

Research Article

Immunohistochemical Expression of P63 and P40 in Squamous Cell Carcinoma of Head and Neck

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Abstract

Background: Squamous cell carcinoma (SCC) in head and neck region has a high worldwide prevalence. Increased accuracy in diagnosis is achieved by immunohistochemical markers.

Objectives: To evaluate the immunohistochemical expression of p63 and p40 in SCC and correlate with histological grades to determine p40 as a better potential diagnostic immunohistochemical marker for SCC.

Methods: Descriptive cross-sectional study was conducted on eighty-three cases of SCC of head and neck from March 2020 to February 2022 at Pathology Department, KEMU, Lahore. Data including age, sex, risk factors and tumor site was collected. SCC grading was done on H&E stained tissue sections. Staining with p63 and p40 was performed and independently scored for percentage of tumor cells stained and intensity of nuclear staining. The results were compared using H-scores in all grades of SCC. Data was entered in Statistical Package for Social Sciences (SPSS-26). Quantitative variables like age were presented as mean \pm SD. Qualitative variables as frequency and percentages. Correlation of two groups was done by applying Chi-square test. P value \leq 0.05 was taken as significant.

Results: SCC was graded according to the W.H.O. grading system. All cases stained positive for p63 and p40. Strong nuclear staining by p40 was observed in 65.02% cases versus 42.17% by p63. The mean tumor area stained by p40 was greater than p63 (90.35% vs 75.5%) in all cases. A significant higher mean H-score for p40 (9.69 \pm 2.86) versus p63 (7.05 \pm 3.12) was observed as indicated by significant P-value. The mean H-score of p40 immunoreactivity was 10.11 and p63 was 6.00 in poorly differentiated SCC. Males comprised 74.70% cases whereas females 25.30% only. SCC in oral cavity was (96.39%) followed by oropharynx and larynx.

Conclusion: P40 immunohistochemical marker was better compared to P63 for the diagnosis of SCC particularly in poorly differentiated SCC.

Received: 23-08-2023 | **1st Revision:** 11-02-2024 | **2nd Revision:** 14-04-2024 | **Accepted:** 19-06-2024

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Keywords | p40, p63, Squamous cell carcinoma (SCC).

Introduction

Head and neck cancers are the sixth most prevalent cancers, with the incidence of squamous

cell carcinoma of head and neck over 500,000 cases per year all over the world.^{1,2} Ninety percent of the head and neck cancers are squamous cell carcinomas, which is the highest frequency among the malignancies of head and neck region.³

Immunohistochemical analysis by visualization of squamous epithelial specific markers has been carried out by researchers aiming at p63, an isoform of p53



Production and Hosting by KEMU

<https://doi.org/10.21649/akemu.v30i3.5476>

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critical to cellular and genetic stability. Δ Np63, designated as p40 is a shorter isoform of p63 which lacks the transactivation domain at the N-terminal of the protein has been frequently over expressed in various carcinomas, including head and neck squamous cell carcinomas.^{4,5,6} This oncogenic role of p40 has been highlighted and used as high-quality screening antibody for determining squamous origin of carcinomas at various sites.⁷ An antecedent analysis revealed significant expression of p40 in contrast to other markers for the differentiation of poorly differentiated squamous cell carcinoma, non-keratinizing squamous cell carcinoma and neuroendocrine carcinomas.⁸ The diagnostic reliability of p40 is excellent as its specificity is 98% - 100%.^{7,9} To eliminate pitfalls in the diagnosis, this enhanced accuracy of p40 is also utilized where sarcomatoid carcinoma required differentiation from mesenchymal tumors.⁹

A statistically significant over expression of p63 has been associated with higher stage and grade of oral cavity squamous cell carcinoma, hence its poorer prognosis in recent studies conducted in Iran and Indian hospitals.^{10,11} However, no recorded research data of p40 and p63 analysis of Head and neck SCC in the population of Pakistan is available up to date. In the present study, the expression of both markers in all histological grades of squamous cell carcinoma was analysed. We determined the expression of immunomarkers p63 and p40 in all histological grades of head and neck squamous cell carcinomas.

Methods

It was a Descriptive Cross-Sectional Study conducted at Histopathology section of Department of Pathology, King Edward Medical University, Lahore. A total of 83 patients of all ages and gender with histological diagnosis of squamous cell carcinoma of head and neck region were included in the study. Patients having coexisting malignancy other than squamous cell carcinoma of head and neck region and those undergoing chemotherapy/radiation therapy for cancers were excluded. Sample size of 83 was estimated by using 95% confidence level, using 7% absolute precision with expected percentage of P40 as 98.8%⁹ and p63 as 89.5%¹³. Selected sample case tissue blocks, histopathology reports and written informed consent of patient with information on questionnaire form was taken with permission from cancer

department.

One tissue section for haematoxylin and eosin staining and two sections for immunohistochemical staining were taken. Haematoxylin and eosin staining was performed. Two pathologists confirmed and reported the diagnoses and graded tumor according to the histological features of SCC according to W.H.O. grading system as well, moderate and poorly differentiated carcinoma.

Immunohistochemical staining with p63 and p40 was done using standard technique and analysed to record results. The brown nuclear staining intensity profile of p40 and p63 immunohistochemical markers among 83 cases under study was interpreted as: score 0(negative), score 1(weak), score 2(moderate) and score 3(strong). The percentage of immunoreactive tumor cells was interpreted for distribution in quartiles. Score of 1 to 4 was given for expression intensity as follows: Score 1 (1-25%) Score 2 (26-50%), Score 3 (51-75%) and score 4(76-100%). H-score representing the extent of immunoreactive cells multiplied with intensity of brown nuclear staining was calculated for each case.

Results

A total 83 cases diagnosed as SCC of head and neck region subsites including oral cavity, oropharynx, hypopharynx and larynx were included in our study. The mean age of included patients was 49.71±12.17 years with a male to female ratio of 3:1. According to the location, highest frequency was seen in oral cavity (96.39%) whereas no case in hypopharynx region was identified. One case of laryngeal carcinoma and two cases of oropharynx were reported. Oral cavity carcinomas were mostly located on tongue, buccal vestibule, alveolus, palate, lip and mandible. The squamous cell carcinomas of well (n=54), moderate (n=20) and poor (n=9) differentiation were graded on tissue biopsy and IHC slides were examined for staining intensity and the percentage area covered.

All 83 cases under study were positive for the brown nuclear expression of P40 marker. The strong staining intensity of p40 is greater number of patients compared to p63. Whereas, moderate intensity (score 2) and weak intensity (score 1) of p63 tumor marker were observed more frequently in all grades of cancer compared to p40 (Table-1).

Table 1: Illustrates the pattern of brown nuclear staining intensity in the histological grades of squamous cell carcinoma of head and neck region.

Grade of SCC	Staining Intensity	Marker	
		p40	p63
Well	0	0	1
	1	1	4
	2	19	23
	3	34	26
Moderate	1	0	2
	2	7	12
	3	13	6
Poor	1	0	1
	2	2	5
	3	7	3

The mean area of tumor covered by P40 staining is 90.35% (mean score 3.61, SD 0.64) whereas the mean area of tumor covered by P63 staining is 73.5% (mean score 2.94, SD 0.83). The difference in the area stained covered by P40 compared to P63 indicates that P40 can be a better diagnostic marker across all grades of squamous cell carcinoma.

The mean value of p40 in well differentiated carcinoma is (3.56±0.66) compared to p63 (3.02±0.86). The mean value of p40 in moderately differentiated carcinoma is (3.80±0.52) compared to p63 (2.90±0.79). The mean value of p40 in poorly differentiated carcinoma is (3.56±0.73) compared to p63 (2.56±0.73). The expression of p40 is greater than p63 as indicated by significant p-value of <0.05 in all grades of squamous cell carcinoma (Table 2).

Table 2: Comparison of Mean Percentage of Immunoreactive Cells in Both Markers According to Grade Of SCC

Grade of SCC	Marker (mean percentage)				P value
	p40		p63		
	Mean	SD	Mean	SD	
Well	3.56	0.66	3.02	0.86	0.0004
Moderate	3.80	0.52	2.90	0.79	0.0001
Poor	3.56	0.73	2.56	0.73	0.0100

The mean H-score of P40 immunohistochemical stain is 9.69±2.86 and the mean H-score of P63 is 7.05 ± 3.12 (Table 3). The mean H-score of p40 tumor marker is significantly higher than p63 in all grades of squamous cell carcinoma among 83 patients in our study, as indicated by significant P-value. These results also indicate that p40 is a better diagnostic marker for squamous cell carcinoma of head and neck.

Table 3: Comparison of H score of Immunoreactive Cells in Both Markers According to Grade of SCC

Grade of SCC	H-SCORE				P-Value
	P40		P63		
	Mean	SD	Mean	SD	
Well	9.44	2.96	7.46	3.37	0.0016
Moderate	10.15	2.52	6.40	2.35	0.0001
Poor	10.11	3.10	6.00	2.74	0.008

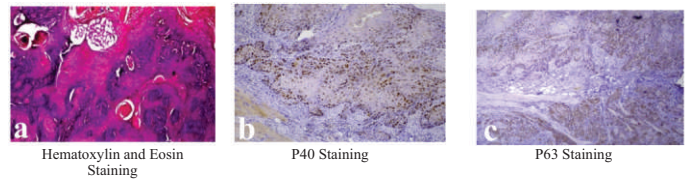


Figure 1 a,b,c: Staining of Well differentiated SCC

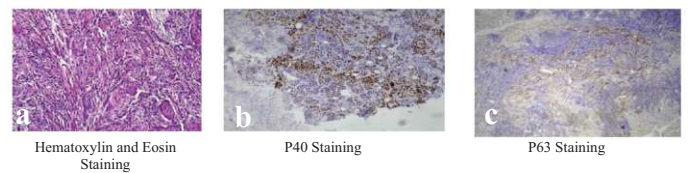


Figure 2 a,b,c: Staining of Moderately Differentiated SCC

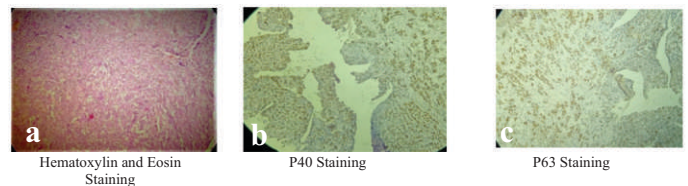


Figure 3 a,b,c: Staining of Poorly Differentiated SCC

Discussion

In a study on Pakistani population, the head and neck squamous cell carcinoma has been reported at a mean age 51.51 ± 12.35 years which is in accordance with our population under study. The average age of 49.71 + 12.17 years was recorded in our study.¹⁴

The frequency of well, moderate and poorly differentiated squamous cell carcinoma of head and neck in the patients was 65.06%, 24.10% and 10.84%. Our study's limitation, due to the low prevalence number of poorly differentiated carcinoma patients can be owing to low turnover of biopsy samples, patients seeking professional second opinion and non-provision of immunohistochemistry for confirmatory diagnosis at Mayo Hospital Lahore.

Induction of tumorigenesis and transition from benign to malignant cells is identified by several biomarkers, particularly p40 and p63 in head and neck squamous cell carcinomas.^{15,16}

Δ NP63 causes the β -catenin to accumulate in the nucleus, enhancing oncogenic potential of cells.¹⁷ Δ Np63 (p40) overexpression in premalignant lesions of oral cancers serves as a potential biomarker for early detection in oncogenesis.¹⁸

Previous studies conducted on squamous cell carcinoma in various tissues of body support the vast usefulness of p63 and p40 immunohistochemical markers.^{19,20} A study conducted on advanced esophageal squamous cell carcinoma reported Δ Np63(p40) was highly expressed in 75.6% of their patients and predicted their poor prognosis.²¹

A wide array of studies on lung tissue used p40 for characterization of lung malignancies as squamous cell carcinoma. Among the several immunomarkers analysed on lung tissue, the incidences of a positive P40 expression were 100 % in squamous cell carcinoma, with specificity of 98.81 %.^{22,23} Of significance is high specificity of p40 isoform for squamous cell carcinoma, with sensitivity similar to p63 expression.^{24,25} These findings are in accordance with our results of H score, p40 displaying a higher mean percentage compared to p63.

The necessity to make accurate histological diagnosis supported by ancillary techniques, such as immunohistochemistry on minimal amount of tissue specimen probed us to study and determine the better marker for squamous cell carcinoma. We verified the superior expression of p40 in detecting squamous cell carcinoma, particularly in poorly differentiated carcinomas in our subset population of Pakistan. H-scores shows significant p-value for p40 in all grades of squamous cell carcinoma when compared to p63 expression, claiming it be a superior diagnostic immunohistochemical stain to support microscopic analysis of pathologists.

Conclusion

It was concluded from our study results that p40 is a better immunohistochemical stain than p63 for diagnosing SCC. This will positively impact accuracy of histological diagnosis of SCC.

Ethical Approval: The Institutional Review Board,

KEMU approved this study vide letter No. 48/ RC/ KEMU/ 2020.

Conflict of Interest: The authors declare no conflict of interest.

Funding Source: None

Authors' Contribution:

AZ: Conception & design, analysis & interpretation of data, drafting of article, critical revision for important intellectual content, final approval

RA: Conception & design, critical revision for important intellectual content, final approval

SH: Conception & design, drafting of article, final approval

ZM: Analysis & interpretation of data, drafting of article

HT: Analysis & interpretation of data, drafting of article

AM: Analysis & interpretation of data, critical revision for important intellectual content, final approval

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