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# Interplay of Epithelial Mesenchyal Transition Markers (Snug and Snail) and Junctional Proteins (Claudin-3) in Infiltrating Ductal Carcinoma of Breast

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## ABSTRACT:

**Background:** Breast cancer is the most common malignancy among women, with infiltrating ductal carcinoma (IDC) being the predominant invasive subtype. EMT markers (Snail and Slug) and Claudin-3 are implicated in tumor invasion, metastasis, and progression.

**Aims:** To evaluate the immunohistochemical expression of Snail, Slug, and Claudin-3 in IDC and determine their association with clinicopathological parameters and molecular subtypes.

**Materials and Methods:** This observational cross-sectional study included 52 histopathologically confirmed IDC cases. Immunohistochemical expression of Snail, Slug, and Claudin-3 was assessed and correlated with tumor grade, lymph node status, lymphovascular invasion, perivascular invasion, and molecular subtype.

**Results:** Grade II tumors and lymph node positivity were most common. Triple-negative tumors showed high Snail/Slug and low Claudin-3 expression, while luminal tumors demonstrated high Claudin-3 with moderate Snail/Slug expression. HER2-positive tumors exhibited high expression of both markers. High Snail/Slug and reduced Claudin-3 expression were significantly associated with adverse pathological features and aggressive molecular subtypes ($p<0.05$).

**Conclusion:** Combined assessment of Snail, Slug, and Claudin-3 may serve as a valuable prognostic biomarker panel for identifying aggressive IDC and improving risk stratification and therapeutic decision-making.

## INTRODUCTION

Breast cancer is the most frequently diagnosed malignancy among women worldwide and remains a leading cause of cancer-related mortality. Infiltrating ductal carcinoma (IDC), also known as invasive carcinoma of no special type (NST), accounts for nearly 70–80% of invasive breast cancers and exhibits marked biological heterogeneity with variable clinical behavior and prognosis.[1,2] Although molecular classification based on estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor-2 (HER2) has substantially improved treatment strategies, considerable differences in clinical outcomes persist even within the same molecular subtype, highlighting the need for additional prognostic biomarkers.[2,3]

Epithelial–mesenchymal transition (EMT) is a critical biological process involved in tumour invasion, metastasis, and therapeutic resistance. During EMT, epithelial cells lose cell polarity and intercellular adhesion while acquiring mesenchymal characteristics that enhance migration and invasion. EMT-regulating transcription factors, particularly Snail (SNAI1) and Slug (SNAI2), repress epithelial adhesion molecules and promote tumour progression. Increased Snail and Slug expression has been associated with higher tumour grade, lymph node metastasis, lymphovascular invasion, and aggressive molecular subtypes, particularly triple-negative breast cancer (TNBC). In contrast, lower or moderate expression of these EMT markers is more frequently observed in luminal breast cancers, indicating that their expression varies according to molecular subtype rather than being uniformly elevated in all breast cancers.[4–6]

Claudin-3 is an important tight-junction protein that maintains epithelial integrity and cell-to-cell adhesion. Loss or reduced expression of Claudin-3 disrupts epithelial architecture, facilitating EMT and tumour progression. Triple-negative breast cancer is widely recognized as belonging to the claudin-low molecular phenotype and typically demonstrates reduced Claudin-3 expression together with increased Snail/Slug expression. Conversely, luminal (ER+/PR+, HER2−) tumours generally retain high Claudin-3 expression with predominantly moderate Snail/Slug expression, while HER2-positive tumours frequently exhibit high expression of both Claudin-3 and Snail/Slug. These findings suggest that the biological association between Claudin-3 and EMT markers is molecular subtype-specific, with a clear inverse relationship being most evident in TNBC rather than across all breast cancer subtypes.[7–10]

Despite growing evidence regarding these biomarkers individually, studies evaluating the combined expression of Claudin-3, Snail, and Slug in IDC and their association with established clinicopathological prognostic factors remain limited, particularly in the Indian population. Therefore, the present study aimed to evaluate the immunohistochemical expression of Claudin-3, Snail, and Slug in infiltrating ductal carcinoma of the breast and to determine their association with tumour grade, lymph node status, lymphovascular invasion, perivascular invasion, and molecular subtypes. Furthermore, the study sought to assess whether the relationship between Claudin-3 and Snail/Slug expression differs among molecular subtypes and to explore their potential role as complementary prognostic biomarkers for risk stratification in breast cancer.

## MATERIALS AND METHODS

**Study design:** Hospital-based, observational, cross-sectional immunohistochemical study.

**Study population:**Histopathologically confirmed infiltrating ductal carcinoma (invasive carcinoma of no special type) patients who underwent surgical excision.

**Sample size:** Fifty-two cases of infiltrating ductal carcinoma.

**Study duration:** Three months.

**Study setting:** Department of Pathology, R.L. Jalappa Hospital and Research Institute, Sri Devaraj Urs Medical College, Kolar, Karnataka, India.

**Inclusion criteria:**

- Histopathologically confirmed IDC cases.
- Lumpectomy or modified radical mastectomy specimens with adequate tissue.
- Complete ER, PR, and HER2 receptor status.
- Adequately preserved tissue for Claudin-3, Snail, and Slug immunohistochemistry.

**Exclusion criteria:**

- Neoadjuvant chemo-, radio-, or targeted therapy before surgery.
- Non-IDC breast tumours.
- Recurrent or metastatic breast carcinoma.
- Inadequate or poorly preserved tissue.

- Incomplete clinicopathological or immunohistochemical data.

**Statistical analysis:** Data were analyzed using IBM SPSS version 27.0. Categorical variables were expressed as frequency and percentage. Associations between Claudin-3, Snail, and Slug expression and clinicopathological parameters were analyzed using the Chi-square test or Fisher's exact test, as appropriate. The association between Claudin-3 and Snail/Slug expression was also evaluated according to molecular subtypes (Luminal, HER2-positive, and Triple-negative). A two-tailed $p<0.05$ was considered statistically significant.

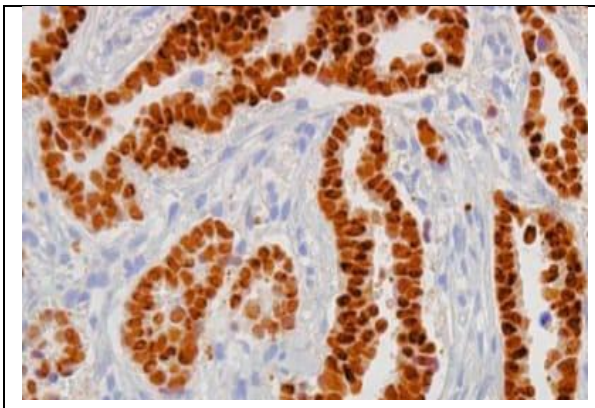

**Fig 1:** Snug snail ihc showing high expression (nuclear stain)

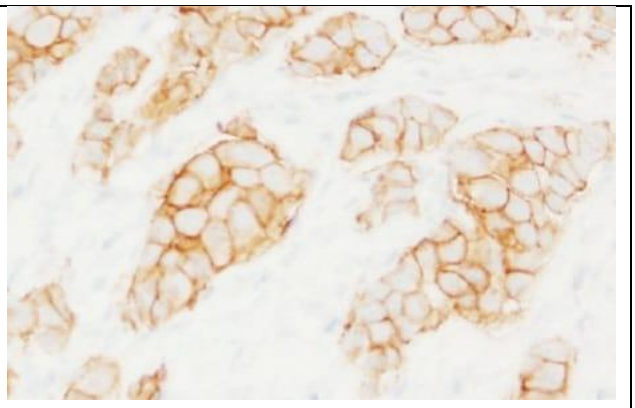

**Fig2:** Claudin3 ihc showing high expression (cytoplasmic staining)

**RESULT**

**Table 1**. Baseline Clinicopathological Characteristics of the Study Population

| Variable | Category | Number of Patients (n) | Percentage (%) | p-value |
|---|---|---|---|---|
| Age (years) | <40 | 8 | 15.4 | **0.041** |
| | 41–50 | 14 | 26.9 | |
| | 51–60 | 18 | 34.6 | |
| | >60 | 12 | 23.1 | |
| | **Total** | **52** | **100** | |
| Tumor Grade | Grade I | 7 | 13.5 | **0.018** |
| | Grade II | 28 | 53.8 | |
| | Grade III | 17 | 32.7 | |
| Lymph Node Status | Negative | 20 | 38.5 | **0.029** |
| | Positive | 32 | 61.5 | |
| Lymphovascular Invasion | Absent | 22 | 42.3 | **0.022** |
| | Present | 30 | 57.7 | |
| Perivascular Invasion | Absent | 37 | 71.2 | **0.047** |
| | Present | 15 | 28.8 | |

**Table 2.** Molecular Subtype-wise Expression Pattern of Claudin-3 and Snail/Slug

| Molecular Subtype | n (%) | High Claudin-3 | Low Claudin-3 | High Snail/Slug | Moderate Snail/Slug | p-value |
|---|---|---|---|---|---|---|
| ER+/PR+, HER2− | 24 (46.2) | 20 | 4 | 8 | 16 | **0.001** |
| HER2 Positive | 12 (23.1) | 10 | 2 | 10 | 2 | |
| Triple Negative | 16 (30.7) | 3 | 13 | 15 | 1 | |
| **Total** | **52 (100)** | **33** | **19** | **33** | **19** | |

**Table 3.** Association of Claudin-3 Expression with Clinicopathological Parameters and Molecular Subtypes

| Variable | High Claudin-3 (n=33) | Low Claudin-3 (n=19) | p-value |
|---|---|---|---|
| Grade I–II | 28 | 7 | **0.003** |
| Grade III | 5 | 12 | |
| Lymph Node Positive | 16 | 16 | **0.018** |
| Lymphovascular Invasion Present | 15 | 15 | **0.026** |
| Perivascular Invasion Present | 7 | 8 | **0.041** |
| Triple-negative subtype | 3 | 13 | **<0.001** |
| HER2-positive subtype | 10 | 2 | **0.017** |

**Table 4.** Association of Snail/Slug Expression with Clinicopathological Parameters and Molecular Subtypes

| Variable | High Snail/Slug (n=33) | Moderate Snail/Slug (n=19) | p-value |
|---|---|---|---|
| Grade III | 14 | 3 | **0.002** |
| Grade I–II | 19 | 16 | |
| Lymph Node Positive | 25 | 7 | **0.001** |
| Lymphovascular Invasion Present | 23 | 7 | **0.004** |
| Perivascular Invasion Present | 12 | 3 | **0.031** |
| Triple-negative subtype | 15 | 1 | **<0.001** |
| HER2-positive subtype | 10 | 2 | **0.011** |
| ER+/PR+, HER2− subtype | 8 | 16 | **0.002** |

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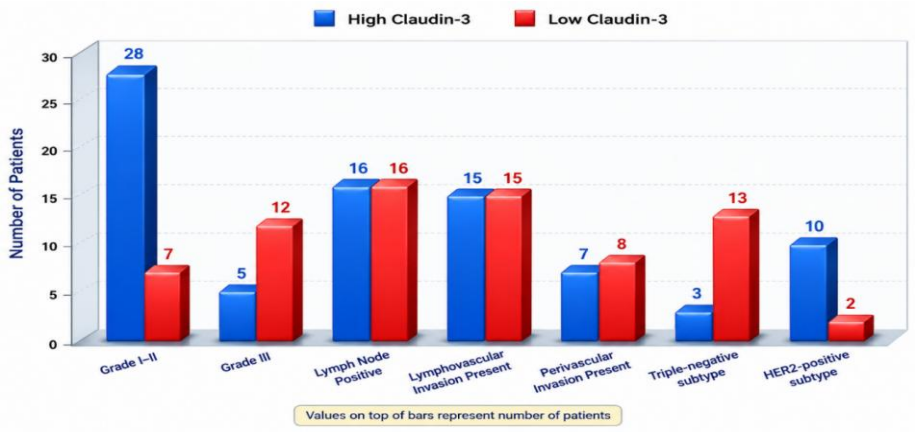


**Figure: 1.** Association of Claudin-3 Expression with Clinicopathological Parameters

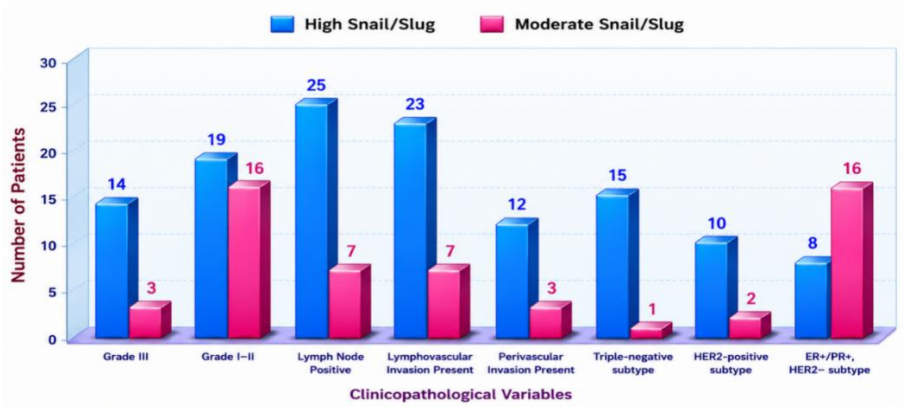


**Figure: 2.** Association of Snail/Slug Expression with Clinicopathological Parameters

A total of 52 patients with infiltrating ductal carcinoma of the breast were included in the study. Most patients were aged 51–60 years (34.6%), followed by 41–50 years (26.9%), >60 years (23.1%), and <40 years (15.4%) (p=0.041). Grade II tumours were the most common (53.8%), followed by Grade III (32.7%) and Grade I (13.5%) (p=0.018). Lymph node metastasis, lymphovascular invasion, and perivascular invasion were present in 61.5%, 57.7%, and 28.8% of cases, respectively (all $p<0.05$).

The luminal (ER+/PR+, HER2−) subtype constituted 46.2% of cases, followed by triple-negative breast carcinoma (30.7%) and HER2-positive carcinoma (23.1%). Luminal tumours predominantly showed high Claudin-3 expression (20/24) with moderate Snail/Slug expression (16/24), whereas HER2-positive tumours demonstrated high expression of both Claudin-3 (10/12) and Snail/Slug (10/12). In contrast, triple-negative breast carcinoma exhibited the characteristic claudin-low phenotype, with low Claudin-3 expression (13/16) and high Snail/Slug expression (15/16) (p=0.001).

Low Claudin-3 expression was significantly associated with Grade III tumours, lymph node metastasis, lymphovascular invasion, perivascular invasion, and the triple-negative subtype (all $p<0.05$). Although some Grade III tumours retained high Claudin-3 expression, reduced Claudin-3 expression was predominantly observed in the triple-negative subtype.

High Snail/Slug expression showed significant associations with higher tumour grade, lymph node metastasis, lymphovascular invasion, perivascular invasion, and aggressive molecular subtypes, particularly triple-negative and HER2-positive breast carcinomas (all $p<0.05$). An inverse association between Claudin-3 and Snail/Slug expression was predominantly evident in the triple-negative subtype, whereas luminal and HER2-positive tumours demonstrated distinct expression patterns rather than a consistent inverse relationship.

## DISCUSSION

In the present study, the majority of patients belonged to the 51–60-year age group, with Grade II tumours being the most common. Lymph node metastasis and lymphovascular invasion were frequently observed, indicating an adverse clinicopathological profile. These findings are consistent with previous studies reporting that infiltrating ductal carcinoma predominantly affects middle-aged and older women, with Grade II tumours being the most frequent and lymph node metastasis representing an important adverse prognostic factor.[11–14]

Distinct immunohistochemical expression patterns of Claudin-3, Snail, and Slug were observed across different molecular subtypes. Triple-negative breast carcinoma demonstrated the characteristic claudin-low phenotype, with predominantly low Claudin-3 expression and high Snail/Slug expression. In contrast, luminal (ER+/PR+, HER2−) tumours retained high Claudin-3 expression with predominantly moderate Snail/Slug expression, reflecting preservation of epithelial differentiation. HER2-positive tumours exhibited high expression of both Claudin-3 and Snail/Slug, suggesting activation of EMT despite maintained expression of tight-junction proteins. Similar subtype-specific expression patterns have been reported by Jacob et al. and other investigators, supporting the biological heterogeneity of breast cancer and the variable interaction between EMT markers and tight-junction proteins across molecular subtypes.[15,16]

The present study further demonstrated that the association between Claudin-3 and Snail/Slug expression was molecular subtype-dependent. A clear inverse relationship was predominantly observed in triple-negative breast carcinoma, where reduced Claudin-3 expression was accompanied by high Snail/Slug expression. However, this inverse relationship was not consistently observed in luminal and HER2-positive tumours, both of which retained high Claudin-3 expression despite differing levels of Snail/Slug expression. These findings suggest that the interaction between Claudin-3 and EMT markers should not be generalized across all breast cancer subtypes.

Low Claudin-3 expression was significantly associated with Grade III tumours, lymph node metastasis, lymphovascular invasion, perivascular invasion, and triple-negative breast carcinoma. Although a proportion of high-grade tumours retained high Claudin-3 expression, reduced Claudin-3 expression was predominantly confined to the triple-negative subtype, indicating that Claudin-3 downregulation is more closely related to molecular subtype than histological grade alone. These findings are consistent with reports by Yadav et al. and Yang et al., who demonstrated that altered Claudin-3 expression is associated with aggressive clinicopathological characteristics and poor prognosis in breast cancer.[17,18]

Similarly, high Snail/Slug expression was significantly associated with higher histological grade, lymph node metastasis, lymphovascular invasion, perivascular invasion, and aggressive molecular subtypes. The strongest expression was observed in triple-negative breast carcinoma, followed by HER2-positive tumours, whereas luminal tumours predominantly demonstrated moderate Snail/Slug expression. These findings support previous studies showing that Snail and Slug are key regulators of epithelial–mesenchymal transition and contribute to tumour invasion, metastasis, and disease progression, particularly in biologically aggressive breast cancer subtypes.[19,20]

## CONCLUSION

The present study demonstrated distinct molecular subtype-specific expression patterns of Claudin-3, Snail, and Slug in infiltrating ductal carcinoma of the breast. Triple-negative breast carcinoma exhibited the characteristic claudin-low phenotype, with low Claudin-3 expression and high Snail/Slug expression, whereas luminal (ER+/PR+, HER2−) tumours showed high Claudin-3 expression with predominantly moderate Snail/Slug expression, and HER2-positive tumours demonstrated high expression of both Claudin-3 and Snail/Slug. An inverse association between Claudin-3 and Snail/Slug expression was predominantly observed in the triple-negative subtype but was not consistently evident in luminal and HER2-positive tumours. These findings suggest that the interaction between EMT markers and Claudin-3 is molecular subtype-dependent rather than universal across all infiltrating ductal carcinomas. Combined immunohistochemical assessment of Claudin-3, Snail, and Slug may serve as a valuable prognostic biomarker panel for identifying biologically aggressive breast cancers and improving prognostic stratification. Further large-scale, multicentre prospective studies are warranted to validate these findings and to explore their potential therapeutic implications.

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