Journal of Oral Science, Vol. 48, No. 4, 177-183, 2006

Original

Screening of periodontitis with salivary enzyme tests

Yoshiaki Nomura[1,2], Yoh Tamaki[1,2,3], Tomoko Tanaka[4], Hirohisa Arakawa[3], Akihisa Tsurumoto[1], Kazuko Kirimura[5], Tsutomu Sato[4], Nobuhiro Hanada[2] and Kyuichi Kamoi[2,6]

[1]Department of Preventive Dentistry and Public Health, Tsurumi University School of Dental Medicine, Kanagawa, Japan
[2]Department of Oral Health, National Institute of Public Health, Saitama, Japan
[3]Division of Oral Health, Department of Health Science, Kanagawa Dental College, Kanagawa, Japan
[4]Department of Oral Health, Nippon Dental University School of Life Dentistry at Tokyo, Tokyo, Japan
[5]Research Center for Odontology, Nippon Dental University School of Life Dentistry at Tokyo, Tokyo, Japan
[6]Nippon Dental University School of Life Dentistry at Tokyo, Tokyo, Japan

(Received 18 July and accepted 1 September 2006)

Abstract: The purpose of this study was to determine the usefulness of salivary biochemical markers for the screening of periodontal disease and examine the agreement between the results of saliva enzyme tests and those of probing depth. The present study included a total of 187 subjects who underwent annual medical check-ups at the Comprehensive Health Care Center, Honjo, Saitama Prefecture, Japan. Periodontal pocket probing was performed with a WHO probe, and various enzymes and biochemical parameters in saliva were measured. For lactate dehydrogenase (LDH), the proportions of the five isoenzymes were calculated. To decide the cut-off point for each enzymatic activity, receiver operating characteristic curves (ROC curves) were constructed and the points of minimum difference between sensitivity and specificity were decided. Among the biochemical markers tested, salivary LDH level had the highest sensitivity and specificity (sensitivity 0.66, specificity 0.67), while salivary levels of aspartate aminotransferase (AST) and blood urea nitrogen (BUN) also had sensitivity and specificity above 0.60. Among the LDH isoenzymes, LDH4 and LDH5 dominated in whole saliva samples. Salivary LDH may be a feasible and useful parameter for the screening of periodontal disease, while salivary AST and BUN also appear to be potentially useful for this purpose. (J. Oral Sci. 48, 177-183, 2006)

Keywords: periodontal disease; periodontal pocket probing; community periodontal index; lactate dehydrogenase; saliva.

Correspondence to Dr. Yoh Tamaki, Department of Preventive Dentistry and Public Health, Tsurumi University School of Dental Medicine, 2-1-3 Tsurumi, Tsurumi-ku, Yokohama, Kanagawa 230-8501, Japan
Tel: +81-45-581-8379
Fax: +81-45-573-9599
E-mail: pxz11337@nifty.com

Introduction

Periodontal diseases are commonly diagnosed on the basis of clinical parameters such as periodontal pocket probing depth, clinical attachment level, bleeding of pockets, and bone absorption determined by radiography. Among them, pocket probing methods such as the community periodontal index (CPI), in which representative teeth are examined, are often used for the screening of periodontal disease (1,2). The CPI was developed as an initiative of the World Health Organization (WHO), and has been reported to be a practical method for routine screening and recording of periodontal disease indicators that were not previously available (3). The advantages of this method are its simplicity and uniformity. The dental associations of several countries such as Japan, the United Kingdom, Australia and Finland have recommended its use in dental practice, health services and epidemiology.

Even though the simple CPI is very useful and rational,

it has some shortcomings. Although CPI was developed for use in the community, only a limited number of qualified examiners are competent at performing this test. It is preferable if screening can be performed easily by nonexpert examiners. Furthermore, when screening for periodontal disease in a large number of subjects, considerable time and effort are needed to evaluate the attachment level and probing depth of one index tooth in each of the sextants for each individual. Consequently, it is impossible to screen many persons in one day. Considering the cost-effectiveness of screening for periodontal disease, the fee of the dentist would increase the total expense because the test must be carried out by a trained dentist. In addition, the results of probing do not indicate whether periodontal disease has been arrested or is still active because pocket depth is not an index of disease activity.

In general medicine, many systemic diseases can be screened by blood or urine tests. If these clinical diagnostic tests could be applied to the screening of periodontal disease, the problems described above could be overcome (4-9). Some reported studies have demonstrated a correlation between blood test parameters and periodontal status. Among them, serum C-reactive protein (CRP) appears to show such a correlation, patients with periodontal disease having high levels of serum CRP (4-6). However, serum CRP is not specifically increased due to periodontal disease, but is increased due to inflammatory conditions caused by many other systemic diseases. Therefore, if CRP is used for screening of periodontal disease, there is the risk of a crossover effect against a background of systemic disease. Thus, there are no known blood parameters that exhibit high levels specifically as a result of periodontal disease.

Gingival crevicular fluid (GCF) may be a potential candidate clinical sample for the screening of periodontal disease. A specific relationship between periodontal disease and parameters related to gingival crevicular fluid has been reported (10-12). However, there are many potential sampling sites in the oral cavity, and differences in results among sampling sites must be considered (10). In addition, the sampling technique is not easy and a long time is needed for sample collection. Thus, GCF is not suitable for mass screening.

In the present study, we investigated candidate biochemical markers in saliva samples for the screening of periodontal disease. Saliva contains many enzymes and some inflammatory markers (13-15). These enzymes in serum have been routinely examined for the screening of systemic disease. Therefore, no specific laboratory devices are necessary, and this approach may be suitable for public health use. Therefore, we tried to apply these conventional clinical laboratory tests to saliva samples, and examined the feasibility and reliability of these methods for the screening of periodontal disease.

Materials and Methods

Study population

Subjects working at the municipal office of Honjo City, Saitama Prefecture, Japan, who underwent annual medical check-ups at Honjo Comprehensive Health Care Center were enrolled in the study. The study population comprised 137 males and 50 females with a mean age of 37.2 ± 9.6 (range; 21-60) years. The mean number of remaining teeth in each subject was 27.5 ± 1.06 (range; 21-32). Smoking status was investigated by interview. Among the subjects, 46 (24.6%) were current smokers, 41 (21.9%) were previous smokers, and 100 (53.5%) had never smoked. Eighteen (9.6%) of the subjects had systemic diseases; however, diseases that affect periodontal conditions, such as diabetes, were not included. Therefore, all these subjects were included in the analysis.

Clinical examination, diagnosis, and saliva sampling

Before the clinical examination, a 5-min stimulated saliva sample was collected by chewing a gum base containing no fragrant or taste ingredients. Six dentists conducted periodontal pocket probing on all the subjects using disposable WHO probes. Before the examination, all the participating dentists were obliged to attend a training course in probing depth determination. The kappa value for probing depth was between 0.67 and 0.80. The diagnostic criteria used in this study were as follows: Gingivitis was diagnosed when bleeding was observed after periodontal pocket probing; moderate periodontitis was diagnosed when at least one probing depth was 4-5 mm; and severe periodontitis was diagnosed when at least one probing depth was 6 mm or more.

Before sampling, informed written consent was obtained from every subject for analysis of the saliva samples. This study was approved by the ethics committee of Nippon Dental College. Saliva samples were immediately transferred to a refrigerated container at 4°C and then transported to the laboratory. The following measurements were carried out on the same day.

Measurement of salivary enzymes

In the present study, the salivary levels of 8 related enzymes or biochemical parameters; aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), creatinine (CRE), blood urea nitrogen (BUN), urea (UA)

and free hemoglobin (f-Hb) were measured using commercially available kits developed for routine blood tests.

The kits used in this study were AST (Apia auto S AST; Daiichi Chemical Co. Ltd., Tokyo Japan), ALT (Apia auto S ALT; Daiichi Chemical), LDH (L type Wako LDH J; Wako Chemical Industry, Osaka, Japan), ALP (L type Wako ALP J; Wako Chemical Industry), CRE (Shika liquid -S CRE; Kanto Chemical, Tokyo, Japan), BUN (L type Wako UN; Wako Chemical Industry), and UA (Apia auto S AST; Daiichi Chemical).

Free-Hb was measured by colorimetric analysis. Briefly, 20 μl of the sample and 1 ml of ortho-toluidine were mixed and incubated for 2 min. Then 1 ml of H_2O_2 was added, followed by incubation for 12 min. Finally, 1 ml of 10% acetic acid was added and the f-HB concentration was measured by colorimetry.

In addition, Titan LDH isoenzyme kits (Helena Laboratories, Saitama, Japan) were used for measuring LDH isoenzymes 1 to 5 and the H or M subunit. Briefly, 25 μl of the sample was electrophoresed on cellulose acetate membrane at 150 V for 13 min in Tris-barbital buffer. Then the membrane was stained with 480 nmol/L DL lactic lithium, 20 nmol/L NAD, 24 KU/L diaphorase, 2.4 nmol/L NTB and 8 nmol/L MTT. The stained bands were quantified using a densitometer. The proportion of each isoenzyme (LDH1-5, H subunit, M subunit) was then calculated.

Statistical analysis

To compare the enzymatic activities among subjects without periodontal disease, subjects with gingivitis and subjects with periodontal disease, one-way analysis of variance (ANOVA) was used. To determine the significance of differences among groups, the *P* values were adjusted by the Bonferroni method for multiple comparisons.

To set the cut-off points for the biochemical markers, receiver operating characteristic curves (ROC curves) were constructed and the points showing minimum difference between sensitivity and specificity were decided for gingivitis or periodontitis. The chi-squared test was carried out to confirm the statistical significance, and positive or negative predictive values were calculated for the biochemical markers. These analyses were conducted using SPSS ver. 14.0 (SPSS, Tokyo, Japan).

Results

We first compared the salivary levels of the biochemical markers among the gingivitis, moderate periodontitis, severe periodontitis and no periodontal disease groups. As shown in Table 1, AST, LDH, ALP, BUN and f-Hb showed statistically significant differences among the four groups by one-way ANOVA. However, the levels differed only slightly between gingivitis and moderate periodontitis. To confirm the differences described above, *P* values were adjusted by the Bonferroni multiple comparison post-hoc test. After adjustment, the differences remained significant for LDH (control vs. severe periodontitis; $P < 0.01$, moderate periodontitis vs. severe periodontitis; $P = 0.01$) and for f-Hb (severe periodontitis vs. control; $P < 0.01$, severe periodontitis vs. gingivitis; $P = 0.05$, severe periodontitis vs. moderate periodontitis: $P < 0.01$). When the salivary levels of these enzymes were compared according to smoking status, no statistically significant differences were observed (data not shown).

Among the LDH isoenzymes, LDH4 and LDH5 were dominant. As shown in Table 1, even though the differences between the groups were not statistically significant for all the isoenzymes, dose response tendencies were observed for LDH2 and LDH5 according to the severity of periodontal disease.

Figures 1 and 2 show the ROC curves of various biochemical markers and LDH isoenzymes. From each ROC curve, the point of minimum difference between the sensitivity and specificity was decided as the cut-off point. Tables 2 and 3 show the cut-off points, sensitivity and specificity for the biochemical markers tested and LDH isoenzymes, respectively. As shown in Table 2, LDH had the highest sensitivity (0.66) and specificity (0.67) among the biochemical parameters tested, while AST and BUN had sensitivity and specificity above 0.60.

As seen in Table 3, LDH2 and LDH5 showed statistically significant differences between healthy subjects and subjects with periodontal disease. LDH2 had sensitivity of 0.71 and specificity of 0.51. For LDH5, both sensitivity and specificity were less than 0.5. This means that evaluation using lower levels of LDH had sensitivity of 0.62 and specificity of 0.56.

Discussion

Body fluids have been used for the screening of systemic diseases and periodontitis. Some studies have demonstrated the usefulness of GCF (10-12) and saliva for the diagnosis of periodontitis (13). Although a lot of evidence has accumulated for the use of GCF for diagnosis of periodontitis, this approach has a demerit in that a special technique is necessary for sampling. In addition, it is difficult to obtain GCF from all sites of the dentition. Smith et al. (10) have shown that GCF volume and enzyme activities differed among 6 sites sampled. Thus, it is difficult to present values representative of a subject's oral cavity or even of one tooth. Thus, GCF may be

Table 1 Comparison of salivary levels of various biochemical markers between subjects without periodontal disease, subjects with gingivitis, subjects with moderate periodontitis and subjects with severe periodontitis

	No periodontal disease (n = 63)		Gingivitis (n = 23)		Moderate periodontitis (n = 70)		Severe periodontitis (n = 31)		
	mean	SD	mean	SD	mean	SD	mean	SD	*P* value*
AST (IU/L)	33.8	28.0	43.4	27.5	49.0	37.5	53.9	43.9	0.03
ALT (IU/L)	14.9	18.8	20.8	21.5	23.0	24.4	24.0	26.3	0.15
LDH (IU/L)	345.1	167.5	569.4	330.7	524.3	266.4	855.9	1061.4	< 0.01
ALP (IU/L)	11.0	5.7	14.1	9.8	10.7	5.2	17.5	26.3	0.04
CRE (IU/L)	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.27
BUN (IU/L)	16.6	6.1	19.6	7.4	19.8	6.8	20.3	7.4	0.02
UA (IU/L)	1.5	1.0	1.4	0.8	1.2	1.0	1.4	1.1	0.46
f-Hb (mg/dl)	0.5	2.1	0.4	1.0	0.7	2.1	5.6	16.4	< 0.01
LDH isoenzymes (%)									
LDH 1	1.5	1.2	1.2	0.4	1.5	0.8	2.1	2.5	0.07
LDH 2	6.7	2.5	6.9	0.8	7.2	1.3	7.6	2.0	0.13
LDH 3	14.3	2.8	14.5	1.3	14.6	1.5	14.7	1.6	0.70
LDH 4	25.3	1.8	25.6	1.1	25.5	1.4	25.4	1.8	0.87
LDH 5	52.2	6.4	51.8	2.5	51.2	3.5	50.1	4.6	0.24
H subunit	19.9	4.2	19.8	1.4	20.5	2.2	21.4	3.9	0.19
M subunit	80.1	4.2	80.2	1.4	79.5	2.2	78.6	3.9	0.19

*: *P* values calculated by one-way ANOVA, AST: aspartate aminotransferase, ALT: alanine aminotransferase, LDH: lactate dehydrogenase, ALP: alkaline phosphatase, CRE: creatinine, BUN: blood urea nitrogen, UA: urea, f-Hb: free hemoglobin

acceptable for clinical use but not for epidemiological purposes, and especially not for mass screening. In contrast, whole saliva sampling is far easier, noninvasive, and cheaper than GCF collection. Furthermore, collection of saliva is less costly and time-consuming because the dental examiner needs only to instruct the subject to chew a piece of gum or wax.

In the present study, salivary LDH was found to be the most useful enzyme for the screening of periodontitis. LDH is a ubiquitous enzyme that plays a significant role in the clinical diagnosis of pathologic processes. Some studies have compared the LDH levels in GCF between subjects with periodontal pockets and those with healthy pockets (10-12). Smith et al. (11) have shown that LDH activity is higher in subjects with increased probing depth (PD) than in individuals with healthy PD. Furthermore Atici et al. (12) have shown that the progression of periodontal disease may be associated with the level of LDH in GCF.

Nagler et al. (15) measured the LDH levels in parotid and submandibular/sublingual salivary secretions as well as in whole saliva, and concluded that 75% of whole-saliva LDH originated from an extra-salivary gland source. Other sources of LDH in saliva could include serum or bacteria (16). In the present study, we used samples of whole saliva, which has highly complex components. By chewing the gum base, inflamed periodontal tissue may be damaged, creating a mixture of blood or exudates in the saliva. The subjects who participated in this study were healthy, and we did not investigate their serum enzyme levels. Thus, the possibility that subjects with high enzyme levels in their serum were included, and that this affected the results, could not be excluded. Further study is needed to confirm the origin of the enzymes, and to determine whether subjects without periodontal disease and high levels of enzymes in

their serum show normal levels of the enzymes in saliva.

Most tissues contain five LDH isoenzymes that, while catalyzing the same reaction, have multiple molecular forms. The isoenzymes are composed of two different types of subunits, called M and H, which combine randomly to form tetrameric structures. Thus there are five component

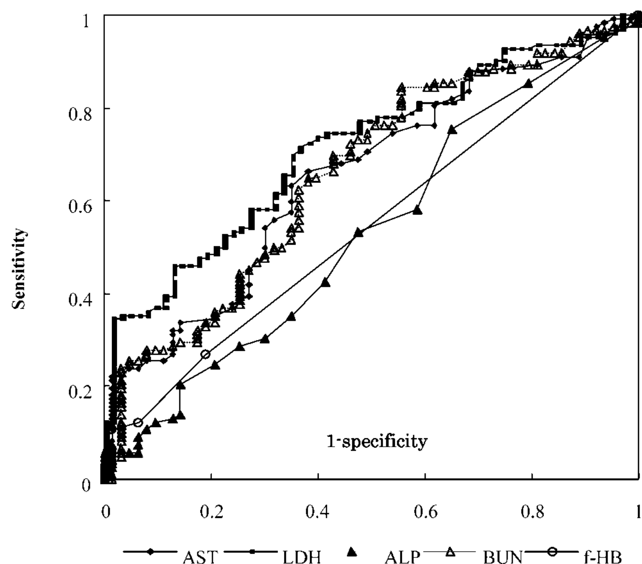

Fig. 1 ROC curves of biochemical markers tested for the screening of periodontitis.
AST: aspartate aminotransferase, LDH: lactate dehydrogenase, ALP: alkaline phosphatase, BUN: blood urea nitrogen, f-Hb: free hemoglobin.

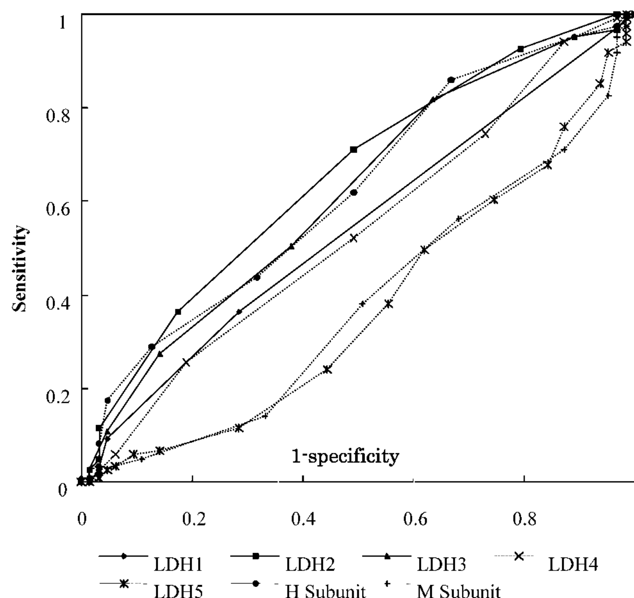

Fig. 2 ROC curves of the lactate dehydrogenase (LDH) isoenzymes and subunits for the screening of periodontal disease.

Table 2 Cut-off point, sensitivity and specificity of various biochemical markers in saliva

	Cut-off point (IU/L)	Gingivitis or Periodontitis			*P* value*	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value
			-	+					
AST	31.5	< 31.5	41†	46	< 0.001	0.63	0.65	0.47	0.78
		≥ 31.5	22	78					
ALT	12.5	< 12.5	36	55	0.098	0.57	0.57	0.40	0.72
		≥ 12.5	27	69					
LDH	371	< 371	42	42	< 0.001	0.66	0.67	0.50	0.80
		≥ 371	21	82					
ALP	9.5	< 9.5	33	57	0.407	0.53	0.52	0.37	0.69
		≥ 9.5	30	67					
BUN	16.95	< 16.95	40	47	< 0.001	0.62	0.63	0.46	0.77
		≥ 16.95	23	77					
f-HB	0.5	< 0.5	51	89	0.229	0.27	0.81	0.36	0.73
		≥ 0.5	12	33					

†: figures are number of cases, *: *P* values calculated by chi-square test.
AST: aspartate aminotransferase, ALT: alanine aminotransferase, LDH: lactate dehydrogenase,
ALP: alkaline phosphatase, CRE: creatinine, BUN: blood urea nitrogen,
UA: urea, f-Hb: free hemoglobin

Table 3 Cut-off point, sensitivity and specificity of lactate dehydrogenase isoenzymes in saliva

	Cut-off point (%)	Gingivitis or Periodontitis			*P* value*	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value
			-	+					
LDH1	1.5	< 1.5	45†	77	0.289	0.36	0.71	0.71	0.63
		≥ 1.5	18	44					
LDH2	6.5	< 6.5	32	35	0.003	0.71	0.51	0.74	0.52
		≥ 6.5	31	86					
LDH3	14.5	< 14.5	39	60	0.112	0.50	0.62	0.72	0.61
		≥ 14.5	24	61					
LDH4	25.5	< 25.5	32	58	0.713	0.52	0.51	0.67	0.64
		≥ 25.5	31	63					
LDH5	52.5	< 52.5	28	75	0.023	0.38	0.44	0.57	0.73
		≥ 52.5	35	46					
H subunit	19.5	<19.5	32	46	0.096	0.62	0.51	0.71	0.59
		≥19.5	31	75					
M subunit	80.5	< 80.5	31	75	0.096	0.38	0.49	0.71	0.71
		≥ 80.5	32	46					

†: figures are number of cases, *: *P* values calculated by chi-square test.
LDH: lactate dehydrogenase

isoenzymes as a result of the five different combinations produced by the subunits (for example, LDH1 has four H subunits, and LDH5 has four M subunits). It is well known that the distribution of the five isoenzymes differs according to tissue type (17). All five LDH isoenzymes have been detected in human saliva. In this study, LDH4 and LDH5 were dominant in samples of whole saliva, consistent with previous studies of LDH levels in whole saliva (15). Huang et al. (18) have reported that LDH4 and LDH5 are produced predominantly by gingival fibroblasts. Thus, further study is needed to determine the origin of LDH in whole saliva.

In this study, the sensitivity and specificity of LDH for screening periodontitis as diagnosed by probing depth were greater than 0.65. Values above 0.65 are acceptable considering the fact that probing depth does not directly reflect the activity of periodontal disease. These results indicate that determination of salivary LDH level is useful for the screening of periodontal disease.

For the other biochemical markers tested, the sensitivity and specificity of AST and BUN were both above 0.60, and therefore these parameters seem to be potentially useful for the screening of periodontitis. A correlation between AST and the progression of periodontal disease has been reported (12). The present study suggests that salivary AST may also be a candidate for the screening of periodontitis (sensitivity 0.63, specificity 0.65), possibly due to the fact that salivary AST may reflect tissue necrosis.

In conclusion, screening of periodontal disease by measuring salivary levels of LDH, AST and BUN may be a feasible, simple and convenient approach that does not require expert examiners. Further study is warranted to confirm the reliability of these parameters for the screening of periodontal diseases.

Acknowledgments

This investigation was supported by a Grant-in-Aid from the Ministry of Health, Labour and Welfare of Japan (H15-Iryo-018).

References

1. Cutress TW, Ainamo J, Sardo-Infirri J (1987) The community periodontal index of treatment needs (CPITN) procedure for population groups and individuals. Int Dent J 37, 222-233
2. Croxson LJ (1984) A simplified periodontal screening examination: the community periodontal index of treatment needs (WHO) in general practice. Int Dent J 34, 28-34
3. Ainamo J, Barmes D, Beagrie G, Cutress T, Martin J, Sardo-Infirri J (1982) Development of the World Health Organization (WHO) community periodontal index of treatment needs (CPITN). Int Dent J 32, 281-291
4. Takami Y, Nakagaki H, Morita I, Tsuboi S, Takami S, Suzuki N, Niwa H, Ogura Y (2003) Blood test values and community periodontal index scores in medical checkup recipients. J Periodontol 74, 1778-1784
5. D'Aiuto F, Ready D, Tonetti MS (2004) Periodontal disease and C-reactive protein-associated cardiovascular risk. J Periodontal Res 39, 236-241
6. Wakai K, Kawamura T, Umemura O, Hara Y, Machida J, Anno T, Ichihara Y, Mizuno Y, Tamakoshi A, Lin Y, Nakayama T, Ohno Y (1999) Associations of medical status and physical fitness with periodontal disease. J Clin Periodontol 26,664-672
7. Morita M, Horiuchi M, Kinoshita Y, Yamamoto T, Watanabe T (2004) Relationship between blood triglyceride levels and periodontal status. Community Dent Health 21, 32-36
8. Katz J (2001) Elevated blood glucose levels in patients with severe periodontal disease. J Clin Periodontol 28, 710-712
9. Grossi SG, Skrepcinski FB, DeCaro T, Robertson DC, Ho AW, Dunford RG, Genco RJ (1997) Treatment of periodontal disease in diabetics reduces glycated hemoglobin. J Periodontol 68, 713-719
10. Smith QT, Geegan SJ (1991) Repeated measurement of crevicular fluid parameters at different sites. J Clin Periodontol 18, 171-176
11. Smith QT, Au GS, Freese PL, Osborn JB, Stoltenberg JL (1992) Five parameters of gingival crevicular fluid from eight surfaces in periodontal health and disease. J Periodontal Res 27, 466-475
12. Atici K, Yamalik N, Eratalay K, Etikan I (1998) Analysis of gingival crevicular fluid intracytoplasmic enzyme activity in patients with adult periodontitis and rapidly progressive periodontitis. A longitudinal study model with periodontal treatment. J Periodontol 69, 1155-1163
13. Pederson ED, Stanke SR, Whitener SJ, Sebastiani PT, Lamberts BL, Turner DW (1995) Salivary levels of α_2-macroglobulin, α_1-antitrypsin, C-reactive protein, cathepsin G and elastase in humans with or without destructive periodontal disease. Arch Oral Biol 40, 1151-1155
14. Alonso de la Pena V, Diz Dios P, Lojo Rocamonde S, Tojo Sierra R, Rodriguez-Segade S (2004) A standardised protocol for the quantification of lactate dehydrogenase activity in saliva. Arch Oral Biol 49, 23-27
15. Nagler RM, Lischinsky S, Diamond E, Klein I, Reznick AZ (2001) New insights into salivary lactate dehydrogenase of human subjects. J Lab Clin Med 137, 363-369
16. Chen A, Hillman JD, Duncan M (1994) L-(+)-lactate dehydrogenase deficiency is lethal in Streptococcus mutans. J Bacteriol 176, 1542-1545
17. Maekawa M (2004) Lactate dehydrogenase (LD, LDH). Nippon Rinsho 62, 352-355 (in Japanese)
18. Huang JS, Liu TZ, Bhatnagar RS (1990) Creatine kinase and lactate dehydrogenase isoenzyme patterns in cultured normal and pathological gingival fibroblasts. J Formos Med Assoc 89, 126-131