2.5±1.8%，相関係数-0.119)、好塩基球率(低；0.8±0.3%，高；0.5±1.3%、相関係数-0.195）において5%危険率で有意差が認められた。

考察：PAc（361－386）ペプチドに対する唾液IgA抗体を有する高齢者では、付着歯肉の遊離量が減少傾向であることが明らかとなった。女性においては、ALに加えPDやBOPの減少にも抗体が関係することが明らかとなった。さらに、自己免疫疾患の指標にもなる好酸球と好塩基球とも抗体は逆相関を示した。この抗体は唾液*S. mutans*量を減少させることが明らかになっているが、この高齢者群ではそのような傾向は認められなかった。本研究の抗体のある高齢者群は、抗体が少しの被験者でもその群に含まれているため、*S. mutans*の感染量に比例して抗体が誘導された被験者と抗体による*S. mutans*の減少した被験者の混合した群になっていると考えられる。よって、抗体の付着歯肉に対する関係は、*S. mutans*の量に依存しないことが示唆され、別の理由があることが考えられる。一般的にも、歯

周ポケットの形成や歯周組織の炎症には、*S. mutans* 依存的なバイオフィルムが関与していないと考えられる。一方、女性において抗体が好酸球や好塩基球と逆相関を示したことは、女性に特有の全身状態に抗体誘導が複雑に関与した可能性が考えられる。歯科臨床において、う蝕や歯周病予防を効率よく行うためには口腔疾患の予知予後がわかるような検査方法が求められ、それを開発する必要がある。いずれにしても、本研究の唾液抗体を検査することは、高齢者の口腔疾患の状態を把握するために有用となるかもしれない。

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投稿論文

1. Yasuhiko Saotome, Akio Tada, Nobuhiro Hanada, Akihiro Yoshihara, Hiroshi Uematsu, Hideo Miyazaki and Hidenobu Senpuku Role of cariogenic bacteria in periodontal status and root surface caries in Japanese elderly
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2. Hidenobu Senpuku, Akio Tada, Ryoma Nakao, Hideo Yonezawa, Saori Yoneda, Akihiro Yoshihara and Hideo Miyazaki. Relationships of anti-PAc (361-386) peptide salivary IgA antibody, eosinophils, and basophils with periodontal status in elderly. Submitting to FEMS Immunology and Medical Microbiology.

Relationships of anti-PAc (361-386) peptide salivary IgA antibody, eosinophils, and basophils with periodontal status in elderly

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Running title: Role of anti-PAc (361-386) peptide antibody

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Abstract

The amino acid residue 361-386 of *Streptococcus mutans* PAc includes an important region associated with the interaction between *S. mutans* and the salivary components. We investigated the relationships between levels of the anti-PAc (361-386) peptide antibody (PPA) in saliva and periodontal status in 281 elderly subjects (mean age, 77 years old; 118 females, 163 males) by assessing dental calculus (CA), attachment loss (AL), pocket depth (PD), bleeding on probing (BOP), and various blood parameters. ELISA results showed that subjects with a PPA level greater than 0.1 (PPA detected group) showed a lower average value for number of sites with more than 6 mm of AL/6 points x 100/tooth (rAL6) than those with a PPA level less than 0.1 (PPA not detected group). Further, the average values for BOP, AL, and rAL6 were significantly lower in the PPA detected group, and correlated positively with the percentage of eosinophils present in leukocytes in female subjects in both groups. PPA level had a negative correlation with the percentages of basophils and eosinophils. Our results indicate that systemic increases in numbers of eosinophils and basophils are associated with the development of periodontal diseases, while PPA level may be a useful indicator of periodontal status.

Introduction

In Japan, the majority of elderly people suffer from periodontitis (3), which is a major factor in tooth loss (1, 2). However, at present there is no known useful indicator or predictor of periodontal status. Periodontal diseases are chronic inflammatory conditions that affect the well-vascularized connective tissues of the periodontium (4). At the end of the previous decade, aggressive periodontitis was redefined as a complex disease exhibiting microbial alterations and cellular dysfunction that is differentiated from chronic periodontal disease by the underlying molecular mechanisms of its pathogenesis (5). Periodontal inflammation may have an effect on and worsen systemic conditions associated with leukocyte migration from the blood stream into tissues at the site of inflammation, and several prominent neutrophils have been implicated as amplifiers of that inflammatory response (6, 7).

In the human oral cavity, gram negative anaerobic organisms reside in a complex mixed-species biofilm that forms on tooth surfaces and in periodontal pockets, with *Porphyromonas gingivalis*, a gram-negative anaerobe, recognized as one of the primary pathogens in severe manifestations of adult periodontitis (8, 9). Recently, Lamont *et al.* reported that the surface protein antigen of *Streptococcus gordonii* (SspB), which is also a member of the highly conserved PAc (10) in *Streptococcus mutans*, interacted with

fimbriae from *P. gingivalis*, as shown by the results of *in vitro* assays (11, 12). *S. gordonii* and *Streptococcus sanguinis* are early colonizers of the salivary pellicle, while *S. mutans* colonizes later. However, the abilities of each to bind to salivary proteins and glycoproteins as well as the differences in affinity to the salivary pellicle between *P. gingivalis* and *S. mutans* may be related to the virulence of supra- or sub-gingival micro flora in dental diseases.

S. mutans has been reported to have an association with the development of biofilm and dental caries on tooth surfaces (13, 14). The function of the cell surface protein antigen of *S. mutans*, also known as PAc (15), Ag I/II (16), PI (17), and B (18), is essential for colonization by the bacterium on tooth surfaces as well as its interaction with the salivary pellicle that coats dental enamel (19, 20, 21). The alanine-rich repeating region (residue 219-464, A-region) of the PAc molecule, which is important for bacterial interaction with the salivary pellicle (22, 23, 24), has a strong immunogenicity in humans (25) and has been proposed as a candidate antigen for inducing the production of antibodies that inhibit the adherence of *S. mutans* to tooth surfaces (26, 27). The PAc (361-377) peptide in the A-region containing the epitope has also been shown to induce an antibody that inhibits the interactions of *S. mutans* with salivary components on tooth surfaces and is considered important for the adherence of

S. mutans to tooth surfaces (26, 28). The overlapped area of the PAc (370-386) peptide to the PAc (361-377) peptide includes a multiple binding motif (L - - V - K - A) that reacts with human leukocyte antigen (HLA)-*DRB1*0802, *1101, *1402*, and **1405* genotypes, and is also found in the A-region (29). The high production of salivary IgA antibody levels in reaction to the coupled PAc (361-386) peptide from residues 361-377 and 370-386 was reported to be a unique indicator of population and proportion of mutans streptococci (mS), such as *S. mutans* and *Streptococcus sobrinus*, because low and high concentrations of the salivary antibody were found to be correlated positively and negatively, respectively, with the concentrations of mS in saliva from human subjects (30).

In our search for a new indicator or predictor of periodontal diseases, the present study analyzed the relationships between the production of anti-PAc (361-386) peptide salivary IgA antibody (PPA), blood status, and periodontal status. PPA level was found to be associated with attachment loss (AL) in both males and females, and bleeding on probing (BOP) and pocket depth (PD), as well as concentrations of total streptococci (tS), lactobacillus (LB), and basophil in females. Our results may provide important information of PPA for the development of preventive medicines for periodontal diseases.

Materials and Methods

Human subjects

In 1998, a longitudinal interdisciplinary study of aging was initiated to evaluate the relationships between health status and dental diseases, such as root caries and periodontal disease, in Japan. Initially, questionnaires were sent to all 4542 residents aged 70 years old (born in 1927) in Niigata City. After dividing by sex, 600 subjects were selected randomly, with approximately the same numbers of each sex chosen for the baseline survey (31, 32). The participants agreed to undergo medical and dental examinations, and signed informed consent forms regarding the protocol, which was approved by the Ethics Committee of Niigata University Graduate School of Medical Dental Science. The study was carried out according to the Helsinki Declarations. Follow-up surveys have been carried out every year in June using the same methods as in the baseline survey. Among the participants (n=399) in the follow-up survey conducted in June 2005, 281 subjects (average age, 77 years old; 118 females, 163 males) participated in the present measurement of salivary antibodies.

Dental examinations were conducted under artificial white light by trained dentists. According to WHO criteria (33), decayed teeth (DT), missing teeth (MT), and filled teeth (FT) (DMFT) scores were recorded along with findings of dental caries.

Four calibrated dentists assessed subject periodontal conditions based on the results from 6 measurements points (mesio-buccal, buccal, disto-buccal, mesio-lingual, lingual, disto-lingual) around each tooth. Intra- and inter-examiner reliability was confirmed using a kappa statistic (k=0.56-0.92 for AL). To estimate periodontal status, dental calculus (CA), AL, BOP, and PD were also measured at the same 6 points of each tooth (34). Thereafter, the indicators were assessed and used to estimate the periodontal status of each subject, according to the methods explained below.

Synthetic peptide

The sequences of PAc (361-386) (NAKATYEAALKQYEADLAAVKKANAA) and PAc (346-364) (AALTAENTAIKQRNENAKA) were derived from the sequence of the PAc gene from *S. mutans* MT8148, as reported by Okahashi *et al.* (10). The peptide was synthesized using a stepwise solid phase procedure at Asahi Techno Glass Co. Inc. (Tokyo, Japan). Synthesized peptide samples were subsequently purified by reversed-phase high-performance liquid chromatography (HPLC) on a TSK-GEL column (1 x 30 cm) (TOSO, Tokyo, Japan) with a 10% to 45% acetonitrile gradient in 0.1% trifluoroacetic acid (TFA) and developed over 50 minutes at a flow rate of 5 ml/minute. Purity was