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PECULIARITIES OF PORCINE PERIODONTIUM IN NORMAL CONDITION AND IN GENERALIZED PERIODONTITIS DURING DENTAL RESTORATION OF VARIOUS TYPES

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The paper was aimed at determining the structural features of porcine gums in normal conditions and experimental generalized periodontitis in the presence of adhesive bridges and dental implants. It is established, that impaired differentiation in the form of dyskeratosis occurs in the epithelium of the gingival mucosa of pigs with generalized periodontitis; it is manifested by the appearance of basal cells in the cytograms that are absent in the control group of animals, and 4-fold increase in the number of parabasal epitheliocytes. Histological study of the mucous membrane of the epithelial plate revealed vacuolar dystrophy of the cells in the spinous layer and local phenomena of spongiosis. In their lamina propria, disorders of microcirculation and connective tissue edema were noted. Changes in the lamina propria during dental restoration of different types were common. In the epithelium, during restoration with dental implant, the changes corresponded to the control group: vacuole dystrophy and local spongiosis. Marked hyperkeratosis in the epithelial plate was noted in restoration with bridge denture.

Key words: experimental generalized periodontitis, adhesive bridge-like structures, dental implants, cytograms, gums, pigs.

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Currently, periodontal diseases are referred to the most prevalent conditions. [6, 9, 11, 12]. The most common is generalized periodontitis, ranking first among all dental diseases at the age of 40 and above. [1, 2, 5].

Publications report, that prolonged inflammatory process in periodontium causes disturbances in keratinization of the gingival epithelium, manifested by the altered cell ratio. The above indicates about strong correlation between the cellular and humoral immunity of periodontal pockets, primarily due to polymorphonuclear leukocytes and macrophages. These cells play a leading role in inflammatory responses and in protecting the body from the effect of foreign factors, including bacteria and their toxins [7, 8, 10].

The main symptoms of generalized periodontitis are the presence of symptomatic gingivitis, periodontal pockets, traumatic occlusion, and progressive resorption of bone tissues of the alveolar process. All this in turn leads to the loss of teeth and dentition defects. The restoration of the dentition in the presence of the bounded edentulous space can be done using the adhesive bridge and dental implantation method.

The purpose of the work was to determine the structural features of porcine gums in normal conditions and in experimental generalized periodontitis in the presence of adhesive bridges and dental implants.

Materials and methods. The study was carried out on 10 castrated male pigs of the Ukrainian Large White breed, weighing 70±4,5 kg, aged 6±1 month old, kept individually on the conventional system of breeding at the Institute of Pig Breeding and Agricultural Production of the National Academy of Agrarian Sciences of Ukraine.

The experimental study was performed in several stages. At the first stage generalized periodontitis was simulated by destruction of the dentogingival junction using micro-abrasor and insertion of crushed tartar into the created periodontal pocket with its subsequent closure with liquid composite material. Following three weeks the development of generalized periodontitis in pigs was verified.

Before modeling generalized periodontitis and following three weeks thereafter two smears per animal were collected for cytological examination. The samples were obtained by the scraping from the mucous membrane of the gums of the lower and upper jaws using a sickle burnisher.

Subsequently, the collected material was applied to a sterile slide. Drying of the smears was performed by the method of dry fixation at room temperature with open air access. The smears were stained using May-Grünwald method [10]. Cytograms were analyzed using the Biorex-3 BM-500T microscope equipped with DCM-900 digital microphoto attachment with software adapted for the studies in 1000×magnification. Quantitative parameters were determined by counting cellular elements in 5 FOV [10]; the amount was recorded in absolute figures and mean values were calculated in Exel software [3]. The latter were used to determine the ratio of different classes of epithelial cells to establish the reference values.

At the next stage, following three weeks, tooth extraction was performed in a pig with simultaneous replacing the missing tooth with *Alpha dent active* dental implant. Following 3 months, the animals were sedated with ketamine (5 mg / kg intramuscularly). The mucous membrane was separated from the extracted fragments of jaws, where edentulous space was restored with dental implant or adhesive bridge,

as well as from the portion of jaw with preserved dentition, and placed in 10% buffered formalin for 24 hours. The material was embedded in paraffin according to the conventional technique and sections of 5 μm thick were made and stained with hematoxylin and eosin.

All animals had no clinical signs of infection or any other oral diseases. Experimental manipulations have been made in compliance with the requirements of international principals of the “European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes” (Strasbourg, 1986), the “General Ethic Rules for Conducting Experiments on Animals”, adopted by the I National Congress on Bioethics [4] and the requirements of the “Procedure for conducting tests, experiments on animals by research institutions” (2012).

The study and imaging of the sections was made on the Biorex–3 BM–500T microscope equipped with DCM-900 digital microphoto attachment with software adapted for the studies in 400×magnification.

Results of the study and their discussion. The study of the scrape smears of mucous membranes of porcine gums in experimental generalized periodontitis has found that cells of the intermediate layer prevailed in the cytograms, similar to the control group of animals, though their average number in the field of view significantly decreased by 29.25% compared to controls (table).

Table

Changes of the ratio of different classes of cells of stratified squamous epithelium of porcine gums in generalized periodontitis

	Types of cells in the cytograms				
	basal	parabasal	intermediate	superficial	horny scales
Control	0	3.43	6.24	89.55	0.78
Generalized periodontitis	2.10*	12.41*	24.63*	60.30*	0.56

The number of intermediate cells increased by 18.39% and was by 4 times significantly higher than in the control group. The average number of parabasal cells also increased by 8.98% (Table). On the part of the horny scales a decrease by 0.22% was established. In contrast to cytograms in the control group of animals with generalized periodontitis, we found basal cells, the average number of which was 2.10%.

The established changes in the ratio of the epithelial cells in the porcine gums indicate a disorder of the processes of differentiation of the epithelium in the form of dyskeratosis due to inflammatory changes of the mucous membrane of the gums caused by experimental generalized periodontitis. The increase in number of parabasal cells reflects an increase in mitotic activity in the epithelial plate as the manifestation of the activation of adaptive mechanisms. The appearance of basal epitheliocytes indicates impaired epithelial barrier function due to hyperhydration and inflammatory changes in the lamina propria.

Cytological study showed that the basal cells had a rounded shape and small size, compared to other elements of the epithelial diferon. Their cytoplasm was basophilic, lumpy condensed chromatin was clearly visualized in the nuclei. The nuclear to cytoplasmic ratio was high.

A significant number of microorganisms, detected in the cytograms, led to the development of necrobiosis of epitheliocytes. Coccal flora and sporadic filaments of fungi were mainly detected in scrape smears (fig. 1a).

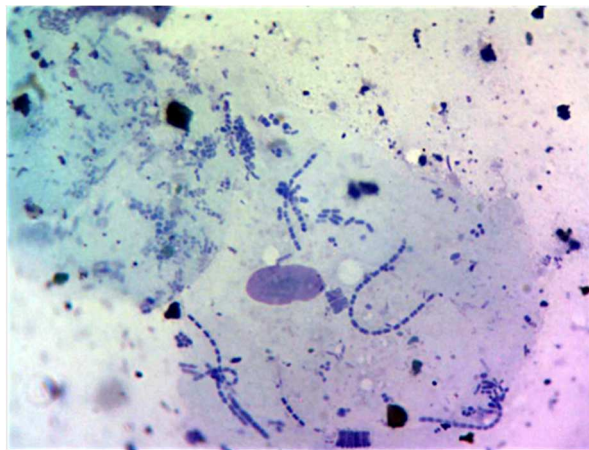


Fig. 1a. Contamination of microorganisms on superficial epitheliocytes of porcine gums in generalized periodontitis. May-Grünwald stain. Lens: 100×magnification, ocular lens: 10× magnification.

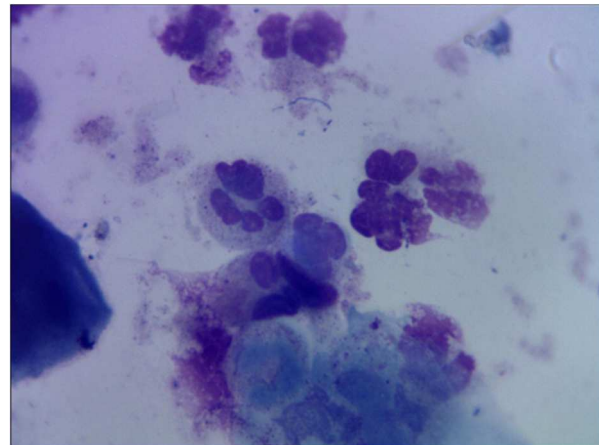


Fig. 1b. Segmentonuclear neutrophils in the cytograms of the porcine gingival mucosa in generalized periodontitis. May-Grünwald stain. Lens: 100×magnification, ocular lens: 10× magnification.

Coccal flora contaminated on segmentonuclear leukocytes. Thus, the activity of phagocytosis of segmentonuclear neutrophils increased, which led to incomplete phagocytosis and the formation of pyocytes (fig. 1b).

Epitheliocytes with signs of irritation were found in the cytograms of the gingival mucosa of pigs with generalized periodontitis. By morphological features, they referred to the cells of the intermediate layer. In this case, enlargement of the cells was observed; vacuoles filled with optically clear content were detected in the cytoplasm (fig. 2a).



Fig. 2a. Intermediate epitheliocyte of porcine gingival mucosa in generalized periodontitis. May-Grünwald stain. Lens: 100×magnification, ocular lens: 10×magnification.

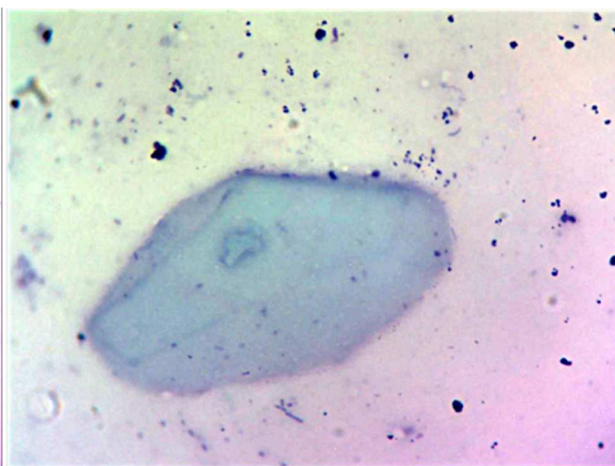


Fig. 2b. Karyopyknosis of the nucleus of the superficial epitheliocyte of the porcine gingival mucosa in generalized periodontitis. May-Grünwald stain. Lens: 100×magnification, ocular lens: 10×magnification.

In some epithelial cells the nuclei were eccentric, shrink, being at the initial stages of karyorhexis (fig. 2b). These changes are the manifestation of the metabolic disorders of cells, resulted from inflammatory changes caused by inflammatory generalized periodontitis.

The analysis of the cytograms has established that epithelial cells of the gums were exposed to higher destruction, accompanied by karyopyknosis and karyorhexis of the nucleus as well as homogenization of the cytoplasm.

The number of horny scales decreased compared to intact cytograms. They had a polygonal shape and were sporadic.

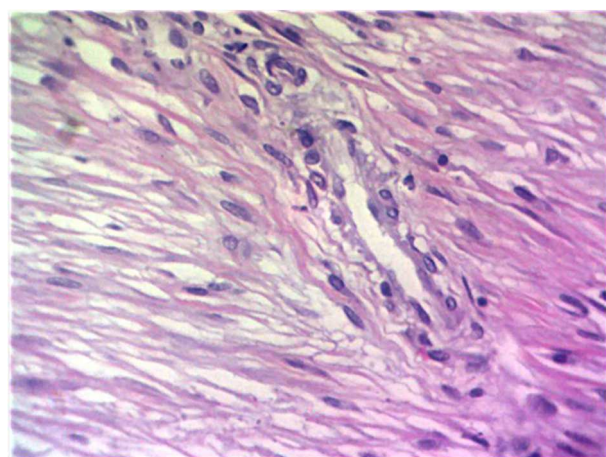


Fig. 3a. Arteriola in the lamina propria of the porcine gingival mucosa in generalized periodontitis. H&E stain. Lens: 40×magnification; ocular lens: 10×magnification.

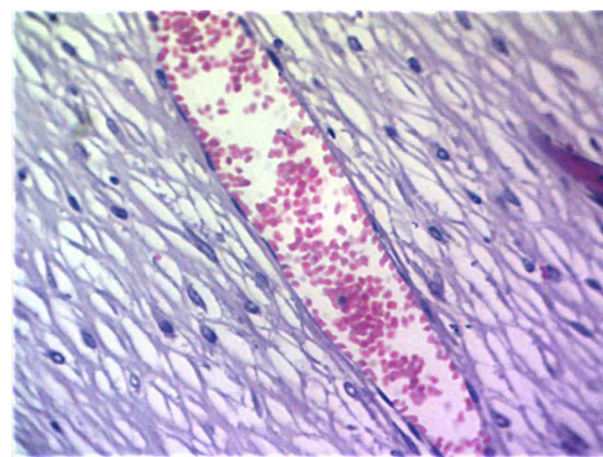


Fig. 3b. Venula in the lamina propria of the porcine gingival mucosa in generalized periodontitis. H&E stain. Lens: 40×magnification; ocular lens: 10×magnification.

The analysis of histological sections of the gingival mucosa of gums of pigs with generalized periodontitis, when the edentulous space was restored by dental implant, has found the impaired epithelial differentiation in the epithelial plate in the form of dyskeratosis. In the spinous layer epitheliocytes showed signs of vacuolar dystrophy, manifested by their enlargement, presence of numerous vacuoles in the cytoplasm, and shrink nuclei. The phenomena of spongiosis were visualized locally. The basement membrane was thickened. The above changes of the epithelial plate were caused by the disorders of

microcirculation with its subsequent reduction and the phenomena of hypoxia. Perivascular edema was developed due to dyscirculatory disorders and increased vascular-tissue permeability. The arteries were spasmodic; no blood corpuscles were detected in the lumina (Fig. 3a). The lumina of venules were enlarged and filled with red blood cells. Adhesion of erythrocytes and sludge syndrome was established (Fig. 3b). Collagen fibers of lamina propria were stratified by edema fluid; lysis and destruction of fibrous structures, as well as diffuse leukocytic infiltrations were locally visualized.

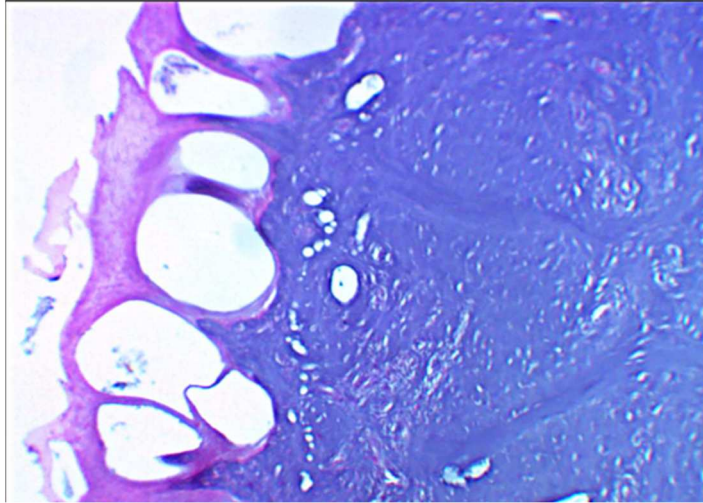


Fig. 4. Epithelium of the porcine gingival mucosa in generalized periodontitis. H&E stain. Lens: 10×magnification; ocular lens: 10×magnification.

The study of the peculiarities of the structural organization of the mucous membrane of the gums of pigs after restoration with adhesive bridge construction has found that changes in the lamina propria were similar to restoration with dental implant. However, marked hyperkeratosis was noted in the epithelial plate. The horny layer was significantly thickened. The basal, spinous and granular layers were thinned and pressed to the basement membrane. Between the above layers and the horny layer, balloon-shaped hollow spaces were identified, which were joined by thin strands of the horny scales (fig. 4).

The established changes in the cellular composition of cytograms in generalized periodontitis are caused by the intensity of inflammatory-dystrophic processes in periodontal tissues. The obtained digital data are significantly different from the ratio of epithelial cells of the stratified squamous epithelium of the gums in pigs of the control group and correlate with the data of other researchers in the study of cytograms of patients with periodontitis [10]. Desquamation disorders of the epitheliocytes, which provide barrier function of the gums are caused by impaired blood microcirculation. The appearance of basal and parabasal cells in the cytograms can be explained by the activation of adaptive mechanisms due to enhanced mitotic activity of the gingival epitheliocytes. Thus, chronic inflammation in the periodontium is the entry of infection that can infect not only the tissues of the maxillofacial area, but also lead to infection of the whole organism, contributing to the formation of foci of chronic infection and intoxication in organs of different systems [2].

Conclusion

Impaired differentiation in the form of dyskeratosis occurs in the epithelium of the gingival mucosa of pigs with generalized periodontitis; it is manifested by the appearance of basal cells in the cytograms that are absent in the control group of animals, and 4-fold increase in the number of parabasal epitheliocytes. Histological study of the mucous membrane of the epithelial plate revealed vacuolar dystrophy of the cells in the spinous layer and local phenomena of spongiosis. In their lamina propria, disorders of microcirculation and connective tissue edema were noted. Changes in the lamina propria during dental restoration of different types were common. In the epithelium, during restoration with dental implant, the changes corresponded to the control group: vacuole dystrophy and local spongiosis. Marked hyperkeratosis in the epithelial plate was noted in restoration with bridge denture.

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Реферати

ОСОБЛИВОСТІ ПАРОДОНТУ СВИНЕЙ В НОРМІ ТА ПРИ ГЕНЕРАЛІЗОВАНОМУ ПАРОДОНТИТІ ЗА УМОВ ВІДНОВЛЕННЯ ВКЛЮЧЕНИХ ДЕФЕКТІВ ЗУБНИХ РЯДІВ РІЗНИМИ СПОСОБАМИ

Попович І.Ю., Петрушанко Т.О., Єрошенко Г.А.

Метою роботи було встановити структурні особливості ясен свиней в нормі та при експериментальному генералізованому пародонтиті за умов наявності адгезивних мостоподібних конструкцій та дентальних імплантатів. Встановлено, що при генералізованому пародонтиті в епітелії слизової оболонки ясен свиней визначається порушення диференціювання у вигляді дискератозу, яке проявляється появою базальних клітин в цитограмах, які в контрольній групі тварин відсутні, та збільшенням в 4 рази кількості парабазальних епітеліоцитів. При гістологічному дослідженні слизової оболонки в епітеліальній пластинці виявлена вакуольна дистрофія клітин шипуватого шару і локальні явища спонгіозу. У власній пластинці визначались розлади мікроциркуляції на набряк сполучної тканини. При відновленні дефектів зубних рядів різними способами зміни у власній пластинці були стереотипними. В епітелії, за умов відновлення дефекту зубного ряду за допомогою дентального імпланту, зміни відповідали контрольній групі – вакуольна дистрофія і локальний спонгіоз. При відновлення дефекту зубного ряду за допомогою мостоподібною конструкції в епітеліальній пластинці встановлений виражений гіперкератоз.

Ключові слова: експериментальний генералізований пародонтит, адгезивні мостоподібні конструкції, дентальні імплантати, цитограми, ясна, свині.

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ОСОБЕННОСТИ ПАРОДОНТА СВИНЕЙ В НОРМЕ И ПРИ ГЕНЕРАЛИЗОВАННОМ ПАРОДОНТИТЕ ПРИ УСЛОВИИ ВОССТАНОВЛЕНИЯ ВКЛЮЧЕННЫХ ДЕФЕКТОВ ЗУБНЫХ РЯДОВ РАЗНЫМИ СПОСОБАМИ

Попович И.Ю., Петрушанко Т.А., Ерошенко Г.А.

Целью работы было установить структурные особенности десен свиней в норме и при экспериментальном генерализованном пародонтите при наличии адгезивных мостовидных конструкций и дентальных имплантатов. Установлено, что при генерализованном пародонтите в эпителии слизистой оболочки десны свиней определяется нарушение дифференцировки в виде дискератоза, которое проявляется появлением базальных клеток в цитограммах, которые в контрольной группе животных отсутствуют, и увеличением в 4 раза количества парабазальных эпителиоцитов. При гистологическом исследовании слизистой оболочки в эпителиальной пластинке обнаружена вакуольная дистрофия клеток шиповатого слоя и локальные явления спонгиоза. В собственной пластинке определялись расстройства микроциркуляции на отек соединительной ткани. При восстановлении дефектов зубных рядов различными способами изменения в собственной пластинке были стереотипными. В эпителии, в условиях восстановления дефекта зубного ряда с помощью дентального импланта, изменения соответствовали контрольной группе – вакуольная дистрофия и локальный спонгиоз. При восстановлении дефекта зубного ряда с помощью мостовидных конструкций в эпителиальной пластинке выявлен выраженный гиперкератоз.

Ключевые слова: экспериментальный генерализованный пародонтит, адгезивные мостовидные конструкции, дентальные имплантаты, цитограммы, десна, свиньи.

Рецензент Старченко И.И.