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Interleukin-1β Level in Gingival Crevicular Fluid of Patients with Active Periodontitis

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SUMMARY **Previous studies revealed that interleukin-1beta (IL-1β) was detectable in gingival crevicular fluid (GCF) of patients with periodontitis, and the level was increased in level in gingival tissue extracts of active periodontal disease sites (defined as attachment loss ≥ 2.5 mm over the preceding 2 months) compared to inactive sites or healthy sites. The present study evaluated the relationship of IL-1β level in GCF and periodontal disease status. GCF was collected with Periopaper strips from 34 disease-active and 45 disease-inactive teeth in 11 untreated periodontitis patients and from 60 teeth in 15 healthy control subjects. Disease activity was defined as attachment loss of ≥ 2.5 mm in at least one site of a tooth as determined by sequential probing. The absorbed GCF volume was determined using a Periotron 6000 and the crevicular IL-1β level was determined using IL-1β monoclonal antibody (Otsuka Pharmaceutical, Japan). IL-1β was below the detection level of the assay (6 pg/ml) in the healthy control group but was detected in most teeth of the periodontitis group. However, disease-active teeth had higher IL-1β level (Mann-Whitney U-test, $p < 0.05$) than disease-inactive teeth (mean total IL-1β of 5.89 ± 7.88 pg/tooth and 1.72 ± 2.28 pg/tooth; mean concentration of 1.6 ± 2.5 ng/ml and 0.6 ± 0.83 ng/ml, respectively). The level of IL-1β showed no correlation with probing depth, but had significant correlation ($p < 0.05$) with the extent of attachment loss. This study suggests that the level of IL-1β in GCF may have a predictive value for determining active and inactive periodontal status.**

Periodontal diseases have been generally assessed using visual inspection, periodontal probing and radiographic interpretation. However, all these techniques are static determinations of the disease severity and have only limited value for identifying future disease progression or loss of clinical attachment (disease activity).[1-5] Therefore, other aspects of the periodontal lesion have been explored that may be useful for developing tests for periodontal disease activity. These include tests to detect causative factors, such as putative periodontopathic bacteria in the subgingival plaque,[6] as well as tests to assess host response.[7] More attention is now being focused on the host due to the significance of host-bacteria interactions on the destructive process. For example, the reaction of the host to the bacteria may be the actual cause of tissue destruction.

A number of host factors have been tested or proposed as diagnostic indicators of disease activity. Among them, interleukin-1 (IL-1β), a cytokine with known inflammatory and bone resorptive effects, appears to be promising.[8-12] Several investigators have shown that significant amounts of IL-1β are present in the gingival crevicular fluid (GCF) at disease sites[13-20] and these amounts are remarkably reduced after therapy.[16,17] This

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might reflect differences in disease activity as shown by Stashenko *et al.*[18] who found that IL-1β in the gingival tissue extract was higher at the active periodontal disease sites (defined as attachment loss ≥ 2.5 mm over the preceding 2 months) compared to inactive sites or healthy sites. There is little data as yet to demonstrate the relationship of IL-1β in GCF and periodontal disease activity. Recently, Lee *et al.*[21] have examined the relationship between GCF levels of IL-1β and progression of refractory periodontitis. Although this study suggested that GCF from progressing sites have elevated levels of IL-1β compared to that of non-progressing sites in the same patients, it included only 8 progressing sites from 10 patients.

This study has assessed IL-1β levels in GCF from healthy subjects and periodontitis patients to see whether crevicular IL-1β level is correlating with clinical parameters, and to compare crevicular IL-1β levels in active and inactive states of the disease.

MATERIALS AND METHODS

Study subjects

Subjects included healthy controls and periodontitis patients. None had diabetes mellitus or were taking medications such as anti-inflammatory agents, antibiotics or other drugs that could affect the periodontium, for at least 4 months prior to and during the study.

The sample size in this study was estimated by using the standard deviation from a previous study of Stashenko *et al.*[18] on the level of interleukin-1β in tissues from sites of active periodontal disease. In this calculation the confidence coefficient is 0.95 and the margin of sampling error is ± 5.

The healthy control group consisted of 60 teeth in 15 clinically healthy adult volunteers, aged 22 to 60 years (mean age 32.9 years), each having at least 20 permanent teeth excluding third molars. None of them had pocket depth (PD) greater than 3 mm nor a gingival index (GI)[22] greater than 1.

The periodontitis group consisted of 34 disease-active teeth and 45 disease-inactive teeth in 11 untreated periodontitis patients, aged 30 to 54 year (mean age 46.3 years). All patients had PD of 5 mm or greater and radiographic bone loss on at least two teeth in each quadrant.

All subjects were given explanations on all procedures and informed consent was signed before starting the study.

Clinical protocol

Healthy control group

A plaque control program was performed in the pre-study period to ensure that a GI of 0 was achieved at all teeth. It consisted of a full mouth scaling and a professional tooth cleaning every week for two weeks for every subject. All subjects were asked to clean their teeth after each meal, at least 3 times a day by Modified Bass brushing technique.[23] Interdental sites were flossed at least once daily and this was stopped 2 days before gingival crevicular fluid sample collection. GCF of each subject was taken from 4 randomly selected teeth. All clinical data were assessed after fluid sampling.

Periodontitis group

All subjects were examined for baseline clinical data. Two months later, they were re-examined and the disease activity of each tooth was determined by the tolerance method.[24] During calibration exercises, measurements were made of attachment level (the distance from the lower border of occlusal stent to the most apical penetration of the probe, AL) using a manual probe. The average standard deviation of repeated measurements was 0.8 mm. Using these standard deviations to estimate significant difference, a tooth with clinical attachment loss in at least one site equal to or greater than 2.5 mm was designated as a disease-active tooth.[18] In contrast, a tooth that had clinical attachment loss of less than 2.5 mm or no further loss was designated as a disease-inactive tooth.[18] GCF samples were taken from all disease-active teeth and from other disease-inactive teeth which had pocket depth equal to or greater than 6 mm.

Clinical data

Clinical data were assessed at 4 locations on each tooth (mesiobuccal, midbuccal, distobuccal, and midlingual). These data were GI, PD and AL. In the periodontitis groups, clinical attachment loss (CAL) was determined as an increase in the attachment level compared to a baseline in at least one site of a tooth. All clinical assessments were carried out by the same investigator.

Collection of gingival crevicular fluid

To avoid contamination with saliva, only teeth of the upper jaw were selected. Immediately following isolation and removal of supragingival plaque, precut Perio-paper strips (Harco Electronics, Tustin, CA, U.S.A.) were inserted

into the gingival crevice until mild resistance was felt, and kept in place for 30 seconds.[16] The fluid volume was determined using a Periotron 6000 (Harco Electronics, Irvine, CA, U.S.A.).

Four samples per tooth (mesiobuccal, midbuccal, distobuccal, and midlingual) were collected and pooled in one vial containing 70 µl Hanks' balanced salt solution, 0.5% bovine serum albumin.[14] All samples were stored at -70°C until assayed.

Sample preparation and IL-1β assay[14]

GCF was eluted from Periopaper strips by centrifugation at 1,090 x g, 4°C for 15 minutes.[14] A further 70 µl of Hanks' balance salt solution containing 0.5% bovine serum albumin was applied and the centrifugation was repeated. The strips were discarded.[14] The amount of IL-1β in GCF sample was assayed by ELISA with IL-1β monoclonal antibody (Otsuka Pharmaceutical, Japan) according to the manufacturer's instruction. Absorbances were measured in an ELISA reader (Turnhout, Belgium, Model 311 Co serial number 3000064) at 492 nm. The total amount of crevicular IL-1β (pg/tooth) in each sample was determined and the concentration of crevicular IL-1β was expressed as ng/ml GCF. The sample and standard specimens were tested in duplicate. The sensitivity of the ELISA assay for GCF was 6 pg/ml.

Statistical analysis

Data analysis was performed using the statistical package SPSS for Windows (version 6.0). Differences in IL-1β levels and clinical parameters were analyzed using the Mann-Whitney U-test. Correlation between the levels of IL-1β and the clinical parameters were assessed using Spearmann's rank correlation coefficient.

RESULTS

Clinical parameters and gingival crevicular fluid volume

In the healthy control group, no clinical sign of gingival inflammation was present at the time of GCF collection. Of the 60 teeth selected for testing, the mean PD and GCF volume were 2.62 ± 0.55 mm and 0.59 ± 0.34 µl, respectively.

In contrast, in the periodontitis group (11 patients), it was found that 119 maxillary teeth had a PD equal to or greater than 5 mm with radiographic bone loss at the baseline. Evaluation of the AL of these teeth during two months indicated that periodontal disease activity (CAL) was found in 34 teeth from 9 patients. These teeth were designated disease-active teeth. Eighty-five teeth were designated as disease-inactive teeth out of which 45 teeth that had the deepest pockets were selected for further testing. The clinical parameters and GCF volume of these teeth are presented in Table 1. Mean GI, PD, CAL and GCF volume were significantly greater in the disease-

Table 1 Mean value and standard deviation of clinical parameters and gingival crevicular fluid volume of periodontitis disease-active and disease-inactive teeth

Clinical parameter (Mean ± SD)	Periodontitis		*p*-value[1]
	Disease inactive (n = 45)	Disease active (n = 34)	
GI	2.07 ± 0.39	2.29 ± 0.46	< 0.05
PD (mm)	6.31 ± 1.69	8.00 ± 1.48	< 0.05
CAL (mm)	1.31 ± 0.63	3.41 ± 0.61	< 0.05
GCF(µl)	2.93 ± 1.07	4.05 ± 0.77	< 0.05

[1]Significant difference using the Mann-Whitney U test ($p < 0.05$)

Table 2 Crevicular IL-1β levels in the peridontal disease group

Tooth designation	Percent of detectable samples	Mean ± SD[3]
Disease-active (n = 34)	85	
IL-1β (pg/tooth)[1]		5.89 ± 7.88[4]
IL-1β (ng/ml)[2]		1.6 ± 2.5[5]
Disease-inactive (n=45)	53	
IL-1β (pg/tooth)[1]		1.72 ± 2.28[4]
IL-1β (ng/ml)[2]		0.6 ± 0.83[5]

[1]Total amount of IL-1β (pg/tooth)
[2]Concentration of IL-1β (ng/ml) = Total amount of IL-1β/GCF volume
[3]Mean of disease-active teeth (n = 34) and disease-inactive teeth (n = 45)
[4, 5]Significant difference using the Mann-Whitney U test ($p < 0.05$)

active teeth than in the disease-inactive teeth ($p < 0.05$). The GCF volume was found to correlate positively with the GI and PD ($r = 0.32$ and 0.61, respectively, $p < 0.05$).

IL-1β levels in the healthy controls and periodontitis group

In the healthy control group, the IL-1β level was not detectable in any GCF sample. Table 2 shows the crevicular IL-1β levels in the periodontitis group. IL-1β was detected in 29/34 (85%) samples of disease-active teeth and in

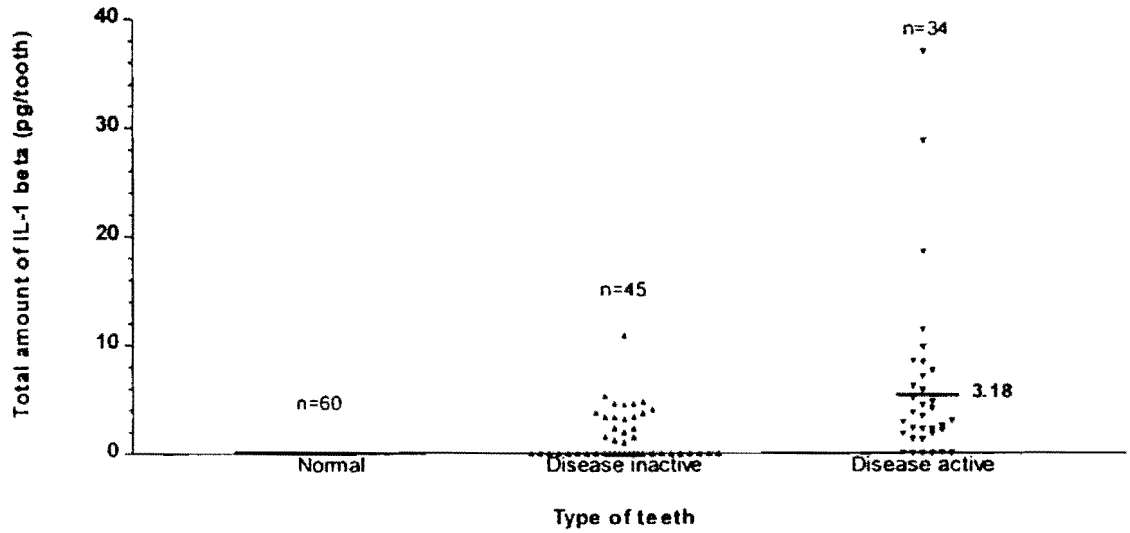

Fig. 1 Total amount of IL-1β in gingival crevicular fluid (pg/tooth) of normal, disease-inactive and disease-active teeth (the bar represents median value).

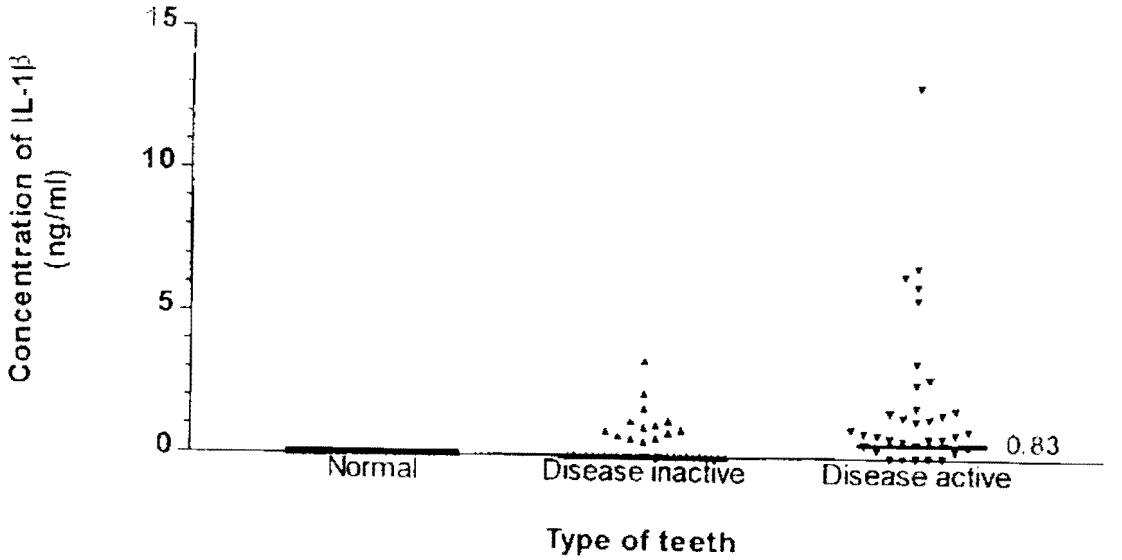

Fig. 2 Concentration of IL-1β in gingival crevicular fluid (ng/ml) of normal, disease-inactive and disease-active teeth (the bar represents median value)

Table 3 Spearman's rank correlation coefficient (r_s) and p-value between the level of IL-1β and clinical parameters and gingival crevicular fluid volume (number of teeth = 79)

	IL-1β			
	Total amount		Concentration	
	r_s	p-value	r_s	p-value
Pocket depth	0.04	0.708	-0.03	0.762
Gingival index	0.26	0.021	0.17	0.127
Clinical attachment loss	0.36	0.001	0.27	0.015
Gingival crevicular fluid	0.17	0.14	0.01	0.91

Significant difference threshold was $p < 0.05$

22/45 (53%) of samples of disease-inactive teeth. Interestingly, crevicular IL-1β was highest in disease-active teeth. Both the concentrations and the total amounts of IL-1β in disease-active teeth were significantly different from those of the disease-inactive teeth as analyzed by the Mann-Whitney U-test, ($p < 0.05$, Figs. 1, 2).

Correlation among the levels of IL-1β and clinical parameters

To determine the correlation between the levels of IL-1β and clinical parameters, the data of disease-active and disease-inactive teeth were combined (Table 3). The concentration of IL-1β was not correlated with any clinical parameter except for the amount of clinical attachment loss ($r = 0.27$, $p < 0.05$). However, the total amount of IL-1β was also positively correlated with both the amount of attachment loss ($r = 0.36$, $p < 0.01$) and GI ($r = 0.26$, $p < 0.05$).

DISCUSSION

The present study has measured IL-1β levels in gingival crevicular fluid by using ELISA. Sampling of GCF provides ease of access and is relatively atraumatic. However, accurate and reproducible sampling of fluid is essential.[25] A variety of protocols have been used for collection of GCF and there is still no consensus on which protocol(s) exhibit(s) the lowest bias, the highest reproducibility and the strongest validity. In this study, the method of GCF sampling suggested by Wilton *et al.*[14] was used, where four samples were collected per tooth and pooled.

In this study, IL-1β was not detected in any healthy control subject, in contrast to the report by Preiss and Meyle[15] describing the presence of IL-1β in all healthy control subjects with a IL-1β concentration range of 22.8-150 ng/ml. GCF samples in our and their studies were taken after a hygiene regimen of two weeks with no clinical signs of gingival inflammation at the time of GCF collection. Differences in the results might be due to differences in preparing GCF samples and different recovery rates. In addition, differences due to populations studied cannot be ruled out, since differences have been reported in serum levels of other cytokines in different populations.[26] Indeed it has been shown that there is genetic polymorphism of the IL-1 gene, so there may be genetic differences in IL-1β levels and this could give rise to greater variation in IL-1β levels.[19,27-28]

In the periodontitis group, all subjects were monitored for attachment loss during the two month period before GCF collection. Teeth that had clinical attachment loss equal to or greater than 2.5 mm were designated as disease-active. Crevicular IL-1β was present at a detectable level in 85% of the disease-active teeth but only in 53% of the disease-inactive teeth. The disease-active teeth had IL-1β concentrations which were about 2.7 times higher than that of the disease-inactive teeth. This is in agreement with the result of Stashenko *et al.*,[18] reporting that diseased sites with smaller amounts of attachment loss (0.5-2.0 mm) also contained more IL-1β than the sites which remained stable. In both studies attachment levels were measured by manual probes and the use of a relatively large attachment loss (≥ 2.5 mm) over a two-month period of examination resulted in the identification of active sites with very few false positives.[24] It is possible that some

teeth in the "diseased but inactive" group were active, but were not detectable under our stringent criteria for attachment level change. Therefore, more sensitive methods such as computer-processed radiography[29] or computer-linked periodontal probing[30] should be employed to improve sensitivity in detection of clinical attachment loss.

Wilton *et al.*[14] and Massada *et al.*[16] reported a lack of correlation between IL-1β concentration and clinical indices, including probing depth, plaque index and bleeding index or attachment loss. On the contrary, Hou *et al.*[17] found that the total amount of IL-1β, but not IL-1β concentration, was positively correlated with gingival index scores and pocket depths. Cavanaugh *et al.*[19] found that there was a correlation between GCF IL-1β levels and maximum bone height loss. In the present study, neither the concentration nor the total amount of IL-1β correlated with the PD. However, the total amount of IL-1β but not the concentration was found to be correlated directly with the GI score. The lack of correlation between the IL-1β concentration and the GI was expected since the GCF volume correlated with the degree of gingival inflammation, so that a large volume of GCF could decrease the concentration of IL-1β in the samples. In addition, vascular changes in the gingiva depend on many other factors.[15] So it is not surprising that the total amount and the concentration of IL-1β were not correlated with pocket depth since the latter reflects the cumulative effect of the past and the present conditions and does not necessarily reflect the current disease activity.[31]

At present, there is no absolute criterion or a "gold standard" to identify active periodontal disease against which various diagnostic methods can be compared. The most promising criterion thus far appears to be the attachment loss, as measured by periodontal probing.[32] In this study, the progressive loss of attachment calculated from the difference in attachment between two successive examinations was used. The two-month interval between these examinations was arbitrarily chosen for practical purposes. If episodes of active disease last a few days or even two or three weeks, the disease may not be active when the actual examinations or tests are performed. The only conclusion that can be drawn if a loss of attachment or bone level is noted between two examinations is that the disease was active at some point between the first and second examination. As a result, it is not possible to determine the sensitivity and specificity of the test or how frequently false negative and false positive test outcomes occur. Prospective longitudinal monitoring of the crevicular IL-1β level in a more narrow time frame should be performed to obtain a more accurate result.

Although the r values (< 0.36) seemed to be very weak, there is a significant difference between the levels of this cytokine and the stages of periodontal disease as seen in the results which may not necessarily represent a statistically strong correlation. This study suggests that the level of crevicular IL-1β may have a predictive value for determining active periodontal disease status. However, in this study, the test outcomes are not simply dichotomous, but rather consist of a continuum or interval scale of values. The detected total amount of IL-1β levels of periodontal diseased teeth (both active and inactive) varied greatly (0-37 pg/tooth). Therefore further studies are needed to evaluate the appropriate cut-off levels of IL-1β.

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REFERENCES

1. Badersten A, Nilveus R, Egelberg J. Effect of nonsurgical periodontal therapy (VII). Bleeding, suppuration and probing depth in sites with probing attachment loss. J Clin Periodontol 1985; 12: 432-40.
2. Lang NP, Joss A, Orsanic T, Gusberti FA, Siegrist BE. Bleeding on probing. A predictor for the progression of periodontal disease. J Clin Periodontol 1986; 13: 590-603.
3. Griffiths GS, Wilton JMA, Curtis MA, *et al.* Detection of high risk groups and individuals for periodontal diseases. Clinical assessment of the periodontium. J Clin Periodontol 1988; 15: 403-10.
4. Caton J. Periodontal diagnosis and diagnosis aids. In: Proceeding of the World Workshop in Clinical Periodontics Nevins M, Becker W, Kornman K, eds. Chicago, American Academy of Periodontology, 1989; pp. 1-32.
5. Badersten A, Nilvius R, Egelberg J. Scores of plaque, bleeding, suppuration and probing depth to predict probing attachment loss. Five years of observation following nonsurgical periodontal therapy. J Clin Periodontol 1990; 17: 102-7.
6. Listgarten MA. Microbiological testing in the diagnosis of periodontal disease. J Periodontol 1992; 63: 332-7.
7. Page RC. Host response tests for diagnosing periodontal diseases. J Periodontol 1992; 63: 356-66.
8. Alexander MB, Damoulis PD. The role of cytokines in the pathogenesis of periodontal disease. Current Opinion in Periodontology 1994; 39-53.
9. Kjeldsen M, Holmstrup P, Bendtzen K. Marginal periodontitis and cytokines: A

review of the literature. J Periodontol 1993; 64: 1013-22.
10. Tatakis DN. Interleukin-1 and bone metabolism: A review. J Periodontol 1993; 64: 416-31.
11. Dinarello CA. Interleukin-1 and interleukin-1 antagonism. Blood 1991; 77: 1627-52.
12. Stashenko P, Jandinski JJ, Fujiyoshi P, Rynar J, Socransky SS. Tissue levels of bone resorptive cytokines in periodontal disease. J Periodontol 1991; 62: 504-9.
13. Figueredo CM, Ribeiro MS, Fischer RG, Gustafsson A. Increased interleukin-1beta concentration in gingival crevicular fluid as a characteristic of periodontitis. J Periodontol 1999;70: 1457-63.
14. Wilton JMA, Bampton JLM, Griffiths GS, *et al.* Interleukin-1 beta (IL-1β) levels in gingival crevicular fluid from adults with previous evidence of destructive periodontitis. A cross sectional study. J. Clin. Periodontol 1992; 19: 53-7.
15. Preiss D, Meyle J. Interleukin 1β concentration of gingival crevicular fluid. J Periodontol 1994; 65: 423-8.
16. Masada MP, Persson R, Kenney JS, Lee SW, Page RC, Allison AC. Measurement of interleukin-1α and-1β in gingival crevicular fluid: Implications for the pathogenesis of periodontal disease. J Periodont Res 1990; 25: 156-63.
17. Hou LT, Liu CM, Rossomando EF. Crevicular interleukin-1β in moderate and severe periodontitis patients and the effect of phase I periodontal treatment. J Clin Periodontol 1995; 22: 162-7.
18. Stashenko P, Fujiyoshi P, Obernesser MS, Prostak L, Haffajee AD, Socransky SS. Levels of interleukin-1β in tissue from sites of active periodontal disease. J Clin Periodontol 1991; 18: 548-54.
19. Cavanaugh PF, Meredith MP, Buchanan W, Doyle MJ, Reddy MS, Jeffcoat, MK. Coordinate production of PGE2 and IL-1β in the gingival crevicular fluid in adults with periodontitis: Its relationship to alveolar bone loss and disruption by twice daily treatment with ketolactromethamine oral rinse. J Periodont Res 1998; 33: 75-82.
20. Engebertson SP, Lamstu LB, Herrara-Abreu M, *et al.* The influence of interleukin gene polymorphisms on expression of interleukin-1β and tumor necrosis factor-α in periodontal tissue and gingival crevicular fluid. J Periodontol 1999; 70: 567-73.
21. Lee HJ, Kang IK, Chung CP, Choi SM. The subgingival microflora and gingival crevicular fluid cytokines in refractory periodontitis. J Clin Periodontol 1995; 22: 885-90.
22. Loe H, Silness J. Periodontal disease in Pregnancy. Prevalence and severity. Acta Odont Scand 1963; 21: 532-51.
23. Bass CC. An effective method of personal oral hygiene. J Louis Med Soc 1954; 106: 100-12.
24. Haffajee AD, Socransky SS, Goodson JM. Comparison of different data analyses for detecting changes in attachment level. J Clin Periodontol 1983; 10: 298-310.
25. McCulloch CAG. Host enzymes in gingival crevicular fluid as diagnostic indicators of periodontits. J Clin Periodontal 1994; 21: 497-506.
26. Kalinkovich A, Maayan S, Weisman Z, Harpaz N, Bentwich Z. Immune activation, a co-factor for HIV transmission in Thailand. Lancet 1994; 343: 1506-7.
27. Lang NP, Tonetti MS, Suter J, Sorrell J, Duff GW, Kornman KS. Effect of interleukin-1 gene polymorphisms on gingival inflammation assessed by bleeding on probing in periodontal maintenance population. J Periodont Res 2000; 35: 102-7.
28. Figueredo CMS, Ribeiro MSM, Fischer RG, Gustafsson A. Increased interleukin-1β concentration in gingival crevicular fluid as a characteristic of periodontitis. J Periodontol 1999; 70: 1457-63.
29. Jeffcoat MK, Williams RC, Reddy MS, *et al.* Flurbiprofen treatment of human periodontitis: Effect on alveolar bone height and metabolism. J Periodont Res 1988; 23: 381-8.
30. Jeffcoat MK, Jeffcoat RL, Jens SC, Captain K. A new periodontal probe with automated cementoenamel junction detection. J Clin Periodontol 1986; 13: 276-80.
31. Jeffcoat MK. Current concepts in periodontal disease testing. J Am Dent Assoc 1994; 125: 1071-8.
32. Listgarten MA. A prospective on periodontal diagnosis. J Clin Periodontol 1986; 13: 175-81.