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MORPHOLOGICAL FEATURES OF WOUND PROCESS IN PATIENTS WITH INFLAMMATORY AND DESTRUCTIVE PERIODONTAL DISEASES IN CASE OF DIABETES MELLITUS

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ABSTRACT — The aim of this study implied comparing the morphology of periodontal tissues in patients with diabetes mellitus following the results of surgical intervention due to inflammatory and destructive periodontal diseases. We used an erbium laser and a standard surgical method. For this, histological samples obtained from 34 patients were examined. The biopsy samples in Group 1 were studied prior to the surgical treatment of inflammatory and destructive periodontal diseases. Group 2 included histological samples of patients following surgeries employing conventional surgical techniques. Group 3 included gum biopsies obtained after surgical interventions performed with the erbium laser. The research outcomes revealed that when performed in patients with diabetes mellitus complicated with diabetic microangiopathy of the gingival mucosa, surgical curettage or a standard surgical operation with a scalpel did not lead to any significant decrease in the chronic inflammatory process. The morphological picture of the gum soft tissues in patients with Type I and II diabetes mellitus, 3 months after the treatment with the erbium laser as a cutting tool, matched the usual structure of the mucous membrane with no obvious signs of inflammation.

KEYWORDS — destructive and inflammatory periodontal diseases, diabetes mellitus, biopsy, erbium laser, surgical stage of treatment.

INTRODUCTION

The treatment effectiveness of inflammatory and destructive periodontal diseases still remains one of the most serious issues faced by dentistry nowadays. Periodontitis is a multifactorial disease and can be due to various reasons [1]. The effect caused by numerous internal and external factors entails a wide range of clinical manifestations of gingivitis and periodontitis. Diabetes mellitus [DM] is a risk factor for the development of inflammatory periodontal diseases [2, 3, 37–39] and exacerbates the severity of their course, also contributing to the development of aggressive forms. DM is a systemic disease and is recognized as a risk factor behind the onset and progress of periodontitis. An important point is that periodontal diseases, apart from all, affect the pathogenesis of numerous systemic diseases, including DM [4]. DM has an adverse effect on periodontal tissues both at the level of tissue and cells [5]. Chronic hyperglycemia contributes to the disruption of the neutrophils function while stimulating the production of inflammatory mediators, and also promotes change in the periodontal vessels vascular endothelium [6, 7]. Metabolic disorders typical of DM result in numerous cellular and molecular changes in periodontal tissues. DM has a negative impact on the course of the wound process in periodontal surgery, while slowing down the healing of wounds making it recurrent. The specific features of the wound process are due to a violated tissue blood circulation in the affected areas, disturbances in all types of the body metabolism, and an anaerobic-aerobic microflora in the purulent inflammation foci. The mechanisms involve numerous factors and include the host's altered inflammatory response, the development of glycation end products, changed bone resorption and bone tissue development, as well as altered subgingival microflora [8–14]. The microbial etiology of periodontal diseases correlates with certain types of microorganisms [15]. There is a link between the types of periodontal pathogenic agents and various subgingival microbial complexes featuring the clinical parameters pertaining to the periodontium [16]. Mechanical removal of bacterial biofilms and deposits from the root surface is the most common treatment method (the "gold standard") for cases of gingivitis and periodontitis [17]. The surgical stage is an important part of the comprehensive treatment offered to cases involving periodontal issues. Gingivectomy, vestibuloplasty, periodontal pocket closed curettage, periodontal pocket open curettage, flap surgeries, frenulotomy, frenulectomy and other invasions are traditionally performed using conventional sets of surgical instruments, namely scalpel, curettage spoons, borers, cutters, suture material, etc. However, there are a number of factors that will reduce the effectiveness of the procedure in terms of complete bacterial elimination, such as unfavorable anatomical conditions [18], invasion of periodontal pathogens into the gingival tissue [19], and related diseases such as DM. The postoperative period includes a decrease in the soft and bone tissues, as well as the occurrence of purulent-inflammatory complications and relapses. Surgical infection in the inflammation foci complicates the course of purulent diseases, slowing down the reparation. The traumatic nature of surgical interventions, postoperative complications and the need to preserve the tissues involved in the pathological process as much as possible demand new treatment options, surgical laser technologies being one of them.

As far as the cutting tool is concerned, the Er: YAG laser offers an alternative to a conventional scalpel. Laser is good when treating patients with systemic factors that can change periodontal tissues biological response through regeneration after mechanical treatment and surgery, or may contribute to the disease progression. Speaking of these modifying factors, an important role here belongs to diabetes mellitus [20, 21]. The Er: YAG lasers are used for the sanation of the dentoalveolar pocket, the elimination of periodontal pathogens, as well as for scaling and root planing [22]. The effect of the Er: YAG laser is similar to that of scaling with root planing [27–30], with a more prominent reduction in the microbial population in the long term, when working with the laser [31]. The clinical effect of the laser is comparable to scaling with root planing when treating periodontitis [30–32]. The Er: YAG laser, unlike other lasers, is effective for surgery involving soft tissues and mineralized tissues. The laser ensures ablation with the required hemostasis and a decrease in the bacterial load [23]. Procedures for lengthening the clinical crown of a tooth, frenulectomy, vestibuloplasty, preparation of the bed tissues for prosthetics, removal of excess gum tissue arising through injury and the use of medications, gingivectomy or gingivotomy, are performed using powerful lasers featuring sufficient hemostasis, while the clinical procedure is more conservative [24]. Lasers shorten the procedure duration, reducing the need for flaps and sutures. Employing the Er: YAG lasers for periodontal surgery allows enhancing tissue regeneration, helps perform surgeries without any discomfort caused during or following the treatment, and with no postoperative sensitivity, pain or bleeding. Activated metabolic processes of cells and improved microcirculation after laser treatment, promotes reparative processes in the area of the surgical wound [33]. The Er: YAG lasers allow making incisions as well as excision of the gum soft tissues and mucous membrane, yet, their use is limited to flap surgery [25]. Dental lasers have been developed using fiber optics helping gain access to periodontal pockets, which, in turn, ensures a powerful bactericidal effect. The ability of water molecules to absorb laser radiation is the factor that determines its penetration into tissues, as well as its capacity to cause thermal damage to surrounding tissues [26]. The Er: YAG laser radiation (2940 nm) makes it effective both for operating on soft tissues and on mineralized tissues. The laser energy is absorbed into the respective gum tissue, while there is no unwanted thermal damage affecting the adjacent tissues (enamel, dentin and bone). This factor makes the laser-involving procedure easy to perform, also allowing conservative and precise removal of the target tissue, especially in areas where periodontal aesthetics is something to be taken into consideration. Using the erbium laser in periodontal surgery in an outpatient setting offers a number of intraoperative and postoperative advantages, namely, reduced tissue traumatization through the surgery, high-quality hemostasis, shorter tissue regeneration time, and prevention of postoperative edema.

Aim of study:

to carry out a comparative morphological investigation into the wound process in patients with inflammatory and destructive periodontal diseases against Type I and II diabetes mellitus following surgical intervention involving the use of both the erbium laser and a scalpel.

MATERIALS AND METHODS

The study was conducted at the Department of General and Clinical Pathology: Pathological Anatomy, Pathological Physiology (Samara State Medical University, Russia) and in the histological laboratory of the clinic pathology department (Samara State Medical University, Russia).

The clinical data is based on what was obtained through surgical treatment offered to patients with infection purulent foci affecting periodontal tissues. The inclusion criteria for the study were chronic types of periodontitis against Type I and II diabetes mellitus. There were no differences made as to the form of diabetes mellitus, since all patients reveal identical clinical signs of the diseases. A total of 34 patients underwent surgeries and were observed subject to the indications (26 — females; 8 — males). The average age of all the patients was 56±5. The average age, gender, the nature of the purulent-necrotic process, as well as other factors were comparable in the 3 clinical groups that the patients had been broken into. The inclusion in each group was based on a voluntary and informed consent. The study meets the requirements set by the Committee for Bioethics functioning at the Samara State Medical University (of 27/01/2010).

To study the morphological features of the gum tissue, biopsy samples were taken at different stages of the surgical treatment. The entire body of samples through the study included 136 histological preparations. The morphological material was studied in 3 groups. The first — control — group, included fragments of the gingival mucosa tissues obtained from patients prior to the start of treatment. The samples in the group were examined straight after taking biopsies. The second group included biopsies of patients following surgeries performed with conventional surgical techniques using a scalpel, curette spoons, borers, cutters, etc. The sample collection as well as the study were done 7–10 days and 1–3 months after surgeries at the stages of comprehensive treatment. The third group — the one under study — had gingival mucosa tissues obtained after surgical interventions on periodontal tissues, with the Er: YAG laser (2940 nm) used (KEY Laser ® 3; KaVo, Biberach, Germany) as the cutting tool with the parameters of 120 mJ, 10 Hz, and the supply of an aqueous spray to the target tissues. The sampling and the study were carried out 7–10 days and 1–3 months following operations on periodontal tissues. The morphological studies were carried out using biopsy samples for histological analysis. The samples included soft tissue fragments of marginal periodontium (volume — 0.2×0.5 cm; 2–4 pieces as per each observation). The samples were fixed in 10% neutral formalin and, according to the commonly accepted method, poured into paraffin blocks. Histological sections made of paraffin blocks (4–5 microns) were stained with hematoxylin and eosin, picrofuxin. The completed preparations were examined with a photomicroscope, analyzed and photographed.

This work relied on the calculation of the percentage ratio (volume density) for epithelium, connective tissue, inflammatory infiltrate on preparations stained with picrofuxin subject to the method of point count based on the Videotest-morpho image visualization software. The statistical processing of the respective data was done on the Statistica standard software package in Windows. The histometric study algorithm included carrying out the measurements themselves (12 in each histological sample), preliminary measurements with the identification of the required number of counting signs, the identification of the correction factor for the tissue shrinkage, and checking the hypothesis for the distribution normality.

RESULTS

The first group's histological samples obtained prior to surgery, featured morphological signs of chronic inflammatory periodontal diseases in the acute stage. Microscopic examination of the bloodstream revealed signs of diabetic microangiopathy in biopsies. The capillary vessels were found to have thickened walls, which was due to puffiness and the development of protein lumpy masses. Congestion of small-size vessels also featuring signs of exudative reactions was observed against morphological changes in connective tissue and multilayer squamous epithelium. The typical polymorphocellular infiltration of connective tissue with the plasma cells to be observed, as well as diffuse lymphoid tissue, point at the inflammation chronization in the studied gingival fragments. The evidence of a high inflammatory activity can be found in leukostases and leukocyte infiltration in the areas of necrotically altered epithelium within the pathological focus. The materials contained typical signs of coarsening collagen fibers in connective tissue with a dominating cellular component, dysplasia and metaplasia of the epithelial layer. Collagen fibers, as well as vessel walls in the preparations, were observed to be of different thickness with varying tinctorial properties when stained with picrofuxin (Fig. 1).

The gingival mucosa preparations that were studied, were observed to bear features typical of dysregulation against the background of proliferative processes. The presence of a granular layer in a multilayer flat non-keratinizing epithelium meant a tendency towards keratinization. In the histological preparations, we could observe thinning the epithelial layer with smoothed papillae. A significant loss of vertical anisomorphy was to be seen in the biopsies, with a mild degree of epithelial cell dysplasia present. There were certain features of leukocyte infiltration of the basal and spinous epithelial layers with areas of intraepithelial desquamation. In addition, acanthosis with edema, a changed structure of epithelial cell contacts and vacuolization of the Malpighian layer elements were obvious in the epithelial lining (Fig. 2).

In Group 2 samples obtained one month following periodontal surgery, blood vessels with changed wall thickness, puffiness and endothelium swelling remained (Fig. 3).

Proliferative-reparative changes were observed in the preparations; fibrous changes could be seen in the connective tissue, while granulation tissue prevailed over mature collagen fibers. In the histological preparations, dysregeneration signs were to be seen in the stromal structures and epithelium. The collagen fibers in the examined fragments differed in maturity and tinctorial properties (Fig. 4).

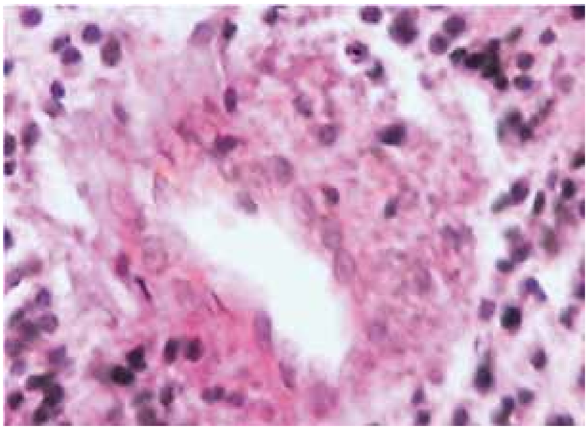

Fig. 1. Deposition of lumpy protein masses in an arteriole with a swollen wall. Staining: picrofuxin × 900

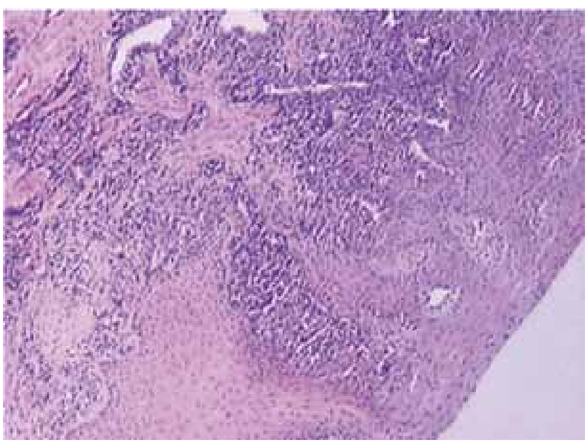

Fig. 2. Acanthosis symptoms in the multilayered flat nonkeratinizing epithelium, inflammatory infiltration in the underlying tissue. Staining: hematoxylin and eosin × 100

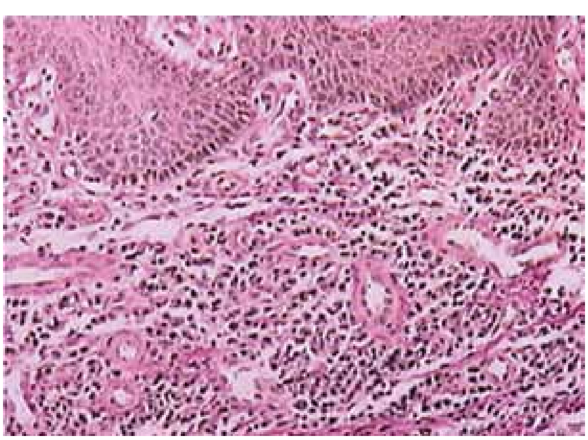

Fig. 3. Gum histological sample, 1 month following the periodontal surgery. Multilayered squamous epithelium, granulation tissue, stroma edema, small vessels with swelling endothelium and thickened wall. Staining: picrofuxin × 300

The third group of biopsies obtained 7–10 days following the surgery using laser technology, revealed no obvious signs of diabetic microangiopathy affecting small arterial vessels. The histological preparations showed no signs of acute inflammation manifested as leukocyte infiltration or necrotic component. Unlike the other two groups, the multilayered flat non-keratinizing epithelium had a normal structure of the oral mucosa. The spinous layer was thicker while acanthotic papillae appeared elongated. The connective tissue stroma had a fibrous component prevailing, which had varying degrees of collagen fibers maturity. The signs of dysregeneration persisted, evidence to that being thin collagen fibers and lumps of collagen surrounded by developed fibrous tissue. The preparations in the said group taken 3 months later, had mature fibrous connective tissue and multilayer flat non-keratinizing epithelium with no signs of inflammation. The examined fragments had a structure that virtually never differed from the normal structure of the gingival mucosa (Fig. 5).

The examined morphological material showed no signs of diabetic microangiopathy, the blood vessels had differentiated walls, with neither inclusions nor thickening. The epithelium was located above the connective tissue base. The epithelial lining thickness, the size and location of the papillae featured no disturbance. The spinous layer had no signs of pathology. Epithelial cells revealed no signs typical of metaplasia, dysplasia, necrobiotic or dystrophic issues. Mature or tough collagen fibers prevailed in the connective tissue. Single focal perivascular lymphocytic infiltrates could be observed. No sign of inflammatory infiltration was odentified (Fig. 6).

Histometric examination suggests signs of subsiding inflammation. The laser technologies, when used for the treatment of tissues at the operated area, left certain signs of reparation, with developing connective tissue and the restoration of the epithelial lining structure. As soon as seven days following the surgery, the area of the pathological periodontal focus where the erbium laser was used, showed a significant increase in the volume density of the vascular component and connective tissue against a decrease in the inflammatory infiltrate volume density. The volume density of the epithelium revealed basically no change. The epithelial lining thickness was not affected by any decrease in the edema or a lower number of intraepithelial leukocytes and microvessels (Table 1).

DISCUSSION

Inflammation affecting periodontal tissues reveals irreversible loss of the tooth-supporting structures, including the gum connective tissue fibers, the

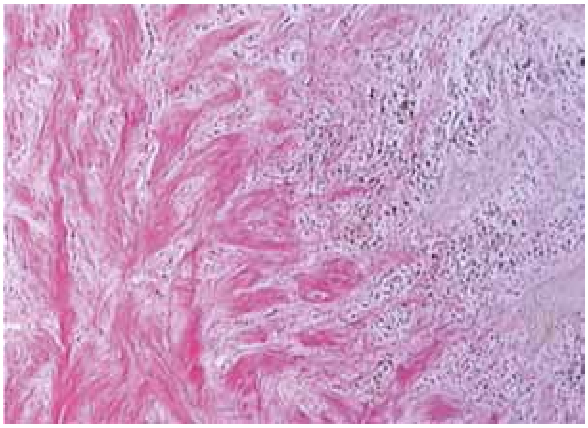

Fig. 4. Connective tissue proliferation. Collagen fibers featuring various thicknesses and tinctorial properties. Staining: picrofuxin × 100

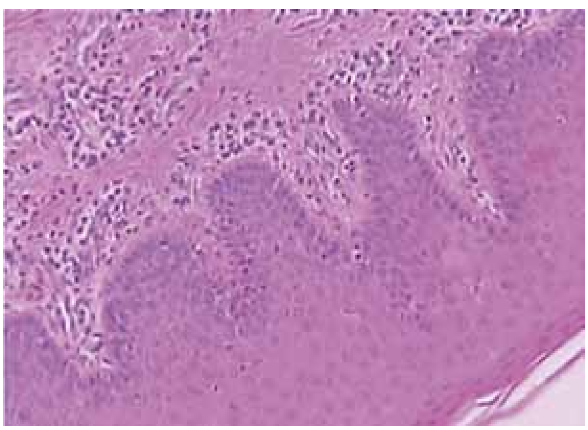

Fig. 5. 3 m following laser treatment. Normal structure of the mucous membrane. Staining: hematoxylin and eosin × 300

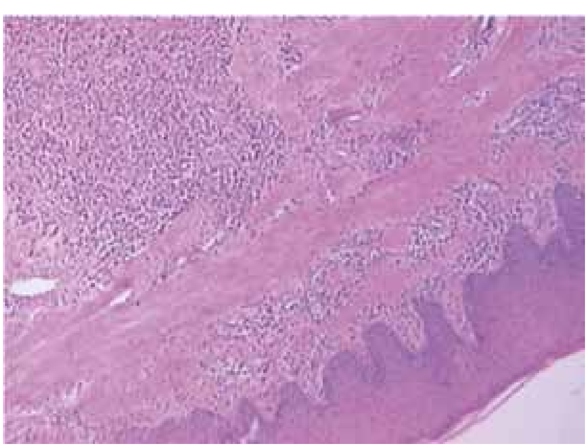

Fig. 6. 3 m following laser treatment. Multilayered flat non-keratinizing epithelium with no signs of acanthosis; moderate fibrosis seen in the underlying tissue. Staining: hematoxylin and eosin × 100

Table 1. Tissue volume density ratio vs. inflammatory infiltrate in gum biopsies, patients with Type I and II diabetes mellitus through surgical curettage and laser treatment following treatment for inflammatory and destructive periodontal diseases, (M±m)

Observation term	Volume density index (%)	
	Connective tissue	Epithelium
Surgery with a scalpel	17.3 ± 3.8	42.3 ± 3.6
1 month following the surgery (scalpel)	29.7 ± 2.8	36.4 ± 4.1
7–10 days following treatment with laser	56.1* ± 3.6	27.5* ± 2.3
1–3 months following treatment with laser	66.2* ± 3.7	24.5* ± 2.6

*Note: * — statistically meaningful difference, $p<0.05$*

periodontal ligament and the alveolar bone. Local irreversible tissue destruction leads eventually to partial or complete loss of teeth [34, 35]. The link between DM and periodontitis is actually bilateral [8], which means there is a need for ways to improve the treatment effectiveness when dealing with these diseases. The treatment outcomes for periodontitis in patients with DM, when low-power lasers are used, are available [36]. Employing the Er: YAG laser (2940 nm) to treat inflammatory periodontitis issues against DM allows obtaining outcomes that differ from those offered by standard approaches. The research described above proves the effectiveness of the Er: YAG laser (2940 nm) in periodontal surgery, when used as a cutting tool, as well as a means of tissue biostimulation. The Er: YAG laser irradiation has proved to be a comfortable choice for patients, which offers a high therapeutic effect causing no thermal damage to the surrounding tissues, unlike other lasers. Compared with standard methods, this type of laser is more conservative and gentler when it comes to periodontal tissues, while there have been no side effects reported when assessing the safety of using a laser at respectively specified wavelength and power.

CONCLUSION

Taking into account the morphological features pertaining to periodontal tissues in patients with Type I and type II diabetes mellitus after surgical intervention the use of the erbium laser proves to have certain advantage over conventional scalpels. Using a dental laser to treat complex treatment of inflammatory and destructive periodontal diseases entailed no signs of diabetic microangiopathy in histological samples. The morphological presentation of the gum tissues reflected the common structure of the mucous membrane with restoring epithelial-stromal ration and an increase in fibrosis without any distinct signs of inflammation. The use of the erbium laser as a cutting tool offers a completely new potential for the rehabilitation of chronic infection foci in the oral cavity, and ultimately improves the quality of life of patients suffering from diabetes mellitus.

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