Correlation of Levels of Interleukin-1β in Gingival Crevicular Fluid to the Clinical Parameters of Chronic Periodontitis

AU Chaudhari, GN Byakod, PF Waghmare, VM Karhadkar

ABSTRACT

Aim: Background and objectives: IL-1β is a potent stimulator of bone resorption and has been implicated in the pathogenesis of periodontal destruction. Therefore, this study was designed to compare the levels of IL-1β of chronic periodontitis patients with the healthy subjects. Another objective of this study was to correlate IL-1β levels with the clinical parameters of the periodontal disease progression.

Methods: For this study, total 60 subjects were chosen (30-healthy and 30-chronic periodontitis). Simplified oral hygiene index (OHI-S), gingival index (GI), periodontal disease index (PDI), probing depth (PD), tooth mobility, bleeding on probing (BOP) were recorded for all the subject. Gingival crevicular fluid (GCF) was collected and subjected for ELISA for estimation of IL-1β.

Results: At the periodontal diseased sites, the IL-1β levels increased at least 2-fold as compared with healthy subjects. This increase was highly significant (p = 0.0000). Within the test group, IL-1β levels correlated positively and significantly with PDI, PD, BOP and tooth mobility. The correlations of IL-1β with PD (p = 0.000) and IL-1β with BOP (p = 0.0004) were highly significant.

Interpretation and conclusion: These data suggest that amount of GCF IL-1β is closely associated with periodontal status. This relationship may be valuable in monitoring periodontal disease activity.

Clinical significance: It could be stated from this study on IL-1β that there seem to be a strong correlation between periodontal tissue destruction and IL-1β. Furthermore IL-1β level could also differentiate between active and inactive periodontal lesions.

Keywords: Interleukin-1β, GCF, ELISA, Chronic periodontitis, Cytokine.

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INTRODUCTION

Periodontal disease is a multifactorial condition where determining relative importance of different risk factors is difficult. It results from the interaction of the host defense mechanism with microorganisms in plaque. The susceptibility of an individual to the periodontal tissue destruction could be due to variable causes. This susceptibility is determined by the host factors, microbiological factors and environment in which the organisms and the host tissue dwell.

The type of immune response that occurs on exposure to pathogen is vital in determining the resistance or susceptibility of the individual to the disease. The host factors that determine this susceptibility are proinflammatory cytokines, matrix metalloproteinases, arachidonic acid derivatives and tissue inhibitors of matrix metalloproteinases, to name a few.

Interleukin-1 is one of the major proinflammatory cytokine involved in the pathogenesis of periodontitis. Its effects range from regulation of inflammatory mediators to catabolic effects on osteoclasts and activation of matrix metalloproteinases. The spectrum of immunostimulating activities and lipopolysaccharide elicited production made IL-1 a very attractive candidate as an important host factor in periodontal diseases.

Neutrophils, which are the first line of defense of periodontal tissues, rapidly accumulate in the infected periodontal tissues in large numbers along with macrophages and migrate into pocket to combat with the microorganisms. These neutrophils along with macrophages and other cells in periodontal tissues produce interleukin-1 in large quantities.

IL-1 has systemic as well as local effects on immunocompetent cells and other cells that are involved in the inflammatory reactions. Some of its effects include activation of T lymphocytes, proliferation of β cells and stimulation of antibody production. IL-1 also has effects on chemotaxis of neutrophils and mononuclear cells. Another important function is modulation of endothelial function that includes the release of granulocyte macrophage colony stimulating factor (GM-CSF), prostacycline (PGI₂),...
prostaglandin E₂ (PGE₂) and synthesis of platelet activating factor (PAF). Plasminogen activator inhibitor (PA-I) and tissue procoagulant activity (PCA) are enhanced by IL-1. It increases the adhesion of polymorphonuclear leukocytes (PMN), monocytes and related leukocyte cell lines. IL-1 modulates bone resorption by activating osteoclasts and by stimulating PGE₂ synthesis. Upon stimulation with IL-1, periodontal and gingival fibroblasts proliferate and release more PGE₂. The synthesis of collagens and hyaluronate synthetase activity are enhanced.⁷

Based on these findings, it seems reasonable to speculate that IL-1 may be relevant in the initiation and progression of periodontitis. Hence, this study is undertaken to correlate the levels of IL-1β with the clinical parameters in chronic periodontitis.

**MATERIALS AND METHODS**

A total of 60 patients were involved in the study. Thirty patients, who were diagnosed as chronic periodontitis based on clinical findings, formed the test group. Thirty individuals who had clinically healthy gingiva formed the control group. Subjects who have undergone any kind of intervention at least one year prior to the study that may affect periodontal condition, those having systemic conditions like diabetes and those having habits like smoking were excluded from the study. All the individuals participating in the study were examined with simplified oral hygiene index by Greene and Vermillion (OHI-S) periodontal disease index by Ramfjord (PDI), Gingival index by Loe and Silness (GI). Probing depth (PD) and tooth mobility and bleeding on probing (BOP) were noted at the sites from which crevicular fluid sample was collected. All the above-mentioned findings were recorded in patients’ first visit and before any periodontal treatment was started.

GCF samples were collected from any of the maxillary anterior teeth for the ease of the procedure and minimal contamination with saliva in the maxillary anterior region. Supragingival plaque and calculus were removed, with hand instrumentation, prior to collection of GCF. Care was taken not to touch gingival margin while scaling, to prevent stimulated flow of GCF. Test sites were carefully dried with a gentle stream of compressed air. Absorbent cotton rolls and saliva ejector were used to maintain isolation during sampling procedure.

The calibrated microcapillary tubes (0.5 ml range) were placed extra crevicularly at the mesiofacial, distofacial or midfacial surface of the tooth. A standardized volume of 20 µl of the gingival crevicular fluid was collected. The entire volume is collected from one site only. Test sites, that did not express adequate volume of fluid and microcapillary tubes contaminated with blood, were discarded. The collected GCF was immediately transferred to the sterilized microcentrifuge (Eppendorff) tubes and stored at 4°C. These tubes were then transported for analysis, which was done within 2 hours of collection of fluid. The GCF fluid was diluted 5 times with normal saline.

The collected GCF sample was used for ELISA (Immunotech, France) specific for human recombinant IL-1β.

**Preparation of Reagents**

Standard diluent buffer 10X concentrate: Distilled water was used to dilute the solution 10 times.

**Standards**

Depending on the type of samples, the kit included two standard diluents. Standard vials were reconstituted with most appropriate standard diluent because biological fluids might contain proteases as cytokine-binding proteins that could modify the recognition of the cytokine.

For serum and plasma samples, human serum was used as preferred standard diluent. The standard was reconstituted with 25 ml of standard buffer diluent. This produces a stock solution of 500 pg/ml IL-1β. Standard was allowed to stand for 5 minutes with gentle swirling prior to making dilutions. Serial dilutions of standard were made before each assay.

**Controls**

For serum and plasma, the preferred standard diluent was human serum. The control was reconstituted with 25 ml of standard diluent buffer. This was allowed to stand for 5 minutes with gentle swirling prior to distribution in control wells.

**Dilution of Biotinylated anti-IL-1β**

Dilution was done immediately before the use. Biotinylated anti-IL-1β was diluted with biotinylated antibody diluent in a clean glass vial. For 16 wells, 40 ml biotinylated antibody was mixed with 1060 ml diluent.

**Dilution of Streptavidin-HRP**

Streptavidin-HRP was diluted with HRP diluent. For 16 wells, 30 ml of streptavidin-HRP was mixed with 2 ml of HRP diluent.

**Washing Buffer 200× Concentrate**

Washing buffer was diluted 200 times in distilled water.

**Assay Method**

Before use, reagents were mixed appropriately without making foam. The number of microwell strips required to test the desired number of samples, and appropriate number of wells needed for running blanks, standards and controls
were determined. Each sample, standard, blank and control samples was assayed in duplicate.

Hundred milliliter of standard diluent was added to standard wells B1, B2, C1, C2, D1, E1, E2, F1 and F2. Standard vial with appropriate volume was reconstituted as described in reagent preparation. Two hundred milliliter of standard was pipetted into wells A1 and A2. Hundred milliliter of solution in A1 and A2 was transferred to B1 and B2. Contents were mixed by repeated aspiration and ejections. This procedure was repeated from B1 and B2 to C1, C2 and from C1 and C2 to D1, D2 and so on, creating two parallel rows of IL-1β standard dilutions ranging from 500 to 15.6 pg/ml, 100 ml from the content of last microwells used (F1, F2) was discarded.

Hundred milliliter of appropriate standard diluent was added to blank wells (G1-G2). Fifty milliliter were added to sample wells and 100 ml of reconstitution added to blank wells (G1-G2). Hundred milliliter of sample used (F1, F2) was discarded.

500 to 15.6 pg/ml, 100 ml from the content of last microwells

The Student’s t-test was employed to compare the IL-1β levels of the test group and control group. The p-value was determined to be 0.0032. PDI values were 4.0333 (±1.1592). PDI and IL-1β showed positive correlation with the p-value of 0.0000. Thus, the results were highly statistically significant.

IL-1β levels within the test group were correlated with each of OHI-S, GI, PDI, PD, mobility and BOP (Table 2).

The mean IL-1β level of test group was 409.2733 (±98.0503) pg/ml. The mean OHI-S level of test group was 5.1567 (±1.4343). The correlation coefficient \( r(X,Y) \) of OHI-S and IL-1β was 0.0772 which was not significant with the p-value of 0.6850. The mean GI score was 1.7793 (±0.4253). The correlation coefficient of GI and IL-1β was 0.3356. This was not significant as the p-value was 0.0698.

Bleeding on probing was determined on the basis of scoring criteria of gingival index by Loe and Silness (GI) and simplified oral hygiene index (OHI-S). Healthy individuals formed the control group (Fig. 1).

The mean IL-1β levels of test group were 409.2733 (±98.0503) pg/ml. Mean IL-1β levels of control group were 195.7700 (±80.0795) pg/ml. When Student’s t-test was applied to these scores, t-value obtained was 9.2373 with p of 0.0000. Thus, the results were highly statistically significant.

Results
Table 1 depicts the comparison between test and control groups for the levels of IL-1β. Both the groups consisted of 30 samples each. Test group was formed of chronic periodontitis cases. All these cases were diagnosed as chronic periodontitis depending on the clinical parameters like probing depth (PD), bleeding on probing (BOP) and tooth mobility and clinical indices, such as periodontal disease index by Ramfjord (PDI), gingival index by Loe and Silness (GI) and simplified oral hygiene index (OHI-S). Healthy individuals formed the control group (Fig. 1).

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### Statistical Analysis

The Student’s t-test was employed to compare the IL-1β levels in different groups. The Pearson’s correlation coefficient \( r \) was utilized to find the correlation between IL-1β levels and various clinical parameters.

The significance of p-value is calibrated as Not significant (NS) = \( p > 0.05 \); Significant (S) = \( 0.01 < p < 0.05 \); Very significant (VS) = \( 0.001 < p < 0.01 \); Highly significant (HS) = \( p < 0.001 \).

### Table 1: Comparison of test and control groups by IL-1β values

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean</th>
<th>SD</th>
<th>t-value</th>
<th>p-value</th>
<th>Significance</th>
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<td>Control</td>
<td>195.7700</td>
<td>80.0795</td>
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</tbody>
</table>

**Table 1:** Comparison of test and control groups by IL-1β values

**Groups**: Test vs. Control

**Mean**: 409.2733 vs. 195.7700

**SD**: 98.0503 vs. 80.0795

**t-value**: 9.2373

**p-value**: 0.0000

**Significance**: HS (Highly Significant)
Correlation of Levels of Interleukin-1β in Gingival Crevicular Fluid to the Clinical Parameters of Chronic Periodontitis


JCDP

The correlation coefficient was –0.2912 and was not significant as p-value was 0.1185. The mean PDI was 0.2333 (±0.4302). This was also a nonsignificant correlation with p-value of 0.5186 and correlation coefficient –0.1226. The PD, mobility and BOP were scored 0 in control group and hence no correlation could be determined (Fig. 3).

DISCUSSION

Chronic periodontitis is characterized by inflammatory destruction of connective tissues, loss of periodontal attachment and resorption of alveolar bone. Cytokine synthesis and release by cells in affected areas may be relevant in the development of these alterations. One of such cytokines is IL-1β.

IL-1β is a highly potent bone resorptive cytokine and was formerly referred to as osteoclast activating factor. It exerts other biological activities consistent with its potential role as a local mediator of tissue destruction in human periodontitis. These include inhibition of bone formation, stimulation of prostaglandin and thromboxane synthesis, stimulation of collagenase and protease production,

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean</th>
<th>SD</th>
<th>r (X,Y)</th>
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<th>p-value</th>
<th>Significance</th>
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<td>0.0004</td>
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</tbody>
</table>

NS – not significance, S – significance, HS – highly significance
potentiation of neutrophil degranulation and superoxide production, enhancement of endothelial cell-leukocyte adhesion and stimulation of fibroblast and keratinocyte proliferation.

Therefore, the levels of IL-1β were evaluated and compared in healthy and chronic periodontitis patients. Also the variations in IL-1β levels were correlated with the clinical parameters of the disease process.

The clinical signs or parameters that were considered in this study are: simplified oral hygiene index by Greene and Vermillion (OHI-S),\(^8\) gingival index by Loe and Silness (GI),\(^9\) periodontal disease index by Ramfjord (PDI),\(^9\) probing depth (PD), tooth mobility and bleeding on probing (BOP). OHI-S was considered to assess the oral hygiene status of the individuals and to keep an account of plaque being the main etiologic factor for initiation of the periodontal disease. Gingival index and bleeding on probing were considered as the indicators of inflammatory conditions. While gingival index was used to assess the inflammatory process throughout the oral cavity, bleeding on probing was used to assess inflammatory status at the site from where GCF was collected for IL-1β estimation. Criteria used for measuring bleeding on probing were similar to the scoring criteria of gingival index. The periodontal disease index and probing depth were taken into consideration to assess the connective tissue destruction and bone resorption. Periodontal disease index gave an idea of the overall periodontal tissue destruction, while probing depth was used as indicator of site-specific tissue destruction at the site from which GCF sampling was done.

The IL-1β levels were estimated from the samples of GCF taken from both healthy individuals as well as chronic periodontitis patients. Charon et al (1982),\(^10\) first showed that IL-1β can be detected in the GCF sample from the gingival inflammation cases. For the estimation of IL-1β, double sandwich antibody ELISA technique was used. This ELISA technique was first used by Honig et al (1989)\(^11\) and Masada et al (1990).\(^12\) This type of assay detects both biologically active and inactive material. Therefore, this assay technique was chosen.

In this study, IL-1β concentration in diseased periodontal tissues when estimated had a mean of 409.2733 pg/ml with a standard deviation of 98.0503, while it was 195.7700 pg/ml in the healthy tissues. On comparison between the two groups, the increase in IL-1β concentration in chronic periodontitis was highly significant (\(p = 0.0000\)). IL-1β is a major proinflammatory as well as host destructive cytokine. The major effect of this cytokine is osteoclast activation and hence the bone resorption. It also causes connective tissue destruction by various mechanisms like activation of MMP, PMN serine protease, phagocytic pathway, plasminogen dependent pathway. IL-1β has been found to be 15-fold more potent than IL-1α and 500-fold more potent than TNFα in stimulating bone resorption. Therefore, it is evident that IL-1β levels increase in chronic periodontitis. Preiss and Myele (1999),\(^13\) Ebersole JL et al (1993),\(^14\) Reinhardt et al (1993b),\(^15\) Matsuki et al (1993),\(^16\) Jandinsky et al (1991),\(^17\) Heasman et al (1993)\(^18\) also support the increase in IL-1β levels in periodontitis cases.

Within the control group, when IL-1β concentration was correlated with the clinical parameters like simplified oral hygiene index by Greene and Vermillion (OHI-S), gingival index by Loe and Silness (GI), periodontal disease index by Ramfjord (PDI), probing depth (PD), tooth mobility and bleeding on probing (BOP), OHI-S was considered to assess the oral hygiene status of the individuals and to keep an account of plaque being the main etiologic factor for initiation of the periodontal disease. Gingival index and bleeding on probing were considered as the indicators of inflammatory conditions. While gingival index was used to assess the inflammatory process throughout the oral cavity, bleeding on probing was used to assess inflammatory status at the site from where GCF was collected for IL-1β estimation. Criteria used for measuring bleeding on probing were similar to the scoring criteria of gingival index. The periodontal disease index and probing depth were taken into consideration to assess the connective tissue destruction and bone resorption. Periodontal disease index gave an idea of the overall periodontal tissue destruction, while probing depth was used as indicator of site-specific tissue destruction at the site from which GCF sampling was done.

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NS – not significance
PDI scores also showed minimal change in some of these subjects. The GI and PDI scores were recorded using a manual 1st generation probe and hence the pressure and direction of probing could not be standardized. Moreover, this method is technique and operator-sensitive. This could have resulted in the variation in GI and PDI scores. But when compared with IL-1β levels, OHI-S, GI and PDI scores could not be correlated as the scores of these clinical parameters were very minimal. PD, mobility and BOP were completely absent in the control group and hence no correlation with IL-1β could be derived. These findings are consistent with other studies done by Stashenko et al (1991b) and Reinhardt et al (1993b). Also reported that supragingival plaque, which contains predominantly gram-positive flora, may not effectively stimulate IL-1β production. This is in conjunction with our findings that OHI-S is not correlating with IL-1β levels.

Within the test group, when IL-1β concentration is compared with OHI-S, the correlation coefficient was 0.0772 that was indicative of nonsignificant correlation (p-value 0.6850). Thus, this correlation could not be established. The oral hygiene index gives the score of general full mouth local factors, whereas IL-1β is site specific. Its levels differ at different sites. Thus, a positive correlation between levels of IL-1β and OHI-S scores need not exist. Second, of more than 300 bacterial species identified in dental plaque, a selected few have been closely associated with active periodontitis. Thus, it can be stated that quality of the plaque rather than the quantity is important in activation of immune response along with IL-1β production. Some authors report that there is no correlation between IL-1β and plaque scores (Masada et al (1990); Wilton et al (1992): Stashenko et al (1991b) and Reinhardt et al (1993b)). However, Tsai, Ho, Chen (1995) found that IL-1β levels positively correlated with the plaque index. They related this to the activation of host immune system against plaque in general.

When IL-1β concentration was compared with GI score, the correlation could not be derived. Mean GI was 1.7793 and correlation coefficient was 0.3356 that was not significant (p = 0.0698). The mean of all the scores from different sites is considered in GI index, but the GCF was collected from one single site for IL-1β estimation. As the GI gives a mean score of all the areas, it might not have correlated with this site-specific cytokine. The local production of IL-1β by macrophages may also be the reason for site specificity of IL-1β. Studies have reported changes in IL-1β levels at different sites (Stashenko et al 1991b), hence the site specificity of IL-1β. Masada et al (1990); Wilton et al (1992); Stashenko et al (1991b) have reported no correlation between them. However, Hou et al (1995); Tsai, Ho, Chen (1995) have reported positive correlation of gingival signs with IL-1β levels attributing this to the degree of inflammation at the time of sampling.

The correlation between IL-1β and BOP was highly significant (p = 0.0004). The correlation coefficient was 0.8080 which meant that there was positive correlation between IL-1β and BOP. For the measurement of bleeding on probing, the scoring criteria of GI were considered. For bleeding on probing the site from which GCF was collected, was considered and as IL-1β is a locally acting cytokine, its levels correlated best with the bleeding on probing. IL-1β being a proinflammatory cytokine, its level increases during active disease process and since bleeding on probing being an indicator of ongoing active disease process, its correlation with IL-1β levels is justifiable. These findings are consistent with Hou et al (1995) and Tsai, Ho, Chen (1995).

As the scoring criteria for GI and BOP were similar, both these should have equally correlated with the IL-1β concentration. But GI did not correlate with IL-1β concentration, whereas BOP positively correlated. Possible reason for this difference might be that IL-1β is produced locally and also acts on the local environment. Therefore, its concentration best correlated with BOP which is an indicator of local inflammatory activity and it did not correlate with GI which is an indicator of general inflammatory activity throughout the oral cavity. IL-1β and periodontal disease index by Ramfjord correlated positively in our study (correlation coefficient 0.5210). This correlation was very significant (p = 0.0032). PDI gives the score of oral cavity for the inflammatory activity as well as the host tissue destruction in the form of clinical attachment loss. Thus, this index gives an overall idea of periodontal disease progression. IL-1β is a proinflammatory cytokine. Its inflammatory activity has been demonstrated by many authors like Heasman et al (1993); Ishida et al (1993); Saito et al (1991); Unemori et al (1994). These activities involve activation of PGE2 synthesis, activation of phospholipase A2, leukotriene B4 and thromboxane B2 production. It also activates the host destructive mechanisms like fibroblast proliferation, stimulation of metalloproteinases, activation of collagen degradation and activation of plasminogen-dependent pathway, PMN-serine proteinase pathway and phagocytic pathway, as studied by Birkedal-Hansen H (1993); Feldner et al (1994); Unemori et al (1994). It also has bone-resorbing activity by activation of osteoclasts which has been reported by Saito et al (1991); Gowen et al (1983); Birkedal – Hansen H (1993) and Stashenko et al (1991a).
Hence, this IL-1β concentration is in positive correlation with the PDI score. These results are in conjunction with Stashenko et al (1991a);30 Stashenko et al (1991b);19 Hou et al (1995);23 Ebersole et al (1993);14 Tsai, Ho, Chen (1995).22

IL-1β levels and probing depth correlated with each other positively (correlation coefficient 0.8866) with high significance (p-value 0.0000). It was also found that the levels of IL-1β increased as the probing depth increased. IL-1β causes bone resorption and connective tissue destruction and, therefore, its levels best correlated with the probing depth which is the outcome of the host tissue destruction. These findings are consistent with the studies done by Reinhardt et al (1993b);15 Hou et al (1995);23 Stashenko et al (1991a,b).19,30

Although both PDI and PD showed correlation with IL-1β, there was difference in the significance level of both these parameters. Probing depth showed a very strong significance in the correlation. This can be explained with two possible reasons. First, PDI gives the overall score of the oral cavity, while probing depth was measured at the site of GCF sampling. Second, in PDI, six index teeth are used for scoring. Thus, the tooth from which GCF sample was collected may not have been considered. This could have affected the significance level.

Mobility also showed positive correlation with IL-1β (correlation coefficient 0.4581). This correlation was significant (p = 0.0109). In most of the cases of periodontitis, tooth mobility is usually associated with the disease progression. Hence, there could be a positive correlation between mobility and IL-1β. But, there are many other causes of mobility like trauma, hormonal variations, systemic diseases, trauma from occlusion, habits like bruxism, tongue thrusting. These factors might have affected the significance level of the correlation between IL-1β and mobility. Tsai, Ho, Chen (1995)22 also found only a borderline correlation between mobility and IL-1β.

CONCLUSION

It could be stated from this study on IL-1β that there seem to be a strong correlation between periodontal tissue destruction and IL-1β. Furthermore, IL-1β level could also differentiate between active and inactive periodontal lesions.

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Correlation of Levels of Interleukin-1β in Gingival Crevicular Fluid to the Clinical Parameters of Chronic Periodontitis

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