



IMMUNOHISTOCHEMICAL STUDY OF PODOPLANIN IN SQUAMOUS CELL CARCINOMA

Pathology

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ABSTRACT

Introduction: Squamous cell carcinoma is a significantly prevalent cause for morbidity/mortality. Podoplanin is an immunohistochemical marker expressed by squamous cell carcinoma of various organs. Present study has been carried out to study podoplanin as a prognostic marker in squamous cell carcinoma in various organs. **Materials And Methods:** The present study included a total of 102 specimens coming to histopathology laboratory from 1st September 2019 to 31st August 2022. **Results:** In our study the mean age of the patients was 50.5 years. Majority of cases were in the 61-70 years age group (46.07%). Out of the 102 cases studied, ulceroproliferative growth was most common clinical presentation (33.33%). In the present study maximum cases were of squamous cell carcinoma of tongue (33.33%) followed by buccal mucosa (18.62%), esophagus (9.80%), epiglottis (5.88%), pyriform fossa (5.88%), cervix (5.88%), tonsil (4.90%), cricoid (4.90%), hard palate (0.98%), uvula (0.98%), gingiva (0.98%), vocal cord (0.98%), pre auricular (0.98%), penis (0.98%), vaginal vault (0.98%) and gluteal region (0.98%). Majority of the cases were moderately differentiated squamous cell carcinoma (75 cases-73.52%). All well differentiated squamous cell carcinoma expressing podoplanin showed membranous positivity. Maximum cases of moderately differentiated squamous cell carcinoma showed both membranous (79.78%) and cytoplasmic positivity (70.21%) and all poorly differentiated squamous cell carcinoma showed membranous (6.38%) and cytoplasmic (6.38%) positivity. All well differentiated squamous cell carcinoma showed German immunoreactive score of weak reactivity, all poorly differentiated squamous cell carcinoma showed high reactivity and 85.33% cases of moderately differentiated squamous cell carcinoma showed high reactivity. **Conclusion:** Our study demonstrates that podoplanin has prognostic significance. Podoplanin could be a potent biomarker in assessing the cytoplasm/membrane staining of tumor cells which correlates with grades of squamous cell carcinoma. High reactivity of podoplanin expression by German immunoreactive score is suggestive of high grade status in the differentiation of squamous cell carcinoma

KEYWORDS

Squamous cell carcinoma, Podoplanin

INTRODUCTION

Squamous cell carcinoma a very common malignancy worldwide. Patients with squamous cell carcinoma have benefited greatly from the latest advances in surgical techniques, radiation therapy and chemotherapy. However, despite the advancements overall quality-of-life achieved with the use of combined modality therapies, the survival rates for squamous cell carcinoma have not improved significantly over the past two decades¹. In most solid tumors, the spread of cancer cells via the lymphatics to regional lymph nodes is an important early event in metastatic disease².

Very recently, expression of the 38-kDa Type 1 transmembrane glycoprotein podoplanin has been shown to induce tumor lymphangiogenesis and enhance metastasis to lymph nodes. Podoplanin is highly and specifically expressed in lymphatic endothelial cells, but not in blood vessel endothelium^{3,4}. It is widely used as a specific marker for lymphatic endothelial cells and lymphangiogenesis⁵. Podoplanin is also expressed by tumor cells. The tumor cells expressing podoplanin promote tumor cell motility and tumor lymphangiogenesis. Therefore cancer cell-expressed podoplanin correlates with higher incidence of lymph node metastasis and can be identified as a marker of poor prognosis^{5,6}. Hence present study is being carried out to study podoplanin expression in squamous cell carcinoma in various organs.

Objectives Of The Study

1. To study the expression of podoplanin in squamous cell carcinoma of various organs.
2. To study the association of podoplanin expression with differential grading of squamous cell carcinoma

MATERIALS & METHODS

A descriptive study was conducted at Basaveshwar Teaching and General Hospital attached to Mahadevappa Rampure Medical College, Kalaburagi. A 3 years study was carried out, 18 months retrospective and 18 months prospective (from 1st September 2019 to 31st August 2022). In the present study we came across 102 cases of squamous cell carcinoma in our tertiary care hospital and other private hospitals and diagnostic laboratories in and around Kalaburagi, Karnataka. All surgical specimens of all ages diagnosed as squamous cell carcinoma

by routine histopathology were included in the present study. The specimens with extensive tumor necrosis and recurrent malignancy were excluded.

A total of 102 cases were examined in the study. Clinical data was obtained from the medical records. The surgical specimens were collected, routinely processed. H&E sections were studied. Then Immuno-histochemistry was carried out as per standard procedure on histopathologically diagnosed cases of squamous cell carcinoma.

For podoplanin immunostaining, sections were de-paraffinized, rehydrated, and subjected to microwaving in EDTA buffer (as per vendor specifications and product instruction manual, including microwaving wattage and duration). Slides were allowed to cool at room temperature for 30 minutes. The D2-40 antibody was applied at room temperature for 2 hours in an automated stainer. Detection steps was performed using the Detection Kit and Standard Protocol. Standardized development time periods allowed accurate comparison of all samples.

Table 1 Criteria For Assessing Podoplanin Staining- German Immunoreactive Score⁶

Quantity scores	Percentage of tumor cells that are positive
0	0%
1	1% to 10%
2	11% to 30%
3	31% to 50%
4	51% to 80%
5	81% to 100%

Staining Intensity:

0	Negative
1	Weak
2	Moderate
3	Strong

RESULTS

A total of 102 squamous cell carcinoma were studied as per the aforementioned inclusion and exclusion criteria from 1st September

2019 to 31st August 2022. Majority of cases encountered (47 cases, 46.07%) were in the 61-70 years age group. In the present study 59 were male and 43 were female. Out of the 102 cases studied, most common, predominant clinical presentation was ulceroproliferative growth. In the present study maximum cases were of squamous cell carcinoma of tongue (33.33%)

Table 2 Correlation Of Podoplanin Expression With Grades Of Squamous Cell Carcinoma

Histopathological Grade	Number of cases	Quantity score : No of cases	Staining Intensity: Number of cases	German immunoreactive score : Number of cases
Well differentiated SCC	21	0%: 8 1%-10%: 5 11%-30%: 8 31%- 50%: 0 51%-80%: 0 81%-100%: 0	Negative: 8 Weak: 12 Moderate: 1 Strong: 0	High reactivity: 0 Weak reactivity: 21
Moderately differentiated SCC	75	0%: 0 1%-10%: 6 11%-30%: 3 31%- 50%: 5 51%-80%: 58 81%-100%: 6	Negative: 0 Weak: 9 Moderate: 62 Strong: 4	High reactivity: 64 Weak reactivity: 11
Poorly differentiated SCC	6	0%: 0 1%-10%: 0 11%-30%: 0 31%- 50%: 5 51%-80%: 1 81%-100%: 0	Negative: 0 Weak: 0 Moderate: 1 Strong: 5	High reactivity: 6 Weak reactivity: 0
Total	102			

Out of 21 cases of well differentiated squamous cell carcinoma 12 cases showed weak staining intensity, 8 cases were negative and 1 case showed moderate staining intensity. All 21 cases showed weak reactivity. Out of 75 cases of moderately differentiated squamous cell carcinoma 62 cases showed moderate staining intensity, 9 cases weak and 4 cases showed strong staining intensity. 64 cases showed high reactivity. Out of 6 cases of poorly differentiated squamous cell carcinoma 5 cases showed strong staining intensity and 1 case was moderate staining. All 6 cases showed high reactivity.

Table 3 Membranous And Cytoplasmic Podoplanin Expression In Squamous Cell Carcinoma According To Grades

Grade	Num-ber of cases	%	Podoplanin expres-sion	%	Mem-branous staining	%	Cytop-lasmic staining	%
Well differentiated scc	21	20.58	13	13.82	13	13.82	0	0
Moderately differentiated scc	75	73.52	75	79.78	75	79.78	66	72.21
Poorly differentiated scc	6	5.88	6	6.38	6	6.38	6	6.38
Total	102	100	94	100	94	100	72	76.59

Out of the 102 cases studied, the predominant histopathological grade was moderately differentiated squamous cell carcinoma which consisted of 75 cases (73.52%). All well differentiated squamous cell carcinoma expressing podoplanin showed membranous positivity, maximum cases of moderately differentiated squamous cell carcinoma showed both membranous (79.78%) and cytoplasmic positivity (70.21%) and all poorly differentiated squamous cell carcinoma showed membranous (6.38%) and cytoplasmic (6.38%) positivity. All the well differentiated squamous cell carcinoma cases showed weak reactivity and all the poorly differentiated squamous cell carcinoma showed high reactivity as per German immunoreactive score for podoplanin expression. Podoplanin shows more reactivity to cervix as compared to other organs. Out of 32 cases of moderately differentiated squamous cell carcinoma 23 cases showed quantity score of 4, 23 cases showed moderate staining intensity, 27 cases showed high reactivity (84.37%). Podoplanin shows more reactivity to cervix as compared to other organs.

DISCUSSION

Table 4 Comparison Of Podoplanin Expression

Study	Well differentiated scc	Moderately differentiated scc	Poorly differentiated scc
Schacht et al (2005) ⁷	No expression	Enhanced membranous expression	Predominant cytoplasmic expression
Present study (2022)	Weak membranous expression	Membranous expression	Strong cytoplasmic and membranous expression

Schacht et al. in his study showed predominant cytoplasmic podoplanin positivity in poorly differentiated squamous cell carcinoma, enhanced membrane podoplanin expression in moderately differentiated squamous cell carcinoma and no expression in well differentiated squamous cell carcinoma⁷. Our study correlates with Schacht et al. in the cytoplasmic podoplanin expression which was high in poorly differentiated squamous cell carcinoma.

Table 5 Comparison Of Quantity Score And Staining Intensity With Anjali Et Al

Study	Grading of squamous cell carcinoma	Quantity score	Staining intensity	Total
Anjali et al ⁸ (2022)	Well differentiated: 5 Moderately differentiated: 36 Poorly differentiated: 4	31-50% : 18 51-80%: 11 1-10%: 7 11-30%: 5 81-100%: 2 Negative : 2	Strong : 19 Moderate : 16 Weak: 8 Negative: 2	45
Present study (2022)	Well differentiated: 21 Moderately differentiated: 75 Poorly differentiated: 6	31-50% : 7 51-80%: 59 1-10%: 11 11-30%: 11 81-100%: 6 Negative : 8	Strong : 9 Moderate : 64 Weak: 17 Negative: 8	102

Anjali Mary Jibi and Vijaya Basawaraj studies have shown that poorly differentiated carcinoma with high reactivity podoplanin expression shows lymph node involvement i.e. higher stage of malignancy⁸.

Prasad et al showed podoplanin staining reactivity increases with increasing grade of squamous cell carcinoma and the present study results also correlates with it⁹.

Ashok Patil et al have studied podoplanin expression in dysplastic stratified squamous epithelium to suspect presence of malignancy. We have studied podoplanin only in malignancy to show its prognostic significance¹⁰.

The present study also shows the prognostic significance of podoplanin expression as per previous studies. Study of correlation of podoplanin expression with lymph node status can be continued afterwards.

CONCLUSION

Our study demonstrates that podoplanin has prognostic significance. Podoplanin could be a potent biomarker which correlates with grades of squamous cell carcinoma. As per German immunoreactive score high reactivity of podoplanin expression is suggestive of high grade status squamous cell carcinoma

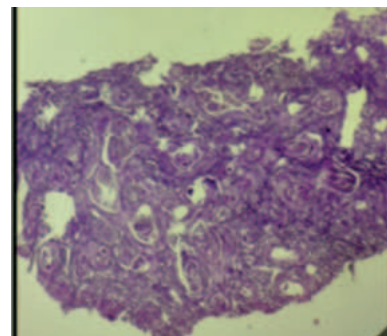


Fig 1 A: H&E Scanner View Showing Well Differentiated

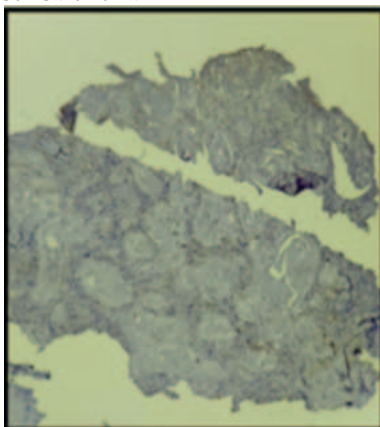
Squamous Cell Carcinoma

Fig 1 B: Podoplanin IHC Scanner View Showing Well Differentiated Squamous Cell Carcinoma With Quantity Score 1, Weak Intensity And Immunoreactivity Score For Podoplanin 1 (Weak Reactivity)

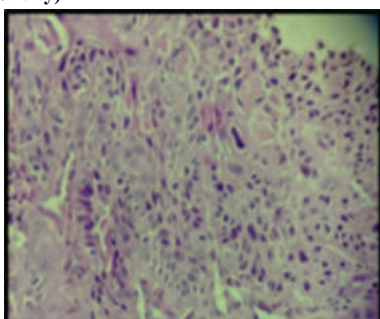


Fig 2 A: H&E High Power View Showing Moderately Differentiated Squamous Cell Carcinoma

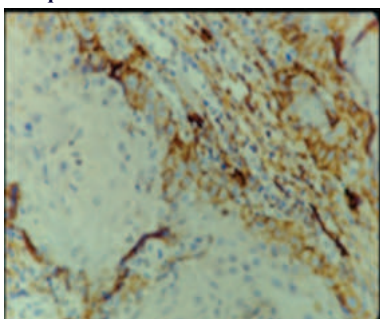


Fig 2 B: Podoplanin IHC Showing Membranous Positivity In Moderately Differentiated Squamous Cell Carcinoma With Quantity Score 2, Moderate Intensity And Immunoreactivity Score For Podoplanin 4 (Weak Reactivity).

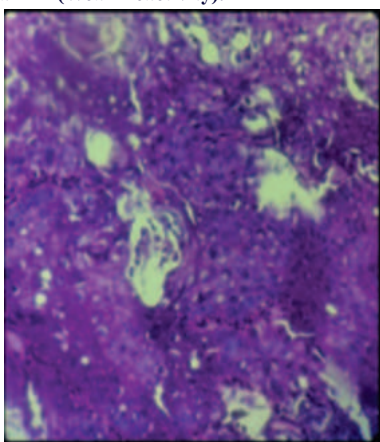


Fig 3a: H&E High Power View Showing Poorly Differentiated Squamous Cell Carcinoma

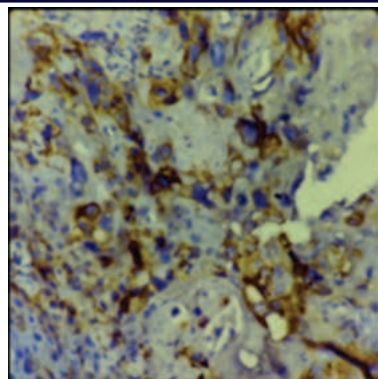


Fig 3b: Podoplanin IHC High Power View Showing Membranous And Cytoplasmic Staining In Poorly Differentiated Squamous Cell Carcinoma With Quantity Score 3, Strong Intensity And Immunoreactivity Score For Podoplanin 9 (High Reactivity)

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