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Matrix Metalloproteinase-8 – A Biomarker For Plaque-Induced Gingivitis

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Abstract

Matrix metalloproteinases play a fundamental role in tissue physiology, and in host responce and MMP-8 is considered to be indicator of periodontal diseases. This study aims to compare MMP-8 levels in saliva and Gingival crevicular fluid (GCF) in patients with periodontal health, plaque-induced gingivitis, and periodontitis to determine the potential of MMP-8 as a non-invasive marker for early diagnosis of periodontal disease. 62 individuals have been involved in the study, divided into three groups based on clinical and radiographic criteria – 19 healthy subjects (H), 19 patients with plaque-induced gingivitis (G) and 24 – with periodontitis (P). GCF from 6 sites and saliva were collected respectively in micropipettes and sterile tubes. The volume was determined. MMP-8 concentration was measured using ELISA. We found significant difference between the mean range of concentrations of MMP-8 in saliva in H and patients with G ($p = 0.008$). We determine MMP-8 in saliva as convenient for differentiation of periodontal health from plaque-induced gingivitis by logistic regression analysis. At the determined cut-off value of the biomarker MMP-8 in saliva – 134 ng/ml, OR = 11.7 (95% confidence interval [CI]: 2.082 to 65.605) ($p = 0.005$), the sensitivity is 57.9%, and the specificity is 89.5%. The established data identify MMP-8 in saliva as a significant marker for the diagnosis of gingivitis. We recommend determining the concentration of salivary MMP-8 as a test that would provide objective data for differentiation plaque-induced gingivitis from periodontal health, based on the determined cut-off value.

Keywords: periodontal diseases, saliva, gingival crevicular fluid, matrix metalloproteinase 8

Introduction

The modern periodontal practice uses clinical parameters to differentiate periodontal health from gingivitis and these two conditions from periodontitis, measuring the thresholds of tissue inflammation and clinically measurable tissue destruction. However, these methods do not provide accurate information about the presence of subclinical inflammation, the onset of tissue destruction, and cannot predict the risk of periodontitis progression.

In general, the development of the periodontal disease is caused by bacterial products, mainly lipopolysaccharides (LPS), which cause activation of monocytes/macrophages, resulting in increased secretion of cytokines and inflammatory mediators such as IL-1β, IL-6 and TNF-α and release matrix metalloproteinases (MMPs), leading to subsequent tissue destruction (1). It is believed that the different response of the organism underlies the different individual sensitivity to the transition of the inflammatory lesion into a destructive manifestation of periodontitis (2). Furthermore, studies of experimental gingivitis conducted by H. Löe , E. Theilade, S. B. Jensen show that there are individual variations in the time required for the development of clinically detectable gingivitis as a result of the action of bacterial plaque. According to the authors, these variations could be a reflection of variations in the mechanisms of the organism's reaction to the etiological factor (3).

Many proinflammatory molecules are found in oral fluids – saliva and gingival crevicular fluid (GCF) (4, 5). MMP-8 and MMP-9 are the major collagen-breakdown enzymes that can be detected in GCF and saliva of patients with chronic periodontitis (6). They are thought to be primarily responsible for the breakdown of collagen in inflamed tissues in gingivitis and periodontitis (7) and are considered to be good indicators of periodontal inflammation (8). Analysis of MMP-8 and interleukin-1β in gingival fluid samples is a potentially non-invasive method that can provide information on periodontal remodeling processes during orthodontic treatment as well (9).

Aim

This study aims to compare MMP-8 levels in saliva and GCF in patients with periodontal health, plaque-induced gingivitis, and periodontitis to determine the potential of MMP-8 as a non-invasive marker for early diagnosis of periodontal disease.

Materials and Methods

62 individuals have been involved in the study (including 24 men and 38 females), with a mean age of 35.55 (SD±13.782) who were admitted for professional prophylaxis and treatment in the Department of Periodontology at the Faculty of Dental Medicine, Medical University of Sofia. The patients who are included in the study are over 18 years old, in good general health, have at least 18 teeth in their mouth, and have not undergone periodontal treatment in the last six months. The research was ethically conducted according to the Helsinki Declaration of the World Medical Association. All protocols had been approved by the institutional ethics committees, including the recording of clinical measurements and collection of GCF and saliva samples. All subjects signed informed consent prior to entry into the study. All participants receive a clinical periodontal examination, which includes: Papillary bleeding index (PBI), Hygiene index (HI), Periodontal pocket depth (PPD), Clinical attachment loss (CAL), presence of Furcation defect (F), Bleeding on probing (BOP) and orthopantomography.

The participants in the study are divided by clinical and radiological criteria into three groups – patients with periodontitis (P – 24 people), patients with gingivitis (G – 19 people) and periodontally healthy people (H – 19 people).
Patients with periodontitis have at least six sites with at least six affected teeth in the six sextants of the dentition with clinical indicators for periodontitis. Each affected site has PPD ≥ 5 mm and CAL ≥ 2 mm. BOP ≥ 20% from all investigated sites in a patient (six sites per tooth were investigated). Radiological evidence for bone loss is interdental alveolar crest level's localization on more than 2 mm apically from the cementoenamel junction of the teeth (2 mm apically from the cementoenamel junction of the teeth is normal level) (10).
Patients from the gingivitis group – have a PBI > 30% from all examined sites, which corresponds with generalized gingivitis and an average value of PBI > 1, have PPD ≤ 4 mm in every site, without any sites with attachment loss, and without any radiological pronounced bone loss.
Healthy patients – have a PBI ≤ 10% from all examined sites and the average value of PBI ≤ 0.1, PPD ≤ 4 mm in every site, lack of sites with attachment loss, lack of bone loss.

Saliva Samples Collection

Saliva samples are taken from all participants. No interventions were done in the mouth before the saliva sample was gathered. Non-stimulated whole saliva is gathered in sterile tubes in accordance with Navazesh's method (11) and is modified in accordance with IARC – International Agency for Research on Cancer (Collecting and Processing Saliva. The Molecular Methods database. Wed, 12/19/2012). Around 6 ml of the whole saliva is collected. More detailed information on saliva samples collection procedures can be found elsewhere (12). All samples are processed in a laboratory and stored in a freezer at temperatures of – 80°C until the time for analysis of the quantity of MMP-8 through enzyme-linked immunosorbent assay (ELISA) (Human MMP-8 Quantikine ELISA, R&D Systems, USA).

Gingival Crevicular Fluid Samples Collection

During the same visit, but after saliva is collected, GCF from six periodontal sites from every person are also obtained in micropipettes. The predetermined sites have standard parameters, that were defined for each clinical group. 1 μL is required to be collected from each site. More detailed information on gingival crevicular fluid samples collection procedures can be found elsewhere (12). This is followed by processing of the materials in a laboratory and freezing in a freezer at – 80°C until the moment of the study. The laboratory study includes a quantitative determination of MMP-8 through the ELISA method (Human MMP-8 Quantikine ELISA, R&D Systems, USA).

Results

Levels of examined biomarkers

In healthy subjects and in patients with gingivitis, MMP-8 concentrations in GCF, respectively 5873.68 pg/ml (SD±6212.70) and 8884.21 pg/ml (6405.49), did not differ significantly (p = 0.154) (Table 1) (Fig. 1). In patients with gingivitis, MMP-8 concentrations in GCF tend to be higher than in healthy subjects. In healthy subjects and in patients with periodontitis, the concentrations of MMP-8 in GCF respectively 5873.68 pg/ml (SD±6212.70) and 6929.17 pg/ml (SD±5634.25), did not differ significantly (p = 0.590) (Table 1) (Fig. 1). The non-parametric Mann-Whitney test was used for statistical data processing, as the distribution of the variables is not normal.

Table 1. Concentrations of MMP-8 (pg/ml) in gingival crevicular fluid (GCF)

Diagnose	N	Mean	SD
Healthy persons	19	5873.68	6212.697
Patients with gingivitis	19	8884.21	6405.489
Patients with periodontitis	24	6929.17	5634.249

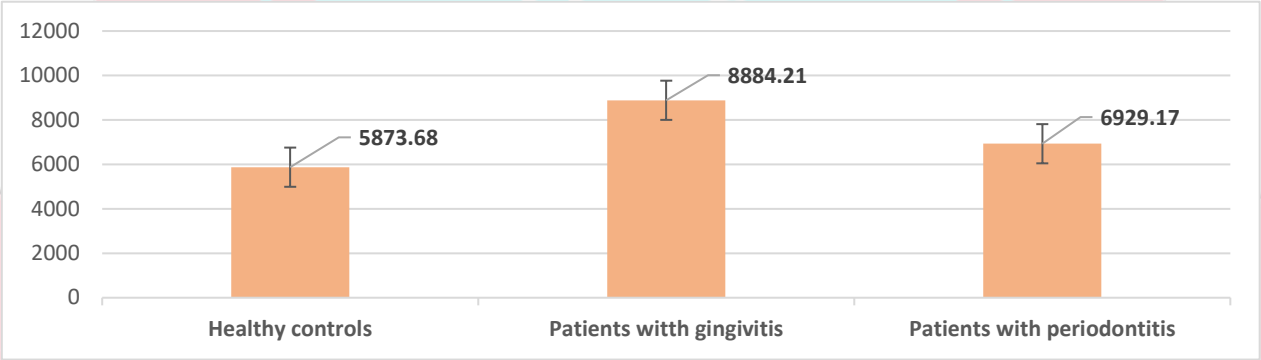

Fig. 1. Mean values of MMP-8 pg/ml concentrations in GCF in healthy subjects and patients with gingivitis and periodontitis.

There was a significant difference between the mean values of MMP-8 concentrations in saliva in healthy subjects (86.53 ng/ml; SD±59.77) and in patients with gingivitis (172 ng/ml; SD±114.45) (p = 0.008) (Table 2) (Fig. 2). The variable MMP-8 in saliva has a normal distribution in healthy subjects and patients with gingivitis, which justifies a comparison of the mean values in the two groups of patients with the Student's T-test. In the studied patients, we did not find a significant difference between the levels of MMP-8 in saliva in healthy individuals (86.53 ng/ml; SD±59.77) and patients with periodontitis (173.17 ng/ml; SD±191.38) (p = 0.471) (Table 2) (Fig. 2). The distribution of salivary MMP-8 values in patients with periodontitis is not normal, which justifies a comparison of the mean values in the two groups of patients with the non-parametric Mann-Whitney test. We did not find significant differences in salivary MMP-8 concentrations between gingivitis patients and periodontitis patients (p = 0.121).

Table. 2. Concentrations of MMP8 (ng/ml) in saliva.

Diagnose	N	Mean (ng/ml)	SD	Median	Minimum	Maximum
Healthy persons	19	86.53	59.766	84.00	0	236
Patients with gingivitis	19	172.00	114.449	164.00	24	396

Patients with periodontitis	24	173.17	191.378	104.00	8	720

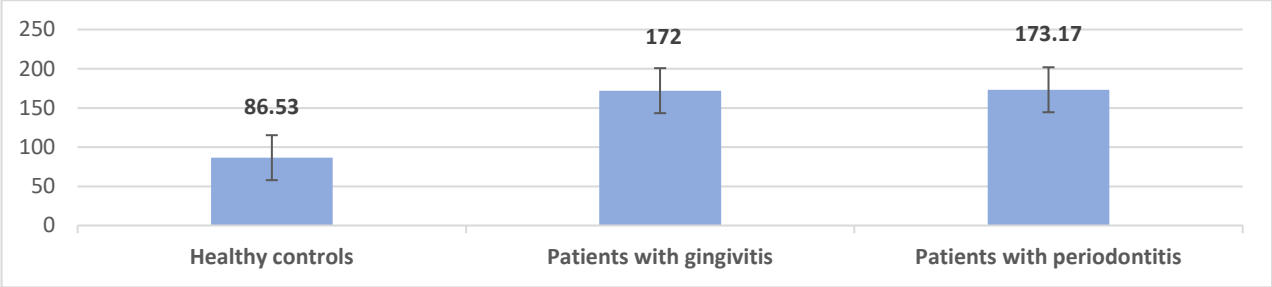

Fig. 2. Mean values of MMP-8 ng/ml concentrations in saliva in healthy subjects and patients with gingivitis and periodontitis.

Determination of threshold value

To determine whether the biomarker MMP-8 in the saliva is convenient for differentiation of periodontal health from disease, a logistic regression analysis was performed, applying the found cut-off value. ROC analysis was applied to the studied biomarker. The area under the curve (AUC) was used to determine the cut-off value, which is with the highest sum of diagnostic sensitivity and specificity. This value is 134 ng/ml, OR = 11.7 (95% confidence interval [CI]: 2.082 to 65.605) (p = 0.005).

Determination of specificity and sensitivity of the biomarker

The cut-off salivary MMP-8 value found in this study was used to test its sensitivity and specificity for differentiating periodontal health from plaque-induced gingivitis. ROC curves were used again. At the determined cut-off value of the biomarker MMP-8 in saliva – 134 ng/ml, the sensitivity is 57.9%, and the specificity is significantly higher – 89.5%.

Discussion

According to published studies, MMP-8 levels in GCF correlate with the severity of periodontal disease, and these levels decrease with the successful performance of periodontal therapy (13, 14).

Our study did not find significant differences in the concentrations of MMP-8 in GCF between healthy individuals and patients with gingivitis, but there is a tendency for higher concentrations in patients with gingivitis. Our study found no significant differences in MMP-8 concentrations in GCF between healthy individuals and patients with periodontitis. We associate the lack of significant differences with the not-normal distribution of variables and the need for a larger number of tests to establish dependencies.

However, we did not find data in the available literature to differentiate periodontal health from gingivitis based on a quantitative difference in MMP-8 levels in saliva. Our results show a significantly higher concentration of MMP-8 in saliva in patients with gingivitis than in healthy individuals, which we consider as a result of increasing the number and activity of polymorphonuclear cells as protecting cells against bacterial biofilm and increasing the amount of synthesized and activated MMP-8, which enters the saliva.

Using a statistical method, we determined that the values of the concentration of MMP-8 in saliva above the cut-off value – 134 ng/ml are associated with gingivitis (sensitivity – 57.9% and specificity – 89.5%), and values below are associated with periodontal health.
The determined cut-off value for the level of MMP-8 in saliva in clinical practice can be applied for a more objective early diagnosis of plaque-induced gingivitis, as well as gingival inflammation in already treated periodontal disease in combination with other biomarkers or clinical methods due to its lower sensitivity.
We did not find a significant difference in the mean values of MMP-8 in saliva in healthy subjects and patients with periodontitis, perhaps due to the not-normal distribution of the mean values of MMP-8 in saliva in the studied patients.
Given the fact that in our study, we did not find a significant difference in the mean concentrations of MMP-8 in saliva and in GCF in patients with gingivitis and those with periodontitis, we consider that these indicators cannot be used to differentiate the mentioned diseases. This is probably due to the fact that there is an increased concentration of MMP-8 in both gingivitis and periodontitis, and it may be different at the time of the study depending on the activity of the disease, regardless of the presence or absence of previous tissue loss.

Conclusion

The established data identify MMP-8 from saliva as a significant indicator for the diagnosis of gingivitis. Due to the high specificity and relatively low sensitivity of the MMP-8 test in saliva for the determined cut-off value for gingivitis, we recommend its use in clinical practice in combination with other biomarkers or clinical methods to differentiate plaque-induced gingivitis from periodontal health. Our data support the possibility of using biological fluid saliva in the diagnosis of gingivitis, which has the advantage of being relatively easy and non-invasive to collect.

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