ROLE OF INTERLEUKIN-8 IN PERIODONTAL HEALTH AND DISEASE

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ABSTRACT

Interleukin-8 is a proinflammatory cytokine, a potent chemoattractant and activator of neutrophils and other immune cells. The present study was undertaken to estimate IL-8 concentration in GCF of healthy, gingivitis and periodontitis patients and also to evaluate any association between IL-8 concentrations in GCF with the amount of periodontal tissue destruction. IL-8 levels were evaluated for three groups consisting of 25 subjects each, aged 25 to 50 years. The subjects were assigned to different groups based on both clinical and radiological findings. The GCF samples were collected with micropipettes and IL-8 concentration was measured using a commercially available ELISA kit. The results indicate that IL-8 concentration increased progressively from health controls to gingivitis and then to periodontitis. There was statistically significant difference in the mean ranks of health, gingivitis and periodontitis. However, there is a high degree of variability in IL-8 concentration from one subject to another in the different groups. Whereas evaluation of IL-8 may indicate the presence of disease, it may not demonstrate those sites which are susceptible for further periodontal breakdown. Future studies employing more samples with longitudinal time frame along with microbiological evaluation are required to assess the diagnostic potential of IL-8 levels in periodontal tissues.

KEYWORDS: IL-8, periodontitis, neutrophils, cytokines.

INTRODUCTION

Periodontal diseases are comprised of a group of inflammatory conditions of microbial origin that result in the destruction of the supporting structures of the teeth. These diseases are
caused by predominantly gram-negative anaerobic micro-organisms localized in the subgingival region and include typically Porphyromonas gingivalis, Prevotella intermedia, Actinobacillus actinomycetemcomitans and Tannerella forsythus.\(^1\) The microbial challenge results in activation of the host response. The host responds with an immediate inflammatory and immune response through the activation of complement, resident leukocytes and mast cells. Neutrophils function primarily as antimicrobial cells, whereas the chronic inflammatory cells orchestrate adaptive responses.\(^2\) The activated immunocompetent cells produce and secrete cytokines. Cytokines are soluble proteins, secreted by cells and act as messenger molecules that transmit signals to other cells. The interleukins are important members of the cytokine family and are primarily involved in communication between leukocytes and other cells, such as epithelia, endothelia and other cells. IL-8 is one such cytokine that acts as powerful chemoattractants for neutrophils and fibroblasts.\(^2\) IL-8 is secreted by monocytes, lymphocytes, keratinocytes, endothelial cells and fibroblasts upon stimulation with IL-1, TNF-\(\alpha\), LPS and other agents.\(^3,4\) Interestingly enough, neutrophils themselves have been shown to produce IL-8 when stimulated with LPS. IL-1, TNF-\(\alpha\) and LPS are the most potent inducers of IL-8 secretion.\(^3\) High levels of IL-8 have been detected in the junctional epithelium and inflammatory cell infiltrate of periodontal lesions.\(^5\) Gingival epithelial cells are able to regulate IL-8 expression in response to A. actinomycetemcomitans and enhance the neutrophil response in defense of the host.\(^6\) IL-8 attracts neutrophils in a similar manner to leukotriene B4, but promotes the activation of different pathways within the neutrophils.\(^6\)

IL-8 could be the most important neutrophil chemotactic factor during the early stages of periodontitis. The biological activities of IL-8 are similar to NAP-2 (neutrophil activating protein-2). It differs from other cytokines in its ability to specifically activate neutrophil granulocytes where it causes transient increase in cytosolic calcium levels, exocytosis of storage granule proteins and respiratory burst. IL-8 increases chemotaxis and enhanced expression of adhesion molecules. IL-8 induces the release of vitamin B12-binding protein and gelatinase from the specific granules and secretory vesicles, respectively. IL-8 stimulates MMP-8 release by neutrophils.\(^7\) The binding activity of leukocyte adhesion receptor is enhanced after stimulation of neutrophils with IL-8.\(^8\) Exposure of PMNs to IL-8 induces upregulation of CD18 (LFA-1) expression.\(^9\) as well as integrin receptor activation.\(^10\) IL-8 exposure also induces rapid shedding of L-selectin by human PMNs.\(^9\) The respiratory burst is a typical response of phagocytes. Like other chemotactic agonists, IL-8 elicits a transient production of superoxide and H2O2. In terms of duration and intensity, NADPH-oxidase
activation induced by IL-8 is intermediate between that induced with FMLP or C5a and that induced with LTB4.\textsuperscript{[3, 11]}

Chemokines attract and activate leukocyte populations through interaction with specific receptors that are members of the seven transmembrane-spanning G-protein coupled β-adrenergic rhodopsin receptor superfamily. On average, human PMN possess 64,500±14,000 receptors for IL-8 on their plasma membranes. IL-8 receptors are glycoproteins and have been designated as CXCR1 and CXCR2.\textsuperscript{[12]} previously called as IL-8 receptor A (IL8RA) and IL-8 receptor B (IL8RB) respectively.\textsuperscript{[13]} The presence of IL-8 receptors have been demonstrated in gingival epithelium, microvascular endothelial cells and vascular smooth muscle cells.\textsuperscript{[14]}

IL-8 is a known leukocyte chemotactic cytokine that has also been implicated in angiogenesis and may play a role in angiogenesis-dependent conditions such as rheumatoid arthritis, tumour growth and wound healing.\textsuperscript{[15]} as well as act as a marker of inflammatory diseases such as psoriasis.\textsuperscript{[16]} There are many studies that evaluated the role of IL-8 in periodontal disease.\textsuperscript{[17-28]} yet there is little information on the association of IL-8 with the progression of disease. Since the local production of IL-8 may be associated with an increased neutrophil migration in the periodontal tissues, it seems reasonable to speculate that IL-8 may be relevant in the initiation and progression of periodontitis. The following study is designed to quantitate IL-8 levels in GCF from patients with chronic periodontitis, chronic gingivitis and subjects with healthy gingiva.

**MATERIAL AND METHODS**

A total number of 75 subjects, comprising of 39 males and 36 females from the outpatient department of Oxford dental college participated in this study. The investigation was approved and informed consent was obtained. The age of the participants ranged between 25 and 50 years. None of the patients had any known systemic conditions or infections that could affect IL-8 levels, either in serum or in gingival tissues. Pregnant and lactating women, smokers, patients with chronic inflammatory diseases and patients with acute infections were excluded from the study.

Subjects were classified into three groups based on clinical parameters such as plaque index, gingival index, bleeding on probing, probing pocket depth (PD), attachment loss (AL) and bone loss. Clinical examination was done using a sterile mouth mirror and a William’s
graduated periodontal probe. Group 1 (Control) consisted of 25 subjects with clinically healthy periodontium and with no evidence of disease. The gingiva was characterized clinically by its pink colour, firm consistency and scalloped margins. The interdental papillae were firm, did not bleed on gentle probing and filled the space below the contact areas. The gingiva exhibited a stippled appearance and there was a knife edge margin between the tooth and soft tissues. Group 2 (Gingivitis) consisted of 25 subjects whose gingivae showed clinical signs of inflammation but there was no evidence of attachment loss. Clinical findings included erythema, edema, bleeding, sensitivity, tenderness and enlargement of the gingiva. Radiographic analysis and/or probing attachment levels did not indicate loss of supporting structures. Group 3 (Periodontitis) consisted of 25 subjects who showed clinical signs of gingival inflammation with clinically detectable attachment loss and bone loss. This was characterized by periodontal pocket formation and/or gingival recession and changes in density and height of subjacent alveolar bone as detected on the bitewing radiograph. Clinical parameters assessed for the study were Calculus Index, Plaque Index, Gingival Index, Bleeding on probing, probing pocket depth and clinical attachment level and radiographic bone level.

Subjects selected for the study were made to sit comfortably in an upright position on the dental chair with proper illumination. Prior to gingival crevicular fluid collection, the supragingival plaque was scored. Based on the periodontal status, the site with the maximum probing depth was selected for sampling. The site to be sampled was isolated with cotton rolls, air dried gently and supragingival plaque was carefully removed. Crevicular fluid was obtained before probing the site by placing colour-coded 1 to 5 microlitre calibrated volumetric micro-capillary pipettes at the gingival margin. The pipettes were obtained from SIGMA-ALDRICH Chemical Company. A standardized volume of 1 microlitre of crevicular fluid was collected by placing the tip of the pipette extracrevicularly. Samples of gingival crevicular fluid contaminated by blood or saliva were discarded. The samples were assayed for IL-8 concentrations by using the QUANTIKINE HUMAN IL-8 ELISA kit obtained from R & D Systems, Minneapolis, MN, USA.

**Statistical Analysis**

Student t test has been used to find the significance of study parameters between Group II and Group III, Analysis of variance has been used to find the significance of study parameters between three groups. Post-hoc Tukey test has been used to find the pair wise significance of
study parameters. The Statistical software namely SPSS 11.0 and Systat 8.0 were used for the analysis of the data and Microsoft word and Excel have been used to generate graphs, tables etc.

Fig.1: Clinical Picture of a Patient of group I showing probing depth of 1 mm on mid-labial aspect of 22.

Fig. 2: Bitewing radiograph showing absence of bone loss in a patient of Group I.

Fig.3: Clinical Picture of a Patient of group II showing probing depth of 3mm on mesial aspect of 12.
Fig. 4: Bitewing radiograph showing absence of bone loss in a patient of Group II.

Fig. 5: Clinical Picture of a Patient of Group III showing probing depth and attachment loss of 5mm on mesial aspect of 13.

Fig. 6: Bitewing radiograph showing bone loss in a patient of Group III.

Fig. 7: Collection of GCF using extra crevicular positioning of microcapillary pipette from 12.
Fig. 8: aspirated GCF samples being added to the wells of ELISA kit.

RESULTS

In this study a highly significant (p<0.001) increase in the IL-8 levels were found in the gingival crevicular fluid of gingivitis and periodontitis patients. The mean IL-8 concentration was 499.12±137.50 pg/μl in the control group, 1144.96±365.30 pg/μl in the gingivitis group and 2100.42±414.96 pg/μl in the periodontitis group.

Table 1: Basic characteristics of the study.

<table>
<thead>
<tr>
<th>Basic characteristics</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Age in years (Mean ± SD)</td>
<td>28.48±4.21</td>
<td>29.04±4.07</td>
<td>40.68±5.48</td>
</tr>
<tr>
<td>Sex</td>
<td>Male (64.0%)</td>
<td>Male (76.0%)</td>
<td>Male (48.0%)</td>
</tr>
<tr>
<td></td>
<td>Female (36.0%)</td>
<td>Female (24.0%)</td>
<td>Female (52.0%)</td>
</tr>
</tbody>
</table>

Table 2: Comparison of study parameters.

<table>
<thead>
<tr>
<th>Study parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plaque Index</td>
<td>0.13±0.08(^a) (0.03-0.42)</td>
<td>1.67±0.42(^b) (1.0-2.45)</td>
<td>1.76±0.49(^b) (0.71-2.83)</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Gingival Index</td>
<td>-</td>
<td>1.80±0.44 (1.04-2.96)</td>
<td>1.81±0.41 (0.75-2.58)</td>
<td>0.941</td>
</tr>
<tr>
<td>Calculus Index</td>
<td>-</td>
<td>1.41±0.48 (0.63-2.00)</td>
<td>1.84±0.26 (0.94-2.00)</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>BOP</td>
<td>-</td>
<td>87.56±10.71 (67-100)</td>
<td>88.64±12.22 (61.20-100)</td>
<td>0.588</td>
</tr>
<tr>
<td>Mean-Probing depth</td>
<td>1.27±0.19(^a) (0.80-1.65)</td>
<td>2.27±0.25(^b) (1.85-2.68)</td>
<td>3.44±0.80(^c) (2.23-6.00)</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Mean CAL</td>
<td>-</td>
<td>-</td>
<td>2.61±1.74 (0.47-6.92)</td>
<td>-</td>
</tr>
<tr>
<td>Probing depth-site</td>
<td>1.24±0.27(^a) (1.00-1.75)</td>
<td>3.38±0.41(^b) (3.00-4.00)</td>
<td>7.72±1.84(^c) (5.0-10.0)</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>CAL-site</td>
<td>-</td>
<td>-</td>
<td>6.84±2.25 (3.00-10.0)</td>
<td>-</td>
</tr>
<tr>
<td>IL-8</td>
<td>0.499.12±137.50(^a) (279-748)</td>
<td>1144.96±365.30(^b) (618-1917)</td>
<td>2100.42±414.96(^c) (1478-2879)</td>
<td>&lt;0.001**</td>
</tr>
</tbody>
</table>

Results are presented in Mean ± SD (Min-Max). Non-identical superscripts are significant at P<0.05 by Post-hoc Tukey test.
Table 3: Pearson correlation co-efficient between IL-8 and study parameters.

<table>
<thead>
<tr>
<th>Pair</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI vs IL-8</td>
<td>-0.114 (0.588)</td>
<td>-0.322 (0.116)</td>
<td>0.137 (0.515)</td>
</tr>
<tr>
<td>GI vs IL-8</td>
<td>-</td>
<td>-0.312 (0.129)</td>
<td>-0.107 (0.610)</td>
</tr>
<tr>
<td>CI vs IL-8</td>
<td>-</td>
<td>0.306 (0.136)</td>
<td>-0.068 (0.747)</td>
</tr>
<tr>
<td>BI vs IL-8</td>
<td>-</td>
<td>0.098</td>
<td>-0.050</td>
</tr>
<tr>
<td>PD mean vs IL-8</td>
<td>0.023 (0.915)</td>
<td>-0.122 (0.563)</td>
<td>0.163 (0.436)</td>
</tr>
<tr>
<td>CAL mean vs IL-8</td>
<td>-</td>
<td>-</td>
<td>0.193 (0.356)</td>
</tr>
<tr>
<td>PD site vs IL-8</td>
<td>0.250 (0.227)</td>
<td>-0.012 (0.954)</td>
<td>0.596** (0.002**)</td>
</tr>
<tr>
<td>CAL site vs IL-8</td>
<td>-</td>
<td>-</td>
<td>0.625** (0.001)</td>
</tr>
</tbody>
</table>

Fig.9: Basic characteristics of the study age wise

Fig.10: Basic characteristics of the study in sex wise.
Fig. 11: Comparison of Plaque Index.

Fig. 12: Comparison of Gingival Index.

Fig. 13: Comparison of Calculus Index.
Fig. 14: Comparison of Bleeding Index.

Fig. 15: Comparison of Mean Probing depth.

Fig. 16: Comparison of Mean CAL.
Fig. 17: Comparison of Probing depth site.

Fig. 18: Comparison of CAL-site.

Fig. 19: Comparison of IL-8.
DISCUSSION

Current understanding of the pathogenesis of periodontitis suggests that it is of a multifactorial nature, being a result of complex interactions between pathogenic subgingival microorganisms and the host response.\cite{33} Increasing knowledge of this complexity provides new opportunities for diagnostic strategies to be investigated.\cite{34} The local host response in periodontal disease can be evaluated by immunological and biochemical analysis of GCF. IL-1β, TNF-α, IL-6 and IL-8 are some of the inflammatory and immune mediators identified in GCF.\cite{35-37}

IL-8 plays an important role in the maintenance of local host-parasite equilibrium and in the limitation of neutrophil-associated tissue destruction.\cite{38} Under normal conditions, IL-8 expression relates well to the pattern of neutrophil infiltration and appropriate release of IL-8 contributes to eliminating the infecting bacteria by neutrophils. Conversely, an uncontrolled release of IL-8 and resultant hyperactivity of neutrophils may cause tissue destruction.\cite{39} IL-8 is considered to be the most important mediator for the recruitment and activation of polymorphonuclear leukocytes.\cite{40} It can induce the release of matrix metalloproteinase-8 (MMP-8) by neutrophils,\cite{7} a potent collagenase for degrading host connective tissues at sites of inflammation. It has been hypothesized that IL-8 elicited neutrophils in periodontitis may have a different phenotype from that elicited by bacterial chemotactic peptides. The former phenotype would result in a tissue-destructive neutrophil phenotype as opposed to the latter, which would be a more antimicrobial neutrophil.\cite{41}

The objective of the present study was to compare the concentration of IL-8 in patients with chronic periodontitis, chronic gingivitis and periodontal health. The presence in of IL-8 in healthy individuals could be related to the steady state of gingiva, considering this is a site of permanent antigenic insult, requiring the presence of neutrophils, macrophages and antigen presenting cells, which could be chemoattracted towards the gingival micro-environment by IL-8. The level of this cytokine exhibited a statistically significant difference between all the three groups, being highest in the periodontitis group and lowest in healthy controls.

IL-8 level in GCF is highly related to the inflammatory status of the periodontium. However, conflicting results concerning the association of IL-8 in GCF and the severity of periodontitis have been reported. Several studies suggest a positive relationship between GCF IL-8 activity and periodontal disease. According to Mathur et al., total amounts of IL-8 were significantly higher in GCF from diseased sites of patients with chronic periodontitis as compared to GCF
from healthy sites of control patients. Moreover, Tsai et al. observed that total amounts of GCF IL-8 significantly decreased after therapy in chronic periodontitis patients. Similar results were found by Giannopoulou et al. where IL-8 levels were significantly elevated in diseased sites compared to healthy sites. On the contrary, other studies suggest an inverse relationship between IL-8 activity and PMN recruitment. Jin et al. showed lower concentrations of IL-8 in patients with periodontitis as compared to healthy controls. Furthermore, Chung et al. observed that healthy subjects demonstrated a significantly higher IL-8 concentration than chronic periodontitis patients. The investigators hypothesized that the decreased concentration of IL-8 in diseased sites could be due to increased binding and interaction of IL-8 with the receptors on the surface of granulocytes. A study in Localized Aggressive Periodontitis patients by Ozmeric et al. showed lower GCF levels of IL-8 as compared to healthy subjects. Since the pathogenesis of Localized Aggressive Periodontitis is completely different from Chronic Periodontitis, the variation in the data may be due to the difference in the concentration of inflammatory mediators including IL-8 secretion and factors besides IL-8 contributing to disease progression.

Such conflicting results may be related to varying factors. One possible explanation could be attributed to as yet unidentified mediators of IL-8 secretion accounting for the difference in concentration observed. Additionally, it could be due to the increased GCF volumes found in diseased sites. It should be borne in mind that in the presence of inflammation, lower concentrations of a given parameter may correspond to a significant increase of crevicular fluid volume, whereas in health, higher concentrations may only reflect the minimal amounts of crevicular fluid available. The difference in GCF volume also depends on the duration of sample collection. Another important factor is the method used for collection of the gingival fluid. In most of the other studies, filter paper strips have been used to collect GCF which was subsequently measured and assayed. In the present study, microcapillary pipettes have been placed used to collect GCF for two main reasons. One because of unavailability of Periotron™ 6000 or an equivalent apparatus to measure the quantity of GCF collected and second to rule out the possibility of polypeptides getting attached to cellulose which is a main component of filter paper. IL-8 mRNA in gingival tissues was not estimated as the procedure would have been unethical.

The present study showed a strong positive association between the concentration of IL-8 in GCF and the periodontal disease status. There is also a great deal of variation in host
inflammatory response to the microbial challenge in terms of intensity of IL-8 related granulocyte activity among individuals. On the basis of the present study, it can be speculated that in a susceptible individual, significantly increased levels of IL-8 in GCF may reflect a stage of inflammatory response that is associated with destructive events.

Questions such as which patient or site is at a greater risk for future breakdown, how aggressive is the disease process, whether the disease is in a state of remission or active and whether the attachment loss is progressive or the condition is stable, have plagued the minds of Periodontists from time immemorial. The findings of this study lead to believe that IL-8 concentration in GCF could assist the clinician in answering some of the above questions but a major question that remains to be answered is the identification of sites at risk for future disease progression. The study could be improved in future by evaluating pre-treatment and post-treatment change in IL-8 levels with microbial analysis and longitudinal studies on gingivitis sites progressing to periodontitis. It would be more beneficial if GCF IL-8 concentration is correlated with IL-8 mRNA profile in tissue biopsies from healthy and diseased sites. Such an approach may lead to strategies for altering cytokine profiles associated with destructive periodontitis lesions.

**CONCLUSION**

Based on the findings from this study, it can be concluded that IL-8 concentration in the gingival crevicular fluid increases significantly with the severity of disease. As IL-8 has pro-inflammatory effects, its presence in localized sites causes an imbalance in the immune response and an enhanced production of inflammatory mediators. However, due to the limited sample size and the cross-sectional design of the study, IL-8 can at best be considered as an “associated factor” of periodontal tissue destruction and not a “predictor” of periodontal disease. Further research through longitudinal studies is required to implicate IL-8 as a “predictor” of further periodontal breakdown.

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