Periodontitis is defined as “inflammatory disease of supportive tissue of teeth caused by specific microorganisms which lead to progressive destruction of periodontal membrane and alveolar bone, with formation of periodontal pockets and gingival recession”. The microorganisms found in the gingival sulcus are responsible for the first pathological changes in the inflamed gingiva. The initial damage consists of widening of the intracellular spaces, which, during the early phase of gingival inflammation, enables aggressive bacteria and their products to penetrate into connective gingival tissue.

In response to dental plaque, healthy periodontium brings about significant changes in the tissue. The changes depend on the interaction between the host response (defense mechanisms) and microbial flora in the plaque, which causes the appearance of various clinical forms of periodontal disease. However, this interaction does not greatly affect the histological changes in the periodontal tissue.

The disease starts with gingival inflammation, which develops through several phases, so that it further affects the other parts of periodontium, and finally turns into periodontitis. The changes at the level of blood vessels appear as the first manifestations of gingival inflammation (initial lesion). There is dilation of capillaries and increase of blood flow. This acute inflammation phase, depending on the host defense mechanisms, may be terminated by tissue restitution or may turn into chronic inflammatory lesions. In the early lesion, the changes noted in the initial lesion become more marked, and inflammatory infiltrate also in-
creases, which clinically brings about the enlargement of gingiva. This is followed by changes in the cell population, with an increase in the number of lymphocytes and macrophages. The sulcus exudate increases as well. The gingival tissue is edematous and red. If plaque is removed at this stage, inflammatory reactions will be eliminated and the tissue will heal. A developed lesion occurs as the consequence of dental plaque persistence, when some of the bacteria may penetrate into the host tissue. Perivascular accumulation of chronic inflammatory cells is evident at this stage. The increasing number of cells in the chronic inflammatory content is followed by the loss of collagen in the affected connective tissue. However, at this stage, there is still no loss of the bone or connective tissue attachment. Chronic periodontitis is characterized by the loss of the connective tissue attachment and alveolar bone. Clinically, the process is manifested as formation of a periodontal pocket.6

Periodontitis is a chronic inflammation with changes which are not a part of a typical inflammatory reaction, such as the epithelial detachment, formation of periodontal pockets, and finally edentulism as its consequence. Periodontitis manifests as a disease which cannot develop without the associated inflammation, because it is always preceded by gingivitis with marked inflammation.

The purpose of this study was to histologically compare inflamed gingiva treated with conventional methods plus low-level laser irradiation as an adjunct vs conventional scaling and planing alone.

MATERIAL AND METHODS

All the patients were registered at the Department of Periodontology and Oral Medicine of the Clinic of Dentistry in Nis. Thirty patients gave their written informed consent to participate in the study. The included patients suffered from chronic periodontitis with marked clinical symptoms of gingival inflammation. The diagnosis of periodontitis was made on the basis of classical diagnostic methods: anamnesis, clinical presentation, an index of gingival oral hygiene, and radiographs. The patients were up to 60 years of age. They were divided into two groups of 15 patients each. The first group of patients was the experimental group given combined therapy, which means that conventional treatment (scaling and root planing) preceded the low-level laser. The second group also comprised 15 patients (control) treated only with conventional methods. They were treated following the same principles and schedule as the experimental group, but without additional application of the low-level laser.

After conventional therapy, which comprised 3, 6, and 10 treatment sessions, the patients in the experimental group were treated with the low-power laser according to the following procedure and parameters.

The laser used in the study was a Scorpion SM-405-7A system (OPTIKA-LASER; Sofia, Bulgaria). The system is a semiconductive laser for oral application, with a wavelength of 670 nm. Laser radiation is applied via the optic fiber, with a spot width of 3 mm, power 100 to 200 mW/cm², and a power output of 4 to 15 mW. Laser irradiation was conducted almost every day after classical instrumental treatment of periodontal pockets for 5 days. The area of inflamed gingiva was irradiated for 2 min, from both the vestibular and oral sides. The laser beam was directed at an angle of 90 degrees in relation to the gingival surface with the laser tip 2 mm from the surface.

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Gingivae of all patients were examined histologically. After administering local anesthesia, a biopsy was taken from the teeth previously marked for extraction. Fixation of samples was done in 10% formaldehyde and lasted 24 h. The tissue was routinely processed, and embedded in paraffin on the microtome up to a thickness of 4 μm. After deparaffinization and processing of the selected samples in a graduated series of alcohol, the following methods of staining were applied.

1. Hematoxylin-eosin (HE) for verification of histopathological processes.
2. Histochemical Alcianblue-Periodic acid Schiff (AB-PAS [pH-2.5]) method for detection of neutral and sialomucins.

RESULTS

With the use of the micromorphological HE method and the histochemical AB-PAS and Van-Gieson methods, the 30 gingival samples were analyzed in detail.

Histological findings of the inflamed gingiva before treatment showed:

- thickening of the basal layer of gingival epithelium with partial damage
- marked inflammation in the connective tissue, consisting of lymphocytes, plasma cells, macrophages and polymorphonuclear leukocytes, whereupon inflammatory cells infiltrate into gingival epithelium in certain places
• numerous blood vessels, some of which have thickened walls (Figs 1 and 2).

After conventional treatment, histopathological findings of the treated gingivae showed an evident improvement of the tissue in terms of epithelial regeneration, decreasing of number of inflamed cells, and partial stroma collagenization (Figs 3 and 4).

Histopathological analysis of gingivae treated with the conventional method plus low-level laser yielded different results. Depending on the number of laser treatments, there was a certain histological presentation of the gingiva.

Gingivae treated 3 times with the laser showed:

• thickened gingival epithelium pervaded with inflamed cells of spinous layer
• marked inflammatory infiltrate in stroma, consisting of the acute and chronic inflammation cells
• dilated blood vessels, some of which exhibited dilated lumen and some showing thickened walls (Figs 5 and 6).
After 6 laser treatments, gingivae showed:
• preserved gingival epithelium
• very small number of lymphocytes and plasma cells
  with the presence of the minimal number of leukocytes
• small number of blood vessels
• marked stroma collagenization (Figs 7 and 8).

The tissue receiving 10 laser applications showed:
• completely normalized gingival tissue with regenerated
  surface epithelial layer and preserved basal layer
• minimal number of chronic inflammation cells
• normal blood vessels
• homogenization, i.e., collagenization of stroma (Figs
  9 and 10).

**DISCUSSION**

Periodontal disease belongs to the group of inflammatory conditions of supportive tissue of teeth caused by bacteria. The clinical manifestation of periodontal disease is the result of a complex interaction between etiologic factors, primarily consisting of specific bacteria in dental plaque and the host’s defense mechanisms. The accumulated dental plaque in persons with poor oral hygiene causes damage of the surface gingival tissue, thus leading to the appearance of gingivitis. The inflammation of gingiva, as a host’s response to bacterial challenge, causes detachment of soft tissue from teeth, making the gingival sulcus deeper, which further leads to formation of a periodontal pocket. If gingivitis is not treated or treated inadequately, then the inflammation spreads into the periodontium, where the supportive
tissue of teeth is destroyed. The loss of support of teeth brings about the loosening of teeth and their early loss.

Periodontal treatment eliminates inflammation and contributes to amelioration of the disease. Besides conventional periodontal therapy, chiefly comprising mechanical treatment of periodontal pockets, antibiotics, and surgery, lasers (high-level lasers in periodontal surgery and low-level lasers for their biostimulating effects) are currently very successfully applied as adjunct therapeutic devices in the treatment of periodontal disease.

Low-level lasers do not have destructive effects, but develop biostimulating effects to enhance repair processes in the damaged or diseased tissue perhaps by restoring bioenergetic balance and stimulating acupuncture points.

An important property of laser irradiation in medical applications is its biological effect. The result of the biological effect depends on the capacity of light energy absorption by the irradiated tissue. The low-level laser has been observed to produce the following therapeutic effects, such as:

1. anti-inflammatory effect
2. biostimulating effect
3. analgesic effect

Inflammation of the gingiva, as an early clinical symptom of periodontitis, is considered to be a risk factor which increases susceptibility of persons to the onset and progression of periodontal disease. That is why, besides gingival curettage, the low-level laser is used to prevent and attenuate the inflammatory process and facilitate recovery.

The laser’s anti-inflammatory effect is reflected in:

- effects on cell elements of inflammation – the laser energy has a positive effect on tissue metabolism followed by normalization of leukocyte chemotaxis.
- effects on blood vessels – the anti-inflammatory effect and edema reduction can be explained by increasing circulation; when vasodilation of blood vessels decreases, vasodilation and normalization of blood vessel permeability occur, as does a decrease in exudate.
- inhibition of production of inflammatory reaction mediators
- inhibition of T and B lymphocytes

The anti-inflammatory effect of the low-level laser can be seen in histopathological changes of inflamed gingiva: after conventional treatment in this study, a decreased number of inflammatory cells and partial stroma collagenization were observed.
The histological condition of gingiva after three treatments with the laser can be explained by the effects of laser radiation on blood vessels (the microcirculation is improved) and on mediators of acute inflammation which occur in the gingival tissue. The results show that due to a decrease in the number of inflammation cells and consolidation of blood vessels, the process of inflammation is reduced. At the same time, tissue is regenerated due to biostimulation.

The histological analysis of the patients’ gingiva treated 6 times with the laser shows an evident subsiding of gingival inflammation with marked repair of collagen tissue filling the parts of tissue that were previously of cellular type.

After 10 laser treatments, the results show the reduction of inflammation, which is based on decreasing number of acute and chronic inflammatory cells, followed by formation of new collagen tissue which indicates repair processes.

Our results showed a clinical improvement in inflamed gingiva in both the experimental and the control group. However, considerably better results were obtained in the experimental group after the maximum number of laser irradiations, demonstrating regenerated gingival epithelium, a low number of inflammatory cells, scarce blood vessels, and stroma homogenization when compared to the control group. Even after therapy in the control group, a considerable number of inflammatory cells was still present and a moderate level of stroma collagenization occurred.

The difference between the results obtained can be explained by the treatment method, with special emphasis on the laser irradiation regime. Histopathological examination in this study verified that the therapy of inflamed gingiva with 10 applications of laser irradiation provided significantly better results compared to conventional treatment, because of a possible cumulative effect of the laser therapy, as described by other authors.

There are still only a few papers presenting the histological effect of laser irradiation on inflamed tissue. Macroscopically, the inflamed gingiva appears less edematous, which points to an anti-inflammatory effect.

More detailed descriptions of histopathological changes after low-level laser (He-Ne, 632.8 nm) treatment have been presented by Zivkovic after cutting the skin, the reactions of inflammation and regeneration (reparation) occurred. In his paper, he followed vascularization, cell infiltration, features of young connective tissue, enzyme cell activity, as well as ultrastructural changes. After 7 days, the author found that in the laser experiment, there were no leukocytes (PMN), blood vessels were reduced, and collagen fibers thickened. Unlike the experimental group, the number of inflammatory cells is reduced in the control group, but to the same degree as in the experimental group, the capillaries are present with minor fibrosis, that is, small, well-formed collagen bundles. The results obtained in this research are almost identical to those obtained by Zivkovic.

Low-level laser is believed to stimulate or correct impaired cellular function. The laser effect improves functional features of granulocytes and normalizes hematoprotective characteristics of patients, which is in accord with findings on anti-inflammatory and regenerative effects of laser beams. The laser effects on the tissue bring about an increase in functional activities of neutrophil granulocytes, which stimulates phagocytosis. In addition, the laser has effects on humoral and cellular immunity, thus strengthening the defense mechanisms.

The therapy conducted with low-level laser compared to conventional methods is more favorable. Its advantage is observed in the effects manifested on tissue, primarily anti-inflammatory as well as stimulative effects on reparative and regenerative processes in inflamed gingiva.

This study clearly shows that the number of laser irradiation applications is important in order to obtain better effects on irradiated tissue. It has been found that the application of low-level laser, according to constant laser parameters, is more effective if the number of applications is greater. After the 6th application, a better anti-inflammatory effect is found, followed by stimulative changes in fibroblast function and inflamed gingiva collagenization as a proof of tissue regeneration and repair.

**CONCLUSION**

Based on histological findings of the examined gingiva, we can say that it is justifiable to use the low-level laser as additional, highly successful physical method in the therapy of periodontitis, since the reduction of tissue inflammation correlates positively with histopathological changes of the gingival tissue.

**REFERENCES**


