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Original Research Article

CORTACTIN IMMUNOEXPRESSION AND CORRELATION WITH WORST PATTERN OF INVASION IN HEAD AND NECK SQUAMOUS CELL CARCINOMAS

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ABSTRACT

Background: Cortactin, an actin-binding protein encoded by the EMS1/CTTN locus on chromosome 11q13, promotes invadopodia formation and tumour invasion. Overexpression of cortactin has been linked to aggressive clinicopathological behaviour, and the worst pattern of invasion (WPOI) is a well-established histological marker of aggressiveness. **Aim:** To estimate the immunoexpression of cortactin in biopsies and resected specimens of histologically proven HNSCC and correlate it with the worst pattern of invasion. **Materials and Methods:** This cross-sectional study included patients with head and neck squamous cell carcinoma (HNSCC) at Tirunelveli Medical College over 18 months. Formalin-fixed paraffin-embedded tissue sections were processed for cortactin immunohistochemistry using a rabbit polyclonal anti-cortactin antibody. Expression was assessed using the Intensity Response Score (IRS), which combines staining intensity and percentage positivity. Weak expression (IRS 1–4) was considered negative, while moderate to strong expression (IRS 6–12) was considered positive. WPOI was classified as 1–4 or 5, respectively. **Result:** Of the 35 cases, 23 (65.71%) showed WPOI 1–4 and 12 (34.28%) showed WPOI 5. Cortactin positivity was observed in 52.17% of WPOI 1–4 cases and 91.66% of WPOI 5 cases, while cortactin negativity was more frequent in WPOI 1–4 (47.82%) than in WPOI 5 (8.33%). The association between cortactin expression and WPOI was statistically significant ($p = 0.019$). **Conclusion:** Cortactin overexpression is significantly associated with WPOI 5 in HNSCC, supporting its role as a marker of aggressive tumour invasion. The combined evaluation of cortactin and WPOI may enhance prognostic assessment and guide treatment planning.

INTRODUCTION

Head and neck squamous cell carcinoma (HNSCC) is a group of malignancies involving the oral cavity, pharynx, hypopharynx, larynx, and salivary glands. HNSCC is the seventh most common cancer worldwide, accounting for 4.5% of all cancer diagnoses.[1] It is a biologically heterogeneous disease, and its local invasiveness and tendency for regional metastasis determine the patient outcomes. Chromosome 11q13 amplification is a recurrent genomic event in HNSCC and includes the EMS1/CTTN locus that encodes cortactin, an actin-binding protein implicated in cytoskeletal remodelling and cell motility.[2] Incidence rate is higher in South and Southeast Asia, particularly India, where head and neck cancers account for 30% of all cancers.[1] High incidence rate is due to chewing tobacco, areca nut, smoking, consumption of alcohol and recently due to high prevalence of human papilloma virus.[3] Early cytogenetic and molecular analyses identified EMS1 (cortactin) amplification in a substantial subset of primary head and neck tumours, linking gene copy number gain to increased cortactin protein levels in tumour cells.[4] HNSCCs are aggressive tumours with high invasive capacity due to phenotypic alterations between neoplastic cells and the surrounding environment.

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Cortactin promotes the formation and maturation of invadopodia, an actin-rich protrusion on the surface of invasive neoplastic cells that promotes degradation of the extracellular matrix.[5] Studies across various cancer types have described cortactin as a core regulator of invadopodia dynamics and matrix degradation, thereby enabling local tissue penetration and metastatic dissemination.[6,7] The overexpression of cortactin is associated with aggressive clinicopathologic behaviour in HNSCC and other related squamous carcinomas.[7]

Early diagnosis of HNSCC is necessary because, despite the advancement of cancer management, the overall 5-year survival rate is still the lowest among all malignancies and has a poor prognosis.[6]Immunohistochemical studies have reported that elevated cortactin protein levels correlate with advanced stage, lymph node metastasis, higher recurrence rates, and poorer disease-specific and overall survival in laryngeal and oral cavity cancers. Studies have found cortactin to be an independent prognostic factor and even suggest it as a molecular marker of aggressiveness.[7,8]

A study on oral squamous cell carcinoma (OSCC) reported that cortactin might help identify patients who are at higher risk of having lymph node spread and decide on further procedures, such as removing neck lymph nodes or performing a sentinel node biopsy.[9] Although cortactin is a good prognostic and immunohistochemical biomarker in OSCC, its use alone may not capture the full spectrum of tumour behaviour, since patient outcomes can be influenced by additional histopathological factors. Thus, recent studies have emphasised the "worst pattern of invasion" (WPOI) classification. This WPOI system identifies the most aggressive form of tumour spread at the invasive front, with categories 4 and 5 (characterised by small isolated tumour nests or satellite islands) consistently associated with higher risks of nodal metastasis, extranodal extension, recurrence, and poorer survival.[10] Recent clinicopathologic studies, therefore, promote the inclusion of WPOI in routine pathology reporting and risk stratification.[10,11]

Since cortactin is responsible for tumour invasion through invadopodia formation and WPOI captures the morphological manifestation of aggressive invasion at the tumour front, correlating the two parameters might result in a better prognostic tool. However, few studies have explored their relationship and potential combined utility. Hence, this study aimed to estimate the immunoexpression of cortactin in biopsies and resected specimens of histologically proven HNSCC and correlate it with the worst pattern of invasion.

MATERIALS AND METHODS

This hospital-based cross-sectional study was conducted on 35 patients at the Department of Pathology, Tirunelveli Medical College, over a period of 18 months (December 2022 to May 2024). Ethical committee approval was obtained.

Inclusion Criteria

Adult patients with histopathologically confirmed HNSCC from biopsy or resection specimens. Primary tumour patients with adequate formalin-fixed paraffin-embedded (FFPE) tissue blocks containing sufficient viable tumour and invasive front for assessment. Patients with complete clinicopathological data were included.

Exclusion Criteria

Patients who underwent prior treatment (surgery, radiotherapy, or chemotherapy), had non-squamous histology, or presented with recurrent or metastatic tumours. Patients with inadequate or poor-quality tissue (extensive necrosis, artefacts, or insufficient viable tumour cells) or very small biopsies without an invasive front.

Methods

Tissue sections of histopathologically proven HNSCC were obtained from the archival blocks of Tirunelveli Medical College. From the formalin-fixed paraffin-embedded (FFPE) tissues, 3 µm thick sections were cut and transferred onto positively charged slides from a tissue float bath. The slides were dewaxed in a hot air oven, treated with two changes of xylene (15 and 5 min), passed through descending grades of alcohol (100% and 90% for 5 min each), and washed in water for 2 min. Antigen retrieval was performed in citrate buffer (pH 6.0) using a pressure cooker for 10 min, followed by 20 min of cooling. The sections were then washed with distilled water and treated with Tris-EDTA buffer. To block endogenous peroxidase activity, 3% hydrogen peroxide was applied for 5 min. A rabbit polyclonal primary anti-cortactin antibody was applied for 1 hour, followed by washing and detection using the Poly Excel HRP/DAB IHC detection system. The sections were incubated sequentially with the target binder (12 min) and Poly Excel HRP reagent (12 min), washed, and treated with DAB substrate for 10 min. After washing in distilled water, counterstaining with haematoxylin for 30 seconds was performed, followed by dehydration in ethanol and xylene and permanent mounting with DPX. The slides were then examined under a light microscope.

Cortactin expression in tumour cells was assessed using the Intensity Response Score (IRS), based on both staining intensity and the percentage of positive cells. Staining was localised to the cytoplasm and cell-substratum contact areas. The intensity was graded as weak (1 point), moderate (2 points), or strong (3 points), and percentage positivity was scored as <10% (1 point), 10–50% (2 points), 51-80% (3 points), or >80% (4 points). The IRS was obtained by multiplying the two scores, yielding values between 1 and 12. Scores of 1-4 were classified as weak expression, 6-8 as moderate, and 9-12 as strong expression. For statistical analysis, weak expression (IRS 1-4) was considered negative,

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while moderate and strong expression (IRS 6-12) were grouped as positive immunoexpression of cortactin. IRS 5 is not included in the standard categorisation and was not observed.

The pattern of invasion was assessed using the Brandwein-Gensler system. In this system, Patterns 1-4 range from broad pushing fronts and large stellate islands to smaller nests and single-cell infiltration, whereas Pattern 5 is defined by tumour satellites separated by ≥1 mm from the main tumour without intervening stromal fibrosis. Cases were grouped into two categories: WPOI 1-4, representing less aggressive patterns, and WPOI 5, representing the most aggressive pattern. This approach was chosen because WPOI-5 carry the highest risk of nodal metastasis, extranodal extension, and recurrence.

Statistical Analysis

Data were analysed using SPSS (v25), and data were presented in frequencies and percentages. The Pearson chi-square test was used to find the association between two categorical variables, and the level of significance was set at a value of < 0.05.

RESULTS

Of the 35 cases, 12 (34.28%) had WPOI 5 and 23 (65.71%) had WPOI 1-4. [Table 1]

Table1:Distribution of WPOI

WORSTPATTERNOF INVASION(WPOI)	NOOF CASES	PERCENTAGE (%)
WPOI1-4	23	65.71%
WPOI 5	12	34.28%
TOTAL	35	100%

Cortactin positivity was observed in 52.17% of the WPOI 1-4 category, and 91.66% of the WPOI 5 category. Cortactin negativity was more frequent in WPOI 1-4 (47.82%) than in WPOI 5 (8.33%), and these associations were significant ($p = 0.019$). [Table 2]

Table 2: Association of immunoexpression of cortactin with WPOI

Cortactinimmunoexpression	WPOI category		p-value
	1-4	5	
Positive	12 (52.17%)	11 (91.66%)	0.019
Negative	11 (47.82%)	1 (8.33%)	

DISCUSSION

Head and neck squamous cell carcinoma shows diverse variations based on individuals, with outcomes mostly determined by local invasion and metastatic potential. Both cortactin expression and WPOI are important predictors of tumour aggressiveness. In our study, we aimed to estimate the immunoexpression of cortactin in biopsies and resected specimens of histologically proven HNSCC and correlate it with WPOI.

Of the 35 cases, 12 (34.28%) had WPOI 5 and 23 (65.71%) had WPOI 1-4. Similarly, Rahat et al. studied 81 OSCC cases and reported that only 14.8% belonged to the WPOI 5 category and 85.2% belonged to the WPOI 1-4 category.[12] In another study on OSCC cases, Narayanan et al. found that 23.4% of the cases belonged to the WPOI 5 category and 52.5% belonged to the WPOI 4 category.[13] Yamauchi et al. observed 38 oral tongue squamous cell carcinoma cases and reported that only 2.6% of cases were WPOI 5 and all the other cases belonged to the WPOI 1-4 category.[14] Thus, indicating that WPOI 5 cases are rare among the tumour cases compared to the WPOI 1-4 category.

In our study, cortactin positivity was observed in 52.17% of the WPOI 1-4 category, and 91.66% of the WPOI 5 category. Cortactin negativity was more frequent in WPOI 1–4 (47.82%) than in WPOI 5 (8.33%), and this association was significant ($p = 0.019$). Horn et al. concluded that cortactin is associated with the progression of non-HPV-related OSCC, which supports our findings. They also emphasised that cortactin influences migration and invasion in non-HPV-related HNSCC cell lines.[15]

Gibcus et al. reported that carcinomas with 11q13 amplification have high protein expression levels of cortactin. They also found that cortactin was associated with disease-specific mortality, while cortactin-positive cases were significantly associated with higher mortality ($p < 0.01$). Furthermore, they concluded that cortactin is a good predictor of disease-related mortality in laryngeal squamous cell carcinomas.[16] Further strengthening our study, Kopecka et al. reported that cortactin synthesis is molecularly linked to aggressive behaviour and poor prognosis, and that cortactin is elevated in tumours under the WPOI 5 category. They further concluded that patients with cortactin-positive tumours in high WPOI categories have reduced survival and higher recurrence rates.[17] Thus, indicating that cortactin is associated with WPOI 5, high mortality, and high recurrence rates.

Cortactin overexpression is strongly associated with WPOI 5, emphasising its role as a prognostic marker

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of aggressive invasion in HNSCC. When used alongside WPOI, cortactin immunoexpression may provide a more detailed assessment of tumour behaviour and help in treatment planning. Future studies with larger samples and long-term follow-ups are needed to validate the prognostic utility of cortactin along with WPOI in routine clinical practice.

Limitations

The relatively small sample size from a single centre may restrict the generalisability of the findings. In addition, the cross-sectional design and lack of long-term follow-up data prevented the correlation of cortactin expression and WPOI with survival outcomes.

CONCLUSION

The immunoexpression of cortactin was significantly associated with WPOI in HNSCC, and the tumours with WPOI 5 had a higher cortactin positivity compared to those in the WPOI 1-4 category. Therefore, cortactin immunoexpression, when evaluated alongside WPOI, may serve as a combined marker of tumour aggressiveness. Future studies evaluating cortactin as a predictive marker for response to targeted or immunotherapy may open new paths for personalised treatment.

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