RANKL and MMP8 levels in Gingival Crevicular Fluid (GCF) as oral biomarker for periodontal condition

Ariyati R. Pratiwi,* Novi K. Firani,1,2 Neny Roeswahjuni,3 Rahmavidy Priyanto,4 Malianawati Fauziah5

Abstract

Objective: We assessed the diagnostic potential of RANKL and MMP8 in gingivitis and periodontitis patients.

Material and Methods: Sixty gingivitis and periodontitis patients were enrolled at Faculty of Dentistry Universitas Brawijaya and Janti Health Service Malang. Gingival Crevicular Fluid (GCF) was sampled modified gingival index including normal (n=26), mild inflammation (n=25), moderate (n=10), and severe inflammation (n=6). For periodontitis parameter, GCF was sampled pocket depth such as pocket depth 0-3.5 mm (n=49), pocket depth 3.6-5.5 mm (n=11), pocket depth >5.5 mm (n=7). RANKL and MMP8 were determined by ELISA (Enzyme-linked immunosorbent assay). Then, One-way ANOVA analysis was used.

Results: RANKL showed a significant difference in gingivitis patients (p<0.05), but not in periodontitis patients (p>0.05). However, MMP8 did not show any significant difference (p>0.05) between gingivitis and periodontitis. RANKL showed a good discrimination performance. RANKL and MMP8 are present in the identification of periodontal disease.

Conclusion: RANKL presented a more accurate diagnostic marker for periodontitis than gingivitis.

Keywords: GCF, Gingivitis, MMP8, Periodontitis, RANKL
DOI: 10.15562/jdmfs.v8i3.1482

Introduction

Periodontitis is an inflammation of periodontal tissue caused by specific microorganisms and systemic immune responses that can cause tooth loss in adults. The role of oral pathogenic bacteria is to develop systemic disease.1 To diagnosis periodontitis, it can be done by clinical examination and radiography. The weakness of cellular examination in confirming the diagnosis of periodontitis (clinical examination with examination and radiographic examination), can be corrected through examination of the activity of the Gingival Crevicular Fluid (GCF), thus improving the type of therapy. GCF is a physiological fluid located in the gingival sulcus. The volume of GCF in each individual can vary with the composition consisting of electrolytes, cytokines, proteins, and specific antibodies. This composition will change along with the presence of infection and is also influenced by the type of microbe causing the infection and the level of disease progression. Therefore, GCF has the potential to be used as a diagnostic instrument for periodontal disorders.2,3

The presence of cellular and humoral activity in the gingival crevicular fluid is thought to be a simple marker to determine the inflammatory status of the periodontal tissue. Cellular immune response shows the role of cytokines in the process of periodontitis. Several cytokines have been associated with periodontitis, including IL-1β, tumor necrosis factor-alpha (TNF-α), IL-8, PGE2, Osteocalcin, Matrix metalloproteinase-8 (MMP-8) and MMP-9. Those levels were identified in GCF by the ELISA method. Matrix metalloproteinases (MMPs) are a major group of enzymes derived mostly from polymorphonuclear leukocytes during the acute stages of periodontal disease, which are responsible for the degradation of the extracellular collagen matrix. The MMP-8 has a distinctive ability to decompose type I and type III collagen. Elevated MMP-8 levels were strongly correlated with probing depth, clinical attachment loss, and bleeding on probing, where these correlations were consistent with the characteristics of periodontal disease.2,4

Cellular activity in gingival crevicular fluid and blood as biomarkers has the potential to be further developed as a tool for early detection of periodontitis and measuring the severity of periodontitis. Until now, the measurement of the severity of periodontitis is still using probing and radiography methods, which have many disadvantages. Furthermore, there is currently no early screening method for periodontitis, thus, the evidence discovered in this study represents a breakthrough for the dental sector, particularly in the area of periodontics.
Table 1. Anova testing between healthy individuals and gingivitis patients

<table>
<thead>
<tr>
<th></th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>RANKL</td>
<td>11.760</td>
<td>4.843</td>
<td>.004</td>
</tr>
<tr>
<td>MMP8</td>
<td>.010</td>
<td>.523</td>
<td>.668</td>
</tr>
</tbody>
</table>

Table 2. Anova testing between healthy individuals and periodontitis patients

<table>
<thead>
<tr>
<th></th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>RANKL</td>
<td>7.290</td>
<td>2.686</td>
<td>.076</td>
</tr>
<tr>
<td>MMP8</td>
<td>.012</td>
<td>.616</td>
<td>.543</td>
</tr>
</tbody>
</table>

Figure 1. The level of RANKL and MMP8 in gingival crevicular fluid (GCF) specimens from healthy individuals and gingivitis patients were measured using an ELISA. *p<0.001.

Figure 2. The level of RANKL and MMP8 in GCF specimens from healthy individuals and gingivitis patients were measured using an ELISA. *p<0.001.

Material and Methods

Gingival Crevicular Collection

This study obtained ethical clearance No 598/KEPK-POLKESMA/2020. This study was conducted at Health Research Ethical Committee State Politechnic of Health Malang. Gingival crevicular fluid samples were collected from healthy individuals (n=67) and periodontitis patients (n=67) at Faculty of Dentistry Universitas Brawijaya and Janti Health Service Malang. GCF was sampled modified gingival index include normal (n= 26), mild inflammation (n= 25), moderate (n=10), and severe inflammation (n=6). For periodontitis parameter, GCF was sampled pocket depth such as pocket depth 0−3.5 mm (n=49), pocket depth 3.6−5.5 mm (n=11), pocket depth >5.5 mm (n=7).

GCF samples were collected non-invasively from the periodontal pocket using paper point (periostrip). Firstly, informed consent written by healthy individuals and periodontitis patient. Sample sites (periodontal pocket) were dried and isolated using cotton rolls. The paper points were gently inserted into the sulcus and left in for 30 seconds. Then the paper points inserted the tube which filled of PBS. The samples contaminated with blood were discarded.

Enzyme-linked immunosorbent testing

RANKL and MMP8 levels in equal amounts of the GCF specimens were examined using ELISA. The ELISA examination procedure is that first a standard tool from the existing samples is prepared, then incubated at 37oC for 60 minutes. After the incubation is complete, cleaning is carried out, then colored according to the standard and the sample is using chromagen. After that, it was incubated for 10 minutes at 37oC and kept away from lighting. After that, add a stop solution, then the color which was originally blue turns yellow, then measurements are taken using a micro analyzer and then the results of the research come out.

Results

The analysis of GCF specimens from healthy individuals and gingivitis patient. According figure 1, the level of RANKL and MMP8 in the GCF samples from healthy individuals were higher than those in the GCF samples from severe gingiva inflammation. However, the MMP8 level between the GCF samples were not significantly different (p<0.05) table 1.

As shown in figure 2, the level of RANKL samples from pocket depth 0−3.5 mm patients were higher than those in the GCF samples from pocket depth 3.6−5.5 mm and pocket depth >5.5 mm. The level of MMP8 samples from pocket depth >5.5 mm were higher than GCF samples. However, the RANKL level and MMP8 level between other GCF samples were not significantly different table 2.

Discussion

Several proinflammatory cytokines found in GCF include actin, keratin, histones, annexins, albumin, macrophages, matrix metalloproteinases and several cytokines such as IgG, IgA, IgM, TNF-α, interleukins-6 (IL-6), prostaglandin E-2 (PGE-2), osteocalcin. Apart from inflammation, there are several risk factors that cause an increase in the volume of GCF including smoking, circadian periodicity, sex hormones, oral contraceptives, orthodontic and periodontal treatment, and others. The results of the observation of RANKL and MMP8 proteins in periodontitis cases showed that there was no significant difference.
While observing the protein in gingivitis cases, it was found that there were significant differences in RANKL. However, there was no significant difference in the association of MMP8 protein with gingivitis. GCF is an inflammatory exudate from the gingival microcirculation that traverses the inflamed periodontal tissue. GCF comes from various sources. GCF contains substances from microorganisms in subgingival and supragingival plaque. The cellular components of GCF are 5% T lymphocytes, 10–20% monocytes, 5% mast cells, and 70–80% granulocytes. Based on the result, the average of RANKL score on healthy individuals were higher than mild gingivitis and severe gingivitis. It may occur because a lot of osteoprotegerin production so that were not bone destruction. The receptor activator of NF-κB ligand (RANKL) promotes osteoclast differentiation. RANKL also inhibit osteoclast hormone such as Parathyroid Hormone (PTH). However, osteoprotegerin (OPG) is produced by fibroblasts apoptosis. RANKL binds to RANK on the surface of osteoclast precursors. RANK is up-regulated by PTH. The OPG produced by fibroblasts is an important regulator of bone resorption. It acts locally and systemically by blocking its interaction with RANK in order to inhibit osteoclast differentiation and also binding to RANK-L. The proportion of interaction between these molecules play in the regulation of bone resorption and osteoclastogenesis.

Matrix metalloproteinases (MMPs) are a group of proteinases involved in the destructive process. MMP8 also can be measured in GCF. The MMP8 levels diagnose gingivitis effectively. It is an early inflammatory prerequisite state to periodontitis. So this approach can prevent periodontitis effectively. Neutrophils responsible for the release of MMPs at the infected site, specifically collagenase-2 (MMP8) and gelatinase-B (MMP9). MMP8 belongs to collagenase group, which can decompose type I and III collagen. MMP8 is able to degrade interstitial collagen, however MMP9 degrades extracellular matrix proteins. Matrix metalloproteinase-8 in GCF showed a good discrimination performance at the periodontal condition. This collagenase, together with MMP9, degrade collagen types I and III in periodontal tissue. Pathologically-levated levels of MMP8 has been reported in periodontal disease in comparison to healthy patients. MMP8 was able to identify periodontitis and its severity. MMP8 found in periodontal disease and GCF. There is no found difference in MMP8 levels from patients with periodontal disease when compared to patients with gingivitis. Collagenase activity have been shown to increasing severity of inflammation and increasing pocket depth. Therefore, it can be hypothesized that MMP8 acts as a biomarker in periodontitis.

Conclusion

From this investigation, it was concluded that MMP8 and RANKL may serve as periodontitis and proinflammatory marker.

Acknowledgment

This work was supported by the PNBP Faculty of Dentistry Universitas Brawijaya.

Conflict of Interest

The authors report no conflict of interest

References