MATRiX METALLOPROTEINASE-8 - A SALIVARY DIAGNOSTIC BIOMARKER RELATED TO SOFT TISSUE DESTRUCTION IN CHRONIC PERIODONTITIS

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Summary

Periodontitis is a bacteria-induced chronic inflammation affecting the tooth-supporting structures. A major challenge in clinical periodontitis is to find a novel diagnostic tool for an objective evaluation of the periodontal status. The aim of this study is to assess the value of salivary Matrix metalloproteinase (MMP)-8, a neutrophil-derived proteolytic enzyme, as an indicator of the severity of periodontal tissues destructions. The study included 11 chronic periodontitis patients and 10 healthy controls. Clinical parameters including periodontal index (PI), bleeding on probing index (BPI), probing depth (PD) and clinical attachment level (CAL) were recorded, and saliva samples were collected. MMP-8 salivary levels were measured using an ELISA quantitative colorimetric assay. The chronic periodontitis patients underwent initial therapy (scaling and root planning) followed by periodontal surgery. The soft tissue wall of the pathologic periodontal pocket was excised and examined microscopically. Statistical analysis (the independent samples test and Pearson correlation coefficient) was employed to compare the clinical parameters with MMP-8 salivary levels. We also compared these data with the histopathological findings.

Our results indicated that there was a correlation between the periodontal status evaluated using clinical parameters, MMP-8 salivary levels and the histopathological changes. Salivary MMP-8 can be taken into consideration as a biomarker of periodontitis and could be used as a valuable indicator of health and pathologic process.

Key words: periodontitis, Matrix metalloproteinase-8, biomarker

Introduction

Periodontitis is the major cause of tooth loss and is also associated with various systemic conditions (Seymour et al. 2007, Bensley et al. 2011); hence it can be considered an important global health problem in terms of quality of life. Therefore, the early diagnosis and the efficient control of disease is the most important goal for periodontists (Nomura et al. 2006).

At present, the diagnosis of periodontitis relies almost entirely on the measurement of clinical parameters including periodontal index (PI), bleeding on probing index (BPI), probing depth (PD), clinical attachment level (CAL) and radiographical findings (Teles et al. 2010).

These procedures can be supplemented by microbial analysis (Teles et al. 2010, Listgarten et al. 2003). Although these measurements are useful, they determine mainly the past history of the disease rather than present disease activity (Buduneli et al. 2011). Knowing the disease activity state might be critical to clinical decision; therefore, it is necessary to find a method to indicate the current state of periodontal tissue destruction. (Ozmeric et al. 2004)

A diagnostic tool should provide reliable information to assess presence, severity and prognosis of a disease (Sexton et al. 2011, Buduneli et al. 2011).
Periodontitis is described as a multifactorial, irreversible and cumulative condition initiated and propagated by both bacteria and host factors (Bascones et al. 2005). Due to the complexity of periodontitis, one single clinical or laboratory examination cannot cover all the mechanisms implicated in its pathogenesis. Though, proteins derived from inflamed host tissue and pathogenic bacteria have the potential of reflecting the severity of periodontitis and could be used as biomarkers of the disease. These specific markers are present in the oral fluids: gingival crevicular fluid and saliva and also in the blood circulation and can be evaluated using immunological and biochemical methods (Sexton et al. 2011).

Periodontitis begins with a microbial infection followed by a host-mediated destruction of periodontal tissues caused by hyperactivated or primed leucocytes and the generation of cytokines, eicosanoids and matrix metalloproteinases that cause clinically significant connective tissue and bone destruction (Bascones et al. 2005, Nussbaum et al. 2011, Preshaw et al. 2011). A specific proteolytic enzyme secreted by neutrophils and macrophages, the collagenase 2 also called matrix metalloproteinase (MMP)-8 plays an important role in the pathogenesis of periodontal disease (Sorsa et al. 2004, Sorsa et al. 2006, Gursoy ez al. 2010). MMP-8 is catalytically the most competent proteinase to initiate type I collagen and extracellular matrix degradation associated with periodontal tissue destruction leading to tooth loss (Gursoy et al. 2010). During the initiation and course of inflammatory responses in periodontitis, pro-inflammatory mediators including especially MMP-8 are up-regulated not only in affected tissues, but also in the secreted, disease affected oral fluids: gingival crevicular fluid and saliva, as well as in serum and plasma (Sexton et al. 2011, Herr et al. 2007, Miller et al. 2006).

Regarding the novel diagnostic tools used in periodontitis, the oral fluid and serum MMP-8 analysis has been suggested to be a potential biomarker as an indicator of health and pathologic process (Miller et al. 2006, Todorovic et al. 2006).

The aim of this study was to investigate a possible correlation between MMP-8 salivary levels, periodontal status evaluated by clinical parameters and the histopathological features of the disease affected periodontal soft tissues.

Material and methods

Subjects: The study included 21 subjects: 11 chronic periodontitis patients assigned to the periodontitis (P) group and 10 healthy subjects assigned to the control (C) group.

Location of the study: Faculty of Dental Medicine, “Iuliu Hatieganu” U.M.Ph. Cluj-Napoca

Ethical aspects: Prior to proceeding with the study, both the Ethics Commission approval and the patient’s informed consent were obtained.

Work protocol includes the following steps: clinical examination, saliva sampling, immunological determination of salivary MMP-8, treatment: initial therapy and periodontal surgery and histopathological examination. All subjects underwent clinical examination and saliva sampling, and P (periodontitis) group also underwent treatment.

1. Clinical examination: periodontal parameters measured were: the Russel Periodontal Index (PI), the Bleeding on Probing Index (BPI), the Probing Depth (PD) and the Clinical Attachment Level (CAL).

PI evaluated the periodontal tissues inflammation, BPI indicated the presence or absence of bleeding on probing, PD was measured as the distance in mm between the base of the pocket and the gingival margin, CAL was measured as the distance in mm between the base of the pocket and the cemento-enamel junction.

2. Saliva samples collection: we used sterile calibrated absorbent strips placed into the sub-lingual space. Saliva
samples were taken before periodontal examination, aliquoted in Eppendorf tubes and stored at -20°C. Salivary MMP-8 levels were measured using the Quantikine Human Total MMP-8 Immunoassay employing an ELISA technique, provided by R&D Systems.

3. Treatment: the C (control) group needed no periodontal treatment, they received only oral hygiene instructions. The P group underwent both initial therapy and surgical treatment. The initial, cause-related therapy included scaling and root planning, professional cleaning, removal of irritating iatrogenic factors (overhanging restorations) and carious cavities treatment. Periodontal surgery (gingivectomy) consisted of the excision of the soft tissue wall of the pathologic periodontal pocket, scaling of the root surface and the placement of a periodontal dressing to protect the incised area during the period of healing.

4. Histopathological examination. The pathologic periodontal tissues were prepared: gingiva fragments were initially treated with the regular method of paraffin inclusion and resulting sections were stained with Masson’s trichrome.

Statistical analysis of data: the clinical parameters values and the MMP-8 salivary levels were measured for the 2 study groups, then we determined a mean value with a standard deviation. The independent samples test was employed to compare the 2 study groups and Pearson’s correlation coefficient was calculated in order to assess the correlation between the clinical parameters and the MMP-8 salivary levels.

The histological sections were stained with Goldner’s trichrome examined at light microscope to observe the pathological modifications.

Results
Results of the clinical examination and immunological determinations:

The clinical parameters values and the MMP-8 salivary levels are presented in table 1.

The clinical parameters (PI, BPI, PD and CAL) and MMP-8 salivary values were significantly higher in chronic periodontal patients compared to the healthy controls. (p<0.001) (the independent samples test).

Table 1: Comparison between the study groups regarding the clinical parameters values and the salivary MMP-8 levels.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>P group (n=11)</th>
<th>C group (n=10)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI</td>
<td>3,28±0,44</td>
<td>0,43±0,20</td>
<td>0.001</td>
</tr>
<tr>
<td>BPI</td>
<td>75,02±7,85</td>
<td>24,95±10,48</td>
<td>0.001</td>
</tr>
<tr>
<td>PD</td>
<td>4,34±0,35</td>
<td>1,55±0,18</td>
<td>0.001</td>
</tr>
<tr>
<td>CAL</td>
<td>3,43±0,25</td>
<td>0,03±0,01</td>
<td>0.001</td>
</tr>
<tr>
<td>MMP-8</td>
<td>577,81±57,35</td>
<td>92,59±24,05</td>
<td>0.001</td>
</tr>
</tbody>
</table>

There is a significant correlation between the MMP-8 salivary levels and the clinical parameters values: PI (r = 0.96), BDI (r=0.96), PD (r=0.97), CAL (r=0.97). (Pearson’s correlation coefficient)

Results of the histopathological examination:

Surgical samples taken from the pathologic wall of the periodontal pocket included soft periodontal tissues: the gingiva consisting of sulcular epithelium, oral epithelium and lamina propria.

In the gingiva, both the epithelium and lamina propria showed evidence of inflammatory modifications.

The pathologic changes in the sulcular epithelium included the followings: in some instances the epithelium was thinned and even ulcerated, while in others there was an epithelial proliferation into the lamina propria. (Figures 1, 2, 3)
The oral epithelium exhibited varying degrees of hyperplasia and keratosis. The inflammatory process caused a proliferation of the basal cells into the lamina propria and an increased width of the spinous layer. Inflammatory cells were found in the epithelium. Hornification of the epithelium was also apparent. (Figures 4, 5, 6, 7)

**Figure 1** - The sulcular epithelium was thinned and consisted of basal cells and a few degenerated spinous cells (20x).

**Figure 2** - Ulceration of the sulcular epithelium (10x).

**Figure 3** - Hyperplasia of the epithelium exhibiting elongated rete pegs with a tendency to anastomose with one another (10x).

**Figure 4** - Proliferation of the epithelial cells in the basal layer (20x).

**Figure 5** - The oral epithelium proliferation into the lamina propria in fingerlike projections which anastomosed and enclosed bits of inflamed connective tissue (10x).
Figure 6- The outer border of the epithelium was distinctly hornified (10x).

Figure 7- Neutrophils located in the spinous layer (20x).

Figure 8- The group of fibers which usually run parallel to the sulcular epithelium was replaced by a dense inflammatory infiltrate; remnants of a subjacent group were discerned (10x).

Figure 9- Inflammatory cells: lymphocytes, plasma cells, macrophages and some degenerated neutrophils can also be distinguished (20x).

Figure 10- Many capillaries may be noted; a perivascular inflammatory infiltrate was found (20x).

The lamina propria was composed of dense inflammatory infiltrate interspersed between the collagen fibers. Inflammatory cells were spread diffusely or localized to focal accumulations. (Figures 8, 9, 11) The inflammatory cells were: chiefly lymphocytes, plasma cells and macrophages and some degenerated neutrophils. (Figure 9) The inflammatory process was more intense near the sulcular epithelium: the fibers were distinctly destroyed and the inflammatory infiltrate was diffusely spread through the gingival tissue; the profound group of gingival fibers was still relatively intact. (Figures 8, 9) The blood vessels are dilated and irregular. (Figure 10) Varying degrees of repair were seen: a fibrous connective tissue, consisting mainly of collagen fibers arranged in irregular bundles. (Figure 11)
Discussions

Our results showed a correlation between the MMP-8 salivary levels and the severity of periodontitis evaluated by the clinical parameters (PI, BPI, PD, CAL) and the periodontal tissues destructions assessed by the histopathological examination. These data are consistent with the results of other studies (Teles et al. 2010, Pozo et al. 2005).

Our histopathological findings are compatible with the characteristic features of periodontitis described by other authors (Williams et al. 1998). The chronic inflammatory process is a balance between destruction and repair; it includes both the tissue degradation: epithelial ulcerations, collagen lysis, and the attempts of regeneration: epithelial proliferation, keratosis and fibrosis.

The morphological modifications identified in the soft periodontal tissues were consistent with the clinical parameters: the inflammatory state of the periodontium was reflected by the periodontal index (PI), the ulcerations of the sulcular epithelium and the vascular changes in the lamina propria caused bleeding on probing (BPI) and the destruction of collagen fibers resulted in apical migration of the clinical attachment level (CAL) and an increase of probing depth (PD).

The association we found between MMP-8 salivary levels and both clinical parameters and the pathological modifications in the soft periodontal tissues was justified by the pathogenesis of periodontitis.

Periodontitis was a chronic inflammatory reaction triggered by the periodontopathogenic microorganisms (Bascones et al. 2005). As periodontitis progressed, the ulcerated epithelium allowed the permanent bacterial invasion into the periodontal tissues. Therefore, numerous immunocompetent cells were recruited in the lamina propria, adjacent to the sulcular epithelium. Neutrophils predominated in the early stages of gingivitis; however, the relative proportion of neutrophils and macrophages decreased during the transition to periodontitis (Lindhe et al. 2008), in which lymphocytes and plasma cells were dominant. Neutrophils and macrophages persistent at the site of inflammation became chronically activated by bacteria and their products discharged large amounts of proteolytic enzymes. The most potent enzyme derived from neutrophils and responsible for significant degradation of periodontal collagen fibers is MMP-8 (Sorsa et al. 2006). This collagenase is present in the inflammatory exsudate within the gingiva and also in the oral fluids (gingival crevicular fluid and saliva) due to the permeability of the sulcular epithelium (Miller et al. 2006). Thus, the MMP-8 salivary levels were correlated with the histopathological changes in the soft periodontal tissues and reflected the severity of the periodontitis.

Conclusions

1. MMP-8 salivary levels were significantly higher in chronic periodontitis patients compared to the healthy individuals.
2. There was a correlation between the increased MMP-8 salivary levels and the periodontitis severity assessed with the clinical parameters and the histopathological exam.
3. Salivary MMP-8 reflects the soft periodontal tissues degradation, therefore it can be considered as a biomarker for periodontitis.
4. Measurement of the salivary MMP-8 provides additional information to the traditional clinical assessments and can be used as a valuable diagnostic tool for periodontitis.
References
Preshaw P.M., Taylor J.J. How has research into cytokine interactions and their role in driving immune responses impacted our understanding of periodontitis? Journal of Clinical Periodontology; 38 (Suppl. 11): 60–84. 2011