

Comparative Morphological Study of Flat and Verrucous Forms of Oral Leukoplakia

Rizaev J. A.¹, Absalamova N. F.², Akhmedova S. M.³

¹Professor, Rector of Samarkand State Medical University, Doctor of Medical Sciences, Uzbekistan

²Independent Researcher, Samarkand State Medical University, Associate Professor of the Department of Clinical Disciplines Alfraganus University, PhD, Uzbekistan

³Professor, Department of Human Anatomy of Tashkent State Medical University, Doctor of Medical Sciences, Uzbekistan

Abstract This study included 92 individuals. OCT images of different anatomical regions of the oral mucosa were obtained in all subjects and compared with morphological analysis results. Morphometric parameters assessed included epithelial thickness, epithelial papilla height, number and diameter of blood vessels, basement membrane thickness, and collagen fiber density. Compared with the control group, leukoplakia cases showed a significant increase in epithelial thickness and papilla height. The number of blood vessels also increased. OCT images demonstrated epithelial thickening and increased brightness in the flat form, while the verrucous form showed irregular margins and verrucous projections. In cases of malignant transformation, the layered structure disappeared, the image became homogeneous, and the informative depth was less than 1 mm.

Keywords Oral mucosa, Leukoplakia, Precancerous lesion, Optical coherence tomography (OCT), Hyperkeratosis, Acanthosis, Malignant transformation

1. Introduction

Leukoplakia is one of the most common and clinically significant precancerous disorders of the oral mucosa. According to the World Health Organization, a considerable proportion of precancerous lesions in the oral cavity are attributed to leukoplakia, with malignant transformation observed in approximately 3–20% of cases [1]. Hence, its early detection and continuous monitoring remain critical challenges in modern dentistry and oncology. Clinically, leukoplakia presents in several forms—flat, verrucous, and erosive—each demonstrating distinct morphological and morphometric features of the epithelium [3]. Conventional diagnostic approaches, including cytology and biopsy, remain the gold standard; however, they are invasive and often limited to late-stage detection [2].

Recently, optical coherence tomography (OCT) has emerged as a valuable non-invasive diagnostic tool in dental practice. It enables the assessment of the epithelial and stromal layers and the identification of early pathological changes such as hyperkeratosis and acanthosis without requiring tissue sampling [4]. Correlating OCT findings with histological and morphometric data enhances diagnostic accuracy, contributing to early identification of at-risk patients [7]. Thus, the integration of OCT data with morphological and morphometric

analysis represents a scientifically and clinically significant advancement, improving the early diagnosis, monitoring, and prevention of precancerous lesions of the oral mucosa. [5]

The purpose of this study is to compare images obtained by optical coherence tomography with morphological and morphometric indicators in cases of oral mucosal leukoplakia.

Materials and methods. The study was conducted at the dental clinic of Samarkand State Medical University. A total of 92 patients were involved in the examination, aged 19 to 70 years, including 55 women and 37 men. The main group included 76 patients with clinically and histologically confirmed leukoplakia of the oral mucosa. The control group consisted of 16 clinically healthy individuals with no pathological changes in the oral mucosa and free from dental and somatic diseases. [6]

Optical coherence tomography was used in all examinations. For OCT diagnostics, a Swept-Source OCT (SS-OCT) system from Novacam Technologies (Canada) was used, with a signal wavelength of 1300 nm and a tissue depth resolution of 7–10 μm . OCT images were obtained from various anatomical regions of the oral cavity: the labial mucosa, cheeks, dorsal surface of the tongue, hard palate, and gingiva. At least 5 different zones were imaged in each patient, and they were comparatively analyzed for normal and pathological changes.

For morphological analysis, biopsy materials were taken from patients in the main group based on clinical indications.

In the control group, biopsy was not performed for ethical reasons; for morphological comparison, normal histological preparations from the pathoanatomical archive and excess tissue fragments removed during surgical procedures were used. [8]

The obtained biopsies were fixed in 10% formalin, and paraffin blocks were prepared. Histological slides were stained with hematoxylin-eosin, and in additional cases, with the Van Gieson method. Morphometric analysis was performed using a microscope (Olympus BX51), images were digitized, and analyzed using the ImageJ software.

The following were studied as morphometric indicators:

- Epithelial thickness (μm);
- Basement membrane thickness (μm);
- Epithelial papilla height (μm);
- Number and diameter of blood vessels (per 1 mm² area);
- Collagen fiber density (arbitrary units);
- Average diameter of glandular acinar structures (μm).

All data were processed using the Statistica 10.0 software. Mean value (M), standard deviation (SD), and confidence intervals (CI) were calculated. Differences between groups were assessed using Student's t-test and the Mann-Whitney criterion. The significance level was accepted as $p < 0.05$.

2. Materials and Methods

In the control group, OCT tomograms of the oral mucosa demonstrated a distinct two-layered structure. The upper layer corresponded to the stratified squamous epithelium, showing moderate brightness and uniform optical density, while the lower layer represented the connective tissue with higher brightness and heterogeneous signal intensity. The boundary between the epithelial and stromal layers was clear, smooth, and continuous, serving as a normal morphological standard. Within the lower layer, small hypoechoic regions of variable brightness were detected, corresponding to blood vessels and mucosal glands. The informative imaging depth was typically 1.0–1.2 mm and approximately 0.5–0.6 mm in the alveolar mucosa. Histological analysis fully corroborated the OCT findings. Microscopic examination revealed stratified squamous hyperkeratotic epithelium with an average thickness of 250–320 μm , while in the hard palate and gingiva, this thickness reached 400–450 μm . The basement membrane remained intact, and the epithelial-stromal boundary was distinctly visible. In the stroma, collagen fibers were densely packed and predominantly oriented horizontally, with deep connections to the epithelial ridges. The mean vessel diameter ranged from 18–26 μm , with an average of 22 ± 3 vessels per 1 mm². Glandular acinar structures appeared intact, averaging 28 ± 4 μm in diameter. Morphometric evaluation indicated that the mean epithelial thickness was 280 ± 25 μm , 410 ± 30 μm in the gingiva, and 260 ± 20 μm in the labial mucosa. The basement membrane thickness ranged between 0.8–1.2 μm , and epithelial ridge height averaged 65 ± 10 μm .

The collagen fiber density was calculated at $0,68 \pm 0,05$ arbitrary units. These findings established the morphological and morphometric reference parameters for the oral mucosa in the control group. The consistency between OCT imaging and histological analysis confirmed the two-layered architecture of the normal mucosa, the integrity of the basement membrane, and the structural harmony between the epithelium and stroma. This dataset serves as a reliable baseline for comparison with leukoplakia cases in the main group.

Comparative evaluation of OCT and histological data revealed that leukoplakia, a common precancerous lesion of the oral mucosa, presents with distinct clinical and morphological variants. Flat leukoplakia predominantly occurs in regions of hyperkeratotic epithelium, maintaining a relatively preserved layered structure. OCT scans showed two horizontal layers: the upper layer—representing hyperplastic, stratified squamous epithelium—appeared brighter and thicker than normal, while the lower layer corresponded to connective tissue with increased reflectivity. Histological findings included acanthosis, parakeratosis, irregular epithelial ridges, local proliferative activity in the basal layer, and focal epithelial atypia. The subepithelial stroma exhibited diffuse or focal lymphomacrophagal infiltration.

Verrucous leukoplakia demonstrated more pronounced pathological alterations in both OCT and histological assessments. Tomograms revealed an uneven, elevated upper boundary due to verrucous epithelial projections, with partial disturbance of the normal layered pattern. The upper layer displayed marked hyperkeratosis and acanthosis, while the lower layer contained hypoechoic regions corresponding to vascular and inflammatory elements. Histological sections showed a markedly thickened keratin layer, epithelial hyperplasia, and dyskeratosis in individual cells. The stratified squamous epithelium exhibited irregular thickening and focal distortions. Epithelial cells displayed nuclear enlargement, hyperchromasia, and an increased nuclear-to-cytoplasmic ratio, indicative of dysplastic transformation. The basement membrane was preserved but locally thickened. The underlying stroma revealed dense fibrosis with thick collagen bundles and sclerosis. Chronic inflammatory infiltrates—mainly lymphocytes with occasional neutrophils—were concentrated around dilated blood vessels, forming perivascular cuffs (Figure 1).

Histological examination (H&E staining) revealed pronounced inflammatory infiltration within the connective tissue, predominantly composed of lymphocytes and macrophages with scattered neutrophils. Focal epithelial proliferation and mild dystrophic changes were observed. Dense collagen fibers and perivascular lymphocytic cuffs were evident in the stroma, indicating chronic inflammatory processes. In some regions, focal epithelial hyperplasia and local necrotic changes were noted.

3. Results and Discussions

These morphological features are consistent with chronic inflammation of the oral mucosa associated with verrucous

or erosive forms of leukoplakia, where prolonged irritation and epithelial remodeling are accompanied by stromal fibrosis and cellular infiltration (Figure 2).

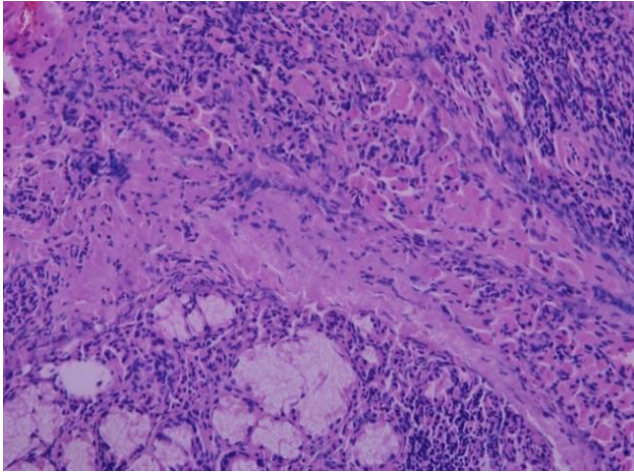


Figure 1. The epithelial cells show nuclear enlargement, hyperchromasia, and an increased nuclear-to-cytoplasmic ratio, suggestive of dysplastic alterations. The basement membrane is preserved but focally thickened. Stain H.E. Magnification 40x10

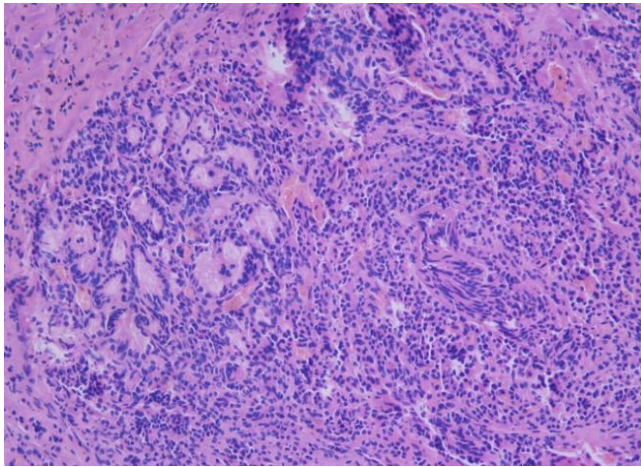


Figure 2. Histological section of the oral mucosa with leukoplakia/ Pronounced lymphomacrophagal infiltration, focal epithelial hyperplasia, and perivascular inflammatory cuffs indicate chronic inflammatory changes typical for verrucous leukoplakia. Stain H.E. Magnification 40x10

Flat and verrucous leukoplakia showed similar optical

phenomena in hyperkeratotic epithelium. The main differences were observed in the degree of brightness and height of the epithelial layer, which were related to the degree of hyperkeratosis and hyperplasia.

In cases of malignization, a new appearance was noted in OCT images: the layered structure disappeared, the image became bright, uniform, and structureless, with depth less than 1 mm. These changes were explained by the loss of cell and tissue differentiation, and increased density of cellular components in the carcinogenesis process. In histological sections, complexes of atypical cells were observed spreading from coarsely layered epithelium through the basement membrane into the stroma, indicating the development of squamous cell carcinoma.

Thus, in cases of flat and verrucous leukoplakia, OCT images and morphological analysis results were mutually consistent, expressed with specific signs depending on the degree of hyperkeratosis, acanthosis, infiltration, and epithelial hyperplasia (Figure 3).

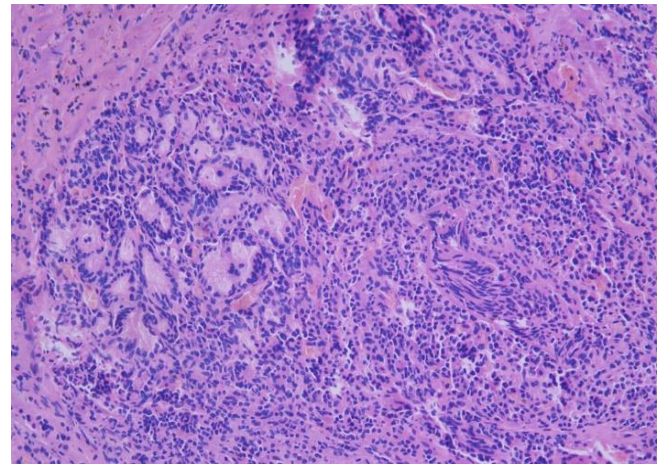


Figure 3. Leukoplakia in the oral mucosa. Lymphomacrophagal infiltration under the epithelium, presence of dyskeratosis in some cells, and signs of atypia are noticeable. Accumulation of inflammatory cells in the stroma and infiltration around blood vessels are noted. Stain H.E. Magnification 40x10

In the process of malignization, the loss of layered structure and uniformity of the signal via OCT were evaluated as reliable signs of cancer transformation from a morphological perspective.

Table 1. Morphometric characteristics of the oral mucosa in control and leukoplakia groups

Parameter	Control (Mean ± SD)	Flat L. (Mean ± SD)	Verruc. L. (Mean ± SD)	p-value
Epithelial thickness (µm)	280 ± 25	360 ± 40	520 ± 60	p < 0.001
Basement membrane thickness (µm)	1.0 ± 0.2	1.1 ± 0.2	1.2 ± 0.3	p < 0.05
Epithelial ridge height (µm)	65 ± 10	80 ± 12	110 ± 18	p < 0.01
Number of blood vessels (per 1 mm ²)	22 ± 3	26 ± 4	30 ± 5	p < 0.05
Vessel diameter (µm)	22 ± 2	25 ± 3	27 ± 4	p < 0.05
Collagen fiber density (a.u.)	0.68 ± 0.05	0.70 ± 0.06	0.75 ± 0.07	p < 0.05

Note: Statistical analysis was performed using Student's t-test. Differences were considered statistically significant at p < 0.05.

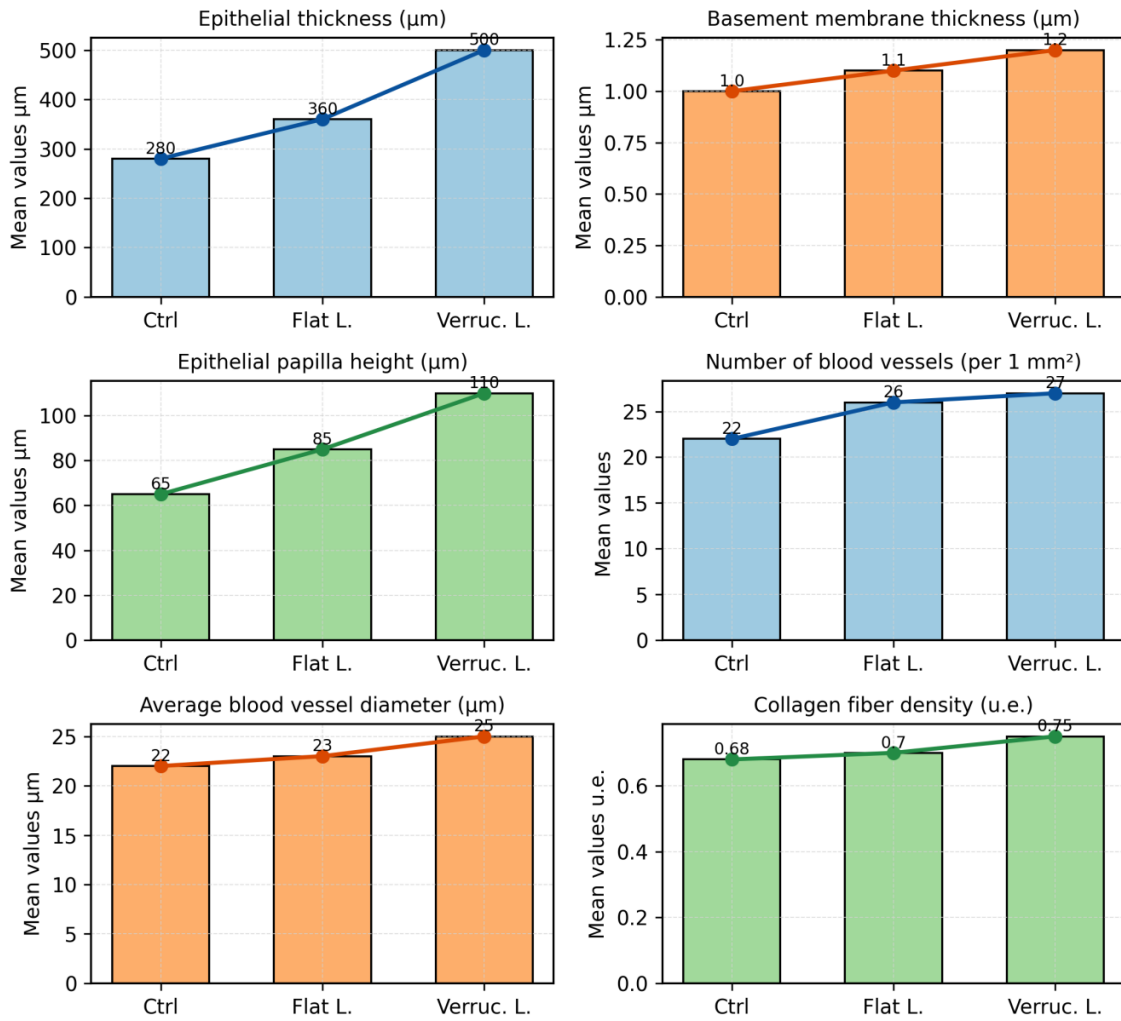


Figure 4. Morphometric indicators of the oral mucosa (mean ±SD)

According to the data presented in Table 1, epithelial thickness averaged $280 \pm 25 \mu\text{m}$ in the control group, while in flat leukoplakia this indicator increased to $360 \pm 40 \mu\text{m}$, and in verrucous leukoplakia up to $520 \pm 60 \mu\text{m}$. Epithelial ridge height was $65 \pm 10 \mu\text{m}$ in the control, $80 \pm 12 \mu\text{m}$ in flat leukoplakia, and $110 \pm 18 \mu\text{m}$ in the verrucous form. The number of blood vessels also increased in pathological conditions: 22 ± 3 per mm^2 in the control, 26 ± 4 per mm^2 in the flat form, and 30 ± 5 per mm^2 in the verrucous form. At the same time, collagen fiber density and basement membrane thickness also showed an increase in the pathological groups.

The diagram presented in Figure 4, visually reflects the dynamics of morphometric indicators. According to it, verrucous leukoplakia is characterized by the strongest expression of morphological changes, while the flat form occupies an intermediate position between the control group and the verrucous form. OCT diagnostic results also confirmed these morphometric data.

In the control group, the layered structure was clearly preserved, and the boundary between the epithelium and stroma was continuous and smooth. In flat leukoplakia, the epithelial layer was thickened and noted with increased

brightness; in the verrucous form, the upper boundary was uneven and appeared elevated due to verrucous growths (Figure 5). In cases of malignization, the layered structure disappeared, the image became uniform and bright, and the informative depth decreased to less than 1 mm.

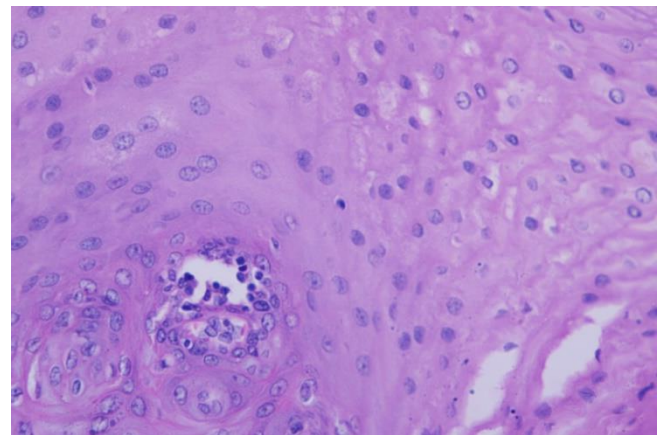


Figure 5. Flat leukoplakia in the oral mucosa. The layers of multilayered squamous epithelium are thickened, signs of hyperkeratosis between cells and dyskeratosis in some cells are observed. Stain H.E. Magnification 40x10

Thus, the obtained morphological and OCT diagnostic data were mutually consistent, providing the opportunity to accurately evaluate changes in various forms of leukoplakia on a comparative basis. These results are of significant importance in the early detection of precancerous processes in the oral mucosa and in identifying risk groups.

The comparative analysis of OCT images and histological sections revealed a strong correlation between non-invasive optical findings and morphological features of leukoplakia. In the control group, OCT demonstrated a well-preserved two-layered structure with a smooth epithelial-stromal boundary, corresponding to normal histological architecture.

In flat leukoplakia, OCT showed increased brightness and thickening of the epithelial layer, which matched the morphological findings of hyperkeratosis, acanthosis, and regular but elongated epithelial ridges. In verrucous leukoplakia, OCT revealed an uneven and elevated surface contour, partial disruption of the layered structure, and hypoechoic areas in the stroma. Histological analysis confirmed these changes, showing coarse hyperkeratosis, irregular epithelial ridges, dyskeratosis, and lymphomacrophagal infiltration of the stroma.

In cases of malignant transformation, OCT findings demonstrated a complete loss of layered structure, with a bright, homogeneous, structureless image and reduced diagnostic depth (<1 mm). Morphologically, this corresponded to the invasion of atypical epithelial cell complexes through the basement membrane into the stroma, consistent with squamous cell carcinoma.

Thus, OCT findings were consistent with histological results and reflected the progressive morphological changes in flat, verrucous, and malignant forms of oral mucosal leukoplakia.

Our study results demonstrated the presence of significant morphological and morphometric changes in cases of leukoplakia in the oral mucosa. In particular, epithelial thickness, epithelial ridge height, and the number of blood vessels were noted to increase significantly in flat and verrucous forms compared to the control group. These data are consistent with the results presented in the scientific literature. For example, in the studies of A.L. Mashkilleysen and Zakrzewska, various clinical forms of leukoplakia are also characterized morphologically by hyperkeratosis, acanthosis, and irregularity of epithelial papillae. [9]

The images obtained through OCT diagnostics corresponded to the results of morphological and morphometric analysis. In the control group, the layered structure was preserved intact and smooth, while in the flat form, thickening of the epithelium and an increase in its brightness were observed. In the verrucous form, unevenness of the upper boundary and profound changes due to verrucous growths were noted. In cases of malignization, the loss of layered structure and the image becoming uniform and structureless were noted as signs characteristic of malignancy. This is also consistent with the data from Pashkov B.M. and subsequent researchers. At the same time, an important aspect of the study is that the OCT method enabled non-invasive detection of early

changes in leukoplakia cases. This method is less invasive and more convenient for the patient compared to classic biopsy and cytology methods. However, OCT data still requires comparison with histological analysis, as in some cases, the possibility of error in non-invasive diagnosis may persist.

4. Conclusions

According to the obtained results, significant changes in morphometric indicators in the epithelium and stroma were identified in cases of oral mucosal leukoplakia. In flat and verrucous forms, epithelial thickness, papilla height, and the number of blood vessels were higher compared to the control group. OCT diagnostics were consistent with morphological analysis, reflecting the integrity of the layered structure in the control group, and hyperkeratosis, acanthosis, and boundary disruption in leukoplakia cases. In malignization processes, the loss of layered structure and uniformity of the optical signal were noted as reliable signs of cancer transformation.

Thus, the OCT method is an effective non-invasive diagnostic tool for early detection of precancerous processes in the oral mucosa, stratification of patients into risk groups, and dynamic monitoring.

REFERENCES

- [1] Warnakulasuriya S., Kujan O., Aguirre-Urizar J.M., Bagan J.V., González-Moles M.Á., Kerr A.R., Lodi G., Mello F.W., Monteiro L., Ogden G.R., et al. Potentially malignant disorders of the oral cavity: consensus report from an international seminar on nomenclature and classification convened by the WHO Collaborating Centre for Oral Cancer. *Oral Diseases*. 2021; 27: 1862–1880.
- [2] Warnakulasuriya S., Johnson N.W., van der Waal I. Nomenclature and classification of potentially malignant disorders of the oral mucosa. *Journal of Oral Pathology & Medicine*. 2007; 36: 575–580.
- [3] Zhang C., Li B., Zeng X., Hu X., Hua H. Global prevalence of oral leukoplakia: a systematic review and meta-analysis from 1996 to 2022. *BMC Oral Health*. 2023; 23: 645.
- [4] Mello F.W., Miguel A.F.P., Dutra K.L., Porporatti A.L., Warnakulasuriya S., Guerra E.N.S., Rivero E.R.C. Prevalence of oral potentially malignant disorders: a systematic review and meta-analysis. *Journal of Oral Pathology & Medicine*. 2018; 47: 633–640.
- [5] Warnakulasuriya S. Clinical features and presentation of oral potentially malignant disorders. *Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology*. 2018; 125: 582–590.
- [6] Warnakulasuriya S., Ariyawardana A. Malignant transformation of oral leukoplakia: a systematic review of observational studies. *Journal of Oral Pathology & Medicine*. 2015; 45(3): 155–166.
- [7] Jeong W.J., Paik J.H., Cho S.W., Sung M.W., Kim K.H., Ahn

S.H. Excisional biopsy for the treatment of leukoplakia of the lateral tongue. *Journal of Oral Pathology & Medicine*. 2011; 41(5): 384.

[8] Wils L.J., Poell J.B., Evren I., Koopman M.S., Brouns E.R.E.A., de Visscher J.G.A.M., et al. Incorporation of differentiated dysplasia improves prognostic evaluation of oral leukoplakia

at increased risk of malignant progression. *Modern Pathology*. 2020; 33(6).

[9] Li K.K., Almazroua S., Carvo I., Salchines A., Woo S.B. Architectural changes in oral epithelial dysplasia are similar in unifocal and proliferative leukoplakia. *Head and Neck Pathology*. 2020; 15(2): 443.

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