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IMMUNOHISTOCHEMICAL ANALYSIS OF THE PROLIFERATIVE ACTIVITY OF KI-67 IN LICHEN PLANUS OF THE ORAL MUCOSA

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Lichen planus is a chronic inflammatory disease of the skin and mucous membrane of the oral cavity (SOPR), characterized by the appearance of papules on the skin and mucous membrane of the oral cavity and accompanied by itching. In the general structure of dermatological morbidity, the lesion of the skin is 2.5%, and the oral mucosa is 35%. [2,3,4,13]. Morphological elements of CPL can sometimes precede the appearance of skin rashes or may remain the only sign of the disease. When the SOPR is affected, 62-67% of patients are women aged 40-60 years. Modern literature sources [1,15,18] indicate the significant role of various general and local factors in the etiology of lichen planus: anxiety, stress and depressive disorders, medications, human papillomavirus, Epstein—Barr, human herpes, human immunodeficiency virus (HIV), hepatitis B and C, microorganisms (Helicobarter pylori, Candida albicans), periodontal pathogens [5,6,8,19]. Local adverse factors of the oral cavity, such as poor oral hygiene, hyposalivation, and dental row defects, play a major role in the pathogenesis of CPL and the aggravation of its course. CPL is a multifactorial polyethological disease, in the development of which immunological, neuroendocrine, intoxication and metabolic factors are important [7,20,14,17]. There are several theories of the development of CPL: viral (infectious), hereditary, immunological, neurogenic, endocrine and metabolic disorders, traumatic, autoimmune, medications, and others.

According to the international classification of dental diseases ICD 10, the following are distinguished: L43 - Lichen planus erythematosus; L43.1 - Lichen planus bullosa; L43.1X - Manifestations in the oral cavity; L43.2 - Lichenoid reaction to the drug; L42.2X - Manifestations in the oral cavity; L43.8 - Other lichen planus; L43.80 - Papular lichen planus in the oral cavity; L43.81 - Reticular lichen planus in the oral cavity; L43.82 - Atrophic and erosive lichen planus in the oral cavity; L43.83 - Manifestations of lichen planus (typical plaques in the oral cavity); L43.9 – Lichen planus, unspecified.

The classification of CPL according to N.F.Danilevsky and L.I.Urbanovich (1979) is based on parallels between the clinic and the data of stomatoscopic,

luminescent, cytological, histological, and histochemical studies. The authors distinguish five forms of CPL on the SOPR and the red border of the lips: hyperkeratous (typical); warty; erosive; pemphigoid; ulcerative. Some clinical forms of CPL are facultative precancers and can become malignant. In this regard, for

The immunohistochemical method using the Ki67 marker is used to assess the proliferation of epithelial cells of the oral mucosa and predict the course of various forms of CPL. [9,10,11,12,16].

The purpose of the research was to evaluate cell proliferation by analyzing Ki67 expression in 40 cases of lichen planus of the oral mucosa.

Research materials and methods. 40 paraffin blocks of patients with CPL were used and examined for Ki67 expression using immunohistochemical staining. The control consisted of 18 healthy individuals with normal and healthy oral mucosa. After careful examination of the tissue samples, the number of positive cells was calculated to assess the expression pattern. The data obtained were statistically analyzed ($P<0.05$).

The study involved 122 patients with CPL, including 34 men (27.83%) and 88 women (72.1%) aged 20-29 years, 30-39 years, 40-49 years, 50-59 years, 60 years and over. Of the total number of patients, 42 patients were diagnosed with ulcerative form, including 6 men (14.28%), 36 women (85.71%); 24 patients had hyperkeratous form, 4 men (16.66%), 20 women (83.33%); there were 19 patients with erosive form, 9 men (47.36%), 10 women (52.63%); 15 with warty form, 8 men (53.33%), 7 women (46.66%), 22 patients had pemphigoid form, 7 men (31.81%), 15 women (68.18%).

In this scientific work, we conducted studies of 40 histological samples obtained from the tissues of patients affected by CPL SOPR. Of the total number of samples, there were 8 in each of the five clinical forms of CPL: typical - reticular (reticular), warty (verrucosus), erosive (erosive), pemygoid (bullosus) and ulcerative (ulcerative). Each of these forms is characterized by specific clinical manifestations and differs in varying degrees of severity and activity of the inflammatory process.

The results obtained were classified as follows:

- "+" <10% — low proliferative activity,
- "++" 10-20% — moderate proliferative activity,
- "+++" >20% — high proliferative activity.

The process of conducting an immunohistochemical (IHC) study includes several successive stages, each of which is important for qualitative analysis and obtaining reliable results. A histological examination was performed to determine the likelihood of dysplasia and the development of malignant transformation.

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To do this, after local infiltration anesthesia with a scalpel, a tissue sample with a thickness of 4 microns was taken, placed on polylized slides, at the stage of dewaxing, the sections were melted with paraffin in a thermostat at a temperature of 55-60 ° C for 60 minutes, dehydration was carried out using 960 ethanol. The sections are washed with distilled water for 3 minutes, then incubated with primary antibodies to the molecular marker with sections, binding to the antigen. Tris buffer washing was carried out for 5 minutes, DAB was used as a chromogen. The immunohistochemical analysis was performed using an automated Bond immunohistocessor from Leica (Australia), which ensured high accuracy and reproducibility of the data obtained. The research materials were subjected to statistical processing using methods of parametric and nonparametric analysis. The accumulation, correction, systematization of the initial information and visualization of the results were carried out in Microsoft Office Excel 2016 spreadsheets. Statistical analysis was performed using the IBM SPSS Statistics v.26 software (developed by IBM Corporation).

The results obtained and the discussion.

The average age of the patients was 54.3±1.3 years. The duration of CPL is from 3 months to 9 years (on average 2.18± 0.47 years). The number of patients under one year was 28.8%, the number of patients aged 1 to 9 years was 71.2%, which is 2.5 times more. The distribution of patients in the main group was 122. The control group consisted of 18 healthy individuals

Table 1

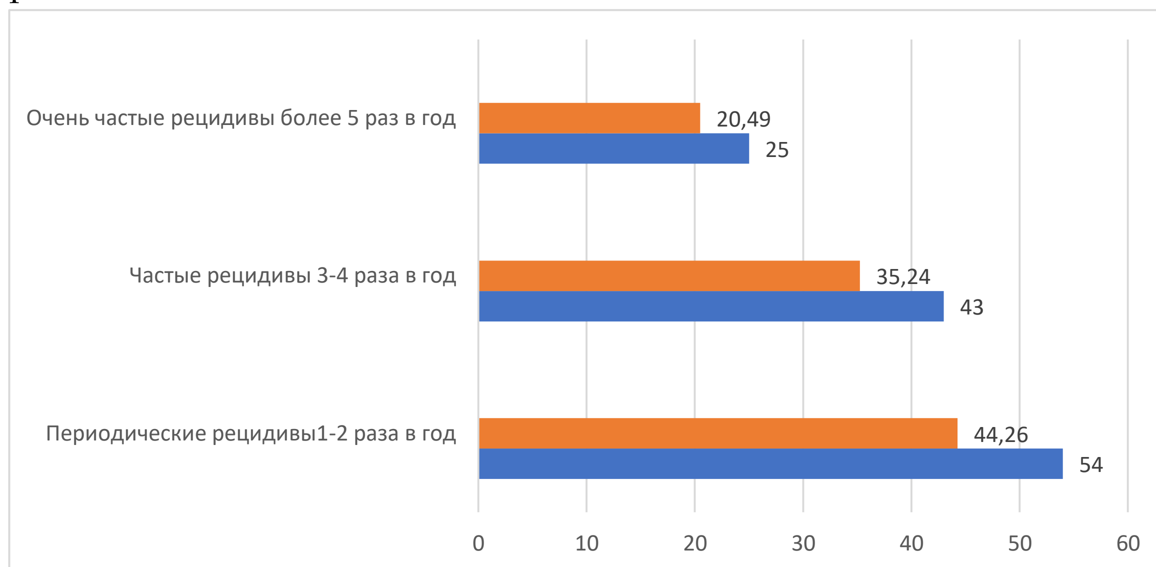
Localization of lesion elements in patients with CPL

Localization	Формы КПЛ										chi squared	
	Hyperkeratotic, n=24		Warty, n=15		erosive, n=19		Pemphigoid, n=22		ulcerous, n=42			
	abs	M±m,%	abs	M±m,%	abs	M±m,%	abs	M±m,%	abs	M±m,%	χ^2	P
cheeks	12	50±10,21	2	13,33±8,78	8	42,11±11,33	10	45,45±10,62	14	33,33±7,27	9,217	0,056
Retromolar region	8	33,33±9,62	2	13,33±8,78	8	42,11±11,33	10	45,45±10,62	16	38,1±7,49	11,455	0,022
Tongue	2	8,33±5,64	9	60±12,65	2	10,53±7,04	1	4,55±4,44	8	19,05±6,06	13,000	0,011
vermilion border of the lip	2	8,33±5,64	2	13,33±8,78	1	5,26±5,12	1	4,55±4,44	4	9,52±4,53	3,000	0,558
P	χ^2= 12,000; p = 0,007		χ^2= 9,800; p = 0,020		χ^2= 9,000; p = 0,029		χ^2= 14,727a; p = 0,002		χ^2= 8,667; p = 0,034			
P	χ^2 Pearson's = 26,833; p = 0,008											

As can be seen from Table 1, the localization of the elements of the lesion of the SOPR demonstrated that the cheeks are more often affected. Thus, in 46 (37.7%) patients with CPF, the mucous membrane of the cheek (18.03%) is affected (χ^2=9.217; p=0.056); the retromolar region is affected in 44 (36,0%) (χ^2=11,455; p=0.022). The tongue is affected in 22 (18.03%) patients (χ^2=13.00; p=0.011); the red border of the lips is affected in 10 (8,19%) (χ^2 ==3,000; p=0.558) of patients with CPR.

As a result of the study of anamnestic and clinical data, the following variants of the course of CPL were identified (Pic.1):

1. Periodic relapses (1-2 times a year) were observed in 54 (44.26%);
2. Frequent relapses (3-4 times a year) were observed in 43 patients (35.24%);
3. Very frequent relapses (more than 5 times a year) were observed in 25 patients (20.49%).



Pic. 1. Recurrence rate of CPL

Table 2.

Clinical forms of CPL SOPR

CPL Forms	Gender				Chi-square	
	Male		Female			
	abs	M±m,%	abs	M±m,%	χ^2	P
Hyperkeratotic	4	16,67±7,61	20	83,33±7,61	10,667	0,001
Warty	8	53,33±12,88	7	46,67±12,88	0,067	0,796
erosive	9	47,37±11,45	10	52,63±11,45	0,053	0,819
Pemphigoid	7	31,82±9,93	15	68,18±9,93	2,909	0,088
ulcerous	6	14,29±5,4	36	85,71±5,4	21,429	0,000
P	χ^2=2,176; p = 0,703		χ^2=29,614; p=0,000			
P	χ^2 Pearson's = 13,956; p = 0,007					

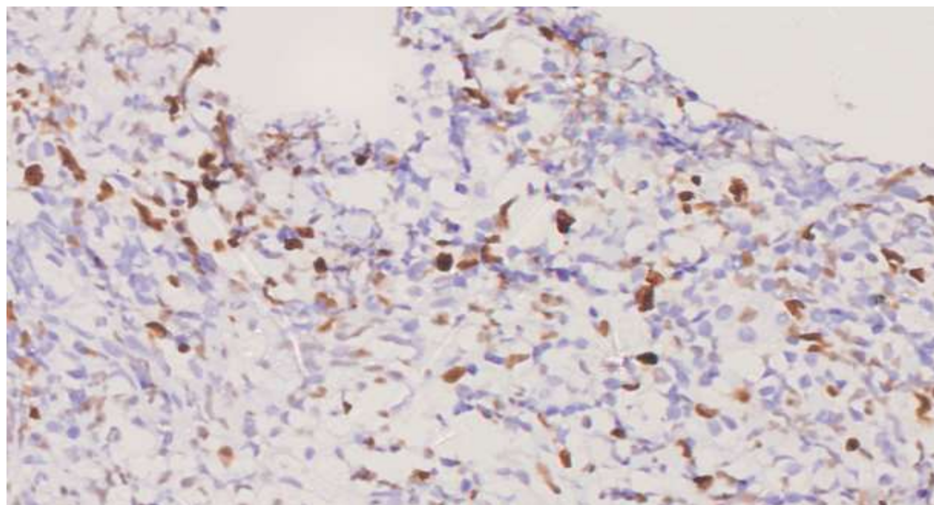
In total:	34	27,87±4,06	88	72,13±4,06		
P	χ^2 = 23,9026; p = 0,000					

Among the patients with CPL studied in this study, the most common form is the ulcerative form. 42 (34,42%) (χ^2=21,429; P=0.000); there were 24 patients with a typical form (29,67%) (χ^2=10,667; P=0.001). The warty form of CPL occurred in 15 patients (12,29%) (χ^2=0,067; P=0.796); erosive form was observed in 19 patients (15,57%) (χ^2=0,053; P=0.819); the pemphigoid form occurred in 22 patients (18,03%) (χ^2=2,909; P=0.088) (Table 2). The WHO criteria (2017) were used to determine edema, while some of the studied material showed signs of dysplasia. Dysplasia was detected in 63.3% of cases with pemphigoid, in 20% with ulcerative, in 10% with erosive, and in 6.7% with warty CPL. Interestingly, dysplastic changes in the form of mild changes in up to two thirds of the epithelium were detected among the studied patients with CPL.

Results of immunohistochemical studies

Mucosal samples showed a positive brown reaction to Ki67. This is a nuclear reaction in the basal cells of the integumentary epithelium and inflammatory cells of the lymphocytic group. In the normal, i.e. healthy oral mucosa, the reaction in the surface epithelium was detected only in the basal and suprabasal layers (Pic. 2).

Pic.2. Ki67 expression is the hyperkeratous (typical) form of Ki67. Staining: immunohistochemistry using DAB chromogen. Ki67 receptor: moderate expression. Scale: 10×, increased to 400×.



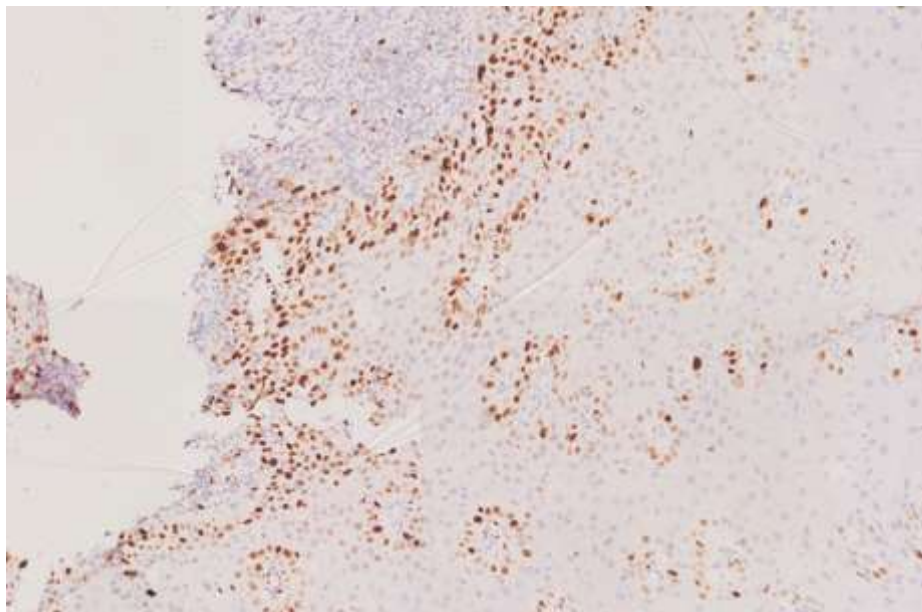
The expression of Ki67, a marker of cell proliferation in the typical form of CDL, was moderately increased in the basal and parabasal layers of the epithelium (approximately 20-30% of positive cells). This indicator indicates the presence of moderate cell hyperplasia, which is characteristic of the hyperkeratous form of the

disease. With relatively stable inflammation, it indicates a progressive, but not aggressive process.

The immunohistochemical features of the warty form of CPL play an important role in clarifying the diagnosis of the disease mechanism, as they allow us to investigate molecular aspects that are not always visible in traditional histological analysis. In particular, the antibodies used in immunohistochemical reactions, in particular Ki67, help to analyze the most important aspects of the pathogenesis of the warty form of KPL. With increased cell proliferation or abnormal maturation of the epithelium, the external structure of the oral epithelium is disrupted in the form of edema or dysplastic manifestations of mild, moderate and severe degrees.

The immunohistochemical features of the warty form of CPL play an important role in clarifying the diagnosis of the disease mechanism, as they allow us to investigate molecular aspects that are not always visible in traditional histological analysis. In particular, antibodies used in immunohistochemical reactions, such as Ki67, help to analyze the most important aspects of the pathogenesis of the warty form of KPL.

Pic. 3. Warty shape of the CPL. Ki67 expression. Staining: immunohistochemistry using DAB chromogen. Ki67 receptor: moderate



expression. Scale: 10×, increased to 40×.

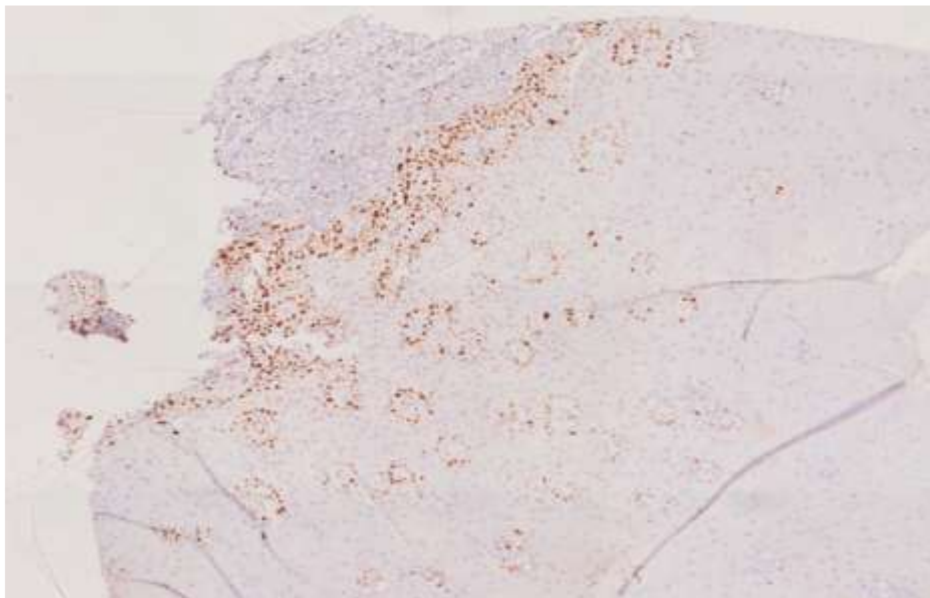
The expression of Ki67, a marker of cell proliferation in the warty form of KPL, is usually moderate (approximately 30-40% of positive cells), however, active cell proliferation in the basal and parabasal layers is observed in the epithelium of the mucous membrane. This may be due to attempts by the epithelium to compensate for cell loss and repair damaged tissue, which leads to the formation of

hypertrophied papillomatous rashes. At the same time, a moderate increase in Ki67 expression indicates regulated cell proliferation, which distinguishes the warty form from more aggressive forms of the disease with severe hyperplasia, for example, ulcerative form (Pic.3).

The erosive form of lichen planus is manifested in the form of erosions, covered with plaque and easily bleeds, often accompanied by a pronounced inflammatory reaction and destruction of the surface layers of the epithelium, which makes it one of the most severe forms of CDL. These changes most often occur on the mucous membrane of the cheeks, tongue, gums and lips, causing pain, especially when eating or talking. Erosions may be limited or spread to significant areas of the mucous membrane.

Pic. 4. Erosive form of CPL. Ki67 expression. Staining: immunohistochemistry using DAB chromogen. Ki67 receptor: low expression. Scale: 10×, increased to 40×.

The expression of Ki67, a marker of cell proliferation, was significantly

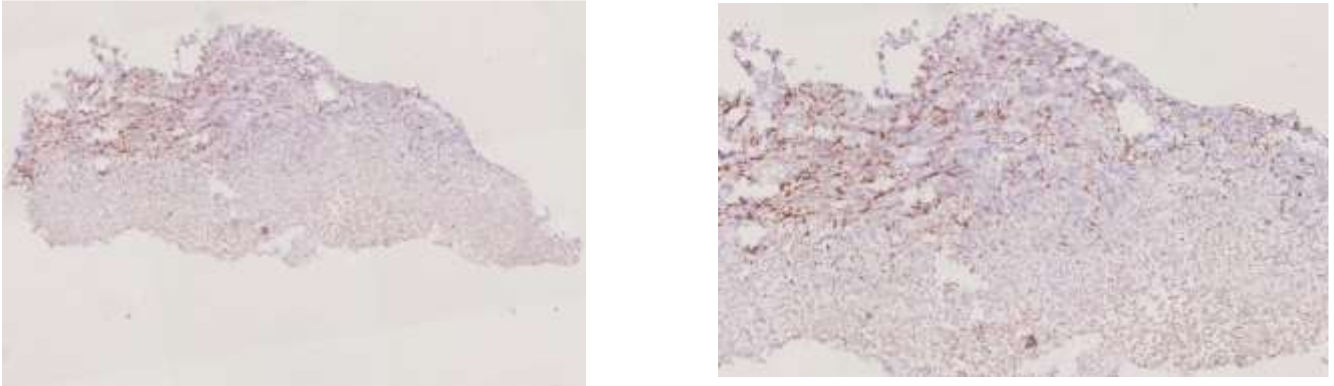


increased in the basal and parabasal layers of the epithelium (approximately 50-65% of positive cells). This indicator confirms a high degree of cell proliferation, which is associated with the body's attempts to repair damaged areas of the epithelium, but the inflammatory process continues to destroy tissues, leading to the formation of erosions (Fig.4).

Thus, the erosive form of lichen planus is characterized by pronounced immune activation, high cell proliferation, and impaired apoptosis. These data confirm that the erosive form is more aggressive and severe than the hyperkeratous form, which requires more intensive treatment and monitoring.

The pemphigoid form of lichen planus is manifested by the formation of large bubbles on the mucous membranes, which can be filled with liquid and located both on the surface of the mucous membrane and in deeper layers of the epithelium. After their rupture, painful erosions and ulcers occur, which is accompanied by acute pain, especially when eating and talking, and reduce the quality of life of patients.

Pic. 5. The pemphigoid shape of Ki67. Staining: immunohistochemistry using DAB chromogen. Ki67 receptor: high expression. Scale: 10×, increased to 40×.



In the pemphigoid form of lichen planus, inflammation affects not only the epithelial layers, but also the subepithelial tissues, which leads to the formation of blisters. These changes are the result of an autoimmune process in which antibodies attack connective tissue and disrupt the integrity of the epithelium and contribute to the formation of blisters. This disease can progress to more severe forms, such as erosive or ulcerative form. The expression of Ki67, a marker of cell proliferation, was significantly increased in the basal and parabasal layers of the epithelium (approximately 60-75% of positive cells). This indicator indicates a high activity of cell proliferation, which contributes to the formation of blisters and erosions, which is characteristic of the pemphigoid form of CPL (Pic.5).

The ulcerative form of CPL can occur on the mucous membrane of the cheek, the lateral surface of the tongue. At the same time, ulcers are noted, the surface of which is covered with a fibrinous film, the edges are uneven, painful on palpation, and moderate infiltration is noted around the ulcer. The ulcer is located around the focus of epithelial dysplasia. The edges of this ulcer can be uneven, very painful on palpation, and there

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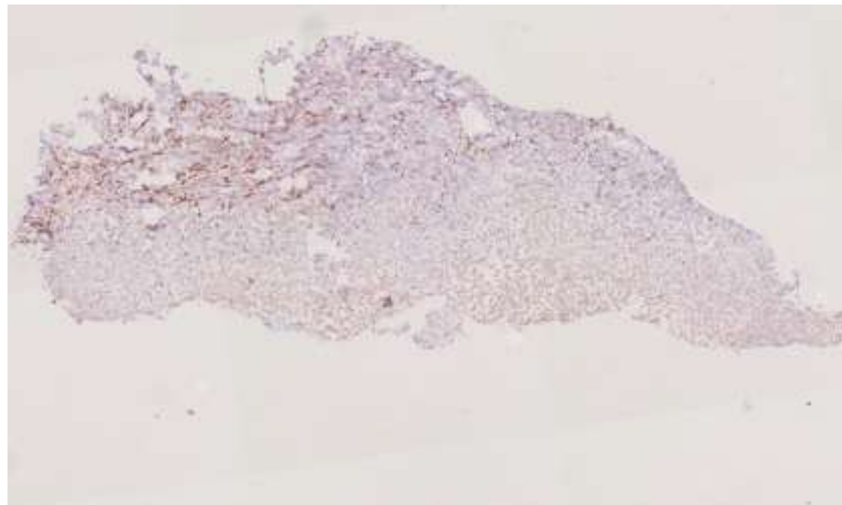
is moderate infiltration around the ulcer (Pic. 6).



Pic. 6. Ulcerative colitis. Ki67 expression. Staining: immunohistochemistry using DAB chromogen. Ki67 receptor: high expression. Scale: 10×, increased to 40×.

The ulcerative form of CPL is the most difficult to treat, and there are cases of this form becoming malignant. Constant irritation of tissues contributes to disruption of the normal regulation of cell division. The expression level is 59-70% of positive cells. There is a high proliferative activity, an attempt to repair damaged tissues against the background of inflammation may indicate insufficient regeneration and maintenance of the ulcerative defect. In response to chronic inflammation, cells begin to divide faster, which increases the likelihood of errors in the division process and mutations and can lead to malignant growth. This is facilitated by chronic inflammatory processes of the epithelium, which can contribute to prolonged cell mutation due to exposure to local trigger factors (dentures, smoking, alcohol, galvanosis), genetic mutations of cells, cellular dysplasia, poor oral hygiene and HPV can contribute to carcinogenesis (Pic.6). Table 3

The expression level of Ki67 of different forms of CPL is high

CPL Forms	Ki67
Hyperkeratous form	25±1,02
Warty form	35±1,33*
Erosive form	$57{,}5\pm1{,}9^{*\Delta}$
The pemphigoid form	$67{,}5\pm2{,}58^{*\Delta xx}$
Ulcerative form	$60\pm2{,}26^{*\Delta ooo}$

Note: * - the reliability of differences in the arithmetic mean values in relation to "Hyperkeratosis" was noted (*** - P<0,05; ** - P<0,01; * - P<0,001); $^{\Delta}$ in relation to «Warty» ($^{\Delta\Delta\Delta}$ - P<0,05; $^{\Delta\Delta}$ - P<0,01; $^{\Delta}$ - P<0,001); ^{x} – $^{\Delta}$ in relation to «Erosive» (^{xxx} - P<0,05; ^{xx} - P<0,01; ^{x} - P<0,001); ^{o} – $^{\Delta}$ in relation

to «Pemphigoid» ($^{\circ\circ\circ}$ - P<0,05; $^{\circ\circ}$ - P<0,01; $^{\circ}$ - P<0,001).

The level of Ki67 expression in various forms of CPL (Table 3) was significantly 25±1.02 in the hyperkeratous (typical) form; 35±1.33 in the warty form (P<0.001); 57.5±1.9 in the erosive form (P<0.001); 67.5±2.58 in the pemphigoid form (P<0.001); 60±2.26 (P<0.001).

Thus, of all 5 forms of lichen planus, the erosive, pemphigoid, and ulcerative forms of COPD are accompanied by increased cell proliferation and enhanced support for cellular survival. These data confirm a more aggressive course of the listed forms of CPL, compared with other forms, and can lead to malignancy and malignancy. The highest degree of Ki67 expression was determined in erosive, ulcerative, and pemphigoid forms of CPL. This is consistent with the data of other authors: Gholizadeh N. et al.,2016 reveals severe degrees of edema with high expression of the Ki67 marker in the basal and parabasal layers of the epithelium [10]; Kitayoshi M. et al.,2023 associate high expression of Ki67 with an increase in the degree of dysplasia [14].

Conclusions. Increased expression of the Ki67 marker may indicate increased proliferation in patients with erosive, ulcerative, and pemphigoid forms of CPL, which is apparently associated with epithelial edema and can be used as a marker to predict the degree of epithelial dysplasia and an increased risk of developing malignant degeneration.

LITERATURE:

1. Грабовская О.В., Каюмова Л.Н., Дамдинова Б.Ш., Шахманова С.И. Проблема диагностики красного плоского лишая при поражении слизистой оболочки полости рта//Российский журнал кожных и венерических болезней. – 2022. – Т. 25. – №. 3. – С.229-238.

2. Камилов Х.П. Адилходжаева З.Х., Ибрагимова М.Х. Изменения слизистой оболочке полости рта при буллезных дерматозах. // Узбекистон тиббиёт журнали. 2017. №4. С.4–8.

3. Камилов Х.П. Адилходжаева З.Х. Патоморфология красного плоского лишая с поражением слизистой оболочки полости рта.//ж. Дерматовенерология и эстетическая медицина №2/2019. С. 63-66.

4. Камилов Х. П. Особенности клинического течения синдрома Гриншпана- Потекаева на слизистой оболочке полости рта: научное издание / Х. П. Камилов, М. Х. Ибрагимова, З. Х. Адылходжаева // Медицинский журнал Узбекистана. - Тошкент, 2015. - N2. С. 8-11.

5. Baek K, Choi Y. The microbiology of oral lichen planus:Is microbial infection the cause of oral lichen planus? Mol Oral Microbiol. 2018;33:22–8.

6. Deng X, Wang Y, Jiang L, Li J, Chen Q. Updates on immunological mechanistic insights and targeting of the oral lichen planus microenvironment. Front Immunol. 2023 Jan 9;13:1023213.

7. El-Howati A, Thornhill MH, Colley HE, Murdoch C. Im- mune mechanisms in oral lichen planus. Oral Dis 2023;29(4): 1400e15.

8. Human papillomavirus linked to auto-immune disease. Immunology, inflammation, infectology and microbiology / M. Viguer [et al.]. – 2014.

9. Isola G, Santonocito S, Leonardi R, Polizzi A. Oral lichen planus and lichenoid lesions: etiopathogenesis, diagnosis and treatment. Cham: Springer; 2023.

10. Kitayoshi M, Tsuji K, Wato M, Ikeda C, Tominaga K, Iseki T. Immunohistochemical study of CK13, CK17, CK19, Ki-67,p53, p63, p21, p27 and Cyclin D1 in oral epithelial dysplasia. J Osaka Dent Univ 2023;57:107e17.

11. Kovesi G, Szende B. Changes in apoptosis and mitotic index, p53 and Ki67 expression in various types of oral leukoplakia. Oncology 2003;65:331e6.

12. Mirmohammadi K, Fattahi S, Tavakoli F, Tashakor A. Prev- alence of dysplasia in oral lichen planus patients. J Res Dent Maxillofac Sci 2024;9(2):94e9

13. Gupta, S. Oral lichen planus: An update on etiology, pathogenesis, clinical presentation, diagnosis and management / S. Gupta, M. K. Jawanda // Indian J Dermatol. – 2015.– №60. – P. 222–229

14. Gholizadeh N, Mehdipour M, Dadgar E, Bahramian A, Moghaddas DE. Immunohistochemical evaluation of Ki-67 expression in erosive andNon-erosive oral lichen planus. Avicenna J Dent Res 2016;8:1

15. Gümrü B. A. Retrospective study of 370 patients with oral lichen planus inTurkey / B. A. Gümrü // Med Oral Patol Oral Cir Bucal. – 2013. – V. 18, N 3. – e427-432.

16. Giuliani M, Troiano G, Cordaro M, Corsalini M, Gioco G, Lo Muzio L, et al. Rate of malignant transformation of oral lichen planus: a systematic review. Oral Dis 2019;25(3): 693e709.

17. Parvini P, Obreja K, Cafferata EA, Aini T, Lermen Y, Begic A, et al. The effect of antiresorptive therapy on the prevalence and severity of oral lichen planus: a retrospective study. BMC Oral Health 2024;24(1):547.

18. Takkem A, Barakat C, Zakaraia S, Zaid K, Najmeh J, Ayoub M, et al. Ki-67 prognostic value in different histo- logical grades of oral epithelial dysplasia and oral squamous cell carcinoma. Asian Pac J Cancer Prev 2018;19(11):3279e86.

19. 128.Wang K, Miao T, Lu W, He J, Cui B, Li J, Li Y, Xiao L. Analysis of oral microbial community and Th17-associated cytokines in saliva of patients with oral lichen planus. Microbiol Immunol. 2015 Mar;59(3):105-13.

20. Yang Q, Sun H, Wang X, Yu X, Zhang J, Guo B, Hexige S. Metabolic changes during malignant transformation in primary cells of oral lichen planus: Succinate accumulation and tumour suppression. J Cell Mol Med. 2020 Jan;24(2):1179-1188.