CAL. The kappa (within plus or minus 1 millimeter) ranged from 0.77 to 1.00 for PPD and from 0.62 to 1.00 for CAL.

Data analysis

Out of 260 non-smokers, 229 subjects who had at least one tooth intact were selected. Consequently, 13,289 sites (5,110 sites in the maxilla and 8,179 sites in the mandible) in the selected subjects were included in the analysis. Periodontal disease progression was defined as a site exhibiting an additional attachment loss of 3 mm or more (AAL $\geq$ 3 mm) from the baseline to final examination. Univariate statistical analyses were performed to describe the prevalence of BOP and PPD of $\geq$ 4 mm at baseline examination, and AAL $\geq$ 3 mm over the 3-year period. Then, BOP frequency was calculated by the total number of sites presented with BOP (+) in the 4 examinations (1 at baseline and 3 times at annual recalls) for all sites and divided into five categories as follows.

0/4 – BOP + at none of 4 examinations

1/4 – BOP+ at 1 out of 4 examinations

2/4 – BOP+ at 2 out of 4 examinations

3/4 – BOP+ at 3 out of 4 examinations

4/4 – BOP+ at all 4 examinations

The proportion of sites with AAL of $\geq$ 3 mm over 3 years by different frequencies of BOP was calculated and Chi-square analysis was used to determine statistical

significance. In addition, the backward stepwise logistic regression analysis was performed to evaluate the relationship between BOP frequency and periodontal disease progression. The dependent variable, periodontal disease progression, was defined as sites exhibiting AAL ≥ 3 mm. The independent variables used were gender, BOP frequency, PPD at baseline, jaw type (Upper or Lower), tooth type, tooth site and the number of missing teeth. The odds ratios with the 95% confidence intervals (CI) were calculated.

In the stepwise logistic regression analysis, $p<0.05$ was used as the entry criterion while $p>0.10$ was the removal criterion. The Hosmer and Lemeshow Goodness-of-Fit test statistic was fixed at $p>0.05$ (Hosmer & Lemeshow. 1982). The software of Statistical Package for Social Sciences (SPSS for Window, Release 11.5) was used for all calculations and analyses.

Results

Out of the total number of subjects selected, 69.2% (Females: 75%, males: 64%, N= 229) exhibited AAL ≥ 3 mm at one or more sites over 3 years. The mean number of sites with AAL ≥ 3 mm per person was 3.48 (SD±3.39) for females and 3.90 (SD±5.29) for males. However, there was no statistically significant difference between the mean number of sites with AAL ≥ 3 mm in females and males (Student t-test $p>0.50$).

Subject-based data analysis.

All baseline parameters have higher average value in subjects who exhibited additional attachment loss (Table 1). However, only subjects with percentage of sites bleeding on probing positive (% BOP positive) and percentage of sites PPD ≥ 4 mm, have significant correlation to AAL ≥ 3 mm (Student t-test, $P<0.001$). As a further analysis, a

multiple linear regression was employed to calculate each site contribution to AAL ≥ 3 mm for each subject as a dependent variable. Table 2 shows multivariate linier regression model for subjects followed over the 3-year period. In this regression model, those subjects with BOP positive; PPD ≥ 4 mm and number of remaining teeth at baseline were significantly associated with AAL ≥ 3 mm during 3 years observation.

Figure shows the relationship between the proportion of sites with AAL ≥ 3 mm and BOP frequency. A highly significant (p<0.001, Pearson Chi-square = 99.575) relation between the increasing frequencies of BOP and AAL ≥ 3 mm was observed. The proportions of disease progression in the 5 categories were 2.9% (0/4), 4.7% (1/4), 8.7% (2/4), 15.8% (3/4) and 26.4% (4/4), respectively.

Table 3 shows the results of the backward stepwise logistic regression analysis. Six variables (BOP frequency, PPD ≥ 4 mm, Upper-lower jaws, Molar, Inter-proximal, and number of missing teeth) remained significant in the final model. As shown in Table, BOP frequency was significantly associated with periodontal disease progression after controlling for 5 other variables. The estimated odds ratios of BOP frequency for periodontal progression were 1.47, 2.54, 4.39, 6.17 for 1/4, 2/4, 3/4, 4/4, respectively.

Discussion

The findings of this longitudinal study revealed a highly significant relationship between the increasing bleeding frequencies and periodontal disease progression in community-dwelling older non-smokers. In the stage of subject-based data analysis, which was showed in Table 1 and 2, BOP positive and PPD ≥ 4 mm had significant correction with periodontal disease progression, as well as the number of teeth.

Figure clearly shows that the sites, which have the highest score of bleeding frequency showed the greatest proportion of sites with periodontal disease progression during the period under study. Sites with a BOP frequency of 4/4 had a 26% chance of periodontal disease progression. This proportion is slightly higher than that observed by Badersten et al. (1990), and almost equal to that reported by Lang et al. (1986). The proportions of BOP frequency in either of these two studies were not more than 30%.

Furthermore, the findings of the backward stepwise logistic regression analysis showed that bleeding frequencies were positively associated with periodontal disease progression after adjusting for five other local factors. The odds ratios increased concomitantly with increasing frequencies of bleeding. The probability to have periodontal disease progression was approximately 2.5 times greater for sites, which experienced BOP 2/4 than for sites with no BOP. Moreover, sites presented with BOP 4/4 had 6.2 times greater chance of periodontal progression compared to sites that showed no BOP. This finding indicates that BOP frequency may be considered as a strong risk factor for periodontal disease progression in older people.

On the other hand, the finding that sites with BOP frequency of 0/4 had only 2.9% chance of periodontal disease progression pointed to the fact that the absence of BOP might be a criterion for periodontal stability. This is in agreement with the previous studies that BOP is a reliable indicator for periodontal stability (Lang et al. 1986, Lang et al. 1990).

We also evaluated the local factors for periodontal disease progression in this study. The prevalence of BOP, PPD $\geq$ 4 mm and AAL $\geq$ 3 mm tended to be higher in maxilla, molar, interproximal sites and lower anterior. The high prevalence of AAL $\geq$ 3 mm at

interproximal sites has also been observed by other studies (Schätzle et al. 2003, Heitz-Mayfield et al. 2003). In a longitudinal study in Chinese adults, similar findings were observed (Baelum et al. 1997).

In this study, although a significant relationship between BOP frequency and periodontal disease progression was seen in backward stepwise logistic regression analyses, Negelkerke *R* square was low. "Negelkerke R square" (pseudo R-square) is standard SPSS output were used as a measure of strength of association. It which similar to R-squared in linear regression models that shows how much of the dependent variable's variation is explained by independent variables. This finding indicates that the variability in periodontal progression explained by the 5 independent variables was low (5.5%) and hence, there may be other explanatory factors, which could not be accounted in the present study. Accordingly, further longitudinal studies that incorporate such unaccounted variables and span over more than 3 years may be required to investigate the influence of BOP on periodontal disease progression in community-dwelling older adults. .

Nevertheless, notwithstanding such limitations, the present study suggested that there was a significant relationship between the BOP frequency and periodontal disease progression and consequently, increasing frequencies of bleeding might increase the probability to have periodontal disease progression in community-dwelling older non-smokers.

Acknowledgments

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Table 1. Relation between baseline parameter and additional attachment loss of 3mm or more at 1 or more sites.

Baseline parameter	Subjects not exhibiting additional attachment loss Mean	SD	Subjects exhibiting additional attachment loss Mean	SD	P value
% BOP positive	5.71	6.30	9.95	11.89	***
% PPD 4+mm	5.17	6.67	10.65	12.38	***
% AL 5+mm	14.52	14.78	19.85	17.91	NS
% of remaining teeth	69.33	30.02	73.00	25.85	NS
% of intact teeth	7.23	5.09	7.64	5.07	NS

*** : P<0.001 NS : Not significant (Student t-test)

Table 2. Subjects base analysis with multiple linier regression and associated *p*-value

Dependent variable
% of sites exhibiting additional attachment loss 3+mm during 3 years

Baseline parameter	Coef.	Std.Err	*P* value	95% CI	
% BOP positive	0.125	1.217	0.002	0.048	0.202
% PPD 4+mm	0.124	0.039	0.001	0.051	0.196
% of remaining teeth	0.0049	0.14	0.001	0.078	0.021
Constant	5.919	0.37	0.000	3.522	8.315

Number of subjects: 260
Prob > F : 0.001
R-squared : 0.165

Table. Backward stepwise logistic regression analysis and associated *p*-values.

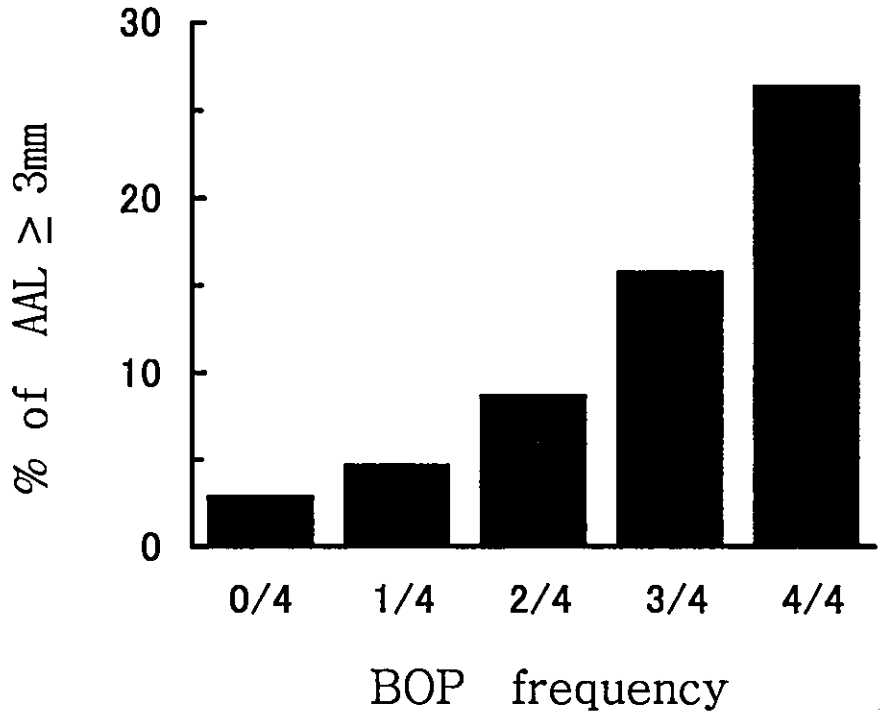
Independent variables	Estimated OR*	95% CI**	SE***	*p* value
Bleeding frequency				
0/4 (Reference)	1.00			0.000
1/4	1.47	1.12 – 1.93	0.14	0.005
2/4	2.54	1.72 – 3.75	0.20	0.000
3/4	4.39	2.50 – 7.70	0.29	0.000
4/4	6.17	2.21 –17.25	0.52	0.001
PPD at baseline ≥ 4mm	1.48	1.05 – 2.09	0.17	0.024
Upper-Lower				
Lower (Reference)	1.00			0.000
Upper	1.32	1.07 – 1.63	0.11	0.011
Molar	1.57	1.18 – 2.07	0.14	0.002
Interproximal	1.38	1.12 – 1.71	0.11	0.003
Number of missing teeth				
1-9 (Reference)	1.00			0.000
10-19	2.00	1.58 – 2.54	0.12	0.000
20+	2.51	1.72 – 3.65	0.19	0.000
Constant	0.02		0.10	0.000

N = 13,289; Chi-square = 24.756, *P*=0.000; Negelkerke R^2 = 0.055;
Hosmer & Lemeshow: *P*= 0.262
* Odds ratio
**Confidence interval
***Standard error

Figure legend

Figure.1. The proportion of sites which had AAL ≥ 3mm over 3 years by different BOP frequency.

Figure Rahardjo *et al.*



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B.指定課題名 ： 平成16年度医療技術評価総合研究事業
「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の歯周疾患に対する役割」

D. 研究協力者：
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E. 研究目的:

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が歯周検査における指標と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の意義について検討することを目的とした。

F. 研究方法:

新潟市で行われている厚労省科学研究の対象者である 73 歳の高齢者 590 名のうち 100 名を無作為に抽出し、調査対象とした。そのうちデータの得られた 87 名（男性 60 名）を分析対象者とした。さらに、3 年後対象者｛69 名（男性 46 名)｝のデータも合わせて調査した。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、OD_{405} で 0.1 を越える 2^n 乗最小希釈値で表した。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)、付着歯肉の減少量 (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。

G. 研究結果および考察:

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、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、BOP や PD 値において低いグループと高いグループに差が認められなかったが、歯石の付着（低；5.4±6.9%，高；1.3±2.0%）と AL 値（4 mm 以上：低: 38.2±27.7%，高；20.4±19.7%、6mm 以上: 低; 11.6±16.6%，高; 2.4±4.6%）においては 5%危険率で有意差が認められた。3 年後のデータにおいては、最大 AL 値（低；7.3±2.2，高；5.6±1.2)、平均 AL 値（低；3.7±1.0%，高；2.9±0.5%)、AL 値（4 mm 以上：低: 48.4±30.0%，高；22.5±15.9%）において 5%危険率で有意差が認められた。

PAc（361－386）ペプチドに対する唾液 IgA 抗体は、抗体価が高いほど歯石付着と付着歯肉の減少に関係しているが、ポケットの深さやプロービング時の出血には関与しないことが明らかとなった。しかし 3 年後のデータにおいては、抗体価が高い程歯石付着程度が低いものの有意差がなかった。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おり、*S. mutans* の減少による口腔バイオフィルム形成への影響が、歯石付着量に影響を与えた可能性がある。しかし、73 歳から 76 歳までの間ではその関係は維持しにくいと考えられる。一方、その抗体は付着歯肉量の減少に関与し 3 年後も持続し減少量が増加したことから、抗体の付着歯肉に対する密接な関与が示唆され、歯周病発症における物理的変化に関わっていると考えられた。一般的に、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。しかし、本研究の結果から *S. mutans* 依存的なバイオフィルムは、歯肉縁上および縁下歯石の形成量に関与し、また持続的に付着歯肉を減少させているの可能性も考えられた。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。この唾液抗体を検査することは、高齢者の口腔疾患の予知予後を的確に把握するために有用となるかもしれない。

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H. 投稿論文：

1. H. Senpuku, A. Tada, Y. Tsuha, M. Kozu, N. Kusumoto, A. Yoshihara and H. Miyazaki. Role of anti-PAc (361-386) peptide antibody for periodontal disease status in elderly. Submitting to Oral Microbiol Immunol.

2. H. Senpuku, A. Tada, Y. Saotome[1], N. Hanada, A. Yoshihara and H. Miyazaki. Role of cariogenic bacteria in periodontal and root status. Submitting to J. Dent. Res.

Role of anti-PAc (361-386) peptide antibody for periodontal disease status in elderly

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Key words: *S. mutans*, PAc, dental calculus, attachment loss, elderly

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

a) H. Senpuku, A. Tada, Y. Tsuha, M. Kozu, N. Kusumoto, A. Yoshihara and H. Miyazaki

b) Role of anti-PAc (361-386) peptide antibody for periodontal disease status in elderly

c) Oral Microbiology and Immunology

d) The amino acid residue 361-386 of *Streptococcus mutans* PAc is an important region of interaction between *S. mutans* and the salivary pellicle on the tooth surface. We investigated the relationship between titers of the anti-PAc (361-386) peptide antibody in saliva, which inhibits the adhesion of *S. mutans* to tooth surfaces, and periodontal status in 87 functionally independent elderly subjects (mean: 73 years old), by assessing dental calculus deposition, attachment loss, pocket depth and anti-PAc (361-386) peptide antibody titer. The subjects were divided into 2 groups according to anti-PAc (361-386) peptide antibody concentration, and those with a titer greater than 2^2 (antibody detected group) showed lower levels of dental calculus deposition than those with a titer less than 2^2 (antibody not detected group). Further, attachment loss was significantly smaller in the antibody detected group. There was no significant difference in pocket depth between the 2 groups. After 3 years, the difference of attachment loss expanded in comparison with identical subjects n the cohort study. Our results suggest that the anti-PAc (361-386) peptide

antibody may be indirectly involved in physical condition for development of periodontal disease, and an useful predictor for diagnosis of periodontal disease risk.

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Introduction

Streptococcus mutans has been reported to have an association with dental caries (8, 9). The function of the cell surface protein antigen of *S. mutans*, known as PAc (15), Ag I/II (17), PI (6), or B (19), is essential for colonization of the bacterium on tooth surfaces and its interaction with the salivary pellicle that coats dental enamel (4, 18, 20). The alanine-rich repeating region (residue 219-464, A-region) of the PAc molecule, which is important for the interaction of the bacterium with the salivary pellicle (2, 12, 27), has a strong immunogenicity in humans (24) and may be a candidate antigen for inducing the production of antibodies that inhibit the adherence of *S. mutans* to tooth surfaces.

Amino acid residue 365 to 377 {PAc(365-377) peptide} in the A-region is an antigenic epitope for the induction of antibodies that inhibit the interaction of *S. mutans* PAc with human salivary components (22). Further, the common epitope (YEA-L-QY) between the surface protein antigen (PAg) of *Streptococcus sobrinus* (15) and PAc, as well as its core B-cell epitope (-Y---L--Y----) are essential sequences in the antigenic epitopes of the PAc protein that are specifically recognized by the antibody (23). The PAc (361-377) peptide containing the epitope has also been shown to induce an antibody to inhibit *S. mutans* colonization and, therefore, is considered important for the

adherence of *S. mutans* to tooth surfaces (21, 26).

The overlapped PAc (370-386) peptide to 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 (25). The high production of salivary IgA antibody to the coupled PAc (361-386) peptide from residues 361-377 and 370-386 inhibited the number of mutans streptococci (MS, *S. mutans* and *S. sobrinus*) and MS/ total streptococci ratio in saliva samples (28). Therefore, the PAc (361-386) peptide is considered to be a minimum antigen of PAc required for induction of the antibody that specifically inhibits colonization of *S. mutans* and *S. sobrinus* on the tooth surfaces. in humans.

The presence of the anti-PAc (361-386) peptide antibody has been suggested indicate an immunological activity to induce antibody to MS after MS infection and to prevent formation of a MS – dependent biofilm that contains insoluble glucan in humans. The function of anti-PAc(361-386) peptide antibodies may be to prevent development of a sticky biofilm that incorporates incorporating various planktonic cells. The antibody may have a direct or indirect association with the growth condition of anaerobic microorganisms which are involved in periodontal disease formation on tooth surfaces. In the present study, we analyzed the relationship between anti-PAc (361-386)