Original Article

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Pathology Section

Cortactin Immunoexpression and its Association with Clinicopathological Parameters in Head and Neck Squamous Cell Carcinoma: A Cross-sectional Study

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ABSTRACT

Introduction: Head and Neck Squamous Cell Carcinoma (HNSCC) is a group of malignancies, involving oral cavity, pharynx, hypopharynx, larynx. HNSCC are aggressive tumours having high invasive capacity which is due to phenotypic alterations between neoplastic cells and surrounding environment. Invadopodia are actin rich protrusions on the surface of invasive neoplastic cells that promotes degradation of extracellular matrix. Cortactin (CTTN), a cytoplasmic protein, promotes polymerisation and rearrangement of actin cytoskeleton plays an important role in predicting tumour progression. It's been suggested that increased expression of cortactin in tumours will help in identifying cell migration, invasion and metastasis thereby predicting tumour progression in HNSCC.

Aim: To evaluate the Immunohistochemical (IHC) expression of cortactin in biopsies and resected specimens of histologically proven HNSCC and to assess its association with tumour grade, Depth of Invasion (DOI), Perineural Invasion (PNI) and Tumour, Nodal, Metastasis (TNM) staging.

Materials and Methods: This cross-sectional study was conducted at the Department of Pathology, Tirunelveli Medical College, Tirunelveli, Tamil Nadu, India, for a period of 18 months, December 2022 to May 2024, on 35 patients of histologically proven HNSCC Cortactin immunoexpression was assessed using Intensity Response Score (IRS) in biopsies and resected specimens of HNSCC. The IRS was calculated as intensity of the staining reaction multiplied by the percentage of positive cells. Based on the IRS, cortactin expression was categorised as negative (weak) immunoexpression, positive (medium and strong) immunoexpression. Positive immunoexpression was correlated with maximum depth of invasion, presence of PNI and higher TNM staging.

Results: In the present study of 35 cases, the most commonly affected age group was 60-69 years. Males constituted the majority of cases 23 cases (65.71%), while females accounted for 12 cases (34.28%). Out of 10 cases (28.57%) with DOI >10 mm, positive cortactin expression was observed in 9 cases (90%). Among the 13 cases exhibiting PNI, cortactin positivity was observed in 12 cases (92.30%). With respect to tumour stage, cortactin positivity was identified in 7 out of 8 cases (87.50%) with the stage T3 disease and in 5 out of 6 cases (83.33%) with the stage T4 disease. Furthermore, cortactin positivity was observed in all cases with nodal involvement, being present in 4 cases (100%) with stage N1 and 6 cases (100%) with stage N2. All associations were statistically significant with p-value <0.05.

Conclusion: Cortactin expression was significantly observed in HNSCC cases with maximum DOI, presence of PNI and higher TNM staging. Thus, cortactin may serve as a relevant prognostic marker in assessing tumour behaviour and prognosis.

Keywords: Biomarker, Cancer staging, Malignancy, Metastasis, Prognosis, Squamous cell carcinoma

INTRODUCTION

The HNSCC is a group of malignancies, involving oral cavity, pharynx, hypopharynx, larynx and salivary glands. It is the seventh most common cancer worldwide, accounting 4.5% of all cancer diagnoses [1]. In India it accounts for 30% of all cancers. High incidence rate is due to chewing tobacco, areca nut, smoking consumption of alcohol and recently due to high prevalence of human papilloma virus [2]. Due to lack of clinical symptoms in early period, patient often presents at advanced stages of tumour. Despite the advancement of cancer management, overall 5-year survival rate remains relatively low compared to many malignancies. This highlights the urgent need of novel marker in earlier detection, prognostication and enabling the treating physician to select more aggressive course of therapy for high-risk patients. This marker should also serve as a molecular target for development of new anticancer drugs. HNSCC has the ability to form the actin rich protrusions called invadopodia on the surface of invasive neoplastic cells, thereby causing degradation of extracellular matrix invasion into the lymphatic vessels and nerves [3]. The genetic alteration involved in HNSCC is amplification of Ch11q13 which is a locus for CTTN gene (previously noted as EMS1) encoding cortactin [4]. Cortactin is ubiquitously expressed in all the cells except for hematopoietic cells. It consists of four major domains: N-Terminal Acidic (NTA), 6.5 tandems repeat, proline-rich domain, and the C-terminal Src homology3 domains (SH3domains). The NTA domain of cortactin plays a critical role in regulating the branched actin assembly. It binds and enhances the activation of Arp2/3 complex by Wiskott-Aldrich Syndrome protein (WASp). It prevents debranching of actin filament networks thereby promoting the cell migration [5]. The changes in the actin cytoskeleton forms two organelles- invadopodia and podosomes. Invadopodia are actin rich protrusion found in the invasive cancer cells which occurs through four stages such as initiation, assembly, maturation and disassembly. Podosomes found in macrophages and helps to cross tissue barriers and extracellular matrix degradation. Cortactin is an important component in both invadopodia and podosomes [5]. The C terminus helps in cortactin to function has a scaffolding protein. Yet another function of cortactin is

bridging the membrane trafficking proteins to the actin cytoskeleton and helps in invasion of tumour cells [6].

The present study simultaneously evaluates cortactin expression with four critical parameters (TNM staging, DOI, PNI and tumour grade) in HNSCC, offering a more comprehensive clinical correlation than many previously published studies where they have demonstrated strong among them [7-10]. Hence, the study aimed to evaluate the immunohistochemical expression of cortactin in HNSCCs using an intensity-based scoring system; And to assess the association between cortactin immunoexpression and tumour grade, depth of invasion, perineural invasion, TNM staging.

MATERIALS AND METHODS

This cross-sectional study was conducted at the Department of Pathology, Tirunelveli Medical College, Tirunelveli, Tamil Nadu, India, for a period of 18 months, December 2022 to May 2024 with a sample size of 35 patients. Ethical committee approval was obtained (ECR/1227/Inst/TN/2019).

Inclusion criteria: Adult patients with HNSCC confirmed by histopathological examination from biopsy or resected specimens. Adequate formalin-fixed paraffin-embedded tissue blocks.

Exclusion criteria: Patients who underwent radiotherapy, chemotherapy; non squamous cell carcinomas of head and neck. Inadequate or poor-quality tissue blocks unsuitable for IHC staining which includes extensive necrosis and insufficient tumour cells. Patients with incomplete clinical or pathological data.

Sample size calculation: The sample size for the present study after applying inclusion and exclusion criteria was 35 cases, determined by census sampling. All eligible primary HNSCC cases received in Department of Pathology during the study period was included. Since the total number of cases meeting the inclusion criteria within this timeframe was limited, a census approach was used. TNM staging and nodal assessment were available for 25 cases only, as 10 cases were small biopsy specimens in which staging could not be assessed.

Study Procedure

Tissue sections of histopathologically proven HNSCC were taken from the archival blocks of Department of Pathology, Tirunelveli Medical College. From the Formalin Fixed Paraffin Embedded tissues, 3 micron thick sections were cut. The ribbons of tissue section were transferred onto the positively charged slides from the tissue float bath. The slides were dewaxed by keeping in the hot air oven and then treated with two changes of xylene for 15 minutes and five minutes. They were put in descending grades of alcohol 100% and 90% for five minutes each followed by water wash for two minutes. Antigen retrieval is done for 10 minutes in the pressure cooker using citrate buffer of pH 6.0 followed by cooling for 20 minutes. Slides were washed using distilled water followed by treating with Tris(hydroxymethyl)aminomethane + Ethylenediaminetetraacetic acid (Tris-EDTA) buffer. Slides are then treated with 3% hydrogen peroxide for five minutes to quench endogenous peroxidase activity of cells that would result in non specific staining. Rabbit polyclonal IgG primary Anticortactin unconjugated antibody with dilution of 1: 200 is added and kept for one hour washed in buffer twice. The primary anticortactin antibody is detected using Poly Excel HRP/ DAB IHC {Poly Excel polymer HRP (Horseradish Peroxidase) DAB (3,3'-Diaminobenzidine)} detection system. The sections are treated with target binder for 12 minutes at room temperature. This is followed by incubation with Poly Excel HRP reagent for 12 minutes at room temperature. After two washes with wash buffer, substrate DAB is added to the sections for 10 minutes. Slides were washed in distilled water to remove excess chromogen and counterstained with haematoxylin for 30 seconds. Dehydrated with ethanol and xylene and mounted permanently with DPX. The slides were observed under the light microscope.

Breast carcinoma- invasive ductal carcinoma- No Specific Type (NOS) was used as positive control as per the instructions provided in IHC test kit instruction manual.

Cortactin expression in the tumour cells was scored based on IRS which includes intensity of staining of tumour cells and percentage of positive tumour cells [8]. Cortactin expression was localised in the cytoplasm and in the areas of cell substratum contacts. The scoring was given by multiplying the intensity and percentage of positive tumour cells and scored according to the below table [Table/Fig-1].

	Score	Intensity of staining		
		Weak 1 Point	Moderate 2 points	Strong 3 points
Positive tumour cells score	<10%=1 point	1	2	3
	10-50%= 2 Points	2	4	6
	51-80%= 3 Points	3	6	9
	>80 %=4 points	4	8	12

Intensity response score (IRS)	Weak (Negative) expression	Moderate (Positive) expression	Strong (Positive) expression
Score	1-4	6-8	9-12

[Table/Fig-1]: Intensity Response Score (IRS) [8].

STATISTICAL ANALYSIS

Data were entered in Microsoft Excel (Windows 10, version 2010) and analysis was done using the Statistical Package for Social Sciences (SPSS) for windows software (version 25; SPSSInc, Chicago). Frequencies and percentages for categorical variable were used. To find association between two categorical variables Pearson's Chi-square test was used. Level of significance (p-value) was set at the value of 0.05.

RESULTS

The most common age distribution among our study population belong to the age group of 60-69 years accounting for 15 cases (42.85%) and the least common age group of the study population is 30-39 years accounting for 3 cases (8.57%). The mean age of the study population was 57 years.

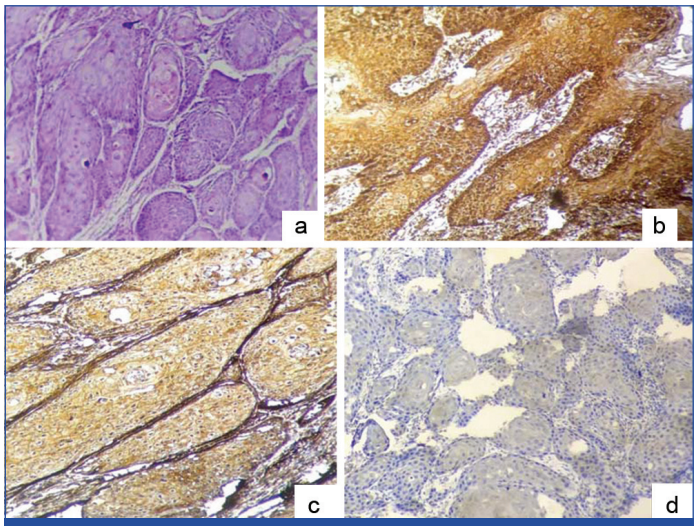
Out of 35 cases, majority were males 23 cases (65.71%), while females were 12 cases (34.28%). The tongue was the most common primary tumour site, accounting for 13 cases (37.14%) followed by buccal mucosa in 8 cases (22.85%), larynx in 6 cases (17.14%), maxilla in 4 cases (11.42%), floor of mouth in 2 cases (5.71%), lower alveolus and lip in 1 case each (2.85%).

Histopathological evaluation revealed moderately-differentiated tumour in 24 cases (68.57%), well-differentiated tumour in 8 cases (22.85%) and poorly-differentiated tumour in 3 cases (8.57%). In the study population, 10 cases (28.57%) of the study population has DOI more than 10 mm, 7 cases (20%) of study population has DOI 5-9 mm followed by 5 cases (14.29%) has DOI 0-4 mm. Three cases were from maxilla where DOI is insignificant and 10 cases were of small biopsies in which DOI could not be assessed.

Out of 35 cases, PNI was identified in 13 cases (37.14%), 22 cases (62.86%) does not show PNI. TNM staging could be assessed in 25 cases, as 10 cases were small biopsies. Among these, 5 cases (20%) belonged to T1 stage, 6 cases (24%) to T2 stage, 8 cases (32%) to T3 stage, and 6 cases (24%) to T4 stage. Among the 25 cases in which nodal staging could be assessed, 15 cases (60%) showed no nodal involvement (N0), while 4 cases (16%) and 6 cases (24%) showed N1 and N2 stage disease, respectively.

Cortactin expression was categorised as negative (weak) immunoexpression, positive (medium and strong) immunoexpression [Table/Fig-2]. It was correlated with multiple clinicopathological parameters. Positive cortactin immunoexpression was observed predominantly in moderately-differentiated tumours (20 cases),

followed by well-differentiated tumours (1 cases), and poorly-differentiated tumours (2 cases), with statistically significant association (p=0.001254). Tumours with DOI greater than 10 mm showed cortactin positivity in nine out of 10 cases, while six of seven cases with a DOI 5-9 mm and one of five cases with DOI 0-4 mm were also positive (p=0.010526). PNI was present in 13 cases, of which 12 cases demonstrated cortactin positivity with p-value of 0.010837 [Table/Fig-3].



[Table/Fig-2]: a) H&E stain of squamous cell carcinoma (10x); b) Strong (positive) cortactin expression (10x); c) Moderate (positive) expression (10x); d) Weak (Negative) expression (10x).

Paramters	Cortactin status			
	Total cases	Positive	Negative	p-value
Grade of differentiation				
Well	8	1	7	0.001254
Moderate	24	20	4	
Poor	3	2	1	
DOI (10 cases are of small biopsies, 3 cases from maxilla where doi: cannot be assesed)				
0-4 mm	5	1	4	0.010526
5-9 mm	7	6	1	
>10 mm	10	9	1	
Perineural invasion				
Present	13	12	1	0.010837
Absent	22	11	11	
TNM Stage – 10 cases were small biopsies, staging could not be assesed				
T1	5	1	4	0.038011
T2	6	5	1	
T3	8	7	1	
T4	6	5	1	
N0	15	8	7	0.0391
N1	4	4	0	
N2	6	6	0	

[Table/Fig-3]: Association of immunoexpression of cortactin with grade, DOI, PNI and TNM staging.
Pearson's Chi-square test was used; p-value less than 0.05-Significant

With respect to tumour stage, cortactin expression was noted in one of five cases with T1 stage, five of six cases with T2 stage, seven of eight cases with stage T3, and five of six cases with T4 disease with statistically significant association (p=0.038011). Among nodal stages, cortactin positivity was observed in all cases with N1 (4 cases) and N2 (6 cases) with p-value of 0.0391. Overall cortactin expression showed significant association with adverse histopathological features and advanced tumour stage [Table/Fig-3].

DISCUSSION

Cortactin encoded by the gene CTTN is an actin binding protein expressed ubiquitously in all tissues except for haematopoietic cells. On the cellular level, cortactin plays an important role in cancer cell migration and invasion through the mechanism of invadopodia and podosomes by degradation of the extracellular matrix and causes lymph node metastasis and other distant metastases, thereby decreasing the survival rate of the patients.

In the present study, cortactin overexpression was observed and shows significant association with aggressive tumour behaviour. A strong correlation between increased cortactin expression and greater DOI suggests its role in promoting tumour cell motility and ability to invade the tissues supporting the hypothesis that cortactin contributes to epithelial mesenchymal transition and extracellular matrix degradation. The study also demonstrated that presence of PNI in tumours showed positive immunoexpression of cortactin indicating the enhanced metastatic potential. Positive association between cortactin and advanced TNM staging reflects its role in tumour progression and disease severity.

Many studies such as Yamada S et al., and Horn D et al., have shown amplification of cortactin in HNSCC [11,12]. Likewise, Wang L et al., assessed overexpression of cortactin is associated with aggressiveness of tumour [13].

Hofman P et al., in their study of prognostic significance of cortactin levels in HNSCC, assessed that overexpression of cortactin was associated with advanced tumour and nodal staging. In his study, 38 out of 77 cortactin overexpressing tumours were of TNM stage IV and 44 out of 77 cortactin - overexpressing tumours were poorly differentiated carcinomas and stated that cortactin is independent prognostic marker for assessing the local recurrence, disease free survival and overall survival rate in oral malignancies [7].

In contrast to the study by Boeve K et al., which did not demonstrate a significant association of cortactin with maximum DOI, our study found a statistically significant correlation between maximum DOI and cortactin expression [8].

In the current study, out of 13 cases with PNI, 92.30% (12 cases) show positive immunoexpression. This is in accordance with study conducted by Ambrosio EP et al., in which out of 71 cases with PNI 58 cases show overexpression and stated that cortactin overexpression was associated with PNI [9]. Comparison of cortactin expression with clinicopathological parameters across Head and Neck Squamous Cell Carcinoma (HNSCC) studies are shown in [Table/Fig-4] [7-9].

Authors and Year of study	Sample size	Parameters evaluated	Significant correlation status with p-value
Present study, 2026	35	Grade, DOI, PNI, TNM staging	Cortactin expression correlated with all parameters (p<0.005)
Hofman P et al., [7] (2008)	176	TNM staging and histologic grade	Yes. Cortactin expression associated with higher TNM, high grade p = 0.005
Boeve K et al., [8] (2021)	87 Early stage oral SCC pT1cN0	DOI	In their study they did not find strong correlation with DOI
Ambrosio EP et al., [9]	47 Laryngeal SCC	PNI and cortactin expression at deep front and invasive areas	Higher cortactin expression at deep invasive front with PNI (p=0.004)

[Table/Fig-4]: Comparison of cortactin expression with clinicopathological parameters across Head and Neck Squamous Cell Carcinoma (HNSCC) studies [7-9].

In the present study, 87.5% cases with the stage T3 and 83.33% of the cases with the stage T2 and T4 shows cortactin positivity, 80% cases with T1 stage shows negative immunoexpression which was statistically significant with p-value >0.05. Further in our

study, 53.33% of cases with N0 staging and 100% of cases with N1 and N2 staging shows positive immunoexpression. This is also accordance with the study, cortactin is a prognostic marker for oral squamous cell carcinoma and its overexpression is involved in oral carcinogenesis done by Liu YC et al., where out of 32 cases with T4 stage, 22 cases show cortactin overexpression. Out of 32 cases with N2 staging 23 cases show cortactin overexpression and infer that higher T staging correlates with positive immunoexpression of cortactin [14].

Limitation(s)

Drawbacks from the current study include the small sample size limiting the statistical power. The results of the present study will need to be validated in a larger population. Follow-up and survival data were not evaluated, preventing a direct assessment of cortactin as a predictor of survival outcomes.

CONCLUSION(S)

Cortactin overexpression in HNSCC showed a significant association with adverse clinicopathological parameters including higher TNM stage, maximum DOI, presence of perineural invasion and higher-grade tumour differentiation. These findings support the role of cortactin as a marker of aggressive tumour biology. Incorporating cortactin in routine histopathology reporting may improve risk stratification and early identification of patients with potentially aggressive disease, thereby guiding timely therapeutic decisions. Therefore, further studies should focus on molecular target for development of new anticancer agents.

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