Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(5), 897-903 | ISSN:2251-6727

# Comparative Evaluation of P21 Marker Detection in Squamous Cell Carcinoma and Leukoplakia Using Enhanced Immunohistochemical Techniques: An Original Research Study

**Dr. Aparna Paliwal[1], Dr. Tanmoy Patra[2], Dr. Ankita Jain[3], Dr. Vineet Tyagi[4], Dr. Sharjeel Anjum Bhat[5], Dr. Biswanath Banerjee[6]**

[1]Professor and Head, Department of Oral Pathology and Microbiology, RKDF Dental College and Research Centre, Bhopal, MP, India (Corresponding Author)

[2]Post Graduate Student (Third Year), Department of Oral Pathology and Microbiology, RKDF Dental College and Research Centre, Bhopal, MP, India

[3]Senior Lecturer, Department of Oral Pathology and Microbiology, RKDF Dental College and Research Centre, Bhopal, MP, India

[4]Senior Lecturer, Department of Oral Pathology and Microbiology, RKDF Dental College and Research Centre, Bhopal, MP, India

[5]Senior Lecturer, Department of Oral Pathology and Microbiology, RKDF Dental College and Research Centre, Bhopal, MP, India

[6]Post Graduate Student (Third Year), Department of Oral Pathology and Microbiology, RKDF Dental College and Research Centre, Bhopal, MP, India

**Corresponding Author:** Dr. Aparna Paliwal

***(Received: 16 July 2025 Revised: 20 August 2025 Accepted: 02 September 2025)***

**KEYWORDS**

Leukoplakia, Squamous Cell Carcinoma, P21 Marker, Enhanced Immunohistochemical Techniques, Oral Potentially Malignant Disorders

**ABSTRACT:**

Aim: This study aims to evaluate the p21 marker detection in Squamous Cell Carcinoma and Leukoplakia using enhanced Immunohistochemical Techniques

Materials and Methods: This study involved 80 cases of predefined lesions, including 40 individuals with Oral Squamous Cell Carcinoma (OSCC) and 40 with Leukoplakia. Strict criteria ensured balanced gender representation and excluded samples with smoking history or necrotic tissue. All patients consented, and none were on medications affecting the study. Samples were prepared as four-micron Paraffin sections, treated with a 3% Peroxidase solution, and stained with Monoclonal Antibodies. After Haematoxylin staining and dehydration, slides were examined microscopically at 100x and 400x magnification, using melanoma tissue as a control. Nuclei were counted and analysed using Broder's Grading system, while staining intensity was assessed by a pathologist as (+) mild, (++) moderate, and (+++) intense.

Statistical Analysis and Results: A study analysed 80 patients with predefined lesions, split into two groups: 40 with Oral Squamous Cell Carcinoma (OSCC) and 40 with Leukoplakia. The findings showed a higher prevalence of males (44 males vs. 36 females). In Group 1 (OSCC), p21 marker expression revealed 5 patients (12.5%) with mild staining, 15 (37.5%) with moderate staining, and 20 (50%) with intense staining, indicating a potential link between high p21 expression and aggressive tumour characteristics. In Group 2 (Leukoplakia), 10 patients (25%) had mild staining, 10 (25%) moderate, and 15 (37.5%) intense staining. A one-way ANOVA assessed the significance of p21 staining intensity across groups, suggesting p21's role as a biomarker for tumour progression in both malignant and precancerous conditions.

Conclusion: This study concluded that p21 expression was moderate in oral SCC patients compared to Leukoplakia, with higher levels indicating greater disease severity. While Leukoplakia showed some p21 expression, it was weaker than in SCC. The research highlighted a significant increase in p21 from normal tissue to Leukoplakia and then to SCC, emphasizing the need for further studies to enhance clinical applications.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(5), 897-903 | ISSN:2251-6727

## Introduction

Oral premalignancy is a critical stage that can lead to oral cancer, including various premalignant lesions that increase cancer risk compared to healthy tissue.[1] The most common type, oral squamous cell carcinoma (OSCC), presents as red and white lesions with irregular surfaces and well-defined borders. Early lesions may be asymptomatic, but can progress to ulceration, nodularity, and pain. OSCC frequently occurs on the posterior lateral tongue and tends to metastasize through lymphatic pathways to distant sites; complicating treatment.[2-5] Patients diagnosed with Oral Potentially Malignant Disorders (OPMDs) are recognized to possess a heightened risk for the development of oral cancer. Initially, these disorders may present without symptoms, but as they progress, individuals may experience clinical signs such as erythema, pain, or ulceration. Proper and timely diagnosis of OPMDs is critical to effectively manage the risk of malignancy. These disorders include various conditions such as oral leukoplakia, oral erythroplakia, oral submucosal fibrosis, and oral lichen planus, each requiring distinct approaches for evaluation and management.[6,7] Oral leukoplakia, characterized by the presence of white patches on the mucosal surface, is particularly associated with tobacco use and other irritants, necessitating thorough evaluation by healthcare professionals. Collaborative efforts among medical and dental professionals are essential to devise appropriate treatment strategies and monitor changes in these lesions over time.[8,9] At the molecular level, the tumour suppressor gene P21, positioned on chromosome 6, plays a pivotal role in curbing tumour proliferation. This essential gene is a key regulator of the cell cycle, acting as a critical checkpoint that prevents unregulated cell growth, which can lead to cancerous development. By inhibiting the activity of cyclin-dependent kinases, P21 ensures that cells do not progress through the cycle in an uncontrolled manner, thereby maintaining the integrity of cellular processes and helping to safeguard against the onset of tumors.[10,11] For the diagnosis of oral cancer and its precursors, a variety of advanced methods are employed, including biosensors, enzyme-linked immunosorbent assays (ELISA), and mass spectrometry. Immunohistochemistry (IHC) techniques are particularly valuable, as they enable the visualization of protein expression within tissue samples through the binding of antibodies to their specific antigens. These IHC methods can utilize either direct or indirect approaches, enhance signal visibility, and provide critical information about the cellular characteristics of lesions, which assists in accurate diagnosis and treatment planning.[12,13] This study aims to evaluate the detection of the p21 marker in Squamous Cell Carcinoma and Leukoplakia using enhanced immunohistochemical techniques.

## Materials and Methods

In this detailed investigation, a total of 80 cases involving patients with predefined lesions were meticulously analyzed. This cohort comprised 40 individuals diagnosed with Oral Squamous Cell Carcinoma (OSCC) and an equal number of 40 patients exhibiting Leukoplakia. Within the study, 80 distinct samples were collected, evenly divided into 40 paraffin blocks representing each of the two lesion types: Leukoplakia and OSCC. The inclusion criteria were carefully set to encompass both male and female patients, ensuring a representative sample for the findings. To uphold the integrity of the research, samples presented with certain risk factors, such as a history of smoking or exhibiting dysplastic changes, were stringently excluded from the study. Additionally, any samples that showed signs of improper fixation, raised suspicion of incorrect diagnosis, or consisted of necrotic tissue were discarded. All participating patients provided informed consent, and a thorough review of medical records confirmed that none had a background of medication usage that might interfere with the study outcomes. The preparation of the samples involved making four-micron paraffin sections, which were precisely mounted on poly-L-lysine slides. These slides were then incubated at a consistent temperature of 58˚C for a full 24 hours and subsequently treated with a 3% Peroxidase solution to enhance staining accuracy. Following this treatment, the slides underwent rinsing with phosphate-buffered saline (PBS) to remove excess reagents. The next critical step involved applying a primary monoclonal antibody, which was incubated under dark, humid conditions to facilitate binding. After several additional rinsing cycles, a secondary antibody and super enhancer were utilized, and Diaminobenzidine (DAB) solution was introduced for Chromogenic staining, resulting in the necessary visualization of cellular activity. For further clarity,

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(5), 897-903 | ISSN:2251-6727

Hematoxylin staining was carried out, and the slides were carefully dehydrated through a sequential Alcohol and Xylene treatment before final mounting. Once prepared, the stained slides were thoroughly examined microscopically at magnifications of both 100x and 400x. Cells exhibiting brown-stained nuclei were classified as positive, with a systematic counting of 1,000 cells conducted per slide to ensure reliable data. In accordance with Broder's grading system, each case of Oral Squamous Cell Carcinoma (OSCC) was systematically classified based on prescribed criteria. A single oral pathologist meticulously reviewed all samples, categorizing the intensity of staining for statistical analysis. Grading for both Leukoplakia and OSCC was defined as follows: (+) indicating mild intensity, (++) reflecting moderate intensity, and (+++) denoting intense staining. This structured approach provided a robust framework for understanding the complexities of these predefined lesions. This study aimed to determine and compare the intensity of the p21 marker in Leukoplakia and Oral Squamous Cell Carcinoma using enhanced Immunohistochemical techniques. By employing a systematic evaluation and comparison of p21 expression levels, the research sought to elucidate the potential role of this marker in distinguishing between these two conditions, thereby contributing valuable insights into their patho-physiological differences and aiding in diagnostic accuracy.

**Statistical Analysis and Results**

In this study, we utilized SPSS software version 29.0 for our statistical analyses due to its strength in the social sciences. To assess the significance of our findings, we applied the chi-square test, which effectively highlights differences in proportions among groups. This method allowed for a rigorous comparison of categorical data, ensuring that our results accurately capture the key trends and relationships within the dataset.

**Results**

A comprehensive analysis was undertaken involving a total of 80 cases of patients diagnosed with predefinedlesions. The cohort was equally divided, featuring 40 individuals diagnosed with Oral Squamous Cell Carcinoma (OSCC) and an additional 40 patients presenting with Leukoplakia. Table 1 offers an in-depth statistical overview of the patients' ages and gender distributions, revealing a notable predominance of males, with 44 male patients compared to 36 females. Graph 1 complements this data by visually illustrating the demographic breakdown and associated characteristics of the patient population, emphasizing the gender disparity. Focusing on Group 1 (n=40), which includes patients diagnosed with Oral Squamous Cell Carcinoma, Table 2 details the comprehensive analysis conducted to identify the expression of the p21 marker utilizing advanced Immunohistochemical techniques. The staining intensity of the p21 marker was meticulously classified into three levels: mild, moderate, and intense. Among the cohort, mild staining was identified in 5 patients (12.5%), indicating a lesser degree of p21 expression. In contrast, moderate staining was observed in 15 patients (37.5%), suggesting a moderate level of marker activity that could have potential implications for tumor behavior. Notably, an impressive 20 patients (50%) exhibited intense staining of the p21 marker, underscoring a significant prevalence of heightened p21 expression within this group. This notable presence of intense staining may correlate with more aggressive tumor characteristics and suggests a potential role for p21 in the pathology of Oral Squamous Cell Carcinoma. Turning to Group 2 (n=40), which consists of patients with Leukoplakia, Table 3 illustrates the examination of the p21 marker using similar advanced Immunohistochemical methods. The analysis revealed a distinct pattern of expression in this cohort. In this group, mild staining was documented in 10 patients (25%), indicating a lower expression level, while 10 additional patients (25%) demonstrated moderate staining. Interestingly, 15 patients (37.5%) displayed intense staining of the p21 marker. This variability in expression levels amongst patients with Leukoplakia highlights the differing biological behavior and potential risk factors associated with the p21 marker in precancerous lesions compared to malignant tumors. In summary, Table 4 provides a comprehensive estimate of the studied groups and employs one-way ANOVA to assess the statistical significance across the various categories of p21 staining intensity. This robust analysis offers valuable insights into the correlation between the levels of p21 expression and the specific types of lesions examined, further enriching our understanding of the p21 marker's role and its potential implications for diagnosis and treatment strategies in oral lesions. The findings contribute to a nuanced perspective on

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(5), 897-903 | ISSN:2251-6727

how p21 may serve as a biomarker for tumor progression and patient management in both carcinogenic and precancerous contexts.

**Table 1:** Age & gender based statistical description of contributing patients

| Age Group (Yrs) | Male | Female | Total | P value |
|---|---|---|---|---|
| 25-30 | 5 | 6 | 11 | 0.02* |
| 31-35 | 7 | 8 | 15 | 0.04* |
| 36-40 | 9 | 6 | 15 | 0.01* |
| 41-45 | 9 | 7 | 16 | 0.50 |
| 46-50 | 14 | 9 | 23 | 0.30 |
| Total | 44 | 36 | 80 | *Sig. |
| *p<0.05 significant | | | | |

**Graph 1:** Patients demographic distribution and associated details

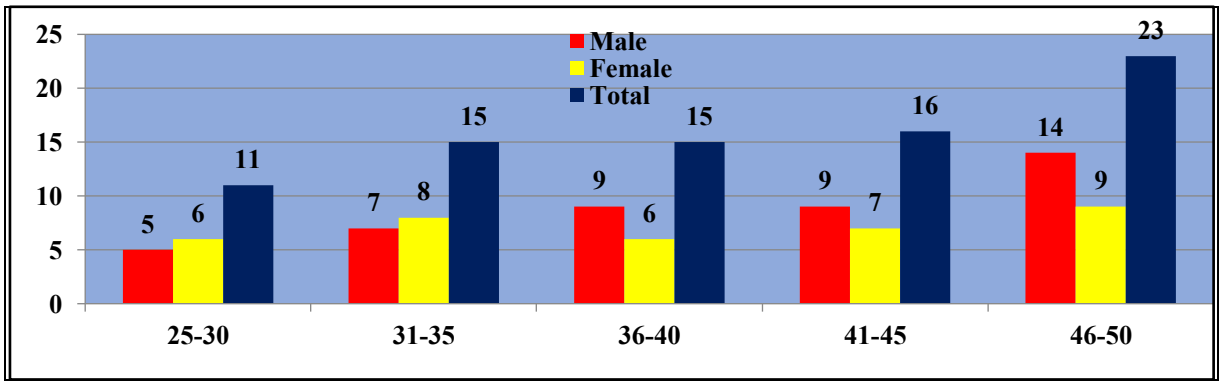


**Table 2:** Group 1 (n=40) Cases of Oral Squamous Cell Carcinoma were analysed to identify the p21 marker, utilizing advanced Immunohistochemical methods, categorized by mild, moderate, and intense staining. The statistical analysis was conducted using the Pearson Chi-Square test

| Grading for Intensity | n | Stat. Mean | Std. Dev. | Std. Error | 95% CI | Pearson Chi-Square Value | df | p value |
|---|---|---|---|---|---|---|---|---|
| Mild (+) | 5 | 1.17 | 1.023 | 1.345 | 1.02 | 1.402 | 1.0 | 0.01* |
| Moderate (++) | 15 | 2.10 | 1.189 | 1.340 | 1.08 | 1.204 | 2.0 | 0.02* |
| Intense (+++) | 20 | 2.24 | 2.234 | 2.308 | 2.48 | 2.012 | 1.0 | 0.40 |
| *p<0.05 significant | | | | | | | | |

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(5), 897-903 | ISSN:2251-6727

**Table 3:** Group 2 (n=40) Cases of Leukoplakia were analysed to identify the p21 marker, utilizing advanced immunohistochemical methods, categorized by mild, moderate, and intense staining. The statistical analysis was conducted using the Pearson Chi-Square test

| Grading for intensity | n | Stat. Mean | Std. Dev. | Std. Error | 95% CI | Pearson Chi-Square Value | df | p value |
|---|---|---|---|---|---|---|---|---|
| Mild (+) | 10 | 2.09 | 1.145 | 1.245 | 1.23 | 1.214 | 1.0 | 0.07 |
| Moderate (++) | 10 | 2.09 | 1.145 | 1.245 | 1.23 | 1.214 | 1.0 | 0.07 |
| Intense (+++) | 15 | 2.10 | 1.189 | 1.340 | 1.08 | 1.204 | 2.0 | 0.02* |
| *p<0.05 significant | | | | | | | | |

**Table 4:** Estimation amongst all studied groups using one-way ANOVA

| Variables | Degree of Freedom | Sum of Squares ∑ | Mean Sum of Squares m∑ | F | Level of Sig. (p) |
|---|---|---|---|---|---|
| Between Groups | 4 | 2.020 | 1.067 | 1.5 | 0.002* |
| Within Groups | 21 | 1.214 | 0.636 | _ | |
| Cumulative | 113.10 | 4.054 | *p<0.05 significant | | |

**Discussion**

Meier J K et al reviewed in their study that oral cancer often originates from precancerous lesions known as potentially premalignant oral epithelial lesions (PPOELs). These lesions pose a significantly greater risk of progression to malignancy compared to normal epithelial tissue. The urgency of early diagnosis and intervention cannot be overstated, as they play a crucial role in preventing the progression to oral squamous cell carcinoma (OSCC). This particular type of cancer is responsible for approximately 90% of all oral cancer cases and has the potential to severely affect essential functions such as speech and swallowing.[14,15] Bhattarai BP et al showed in their study that the development of oral cancer is influenced by several significant risk factors, with tobacco and alcohol consumption being among the most critical. Both habits have been extensively studied and are strongly associated with a variety of oral diseases and conditions, including precancerous lesions and malignancies.[16] Odell E et al included in their study that in addition to these lifestyle choices, certain oral conditions warrant particular attention due to their potential for malignant transformation. Oral leukoplakia, characterized by the appearance of white plaques in the oral cavity, is particularly concerning. Its risk profile remains uncertain, as some lesions may indicate the presence of dysplasia or other underlying pathologies that could escalate to cancer. Therefore, it is essential for healthcare providers to thoroughly investigate and rule out other possible conditions when diagnosing oral leukoplakia.[17,18] Deng J et al reviewed in their study that similarly, oral submucous fibrosis (OSMF) poses a significant risk. This chronic, progressive condition is often linked to the chewing of betel quid or areca nut and leads to fibrotic changes in the oral mucosa, restricting movement and potentially giving rise to malignant transformations over time. Due to the serious implications of these conditions, vigilant monitoring and comprehensive assessment are crucial to mitigate

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(5), 897-903 | ISSN:2251-6727

the risk of progression to oral cancer. Overall, understanding and addressing these risk factors and precancerous lesions are vital for early detection and prevention strategies in oral cancer management.[19] Kumar S et al included in their study that the p21 gene, also known as CDKN1A, plays a multifaceted role in the realm of cancer biology, acting variably as a tumour suppressor and as a promoter of tumorigenesis depending on the specific cellular context and environmental influences. This dual functionality is particularly intriguing, as it underscores the gene's involvement in critical processes such as cell cycle regulation, apoptosis, and cellular senescence. Recent studies have illuminated its significant role in promoting cellular senescence, a state characterized by permanent cell cycle arrest, which can function as a barrier to tumour formation. Consequently, p21 emerges as a promising target for innovative therapeutic strategies aimed at harnessing its senescence-inducing properties to combat cancer more effectively.[20-22] Hussaini HM et al showed in their study that immunohistochemistry (IHC) is an indispensable technique in the field of pathology, allowing for in-depth analysis of tissue samples through the use of specific labelled antibodies. This method provides a detailed visualization of various cellular components, enabling pathologists to discern the intricate cellular architectures and protein expressions within tissue samples. IHC is crucial for accurate pathological assessments, as it facilitates the identification of disease markers and the evaluation of tumour characteristics. Additionally, by employing this technique, researchers can gain a deeper understanding of disease processes, paving the way for potential diagnostic and treatment advancements in oncology.[23]

**Conclusion**

In this study, the authors investigated the detection of the p21 marker in cases of Squamous Cell Carcinoma (SCC) and Leukoplakia through advanced Immunohistochemical techniques. The findings revealed and concluded that p21 expression was moderately higher in patients with Oral Squamous Cell Carcinoma compared to those with Leukoplakia. Typically, the p21 marker is present at elevated levels and with increased intensity in Oral Squamous Cell Carcinoma, suggesting a progression in the disease's severity as indicated by higher expression in malignant tumors. While Leukoplakia may exhibit some p21 expression, this is generally weaker than in SCC. The study noted a significant and progressive increase in p21 expression from normal tissue to Leukoplakia, and subsequently to Oral Squamous cell Carcinoma. The authors emphasized the importance of further comprehensive research to deepen our understanding of these techniques and refine their application in clinical practice.

**References**

1. Vigneswaran N & Williams M D, Epidemiologic trends in head and neck cancer and aids in diagnosis. Oral Maxillofac. Surg. Clin 2014;99(4):123–41.
2. Romano A, Di Stasio D, Petruzzi M, Fiori F, Lajolo C, Santarelli A, Lucchese A, Serpico R, Contaldo M. Noninvasive Imaging Methods to Improve the Diagnosis of Oral Carcinoma and Its Precursors: State of the Art and Proposal of a Three-Step Diagnostic Process. Cancers (Basel). 2021;13(12):2864-9.
3. Safi AF, Kauke M, Grandoch A, Nickenig HJ, Drebber U, Zöller J, Kreppel M. Clinicopathological parameters affecting nodal yields in patients with oral squamous cell carcinoma receiving selective neck dissection. J Craniomaxillofac Surg. 2017;45(12):2092-6.
4. Linsen SS, Gellrich NC, Krüskemper G. Age- and localization-dependent functional and psychosocial impairments and health related quality of life six months after OSCC therapy. Oral Oncol. 2018;8(1):61-8.
5. Narayan TV, Shilpashree S, Meta-analysis on clinicopathologic risk factors of leukoplakias undergoing malignant transformation. J Oral Maxillofac Pathol. 2016;20(3):354-61.
6. Shah S, Dave B, Shah R, Mehta TR, Dave R. Socioeconomic and cultural impact of tobacco in India. J Family Med Prim Care. 2018;7(6):1173-6.
7. Bose SC, Singh M, Vyas P, Singh M. Plasma zinc antioxidant vitamins, glutathione levels and total antioxidant activity in oral leukoplakia. Dent Res J (Isfahan). 2012;9(2):158-61.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(5), 897-903 | ISSN:2251-6727

8. Abbas T, Dutta A. p21 in cancer: intricate networks and multiple activities. Nat Rev Cancer. 2009;9(6):400-14.
9. Allouch A, Voisin L, Zhang Y, Raza SQ, Lecluse Y, Calvo J, Selimoglu-Buet D, de Botton S, Louache F, Pflumio F, Solary E, Perfettini JL. CDKN1A is a target for phagocytosis-mediated cellular immunotherapy in acute leukemia. Nat Commun. 2022;13(1):6739-45.
10. Aprelikova O, Xiong Y, Liu ET. Both p16 and p21 families of cyclin-dependent kinase (CDK) inhibitors block the phosphorylation of cyclin-dependent kinases by the CDK-activating kinase. J Biol Chem. 1995;270(31):18195-7.
11. Bae JH, Jeong HJ, Kim H, Leem YE, Ryu D, Park SC, Lee YI, Cho SC, Kang JS. ZNF746/PARIS overexpression induces cellular senescence through FoxO1/p21 axis activation in myoblasts. Cell Death Dis. 2020;11(5):359-67.
12. Lin F, Chen Z. Standardization of diagnostic immunohistochemistry: literature review and Geisinger experience. Archives of Pathology & Laboratory Medicine 2014;138(12):1564-77.
13. Dabbs DJ, editor. Diagnostic immunohistochemistry e-book: theranostic and genomic applications. Elsevier Health Sciences; 2017 Nov 29.
14. Meier JK, Schuderer JG, Zeman F, Klingelhöffer C, Hullmann M, Spanier G, Reichert TE, Ettl T. Health-related quality of life: a retrospective study on local vs. microvascular reconstruction in patients with oral cancer. BMC Oral Health. 2019;19(1):62-9.
15. Maymone MBC, Greer RO, Kesecker J, Sahitya PC, Burdine LK, Cheng AD, Maymone AC, Vashi NA. Premalignant and malignant oral mucosal lesions: Clinical and pathological findings. J Am Acad Dermatol. 2019;81(1):59-71.
16. Bhattarai BP, Singh AK, Singh RP, Chaulagain R, Søland TM, Hasséus B, Sapkota D. Recurrence in Oral Leukoplakia: A Systematic Review and Meta-analysis. J Dent Res. 2024;103(11):1066-75
17. Odell E, Kujan O, Warnakulasuriya S, Sloan P. Oral epithelial dysplasia: Recognition, grading and clinical significance. Oral Dis. 2021;27(8):1947-76.
18. Das R, Misra SR, Baskaran P, Mohanty N. Oral erythroplakia harbouring a squamous cell carcinoma. BMJ Case Rep. 2022;15(2):e247983.
19. Deng J, Sun C, Xu G, Wang B, Tzortzopoulou E, Deng D, Giovannetti E. The Oral Microbiome and Cancer. Adv Exp Med Biol. 2025;1472:151-70.
20. Kumar S, Singh N, Kataria SP, Kandoi S, Verma M, Sen R. Cyclin-dependent kinase inhibitor p21 and proliferative marker ki67 in colonic carcinoma. J Cancer Res Ther. 2022;18(4):915-20.
21. De Vicente JC, Peña I, Rodrigo JP, Rodríguez-Santamarta T, Lequerica-Fernández P, Suárez-Fernández L, Allonca E, García-Pedrero JM. Phosphorylated ribosomal protein S6 correlation with p21 expression and inverse association with tumor size in oral squamous cell carcinoma. Head Neck. 2017;39(9):1876-87.
22. Wagner KD, Wagner N. The Senescence Markers p16INK4A, p14ARF/p19ARF, and p21 in Organ Development and Homeostasis. Cells. 2022;11(12):59-66.
23. Hussaini HM, Seo B, Rich AM. Immunohistochemistry and Immunofluorescence. Methods Mol Biol. 2023;25:439-50.