Markers of inflammation in periodontal diseases

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Abstract

Periodontitis is a disease of chronic, progressive course and multifactorial etiology. Traditional diagnosis of periodontal disease is based on clinical and radiological examinations. However, clinical experience has shown that such diagnosis is not always sufficient. Numerous studies have attempted to identify – in gingival crevicular fluid, gingival tissue, saliva, and peripheral blood – various substances of both bacterial origin and derived from host cells, which would be pre-clinical indicators of disease progression. A certain level of such markers, or a change in their level within periodontium would be closely correlated with the severity of the disease. In order to determine that a given enzyme or cytokine may be a marker of inflammation, its role in both physiology and pathology of periodontal tissues should be thoroughly understood and analyzed. Currently, interleukin-1β, prostaglandin E_2, elastase and metalloproteinase 8 are considered the most significant immunological indicators. Extending the diagnostic procedures and treatment monitoring by methods based on determination of the level of inflammatory mediators in gingival crevicular fluid or in saliva may facilitate diagnosis, increase treatment effectiveness, and provide data on the pathomechanism of periodontal diseases.

Key words: *periodontitis, inflammation, markers.*

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Introduction

Epidemiological studies have indicated that in Western Europe, 36% of people aged 35-44 years have a moderately severe, and about 10% – a severe form of periodontitis [1]. These proportions are higher in Eastern Europe and equal 45% and 30-40%, respectively. Research conducted by Górska *et al.* [2] showed that advanced periodontitis affects 18% of adult Poles. These data are shocking, particularly when taking into account local effects, such as loss of teeth, as well as systemic implications, such as association of untreated periodontal inflammation with cardiovascular diseases.

Traditional diagnosis of periodontal disease is based on clinical and radiological examinations. However, clinical experience has shown that such diagnosis is not always sufficient. Observation of changes in indicators such as clinical attachment loss (CAL) and radiographic evaluation of alveolar bone loss (BL) over time, is unfortunately possible only after a few millimeters' loss of clinical attachment (retrospective diagnosis). Therefore, for many years studies have been conducted in order to develop diagnostic tests, allowing prediction of disease activity. Such tests would help to identify patients (or specific locations in patient's periodontium) that are particularly vulnerable to occurrence and rapid progression of periodontal disease. Such extended diagnostic procedures are particularly important for patients with possible periodontal lesions which could occur at a young age, or for detection of the disease progression risk at the subclinical level.

Numerous studies concerned determination of specific species (strains) of bacteria present in dental plaque, which would be responsible for a more rapid progression of periodontal disease or its occurrence at a very young age. Research carried out by Sokransky *et al.* [3] showed, however, that in the case of both chronic and aggressive periodontitis we are dealing – at most – with the so-called specific bacterial complexes, but none of the known bacteria is pathognomonic for a given disease. Moreover, in the light of recent studies it is known that anaerobic bacteria such

as *Porphyromonas gingivalis*, *Prevotella intermedia*, and *Treponema denticola* are responsible only for initiating and sustaining inflammation in periodontium. There is no doubt, however, that the mere direct action of the bacterial factor by metabolism products or bacterial enzymes would not be able to cause such significant destruction of epithelium, connective tissue and alveolar bone, which is characteristic in the course of periodontitis. Destruction of periodontal tissues, which are necessary for proper functioning of the tooth in the oral cavity, is largely caused by so-called indirect mechanisms, involving activation of the host immune response, which consequently leads to destruction of periodontal tissues.

Therefore, from a clinical point of view, a more useful method seems to be identification of inflammatory markers in material derived from the patient. A certain level of such markers, or a change in their level within periodontium would be closely correlated with the severity of the disease. In order to determine that a given enzyme or cytokine may be a marker of inflammation, its role in both physiology and pathology of periodontal tissues, should be thoroughly understood and analyzed.

Pathomechanism of periodontal disease

In the latest (1999) valid division of periodontal disease, three main groups of inflammatory periodontal diseases are distinguished: gingivitis caused by dental plaque, chronic periodontitis and aggressive periodontitis. Although each of the diseases is characterized by a different pathomechanism and clinical course, in each case a fundamental role is played by immuno-inflammatory mechanisms constituting a response to the bacterial factor.

According to a theory by Page [4], anaerobic bacteria initiate the inflammatory process in periodontium, by initiating a cascade of immuno-inflammatory reactions. Accumulation of factors of virulent bacteria and bacterial enzymes' products in tissues adjacent to the periodontal pocket (internal epithelium and subepithelial connective tissue) causes vasodilation and congestion of gingival tissue. In such case, redness, swelling, and spontaneous or stimulated bleeding of gingiva are clinically observed. Released blood flow promotes increased exudate – referred to as gingival crevicular fluid (GCF), and migration of inflammatory cells towards the periodontal pocket. Chemotaxis is also promoted by high concentrations of bacterial toxins (including lipopolysaccharides – LPS) as well as products of tissue destruction by either bacterial enzymes or host's enzymes. Bacterial lipopolysaccharides, which can penetrate from the periodontal pocket to tissues surrounding the tooth, are also able to activate complement in an alternative way, whereas bacterial antigens coated by complement components are absorbed by cells of the first line of defense, which are present in periodontium and those which arrived to the site of periodontal inflammation. Virulent factors of

anaerobic bacteria, such as LPS, also have the ability – through CD14 receptors on neutrophils – to initiate degranulation of inflammatory infiltration cells, such as PMN. As a result of this process, enzymes, among other things, are released in the region of tissues of epithelial and connective tissue attachment. These enzymes (**elastase, metalloproteinases**) have the ability to destroy the connective tissue, which – together with the bone – is the primary tissue responsible for the proper maintenance of the tooth in the alveolus. Operation of bacterial virulence factors results also in activation of a cascade of inflammation reactions within the mucous membranes of host cells, which cause changes in arachidonic acid, to typical products of inflammation, such as **prostaglandins** and **leukotrienes**. They are responsible for initiating destruction of the alveolar bone.

All the above processes are regulated by cytokines. They constitute a key element of immunoregulation in the pathomechanism of periodontal disease. Produced in healthy tissue, they condition its proper functioning. They are secreted by most cells present in periodontium and are responsible for transmission of signals between fixed cells and inflammatory infiltration cells. The release of proper cytokines in a determined amount depends not only on stimulating factors such as lipopolysaccharides, but also on activation of appropriate genes. The main mediator of periodontal tissue destruction seems to be, as in other inflammatory diseases, uncontrolled release of IL-1β. It occurs in response to induction by periodontal pathogenic bacteria, present in the periodontal pocket, such as *P. gingivalis* and is considered to be the main factor causing periodontal tissue destruction.

Mediators of inflammation in periodontal diseases

Inflammatory mediators were assessed in various material collected from patients with periodontal disease. Determination of inflammatory mediators in peripheral blood (plasma, serum) allows an analysis of the relationship between periodontal disease with systemic diseases, but does not reflect unambiguously the current status of periodontal tissues. Joshipura *et al.* [5] used a multivariate regression model to associate periodontitis with a high CRP level in serum. Many systemic factors affect the level of inflammatory mediators, such as CRP in peripheral blood. Nonetheless, a study by D'Aiuto *et al.* [6] showed a significant reduction in CRP and IL-6 levels in serum as a result of periodontal treatment. Also own studies by Górska *et al.* [7] showed significant differences in MMP-8 concentration in peripheral blood serum in a group of healthy individuals and patients with chronic periodontitis.

Collecting tissue samples from the periodontal pocket area seems to be a good method in the study on periodontal disease pathomechanism, yet it is not popular in clinical trials. Korostoff *et al.* [8] demonstrated an expression of latent forms of MMP-2 and MMP-9 in gingival biopsies

taken from healthy individuals and patients with periodontitis. In turn, the active form of MMP-2 was present only in material collected from patients with periodontitis [9].

An increased expression of proteolytic enzymes and inflammatory enzymes in periodontal tissues (both periodontal tissue cells and inflammatory infiltration cells) in the course of periodontitis can cause increased levels of these enzymes in body fluids. Gingival crevicular fluid, whose composition reflects the course of inflammation in periodontal tissues, seems to be credible material.

Research by Masada *et al.* [10] demonstrated elevated concentrations of IL-1β in GCF collected from diseased sites in periodontium, compared to concentrations in healthy areas. The concentration of the cytokine decreased after scaling and root surface planing, even if it did not correlate with pocket depth change. Similar results were obtained by Hou *et al.* [11]. They observed that the amount of IL-1β is closely associated with progression of lesions in periodontium. Reinhardt *et al.* [12] demonstrated that an increased level of IL-1β is closely associated with the presence of three bacterial species in the examined pockets: *Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis* and *Eikenella corrodens*.

A study by Preiss *et al.* [13] demonstrated that the amount of IL-1β in gingival crevicular fluid in patients with periodontitis is significantly higher than in healthy individuals. This was confirmed by Tsai *et al.* [14], who also concluded that it is reduced as a result of conducting the initial phase of periodontal treatment. The amount of IL-1β was also correlated with clinical parameters [15]. Another study by Hou *et al.* [11] suggested that the amount of IL-1β in GCF is closely associated with the patient's periodontal status. Engebredson *et al.* [16] observed that increased levels of IL-1β in gingival crevicular fluid in patients with severe periodontal disease, is also found in shallow pockets, which confirms the role of the genetic factor associated with an increased expression of IL-1β in the pathogenesis of chronic periodontitis.

Research by Atilla *et al.* [17] showed that gingival crevicular fluid of patients with periodontitis has significantly higher concentrations of MMP-8 and MMP-9 compared to healthy individuals. Chen *et al.* [18] observed that a concentration of MMP-8 in gingival crevicular fluid is positively correlated with the plaque index and bleeding index, whereas the total amount of MMP-8 in GCF is also correlated with the depth of periodontal pockets in patients with periodontitis.

An increased level of elastase was also observed in gingival crevicular fluid of patients with periodontitis compared with healthy controls. Eley *et al.* and Cox *et al.* [19, 20] demonstrated that elastase concentration in GCF correlates with clinical indicators of progressive periodontitis and decreases significantly after periodontal treatment. In addition, higher elastase levels in gingival crevicular fluid were also found in patients with so-called refractory periodontitis compared to patients with a similar degree of periodontitis severity who responded positively to treatment. Moreover, a significantly higher concentration of elastase was observed in areas demonstrating periodontal disease progression [21] and a risk of the progressive alveolar bone loss [22]. A study by Meile *et al.* [23] showed that intensive hygienic procedures lead to reduced elastase levels in GCF.

Jin *et al.* [24], and Söder *et al.* [25] studied relationships between the elastase activity and PGE$_2$ level in gingival crevicular fluid on one hand, and the presence of periodontal pathogenic bacteria (*Actinobacillus actinomycetemcomitans, Bacteroides forsythus, Porphyromonas gingivalis, Prevotella intermedia, Treponema denticola*) on the other hand, in patients with untreated periodontitis. They demonstrated that a local immune response to periopathogens varies depending on intensity of the inflammatory response measured by elastase and PGE$_2$ levels in gingival crevicular fluid.

According to Offenbacher *et al.* [26], an elevated level of PGE$_2$ in gingival crevicular fluid collected from lesioned sites corresponds to the concentration causing bone tissue resorption *in vitro*. Studies by Haesman *et al.* [27] showed that PGE$_2$ concentrations in GCF of healthy individuals are low and they increase in the course of gingivitis. Moreover, patients with periodontitis demonstrate higher PGE$_2$ levels in GCF than those with gingivitis [28]. Also in untreated sites with a progressive clinical attachment loss, a significant increase in the PGE$_2$ level in GCF was observed [29].

Gingival crevicular fluid, however, is not an easy material to collect, especially for analysis, due to very small volume of fluid intake. Measurement devices for small volumes of liquid which are available on the market, such as Periotron 8000, make it possible to analyze the concentration of selected mediators, however, such methods are very time-consuming and costly.

Saliva seems to be a material which is simple to collect and analyze. Although the concentration of inflammatory mediators in this material is several times lower than in gingival crevicular fluid, sensitivity of presently applied methods makes the issue less critical. Saliva seems to be a very good test material for substances derived from PMN cells. These cells infiltrate tissues surrounding a periodontal pocket, then penetrate into gingival crevicular fluid, and, consequently, to saliva. According to Uitto *et al.* [30], polymorphonuclear leukocytes (PMN) constitute the main source of salivary collagenases, such as MMP-8. Each milliliter of whole saliva contains from 100,000 to 500,000 PMN cells in various stages of degradation. They transfer into the oral cavity through gingival sulcus. Research by Gangbar *et al.* [31] showed that isolated parotid saliva and sublingual saliva virtually contain no collagenases, and only small quantities of them are found in saliva of edentulous patients. Our study [7] demonstrated increased levels of MMP-8 in saliva of patients with advanced periodontitis compared to patients with mild to moderate periodontitis and to healthy

individuals. It was also shown that elastase concentration in saliva of patients with chronic periodontitis is significantly higher compared to those with healthy periodontium.

Multifactorial etiology of periodontitis makes causal treatment of some of its manifestations often impossible and inadequate. Extending the diagnostic procedures and treatment monitoring by methods based on determination of the level of inflammatory mediators in gingival crevicular fluid or in saliva may facilitate diagnosis, increase treatment effectiveness, and provide data on the pathomechanism of periodontal diseases.

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