RANTES comparison in patients with periodontal disease - A prospective clinical study

Ena Sharma a,*, Anuj Sharma a, Manjari Nadelab

a Maharishi Markendeshwar Medical College and Hospital, Solan City, HP state, India
b Oxford Dental College, Bangalore, Karnataka state, India

1. Introduction

Research into the pathogenesis of disease has traditionally involved a reductionist approach in which discrete inflammatory pathways and processes are investigated to elucidate underlying mechanisms. With advances in genomic, epigenetic, proteomic, and metabolomic capabilities, an increased interest has emerged in a biologic systems approach to define the complex regulatory networks that result in health or disease [1] (see Figs. 2–6).

Periodontitis is a complex disease in which disease expression involves intricate interactions of the biofilm with the host immunoinflammatory response and subsequent alterations in bone and connective tissue homeostasis [2–4].

The basic conceptual model of periodontitis was revised in 1997 (Fig. 1) [5], in great part to acknowledge that various risk factors operate by modifying host responses led to changes in disease expression. In this model, host immunoinflammatory mechanisms are activated by bacterial product. In addition, cytokines and prostanoids, as well as matrix metalloproteinases activated through the host response, may stimulate damage to connective tissue and bone and shape the clinical presentation of disease [6].

Though specific microorganisms are cited as a cause for periodontitis, various other aspects of tissue alterations are also known to modify the periodontal status adversely. Based on this concept, presently serum, saliva, tissue biopsy specimens and gingival crevicular fluid have been investigated for their biochemical constituents [7,8].

Gingival crevicular fluid is generally considered as an initial transudate/interstitial fluid which later changes to exudates in the presence of inflammation [9]. It contains a vast array of biochemical factors, offering potential use as a diagnostic or prognostic biomarker of the biologic state of the periodontium in health and disease [10].

Chemokines selectively attract and activate different leukocyte subpopulations which in turn induce inflammation [11]. RANTES, a member of the CC chemokine family, displays a significant chemotactic activity for eosinophils, monocytes and CD + T cells [12].

RANTES expression has also been demonstrated in a variety of other diseases characterized by inflammation, including asthma, atherosclerosis, endometriosis and fibrosis [13].

Considering the multifunctional ability of monocytes/macrophages, these could be directly involved in initiation of development of the inflammatory response and alveolar bone loss observed in periodontitis.

Therefore, analysis of RANTES mechanism that induces monocyte recruitment into periodontal tissues represents an important step towards understanding the pathogenesis of this disease.

This study envisages to determine the presence of RANTES in GCF samples in healthy subjects, patients with gingivitis and periodontitis before and after initial periodontal therapy. The objectives of the study include assessment and comparisons of the levels of RANTES in GCF of healthy subjects, patients with gingivitis, chronic periodontitis and aggressive periodontitis, before and after 2 months following initial periodontal therapy, to correlate the levels of RANTES in GCF with various periodontal parameters, to determine whether RANTES can serve as a marker in the identification of active phase of periodontal disease.

2. Materials and methods

40 subjects who visited the Department of Periodontics, The Oxford Dental College, Hospital and Research center, Bangalore were included in the study. An informed consent was obtained...
from all the subjects. The study period was of 10 months.

Inclusion Criteria: Subjects aged between 18 and 55 years, Systemically healthy subjects, No antibiotic/NSAIDs usage in previous 3 month, No history of periodontal treatment in the last 6 months.

2.1. Exclusion criteria

Patients on medications (eg. Corticosteroids, anti inflammatory drugs, immune modulators), Patients with infectious conditions other than periodontitis, Medically compromised patients.

2.2. Study design

A total of 40 subjects were recruited and were divided into 4 groups.

**Group 1** included 10 patients diagnosed with chronic periodontitis with probing pocket depth > 5 mm and clinical attachment loss > 3 mm with radiographic evidence of bone loss. In this group 5 patients having localized periodontitis and 5 with generalized periodontitis were included. (Photograph 1).

**Group 2** included ten patients with aggressive periodontitis. In this group 5 patients having localized aggressive periodontitis and 5 with generalized aggressive periodontitis were included. (Photograph 2).

**Group 3** included 10 patients with gingivitis. In this group 5 patients having localized gingivitis and 5 with generalized gingivitis were included. (Photograph 3).

**Group 4** consisted of 10 systemically and clinically healthy
sites were gently dried with an air syringe. Gingival crevicular fluid was obtained before probing the site by placing colour coded 1–5 μL calibrated volumetric micro-capillary pipettes and a minimum volume of 3 μL pooled sample was obtained by placing the tip of the pipette extracrevicularly at the gingival margin (Photograph 5). Samples of gingival crevicular fluid contaminated by blood or saliva were discarded. The sample was immediately transferred to an eppendorf tube containing 9 μL of phosphate buffer saline.

This tube was kept in a thermocol transport case with ice packs to maintain temperature at 0°C till it was transferred to the refrigerator. Within 12 h samples were transferred to −80°C refrigerator at St. Johns Medical College till the time of assay.

The patients in the first three groups were then given nonsurgical periodontal therapy (scaling and root planning) in two appointments within a weeks time and then followed for up to 8 weeks. In their visit after 8 weeks all the above data (plaque index, gingival index, probing sulcus depth, clinical attachment level and GCF sample) were collected in the same fashion as in their first visit.

GCF samples were assayed by an enzyme linked immunosorbent assay (ELISA, Autoplex Elisa Workstation) to determine RANTES using matched antibody pairs following manufacturer’s recommendation.

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for RANTES has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any RANTES present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for RANTES is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of RANTES bound in the initial step. The color development is stopped and the intensity of the color is measured.

2.3. Statistical Analysis [14,15,16,17]

Data was tabulated and graphs were made for comparing the mean of three or more groups, ANOVA/Kruskal-Wallis test was used, followed by Bonferroni/Mann-Whitney test for pair-wise comparisons. For comparing the change in a parameter within a group at different time intervals paired t-test/Wilcoxon Signed Ranks test was used.

Karl Pearson’s/Spearman’s Rank Correlation was applied to test the relationship between two variables.

3. Results

Out of the total of 40 subjects, comprising of 23 males and 17 females who participated in this study, the correlation between baseline and 8 weeks post treatment parameters in the first three groups who had undergone nonsurgical periodontal therapy was as follows:

The reduction in PI, GI, PPD, CAL from baseline to post treatment was found to be statistically significant in the first three groups (P < .001)

The reduction in RANTES from baseline to post treatment was also found to be statistically significant in these three groups (P < .001) (Table 1). The mean RANTES in chronic periodontitis group at baseline was 61.03 ± 18.87 and post treatment was 37.97 ± 12.29, while in aggressive periodontitis group it was 82.19 ± 14.63 at baseline and 54.24 ± 10.62 post treatment. In the gingivitis group it was 39.76 ± 6.88 at baseline and 12.34 ± 4.76 post treatment.
3.1. Comparison of RANTES between the four groups at baseline and post treatment

Higher mean RANTES was recorded in aggressive periodontitis group followed by chronic periodontitis group, gingivitis group and healthy group respectively. The difference in mean RANTES between these groups was found to be statistically significant (P < .001). (Table 2 and 3).

3.2. Correlation between RANTES and other parameters in chronic periodontitis group

The correlation between RANTES and PI, GI, PPD, CAL at baseline and post treatment was found to be positive but very weak and not statistically significant (P > .05). (Table 4).

3.3. Correlation between RANTES and other parameters in aggressive periodontitis group

The correlation between PI and RANTES post operatively was found to be strong and statistically significant (P < .05) in comparison to relation between RANTES and PI at baseline. However, there was no correlation between GI, PPD, CAL & RANTES postoperatively and at baseline (P > .05). (Table 5).

3.4. Correlation between RANTES and other parameters in gingivitis group

There was no statistically significant correlation between RANTES and PI, GI, PPD, CAL at baseline and post treatment (P > .05). (Table 6 and 7).

3.5. Correlation between RANTES and other parameters in healthy group

The correlation between RANTES and PI was found to be strong statistically significant (P < .05). Whereas the correlation between GI, PPD, CAL and RANTES was not statistically significant (P > .05).

4. Discussion

The assessment of periodontal disease and the effectiveness of periodontal therapy have been traditionally made using clinical and radiographic parameters. Nevertheless, recent advances in the understanding of natural history of periodontal disease have raised questions about the significance of these diagnostic criteria [18,19].

In our study we used the micropipette methodology as we needed to collect 3 μl of GCF for qualitative analysis. All the samples were collected at 11 a.m. in the morning as GCF is considered to show circadian periodicity. (Bissada et al., 1967) [20].

RANTES is a member of the CC chemokines, with significant chemotactic activity for eosinophils, monocytes and CD45+ T cells. The previous data suggest a role for RANTES in acute and chronic inflammation. The potential value of RANTES as a marker has been identified by many authors like Gamonal J, Acevedo A and Bascones A [12]. Based on their findings, the present study was designed to analyze the levels of RANTES in chronic periodontitis, aggressive periodontitis, gingivitis and healthy subjects. In disease RANTES level was compared before and after periodontal therapy. The efficacy of periodontal therapy in controlling the disease activity was assessed by comparing the levels of RANTES with clinical parameters like plaque index, gingival index, probing pocket depth and clinical attachment level.

In the present study, a total of 40 subjects were recruited and were divided into 4 groups:

The percentage of males and females were 90% and 10% in group...
The reduction in GI from baseline to post treatment was found to be statistically significant in all the three groups. Maximum mean difference was seen in chronic periodontitis (1.340) followed by aggressive periodontitis group (1.202) and gingivitis group (0.462). This can be explained by the fact that chronic periodontitis is an plaque induced condition associated with gingival inflammation and once plaque load was reduced by effective periodontal therapy, gingival inflammation and gingival index was reduced. However in aggressive periodontitis, plaque score does not commensurate with gingival changes and disease activity [7].

The reduction in PPD from baseline to post treatment was found to be statistically significant in all the three groups. Maximum mean difference was seen in aggressive periodontitis group (1.390) followed by chronic periodontitis (1.150) and PPD in gingivitis group was (0.353). This can be attributed to the presence of statistically significant difference in probing pocket depth at baseline within the selected groups and maximum initial probing pocket depth in aggressive periodontitis group.

The reduction in CAL from baseline to post treatment was found to be statistically significant in both the groups. Maximum mean difference was seen in aggressive periodontitis group (1.290) followed by chronic periodontitis (1.820) which is in accordance with consensus reached by other authors like Lindhe and Socransky for outcome of non surgical periodontal therapy [23].

The reduction in RANTES from baseline to post treatment was found to be statistically significant in all the three groups, with maximum decrease in aggressive periodontitis group followed by gingivitis group and chronic periodontitis group.

Higher mean RANTES was recorded in aggressive periodontitis group followed by chronic periodontitis group, gingivitis group and healthy group respectively at baseline and post treatment.

The most common inflammatory cytokines are IL-1ß,IL-6,IL-8 and TNF-α are primarily responsible for disease initiation [24]. If the host response to oral infection is only temporary, the inflammation serves as immune protection and begins the wound healing [25]. However after prolonged exposure to periodontal pathogens, the cytokines released by PMNs, such as IL-1ß,IL-6,IL-8 and TNF-α are responsible for initiating the secondary proinflammatory response, which consists of chemokines as well. During this phase of inflammation considerable tissue damage can potentially occur, leading the host into chronic inflammation and further destruction [26]. Therefore increased chemokines are anticipated in chronic infection but in the present study higher levels of RANTES was found in aggressive periodontitis group which may be attributed to, all the patients being in active phase of disease as suggested by clinical parameters and also aggressive periodontitis is a rapidly progressive disease. As said by Garrison and Nicholas in 1989, hyperinflammatory monocyte phenotype with increased expression of MCP-1 represents a risk factor for aggressive periodontitis. So MCP-1 may function either directly or synergistically with other inflammatory mediators, thereby involving in the amplification and continuation of the inflammation [27].

RANTES have now been implicated in the complex interaction of the several aspects of T-lymphocyte biology that could contribute to the symptoms of periodontal disease (Ward & Westwick 1998). RANTES is an efficient chemoattractant of Th1 cells that predominately control cell-mediated immune responses. Thereby, it was stated that they could mediate the complex network of interactions within the immune system by controlling the balance between proinflammatory and anti-inflammatory T cell subsets (Gamonal et al., 2001) Recent studies have shown the presence of high levels of GCF RANTES in patients with chronic periodontitis and these levels have been shown to be related to the active attachment loss and advanced periodontal destruction (Gamonal et al., 2000a, b, 2000b).

### Table 4

Correlation between RANTES and other parameters in Chronic Periodontitis group.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>Post Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.971</td>
<td>.790</td>
</tr>
<tr>
<td>GI&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-0.215</td>
<td>.550</td>
</tr>
<tr>
<td>PPD&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.579</td>
<td>.079</td>
</tr>
<tr>
<td>CAL&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.116</td>
<td>.751</td>
</tr>
</tbody>
</table>

<sup>a</sup> Karl Pearson’s correlation (r).  
<sup>b</sup> Spearman’s Rank correlation (p).

### Table 5

Correlation between RANTES and other parameters in Aggressive Periodontitis group.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>Post Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-0.550</td>
<td>.100</td>
</tr>
<tr>
<td>GI&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-0.519</td>
<td>.125</td>
</tr>
<tr>
<td>PPD&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.394</td>
<td>.260</td>
</tr>
<tr>
<td>CAL&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.256</td>
<td>.475</td>
</tr>
</tbody>
</table>

<sup>a</sup> Karl Pearson’s correlation (r).  
<sup>b</sup> Spearman’s Rank correlation (p).

### Table 6

Correlation between RANTES and other parameters in Gingivitis group.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>Post Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-0.588</td>
<td>.074</td>
</tr>
<tr>
<td>GI&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-0.434</td>
<td>.210</td>
</tr>
<tr>
<td>PPD&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-0.457</td>
<td>.184</td>
</tr>
<tr>
<td>CAL&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-0.457</td>
<td>.184</td>
</tr>
</tbody>
</table>

<sup>a</sup> Karl Pearson’s correlation (r).  
<sup>b</sup> Spearman’s Rank correlation (p).

### Table 7

RANTES level in GCF.

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline</th>
<th>Post Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic Periodontitis</td>
<td>61.03</td>
<td>37.97</td>
</tr>
<tr>
<td>Aggressive Periodontitis</td>
<td>82.19</td>
<td>54.24</td>
</tr>
<tr>
<td>Gingivitis</td>
<td>39.76</td>
<td>12.34</td>
</tr>
<tr>
<td>Healthy</td>
<td>21.63</td>
<td>6.78</td>
</tr>
</tbody>
</table>
2001). In the present study, elevated RANTES levels were present in aggressive periodontitis. It is possible that MCP-1 releasing from activated monocyte at sites of inflammation could indirectly amplify monocyte functions by recruiting additional cells to the inflammatory site and could contribute to severe periodontal destruction in aggressive periodontitis patients [27].

The presence of RANTES in healthy individuals could be related with the steady state of the gingiva, considering this is a site of permanent antigenic insult, requiring the presence of neutrophils, macrophages and antigen presenting cells [12]. The presence of RANTES in GCF could be involved in the development of the gingival inflammatory response by mediating leukocyte recruitment and activation. This is in agreement with authors like Gamonal J, Bascones A, Jorge O, Silva A, who detected RANTES in sulcus less than 3 mm [28].

The marked reduction of RANTES in GCF following treatment observed in the present study clearly suggests a relationship between disease and chemokine production. The higher decrease in RANTES level in gingivitis group compared to chronic periodontitis group may be because gingivitis is a reversible condition which responds well to periodontal therapy alone where as chronic periodontitis does not always respond to periodontal therapy alone, it further needs surgical intervention for effective results. But the question remains unanswered in our study is the exact cut off value/range for RANTES level in GCF that could help us decide if a particular site needs surgical therapy or only non surgical therapy which includes periodontal therapy followed by maintenance that would be sufficient.

In our study none of the patients belonging to aggressive periodontitis group were prescribed antibiotics post periodontal therapy in all the three groups. Although there was a statistical difference between values in all the three groups, the range was limited. Therefore further research and longitudinal studies with increased sample size are needed before levels of RANTES in GCF can be used accurately as a diagnostic/prognostic marker of disease activity.

Moreover since we could not reach to an exact standard value of GCF RANTES which could be accurate to differentiate between health and disease and active and inactive sites post periodontal therapy, we suggest that reduction in GCF RANTES after therapy is useful only in reflecting short term healing and decisions regarding the need for further treatment and surgical intervention cannot be taken based on the reduced value of GCF RANTES alone.

5. Conclusions

According to the results obtained from the present study, it can be concurred that GCF levels of RANTES were significantly higher in periodontal disease than in healthy subjects. This could be attributed to inflammatory response and host defense mechanism that are instigated in disease.

The RANTES level decreased significantly after periodontal therapy in all the three groups. Although there was a statistical difference between values in all the three groups, the range was limited. Therefore further research and longitudinal studies with increased sample size are needed before levels of RANTES in GCF can be used accurately as a diagnostic/prognostic marker of disease activity.

Ethical approval

This study was ethnically approved by the committee of oxford dental college. As this study was invivo and does not involve any kind of harm to any human/animal.

Conflicts of interest

The authors declare that there is no conflict of interest regarding the publication of this paper We authors also state that we have not received any sort of sponsorship for our research paper.

References


