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Quantification of mast cells in different stages of periodontal disease

Kvantifikacija mastocita u različitim stadijumima parodontalne bolesti

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Abstract

Background/Aim. Mast cells are mononuclear cells originating from bone marrow. They produce various biologically active substances, which allow them to actively participate in immune and inflammatory processes associated with periodontal disease. The study focused on distribution and density of mast cells in healthy gingiva as well as in different stages of periodontal disease. **Methods.** The material used for this purpose was gingival biopsies taken from 96 patients classified into 4 groups: healthy gingiva, gingivitis, initial and severe periodontal disease. Toluidine blue staining according to Spicer was utilized for identifying mast cells. **Results.** Basing on our study, the density of mast cells in the gingival tissue increases with the progression of the infection, which means they are more numerous in gingivitis compared to healthy gingiva, as well as in periodontal disease compared to gingivitis. **Conclusion.** Increase in the number of mast cells in the infected gingiva can be correlated with an increased influx of inflammatory cells from blood circulation into the gingival stroma, as well as with the collagen lysis, since these cells produce substances with collagenolytic potential. Based on the distribution of mast cells, it could be concluded that in the evolution of periodontal disease there are significant dynamic alterations in migration and localization of these cells.

Key words:
periodontal diseases; disease progression; mast cells.

Apstrakt

Uvod/Cilj. Mastociti su mononukleusne ćelije poreklom iz koštane srži. Oni proizvode brojne biološki aktivne supstance, usled čega aktivno učestvuju u imunim i inflamatornim procesima kod parodontalne bolesti. U istraživanju je praćena distribucija i gustina mastocita u zdravoj gingivi i u različitim fazama parodontalne bolesti. **Metode.** Materijal je dobijen gingivalnom biopsijom kod 96 bolesnika razvrstanih u 4 grupe: zdrava gingiva, gingivitis, početna parodontopatija i uznapredovala parodontopatija. Za identifikaciju mastocita korišćeno je toluidin plavo bojenje po Spiceru. **Rezultati.** Prema našem istraživanju gustina mastocita u gingivalnom vezivu raste sa progresijom infekcije, što znači da su mastociti u gingivitisu brojniji u odnosu na zdravu gingivu, a u parodontopatiji brojniji u poređenju sa gingivitisom. **Zaključak.** Povećanje broja mastocita u inficiranoj gingivi može se dovesti u vezu sa pojačanim influksom inflamatornih ćelija iz cirkulacije u stromu gingive, ali i sa lizom kolagena budući da ove ćelije proizvode supstance sa kolagenolitičkim potencijalom. Na osnovu distribucije mastocita može se zaključiti da u evoluciji parodontalne bolesti postoje značajne dinamičke alteracije u migraciji i lokalizaciji ovih ćelija.

Ključne reči:
periodontalne bolesti; bolest, progresija; mast ćelije.

Introduction

Periodontitis or periodontal disease is a chronic inflammatory disease associated with bacterial infection [1]. The disease is characterized by destruction of the periodontal ligament and gingiva and by alveolar bone loss [2]. Periodontal disease goes through various stages – gingivitis, initial and severe periodontal disease [3]. Periodontal disease is one of the most common diseases nowadays, and at the same time it is a risk factor for various systemic diseases such as diabetes, cardiovascular, renal or pulmonary disease [4]. Periodontal pathogens and their products cause pathological changes, but cannot contribute to the comprehensive development of periodontal disease [5]. It has been suggested that the reaction of the patient's immune system has a key role in the destruction of periodontal tissues [6]. Numerous types of cells, including

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mast cells with their important role are involved in the pathogenesis of periodontal disease.

Mast cells (MCs) are mononuclear cells originating from CD34+ precursors from bone marrow [6]. They differentiate and mature in peripheral tissues [7]. MCs are normal residents of the *lamina propria* of human oral mucosa and gingiva [8]. They produce various biologically active substances, which allow them to actively participate in immune and inflammatory processes associated with periodontal disease [9]. In their cytoplasm, they contain about 80 to 300 granules which exhibit metachromasy and toluidine blue staining [10]. Various enzymes and mediators, such as the serine proteases, tryptases, chymases, cathepsin G, acid hydrolases, matrix metalloproteinase, histamine, heparin and serotonin are stored in granules [11]. Based on the content of neutral serine proteases human MCs can be divided into mast cell tryptases (MCT) and mast cell tryptase and chymotryptic proteinase (MCTC). MCTC in their granules contain tryptases, chymases, carboxypeptidases and cathepsin G-like proteases, and mainly can be found in the dermis and intestinal submucosa [12]. Of neutral proteases, MCT contain only tryptases and primarily can be found in red mucous membrane and in alveolar walls [13]. MCs release proinflammatory mediators, promote inflammation and angiogenesis, degeneration of the extracellular matrix and tissue remodeling [10]. These cells are noted in different parts of gingiva, and information on their density in healthy and inflamed tissues is contradictory. The aim of the study was to examine the distribution and density of various stages of periodontal disease in order to assess the importance of this cell population in a specific immune response and related tissue destruction.

Methods

The research was undertaken at the Dental Clinic and Institute of Histology of Faculty of Medicine in Kosovska Mitrovica. This research has been conducted following the principles of the Declaration of Helsinki (retrieved from http://www.wma.net/en/ 30publications/10policies/b3/. Last accessed: 20-12-2014) and approved by the Institutional Review Board of the Faculty of Medicine in Kosovska Mitrovica. Informed consent was issued by all patients after a careful explanation of the aims of the study.

The material consisted of gingival biopsies taken from 96 patients aged 13–68 years. Biopsies were made during extractions of teeth for orthodontic reasons or periodontal disease. Patients included in this study did not suffer from systemic diseases. The following indices: Community Periodontal Index of Treatment Needs (CPITN), Löe-Silness gingival index, Mühlemenn-Sulcus bleeding index, Silness-Löe plaque index were used to assess the clinical stage of the disease. Periodontal pocket depth was measured with a ball tip probe. In accordance with the classification system for periodontal diseases and conditions of the American Academy of Periodontology [14], gingival samples were classified into four main groups: healthy gingiva (11 samples taken from people whose gingiva showed no signs of bleeding, swelling or inflammation, and where gingival groove depth was usual); gingivitis (18 tissue samples taken from patients reported with redness, swelling and bleeding gums, and Löe-Silness gingival index was 2 or 3); moderate periodontitis (36 samples taken from patients with periodontal pocket depth below 6 mm); severe or advanced periodontitis (31 samples taken from patients with periodontal pocket depth over 6 mm).

Tissue samples were fixed in 10% formalin, dehydrated in increasing concentrations of alcohol, lightened in xylene and embedded in paraffin. Serial sections of 5 mm thicknesses were made from the paraffin blocks using Reichert sliding microtome. Spicer's staining technique was used for identification of mast cells [15]. Morphometric analysis was done using previously calibrated mesh whose surface was 0.0145 mm^2 at 400× microscope magnification. For each measurement, the mash was put over the entire tissue sample, whose surface required a certain number of measurements *per* sample. The average number of cells *per* mesh surface was determined for each sample and the number of cells *per* mm^2 of gingival tissue is obtained with its dividing by 0.0145. Statistical analysis was done using MS Office Excel program. The density of MCs *per* unit area (mm^2) was expressed in mean value ± standard deviation. Testing the statistical significance of differences in the mean values of the number of MCs *per* mm^2 of gingival tissue between the experimental groups was done using Student's *t* -test.

Results

A moderate number of round, oval or spindle-shaped MCs can be seen in preparations of healthy gingiva (Figure 1). They can be found as single cells, rarely in smaller groups. MCs were more numerous in the reticular than in papillary layer of gingiva, and most of them were on the border between the two layers and along blood vessels. MCs cannot be found in gingival epithelium. In gingivitis, it is reported that there is an increased number of MCs in reticular gingival layer with the tendency to clustering around arterial and venous blood vessels, around inflammatory infiltrates and within them, whereas they are very rare and usually oblong-shaped between densely organized collagen bundles (Figure 2).

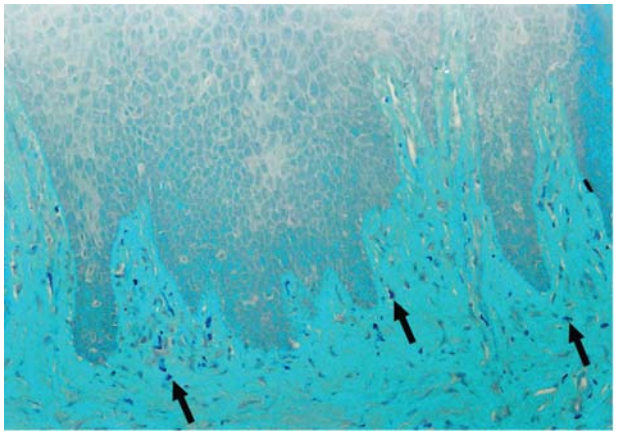

Fig. 1 – Mast cells (black arrows) in healthy gingiva were most numerous on the border between the reticular and papillary layer (Alcian blue, 200 ×).

No significant differences were found in the density and distribution of MCs between moderate and advanced periodontitis, but compared to healthy gingiva and gingivitis, a noticeable increase in their number was reported in both groups. In periodontal disease, MCs were plentiful in collagen bundles and inflammatory foci (Figure 3), but it is evident that most of them were around inflammatory infiltrates and blood vessels (Figure 4). It was notable that MCs within infiltrates were round-shaped and to some extent degranulated, whereas MCs along collagen bundles were by the rule oblong-shaped. In periodontal disease, mast cell density was increased in papillae and usually at their base, rarely at the top. MCs in the basal and spinous layers of the oral gingival epithelium were observed in two samples with initial periodontal disease and in three samples with advanced periodontal disease (Figure 5).

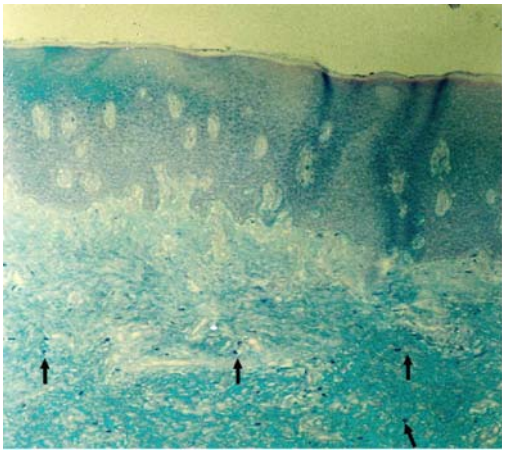

Fig. 2 – In gingivitis, mast cells (arrows) were densest in the reticular layer, much rarer in the papillary layer of gingiva and not found in the epithelium (Alcian blue, 100 ×).

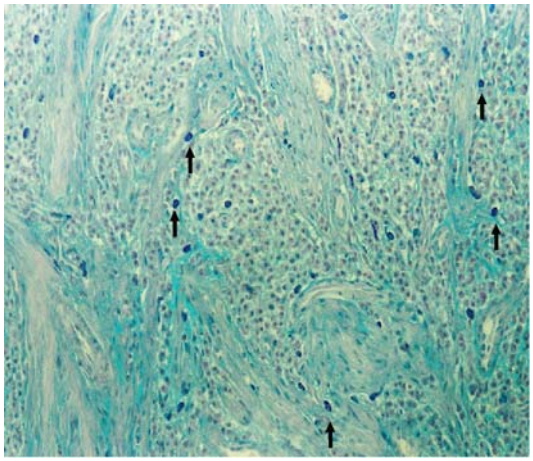

Fig. 3 – In periodontal disease mast cells were particularly numerous on the border of inflammatory foci with collagen bundles (arrows) (Alcian blue, 200 ×).

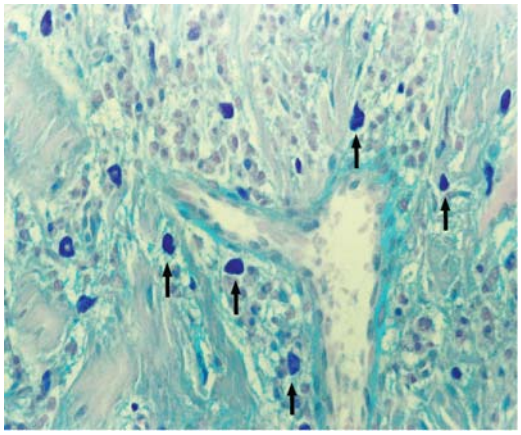

Fig. 4 – A lot of mast cells (arrows) surrounding a blood vessel in advanced periodontal disease (Alcian blue, 400 ×).

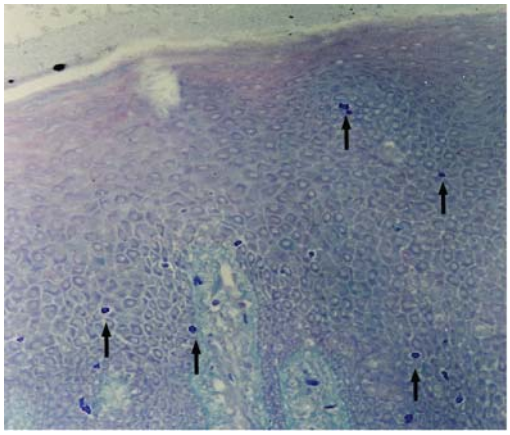

Fig. 5 – In some samples of periodontitis mast cells (arrows) were found in the gingival epithelium (Alcian blue, 200 ×).

By doing stereological study and statistical analysis it was found that the binder in normal gingiva contained an average of 59.43 ± 19.85 mast cells/mm^2, in gingivitis 96.83 ± 25.72 cells/mm2, in initial periodontal disease 136.42 ± 28.52 cells/mm^2, in advanced periodontal disease 128.43 ± 42.89 cells/mm^2. The average number of MCs in the group with initial periodontal disease was statistically significantly higher than in healthy gingiva ($p < 0.001$) and gingivitis ($p < 0.05$). Both gingivitis group ($p < 0.05$) and advanced periodontal disease group ($p < 0.01$) had a higher density of MCs compared to healthy gingiva. The difference in the average number of MCs was not statistically significant between the initial and advanced periodontal disease.

Discussion

MCs secrete primary and secondary inflammatory mediators and have an important role in inflammatory reactions [10]. In our study, detection of MCs was performed using Alcian blue polyvalent basic dye according to Spicer's method. The results suggest that MCs are dispersed throughout the binder of healthy gingiva, mostly as single cells, sometimes in smaller groups. Naesse et al. [11] studied the number of MCs in three randomly selected gingival zones (the zone beneath marginal epithelium, the central zone and the zone beneath oral epithelium) and found no differences in the

density of MCs. The same authors recorded a number of MCs in the gingival epithelium as well. In our material, MCs were found neither in epithelium of healthy gingiva, nor in gingivitis, but in some samples of moderate to advanced periodontal disease they were found in oral epithelium. Similar results were recently published by Popovici et al. [16]. These authors found that the number of intraepithelial MCs increases with the severity of inflammation and intraepithelial MCs could be important in the evaluation of the severity of periodontal disease.

Some authors have recorded a connection between MCs and peripheral nerves which secrete neuropeptides (substance P, vasoactive intestinal peptide). It is well-known that neuropeptides can induce degranulation of MCs [17], and thus affect the course and severity of inflammatory reactions. Huang et al. [18] pointed to the correlation between MCs degranulation and the severity of periodontal disease. In our study, it has been recorded that MCs can often be found on the border of inflammatory infiltrates. Previously, Mekori and Metcalfe [19] recorded similar findings in their studies. They noticed MCs surrounding T-lymphocytes and concluded that it is possible that these cells phagocytize, process and present antigens to T-lymphocytes thus initiating adaptive immune response [20]. In addition, there is proof that MCs secrete both Th1-type and Th2-type cytokines, suggesting that these cells may send an immune response to an inflammatory or anti-inflammatory reaction. It is known that MCs secrete a significant amount of IL-4. Thus favoring the Th2 immune response followed by activation of B-lymphocytes and production of antibodies. Previous observations confirm the existence of functional relations between MCs and immunocompetent cells. Batista et al. [21] observed that MCs are concentrated in a large number not only in the inflammatory focus, but also around it. They recorded that in localized chronic periodontitis the number of MCs was increased by 3% around the inflammatory focus and by about 28% in the focus itself. Quantification of MCs within and around the inflammatory focus was not done in our study, but in many samples of periodontal disease a larger number of these cells were found around the focus than within it. These results suggest that there are significant dynamic alterations in migration and localization of MCs in the evolution of periodontal disease.

It is known that MCs start to develop in bone marrow, then enter the peripheral circulation and mature in tissues. MCs of connective tissue have a long lifetime and require stem factors for their development and survival [22]. Mucosal MCs mature in mucosa of various organs after exposure to Th2-type anti-inflammatory cytokines and their number increases in the case of parasitic infection or allergy [22]. Since MCs do not proliferate locally, their precursors come into gingiva from bone marrow, and thus it can be possible to conclude that there are certain processes that direct MCs precursors to the inflammatory focus [23].

In the literature, there is a strong disagreement about the number of MCs in healthy and unhealthy gingiva. While some authors have recorded an increase in the number of MCs in periodontal disease [24], others have recorded a decrease in their number [25, 26]. Cindrić et al. [27] have recorded an increase in the number of MCs in inflamed gingiva in patients with gingival index 1–2 and a decrease in their number in patients with gingival index 3. According to our research, the binder in normal gingiva contains an average of 59.43 ± 19.85 mast cells/mm^2 of binder, in gingivitis 96.83 ± 25.72 cells/mm^2, in initial periodontal disease 136.42 ± 28.52 cells/mm^2 and in advanced periodontal disease 128.43 ± 42.89 cells/mm^2 of binder. The average number of MCs in healthy gingiva was significantly lower compared to the other tested groups, and in gingivitis was significantly lower than in both forms of periodontal disease. On the other hand, no significant difference was noted in the average number of MCs between initial and advanced periodontal disease. In 1950, Wislocki and Sognnaes [28] reported that the number of MCs *per* mm^2 of healthy gingival tissue ranged from 30 to 60 cells, which is equivalent to our findings, while by doing quantitative analysis, Dummett et al. [29] concluded that the number of MCs in gingiva ranged from 0.47 to 146 cells per mm^2. Batista et al. [21] also performed quantification of MCs in various stages of periodontal disease on preparations stained with toluidine blue. According to their findings, healthy gingival tissue contained an average of 35.73 ± 37.77 mast cells/mm^2, in gingivitis that number increased to 44.54 ± 30.31 cells/mm^2, whereas in the localized chronic periodontitis their density was the highest (71.38 ± 59.15 cells/mm^2). In comparison with our results, it is evident that there is a lower cell density (which can be attributed to different staining techniques), but the trend of increasing cell density is consistent to disease progression.

The increase in the number of MCs in acute and chronic inflammation of gingiva explains the role of these cells in the pathogenesis of the disease. There are results that confirm the correlation between the density of MCs in tissues and hyperemia, as well as the connection between increased vascular permeability and degranulation of MCs accompanied with histamine release [30]. Since MCs serve as a major source of histamine in tissues [31], the increase in their number can be related to an increased influx of leukocytes into the inflammatory focus.

The results by Naesse et al. [11] showed that of all cells of gingival infiltration, MCs express matrix metalloproteinase (MMPs) most prominently. They concluded that the concentration of MCs that express MMPs increases in periodontal disease. Since these enzymes are responsible for the destruction of collagen, it is possible to make conclusions about the participation of MCs in both defense mechanisms and destructive processes during chronic inflammation of periodontal tissues. The destructive effect may be of IL-4 originating from MCs, which directs the immune response towards the production of antibodies, which happens in exacerbations. Further, tryptases also have collagenolytic potential and may contribute to the tissue destruction in periodontal disease [9]. The byproducts of MCs could be correlated with bone resorption since the deficit of MCs is associated with lower levels of bone remodeling, and excess of MCs could lead to acceleration of bone turnover [32].

Conclusion

According to our study, the density of mast cells in the gingival binder increases with the infection progression, so these cells are more numerous in periodontal disease as compared to gingivitis and healthy gingiva, and in gingivitis they are more numerous than in healthy gingiva.

No differences in the density of mast cells were found between initial and advanced periodontal disease. The increase in the number of mast cells in the infected gingiva may be the consequence of the need for increased migration of inflammatory cells from blood circulation into gingival stroma, but it can be correlated with collagen lysis since these cells produce matrix metalloproteinase and tryptases. By inspecting the distribution of mast cells, it can be possible to conclude that there are significant dynamic alterations in migration and localization of these cells in the evolution of periodontal disease.

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