

## ORIGINAL PAPER

# EXPRESSION OF C-TERMINAL TENSIN-LIKE IN BREAST CARCINOMA AND ITS CORRELATION WITH KNOWN PROGNOSTIC FACTORS

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C-terminal tensin-like (Cten) is a marker for poorly differentiated breast cancer. We evaluated the immunohistochemical expression of Cten in invasive breast carcinoma in our population and correlated it with known histopathologic prognostic variables.

Fifty-seven specimens of modified radical mastectomy diagnosed as invasive ductal carcinoma were collected. The histopathologic findings were noted independent of the result of Cten. According to the results of Cten immunohistochemistry, the tumors were categorized as negative/mild, moderate, or high expression and were statistically correlated with histologic findings.

In our study, 47 (82.5%) cases showed negative/mild expression, 2 (3.5%) cases showed moderate staining, and 8 (14%) cases showed strong expression of Cten. Positive Cten was present in pT4 stage tumors. Similarly, grade III tumor showed moderate expression in 2 (3.5%) cases and strong staining in 8 (14%) cases. Positive expression of Cten was observed in cases with lymphovascular invasion (LVI) and high axillary lymph nodal involvement (N3). All these poor prognostic factors were significantly associated with moderate to high expression of Cten.

We found that tumor size and extent, histologic grade, LVI, and lymph node status were significantly associated with Cten expression. C-terminal tensin-like can be used as marker of poor prognosis in breast carcinoma.

**Key words:** breast carcinoma, Cten, immunohistochemistry, lymphovascular invasion, pathological stage.

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## Introduction

Breast cancer is the leading cause of cancer morbidity and mortality in females [1–4]. Although methods for early detection and personalized treatment have been developed, the survival rate is still around 80% for developed countries and below 40% for less developed countries [2]. It is a heterogeneous disease with number of molecular mechanisms involved in tumor initiation, progression and regulation [5, 6]. Inter-

tumoral and intra-tumoral heterogeneity largely affect patient management and outcomes [6]. Tumor heterogeneity leading to metastasis and therapy resistance was reported to be the possible cause of more than 620,000 women succumbing to breast cancer in 2018 [6]. An insight into the molecular mechanisms involved can help us to understand the variable therapy outcomes in patients with similar prognostic features [2, 5]. Moreover, targeted therapies can be developed against the metastatic potential of cancer

cells. Recent research has led to identification of numerous novel biomarkers involved in cancer metastasis including C-terminal tensin-like (Cten) [1, 7, 8].

C-terminal tensin-like is the fourth member of the tensin focal adhesion family and is also designated as tensin4 or TNS4 [1, 9, 10]. Focal adhesions are part of cellular signaling pathways that regulate biological processes, such as cell attachment, apoptosis, growth factor receptor homeostasis, gene expression and differentiation, and invasion [1, 10, 11]. C-terminal tensin-like has the lowest molecular weight among tensins. It is not only localized to the cytoplasmic side of the focal adhesion molecule but is also present in the nucleus [9, 11]. C-terminal tensin-like has oncogenic and tumor suppressor functions that are manifested in various cancers [2, 7, 9].

C-terminal tensin-like is expressed by normal prostate and placenta and is not detectable in other tissues by Northern blot assays [1, 2, 8, 9]. It can be a cancer diagnostic and prognostic biomarker owing to its expression in various cancers. In prostatic cancer, Cten is downregulated, while in breast, colon, lung, and pancreatic cancer, it is upregulated [1, 2, 9, 12]. Moreover, it is involved in cell migration and invasion in these tumors [1, 2, 9, 10, 12, 13].

In breast carcinoma, Cten expression is linked to epidermal growth factor receptor (EGFR) [8, 9, 13]. Epidermal growth factor receptor plays crucial roles in cell proliferation, migration, and differentiation. It causes 'tensin switch' in breast cancer where tensin3 is downregulated and Cten is upregulated [8, 13]. C-terminal tensin-like diminishes the degradation of active EGFR and prolongs its signaling, leading to cell invasion and migration due to disruption of the link between integrin and cortical actin at focal adhesion sites [1, 9, 11]. C-terminal tensin-like has been correlated with poor prognostic variables of breast cancers such as high grade, tumor size, lymph node involvement, local invasion, distant metastasis, expression of EGFR and human epidermal growth factor receptor 2 (HER2), and a poor Nottingham prognostic index [1, 2, 8, 10, 12]. Research on Cten is warranted as it is a potential diagnostic and prognostic marker for poorly differentiated breast cancer and may also prove to be a therapeutic target along with EGFR.

This study was designed to evaluate the immunohistochemical expression of Cten in invasive breast carcinoma in our population and correlate it with known prognostic variables.

## Material and methods

It was a correlational study that was carried out in the central laboratory, King Edward Medical University, Lahore, Pakistan over a period of one year from December 2021 to November 2022. Ethical approval

was obtained from the institutional ethical review board and informed consent was obtained from patients. Fifty-seven specimens of modified radical mastectomy (MRM) were collected using the 95% confidence level, 8% absolute precision, with the expected Cten gene percentage being 90% [1]. Non-probability purposive sampling was carried out over a duration of 6 months. Well-fixed specimens of adult female patients who had undergone an MRM procedure for malignant breast carcinoma were collected. Patients who had a diagnosis other than invasive ductal carcinoma (IDC) and those who had received neoadjuvant therapy (chemotherapy or radiotherapy) were excluded from the study. Specimens were processed and stained with hematoxylin and eosin using routine procedures. The microscopic examination of mastectomy specimens was reported by histopathologists and the paraffin block with the maximum amount of tumor with the highest grade was selected for immunohistochemistry (IHC). The Nottingham grading system was used to grade the breast carcinoma. The American Joint Committee on Cancer tumor-node-metastasis (TNM) staging system was used for staging of breast carcinoma. A routine procedure was carried out for IHC staining using a Cten kit. The rabbit recombinant monoclonal antibody was provided by Abcam.

The histochemical score (H-score) was used to determine the IHC staining of Cten by tumor cells [1]. The intensity of staining was scored 0–3 (0 – no staining, 1 – weak, 2 – moderate, 3 – strong). The percentage of cells (0–100%) showing cytoplasmic positivity was estimated subjectively. The intensity score was multiplied by the percentage of cells showing that intensity and then these were added up to attain the H-score (0–300). Tumors with no or mild (< 85 H-score) staining for Cten were designated as negative/mild Cten expression. Tumors with a score of 85–200 were designated as moderate Cten expression, and tumors with a score > 200 were designated as strong Cten expression. The tumors with negative or mild expression were taken as negative for Cten expression while the tumors with moderate or strong expression were taken as positive for Cten expression. The Pearson test was applied for statistical analysis.

## Results

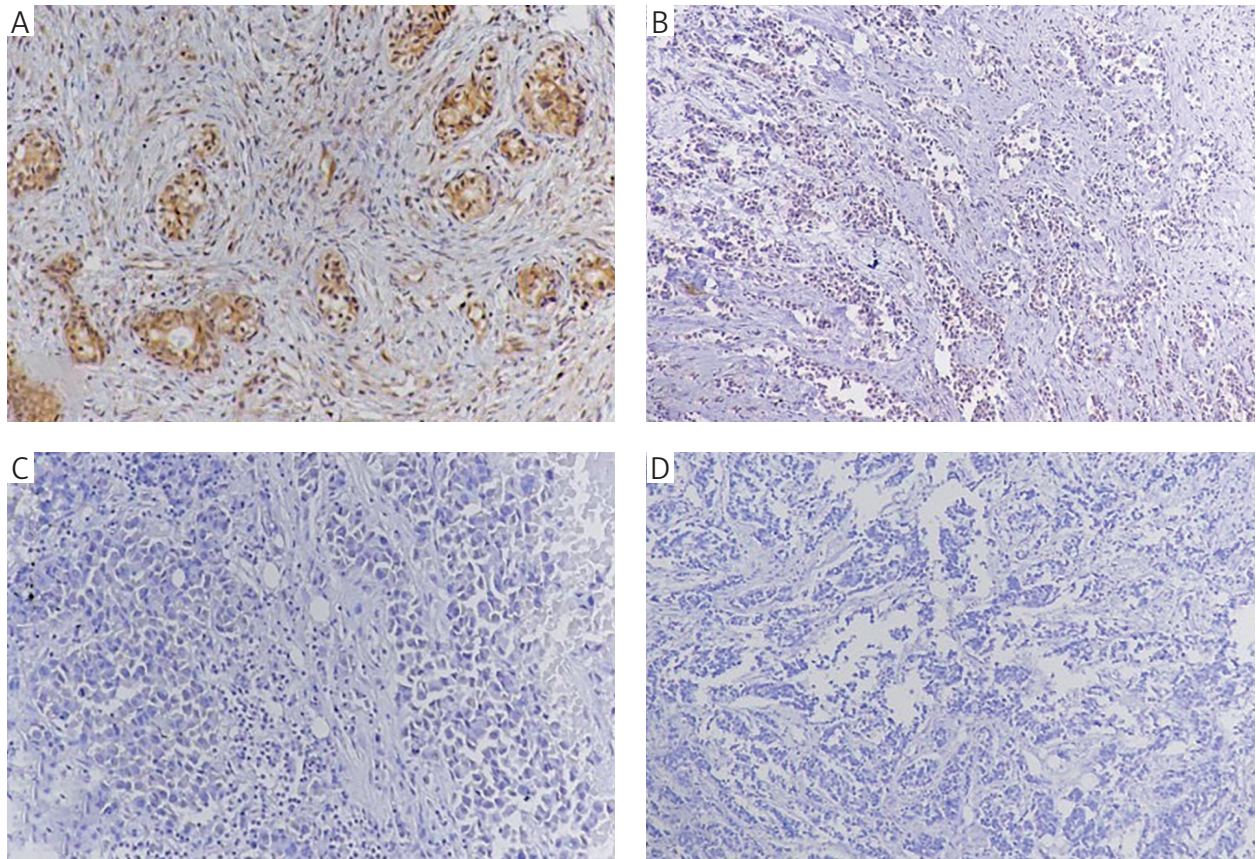
In our study, we collected 57 specimens of MRM diagnosed as invasive ductal carcinoma. All the cases were from females, in the age range 25–70 years, with a mean age of  $45.56 \pm 9.42$  years. The known histologic prognostic parameters such as size and local extent of tumor (pT), histological grade (G), lymphovascular invasion (LVI), and nodal status (N) were noted and recorded. Immunohistochemistry for Cten was performed on all cases. Normal breast epithelium was taken as an internal control. Eight (14.1%) cases

had strong expression, 2 (3.5%) cases showed moderate staining, and 47 (82.5%) cases showed negative/mild expression. These findings were correlated with histopathological parameters, and the results are shown below (Fig. 1).

All (45, 78.9%) cases in stages pT1, pT2, and pT3 showed negative/mild expression for Cten. Out of 12 (21.1%) cases in stage pT4 (tumor of any size with skin or chest wall invasion), 2 (3.5%) cases were negative/mild for Cten, 2 (3.5%) cases showed moderate staining, and 8 (14%) cases had strong expression of Cten. This shows a significant correlation of

size and local extent of tumor with Cten expression ( $p = 0.0001$ ) (Table I).

All the cases were histologically graded by Nottingham histologic grade for invasive ductal carcinoma. Twenty-five (43.9%) cases were grades as moderately differentiated (grade II), and all of them were negative/mild for Cten staining. Out of 32 (56.1%) poorly differentiated (grade III) carcinomas, 22 (38.6%) were negative/mild, 2 (3.5%) showed moderate expression, and 8 (14%) showed high Cten expression. Higher grade was strongly associated with Cten expression ( $p = 0.009$ ) (Table II).



**Fig. 1.** Immunohistochemical (IHC) expression of C-terminal tensin-like (Cten) on invasive breast carcinoma. **A)** Control, normal breast ductolobular units (IHC Cten 100 $\times$ ); **B)** strong expression of Cten (IHC Cten 100 $\times$ ); **C)** moderate expression of Cten (IHC Cten 100 $\times$ ); **D)** negative expression of Cten (IHC Cten 100 $\times$ )

*Cten* – C-terminal tensin-like

**Table I.** Correlation of pathological stage of breast cancer with C-terminal tensin-like expression

PATHOLOGICAL STAGE	CTEN EXPRESSION, N (%)			TOTAL, N (%)	P-VALUE
	NEGATIVE/MILD	MODERATE	STRONG		
pT1	4 (7.0)	0 (0.0)	0 (0.0)	4 (7.0)	0.0001
pT2	22 (38.7)	0 (0.0)	0 (0.0)	22 (38.7)	
pT3	19 (33.3)	0 (0.0)	0 (0.0)	19 (33.3)	
pT4	2 (3.5)	2 (3.5)	8 (14)	12 (21.0)	
Total	47 (82.5)	2 (3.5)	8 (14)	57 (100)	

*Cten* – C-terminal tensin-like

**Table II.** Correlation of histologic grade of breast cancer with C-terminal tensin-like expression

TUMOR GRADE	CTEN EXPRESSION, N (%)			TOTAL, N (%)	P-VALUE
	NEGATIVE/MILD	MODERATE	STRONG		
Grade II	25 (43.9)	0 (0.0)	0 (0.0)	25 (43.9)	0.009
Grade III	22 (38.6)	2 (3.5)	8 (14.0)	32 (56.1)	
Total	47 (82.5)	2 (3.5)	8 (14.0)	57 (100.0)	

*Cten – C-terminal tensin-like*

**Table III.** Correlation of lymphovascular invasion with C-terminal tensin-like expression

LYMPHOVASCULAR INVASION	CTEN EXPRESSION, N (%)			TOTAL, N (%)	P-VALUE
	NEGATIVE/MILD	MODERATE	STRONG		
Absent	36 (63.2)	0 (0.0)	0 (0.0)	36 (63.2)	0.0001
Present	11 (19.3)	2 (3.5)	8 (14.0)	21 (36.8)	
Total	47 (82.5)	2 (3.5)	8 (14.0)	57 (100.0)	

*Cten – C-terminal tensin-like*

**Table IV.** Correlation of lymph nodal involvement and C-terminal tensin-like expression

LYMPH NODE INVOLVEMENT	CTEN EXPRESSION, N (%)			TOTAL, N (%)	P-VALUE
	NEGATIVE/MILD	MODERATE	STRONG		
N0	22 (38.6)	0 (0.0)	0 (0.0)	22 (38.6)	0.001
N1	16 (28.1)	0 (0.0)	0 (0.0)	16 (28.1)	
N2	9 (15.8)	0 (0.0)	0 (0.0)	9 (15.8)	
N3	0 (0.0)	2 (3.5)	8 (14.0)	10 (17.5)	
Total	47 (82.5)	2 (3.5)	8 (14.0)	57 (100)	

*Cten – C-terminal tensin-like*

In our study, LVI was observed in 21 (36.8%) out of 57 cases, while 36 (63.2%) cases did not exhibit this feature. All the cases where no LVI was observed as well as 11 (19.3%) cases with LVI showed negative/mild expression of Cten. Two (3.5%) cases with LVI showed moderate staining for Cten, and 8 (14%) cases showed strong staining. The *p*-value 0.0001 shows that there was a significant association between Cten and LVI (Table III).

The examination of axillary lymph node status revealed 22 (38.6%), 16 (28.1%), and 9 (15.8%) cases in nodal stages N0, N1, and N2. All of them showed negative/mild expression of Cten. Out of 10 (17.5%) cases of stage N3 (≥ 10 lymph nodes involved), 2 (3.5%) showed moderate expression, and 8 (14%) showed strong expression. This result demonstrates a significant association between this nodal involvement and Cten expression (*p* = 0.0001) (Table IV).

## Discussion

Breast cancer is leading cause of cancer-related morbidity and mortality [1, 2]. The prevalence of

breast cancer in Pakistan is highest among the Asian countries [14]. Novel molecular markers such as Cten are being researched to understand the molecular mechanisms of cancer initiation, progression, and metastasis. The aim is to diagnose breast cancer early and develop combined therapies that can target different steps in pathogenesis [1, 7, 11]. Prognosis of breast cancer depends on multiple factors related to tumor biology and extent. Prognostically important histopathological factors include, among others, tumor size (pT), nodal status (N), tumor grade, LVI, and expression of estrogen receptor and HER2 [1, 15].

In our study, we collected 57 specimens of MRM. All the cases were from females, diagnosed as invasive ductal carcinoma. The age range was 25–70 years, with a mean age of 45.56 ± 9.42 years. This is in concordance with studies done on the local population, where the reported mean age was 47.2 ± 10.5 SD and 45 years [13, 14]. Notably, the mean age of the Pakistani population for breast cancer diagnosis is less than 50 years, in contrast to the western population.

C-terminal tensin-like has been introduced as a marker related to poor prognosis in breast cancer

[1, 11]. In our study, the size and extent of tumor (pT), histological grade (G), LVI, and nodal status (N) were examined microscopically and then correlated with Cten expression.

In our study, we divided the Cten expression according to H-score into negative/mild (H-score < 85), moderate (85–200), and strong (> 200) expression categories. Histologically, all our cases were invasive ductal carcinomas. Negative/mild, moderate, and high expression levels were shown by 47 (82.5%), 2 (3.5%), and 8 (14%) cases, respectively. The percentage of positive cases was only 17.5%, which is very low compared to 92.9% positivity in IDC reported by Albasri *et al.* [1]. This may be due to differences in the population and related tumor characteristics.

A significant association was noted in size and local extent of the tumor and Cten expression ( $p = 0.0001$ ). All the Cten positive tumors belonged to the pT4 category in our study, which means that local extent, skin and chest wall invasion were more associated with Cten expression than just the size of the tumor. In a previous reported study, the tumor size was not statistically significantly related to Cten expression ( $p = 0.84$ ) [13]. However, another study reported a significant association ( $p = 0.044$ ) [1]. The difference in population, sample size and categorization of tumor size may have impacted the results.

Twenty-five (43.9%) and 32 (56.1%) cases belonged to histological grade II and grade III, respectively. This is in accordance with local studies, where grade III was reported to be the predominant grade [14, 15]. In our study, all cases in grade II were negative/mild for Cten expression, while out of poorly differentiated (grade III) carcinomas, 22 (38.6%) were negative or showed mild expression, 2 (3.5%) showed moderate expression, and 8 (14%) showed high Cten expression. Higher grade was strongly associated with Cten expression ( $p = 0.009$ ). This is in accordance with the studies done by Katz *et al.* and Albasri *et al.*, where the  $p$ -value for these two variables was 0.001 and 0.019, respectively [1, 13].

Lymphovascular invasion is an important prognostic parameter, as it is linked to tumor recurrence [15]. In our study, LVI was seen in 21 (36.8%) out of 57 cases. Out of these, 11 (19.3%) cases showed negative/mild expression of Cten, 2 (3.5%) cases showed moderate staining for Cten, and 8 (14%) cases showed a strong staining pattern. All cases where LVI was absent did not show Cten expression. The  $p$ -value 0.0001 shows that there was a significant association between Cten expression and LVI. This finding is in contrast to the report of Albasri *et al.*, as they did not find a significant association between Cten and vascular invasion [1].

Presence of lymph node metastasis is an indication for systemic adjuvant therapy and also determines the long-term survival [15]. We found positive

Cten expression only in 10 cases in the N3 category. Two (3.5%) cases showed moderate expression and 8 (14%) showed strong expression. It demonstrates a significant association of Cten with nodal status ( $p = 0.0001$ ). This is in accordance with previous studies showing a significant association between these two variables [1, 13].

## Conclusions

In our study, a significant association was found between Cten expression and prognostically important parameters such as tumor size and extent, histologic grade, LVI, and lymph node status. Cten can be used as a marker of poor prognosis in breast carcinoma.

This study was conducted with a limited number of cases within a single center. Generalization based on this study may not be accurate. Thus, more studies are warranted to ascertain the actual significance.

## Disclosures

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4. Conflicts of interest: None.

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