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ORIGINAL ARTICLE

Screening for periodontal diseases using salivary lactate dehydrogenase, hemoglobin level, and statistical modeling

Yoshiaki Nomura [a], Yoh Tamaki [b*], Akiko Eto [c], Erika Kakuta [a], Daisuke Ogino [b], Yuichi Nakamura [d], Naoki Takahashi [e], Fumihiko Hino [d], Keizo Koresawa [d], Nobuhiro Hanada [a], Keita Shimizu [d]

[a] *Department of Translational Research, Tsurumi University, School of Dental Medicine, Kanagawa, Japan*
[b] *Center for Public Health Informatics, National Institute of Public Health, Saitama, Japan*
[c] *Department of Health Crisis Management, National Institute of Public Health, Saitama, Japan*
[d] *Ehime Dental Association, Ehime, Japan*
[e] *Health Promotion Division, Health and Welfare Department, Ehime Prefecture Government, Ehime, Japan*

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Abstract *Background/Purpose:* The intracellular enzymes present in the saliva have been studied as markers of periodontal disease. The aim of this study was to establish procedures for using salivary biomarkers as an alternative to the Community Periodontal Index (CPI) for community-based screening for periodontal disease.
Materials and methods: The study included 101 adults aged between 19 and 77 years who were undergoing treatment for periodontal disease. We applied analysis of covariance (ANCOVA) to analyze the relationship between Community Periodontal Index (CPI) and levels of salivary factors for the 101 volunteers.
Results: Demographic characteristics, including age, number of remaining teeth, and smoking habits, showed a significant correlation with CPI. An overall correlation was shown between CP and both salivary lactate dehydrogenases (LD) and hemoglobin (Hb) levels, when analyzed using continuous demographic variables as covariates.
Conclusion: Results indicated that screening with statistical modeling can be an effective tool for detecting periodontal disease.

* Corresponding author. Center for Public Health Informatics, National Institute of Public Health, 2-3-6 Minamai, Wako, Saitama 351-0197, Japan.
E-mail address: pxz11337@nifty.com (Y. Tamaki).

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Introduction

Periodontal disease is one of the most important diseases to screen for in community health check-up systems. Because it is a silent disease, it would be ideal to detect it at an early stage, even before the patient becomes aware of it. Conventionally, the methods of screening for periodontal disease are similar to those used for making clinical diagnoses: pocket probing of representative teeth recorded by dental professionals. A representative scoring system for screening for periodontal disease is the Community Periodontal Index (CPI).[1,2] Although the CPI program was developed for use in communities, only a limited number of qualified examiners can competently perform this test. For this reason, it would be better if screening could be done by non-experts. Furthermore, where screening for periodontal disease must be done in large numbers of subjects, considerable time and effort are needed to evaluate the attachment level and probing depth of one index tooth in each sextant. The dentists' fees increase the total expense of such screening because the test must be carried out by a trained dentist, thereby decreasing the overall cost-effectiveness of this approach.

To overcome these problems, we have developed a screening system for periodontal disease that uses salivary molecules.[3,4] Many reagents used for investigating periodontal disease are limited to research use and are generally expensive, although they have high sensitivities or specificities. In previous papers, we tested eight conventional markers that are widely used for routine medical examinations or mass screenings. The costs of measuring these molecules are effective for mass screening purposes. The molecules used in our previous studies were: aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LD), alkaline phosphatase (ALP), creatinine (CRE), blood urea nitrogen (BUN), urea (UA), and hemoglobin (Hb). In particular, LD and Hb in saliva are promising candidate biomarkers for periodontal disease. Both of these two molecules such as Hb and LD reflect, directly or indirectly, certain processes in the development of periodontal disease.

Despite previous screening studies, problems remain, such as low sensitivity and specificity for Hb and LD. For example, they were insufficiently specific and sensitive to fulfill the ideals proposed by Hausen.[5] One reason is that the results of probing used for the gold standard do not indicate whether periodontal disease has been arrested or is still active is because pocket depth is not an index of disease activity. Another is that the population we have investigated is not uniform in terms of demographic characteristics such as gender, age, and number of remaining teeth. However, the latter problem can be solved by statistical modeling. Accordingly, in this study, we screened for periodontal disease using these two markers, analysis of covariance (ANCOVA), and statistical modeling.

Materials and methods

Study populations

Study participants were drawn from populations of patients who sought care at 18 private dental offices belonging to the Ehime Dental Association. The study population consisted of 38 males (37.6%) and 63 females (62.4%) with a mean age of 42.62 $\pm$ 9.84 (range, 19—77) years who had periodontal disease. The participants had not taken antibiotics for at least 4 weeks and had not received any treatment for at least 12 weeks before saliva sampling. We excluded individuals who had systemic diseases, such as diabetes or hypertension, or who were taking medication. We enrolled patients who had more than 15 remaining teeth and lacked oral mucosal disease. The ethics committee of Tsurumi University approved this study.

Oral examination

Oral examinations were carried out by 18 dentists in their private dental offices. Before the start of the study, we held a training course in pocket probing calibration. One colleague who attended the ICS II (International Collaboration Study II) as an examiner was the instructor of the training course. The kappa value for probing depth was between 0.66 and 0.80. The number of remaining teeth and CPI were recorded. The CPI method was based on the standard model recommended by WHO.[6] The salivary biomarkers represent the whole tooth conditions. The highest CPI value of the patient was used for analysis.

The mean number of remaining teeth was 26.66 $\pm$ 2.44 (range, 19—27). CPI was coded from 0 to 4 using a WHO probe.

Questionnaire

The smoking behavior of each participant was investigated by a questionnaire survey. Individuals were categorized into three groups: nonsmoker, previous smoker, and current smoker. We examined correlations between smoking habits and other criteria: CPI and salivary levels of LD and Hb.

Analysis of salivary markers

Saliva was collected after obtaining written informed consent, but prior to the oral clinical examination. After 5 minutes stimulation by chewing on a gum base containing neither fragrance nor taste ingredients, whole saliva was collected from each volunteer and saliva volume was recorded. For the examination of salivary levels of LD and Hb, collected saliva was kept at 4 °C until the examination. Laboratory analysis of each saliva sample was carried out on the day of collection. LD was measured using commercially available kits (L type Wako LDH J; Wako Chemical Industry, Osaka, Japan) as previously described.[4] Salivary hemoglobin was measured using a commercially available kit (NESCAUTO SALIVAHEMO Plus; Alfresa Pharma, Osaka, Japan) and according to the manufacturer's instructions.

Statistical analysis

To investigate the correlation between CPI and continuous variables, including salivary levels of LD and Hb, the Kruskal—Wallis test was used. For categorical variables, the Fisher's Exact test was carried out. For analysis of covariables, salivary levels of LD and Hb were used as

Table 1 Correlation between demographic variables and CPI.

(A). Continuous variables.

CPI	0	1	2	3	4	Total	*P*
n	28	21	18	25	9	101	
Age	35.54 ± 9.17	42.0 ± 9.14	45.28 ± 6.35	44.68 ± 7.48	55.0 ± 9.22	42.62 ± 9.84	<0.001
Number of remaining teeth	27.25 ± 1.38	26.52 ± 2.44	27.17 ± 1.58	27.24 ± 1.45	22.56 ± 3.71	26.66 ± 2.34	0.001
Saliva volume (mL /5min)	7.88 ± 2.59	8.68 ± 2.31	8.29 ± 2.39	8.58 ± 2.83	7.63 ± 2.33	8.27 ± 2.53	0.727

(B). Categorical variables.

		CPI					Total	*P*
		0	1	2	3	4		
Gender	Female	23	14	10	13	3	63	0.047
	Male	5	7	8	12	6	38	
Smoking	Nonsmoker	25	15	12	19	3	74	<0.001
	Previous Smoker	3	0	4	4	0	11	
	Current Smoker	0	6	2	2	6	16	
Total		28	21	18	25	9	101	

P was calculated by the Kruskal—Wallis test.
P was calculated by Fisher's exact test.

objective variables, CPI was used as the fixed variable, and demographic variables that were statistically significant for CPI were used as covariates. To set the cut-off point, receiver operating characteristic (ROC) curves were plotted and the point of minimum difference between the sensitivity and specificity was defined as the cut-off point. We also calculated the area under the ROC curve (AUR), which indicates the precision of the screening. These analyses were carried out using SPSS version 14 (SPSS Japan Inc., Tokyo, Japan).

Results

We first analyzed correlations between CPI and demographic factors: age, number of remaining teeth, saliva volume, gender, and smoking habits (Table 1). Of these, smoking habits had a statistically significant correlation with CPI. Age also had a significant correlation with CPI—generally, older patients had more severe periodontal disease. An exception was that the mean age of patients diagnosed at CPI 2 was higher than for CPI 3. For the number of remaining teeth, differences between groups (CPI 0—3) were slight, except for CPI 4, for which the mean value was drastically decreased. There was a tendency for males to have more severe periodontal disease.

Second, we examined salivary levels of LD and Hb for all patients, and analyzed their relationships with other criteria. In a non-adjusted analysis, salivary levels of LD and Hb had no statistically significant correlation with CPI (Table 2), and sensitivity and specificity for screening did not reflect significantly different values, regardless of the cut-off point chosen (Table 3). We then used ANCOVA to adjust demographic factors for significant correlations with CPI. We applied continuous variables, age and number of remaining teeth as covariates. As shown in Table 2, adjusted LD and Hb showed statistically significant correlations with CPI. Using the reset cut off points, we plotted ROC curves and calculated the areas under the ROC curve (AURs), which indicates the accuracy of the screening (Table 3). The AURs for both LD and Hb were more than 0.7, except for the case of CPI 4, indicating that salivary levels

Table 2 Salivary levels of LD and Hb, crude and adjusted by age and number of the remaining teeth.

	CPI					Total	*P*
	0	1	2	3	4		
Crude LD	171.2 ± 20.6	245.5 ± 37.8	192.9 ± 57.2	271.0 ± 148.4	290.7 ± 188.1	225.7 ± 105.7	0.167
Adjusted LD	171.2 ± 20.5	245.5 ± 32.7	192.9 ± 49.1	271.0 ± 122.3	290.7 ± 184.2	225.7 ± 95.5	<0.001
Crude Hb	1.4 ± 2.4	3.5 ± 9.8	2.5 ± 5.5	6.3 ± 20.2	3.4 ± 8.1	3.4 ± 11.6	0.705
Adjusted Hb	1.4 ± 1.1	3.7 ± 2.2	2.5 ± 1.7	6.3 ± 7.3	3.4 ± 1.6	3.5 ± 4.3	<0.001

P was calculated by the Kruskal—Wallis test. Adjustments were carried out by analysis of covariance (ANCOVA). After adjustment, differences in both LD and Hb were statistically significant. For LD, dose response relationships could not be obtained from CPI 1 to 3, even after adjusting for age and number of remaining teeth. Hb of CPI3 was higher than that of CPI 4, because one patient diagnosed as CPI 3 had high levels of Hb (99.05 μg/mL). This effect is reflected in the standard deviation.

Table 3 Cut off points for LD and Hb for screening for periodontal disease.

	Cut off point	AUR	Sensitivity	Specificity	Positive predictive value	Negative predictive value	Cut off point	AUR	Sensitivity	Specificity	Positive predictive value	Negative predictive value
	Crude LD						Adjusted LD					
CPI 1	177.5	0.624	0.575	0.571	0.778	0.571	204.1	0.963	0.903	0.893	0.956	0.893
CPI 2	182.5	0.511	0.519	0.531	0.540	0.531	201.6	0.713	0.731	0.729	0.745	0.729
CPI 3	182.5	0.533	0.529	0.522	0.360	0.522	208.6	0.807	0.794	0.803	0.675	0.803
CPI 4	217.5	0.617	0.556	0.652	0.135	0.652	229.7	0.713	0.667	0.670	0.167	0.670
	Crude Hb						Adjusted Hb					
CPI 1	2.493	0.546	0.534	0.536	0.750	0.536	2.244	0.872	0.833	0.750	0.896	0.750
CPI 2	2.945	0.529	0.519	0.551	0.551	0.551	2.877	0.737	0.731	0.583	0.655	0.583
CPI 3	3.250	0.571	0.559	0.582	0.404	0.582	3.185	0.755	0.706	0.773	0.615	0.773
CPI 4	3.865	0.607	0.556	0.739	0.172	0.739	3.556	0.640	0.656	0.593	0.069	0.407

AUR = Area Under the ROC curve. After adjustment, sensitivities, specificities, positive predictive values and negative predictive values were all significantly increased. For LD, the cut off point for CPI 2 did not have the dose response relationship.

of LD and Hb may be useful for screening for periodontal disease, especially for CPI 1 and CPI 3. Another factor that correlated with CPI, smoking, was also entered as a covariate, but it improved neither specificity nor sensitivity (data not shown), consistent with the general idea that categorical variables are not suitable for this statistical analysis. We also confirmed that a smoking habit by itself did not affect salivary levels of LD and Hb (Table 4).

Discussion

In this study, we found an overall correlation between salivary factors and CPI. Firstly, demographic characteristics, including age, number of remaining teeth, and smoking habits, showed a significant correlation with CPI. In particular, the influence of smoking on periodontal diseases was explicit, despite the small numbers of smokers. It was consistent with the general idea that smoking is one of the most important risk factors for periodontal disease.[7] We also showed that salivary LD and Hb levels correlated with CPI when they were analyzed using continuous demographic variables as covariates. Hb in saliva may reflect bleeding from the oral cavity. According to Martí GA et al,[8] none of a total of 101 edentulous patients exhibited hemoglobin in saliva. In contrast, among 83 dentulous patients with clinically healthy gums, 67% of the dentulous patients tested positive for hemoglobin in saliva. This study indicated that bleeding from oral mucosa, but not from periodontal tissue, may be rare. LD is a deviation enzyme and is derived from destroyed cells. These molecules could be derived from inflamed periodontal tissue by the stimulation of gum chewing. In this study statistically significant correlation between LD and Hb was not obtained ($r = 0.055$, $P = 0.583$). The mechanisms by which these molecules are released into saliva may involve different pathways. To confirm this hypothesis, further basic studies or animal experiments are necessary.

Table 4 Salivary levels of LD and Hb in groups with different smoking status.

	Smoking status	N	Mean	*P*
LD	No	74	226.7 ± 185.9	0.920
	Previous	11	223.6 ± 145.9	
	Current	16	218.3 ± 98.8	
	Total	101	225.0 ± 169.8	
Hb	No	74	3.3 ± 12.8	0.806
	Previous	11	6.6 ± 9.4	
	Current	16	2.0 ± 6.2	
	Total	101	3.4 ± 11.6	

P was calculated by the Kruskal–Wallis test.

The mean values of LD and Hb were sufficient to distinguish subjects with CPI 1 and CPI 3. In contrast, dose–response relationships were not shown for CPI 2. This may be because calculus, a distinctive feature of CPI 2, may not be a hallmark for the inflammation and progression of periodontal disease. Therefore, it is logical that this factor would show a correlation only with CPI 1 and CPI 3. As for LD, it will increase in saliva in proportion to cell death caused by periodontal disease, as previously described.[9] Other groups also reported a correlation between LD levels in oral fluid and periodontal disease.[10,11]

With improved accuracy, the screening method described here could be an effective tool for detecting periodontal disease prior to a patient's awareness of it. It could thus contribute to promoting primary and secondary prevention, which are important countermeasures against periodontal diseases. However, we employed limited salivary biomarkers and a small sample size in this study. These questions would be better settled by a further study with a larger sample size.

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