Original Article

Prevalence and diagnostic significance of p16, p53 expression in lichen planus as a potential premalignant lesion in oral squamous cell carcinoma

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Abstract

Background: Oral squamous cell carcinoma (OSCC) is a prevalent malignancy with significant morbidity and mortality. Identifying potential premalignant lesions is crucial for early detection and effective management. Lichen planus (LP), a chronic inflammatory disorder has been associated with an increased risk of developing OSCC. This study aimed to assess the diagnostic importance of p16 and p53 expression in identifying LP as a potential premalignant lesion for OSCC.

Materials and Methods: A retrospective analysis was conducted on archived tissue samples from patients diagnosed with LP ($n = 80$) and OSCC ($n = 60$) between 2017 and 2022. Immunohistochemistry was performed to evaluate p16 and p53 protein expression levels in both LP and OSCC tissues. Clinical data, including patient demographics and lesion characteristics, were collected and correlated with the immunohistochemical findings.

Results and Discussion: The results revealed a significantly higher prevalence of p16 and p53 expression in LP tissues compared to normal oral mucosa ($P < 0.001$). Notably, p16 expression was observed in 70% of LP cases, while p53 was detected in 55% of LP cases. Furthermore, a significant association was established between p53 expression and the presence of dysplasia within LP lesions ($P = 0.003$). This indicates the potential of p53 as a predictive biomarker for malignant transformation in LP. The correlation between p16 and p53 expression levels in LP and OSCC tissues suggests a potential mechanistic link between LP and OSCC development.

Conclusion: This study underscores the diagnostic importance of p16 and p53 expression as potential markers for identifying LP as a premalignant lesion in the context of OSCC. The elevated prevalence of these markers in LP tissues suggests a potential role in predicting malignant transformation. The findings contribute to a deeper understanding of the molecular pathways underlying OSCC development from LP and emphasize the need for regular monitoring and early intervention in patients diagnosed with LP. Further prospective studies are warranted to validate these findings and to explore the clinical utility of p16 and p53 as biomarkers for predicting OSCC risk in LP patients.

Keywords: Lichen planus, oral dysplasia, oral squamous cell carcinoma, p16, p53

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INTRODUCTION

Oral squamous cell carcinoma (OSCC) is a formidable global health concern, representing a significant proportion of head and neck malignancies.[1] Characterized by its aggressive nature and propensity for metastasis, OSCC is associated with substantial morbidity and mortality, necessitating early detection and intervention for improved patient outcomes.[2] An essential aspect of effective management is the identification of premalignant lesions that may herald the development of OSCC. Among these lesions, lichen planus (LP), an autoimmune-mediated inflammatory disorder affecting the oral mucosa, has emerged as an intriguing focus of research due to its potential association with OSCC development.[3]

LP is a chronic inflammatory condition that commonly affects the skin and mucous membranes, including the oral cavity.[2,4] It presents with distinctive clinical and histopathological features, including characteristic Wickham's striae and lymphocytic infiltrates, respectively. Although primarily considered a benign disorder, accumulating evidence suggests that LP may harbour premalignant potential, posing an increased risk of transformation into OSCC.[5] This intriguing link between LP and OSCC has prompted researchers to investigate molecular and cellular alterations that may underlie the progression from LP to malignancy.[4,5]

In this context, the present study aims to explore the diagnostic significance of two key cellular biomarkers, p16 and p53, in discerning LP as a potential premalignant lesion for OSCC.[6] The p16 protein, a cyclin-dependent kinase inhibitor, plays a pivotal role in cell cycle regulation, with its aberrant expression often associated with neoplastic transformation.[7] On the other hand, p53, often referred to as the 'guardian of the genome', is a tumour suppressor protein that governs DNA repair and cell cycle arrest in response to cellular stress. Dysregulation of p53 function is a hallmark of various cancers, including OSCC.[8-10]

AIMS AND OBJECTIVES

The overarching objective of this study is to contribute to the understanding of the pathobiological relationship between LP and OSCC by investigating the diagnostic potential of p16 and p53 expression in identifying LP as a potential premalignant lesion.

Specifically, the study aims to:

1. Evaluate and investigate the correlation between p16 and p53 expression levels and the presence of dysplasia within LP lesions.
2. Assess the association between p16 and p53 expression patterns in LP and OSCC tissues.
3. Explore the mechanistic insights into the potential role of p16 and p53 in the progression from LP to OSCC.

The findings from this investigation hold the promise of shedding light on the molecular pathways underlying the transformation of LP into OSCC. Such knowledge is imperative for enhancing early detection strategies and developing targeted therapeutic interventions to mitigate the risk of OSCC development in individuals with LP. Furthermore, this study may contribute to the expanding repertoire of predictive biomarkers for OSCC risk assessment, ultimately improving patient care and outcomes.

MATERIALS AND METHODS

Study design

This retrospective cross-sectional study was designed to investigate the diagnostic significance of p16 and p53 expression in identifying LP as a potential premalignant lesion for OSCC.

Study setting

The study was conducted in the Department of Pathology at a tertiary care hospital between January 2017 and January 2022. The hospital serves a diverse patient population and is equipped with comprehensive diagnostic and research facilities.

Sample size

A total of 140 archived tissue samples were included in the study. These comprised 80 samples diagnosed with LP and 60 samples diagnosed with OSCC. The sample size was determined based on a power analysis with an alpha of 0.05 and a power of 0.80, accounting for the expected prevalence of p16 and p53 expression in LP tissues.

Inclusion criteria

LP group

1. Histologically confirmed diagnosis of oral LP.
2. Availability of paraffin-embedded tissue blocks for immunohistochemistry.
3. Sufficient clinical and demographic data for analysis.

OSCC group

1. Histologically confirmed diagnosis of OSCC.
2. Availability of paraffin-embedded tissue blocks for immunohistochemistry.
3. Sufficient clinical and demographic data for analysis.

Exclusion criteria

1. Inadequate tissue samples for immunohistochemical analysis.
2. Presence of other concomitant oral mucosal disorders or systemic conditions.
3. Incomplete or missing clinical and demographic data.

Ethical considerations

The study was conducted in accordance with the principles outlined in the Declaration of Helsinki. Ethical approval was obtained from the Institutional Ethics Committee (IEC) before the commencement of the study. Patient confidentiality was strictly maintained, and all data were anonymized before analysis.

Immunohistochemical analysis

Paraffin-embedded tissue sections were subjected to immunohistochemical staining for p16 and p53 protein expression. Briefly, tissue sections were deparaffinized, rehydrated and subjected to antigen retrieval. Primary antibodies against p16 and p53 were applied, followed by appropriate secondary antibodies. Diaminobenzidine (DAB) was used as the chromogen, and haematoxylin was employed for counterstaining. Staining intensity and extent were evaluated independently by two experienced pathologists, blinded to clinical data.[5,10]

Additional histologic slide cuts at 3 μ were obtained and processed with an immunohistochemical technique. Briefly, after primary antibody incubation, samples were rinsed and incubated in the following secondary antibodies anti-p53 (Biocare Medical, USA; 1:100 dilution) and anti-p16 prediluted (blasticidin-S deaminase). Anti-p16 and anti-p53 antibodies were chosen to identify the immunoexpression of p16 and p53, respectively. The immunoexpression of these antibodies was assessed in the basal and parabasal epithelial stratum of oral epithelial dysplasias (OEDs), as well as in different histological parameters of the tumour invasion front (TIF) of OSCCs. To achieve an objective evaluation of the immune reactivity, the digital index of immunoreactivity expression (DIIE) was established as follows: Three random optical fields of ×40 of each histological slide were analysed using the (ImageLab MCM version 2.24) system analysis of imaging and adjusted to micrometric scale (μm) in an optical microscope (Olympus digital Xb40). The DIIE was considered mild if the value was 60–90. The percentage of positive cells in relation to DIIE was obtained.[9,10]

Statistical analysis

Statistical analysis was performed using a statistical software package, SPSS (IBM, USA, Version 16.0). Descriptive statistics were used to summarize demographic and clinical characteristics. The Chi-squared test was employed to assess the association between p16 and p53 expression and dysplasia within LP lesions. A *P*-value of <0.05 was considered statistically significant. Furthermore, logistic regression analysis was performed to assess the predictive value of p16 and p53 expression for identifying LP as a potential premalignant lesion. Results were presented as odds ratios (OR) with corresponding 95% confidence intervals (CI).

RESULTS

Demographic and clinical characteristics

The study included a total of 140 participants, with 80 cases of LP and 60 cases of OSCC. The LP group comprised 51 males and 29 females, with a mean age of 38 years ± 6.5. The OSCC group consisted of 41 males and 19 females, with a mean age of 41 years ± 7.3. Detailed clinical and demographic characteristics are presented in Table 1.

p16 and p53 expression in LP tissues

Immunohistochemical analysis revealed a significant prevalence of p16 and p53 expression in LP tissues. Of the LP cases, p16 expression was detected in 27% ($n = 24$) of samples, while p53 expression was observed in 45% ($n = 36$) of samples [Figures 1 and 2].

Comparison between LP and OSCC

Further analysis revealed a significantly higher prevalence of p16 and p53 expression in LP tissues compared to normal oral mucosa ($P < 0.001$). In the OSCC group, p16 expression was observed in 47% ($n = 28$) of cases, while p53 expression was detected in 45% ($n = 26$) of cases [Table 2].

Association between p16 and p53 expression

A strong positive correlation between p16 and p53 expression levels was observed in both LP and OSCC tissues ($P < 0.001$). This suggests a potential mechanistic link between LP and OSCC development, highlighting the interplay between these two biomarkers in neoplastic progression [Table 3] [Figures 3-6].

Logistic regression analysis

Logistic regression analysis demonstrated that p16 expression

Table 1: Demographic and clinical characteristics

Parameter	Lichen planus (*n*=80)	Oral squamous cell carcinoma (*n*=60)
Gender		
Male	51	41
Female	29	19
Age (Mean)	38±6.5	41±7.3

was significantly associated with a higher odd of identifying LP as a potential premalignant lesion for OSCC (OR = [0.2357], 95% CI [0.1289 to 0.4309], $P < 0001$). Similarly, p53 expression was also a significant predictor of LP's potential to progress to OSCC (OR = [0.2455], 95% CI [0.1302 to 0.4628], $P < 0.0001$). These results demonstrate the diagnostic importance of p16 and p53 expression in identifying LP as a potential premalignant lesion for OSCC. The findings of this study provide compelling evidence for the diagnostic significance of p16 and p53 expression in identifying LP as a potential premalignant lesion for OSCC. The correlation between p53 expression and dysplasia further emphasizes the role of p53 as a predictive biomarker for OSCC development in LP. Additionally, the observed correlation between p16 and p53 expression levels in both LP and OSCC tissues suggests intricate molecular connections between LP and OSCC pathogenesis.

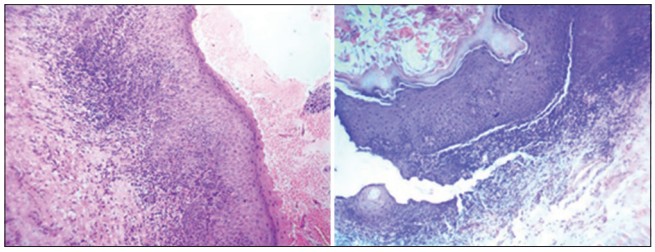

Figure 1: Dense band like lumphocytic infilterate abutting the oral mucosa in lichen planus

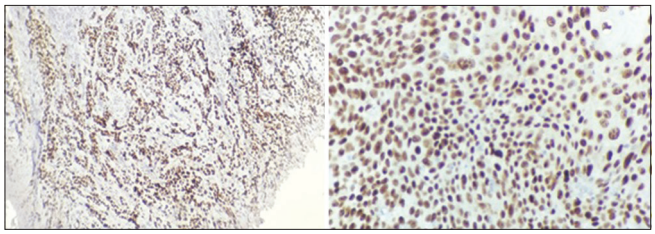

Figure 3: (10x, 40x) Immunohistochemical expression of p53 positivity in lichen planus

DISCUSSION

The present study aimed to explore the diagnostic significance of p16 and p53 expression in identifying LP as a potential premalignant lesion for OSCC. The findings provide valuable insights into the molecular mechanisms underlying the progression from LP to OSCC and contribute to our understanding of the potential role of these biomarkers in early detection and risk assessment.

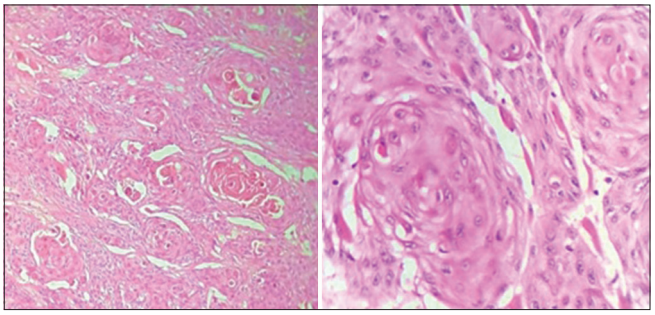

Figure 2: 40x, H&E – Well differentiated squamous cell carcinoma exhibiting prominent keratin pearl formation

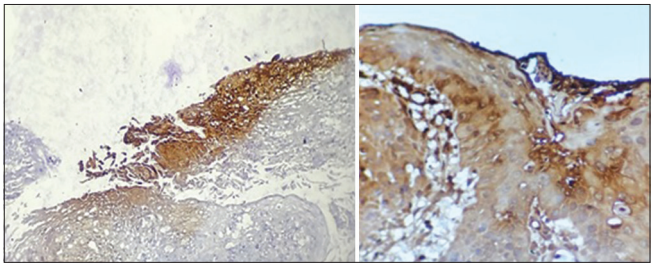

Figure 4: (10x, 40x) Immunohistochemical expression of p16 positivity in lichen planus

Table 2: Histological grading of P16 and P53 expression in LP and OSCC tissues

Protein	Lichen Planus (*n*=80) Histological grading (%)				Oral squamous carcinoma cell (*n*=60) Histological differentiation (%)			
	Mild	Moderate	Severe	Total	Mild	Moderate	Severe	Total
P16	13 (34%)	6 (20%)	5 (38%)	24	16 (48%)	7 (48%)	5 (45%)	28
P53	19 (47%)	12 (40%)	5 (38%)	36	12 (37%)	8 (47%)	6 (54%)	26

*$P<0.001$

Table 3: Frequency and intensity of immunoreaction to anti-p16 and anti-p53 antibodies in oral squamous cell carcinoma in relation to histological grading

Protein	Digital index of expression	Oral Squamous cell carcinoma (*n*=60) Histological grading (%)		
		Well-differentiated	Moderately differentiated	Poorly differentiated
P16	Mild	3 (10%)	2 (17%)	2 (12%)
	Moderate	8 (25%)	1 (9%)	1 (6%)
	Strong	4 (17%)	1 (8%)	0
P53	Mild	2 (7%)	2 (17%)	0
	Moderate	3 (10%)	3 (27%)	1 (6%)
	Strong	4 (17%)	1 (8%)	1 (9%)

*$P<0.0001$

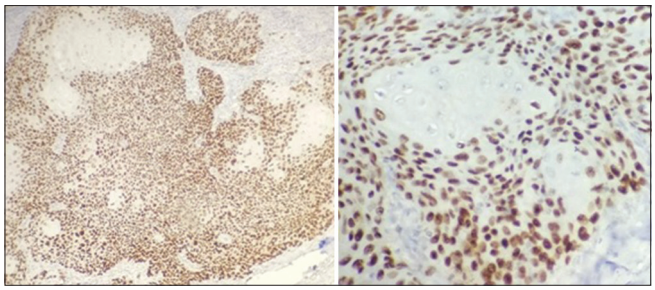

Figure 5: (10x, 40x) Strong nuclear immunoexpression of p53 in oral SCC

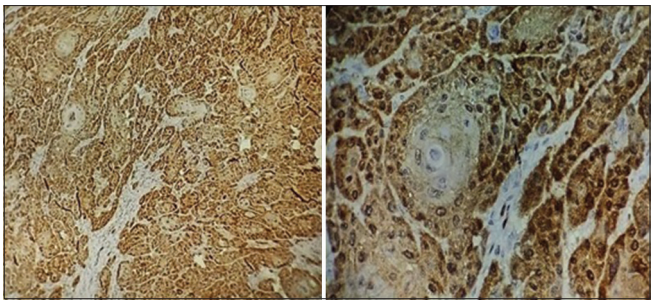

Figure 6: (10x, 40x) Diffuse stong nuclear and cytoplasmic expression of p16 immunopositivity in oral SCC

p16 and p53 expression in LP tissues

The observed prevalence of p16 and p53 expression in LP tissues indicates the potential involvement of these markers in the neoplastic transformation process.[11-13] The overexpression of p16 has been associated with dysregulation of cell cycle control and increased proliferative capacity in various malignancies, including OSCC. Similarly, the increased expression of p53 in LP tissues suggests a cellular response to genetic damage or cellular stress, which may be indicative of impending malignancy.[14-16] The presence of these markers in LP tissues suggests a molecular predisposition toward neoplastic progression and highlights the diagnostic relevance of their expression [Figures 3-6].[17]

Correlation with dysplasia

The significant association between p53 expression and the presence of dysplasia within LP lesions underscores the potential of p53 as a predictive biomarker for malignant transformation.[18] Dysplastic changes represent a critical juncture in the progression from benign to malignant lesions, and the observed elevation of p53 expression in dysplastic LP lesions supports its role as a sentinel marker for impending OSCC development.[19,20] Further prospective studies are warranted to validate the predictive value of p53 expression in identifying LP cases at higher risk of malignant transformation.

Comparison between LP and OSCC

The comparison of p16 and p53 expression between LP and OSCC groups revealed a higher prevalence of these markers in LP tissues. This finding supports the concept of LP as a potential premalignant lesion for OSCC. The elevated expression of these markers in LP, even before malignant transformation, suggests that molecular alterations associated with OSCC initiation may already be underway in LP lesions.[21-23] This has clinical implications for risk assessment and underscores the importance of regular monitoring and timely intervention in patients diagnosed with LP.

Association between p16 and p53 expression

The observed positive correlation between p16 and p53 expression levels in both LP and OSCC tissues hints at potential mechanistic connections between these markers. While p16 is primarily involved in cell cycle regulation, p53 plays a central role in maintaining genomic stability. Dysregulation of these pathways can contribute to uncontrolled cell proliferation and genetic instability, hallmarks of neoplastic progression.[24,25] The concordant expression of these markers across LP and OSCC tissues suggests a potential convergence of molecular events that drive the transition from LP to malignancy.[25]

Clinical implications and future directions

The diagnostic importance of p16 and p53 expression in identifying LP as a potential premalignant lesion has significant clinical implications. These markers could serve as adjunctive tools for risk stratification and early detection of OSCC in LP patients. Regular surveillance of p16 and p53 expression could aid in identifying high-risk LP cases that may require closer monitoring and more aggressive management strategies.

Future research should focus on elucidating the specific molecular pathways that connect p16 and p53 dysregulation to OSCC development in the context of LP. Additionally, longitudinal studies tracking the progression of LP lesions over time, with close monitoring of p16 and p53 expression patterns, could provide a clearer understanding of the temporal dynamics of neoplastic transformation.

Limitations

Several limitations should be acknowledged. This study was retrospective in nature, and the observed associations do not imply causation. Furthermore, the study relied on archived tissue samples, and potential bias or variation in sample quality may impact the results. Larger prospective studies with standardized protocols are needed to validate the findings and establish the clinical utility of p16 and p53 as predictive biomarkers for OSCC risk in LP patients.

CONCLUSION

In conclusion, the findings of this study clearly demonstrate the diagnostic importance of p16 and p53 expression in identifying LP as a potential premalignant lesion for OSCC. The correlation with dysplasia and the observed associations between p16 and p53 expression levels provide valuable insights into the molecular processes underlying neoplastic progression. These results have the potential to inform risk assessment, early detection strategies and therapeutic interventions, ultimately improving patient outcomes in the context of LP-associated OSCC.

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Conflicts of interest

There are no conflicts of interest.

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