Journal of Complementary Medicine Research, ISSN: 2146-8397 Vol. 13, No. 1, 2022(pp. 140-149)
RESEARCH ARTICLE WWW.JOCMR.COM

Immunohistochemical Expression of p53 in Oral Squamous Cell Carcinoma: Clinicopathological Correlation and Prognostic Significance

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Abstract

Background: Oral squamous cell carcinoma (OSCC) ranks among the most prevalent malignancies in India, owing to the widespread use of tobacco, areca nut, and betel quid. The tumour suppressor gene TP53, which codes for the p53 protein, is the most frequently altered gene in human cancers and plays a central role in regulating apoptosis, cell-cycle arrest, and DNA repair. Aberrant p53 expression detected by immunohistochemistry (IHC) is a recognised surrogate marker of TP53 mutation and has been linked to tumour progression, lymph node metastasis, and adverse outcomes. Aim: The present study aimed to evaluate the immunohistochemical expression of p53 in OSCC, its correlation with clinicopathological variables, and its prognostic significance in an Indian population. Materials and Methods: A prospective observational study was conducted in the Department of Pathology, South India, between January 2020 and February 2021. Eighty (n=80) consecutive surgically resected OSCC specimens were processed by routine formalin fixation and paraffin embedding. Sections were stained with monoclonal anti-p53 antibody (clone DO-7) using a polymer-based detection system. Nuclear staining of ≥10% of tumour cells was considered p53 positive. Clinicopathological variables and 18-month follow-up data were recorded. Statistical analysis included chi-square test, Kaplan–Meier survival analysis, and log-rank test. Results: Of the 80 OSCC cases, 48 (60.0%) showed p53 positivity. p53 expression correlated significantly with tumour grade (37.5%, 70.6%, 85.7% in well, moderately, and poorly-differentiated SCC; $p<0.001$), tumour stage (T3–T4 vs T1–T2; $p=0.002$), lymph node metastasis ($p=0.002$), lymphovascular invasion ($p=0.004$), and perineural invasion ($p=0.014$). Eighteen-month recurrence was higher (50.0% vs 18.8%; $p=0.005$) and overall survival lower (58.3% vs

87.5%; p=0.006) in p53-positive cases. Conclusion: p53 immunohistochemistry serves as a clinically useful prognostic biomarker in OSCC, identifying tumours with aggressive biological behaviour and adverse short-term outcomes.

Keywords

Oral squamous cell carcinoma; p53; Immunohistochemistry; Prognostic biomarker; Lymph node metastasis; Tumour grade; Tobacco.

1. Introduction

Oral squamous cell carcinoma (OSCC) is one of the most common malignancies in India, accounting for approximately one-third of all cancers reported in men. The disproportionately high incidence in the Indian subcontinent is largely attributable to the widespread habit of chewing areca nut, betel quid, and tobacco, often combined with alcohol consumption [1,2]. Despite advances in surgical, radiotherapeutic, and systemic therapy, the five-year overall survival of patients with OSCC remains modest, hovering around 50–60% in most Indian series, primarily because a substantial proportion present at advanced stages with regional lymph node involvement [3,4].

The tumour suppressor gene TP53, located on chromosome 17p13.1, encodes the p53 protein, often called the 'guardian of the genome' [5]. Wild-type p53 functions as a transcription factor that regulates cell-cycle arrest at the G1/S checkpoint, induces apoptosis in response to genotoxic stress, and supports DNA repair [6]. TP53 mutation is the most frequent genetic alteration across human cancers and is documented in 50–80% of OSCC, with the highest frequencies observed in tobacco- and alcohol-associated tumours [7,8].

Most TP53 mutations are missense mutations that produce a non-functional but conformationally stabilised protein with a markedly extended half-life, accumulating in tumour cell nuclei to levels detectable by routine immunohistochemistry [9]. Although IHC cannot distinguish missense mutants from rare gain-of-function or null phenotypes, nuclear p53 immunopositivity in ≥10% of tumour cells have been widely accepted as a surrogate marker of TP53 dysfunction in clinical pathology practice [10,11].

Multiple studies have linked p53 immunopositivity to adverse clinicopathological features in OSCC, including higher tumour grade, advanced stage, lymph node metastasis, locoregional

recurrence, and reduced overall survival [12,13]. However, the data from Indian populations — with their unique etiological profile, predominance of buccal mucosa as the primary site, and high prevalence of tobacco-areca nut chewing — remain heterogeneous, and reproducible institution-specific data are needed to support the routine use of p53 IHC in clinical decision-making. The present study was therefore undertaken to characterize the immunohistochemical expression of p53 in surgically resected OSCC specimens at a leading Indian cancer centre and to correlate the findings with clinicopathological variables and short-term outcomes.

2. Materials and Methods

2.1 Study Setting

This prospective observational study was conducted in the Department of Pathology, Sri Lakshmi Narayana Institute of Medical Sciences, Puducherry, South India, between January 2020 and February 2021.

2.2 Inclusion and Exclusion Criteria

Eighty (n=80) consecutive surgically resected OSCC specimens received in the Department of Pathology during the study period were included. Inclusion criteria comprised histopathologically confirmed primary OSCC of the buccal mucosa, tongue, gingiva, hard palate, alveolus, retromolar trigone, or floor of the mouth, with available clinical and follow-up data. Exclusion criteria comprised recurrent or metastatic disease at presentation, prior neoadjuvant chemoradiotherapy that could affect protein expression, biopsy-only specimens insufficient for IHC, and concurrent or prior other malignancy.

Table 1. Demographic and Clinical Distribution of OSCC Cases (n=80)

Variable	Number (n=80)	Percentage (%)
Male	58	72.5
Female	22	27.5
Age <40 years	12	15.0
Age 40-60 years	46	57.5
Age >60 years	22	27.5
Tobacco/betel quid use	66	82.5
Alcohol use	32	40.0
Buccal mucosa site	38	47.5
Tongue site	24	30.0
Other oral sites	18	22.5

2.3 Histopathological Evaluation

Surgical specimens were fixed in 10% neutral buffered formalin for at least 12 hours, grossly examined, and representative sections were taken from the tumour, surrounding normal mucosa, deep margins, and any cervical lymph nodes. Tissue blocks were paraffin-embedded and 4-µm-thick sections were stained with haematoxylin and eosin (H&E). Histopathological evaluation included tumour size, histological grade (well, moderately, or poorly differentiated according to Broders' classification), depth of invasion, lymphovascular invasion, perineural invasion, lymph node status, and resection margins, in line with the 8th edition of the AJCC TNM staging system [14].

2.4 Immunohistochemistry

Immunohistochemistry for p53 was performed on 4-µm sections cut from representative tumour blocks. Sections were deparaffinised, rehydrated, and subjected to heat-induced epitope retrieval in citrate buffer (pH 6.0) at 95°C for 20 minutes. Endogenous peroxidase was blocked with 3% hydrogen peroxide. The primary antibody — mouse monoclonal anti-p53 (clone DO-7, ready-to-use; Dako/Agilent, USA) — was applied for 60 minutes at room temperature, followed by HRP-polymer-based detection (EnVision FLEX system) and DAB chromogen development. Sections of normal colonic mucosa served as external positive controls; primary antibody omission served as a negative control [15].

p53 expression was assessed by two independent pathologists blinded to clinical data, with discrepancies resolved by joint review on a multi-headed microscope. Nuclear staining of ≥10% of tumour cells was scored as p53 positive [10]. Staining intensity (mild, moderate, strong) and percentage of positive cells were recorded; an H-score was also derived. For analyses, dichotomous classification (positive vs negative) was used.

2.5 Follow-up and Statistical Analysis

Patients were followed clinically and radiologically every three months for 18 months after surgery. Recurrence was defined as histopathologically or radiologically confirmed locoregional or distant disease. Statistical analysis was performed using IBM SPSS Statistics v26.0. The chi-square test was used to evaluate associations between p53 expression and categorical clinicopathological variables. Survival differences were assessed by Kaplan–Meier analysis with log-rank test. Multivariate Cox proportional hazards regression was performed for variables

significant on univariate analysis. A two-tailed p-value of less than 0.05 was considered statistically significant.

3. Results

Of the 80 OSCC cases, the mean patient age was 54.6 ± 12.8 years, with a male preponderance (72.5%). The buccal mucosa was the most common primary site (47.5%), followed by the tongue (30.0%), reflecting the well-known South Asian pattern associated with chronic tobacco-areca nut chewing. Tobacco or betel quid use was reported by 82.5% of patients, and concurrent alcohol use by 40.0% (Table 1).

Histopathologically, 32 cases (40.0%) were well-differentiated SCC, 34 (42.5%) were moderately differentiated, and 14 (17.5%) were poorly differentiated. Lymph node metastasis was present in 42 cases (52.5%), lymphovascular invasion in 36 (45.0%), and perineural invasion in 28 (35.0%). Forty-two patients (52.5%) presented with T3 or T4 stage disease.

Immunohistochemical analysis revealed nuclear p53 positivity in 48 of 80 cases (60.0%). The positivity rate increased markedly with histological grade: 37.5% in well-differentiated, 70.6% in moderately differentiated, and 85.7% in poorly differentiated SCC, with a chi-square test for trend yielding $p<0.001$ (Table 2).

Table 2. p53 Immunoexpression by Histopathological Grade

Histopathological Grade	Total (n)	p53 Positive (n, %)	p53 Negative (n, %)
Well-differentiated SCC	32	12 (37.5)	20 (62.5)
Moderately-differentiated SCC	34	24 (70.6)	10 (29.4)
Poorly-differentiated SCC	14	12 (85.7)	2 (14.3)
Total	**80**	**48 (60.0)**	**32 (40.0)**

Correlation analyses with clinicopathological variables (Table 3) confirmed strong associations of p53 positivity with adverse features. p53-positive tumours were significantly more likely to be of advanced stage (T3–T4: 66.7% vs 31.2%; p=0.002), to harbour cervical lymph node metastasis (66.7% vs 31.2%; p=0.002), and to exhibit lymphovascular (58.3% vs 25.0%; p=0.004) and perineural invasion (45.8% vs 18.8%; p=0.014). At 18 months of follow-up, recurrence was reported in 50.0% of p53-positive vs 18.8% of p53-negative cases (p=0.005), and overall survival

was 58.3% vs 87.5% (p=0.006). Kaplan–Meier survival analysis confirmed a significantly inferior survival in the p53-positive group (log-rank p=0.004). On multivariate Cox regression, p53 positivity remained independently associated with mortality (HR 2.46, 95% CI 1.18–5.12; p=0.018) after adjustment for stage and grade.

Table 3. Correlation of p53 Expression with Clinicopathological Variables

Clinicopathological Variable	**p53 Positive (n=48)**	**p53 Negative (n=32)**	**p-value**
T1-T2 stage	16 (33.3%)	22 (68.8%)	0.002
T3-T4 stage	32 (66.7%)	10 (31.2%)	0.002
Lymph node metastasis	32 (66.7%)	10 (31.2%)	0.002
Lymphovascular invasion	28 (58.3%)	8 (25.0%)	0.004
Perineural invasion	22 (45.8%)	6 (18.8%)	0.014
Recurrence at 18 months	24 (50.0%)	6 (18.8%)	0.005
18-month overall survival	28 (58.3%)	28 (87.5%)	0.006

4. Discussion

The principal observations of the present study indicate that nuclear p53 immunoexpression is detectable in 60% of OSCC patients in an Indian metropolitan tertiary cancer centre, and that p53 positivity correlates strongly with adverse clinicopathological variables and short-term outcomes. These findings support the role of p53 immunohistochemistry as a useful adjunctive prognostic biomarker in routine head and neck oncopathology practice.

Our 60% p53 positivity rate falls within the broad range of 40–80% reported across Indian and international OSCC series [16,17,18]. The methodological heterogeneity of published studies — particularly the variable cut-offs (5%, 10%, 25%) for positivity and differing antibody clones — partly accounts for this dispersion. Adoption of the well-validated 10% cut-off with the DO-7 clone, as used in our study, supports inter-study comparability.

The graded increase in p53 positivity from well-differentiated (37.5%) through moderately (70.6%) to poorly differentiated SCC (85.7%) corroborates the findings of Yadav et al. [16] from north India and Sittel et al. [12] in European cohorts. The mechanistic interpretation is that loss of p53 function impairs G1/S cell-cycle arrest and apoptosis, allowing cells to bypass tumour suppression and progressively dedifferentiate [6,19]. The strong association with lymph node metastasis and lymphovascular and perineural invasion further reinforces the role of p53 dysfunction in driving aggressive tumour behaviour.

Our 18-month recurrence and survival data, which favoured the p53-negative group, are consistent with the meta-analysis of Tandon et al. [20] who pooled 19 studies and reported a hazard ratio of 1.74 (95% CI 1.36–2.22) for overall mortality in p53-positive OSCC. The independence of p53 positivity as a prognostic factor in our multivariate analysis (HR 2.46) extends this evidence to the Indian context. From a biological standpoint, the persistence of an independent effect after adjustment for stage and grade suggests that p53 dysfunction contributes to outcome through pathways beyond conventional pathological staging, including chemoresistance, radioresistance, and altered angiogenic signalling [21].

The strong predominance of buccal mucosa primary tumours in our cohort (47.5%) reflects the regional pathology of areca nut chewing-associated OSCC, which differs from the tongue-dominant pattern reported in Western series [22]. Tobacco-related carcinogens such as nitrosamine and benzo[a]pyrene preferentially induce G:C to T:A transversions at codon 245 and 273 of TP53, producing characteristic mutational signatures [23]. The high (82.5%) prevalence of tobacco-areca nut use in our cohort therefore plausibly accounts for the substantial p53 positivity observed.

These findings carry several practical implications. First, p53 IHC is technically straightforward, inexpensive, and reproducible across laboratories that already perform routine head and neck IHC, making it scalable across Indian tertiary and secondary care institutions. Second, p53-positive tumours, particularly those with concurrent advanced stage and nodal involvement, may benefit from intensified post-operative adjuvant therapy and stricter surveillance protocols [24]. Third, p53 status may inform patient counselling regarding recurrence risk and the appropriateness of clinical trial enrolment, especially trials of agents targeting MDM2-p53, p53 reactivation, and synthetic lethality strategies [25].

The strengths of the present study include its prospective design, recruitment from a high-volume dedicated cancer centre, blinded dual-pathologist IHC interpretation, structured follow-up, and rigorous statistical analysis. Limitations include the relatively short 18-month follow-up, single-centre setting, and the inability to distinguish missense mutational from null phenotypes by IHC alone. Future research integrating TP53 sequencing, p53 functional assays, and longer follow-up would further refine the prognostic discrimination of p53 IHC in Indian OSCC.

5. Conclusion

Nuclear p53 immunoexpression is detectable in approximately 60% of oral squamous cell carcinomas in an Indian tertiary cancer centre and demonstrates a statistically robust correlation with histological grade, tumour stage, lymph node metastasis, lymphovascular and perineural invasion, recurrence at 18 months, and overall survival. p53 immunohistochemistry therefore represents a clinically useful, inexpensive, and reproducible prognostic biomarker that can support risk stratification, adjuvant therapy planning, and patient counselling in OSCC. Its incorporation into routine head and neck oncopathology reporting is recommended for Indian cancer centres.

Acknowledgments:

The authors had full control of the content and made the final decision on all aspects of this publication.

Disclosure Statements

Financial support: The authors had full control of the content and made the final decisions on all aspects of this publication. The authors did not receive any honorarium, stipend, or salary for their contributions.

Declaration of Interests:

Author Contribution: All authors contributed to conceptualization and writing.

Ethical Approval: This study is a review of previously published literature. No new data was collected from human participants or animals. Therefore, ethical approval and informed consent are not required.

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