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## Clinical and Experimental Study of the Regenerative Features of Oral Mucosa under Autohemotherapy.

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### ABSTRACT

The urgent matter of the modern dental practice is to study the possibility of applying the platelet autoplasm method in the treatment of erosive and ulcerative lesions of the oral mucosa in order to accelerate regeneration and recovery of reparative functions of the damaged tissue. Experimental part included investigation of the characteristics of connective tissue regeneration in dogs being also divided into two groups. Clinical, morphological, and cytological data suggests the effectiveness of autohemotherapy in patients with erosive and ulcerative lesions of the oral mucosa in comparison with the conventional method, and that is the reason to give our preference to this method of disease treatment. Applying the autohemotherapy allows us to explain the regenerative features of the oral mucosa, indicating the feasibility and justifiability of this method.

**Keywords:** regeneration, erosion, ulcer, autohemotherapy.

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## INTRODUCTION

The problem of high incidence and treatment need of the oral mucosa diseases is one of the priorities for the modern dental practice. WHO data shows that 80 to 100% of the population of different age have some form of the oral mucosa pathology, which leads to significant changes in dental system, affects adversely the digestive process, reduces the resistance of the body, affects the psycho-emotional sphere of a patient, and thus impairs the quality of life.

Despite the wide range of existing drugs and techniques, no significant reduction in the number of patients suffering from diseases of oral mucosa has been observed [2].

One of the most common diseases of the oral mucosa is lichen ruber planus, which is characterized by a variety of forms, the complexity of the diagnosis, and its frequent malignization in the oral cavity [11]. Its erosive and ulcerative form is of particular interest, characterized by a prolonged and severe progress, and, according to various authors, accounting for 23% - 46% of all forms of lichen ruber planus of the oral mucosa [3,8]. It is the most severe and hardly sanable. Its malignization frequency reaches 1.6% [4]. This form is characterized by hyperemic and swollen oral mucosa with erosions and sometimes sores surrounded with arranged in pattern papules, typical of lichen ruber planus, against a strongly pronounced inflammation. Erosion and/or ulceration of irregular shape, covered with fibrinous pellicle, after which removal the bleeding starts easily. They may be single, small, slightly painful, however, can be multiple with pronounced painfulness. Currently, new treatment methods for oral mucosa diseases are investigated and developed due to the wide prevalence of micro-organisms cross-resistant to antibiotics, and the increased allergization of population. Today, this may be the platelet-rich autoplasm injection method. The positive effect of autohemotherapy has been known to doctors since the beginning of the XX century. In 1905, the surgeon August Bier conducted the first experiments on the use of autohemotherapy in treating the fractures, by creating artificial hematomas [9]. Thereafter, doctors have often used autohemotherapy to stimulate protective reactions of patients with infectious diseases, furunculosis, and chronic inflammatory diseases [5].

The experts who had applied the autohemotherapy, noted an intensification of reparative processes such as accelerated wound healing during recovery from injuries and operational interventions, as well as positive dynamics of skin and soft tissues purulent processes, and accelerated healing in case of chronic inflammatory diseases [7].

Last decade is characterized by a significantly grown interest in the use of platelet autoplasm, due to its high efficiency with a simultaneously high safety level and low cost. Platelet-rich autoplasm has a number of useful properties such as acceleration of tissue regeneration, anti-inflammatory effect, and reduction of pain syndrome. Nowadays, a platelet-rich autoplasm is actively used in surgery, dentistry, traumatology, orthopedics, sports medicine, cosmetology, and dermatology [1].

Application of autohemotherapy today is one of the few opportunities to modulate and enhance the regeneration of connective tissue. Platelet autoplasm is a highly active biological stimulator of regenerative processes due to the platelets of various growth factors contained in its alpha granules, which affect all structural units of the surrounding tissue and stimulate the regeneration processes [1, 4, 10].

Currently, there is information occurring in the professional literature about the use of platelet autoplasm in treating the inflammatory diseases of the maxillofacial region. Its applicability in various fields of medicine and dentistry is under consideration, based on the activation of human functional reserves reduced under the influence of either adverse environmental factors or disease [6]. However, clinical studies based on demonstrative data are scarce, and the obtained results require further study.

### Objective of Research

To study the regenerative features of oral mucosa with the application of the autohemotherapy method in treating erosive and ulcerative lesions of the oral mucosa.

## MATERIALS AND METHODS

To achieve the objectives, the clinical and experimental study was conducted. Clinical part included the examination and treatment of 60 people diagnosed with erosive and ulcerative form of lichen ruber planus in age of 40 to 70 years. Depending on the treatment performed, all patients were divided into 2 groups:

Group 1 - a control group (30 patients) who underwent conventional medical treatment.

Group 2 - an experimental group (30 patients), whose treatment included the use of the platelet-rich autoplasm.

All patients had general dental examination, and monitoring of the dynamics of reparative processes with the use of a cytological method.

The patients' examination started from the survey, which included the identification of complaints, and taking of disease and life history. In case of complaints identified, the patients were asked to describe in detail the nature of the sensations, their location, and relation to the meal. During history taking, we found out the nature of the disease progress, possible causes of the pathology and its aggravations, in particular, the presence of stressful situations and psycho-emotional stresses, and physical illnesses. We paid attention to the previous treatment of this disease and its effectiveness. We found out the number of relapses, their frequency and duration.

We conducted a thorough visible examination of the oral cavity by the conventional method, using a set of dental instruments, which included an assessment of hard tooth tissues, periodontal tissue, and oral mucosa. We paid particular attention to dental health and, if necessary, elimination of initiating factors (sharp tooth edges, broken teeth, as well as dentures made of dissimilar metals).

The method of platelet autoplasm treatment was as follows: blood was centrifuged at 2600 rpm, for 10 minutes. The result was three fractions: the top one was plasma with low platelet count, the middle layer was rich in platelets and leukocytes, and the lower was red blood cells. Autoplasm was dissected by sterile forceps and fixed to the lesion via salve film until complete plasma and the salve film dissolution.

Oral mucosa was evaluated on the following criteria: inflammation, hyperemia, bleeding, and continuity disruption (erosion or ulcer). Studying the pathological elements, we focused particularly on the size, depth, and color of the lesion. The area of the oral mucosa lesion was determined with the help of teeth impression. The impression was taken with the use of a sterile rubber and a chemical pencil, and further put on graph paper, where the lesion area was calculated.

We applied cytological method to clarify the diagnosis, and identify both the beginning of the malignization and features of the inflammatory response. The object of cytological research was the scrapings on a cotton swab. The material was further fixed on the sterile glass, and smeared. The dried smears were stained with hematoxylin-eosin and examined under a microscope. Studying the obtained material, we considered the number of leukocytes, histioid elements, and the nucleus structure (nuclear membrane, chromatin). We also considered the amount of coccal flora. We observed the processes of degenerative degradation, and hyperkeratotic effects.

Studying the cytograms, we considered qualitative and quantitative cellular composition (number and ratio of leukocytes, macrophages, histiocytes, etc.), the nature and amount of microflora, as well as assessed the epithelial cells status.

We conducted a cytology, the lesion measurement, and evaluation of the clinical pattern in the two groups of patients with lichen ruber planus at the initial examination, and on the 5th, 10th, 15th, and 30th day of treatment. All information was recorded in the case record of a dental patient.

Experimental part of study was carried out on 32 dogs, each of 10 kg. The experiments were conducted with the permission of the Research Ethics Review Committee of Volgograd State Medical University (protocol No. 214 - 2015 of 04.29.2015).

We performed experimental modeling of the pathological process of periodontal disease by creating 1x1 cm oral mucosa defect on the maxilla left and right, in the region of one premolar in the oral vestibule. Surgery was performed under intravenous anesthesia with Zoletil. The defect was made with a scalpel, forceps, and chisels up to the periosteum.

All animals were divided into 2 groups: Group I - (control) - the defect healed without additional treatment, group II - the animals had platelet autoplasm introduced. To eliminate the influence of additional factors, related to individual characteristics of the animal, on the final result of the experiment, the observational groups were formed of the same animal species.

The experimental dogs had their blood drawing from the vein, in the amount of 7 ml. Blood was sampled by the standard method using a tourniquet, alcohol wipes, a needle, a test-tube holder adapter, plaster, and specialized test-tubes "Plasmolifhting™". After sampling the blood, a test tube was placed into a centrifuge "EVA 20", at 3200 rpm for 5 minutes. Using special test tubes "Plasmolifhting™" allowed us to obtain 1.5±0.5 ml of platelet plasma; in addition, a separating gel ensures high-quality filtration of plasma and good fixation of the erythrocyte clot.

To compare the effectiveness of PRP-therapy, we performed the following procedures. The dogs of group II were introduced 2.0 ml platelet autoplasm in the area of wound defect left along the transitory fold by infiltration. The wound defect right was left for monitoring and comparing the time of the epithelium regeneration. The dogs and the regeneration of the epithelium in the wound defect were controlled daily for 14 days. Results of the study were recorded in written form and by photography.

Data obtained from studies were processed by variation statistical method on the IBM PC/AT Pentium-IV Windows 2000 using the application package Statistica 6 (Statsoft-Russia, 1999) and Microsoft Exsel Windows 2000. Statistical analysis was performed by variation statistical method with defining the average value ( $M$ ), its mean error ( $\pm m$ ), and evaluating the significance of difference in the groups using Student's t test ( $t$ ). The difference between the compared indicators was considered significant at  $p < 0.05$ ,  $t \geq 2$ .

## RESULTS

As we can see from clinical data, in some cases the first therapeutic effect can be observed in the patients' oral cavities after a week, which is manifested by improved hygienic and periodontal indices, decreased gum bleeding and hyperemia, and the recovered physiological color of the gum. Some patients had no complaints, against the positive dynamics of diagnostic methods indicators (OHI-S, GI, KPI, etc.). All examined patients had good oral hygiene (OHI-S= 0.24±0.02). KPI was 0.4±0.05, which corresponds to the risk of periodontal tissue disease. GI was 0.27±0.03 (mild gingivitis), DEF index was 14.5±0.7.

Both groups of patients showed positive changes during treatment. When analyzing the clinical data of therapeutic measures in patients of group 1, we should note that the patients reported a temporary improvement at the end of their treatment (on day 30). However, the mucous membrane still had papular pattern remained, and no complete epithelialization was observed in erosions and ulcers.

The assessment of changes in clinical findings of patients of group II found that all patients reported no discomfort in oral cavity as well as no tightness of mucosa. Two patients of group II showed complete disappearance of papular pattern, and the rest had a significant reduction in its length. There also was a sharp reduction in signs of hyperemia, and mucous membrane swelling in the area of the lesion.

On day 15, the patients of group 2 showed a decrease in both area and depth of the lesion, the ulcer cleansing from necrotic plaque, the disappeared hyperemia and swelling of the mucous membrane, and pale grayish color of the papular pattern. We should note that 5 patients of this group had a complete epithelialization of erosions, and only a gentle papular pattern on the mucous membrane against slightly congested mucosa, which indicated the disease changing into a milder form.

Cytological pattern was characterized by maximal pathological changes and corresponded to the acute purulent inflammation pattern. During treatment, the cytological pattern of patients of group 2 was normalized also in shorter term. These changes were characterized by an earlier appearance of immature and

mature forms of metaplasied cells (indicating an increased activity of the epithelium regeneration process), a decreased amount of neutrophils and coccal microflora, decreased evidences of degenerative changes in the epithelial cells, and subsequently the earlier increase in the number of epithelial cells, arranged in layers, and in the amount of the surface epithelium cells.

Dynamic measurement of the lesion area in patients, carried out for investigation of the reparative effect in the course of different treatment, indicated more pronounced epithelialization in patients of group 2.

By day 30, group 1 showed the average area of lesions equal to  $3.0 \pm 0.2 \text{ mm}^2$ , group 2 -  $1.5 \pm 0.3 \text{ mm}^2$ , and the intensity of shift in average lesion area was 60.16% and 75.34% in group 1 and 2, respectively.

During clinical observation of patients with erosive and ulcerative form of lichen ruber planus we revealed that on day 5 of the treatment of the oral mucosa diseases by platelet autoplasm the patients had no complaints of pain in speaking and eating. We objectively observed the epithelialization of erosive and ulcerative lesions. For example, prior to treatment, the patients of group 1 and 2 had the average area of the lesions equal to  $8.0 \pm 0.7 \text{ mm}^2$ . On day 7, the value in group 1 remained the same, while in group 2 decreased up to  $3.5 \pm 0.7 \text{ mm}^2$  (56.25%). The patients of group 1 had a decrease in lesion area observed on day 10 equal to  $6.0 \pm 0.5 \text{ mm}^2$  (25%), and the average value in group 2 was  $0.5 \text{ mm}^2$ . The patients of group 1 had no complete epithelialization observed by the end of the treatment, in contrast to the second group. One month after the final treatment, the second group showed the improved results of cytological indicators.

The normalization of oral mucosa epithelium in the lesions area of patients treated with platelet-rich plasma proceeded in a shorter time, as evidenced by the data obtained by dynamic cytology, as well as measurement values of the lesions area in patients with erosive and ulcerative form (shift in lesion area average by day 30 in this group was 80%, while the same in the group having only medical treatment was 60.16%).

Microscopic examination of the oral mucosa in control animals allowed us to determine a fragment of mucosa lined with hyperplastic, multilayered, non-squamous epithelium with focal acanthosis, and the underlying tissue with focal sclerosis, foci of chronic inflammation represented by an accumulation of lymphocytes, histiocytes, and fibroblasts with single gigantic debris-type multi-core cells, with areas of maturing granulation tissue.

Histological examination of a fragment of mucosa lined with hyperplastic, multilayered, non-squamous epithelium, conducted on day 14 in group II undergoing autochemotherapy, allowed us to reveal the signs of acanthosis, the underlying tissue with diffuse lymphohistiocytic infiltration with a mixture of neutrophils, and a group of newly formed blood vessels. A moderately severe congestion of the blood vessels was observed more often, which led to an increase in their diameter, i.e. trophic function of the periodontal tissue has recovered.

Thus, the histological changes in the control group indicate the development of destructive and inflammatory changes in the early stages of the experiment (day 14). The group of animals undergoing platelet autoplasm showed the recovery of histological structure of connective tissue, the reduction of the inflammatory response, the earlier maturation of granulation tissue with increasing amount of fibroblasts, lymphocytes, histiocytes, admixed with neutrophils.

#### SUMMARY

Based on the morphological study it was found that the use of autohemotherapy ensures a stable regeneration of connective tissue, lymphohistiocytic infiltration, rapid maturation of the granulation tissue, which indicates a rather quick repair of the oral mucosa, stimulated processes of tissue regeneration, quickly stopped inflammation, and recovery of the reparative function.

Clinical study showed that the complete regeneration of the oral mucosa was observed by the end of week 2, and the repair processes in the lesion area - by the beginning of week 3. The use of platelet autoplasm injections in the treatment of inflammatory diseases of the oral mucosa promotes acceleration of

tissue recovery in erosive and ulcerative processes, reduces the frequency of relapses, stabilizes inflammation processes, and accelerates tissue regeneration and repair.

Thus, the application of the method of platelet autoplasm in treating the erosive and ulcerative forms of lichen ruber planus turned to be more effective than conventional drug treatment, as evidenced by the earlier clinical improvement and increase in intensity and acceleration of reparative processes of the connective tissue in patients with lichen ruber planus, as well as long-term treatment outcomes. The group II showed more pronounced positive dynamics throughout the study as compared to the group I, where autochemotherapy was excluded from treatment. The findings of clinical study of the platelet-rich plasma efficiency in the therapeutic treatment demonstrate convincingly the accelerated regeneration process of the defective area, expressed in a significantly reduced size of the defect by 70% already by the 5th day of observation.

### CONCLUSION

Therefore, the normalization of oral mucosa epithelium in the lesions area of patients treated with autochemotherapy proceeded in a shorter time, as evidenced by the data obtained by dynamic cytology, as well as measurement values of the lesions area in patients with erosive and ulcerative form.

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