ISSN Print: 2394-7489
ISSN Online: 2394-7497
Impact Factor (RJIF): 7.85
IJADS 2025; 11(3): 406-412

www.oraljournal.com
Received: 01-07-2025
Accepted: 03-08-2025

Dr. Richa Singla
PG Student, Department of Oral Pathology, Surendera Dental College & Research Institute Sriganganagar, Rajasthan, India

Dr. Meghanand Nayak T
Professor & Head, Department of Oral Pathology, Surendera Dental College & Research Institute Sriganganagar, Rajasthan, India

Dr. Mahima Chaudhary
PG Student, Department of Oral Pathology, Surendera Dental College & Research Institute, Sriganganagar, Rajasthan, India

Dr. Renu
PG Student, Department of Oral Pathology, Surendera Dental College & Research Institute Sriganganagar, Rajasthan, India

Corresponding Author:
Dr. Richa Singla
PG Student, Department of Oral Pathology, Surendera Dental College & Research Institute Sriganganagar, Rajasthan, India

To detect the potential expression of proteins p53, EGFR & Bcl-2 in oral potentially malignant disorders & their predictive value for the identification of lesions at high risk of progression to invasive carcinoma

Richa Singla, Meghanand Nayak T, Mahima Chaudhary and Renu

DOI: https://www.doi.org/10.22271/oral.2025.v11.i3f.2233

Abstract
Background: Oral potentially malignant disorders (PMDs) carry a variable but significant risk of progression to oral squamous cell carcinoma (OSCC). Traditional histopathological grading remains the diagnostic gold standard; however, its predictive accuracy is limited. Molecular biomarkers reflecting alterations in proliferation, differentiation, and apoptosis may provide valuable adjunctive information. The aim of study is to evaluate the expression of p53, epidermal growth factor receptor (EGFR), and Bcl-2 in PMDs and their predictive potential for malignant transformation.
Materials and Methods: 30 archival formalin-fixed paraffin-embedded (FFPE) tissues were analyzed, comprising 10 hyperplastic tissues (HPTs), 10 PMDs, and 10 well-differentiated squamous cell carcinomas (WDSCCs). Immunohistochemistry was performed using monoclonal antibodies against p53, EGFR, and Bcl-2. Expression patterns were assessed in basal, suprabasal, and keratin layers, as well as in peripheral and central tumor islands. Statistical analyses included Wilcoxon signed-rank test, with $p<0.05$ considered significant.
Results: p53 expression was detected in 30% of HPTs, 70% of PMDs, and 100% of WDSCCs, with a significant increase across the spectrum ($p<0.001$). EGFR expression was membranous in HPTs and PMDs but demonstrated mixed membranous and cytoplasmic localization in WDSCCs ($p<0.001$). Bcl-2 positivity was observed in 40% of HPTs, 60% of PMDs, and 100% of WDSCCs, with a progressive extension from basal to suprabasal layers ($p<0.001$). Both p53 and Bcl-2 showed statistically significant co-expression trends in PMDs and WDSCCs.
Conclusion: The gradual upregulation of p53, EGFR, and Bcl-2 from HPTs to PMDs and OSCC underscores their significance in oral carcinogenesis. These biomarkers may function as significant supplements to histopathological grading, facilitating risk categorisation of PMDs and informing early therapeutic action. It is advisable to conduct larger investigations utilising broader molecular panels to confirm their prognostic efficacy.

Keywords: Bcl-2, biomarkers, EGFR, immunohistochemistry, oral potentially malignant disorders, p53, oral squamous cell carcinoma

Introduction
The oral mucosa, consisting of stratified squamous epithelium and connective tissue, indicates systemic health and frequently displays initial indications of disease. Local irritants, including smoke and alcohol, together with systemic diseases such as diabetes and nutritional deficits, present as mucosal changes. Among oral diseases, potentially malignant disorders (PMDs) are clinically noteworthy due to their propensity to progress to oral squamous cell carcinoma (OSCC), which constitutes approximately 91% of oral malignancies [1-3].
Three primary molecular pathways in tumour biology—differentiation, proliferation, and apoptosis—are often disrupted in OSCC and PMDs. Biomarkers indicative of these pathways show potential in forecasting malignant transformation. The p53 gene, an essential tumour suppressor, governs cell cycle arrest and death in reaction to DNA damage. Mutations result in the accumulation of defective proteins, commonly seen in PMDs and OSCC, establishing p53 as an early molecular marker of oral carcinogenesis.

The epidermal growth factor receptor (EGFR), a transmembrane tyrosine kinase receptor, promotes cell survival and proliferation when overexpressed or mutated. Elevated EGFR expression correlates with aggressive tumour characteristics and unfavourable prognosis. The anti-apoptotic protein Bcl-2, situated in the mitochondrial membrane, obstructs programmed cell death and extends cell viability, thereby facilitating the accumulation of further mutations [4].

Clinically, potentially malignant disorders (PMDs) encompass leukoplakia, erythroplakia, oral submucous fibrosis, and oral lichen planus. Although histopathological grading of dysplasia is the established gold standard, it is plagued by variability and constrained predictive usefulness. Consequently, immunohistochemistry (IHC) has become an essential method for assessing molecular changes in PMDs. Immunohistochemistry (IHC) facilitates the evaluation of tissue antigen expression by specific antibodies, allowing for the assessment of biomarkers such as p53, EGFR, and Bcl-2, which can yield prognostic information that surpasses standard histological analysis [5].

The cumulative acquisition of genetic modifications impacting apoptotic regulators, tumour suppressors, and oncogenes is fundamental to malignant transformation in OPMDs. Assessing the expression levels of p53, EGFR, and Bcl-2 provides a means to categorise lesions into high-risk and low-risk classifications. This study was conducted to examine the potential expression of these proteins in oral potentially malignant disorders (PMDs) and to assess their predictive utility for detecting lesions at elevated risk of development to invasive carcinoma.

Material and Methods

The laboratory based retrospective study was conducted at Surendera Dental College & Research Institute's Department of Oral Pathology & Microbiology in Sri Ganganagar for a time period of one year. Ethical clearance for conducting the research was taken from institutional ethics committee of college and hospital.

Formalin-fixed paraffin embedded (FFPE) tissues of previously diagnosed hyperplastic tissues (HPTs), potentially malignant disorders (PMDs), & well-differentiated squamous cell carcinomas (WDSCCs) were examined. The tissues were taken from the archival files of the respected college.

After examining the microscopic slides & case histories, the diagnosis of 30 FFPE tissues—10 of which were HPTS, PMDs, & WDSCCs—was re-examined & verified using the standards established by the most recent WHO Classification of Tumours.

Materials used were

1. Reagents

- **Primary Antibody**

a) Anti-p53: Primary mouse monoclonal antibody (Ready to use DO7) Quantity 6 ml, Source: BioGenex; Hyderabad

b) Anti-EGFR- Primary rabbit monoclonal antibody (Ready to use EP38Y) Quantity 6 ml, Source: BioGenex; Hyderabad

c) Anti-Bcl-2- Primary mouse monoclonal antibody (Ready to use Bcl-2/100) Quantity 6 ml, Source: BioGenex; Hyderabad

Supersensitive polymer-horseradish peroxidase (Poly-HRP) IHC detection system containing

a) **Peroxide block (to block endogenous peroxidase activity):** 3% hydrogen peroxide in water, Quantity 6 ml, Source:BioGenex; Hyderabad

b) **Power block:** Contains buffered casein with 15mM sodium azide, Quantity 6 ml, Source: BioGenex; Hyderabad

c) **Negative controls:** Rabbit negative control: Non-immune serum or immunoglobulins (Igs) in phosphate buffered saline (PBS) with 0.09% sodium azide, Quantity 6 ml, Source: BioGenex; Hyderabad

Mouse negative control: Non-immune serum or Igs in PBS with 0.09% sodium azide. Quantity 6 ml Source:BioGenex; Hyderabad

d) **Poly-HRP reagent:** Pretitrated anti-species Igs labelled with enzyme polymer in PBS with stabilizers & proclin 300, Quantity 6 ml Source:BioGenex; Hyderabad

e) **Super enhancer Reagent:** A reagent that enhances the signal & is used after the primary antibody incubation, Quantity 6 ml, Source: BioGenex; Hyderabad

f) **Diaminobenzidine (DAB) substrate:** Comprises Tris buffer containing the peroxide & stabilizers, Quantity 6 ml, Source:BioGenex; Hyderabad

g) **Liquid DABchromogen:** Quantity 6 ml, Source:BioGenex; Hyderabad

Preparation of substrate-chromogen solution: 0.5ml of DAB substrate was transferred into the provided calibrated test tube. A drop (20µl) of liquid DAB chromogen was added & mixed immediately.

- Citrate buffer- Ready to use EZ Antigen Retrieval solution (EZ-AR 1 Solution) Quantity: 1litre, Source: BioGenex; Hyderabad
- Phosphate buffer saline pH 7.2-7.4

Reagent preparation: 3.4gm of sodium dihydrogen phosphate, 12.0 gm of disodium hydrogen phosphate & 8.5 gm of sodium chloride were dissolved in 1 litre of deionized water.

- Deionized water

Source: Recombigen Laboratories

Additional materials

- Semiautomatic microtome (Yorco YSI 060)
- Glass slides(75 x 25mm, thickness 1.45mm)
- Glass marking pencils
- Coplin jars
- Isopropyl alcohol (70%, 90% & 100%)
- Xylene
- Hematoxylin & Eosin (H&E) stain
- Non aqueous permanent mounting media: Dibutyl Phthalate Xylene (DPX)
- Cover slips (22 x 50mm, No. 1)
- Absorbent wipes
- Humidifying chamber
- Timer
- Antigen retrieval microwave oven (EZ retriever system BioGenex; Hyderabad)
- Poly-L-Lysine coated (PLL) slides
- Nikon E-200 microscope with digital camera attachment"

Storage & handling

- In accordance with the manufacturer's recommendations, the reagents & the primary antibody were kept in the refrigerator between 2 & 8 degrees Celsius.
- Before immunostaining, all of the reagents were allowed to come to room temperature (25-27 °C).

- Every incubation was carried out in the humidifying chamber at ambient temperature. To avoid more non-specific staining, the slides were not permitted to dry out while being stained. In each set of slides, both positive & negative controls were employed.

Methodology

30 FFPE blocks (10 for each lesion) were chosen from the archives of the college after the slides had been examined & analysed. FFPE blocks were sliced into sections 3-5 µm thick using a semiautomated microtome (Yorco YSI 060). To reevaluate & confirm the diagnosis, sections were taken & stained with routine H&E in accordance with usual methods. From each block, three more pieces were cut out so that the corresponding primary antibodies could be immunolabeled. Every slide has a suitable label.

Controls

The process was standardised between batches through the use of positive & negative controls.

Mouse negative control was used as a non-specific negative reagent control, while tonsil sections were used as positive tissue control for Bcl-2. Breast cancer sections served as EGFR & p53 positive tissue controls, while rabbit & mouse negative controls served as non-specific negative reagent controls.

The IHC Method

1. Rehydration & Deparaffinization

The tissue slides need to be rehydrated & deparaffinized to get rid of embedding media before staining. Avoiding partial paraffin removal is necessary since any remaining embedding media will exacerbate non-specific staining.

1. The slides spent ten minutes each in two changes of xylene-containing coplinjar.
2. After tapping out any extra liquid, slides were submerged in pure iso-propyl alcohol for five minutes.
3. The slides were submerged in 90% & 70% iso-propyl alcohol for five minute each after the extra liquid was tapped off.

After that, the slides were submerged in deionised water for at least 30 seconds.

2. The Peroxide Block

To maintain the reagent in the designated region, any leftover liquid was cleaned away using an absorbent cloth. After that, the specimen was placed in an enclosed, hydrated container, coated with peroxide block, & incubated for ten minutes. After then, the slides were shown on PBS for five minutes.

3. The recovery of antigens

By creating intermolecular cross links, formaldehyde causes conformational changes in the antigen molecules. Excessive formalin fixation can reduce some stains & obscure antigenic sites. Before immunostaining, these sites can be made visible using the heat-induced epitope retrieval (HIER) approach.

The deparaffinized tissue slices were stored in a slide rack with 0.01 M of tri-sodium citrate buffer (retrieval solution) & heated to 950C & 980C for ten minutes each in an antigen retrieval microwave oven. After 30 minutes of cooling to room temperature, the slides were put in PBS for five minutes.

4. The Power Block

A sufficient amount of power block was used to completely cover the portion, & it was then incubated in a moistened container for ten minutes.

5. The main antibody

The section was covered with the required quantity of a ready-to-use primary antibody or non-specific negative control reagent after the power block had been drained off, & it was then incubated for 60 minutes in a hydrated container that was contained. After a quick 15-second PBS rinse, the slides were left in a new buffer for five minutes.

6. Super Enhancer

After applying enough super enhancer to completely cover the portion, it was incubated for 25 minutes in a moistened container that was enclosed. After then, the slides were shown on PBS for five minutes.

7. The Poly HRP

Slides were deleted as usual when any extra buffer was tapped off. After that, the sections were well coated with the proper quantity of Poly HRP (secondary antibody) & allowed to sit in a moistened container for half an hour. After 15 seconds of gentle PBS rinsing, the slides were left in it for five minutes.

8. The Chromol Reagent

Slides were cleaned same like previously. The sections were placed in an enclosed, moistened container, topped with an adequate amount of freshly made chromogen reagent (DAB), & then incubated for five minutes. After that, distilled water was used to gently rinse.

9. Counter Stain with Haematoxylin

After continuously dipping the slides in Harris haematoxylin solution for 30 seconds, they were submerged in flowing tap water for five minutes to counterstain them.

10. Installing

After that, slides were dehydrated by dipping them once in 70% alcohol & then again in 100% alcohol for one minute each. After that, the slides were mounted using DPX after being placed in coplin jars filled with xylene to clean the section.

For every marker, the same process was carried out.

Immunolabeling

Two skilled pathologists independently inspected each stained slide under a Nikon E-200 light microscope. Using low-power magnification (x4 objective), the slice was scanned to identify five typical fields of the lesion (hot spots). After that, the chosen regions were examined using an x40 objective, & digital images were captured & saved as jpeg files. Microsoft Office Power was utilised to open each file.

Point, then overlay the image with a 6X6 grid created with the 'Table' function. To fully cover the immunohistochemistry picture, the grid was extended. Visual exclusion was applied to regions that did not correspond to the tissue of interest. In the upper left frame, cell counting began, & in the upper right frame, it was completed. Each image's overall cell count & positive cell count were manually tallied & scheduled on each frame.

A thorough grading formula assessed the degree of expression in each section:

The immunolabeling of HPTs, PMDs, & WDSCCs was evaluated independently in the basal, suprabasal, & keratin layers. Additionally, epithelial tumour islands in WDSCCs

were evaluated for both central & peripheral immunolabeling. The nuclear morphology served as the criterion for identifying cells in the suprabasal layer. Suprabasal cells were defined as having a spherical nucleus & being directly above the basal layer (4-5 cells above the basal cell layer). Keratin layer cells were identified by their flattened nucleus. Regardless of staining strength, a brown colour was the requirement for positive.

Evaluation of p53

Using the criteria outlined in Appendix D, the percentage of cells that tested positive for p53 were determined by looking for brownness in the nucleus (de souse *et al*.46 2009).

Evaluation of EGFR

The cells' levels of EGFR were evaluated for brownness in the cytoplasm, membrane, & both (both cytoplasmic & membrane staining).

The criterion outlined in Appendix D was used to assess the proportion of positive cells (Sarkis *et al*. 51 2010).

Evaluation of Bcl-2

Cells' levels of Bcl-2 were measured by looking for brown cytoplasm. The criteria outlined in Appendix D (de souse *et al*. 46 2009) was used to assess the proportion of positive cells.

Calibration & statistical analysis

A statistical analysis was performed on the data collected from 30 parts. Cronbach's alpha reliability test was used to statistically analyse the values collected from the two observers. To compare the data between various markers & within different tissue layers, the Wilcoxon signed rank test was employed. Version 25.0 of the Statistical Package for the Social Sciences (SPSS) was used to conduct the statistical analysis. A p-value of less than 0.05 was deemed significant.

Results

It was discovered that the number of cases with positive p53 expression rose from HPTs to PMDs to WDSCCs. While p53 expression is seen in both the basal & suprabasal layers in PMDs & WDSCCs, it was mostly found in the basal layer in HPTs. The difference between HPTs, PMDs, & WDSCCs was determined to be highly significant using the Wilcoxon Signed Rank test as shown in table 1.

Table 1: Comparison of immunohistochemical expression of p53 in hyperplastic tissues (hpts), potentially malignant disorders (PMDS) & well differentiated squamous cell carcinomas (WDSCCS)

Tissue	Layers	Number of cases			
		0	1+	2+	3+
HPTs	Basal	9	1	-	-
	Suprabasal	10	-	-	-
	Keratin	10	-	-	-
PMDs	Basal	3	-	-	7
	Suprabasal	6	-	4	-
	Keratin	10	-	-	-
WDSCCs	Basal	-	-	-	10
	Suprabasal	-	-	-	10
	Keratin	10	-	-	-
P value		.000[a]**	.000[b]**		.000[c]**

"Expression of p53 was scored as follow: 0< 5% cells positive, 1+: 5-25% cells positive, 2+**:** 25-50% cells positive, 3+:> 50% cells positive.***p*<0.001, Highly Significanta: HPTs vs PMDs, b: HPTs vs WDSCCs c: PMDs vs WDSCCs"

Both the basal & suprabasal layers of HPTs & PMDs showed exclusively membrane EGFR expression (Score 4). All of the instances in WDSCCs were primarily mixed (membranous + cytoplasmic) staining (scoring 4). The mean LI difference between the membranous staining of EGFR in the basal layer of HPTs vs. PMDs & the combined staining of EGFR in both the basal & suprabasal layers of HPTs vs. PMDs was determined to be non-significant using the Wilcoxon Signed Rank Test as shown in table 2.

Table 2: Comparison of immunohistochemical expression of EGFR in hyperplastic tissues (HPTD), potentially malignant disorders (PMDS) & well differentiated squamous cell carcinomas (WDSCCS)

Tissue	Layers	Number of cases														
		0			1			2			3			4		
		M	C	M+ C	M	C	M+C	M	C	M+C	M	C	M+C	M	C	M+C
HPTs	Basal	-	10	10	-	-	-	-	-	-	-	-	-	10	-	-
	Suprabasal	-	10	10	-	-	-	-	-	-	-	-	-	10	-	-
	Keratin	10	10	10	-	-	-	-	-	-	-	-	-	-	-	-
PMDs	Basal	-	10	10	-	-	-	-	-	-	-	-	-	10	-	-
	Suprabasal	-	10	10	-	-	-	-	-	-	-	-	-	10	-	-
	Keratin	10	10	10	-	-	-	-	-	-	-	-	-	-	-	-
WDSCCs	Basal	-	10	-	10	-	-	-	-	-	-	-	-	-	-	10
	Suprabasal	-	10	-	10	-	-	-	-	-	-	-	-	-	-	10
	Keratin	10	10	10	-	-	-	-	-	-	-	-	-	-	-	-
p value		1.000[a NS] .000[b]**		.000[c]** .000[d]**			.000[e]** .000[f]**			1.000[gNS] .000[h]**			.000[i]** 1.000[j NS]			.000[k]** .000[l]**

"M: staining in membrane; C: staining in cytoplasm; M+C: staining in both membrane & cytoplasm. Expression of EGFR was scored as follows**:**0: negative staining, 1: < 10% cells positive, 2**:**10-50% cells positive, 3: 51- 80% cells positive,

4:> 80% cells positive a: HPTs Basal M vs PMDs Basal M, b: HPTs Basal M vs WDSCCs Basal M, c: PMDs Basal M vs WDSCCs Basal M, d: HPTs Suprabasal M vs PMDs Suprabasal M, e: HPTs Suprabasal M vs WDSCCs Suprabasal M, f: PMDs Suprabasal M vs WDSCCs Suprabasal M, g: HPTs Basal M+C vs PMDs Basal M+C, h: HPTs Basal M+C vs WDSCCs Basal M+C, i: PMDs Basal M+C vs WDSCCs Basal M+C, j: HPTs Suprabasal M+C vs PMDs Suprabasal M+C, k: HPTs Suprabasal M+Cvs WDSCCs Suprabasal M+C, l: PMDs Suprabasal M+Cvs WDSCCs Suprabasal M +C. NS: Not Significant **p<0.001, Highly Significant"

It was discovered that the percentage of cases with positive Bcl-2 expression rose from 40% of HPTs to 60% of PMDs & 100% of WDSCCs. In HPTs, Bcl-2 expression was primarily found in the basal layer; in PMDs & WDSCCs, however, it is found in both the basal & suprabasal layers. The difference between HPTs, PMDs, & WDSCCs was determined to be highly significant using the Wilcoxon Signed Rank Test as shown in table 3.

Table 3: comparison of immunohistochemical expression of bcl-2 in hyperplastic tissues (HPTs), potentially malignant disorders (PMDs) & well differentiated squamous cell carcinomas (WDSCCs)

Tissue	Layers	Number of cases			
		0	1+	2+	3+
HPTs	Basal	6	3	1	-
	Suprabasal	10	-	-	-
	Keratin	10	-	-	-
PMDs	Basal	4	-	6	-
	Suprabasal	4	4	2	-
	Keratin	10	-	-	-
WDSCCs	Basal	-	-	10	-
	Suprabasal	-	4	6	-
	Keratin	10	-	-	-
P value		.000[a]**	.000[b]**		.000[c]**

"Expression of Bcl-2 was scored as follow: 0< 5% cells positive, 1+: 5-25% cells positive, 2+**:** 25-50% cells positive, 3+:> 50% cells positive, a HPTs vs PMDs b.HPTsvs WDSCCs c PMDs vs WDSCCs**p<0.001, Highly Significant"

From HPTs to PMDs to WDSCCs, the amount of p53 & Bcl-2 immunostaining demonstrated an increasing trend. All of the WDSCC layers showed a highly significant difference in p53 & Bcl-2 comparative expression, but the suprabasal/keratin layer of HPTs & PMDs showed no significant difference as shown in table 4.

Table 4: Comparison of immunohistochemical expression of p53 & bcl-2 in basal, suprabasal & keratin layers of hyperplastic tissues (HPTs), potentially malignant disorders (PMDs) & well differentiated squamous cell carcinoma (WDSCCs)

Lesion	No. of cases	Layer	p53					Bcl-2					p value
			0	1+	2+	3+	Mean	0	1+	2+	3+	Mean	
HPTs	10	Basal	9	1	-	-	1.1418	6	3	1	-	9.8336	.000[a]**
		Suprabasal	10	-	-	-	.414	10	-	-	-	.0000	1.000[b] NS
		Keratin	10	-	-	-	.0000	10	-	-	-	.0000	1.000[c] NS
PMDs	10	Basal	3	-	-	7	50.3064	4		6	-	20.5996	.000[d]**
		Suprabasal	6	-	4	-	14.0078	4	4	2	-	14.2524	.516[e] NS
		Keratin	10	-	-	-	.0000	10	-	-	-	.0000	1.000[f] NS
WDSCCs	10	Basal	-	-	-	10	95.72	-	-	10	-	37.5820	.000[g]**
		Suprabasal	-	-	-	10	92.916	-	4	6	-	27.7666	.000[h]**
		Keratin	10	-	-	-	1.0000	10	-	-	-	.0000	.000[i]**

"Expression of p53 & Bcl-2 was scored as follow: 0< 5% cells positive, 1+: 5-25% cells positive, 2+**:** 25-50% cells positive, 3+:> 50% cells positive, NS: p> 0.05; Not Significant, **p<0.001; Highly significant"

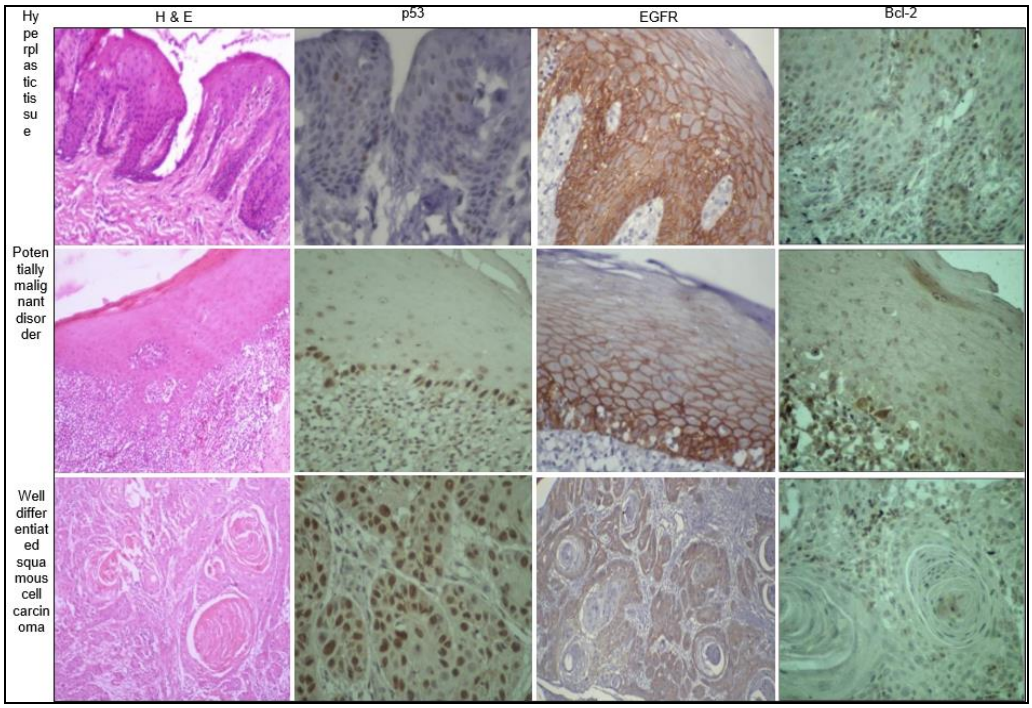

Fig 1: H&E & IHC (p53, EGFR, Bcl-2) stained sections of hyperplastic tissues, potentially malignant disorders & well differentiated squamous cell carcinoma

Discussion

Cancer remains a persistent global health challenge despite advances in detection, prevention, and treatment. Carcinogenesis is a multistep process characterized by tumor suppressor gene inactivation and oncogene activation, leading to disruption of regulatory checkpoints in the cell cycle [7]. The imbalance between cell proliferation and programmed cell death (apoptosis) results in tumorigenesis. While proliferation ensures tissue growth and repair, its dysregulation precedes cancer development. Apoptosis, described as a genetically regulated process, is critical for tissue homeostasis, embryogenesis, and tumor suppression. When apoptotic pathways are disrupted, cells accumulate mutations that favor immortalization [8].

Most oral cancers evolve from hyperplastic tissues (HPTs) through potentially malignant disorders (PMDs) to invasive oral squamous cell carcinomas (OSCCs). Prognosis varies: early-stage tumors without nodal involvement achieve 5-year survival rates of 82%, compared to only 56% with nodal spread and 34% with distant metastases. Unfortunately, late detection of OSCC remains a major barrier to improved survival [9]. Thus, early recognition of PMDs is crucial. While biopsy remains the diagnostic gold standard, molecular alterations precede microscopic changes, underscoring the value of biomarker evaluation.

Proliferation markers such as Ki-67, PCNA, Cyclin D1, and AgNORs, along with tumor suppressors like p53, have been studied extensively in HPTs, PMDs, and OSCCs. Among these, p53 is the most widely investigated due to its role in DNA repair, genomic stability, apoptosis, and malignant transformation [10]. Wild-type p53 has a short half-life and is undetectable by immunohistochemistry, but mutations stabilize the protein, leading to accumulation detectable by IHC.

Growth factor signaling also contributes to oral carcinogenesis. EGFR, a transmembrane tyrosine kinase receptor, mediates responses to ligands such as EGF and TGF-α, triggering proliferative cascades. Overexpression of EGFR in OSCC and PMDs correlates with aggressive behavior and poor outcomes [11].

Apoptotic regulation is another determinant. Bcl-2, a key anti-apoptotic protein, is downregulated in terminally differentiated cells but upregulated in proliferative compartments. Its overexpression in PMDs and OSCC suggests resistance to cell death and enhanced malignant potential [5, 12]. Previous studies have explored apoptosis-proliferation balance using combinations of markers such as p53, Bcl-2, Bax, and PCNA [13]. However, simultaneous evaluation of p53, EGFR, and Bcl-2 remains relatively underexplored [14].

Positive p53 staining was observed in 30% of HPTs, 70% of PMDs, and 100% of WDSCCs. Expression was localized mainly in the basal layers of HPTs but extended into suprabasal layers in PMDs and WDSCCs, indicating abnormal proliferative activity. All WDSCCs demonstrated strong nuclear staining, with peripheral tumor islands showing greater positivity than central cells. These findings highlight a progressive increase in p53 expression across the spectrum from hyperplasia to carcinoma, supporting its role in early oral carcinogenesis [15].

EGFR staining was membranous in HPTs and PMDs but showed mixed membranous and cytoplasmic localization in WDSCCs. Suprabasal layer expression in PMDs and OSCCs suggested heightened proliferative activity outside normal compartments. Peripheral tumor cells in WDSCCs stained more strongly than central cells, consistent with previous reports [16]. These patterns indicate EGFR overexpression as a poor prognostic marker and potential therapeutic target.

Bcl-2 positivity was identified in 40% of HPTs, 60% of PMDs, and 100% of WDSCCs. Expression was primarily basal in HPTs but extended to suprabasal layers in PMDs and OSCCs, reflecting enhanced survival signaling. Stronger peripheral staining in tumor islands was observed, consistent with previous findings [17, 18]. Occasional positivity in lymphocytes and endothelial cells was also noted, aligning with earlier reports [13].

Both p53 and Bcl-2 exhibited increasing expression from HPTs to PMDs to WDSCCs, with statistically significant

differences across groups. EGFR expression also shifted toward mixed cytoplasmic and membranous localization in OSCCs. Collectively, the results highlight a progression pattern where proliferation, growth signaling, and apoptotic resistance act synergistically in malignant transformation

Conclusion

A study found that p53 mutations are common in PMDs and WDSCCs and may be detected by immunohistochemistry, marking an early stage in oral carcinogenesis. Future gene therapy requires immunohistochemistry identification of the p53 protein, which also aids prognosis.

WDSCCs and PMDs express more Bcl-2 than HPTs, which may affect early oral tumour development. WDSCCs' elevation of mixed (cytoplasmic + membranous) EGFR staining in early OSCCs hinted at poor prognosis. WDSCCs are EGFR-overexpressing tumours that can be treated with anti-EGFR drugs when surgery is not an option or has failed.

Changes in biomarker expression may cause PMDs to become invasive carcinomas. However, more study with a larger sample size and a panel of related molecules with different OSCC grades is needed to completely assess the potential of these biomarkers in PMDs and OSCCs.

Conflict of Interest

Not available

Financial Support

Not available

References

1. Alberts B, Johnson A, Lewis J. Molecular Biology of the Cell. 4th ed. New York: Garland Science; 2002.
2. Fink SL, Cookson BT. Apoptosis, Pyroptosis & Necrosis: Mechanistic Description of Dead & Dying Eukaryotic Cells. Infect Immun. 2005;73(4):1907-1916.
3. Elmore S. Apoptosis: A Review of Programmed Cell Death. Toxicol Pathol. 2007;35(4):495-516.
4. Issa HI. Immunoexpression of Epidermal Growth Factor Receptor, Ki-67 & p53 Protein in Squamous Cell Carcinoma of the Head & Neck. Res J Med Med Sci. 2013;8(13):9-15.
5. Koshy VA, Shaikh NA, Kapse SC, Bussari S. Immunohistochemical Evaluation of Bcl-2 oncoprotein in normal oral mucosa, leukoplakia & oral squamous cell carcinoma. Universal Research Journal of Dentistry. 2012;2(1):1-5.
6. Coelho KR. Challenges of the Oral Cancer Burden in India. J Cancer Epidemiol. 2012;2012:701932.
7. Kummoona R, Mohammad Sámi S, Al-Kapptan I, Al-Muala H. Study of antiapoptotic gene of oral carcinoma by using Bcl-2 oncogene. J Oral Pathol Med. 2007;37(6):345-351.
8. Nafarzedeh S, Jafari S, Bijani A. Assessment of Bax & Bcl-2 Immunoexpression in patients with Oral Lichen Planus & Oral Squamous Cell Carcinoma. Int J Mol Cell Med. 2013;2(3):136-142.
9. Bansal S, Sircar K, Joshi SK, Singh S, Rastogi V. A comparative study of p53 expression in hyperplastic, dysplastic epithelium & oral squamous cell carcinoma. Braz J Oral Sci. 2010;9(2):85-88.
10. Pooja N, Farzan R, Shoaib RT, Sameer. Expression of p53 protein in normal oral mucosa, hyperplasia, grades of epithelial dysplasia & oral squamous cell carcinoma: An immunohistochemical study. Indian J Stomatol. 2010;1(2):56-60.
11. Rajeswari MR, Saraswathi TR. Expression of epithelial growth factor receptor in oral epithelial dysplastic lesions. J Oral Maxillofac Pathol. 2012;16(2):183-188.
12. Nair RG, Shameen PM, Varghese I, Sudha S. Immunohistochemical Evaluation of Bcl-2 oncoprotein in Oral Dysplasia & Carcinoma. OMPJ. 2011;2(1):9-15.
13. Freitas RDA, de Amorim RFB, de Queiroz SBF, da Costa Miguel MC, Silveira EJD, Godoy GP. Expression of PCNA, p53 & bcl-2 in oral lichen planus: An immunohistochemical study regarding the potential of malignant transformation. Oral Sci. 2010;2:9-16.
14. Kipli NP, Kumar SKS, Zain RB, Fan CY, Boon PK. Expression of p53, pRb, Ki67 & Bcl-2 in betel quid induced oral mucosal lesions. Oral Oncol. 2005;1:87.
15. Pillay M, Vasudevan DM, Rao CP, Vidya M. p53 Expression in Oral Cancer: Observations of a South Indian Study. J Exp Clin Cancer Res. 2003;22(3):447-451.
16. Monteiro LS, Diniz-Freitas M, Garcia-Caballero T, Warnakulasuriya S, Forteza J, Fraga M. Combined cytoplasmic & membranous EGFR & p53 overexpression is a poor prognostic marker in early stage oral squamous cell carcinoma. J Oral Pathol Med. 2012;41(7):559-567.
17. Shailaja G, Kumar JV, Baghirath PV, Kumar U, Ashalata G, Krishana AB. Estimation of malignant transformation rate in cases of oral epithelial dysplasia & lichen planus using immunohistochemical expression of Ki-67, p53, BCL-2 & BAX markers. Dent Res J (Isfahan). 2015;12(3):235-242.

How to Cite This Article

Singla R, Meghanand Nayak T, Chaudhary M, Renu. To detect the potential expression of proteins p53, EGFR & Bcl-2 in oral potentially malignant disorders & their predictive value for the identification of lesions at high risk of progression to invasive carcinoma. International Journal of Applied Dental Sciences. 2025;11(3):406-412.