Correlation between Circulatory and Salivary IL 10 Levels in Periodontal Health and Disease – A Report

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Abstract Interleukin (IL)-10 is an important immunoregulatory cytokine produced by many cell populations. Its main biological function seems to be the limitation and termination of inflammatory responses and the regulation of differentiation and proliferation of several immune cells such as T cells, B cells, natural killer cells, antigen-presenting cells, mast cells, and granulocytes. The present study has correlated the salivary IL-10 levels with that of the circulatory levels in periodontal health and diseased patients and also brings an attestation for Saliva as point of care diagnosis.

Keywords: interleukins, pro inflammatory cytokines, anti inflammatory cytokines Th1, Th2, GCF

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1. Introduction

Periodontal Disease is a chronic inflammatory condition, characterized by loss of hard and soft tissues of the tooth supporting structures. Although microbial in origin, the destruction of the tissues is the result of an exaggerated host response [2,4]. Complex inflammatory and immune responses are involved in the progression of periodontal disease. B cells and T cells accumulate in large numbers in the periodontal tissues, although until recently we have had little information on cellular synthetic activity and proliferation of these cells. [7] When an assessment is made of the role that different cell types play in the sites of inflammation one must be wary of the limitations imposed by merely observing morphology and phenotypic cell surface markers. Gemmell & Seymour [18] have shown an increased proportion of T cells as the infiltrate increases in diseased gingival tissues. Others have shown an increase in T cell numbers in peripheral blood and it has been suggested that homing of these cells to the gingiva may occur during the disease process [5]. The specific T cells proliferate locally within certain tissues, giving rise to characteristic T cell clones for these regions [1,12,15].

Analysis of the cytokine profiles of the T cell subsets Th1 and Th2 in periodontal tissues have been made using distinctly different strategies. Gemmell & Seymour [17] performed FACs analysis of leucocytes extracted from periodontal tissues, while Yamazaki et al [34] used an Immunochemical method is used to detect the cells in periodontal lesions, and a cell blotting technique to trap and identify IL-2- and IL-4-expressing T cells.

Figure 1. Three – dimensional Structure of IL 10

1.10 & its biological effects:
IL-10 a Negative Feedback Regulator: Immunomodulator
1. Produced mainly by activated macrophages and regulatory T cells
2. Inhibitor of activated macrophages and dendritic cells and is thus involved in the control of Innate Immune Reactions and Cell Mediated Immunity [6,9]
3. Thus serves as Immunoregulatory cytokine stimulating innate immunity and Th2 responses while suppressing Th1 responses (inflammation associated) [14].

2. Cytokines in Serum

1. Up regulation of pro inflammatory cytokines not only lead to tissue destruction in inflammed gingival
tissues but also result in a systemic spill over, and thereby modulate the course of several systemic diseases viz. CVD, RA, diabetes etc. [Offenbacher et al 1998] [30]

2. Elevated levels of several pro-inflammatory cytokines such as TNFα, IL-1, IL-6, have been reported, but the role of the anti-inflammatory cytokines, in circulation has not yet been fully elucidated. [Miller et al] [27].

3. Saliva – A Diagnostic Tool

Saliva has been used as a noninvasive vehicle to monitor progression and treatment outcome of several diseases including periodontal disease. Recently, oral fluid based “point of care” diagnostics, based on microfluidic assays are being adapted for its potential chair side use in periodontal disease

i. **Aim of the Study:**
   1. Evaluation of circulatory levels of IL-10 & hs CRP in Periodontal health and disease
   2. Evaluation of salivary IL-10 levels and its relationship to its circulatory levels

ii. **Materials and Methods**:

Sample population:
   Total 30 patients (Periodontally health & disease)- out patient pool of Ragas Dental College.

Sample Collection:
   a. Blood- from the antecubital fossa, serum separation was done by centrifugation.
   b. Saliva – 5 ml of whole unstimulated saliva was collected by spitting method, centrifuged at 2500 rpm for 10 mins, stored in -80°C till further use.
   c. Both blood and unstimulated saliva samples were obtained prior to treatment.

**Figure 2. Healthy gingiva**

**Figure 3. Healthy gingiva**

**Figure 4. Saliva Sample**

**Figure 5. Saliva Sample**

**Figure 6. Sterile Container to collect saliva**

**Figure 7. Blood Sample Collection from Ante cubetal fossa**

**Figure 8. Armamentarium used for Blood sample collection**

**Figure 9. Armamentarium for centrifuge**

**Figure 10. Centrifuge**
Serum hsCRP levels- evaluated by immunoturbidity method (private laboratory).

ELISA Assay
Serum & salivary IL-10 levels evaluated by sandwich ELISA method.

|                | hs CRP (mg/l) | IL10 (Serum levels) (pg/ml) | IL10 (Salivary Levels) (pg/ml) |
|----------------|---------------|-----------------------------|-------------------------------|
| Health (Mean±SEM) | 1.16±0.033    | 33.7±2.7                    | 88.7±5.1                      |
| Disease (Mean±SEM) | 2.25±0.29     | 26.1±2.3                    | 74.5±3.6                      |
| P Value         | 0.024         | 0.045                        | 0.036                         |

1. A statistically significant (p<0.05) increase of serum hs-CRP levels was observed in of serum hs-CRP levels was observed in periodontal disease when compared to health.
2. Statistically significant (p<0.05) decrease of serum IL-10 levels in periodontal disease when compared to health.
3. Negative correlation between serum hs-CRP and IL-10 levels was observed which was not statistically significant.
4. Statistically significant (p<0.05) decrease of salivary IL-10 levels in periodontal disease when compared to health.

5. Discussion
A systemic inflammatory state has been reported when there is a dysregulation in the balance between the pro inflammatory and anti inflammatory cytokine levels (Ritter et al 1999) [32]. A preponderance of pro inflammatory cytokine in disease has been documented in several studies but the down regulation of the anti inflammatory cytokines has not been widely documented. However decreased levels of IL-4 in the GCF has been reported by Giannobile et al 1998 [22] following periodontal disease. These authors conclude that downregulation of the anti inflammatory IL-4 would have contributed to the prolonged inflammation observed in periodontal tissues. Several lines of evidence suggest a role for IL-10 in periodontal disease pathogenesis:
- Regulate immune responses
- IL-10 gene polymorphism associated with disease severity
IL-10 gene knock out mice exhibit increased osteoclastogenesis and bone loss [28].
This study also shows a down regulation of circulatory anti inflammatory cytokine IL-10 in periodontal disease in accordance with the Giannobile study [21]. This could have contributed to the systemic inflammatory state, as shown by increase of hs-CRP observed in our patients. However as the correlation was not statistically significant other contributing factors may also play a role [20]. There is thus a need for simultaneous assessment of several pro and anti inflammatory cytokines to predict the systemic inflammatory status accurately. There was also a significant decrease of Salivary IL-10 levels in Periodontal disease and this correlates strongly to the circulatory hs-CRP. Saliva may therefore truly reflects the systemic inflammatory state evoked by Periodontal disease [23].

6. Conclusion
Salivary IL-10 levels may reflect the systemic inflammation observed in periodontitis. Hence saliva may be used as a non-invasive chair side diagnostic tool for systemic status evaluation of periodontal patients. A larger sample size with a longitudinal assessment of a cocktail of pro and anti inflammatory cytokines may provide greater strength to this hypothesis.
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