PERIODONTAL DISEASE: CORRELATION WITH HISTOLOGICAL AND IMMUNOLOGICAL PARAMETERS

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ABSTRACT

Periodontal disease is inflammatory pathological conditions in the gingiva and dental support structures that usually results in extracellular matrix and connective tissue destruction. During periodontitis, inflammatory cells facilitate collagen and connective tissue loss, affects the number and activity of fibroblasts and its production of local collagen networks. Aim of this study was to evaluate collagen density and accumulation of collagen producing fibroblast and macrophages in affected tissue of periodontal disease. Histological and immunohistochemical analyzes were performed on paraffin embedded tissue sections of gingival biopsies, obtained from 30 patients with diagnosis of periodontal disease and 10 healthy donors. Tissue sections of gingival of patients with periodontal disease had significantly decreased collagen volume density and visible fragmentation and lysis of the collagen fibers, decreased number of fibroblasts, accompanied with increased accumulation of macrophages. Presented data implicate that macrophages accumulation may be the cause of enzyme mediated collagen destruction

Keywords: *Periodontal disease, collagen, fibroblasts, macrophages.*

INTRODUCTION

Periodontal disease presents group of pathological conditions in the gums and dental support structures, produced mainly by infective agents (1). Approximately 15%–35% of the adult population has periodontal disease (2-3). One of the hallmarks of periodontal disease is inflammation in periodontium (1). During inflammation, immune cells accumulate in gingival tissue in order to control invading micro-organisms. Periodontitis usually results in the destruction of the teeth supporting structures such as alveolar bone and connective tissue attachment to teeth (4).

Extracellular matrix and connective tissue destruction in gingival are sequel of lytic enzymes activity. These enzymes are partly produced by bacteria, but, in general, main source are host cells. During periodontitis, inflammatory cells secrete a variety of substances, such as enzymes. Enzymes responsible for periodontal destruction are matrix metalloproteinases (MMPs) (5). Matrix metalloproteinases play important functions within the setting of inflammation and tissue injury and remodeling (6). The main source of this enzymes are connective tissue cells and inflammatory cells (6,7). Activated inflammatory cells, that infiltrate gingival tissue produce large amounts of metalloproteinases and facilitate collagen and connective tissue destruction (6). Macrophages represent common immune cells that accumulate into affected gingival tissue (8). Bacterial LPS activates macrophages through Toll-like receptors (9). Once activated, macrophages may produce matrix metalloproteinases that contributes to local extracellular matrix destruction (8). Main source of collagen in gingival connective tissue are fibroblast (10-12). Local inflammation affects the number and activity of fibroblasts and its production of local collagen networks.

Main goal of this study was to evaluate collagen density and accumulation of collagen producing fibroblast and macrophages in affected tissue of periodontal disease. Obtained results revealed loss of collagen fibers accompanied with decreased number of fibroblast and increment in macrophages infiltration in affected gingival tissue.

MATERIAL AND METHODS

Human gingival tissues

Each diagnose was estimate according to periodontal disease classification (13-5) and confirmed by a pathologist, on hematoxylin and eosin stained slides, using standard histopathologic criteria. Control group consist of tissue specimens taken from patients who had an indication for dental extraction due to orthodontic reasons. These sections had no visible inflammation and tissue injury observed.

Hystological and immunohistochemical analyzes

Van Gieson staining was performed for collagen fibers detection (16), while immunohistochemical staining was performed for macrophages detection. Immunohistochemistry was performed on a single representative block from each case, as previously described (17). Primary polyclonal antibodies were directed against CD68 (Sigma-Aldrich, USA). The slides were examined and analyzed at x100, x200 and x400 magnification, using conventional light microscopy (Olympus, Japan). Only brightness and contrast were adjusted. Collagen volume density and number of fibroblast and macrophages in tissue sections was estimated using the M42 test system calibrated for proper magnification.

Statistical analysis

The data were analyzed using commercially available software (SPSS version 23.0). All results were analyzed using the Student's t test or Mann-Whitney U test, where appropriate. All reported P values were 2-sided and $p<0.05$ was considered statistically significant and highly significantly different when $p<0.01$. Data are presented as mean ± SEM.

RESULTS

Lower collagen volume density in periodontal lesions

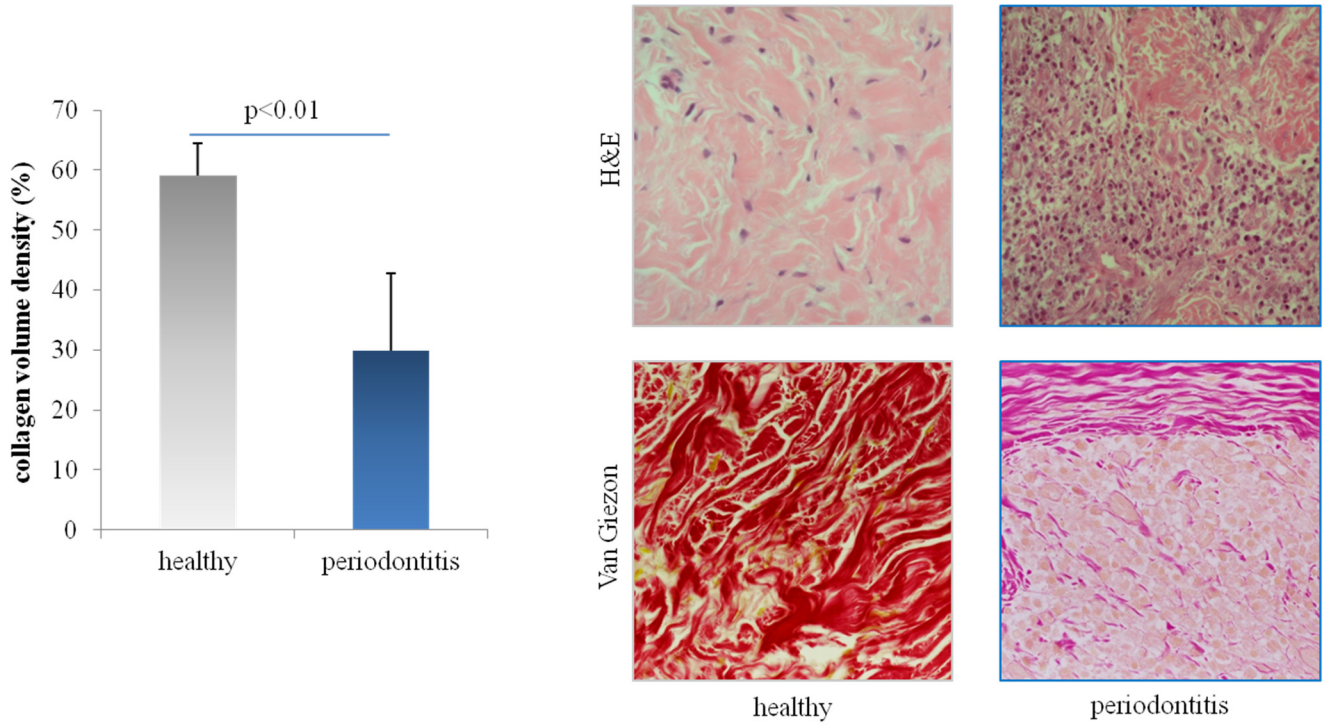
All of recruited patients in experimental group met the criteria for periodontitis, according to periodontal disease classification, while control subjects had no visible inflammation and local tissue injury. There was no significant difference in gender distribution between groups. Patients with diagnosed periodontal disease were significantly elder ($p<0.05$) in comparison to healthy patients (data not shown).

Tissue samples of gingiva derived from patients with periodontal disease had evident destruction of basal lamina with intense accumulation of inflammatory cells (Figure 1, right panel). In the same samples we found significantly decreased collagen volume density ($p<0.01$) and visible fragmentation and lysis of the collagen fibers. This finding is confirmed with pathohistological characteristics shown in figure 1.

Decreased number of fibroblasts is accompanied with increased accumulation of macrophages in periodontal lesions

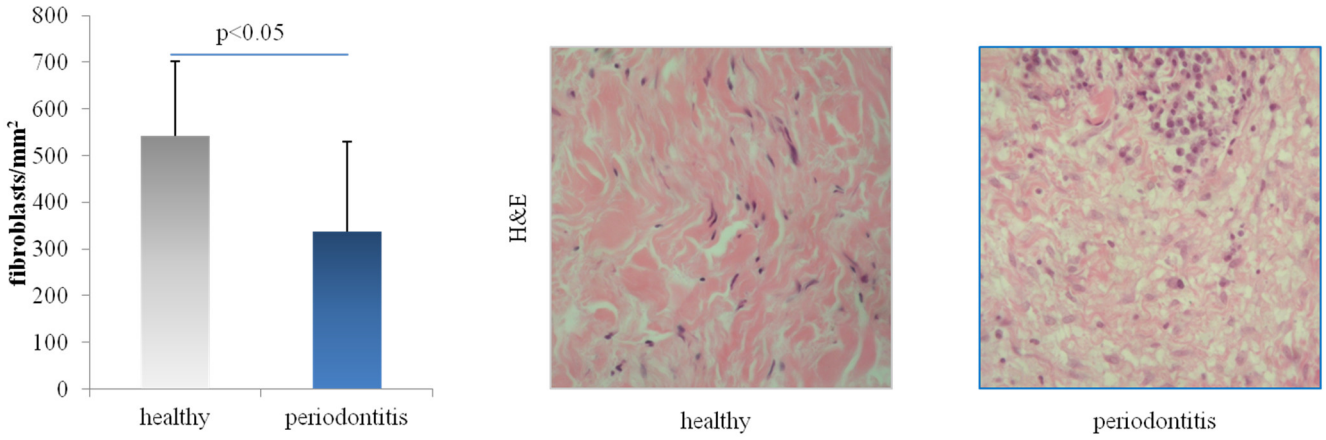
Further histological analyzes of gingiva were focused on fibroblasts. Number of fibroblasts was significantly lower in affected gingival tissue of patients with periodontal disease in comparison to control ($p<0.05$). In addition, in healthy gingiva fibroblasts were present predominantly in papillary layer, while in diseased tissue there was lesser number of fibroblasts, as presented in figure 2, right panel. Immunohistochemical analyzes showed increment of number of $CD68^+$ cells in periodontal lesions, compared to healthy control ($p<0.05$; Figure 3). $CD68^+$ macrophages are rare and evenly distributed throughout the healthy tissue. In affected gingival tissue $CD68^+$ macrophages are located predominantly into inflammatory infiltrates, as shown on figure 3, right panel.

Figure 1. Collagen volume density. Collagen fibers were detected by Van Gieson staining.



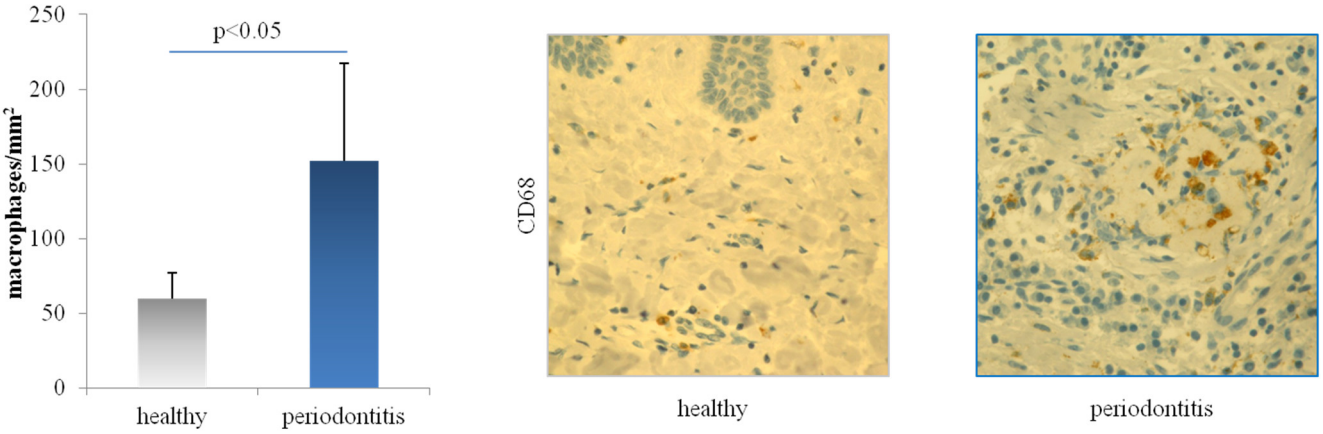
Representative images of human gingival tissue, healthy and periodontitis: upper right panel (H&E, original magnification: x400), lower right panel (Van-Gieson, original magnification: x400).

Figure 2. Number of fibroblasts. Fibroblast were detected by H&E staining.



Number was estimated by using the M42 test system. Representative images of human gingival tissue, healthy and periodontitis (H&E, original magnification: x400).

Figure 3. Number of macrophages.



Macrophages were evaluated by immunohistochemistry for each patient. Number was estimated by using the M42 test system. Representative images of human gingival tissue, healthy and periodontitis (Anti-CD68, original magnification: x400).

DISCUSSION

The aim of this study was to evaluate collagen density and accumulation of collagen producing fibroblast and macrophages in affected tissue of periodontal disease. We found significant decrement in collagen volume density, lower number of fibroblast and higher number of local infiltrating macrophages in affected gingival tissue, compared to healthy gingiva.

Firstly, we investigate extracellular matrix composition and find significantly decreased collagen volume density (Figure 1). In line with this finding, recent studies also revealed loss of the extracellular matrix during periodontitis (18,19). Significant decrement in collagen volume density implicate it`s destruction in affected tissue. Possible explanation for this phenomenon is inflammation as a cause of collagen loss, as recruited leukocytes represent a significant source of MMPs, enzymes that present major factors for collagen lysis (19-21). During inflammation, inflammatory cells accumulate in gingival tissue in order to control invading micro-organisms. Among inflammatory cells, macrophages present important players in establishing inflammation and tissue remodeling (22). Namely, they produce various pro-inflammatory mediators as well as MMPs. They are the main source of several types of MMPs (23). Increment of number of macrophages detected in affected gingival tissue (Figure 3) revealed that enhanced accumulation of macrophages and subsequent secretion of MMPs may be the cause of collagen volume density decrement. This finding can also explain visible fragmentation and lysis of the collagen fibers in same group of tissue samples (Figure 1). Degradation of collagen is probably due to gingival infiltration of inflammatory cell populations, such as macrophages.

Factors that limit collagen repair, such as decrement in number of fibroblasts may be as important as those that initiate its loss. Fibroblasts are known for their role in synthesizing and re-modelling the ECM in tissues (22). It is well established that lysis of collagen fibers is accompanied with the loss of fibroblast (24). Namely, number of fibroblasts was significantly lower in affected gingival tissue (Figure 2). Possible mechanism that underlies this finding is the stimulation of apoptosis in fibroblasts (25). Previous studies revealed that bacteria induce apoptosis of fibroblast *in vitro* (26-29), but inflammation and immune cells have a more prominent role in apoptosis of fibroblasts (30). Study by Moodley Y et al. demonstrated that apoptotic fibroblasts release a potent chemoattractant for macrophages that subsequently phagocytose fibroblast (31). Increment in number of macrophages (Figure 3) accompanied with decrement in number of fibroblasts (Figure 3) supports previous study and implicate that loss of fibroblast is also probably due to gingival infiltration of inflammatory cell populations, such as macrophages.

CONCLUSION

Taken all together, presented data reveal that periodontal disease results in the destruction of the collagen. Collagen loss is accompanied with intense macrophage infiltration, followed by decrement of number of fibroblasts in affected gingival tissue. Possible explanation of this finding is that macrophages accumulation may be the cause of enzyme mediated collagen destruction as well as removal of apoptotic fibroblast. Clarifying of suggested interplay requires further studies.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was conducted in accordance with the ethical standards of the committee responsible for human experimentation (institutional and national) and the Helsinki Declaration of 1975, as revised in 2013. Voluntary written and informed consent was obtained from each participant prior to enrollment in the study. The protocol of the study was approved by the local ethics committees.

DECLARATION OF INTEREST

The authors declare that they have no competing interests.

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