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IMMUNOHISTOCHEMICAL EXPRESSION OF KI67 AND P53 IN ORAL PREMALIGNANT LESIONS AND SQUAMOUS CELL CARCINOMA

Pathology

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ABSTRACT

Background: Oral premalignant lesions (OPMLs), such as leukoplakia and OSMF, affect 2.5% of people and may progress to oral cancer. Early histopathological and molecular diagnosis is vital, especially in India, which reports one-third of global OSCC cases. **Materials and Methods:** A combined retrospective and prospective study was conducted on 63 oral biopsy specimens. Cases with confirmed oral epithelial dysplasia or OSCC and adequate tissue for immunohistochemical analysis were included. Sections were stained for Ki-67 and p53, and their expression was correlated with histopathological diagnosis and tumor grade. **Results:** Ki-67 and p53 expression was observed in 90.48% and 87.30% of cases, respectively. Malignant lesions, particularly non-keratinizing and keratinizing SCC, showed higher biomarker positivity. Although no statistically significant association was found between marker expression and tumor grade or diagnosis type, increased biomarker expression was noted in poorly differentiated tumors. **Conclusion:** Elevated expression of p53 and Ki-67 correlates with malignant transformation in oral lesions, highlighting their role as potential early indicators. Incorporating these markers into routine diagnostics could improve early detection and risk assessment. Further large-scale studies are warranted to validate their prognostic and diagnostic utility.

KEYWORDS

Oral premalignant lesions, oral squamous cell carcinoma, p53, Ki-67, immunohistochemistry, OSMF, biomarker expression.

INTRODUCTION

A Premalignant lesions are conditions with potential to progress into malignancy if untreated, first described by Victor Babeş in 1875. Oral premalignant lesions (OPMLs) affect approximately 2.5% of the population, serving as key indicators for early cancer detection. Major OPMLs include leukoplakia, erythroplakia, erythroleukoplakia, oral submucous fibrosis (OSMF), and lichen planus. Other less significant lesions include leukoedema, leukoderma, and smokeless tobacco keratosis. Histopathology remains the definitive tool for assessing malignant risk.[1]

Leukoplakia, a white patch of uncertain cause, has premalignant potential, with 30% showing dysplasia and 60% of oral cancers arising from such lesions. Proliferative verrucous leukoplakia (PVL) is a high-risk, aggressive variant, often affecting older females.[2]

Erythroplakia, erythroleukoplakia, and OSMF carry high malignant potential, especially with dysplasia or areca nut use. Lichen planus has lower risk but needs monitoring. Frictional keratosis generally poses minimal risk but should still be evaluated.[3]

Oral cancer is a major health concern globally, with ~300,000 new cases annually—62% in developing countries. India contributes to one-third, predominantly oral squamous cell carcinoma (OSCC), which forms 91% of oral malignancies. Risk factors include tobacco, alcohol, areca nut, HPV, and poor oral hygiene. Leukoplakia and OSMF have malignant transformation rates of 5–43% and 7.6% over 10 years, respectively. Histopathology is the gold standard for diagnosis, supported by molecular markers.[4]

The p53 gene, located on chromosome 17p, encodes a tumor suppressor protein. Normally short-lived, mutated p53 accumulates in cells, indicating early carcinogenic change. Mutations mainly occur in exons 5–8 and are found in 40–50% of OSCC cases.[5]

Ki-67 is a proliferation marker expressed in active cell cycle phases. Its overexpression correlates with poor prognosis but may predict better chemotherapy response. In OPMLs, Ki-67 and p53 expression increase with dysplasia severity, indicating malignancy risk. In OSCC, both markers show high expression, especially in poorly differentiated tumors, aiding in diagnosis, prognosis, and treatment planning.[6]

MATERIALAND METHOD

STUDY:

A combined retrospective and prospective study analyzed around 63 oral biopsy specimens. These samples were collected from the oral cavity and examined in the pathology department. The study involved both previously archived and newly received biopsy specimens to evaluate oral lesions, providing insight into the histopathological features of various oral conditions.

INCLUSION CRITERIA:

The study included cases with histologically confirmed oral epithelial dysplasia or squamous cell carcinoma. Only specimens with sufficient tissue for Ki-67 and p53 immunohistochemical staining were selected. Additionally, complete clinical information was required to allow for proper correlation between pathological findings and patient data.

EXCLUSION CRITERIA:

Cases were excluded if they involved jaw metastases originating from non-oral primary cancers, had inadequate or autolyzed tissue samples, or presented with non-neoplastic lesions such as traumatic ulcers or herpetic lesions. These criteria ensured the selection of only relevant and high-quality samples for accurate analysis.

IMMUNOHISTOCHEMISTRY PROTOCOL:

Formalin-fixed paraffin-embedded tissue sections (3–4 µm) were mounted on charged slides, deparaffinized, and antigen retrieval was performed using a pressure cooker. After blocking peroxidase, sections were incubated with Ki-67 or p53 antibodies, followed by HRP and DAB. Slides were counterstained, dehydrated, mounted, and evaluated alongside H&E sections.

STATISTICAL ANALYSIS: Data were analyzed using the chi-square test and Fisher's exact test.

RESULTS AND OBSERVATIONS

Table 1: Distribution Of Patients Based on Immunoexpression of Ki-67 And P-53.

Immuno-expression		No. of Patients	Percentage
ki-67	Positive	57	90.48
	Negative	6	9.52
P-53	Positive	55	87.30
	Negative	8	12.70

Ki-67 was positive in 90.48% and p53 in 87.30% of cases, indicating high cellular proliferation and frequent tumor suppressor gene involvement, with only a small fraction showing negative expression for either marker.

Table 2: Distribution Of Cases According to Ki67 Expression in

Tumour Cells.

% of tumour cells showing ki67 Expression	No. Of cases	Percentage
Low (<10%)	31	49.21
Borderline (10-20%)	20	31.75
Strong (>20%)	12	19.05
Total	63	100.00

In this study, Ki-67 expression was low (<10%) in 49.21% of cases, borderline (10–20%) in 31.75%, and high (>20%) in 19.05%, indicating that nearly half had low proliferative activity, while one-fifth showed elevated proliferation.

Table 3: Correlation Between Histopathological Grade and Ki-67 Immuno-expression.

Diagnosis	Immunoexpression ki-67				P-Value
	Positive (n=47,48)		Negative (n=6,7)		
	No. of Patients	Percentage	No. of Patients	Percentage	
Well Diff.	17	35.42	3	50	0.73
Moderately Diff.	17	35.42	3	50	
Poorly Diff.	14	29.17	0	0	
Well Diff.	17	36.17	3	42.86	0.69
Moderately Diff.	16	34.04	4	57.14	
Poorly Diff.	14	29.79	0	0	

Among 54 patients, Ki-67 expression showed no significant association with tumor grade (p = 0.73). Positive cases were evenly distributed across grades, while negative cases appeared only in well and moderately differentiated tumors, with none poorly differentiated.

DISCUSSION

The immunohistochemical analysis revealed Ki-67 positivity in 90.48% and p53 positivity in 87.30% of cases, indicating a strong association with increased cellular activity and tumor suppressor gene involvement. However, nearly half the tumors showed low Ki-67 expression (<10%), and only 19.05% had strong expression (>20%). Similarly, p53 expression was low (0–10%) in 68.25% of cases, with strong expression (>50%) in just 3.17%, suggesting that marked overexpression was uncommon. In a comparable study, Gupta S et al.[7] found p53 positivity in 66.6% and negativity in 33.4% of cases, while Ki-67 showed higher expression at 85.18%, indicating notable proliferative and genetic activity in oral lesions. Similarly, Angiero F et al.[8] observed varied immune-expression with disease severity. Group 1 (no/mild dysplasia) had no Ki-67 and minimal p53 (6.9%). Groups 2 (moderate/severe dysplasia) and 3 (invasive carcinoma) showed 100% Ki-67 positivity. P53 positivity increased from 64.3% in Group 2 to 81.8% in Group 3, with significant differences ($p \leq 0.001$) between Group 1 and others.

The study outlines the distribution of Ki-67 immunoexpression across different histopathologicalgrades in 54 patients. Among the 48 Ki-67 positive cases, 35.42% were well-differentiated, 35.42% moderately differentiated, and 29.17% poorly differentiated. Conversely, the 6 Ki-67 negative cases were split evenly, with 50% well-differentiated and 50% moderately differentiated, and no poorly differentiated cases. The p-value of 0.73 suggests no significant relationship between Ki-67 expression and tumordifferentiation. In a comparable studyGupta S et al.[7]demonstrated a strong correlation between Ki-67 expression and tumor grade, with only 29.2% positivity in well-differentiated tumors versus 73.3% in moderately and poorly differentiated ones, indicating its role in tumor aggressiveness. Similarly, Raju B et al.[9] found increasing Ki-67 expression with dysplasia severity, showing >10% positivity in 92.8% of mild and 81.8% of moderate/severe dysplasia (p = 0.03), highlighting Ki-67's diagnostic relevance.

CONCLUSION

The study reveals a strong link between oral epithelial changes and increased p53 and Ki-67 expression, highlighting their potential as early markers of malignancy. Their use in screening may aid early detection and risk assessment. However, larger studies are needed to confirm their clinical value and uncover underlying genetic mechanisms for better diagnosis and personalized prevention strategies.

REFERENCES

1. Holmstrup P, Vedtofte P, Reibel J, Stoltze K. Oral premalignant lesions: is a biopsy reliable. J Oral Pathol Med 2007;36:262–266.
2. Cabay RJ, Morton TH, Epstein JB. Proliferative verrucous leukoplakia and its progression to oral carcinoma: a review of the literature. J Oral Pathol Med 2007;36:255–261.
3. Messadi DV, Waibel JS, Mirowski GW. White lesions of the oral cavity. Dermatol Clin 2003;21:63–78.
4. Liu SC, Klein-Szanto AJ. Markers of proliferation in normal and leukoplakic oral epithelia. Oral Oncol 2000;36:145-51.
5. Chandra A, Singh A, Sebastian BT, et al. Oral squamous cell carcinomas in age distinct population: a comparison of p53 immunoexpression. J Cancer Res Ther. 2013;9:587–91.
6. Humayun, S., & Prasad, V. R. "Expression of p53 protein and ki-67 antigen in oral premalignant lesions and oral squamous cell carcinomas: An immunohistochemical study." National Journal of Maxillofacial Surgery, 2011;2(1), 38–46.
7. Gupta S, Khatri SL, Deepak, Khatri J. A study of Ki 67, p53 and their combination as immunomarkers in squamous cell carcinoma of oral cavity. IP Journal of Diagnostic Pathology and Oncology 2020;5(2):179–186.
8. Angiero F, Berenzi A, Benetti A, Rossi E et al. Expression of P16, P53 and Ki-67 Proteins in the Progression of Epithelial Dysplasia of the Oral Cavity. ANTICANCER RESEARCH 2008; 28: 2535-2540.
9. Raju B, Mehrotra R, Øijordsbakken G, Al-Sharabi AK, Vasstrand EN, Ibrahim SO. Expression of p53, Cyclin D1 and Ki-67 in Pre-malignant and Malignant Oral Lesions: Association with Clinicopathological Parameters. Anticancer Research 2005;25: 4699-4706.