Predication of the prognosis after initial periodontal treatment by salivary biomarkers

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Purpose: The aim of this study was to predict the necessity of periodontal pocket curettage by the salivary levels of hemoglobin and lactose dehydrogenase at baseline.
Materials and Methods: The study population was selected from the subjects with chronic periodontitis who attended eight private dental offices. The study population consisted of 14 men and 32 women, and their mean age was 53.9+/-15.4. Patients with periodontal pockets deeper than 4 mm after initial treatment were diagnosed as necessity pocket curettage (Group II: *n*=31). Other patients were once finished periodontal therapy and recommended to visit for the supportive periodontal therapy (SPT) (Group I: *n*=15). Salivary levels of lactose dehydrogenase and hemoglobin were measured using commercially available kits, according to the manufacturer's instructions.
Results: For predict the prognosis of the initial treatment, statistically significant differences were not observed in clinical markers. In contrast both hemoglobin and lactose dehydrogenase, mean values were significantly higher in Group II patients. In addition, 94.4% patients were necessary of the periodontal curettage if the lactose dehydrogenase value was more than 333.3 IU/L at baseline.
Conclusion: Salivary levels of lactose dehydrogenase at baseline may be useful markers for the prediction of the necessity of pocket curettage after initial periodontal therapy.

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Key Words: initial periodontal therapy, lactose dehydrogenase, prognosis, saliva

Introduction

Periodontal disease is one of the most prevalent oral diseases among the middle-aged and elderly population. Diagnosis, assessment, and evaluation of periodontal disease are based on the visual examination and manipulation. This method check the morphologically changed periodontium caused by the inflammation originated from bacterial infection. It is obstacle to detect initial stage of periodontal disease, to check the subtle changes of the inflammation and cost effective screening of periodontal disease. Additionally, plural teeth for the examination make the problem more conflicting. Even the definition of the periodontal disease is still conflicting.[1,2] Therefore, more simple, useful and objective markers should be developed.

Saliva has been used as a diagnostic fluid in medicine and dentistry.[3-5] Salivary level of lactate dehydrogenase (LD) has been suggested to be co-related with several oral diseases.[6-18] Our previous study and other studies demonstrated the usefulness of the levels of LD, in the screening,[19-21] diagnosis, prognosis and evaluation of therapy in periodontal disease.[22-25] LD activity in saliva could constitute a specific indicator of oral mucosal lesions with tissue breakdown including periodontal disease.[20,23,24] Previous studies considered hemoglobin (Hb) as a potential biomarker.[19-21,24,25] Salivary levels of Hb and LD had statistically significant co-relations with the probing pocket depth (PD)[20,25] and CPI.[21] Moreover, we also showed that salivary LD levels were significantly decreased after dental scaling.[24] These biomarkers are thought to reflect the levels of inflammation and destruction of periodontal tissues, thus indicating that they are clinically useful biomarkers for

patient follow-up after periodontal therapy.

Periodontal therapy is consisted of the initial preparation and subsequent pocket curettage and surgical treatment. At the initial stage if the revaluation can predict, more effective therapy can be carried out and patient satisfaction will go up. The aim of this study was to predict the periodontal conditions at the revaluation by the conventional clinical markers and salivary biomarkers and set the cut-off point of these tests.

Materials and Methods

Study population

The study population was selected from the subjects with chronic periodontitis who attended eight private dental offices under the administration of members of the Shimane Dental Association in Japan. Subjects were included if older than 20 and had more than 20 remaining teeth. Current smokers, subject with affected life-related diseases were excluded. Smoking status, medication and with or without life related diseases were checked by interview. The study population consisted of 14 men and 32 women, and their mean age was 53.9+/-15.4.

Clinical examination

Oral examination was carried out by the dentists. The number of remaining teeth, presence or absence of calculus, bleeding on probing (BOP), and pocket depth (PD) were recorded. PD were assessed using a probe at six sites around each tooth; mesiobuccal, midbuccal, distobuccal, mesiolingual, midlingual, and distolingual. Measurements were recorded to the nearest millimeter, and every observation close to 0.5 mm was rounded to the lower whole number.

Clinical treatment

After tooth brushing instruction, whole-mouth scaling and root planing were performed under local anesthesia. Clinical parameters were measured and saliva was collected two weeks after final scaling. Patients with periodontal pockets deeper than 4 mm after initial treatment were diagnosed as necessity pocket curettage. Other patients were once finished periodontal therapy and recommended to visit for the supportive periodontal therapy (SPT) (Group I). These patients (Group II) were treated with subgingival curettage after the initial treatment. After two-week final curettage, clinical parameters were measured and saliva was collected again.

Measuring salivary Hb and LD

Saliva was collected after informed written consent prior to the oral clinical examination. Stimulated whole saliva was collected by chewing on a gum base containing neither fragrant nor taste ingredients for 5 minutes. For the examination of salivary levels of LD and Hb, collected saliva was kept at 4°C. Salivary levels of LD and Hb were measured using commercially available kits; L type Wako LDH J (Wako Chemical Industry, Osaka, Japan) and OC-HEMODIA AUTO S (Eiken Kagaku, Tokyo, Japan) according to the manufacturer's instructions.[19]

Statistical analysis

The changes in clinical parameters and salivary biomarkers were expressed as means ± standard deviation, and Wilcoxon signed-rank test or Friedman's tests were used to calculate *p*-values. The Mann-Whitney *U* test was used for comparison between with or without necessity of subsequent treatment of initial preparation. To set the cut-off points to distinguish these two groups, receiver operating characteristic curves (ROC curves) were

constructed and the points showing minimum difference between sensitivity and specificity were selected. The Fisher's exact tests were carried out to confirm the statistical significance, and positive or negative predictive values were also calculated.

Logistic regression analysis and Classification and Regression Trees analysis (CART) were carried out to find out the effective markers for the prediction of necessity of subsequent treatment of initial preparation. The analyses were carried out using the statistical software package SPSS ver 19.0 software (SPSS Inc., Tokyo, Japan).

Ethics

The study was approved by the Ethics Committee of Tsurumi University School of Dental Medicine (approval number: 430) and was conducted in accordance with the Declaration of Helsinki.

Results

At baseline examination, patients were not diagnosed for the necessity of periodontal curettage. Patients were divided into two groups by revaluation after initial treatment of the periodontal disease with (Group II: n=31) or without (Group I: n=15) periodontal pocket more than 4 mm. For both groups, statistically significant reductions of clinical markers and salivary biomarkers were observed (Fig. 1).

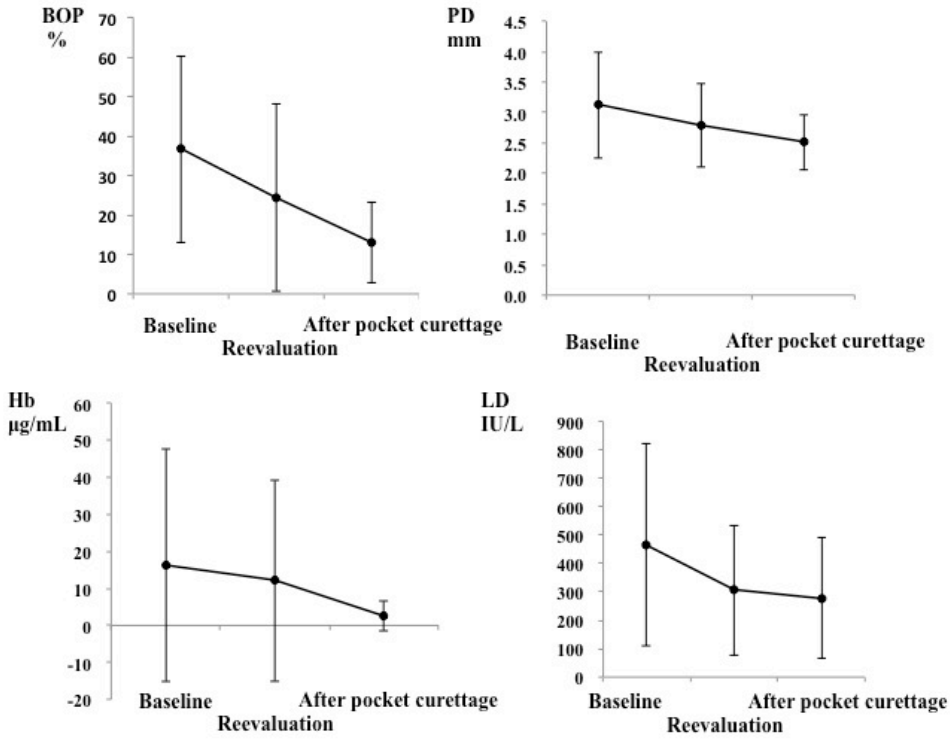

Fig. 1 Changes of clinical markers and salivary biomarkers after initial treatment and subsequent pocket curettage
Patients without probing depth more than 4 mm were finished periodontal therapy (Group I: n=15).
Patients with probing depth more than 4 mm were diagnosed as the necessity of pocket curettage (Group II: n=31).
All change was statistically significant (p<0.05).

To predict the prognosis of the initial treatment of the periodontal disease, baseline clinical markers and salivary biomarkers were compared. For the clinical markers, statistically significant differences were not observed. In contrast both Hb and LD, mean values were significantly higher in patients with the necessity of periodontal curettage (Fig. 2).

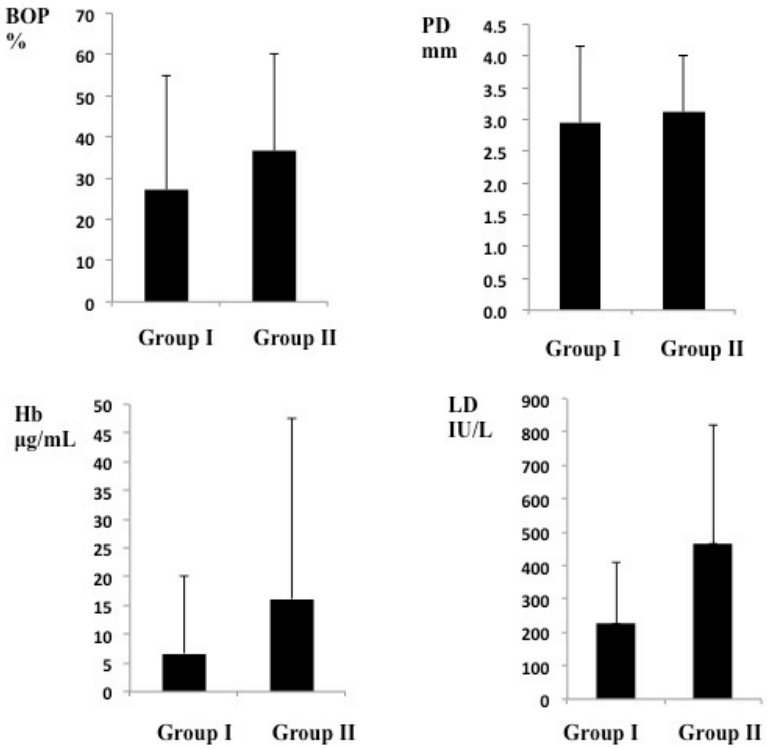

Fig. 2 Comparison clinical markers and salivary biomarkers between the groups with or without periodontal pocket after scaling and root planing
Salivary eves of Hb and LD had statistically significant differences in Groups I and II.

Table 1. Cut off point to predict the necessity of pocket curettage at baseline

	Cut off	Sensitivity	Specificity	Positive predictive value	Negative predicitive value	*p*-value	AUR
BOP	26.3	0.67	0.67	0.8	0.5	0.056	0.64
PD	2.8	0.67	0.67	0.8	0.5	0.056	0.68
Hb	1.4	0.68	0.67	0.81	0.5	0.055	0.69
LD	248.5	0.74	0.73	0.85	0.58	0.004	0.77

Only LD had statistically significant correlation with the necessity of pocket curettage at baseline and its AUR was highest.

Table 2. Crude and multivariate adjusted odds ratios for the prediction of necessity of pocket curettage at baseline

	Crude odds ratio	95% CI		*p*-value	Multivariate adjusted odds ratio	95% CI		*p*-value
BOP %	4	1.07	14.9	0.039	1.92	0.32	11.4	0.475
PD	4	1.07	14.9	0.039	2.94	0.52	16.58	0.221
Hb	4.2	1.13	15.59	0.032	0.97	0.16	5.81	0.971
LD	7.91	1.95	32.02	0.004	7.13	1.45	35.15	0.016
	Crude odds ratio	95% CI		*p*-value	Multivariate adjusted odds ratio	95% CI		*p*-value
BOP %	4	1.07	14.9	0.039	1.92	0.32	11.4	0.475
PD	4	1.07	14.9	0.039	2.94	0.52	16.58	0.221
Hb	4.2	1.13	15.59	0.032	0.97	0.16	5.81	0.971
LD	7.91	1.95	32.02	0.004	7.13	1.45	35.15	0.016

Odds ratios were calculated by logistic regression analysis.
Among the multivariate adjusted odds ratios, only LD was statistically significant and its odds were highest.

To set the cut off point for the prediction of necessity of periodontal curettage, ROC curves were

constructed (Fig. 3). By the cross tabulations only LD were statistically significant. Additionally, to check the importance of the LD (Table 1), logistic regression analysis was carried out by using the positive or negative for these markers as independent variables. By the multivariate adjusted odds ratios, only LD was statistically significant and its odds ratio was highest (Table 2).

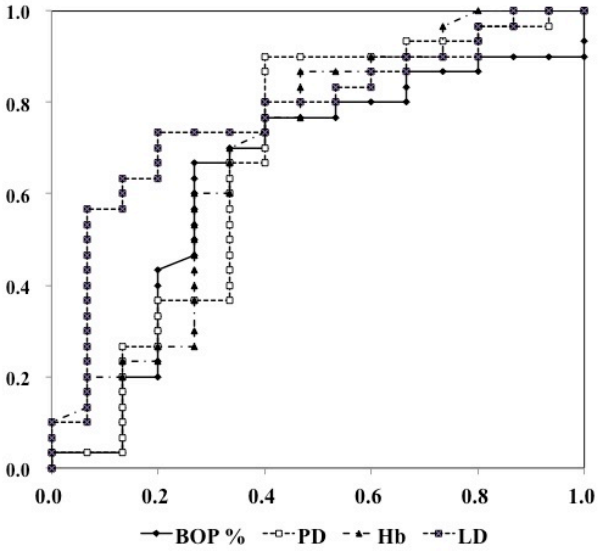

Fig. 3 ROC curves of the clinical markers and salivary biomarkers at baseline to predict the necessity of pocket curettage

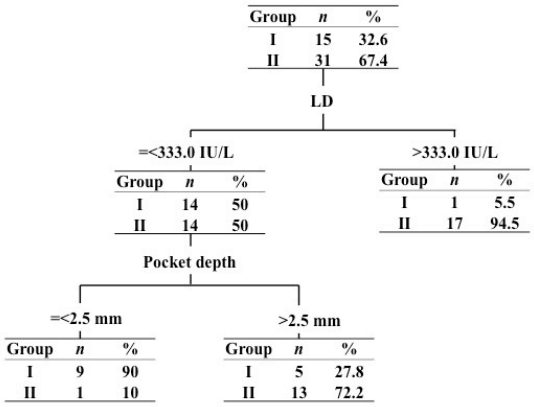

Fig. 4 Diagnostic chart to predict the necessity of pocket curettage at baseline

Finally, diagnostic chart was constructed by the decision analysis. The 94.4% patients were necessary of the periodontal curettage if the LD value was more than 333.3 IU/L at baseline. If the LD value was less than 333.3 IU/L, mean pocket depth was more than 2.5 mm, 72.2% patients were necessary of the periodontal pocket curettage (Fig. 4).

Discussion

Scaling and root planing is traditional periodontal therapy and its effect was reported by many researchers. According to the meta analysis reported by Hung et al.,[26] range of mean reduction of the probing depth by scaling and root planing was -0.15 mm to 0.62 mm for shallow initial pocket, 0.40 mm to 1.70 mm for medium initial pocket and 0.99 mm to 2.80 mm for deep initial pocket. Our results showed that mean reductions were 0.37 mm for group I and 0.33 mm for group II on average. However changes were very slight. Therefore, PD was not suitable for the evaluation of the treatment effects. Mean reductions of BOP % were 13.04% for group I and 12.31% for group II. BOP % is more suitable for the evaluation of treatment effects as a conventional clinical marker. In contrast, reduction of LD were 121.9 IU/L for group I and 160.9 IU/L for group II and those of Hb were 4.2 μg/mL for group I and 4.0 μg/mL. The reductions of biomarkers were also cleared.

Our previous reports have shown that salivary levels of LD and Hb had co-relation with periodontal conditions.[19,21,24,25] For the screening, LD value 345 IU/L is suggested to be the cut-off point for subjects with pocket depth more than 4 mm.[19] The cut-off point to diagnose progressive or no progressive patients after conventional periodontal therapy was 262 IU/L.[25] At that time, hemoglobin was measured by colorimetric detection and only free hemoglobin was measured.[19,24,25] By this method, the contaminated bloods of other species derived from food such as meat or fish were included. Therefore, the diagnostic precision of

hemoglobin was inadequate. Since then, the method of measuring hemoglobin has been improved. Now, two reagents used for the stool occult blood test are commercially available. These reagents can apply for the salivary Hb and they use polyclonal antibody and the reactions were measured by colloidal gold-label or latex beads. With this method, detection of Hb in saliva has become more sensitive.[21] However, data for screening level or cut-off point to diagnose progressive or no progressive patients is missing. Then, we compared the CPI value and Hb and LD. Hb value 3.86 μg/mL and LD value 217 IU/mL corresponded to CPI 4.[21]

In this study, baseline Hb values for the patients with was 6.66 μg/mL and patients with was 16.13 μg/mL. These values were higher than CPI 4 of our previous study. LD values were also 225 IU/mL and 466 IU/mL were higher. After scaling and root planing, values of the subjects without necessity of pocket curettage were decreased to 1.9 μg/mL for Hb and 184 IU/mL for LD.

In contrast, the patients with necessity of pocket curettage were decreased to 12.2 μg/mL for Hb value and 305 μg/mL for LD value. When compared with our previous report, LD values were higher than that of patients with progressive after conventional periodontal therapy. The cut-off point of 248.5 IU/mL for LD were lower than that of the no progressive CP patinents.[25]

In this study, salivary level of LD is useful marker for the prediction of the prognosis after conventional periodontal therapy. Additionally, LD measurement is one of conventional routine bold tests; therefore, test is cost effective. Our results suggested that routine use of salivary levels of LD can be the diagnostic aids, especially for the prediction of the prognosis. And diagnostic chart shown in Fig. 4 may facilitate the diagnosis.

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