Comparative Evaluation of Membrane-Organizing Extension Spike Protein in Gingival Crevicular Fluid in Individuals with and Without Severe Chronic Periodontitis

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Abstract: Background: Gingival Crevicular Fluid (GCF) is the principal target in the search for biomarkers of periodontal disease, because its protein composition may reflect disease pathophysiology. The objective of this study was to evaluate and compare the levels of membrane-organizing extension spike protein (Moesin) in GCF of individuals with and without severe chronic periodontitis. Materials and Methods: Forty systemically healthy subjects satisfying the required inclusion criteria were selected and equally divided into two groups: Group A – with no periodontitis and Group B – with severe chronic periodontitis. Periodontal parameters were recorded at baseline. GCF samples were collected and evaluated for Moesin levels in both the groups using enzyme-linked immunosorbent assay. Scaling and root planing were performed in Group B patients whose GCF samples were collected and assessed again after 4 weeks. Results: At baseline, the mean GCF Moesin level in Group A was 525.30 ± 315.252 pg/ml, while in Group B, it was found to be 25335.40 ± 12251.53 pg/ml, which showed a highly significant statistical difference on comparison. The mean GCF Moesin level in patients with chronic severe periodontitis was 25335.40 ± 12251.53 pg/ml at baseline, and on review 1 month after SRP, it was found to have undergone a statistically significant reduction to 25061.28 ± 12233.33 pg/ml (P = 0.001). Conclusion: Moesin levels were found to be higher in subjects with severe chronic periodontitis as compared to those with no periodontitis. Also there was significant reduction in moesin levels after one month of performing non-surgical periodontal therapy. Thus Moesin can also serve as a potential biomarker for periodontitis.

Keywords: Gingival crevicular fluid, Moesin, Periodontitis.

I. Introduction

Chronic periodontitis is an infectious disease resulting in inflammation within the supporting tissues of the teeth, progressive attachment loss and bone loss1. However, in the presence of systemic or environmental factors that may modify the host response to plaque accumulation, such as diabetes, smoking and stress, disease progression may become aggressive. Gingival crevicular fluid (GCF) is a fluid present in the gingival crevices and periodontal pockets. It consists of both host-derived and bacteria-derived proteins 2, which play an important role in periodontal tissue turnover 3. Many biomarkers of periodontal disease have been identified in the gingival crevicular fluid (GCF) present within the gingival crevice.

Among the various identified proteins in GCF, membrane-organizing extension spike protein (moesin) is a disease-associated protein that belongs to the Ezrin-Radixin-Moesin (ERM) protein family. Moesin is of particular interest because of its role in cell-cell recognition, signaling, and cell movement 4. Amar et al. 5 demonstrated that moesin may play a role in lipopolysaccharide (LPS) signaling via CD14 and toll-like receptor (TLR) pathways. Previous studies have demonstrated that LPS from periodontal pathogens stimulate host cells to secrete pro-inflammatory mediators such as interleukin (IL)-1, IL-6, tumor necrosis factor-alpha, and prostaglandin E2 6-7. The role of inflammatory cytokines in the development of periodontal disease is also well documented 8. Thus, moesin may play an important role in the recognition of oral bacteria and in the consequent development of inflammatory immune responses involved in periodontal disease development.

According to the study conducted by Tsuchida et al., Western blotting revealed that Moesin expression in GCF was higher in patients with severe periodontal disease than healthy controls9. To the best of our knowledge, there is only one study available in literature in which enzyme-linked immunosorbent assay (ELISA) was used to estimate the Moesin levels in GCF. Thus, this study was conducted to evaluate whether Moesin can be used as a biomarker for periodontal disease by comparing its level in GCF in healthy controls.

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and patients with severe chronic periodontitis as well as in the severe chronic periodontitis patients before and after scaling and root planning (SRP).

II. Materials And Methods

Forty subjects (25 males and 15 females) aged between 35–55 years (mean age: 42.6 years) were recruited voluntarily from the outpatient section of the department of Periodontology. A written informed consent was obtained from the individuals after explaining the procedure. The study was conducted in the department of Periodontology of our institution after approval by the Institutional Ethical Committee.

The inclusion criteria of this study were systemically healthy subjects between age group of 35–55 years with presence of at least 16 natural teeth, with chronic periodontitis with >30% sites having clinical attachment loss >5 mm for the test group and subjects with no periodontitis, no clinical attachment loss, no probing depth >3 mm for the control group.

Those having any systemic diseases, with the history of any drug allergies, antimicrobial or anti-inflammatory therapy within the past 6 months, periodontal therapy within the past 6 months, individuals who smoke or chew any form of tobacco or alcoholics, pregnant or lactating women, postmenopausal women and immunocompromised individuals were excluded from the study.

A total of forty subjects selected were equally divided into two groups: Group A – Control group with no periodontitis, no clinical attachment loss, and no probing depth >3 mm and Group B – Test group with severe chronic periodontitis as defined by clinical attachment loss >5 mm (based on the classification given by the 1999 International Workshop for the Classification of Periodontal Diseases organized by the American Academy of Periodontology).

The severity and amount of gingival inflammation were assessed using the Gingival Index (Loe and Silness) and Simplified Oral Hygiene Index (OHI-S) while the clinical attachment level (CAL) and pocket probing depth (PPD) were used to evaluate the periodontal tissue destruction. These parameters were assessed at baseline in Group A and Group B and 1 month after nonsurgical periodontal therapy (SRP) in Group B alone.

In this study, William’s periodontal probe was used to measure the pocket depth and CAL at six sites per tooth (mesiobuccal/labial, mid-buccal/labial, distobuccal/labial, mesiolingual/palatal, mid-lingual/palatal, and distolinguinal/palatal) in all the teeth, excluding third molars. To ensure reproducibility during examinations, a customized acrylic stent was used as a reference to determine the site and angle of insertion of periodontal probe.

In Group A, GCF sample was collected from any one of the maxillary molars. Prior to GCF collection, the site was air-dried and isolated with cotton rolls. Supragingival plaque was removed gently without manipulating the gingival margin, to avoid contamination and obstruction of the micropipette. The site showing the greatest clinical attachment loss was selected for GCF sample collection in Group B.

GCF samples were collected from the gingival sulcus with the help of Thermo Scientific micropipette F2 using extracrevicular method. A standardized volume of 1-μL GCF was collected from each site and those sites which did not express sufficient volume of GCF or micropipettes contaminated with blood/saliva/plaque were disposed. GCF was collected at baseline from both Group A and Group B. Nonsurgical periodontal therapy comprising full-mouth SRP was performed for Group B using ultrasonic scalers and area-specific Gracey curettes. GCF was collected from the same site in Group B individuals 1 month after completion of SRP. To estimate the Moesin levels in the GCF samples, a commercially available Human Moesin ELISA Kit (Cloud - Clone Corp., Houston, USA) was used. Collected samples were immediately transferred to Eppendorf tubes and stored at −20°C until the time of the assay.

The recorded data was compiled and entered in a spreadsheet (Microsoft Excel) and then exported to data editor of SPSS Version 20.0 (SPSS Inc., Chicago, Illinois, USA). For all continuous variables, the results are either given in mean standard deviation and for categorical variables as percentage. To test the statistical significance of the difference in the GCF Moesin levels in Group A and Group B at baseline, the Mann–Whitney U-test was applied. To assess the significance of the Moesin levels as well as the periodontal parameters in the intragroup comparison (in Group B before and 1 month after SRP), the Wilcoxon signed-rank test was applied.

III. Results

At baseline, the mean GCF Moesin level in Group A subjects was 525.30 ± 315.252 pg/ml, while in Group B, it was found to be 25335.40 ± 12251.53 pg/ml. Intergroup comparison using the Mann–Whitney U-test showed a highly significant statistical difference (P < 0.001) [Table 1] and [Figure 1].
Comparative evaluation of membrane-organizing extension spike protein in gingival crevicular fluid... 

Table 1: Intergroup comparison of mean Moesin levels in gingival crevicular fluid

<table>
<thead>
<tr>
<th>Moesin level in GCF (pg/ml)</th>
<th>Mean±SD (Group A)</th>
<th>Mean±SD (Group B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>525.30±315.252</td>
<td>25335.40±12251.53</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Statistical significance is taken as p<0.05. GCF- Gingival crevicular fluid, SD- Standard deviation, p- Probability value

Figure 1: Intergroup comparison of mean Moesin levels in gingival crevicular fluid

All the clinical parameters which were evaluated showed a statistically significant reduction 1 month after SRP. The mean values for OHI-S, gingival index, probing depth, and CAL were reduced from 2.52 ± 0.32, 1.48 ± 0.18, 5.85 ± 0.50, and 7.25 ± 1.20 mm at baseline to 1.40 ± 0.54, 1.16 ± 0.34, 4.29 ± 0.98, and 5.56 ± 1.45 mm, respectively, when assessed 1 month postoperatively [Table 2] and [Figure 2].

Table 2: Comparison of periodontal parameters and gingival crevicular fluid Moesin level in Group B before and after nonsurgical periodontal therapy

<table>
<thead>
<tr>
<th>Group B</th>
<th>Probing depth(mm)</th>
<th>OHI-S</th>
<th>Clinical attachment level(mm)</th>
<th>Gingival index</th>
<th>Moesin level(pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>5.85(0.50)</td>
<td>2.52(0.32)</td>
<td>7.25(1.20)</td>
<td>1.48(0.18)</td>
<td>25,335.40(12,251.53)</td>
</tr>
<tr>
<td>1 month</td>
<td>4.29(0.98)</td>
<td>1.40(0.54)</td>
<td>5.56(1.45)</td>
<td>1.16(0.34)</td>
<td>25,061.28(12233.33)</td>
</tr>
<tr>
<td>p</td>
<td>&lt;0.001</td>
<td>0.001</td>
<td>&lt;0.001</td>
<td>0.001</td>
<td>0.001</td>
</tr>
</tbody>
</table>

*Statistical significance is taken as p<0.05. GCF- Gingival crevicular fluid, SD- Standard deviation, p- Probability value, OHI-S- Simplified oral hygiene index

Figure 2: Comparison of periodontal parameters in Group B before and after nonsurgical periodontal therapy

The mean GCF Moesin level in patients with severe chronic periodontitis was 25335.40 ± 12251.53 pg/ml at baseline. One month following SRP, it was found to have undergone a statistically significant reduction to 25061.28 ± 12233.33 pg/ml (P = 0.001) [Table 2] and [Figure 3].
IV. Discussion

Periodontitis is a multifactorial inflammatory disease caused by interactions between periodontal microflora and host response\(^{10}\). Progressive periodontal destruction is an important sequela of periodontitis that can lead to the loosening and subsequent loss of teeth\(^{11}\).

The most common clinical features of chronic periodontitis are the loss of connective tissue fibers, alveolar bone destruction, the formation of periodontal pockets, and gingival inflammation. Additional signs and symptoms of a chronic periodontal disease are the accumulation of supra- and sub-gingival calculus, gingival enlargement or recession, bleeding, furcation involvement, the mobility of the teeth, and bad odor\(^{12}\).

The aim of nonsurgical periodontal treatment is the elimination of bacterial deposits which adhere to tooth surfaces, primarily by means of dental plaque control performed by the patient, in addition to dental scaling and root surface debridement performed by the dentist\(^{13}\).

Tsuchida et al. developed a novel protocol for effective protein extraction from GCF and then conducted gel-based and gel-free proteome analyses of GCF from patients with chronic severe periodontitis in which several proteins were discovered. Amid these proteins, we focused on Moesin, a disease-associated protein belonging to the Ezrin-Radixin-Moesin family of proteins that have seldom been studied in relation to periodontal disease\(^{14}\).

Moesin, a membrane-organizing extension spike protein, is involved in connections of major cytoskeletal structures to the plasma membrane\(^{15}\). Moesin has been implicated in the development of several human diseases, including cancer and liver injury\(^{16}\). Western blot studies indicated that moesin protein expression was significantly increased in GCF of individuals with periodontal disease. Thus, moesin may either be a byproduct or a mediator of the pathophysiology of periodontal disease. The elevated moesin levels also indicate that moesin could be potentially used as a biomarker for periodontal disease. Furthermore, cell culture stimulation studies demonstrated that moesin expression was increased by LPS stimulation in a dose-dependent manner. Thus, moesin may be involved in the TLR4 mediated responses that induce the production of inflammatory responses, which can exacerbate periodontal disease progression\(^9\).

In this study, at baseline, the mean GCF Moesin levels in systemically healthy controls with no periodontitis (Group A) and patients with severe chronic periodontitis (Group B) showed a highly significant statistical difference between the two groups (\(P < 0.001\)). This is in accordance with the results from the studies conducted by Tsuchida et al. in 2014\(^9\) and Sreedharan et al. in 2019\(^{17}\).

The first phase of periodontal treatment comprising SRP primarily aims at the elimination or reduction of bacterial infection and the control of periodontal plaque-associated inflammation. All the clinical parameters which were evaluated showed a statistically significant reduction 1 month after SRP as expected. This is in accordance with the results of a case report published by Carnio et al. in 2015, which showed a significant reduction in clinical parameters and improvement in the overall as well as individual prognosis of teeth when a patient with chronic severe periodontitis was treated with nonsurgical periodontal therapy and periodic maintenance visits alone\(^{18}\).

The difference between the GCF Moesin levels of healthy controls and patients with severe chronic periodontitis as well as the reduction in the Moesin levels after nonsurgical periodontal therapy indicates that Moesin could be potentially used as a biomarker for periodontal disease. Hence, from the results of the present study, it is observed that the level of Moesin expression in GCF is significantly higher in chronic inflammatory conditions (chronic severe periodontitis) and reduces with decrease in the severity of inflammation (after SRP). In this study, we have used ELISA for estimation of Moesin levels which are much more affordable and cost-effective than Western blot. Furthermore, the ELISA kit for Moesin is economically more feasible than those for many other already discovered biomarkers for periodontal disease. Further studies on a larger scale should be conducted to evaluate the role of Moesin as a biomarker for periodontal disease.
V. Conclusion

Moesin levels were found to be higher in subjects with severe chronic periodontitis as compared to those with no periodontitis. Also there was significant reduction in moesin levels after one month of performing non-surgical periodontal therapy. Thus Moesin can also serve as a potential biomarker for periodontitis.

References


DOI: 10.9790/0853-1901125155 www.iosrjournals.org 55 | Page