

Immunohistochemical Expression of Stem Cell Markers Sox-2 and Oct3/4 and Their Correlation with Clinicopathological Parameters in Breast Carcinoma

Swati Soni Jain¹, Ankit Jain², Alok Jain³

¹Associate Professor, Department of Pathology, Peoples College of Medical Sciences and Research Centre (PCMS&RC), Bhopal, MP, India

²Associate Professor, Department of General Surgery, Mahaveer Institute of Medical Sciences and Research (MIMS & R), Bhopal, MP, India

³Assistant Professor, Department Of Orthopaedics, Mahaveer Institute of Medical Sciences and Research (MIMS & R), Bhopal, MP, India

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Corresponding Author: Dr. Ankit Jain

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Abstract:

Background: The most common malignancy diagnosed in women all over the world are carcinoma of the breasts and is one of the largest cause of cancer related death. Although there have been improvements in molecular profiling and targeted therapy, there are some tumors which are aggressive and not responsive to therapy. Proposed theories The cancer stem cell (CSC) hypothesis proposes that a subpopulation of tumor-initiating cells that possess the characteristic of self- renewal and pluripotentiality propagate the progression, recurrence and metastasis of tumors. SOX-2 and OCT3/4 are transcription factors that play an important role in the maintenance of stem cells in the human embryo, and are increasingly becoming of interest in oncogenesis. In this paper, the authors tried to assess the SOX-2 and OCT3/4 immunohistochemical expression of breast cancer as well as compare its expression with clinicopathological variables and more traditional molecular features (ER, PR, HER2/neu).

Methods: The study was a cross-sectional observational study catering to the requirement of 50 histopathologically confirmed cases of Invasive breast Carcinoma on female patients, National Institute of Pathology, VMMC and safdarjung Hospital, New Delhi. Paraffin embedded tissues were stained with immunohistochemistry for SOX-2 and OCT3/4. Expression was scored semi quantitatively in terms of intensity and magnitude of staining. SPSS v22 was used to do the statistical analysis. Pearson Chi-square test was used to test each correlation and values less than 0.05 were deemed significant.

Results: SOX-2 and OCT3/4 were expressed in 14 percent (7/50) and 8 percent (4/50), respectively. The expression of SOX-2 was significantly correlated with lymph node involvement ($p=0.016$), high histological grade ($p=0.034$), DCIS ($p=0.031$), PR negativity ($p=0.007$) and triple negative molecular subtype ($p=0.020$). Increased tumor grade and metastatic recurrence to the associated regional lymph nodes ($p=0.042$) were significantly correlated with a positive, OCT3/4 staining phenotype but not with ER, PR or HER2/neu expression.

Conclusion: High expression SOX-2 and OCT3/4 in breast carcinoma are associated with clinicopathological aggressiveness and thus are potential biomarkers of tumor aggressiveness and tumor CSC-related phenotypes. They can be further validated in larger cohort studies for improved prognostic and therapeutic value.

Keywords: Breast carcinoma; SOX-2; OCT3/4; Cancer stem cells; Immunohistochemistry; Triple-negative breast cancer.

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Introduction

Breast carcinoma is a health disease of international nature with the second most common cancer frequent in general and the first common cancer frequent in females. GLOBOCAN 2012 estimates that 1.67 million new cases was diagnosed annually across the world as of 2012

which is 20% more than the number that was diagnosed in 2008 [1]. The Indian Council of Medical Research (ICMR) in India estimated that there were about 145,000 new cases in 2016, and this is the most common cancer in Indian women [2]. Despite advancements in screening, molecular

subtyping, and targeted therapies, mortality remains substantial, especially in developing nations [3]. The recent investigations have pointed out the importance of cancer stem cells (CSCs) in the development and development of cancer. CSCs possess the ability to self-renew as well as differentiate, like normal stem cells and are believed to be responsible for tumorigenesis, recurrent-ness and resistance to therapy [4]. In breast carcinoma, a subpopulation of cells expressing stemness markers contributes to tumor heterogeneity and metastasis [5].

Transcription factors such as SOX-2, OCT3/4, and NANOG are key regulators of pluripotency in embryonic stem cells [6]. SOX-2 (SRY-related HMG-box 2), located on chromosome 3q26.33, maintains stem cell self-renewal and is involved in reprogramming somatic cells to pluripotency [7]. Aberrant SOX-2 expression has been documented in several malignancies, including gliomas, lung squamous cell carcinoma, and breast carcinoma, where it is linked with basal-like phenotypes and poor clinical outcomes [8].

OCT3/4 (POU5F1), a member of the POU-domain transcription factor family, acts synergistically with SOX-2 to sustain pluripotency and repress differentiation [9].

OCT3/4 is normally restricted to germ cells and embryonic stem cells, yet its re-expression has been observed in various tumors, including breast, gastric, and pancreatic cancers [10]. Experimental data indicate that OCT3/4 confers resistance to apoptosis, promotes invasion, and supports epithelial-to-mesenchymal transition (EMT) [11–13].

Given the overlapping roles of SOX-2 and OCT3/4 in stemness regulation, their co-expression in breast carcinoma may reflect the activation of stem cell-like transcriptional programs driving tumor aggressiveness [14]. The paper looks at the immunohistochemical RNA-binding protein SOX-2 expression and the OCT3/4 expression in invasive breast carcinoma and their relations to clinicopathological variables (ER, PR, HER2/neu), in order to identify the potential prognostic value of these two proteins.

Materials and Methods

Study Design and Setting: Cross-sectional observational study was done in the National Institute of Pathology (ICMR), VMMC and Safdarjung Hospital, New Delhi, during May 2017 to April 2018.

Sample Size and Selection: Fifty female patients with histopathologically confirmed invasive breast carcinoma were included. Cases with other malignancies or male breast cancers were excluded.

Data Collection: Medical records were used to provide the clinical information (age, size, nodal status, stage and histological grade of the tumor). Pathological examination consisted of tumor type, lymphovascular invasion (LVI) and Ductal Carcinoma in Situ (DCIS). Immunohistochemical evaluation on ER, PR and HER2/neu was done.

Immunohistochemistry: Paraffin embedded blocks were fixed in formalin and 4 μ m sections were cut. Immunohistochemistry Endogenous peroxidase was inhibited following deparaffinization and antigen retrieval with Tris-EDTA buffer pH 9.0.

Primary antibodies against SOX-2 (Rabbit monoclonal, Cell Marque, 1:100) and OCT3/4 (Mouse monoclonal, Biocare, 1:100), were used and then the HRP-polymer detection and DAB visualized. Nuclear staining was considered positive.

Scoring System: Expression intensity (0–3) and extent (0–3) were multiplied to yield a composite score. Grades 0–2 were considered negative/weak, 3–4 moderate, and 6–9 strong.

Statistical Analysis: The data were analyzed with the IBM Statistical Program for Social Sciences v 22.

Correlations effected by Chi squared test with p value less than 0.05 found to be statistically significant.

Results

Clinicopathological Profile: The patients' ages ranged from 21–80 years (mean: 50.2 years). Most cases were within 41–60 years (54%).

Table 1 summarizes the age distribution.

Table 1: Age distribution of breast carcinoma cases

Age Group (years)	Number	Percentage
21–30	2	4%
31–40	11	22%
41–50	14	28%
51–60	13	26%
61–70	8	16%
71–80	2	4%

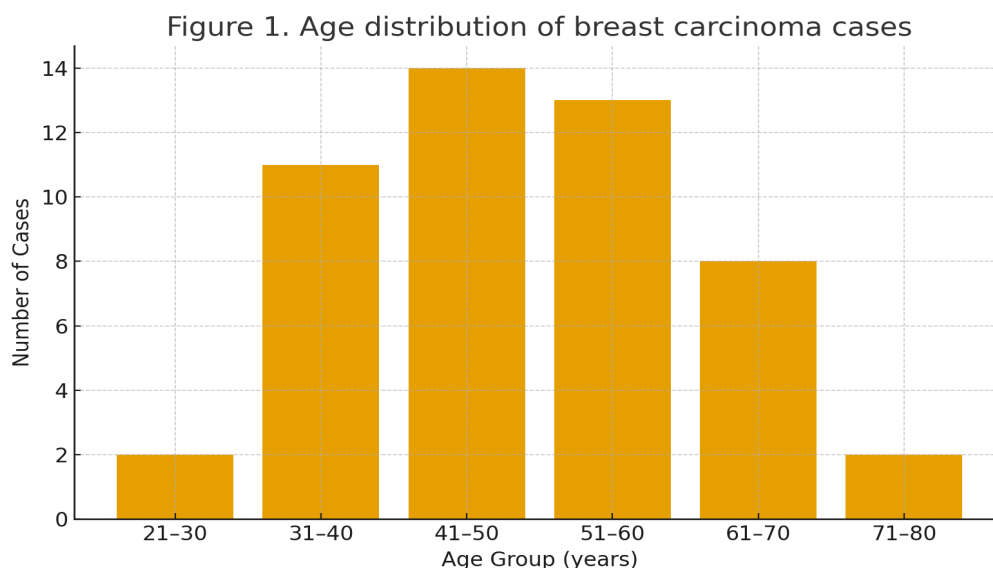


Figure 1: Age Distribution of Breast Carcinoma Cases

Most tumors were of size T2 (40%), and nodal involvement was present in 62%. Stage IIIA was the most common (28%). Grade II predominated (44%), and lymphovascular invasion was present in 60% of cases.

Hormone Receptor and HER2/neu Status: ER positivity was seen in 48%, PR in 40%, and HER2/neu overexpression in 46%. Luminal subtype constituted 48%, HER2-overexpressing 24%, and triple-negative 28%.

Table 2: Hormone receptor and molecular subtype profile

Marker/Subtype	Positive (%)	Negative (%)
ER	48	52
PR	40	60
HER2/neu	46	54
Luminal	48	—
HER2-OE	24	—
TNBC	28	—

Expression of SOX-2 and OCT3/4: SOX-2 positivity was observed in 7 (14%) cases, and OCT3/4 in 4 (8%) cases (Figure 2).

Figure 2. Positive expression of stem cell markers SOX-2 and OCT3/4

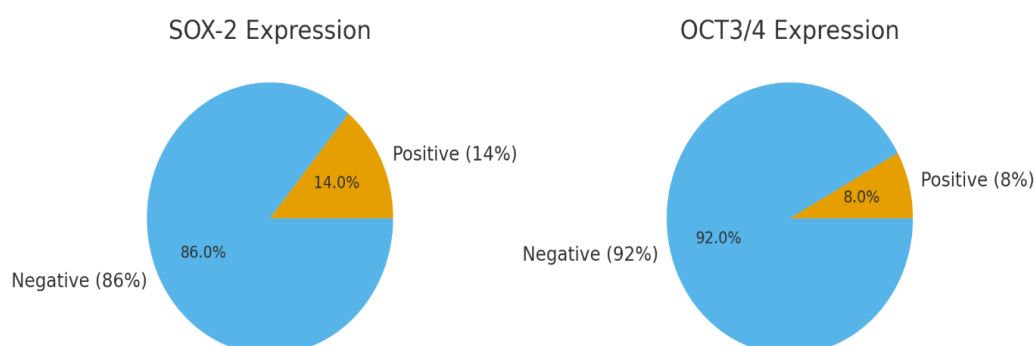


Figure 2: Positive Expression of Stem Cell Markers Sox-2 and Oct3/4

Table 3: SOX-2 and OCT3/4 expression

Marker	Positive	Negative	Percentage Positive
SOX-2	7	43	14%
OCT3/4	4	46	8%

Correlation with Clinicopathological Parameters: SOX-2 was also significantly related to lymph node metastasis ($p=0.016$), elevated grade ($p=0.034$), presence of DCIS ($p=0.031$), the PR negativity ($p=0.007$) and triple-negative subtype

($p=0.020$). There was a correlation between the OCT3/4 positivity and the lymph node ($p=0.042$), high-grade tumor ($p=0.137$, trend). Tumor size or stage also did not reveal any significant relation.

Table 4: Correlation of SOX-2 expression with clinicopathological features

Parameter	Positive (%)	p-value
Lymph node involvement	60% (N3)	0.016
Grade III	33%	0.034
DCIS present	25%	0.031
PR negative	30%	0.007
TNBC subtype	35.7%	0.020

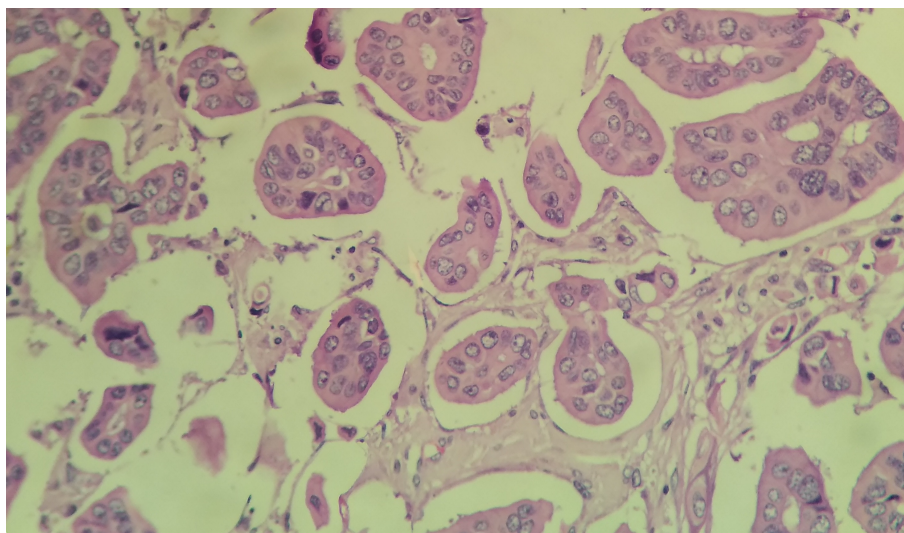


Figure 3: Invasive Ductal Carcinoma, Nst, Grade I (H&E, ×400) — Mild Nuclear Pleomorphism and Minimal Mitotic Activity.

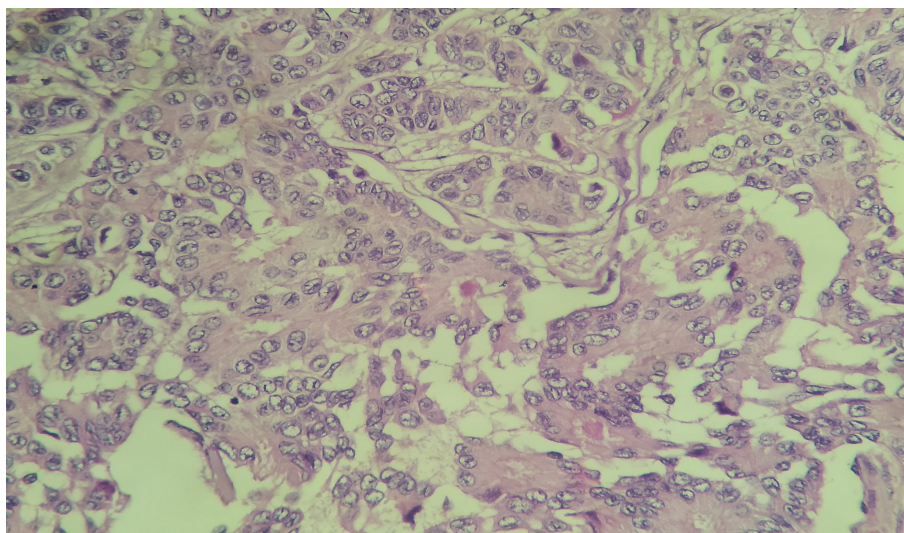


Figure 4: Grade II (H&E, ×400) — Moderate Pleomorphism and Occasional Mitoses.

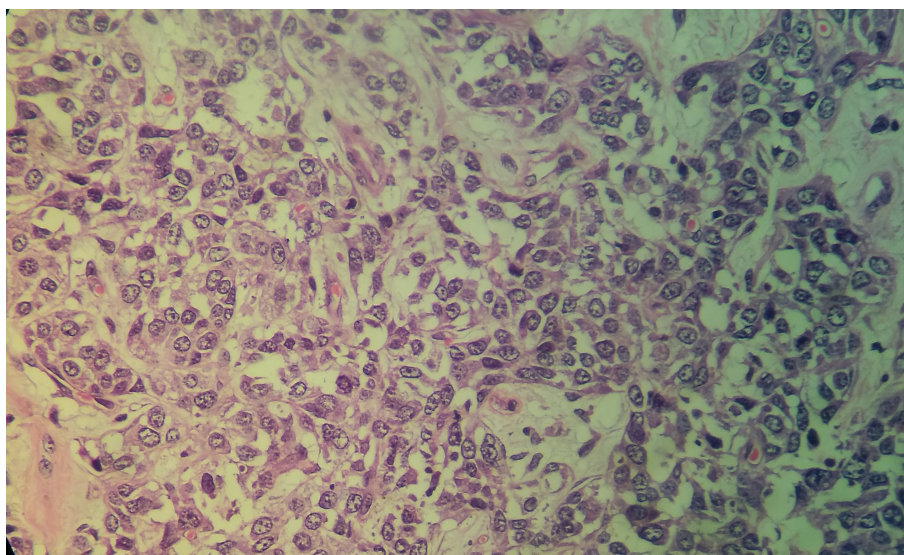


Figure 5: Grade Iii (H&E, ×400) — Highly Pleomorphic, Mitotically Active Cells.

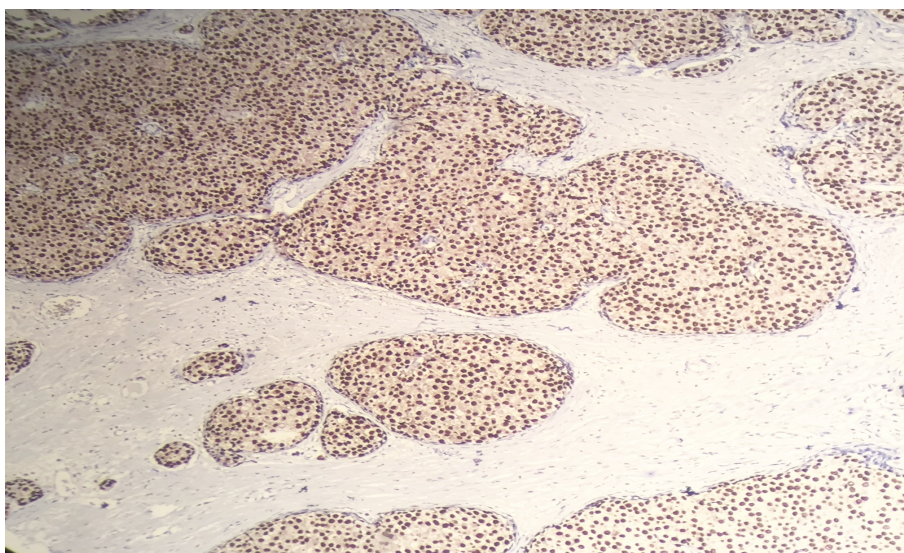


Figure 6: Er (Ihc, ×200) — Diffuse Nuclear Staining.

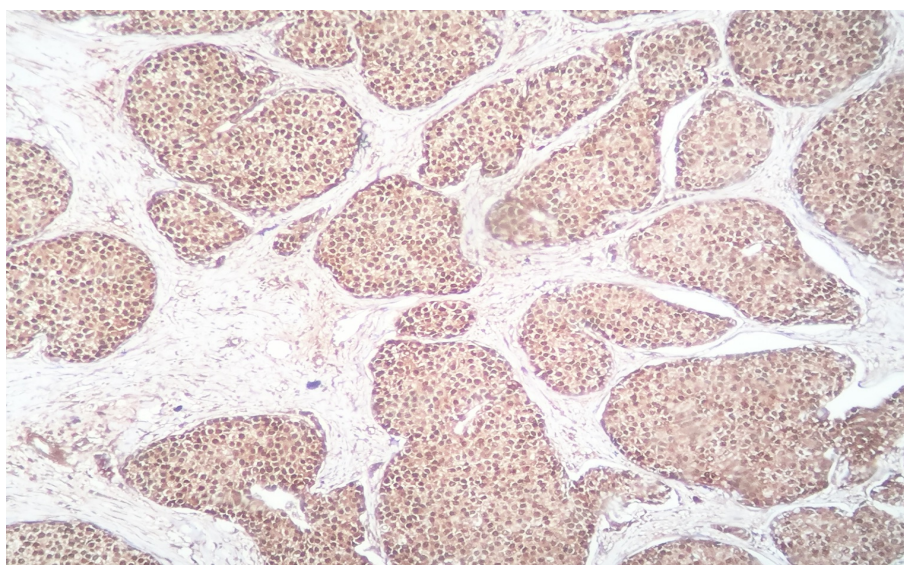


Figure 7: Pr (Ihc, ×200) — Nuclear Staining In Tumour Cells.

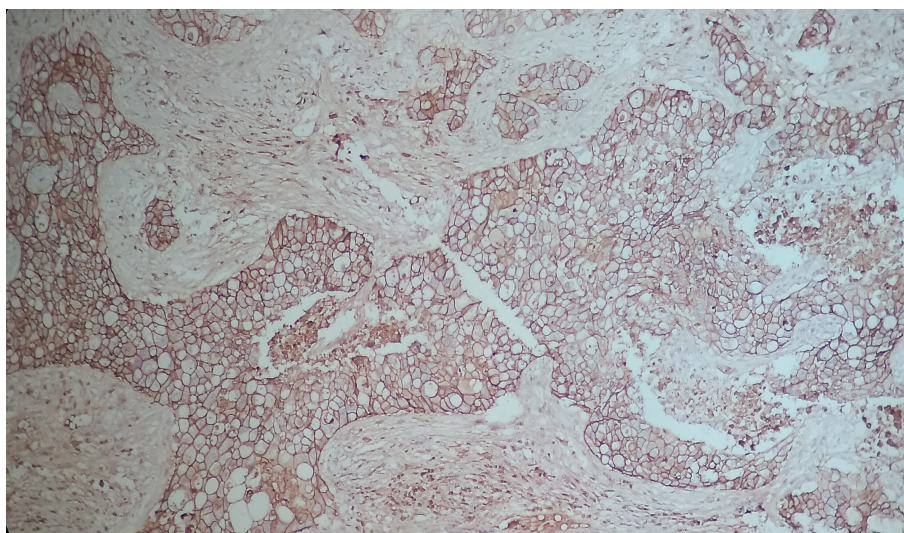


Figure 8: Her2/Neu (Ihc, ×200) — Intense, Complete Membranous Staining (3+).

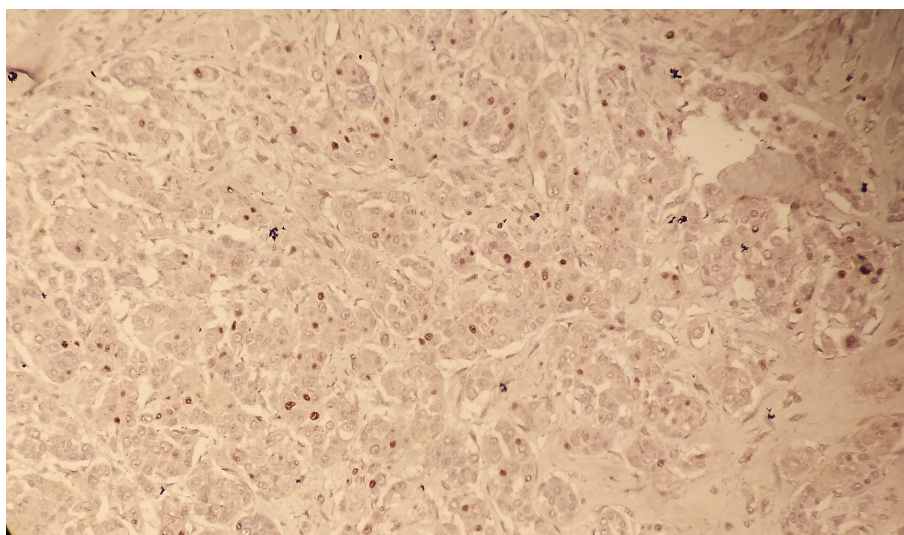


Figure 9: Immunohistochemical Expression of Oct3/4 in Tumour Cells (Ihc, ×100). Strong Nuclear Staining In <10% Of Tumour Cells (Grade 3).

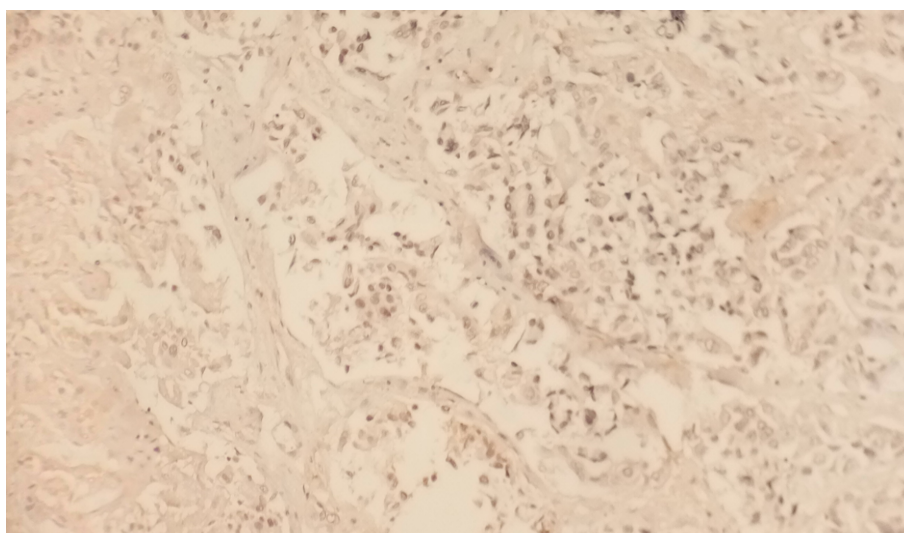


Figure 10: Immunohistochemical Expression of Oct3/4 in Tumour Cells (Ihc, ×100). Intermediate Nuclear Staining In 10–50% Of Tumour Cells (Grade 4).

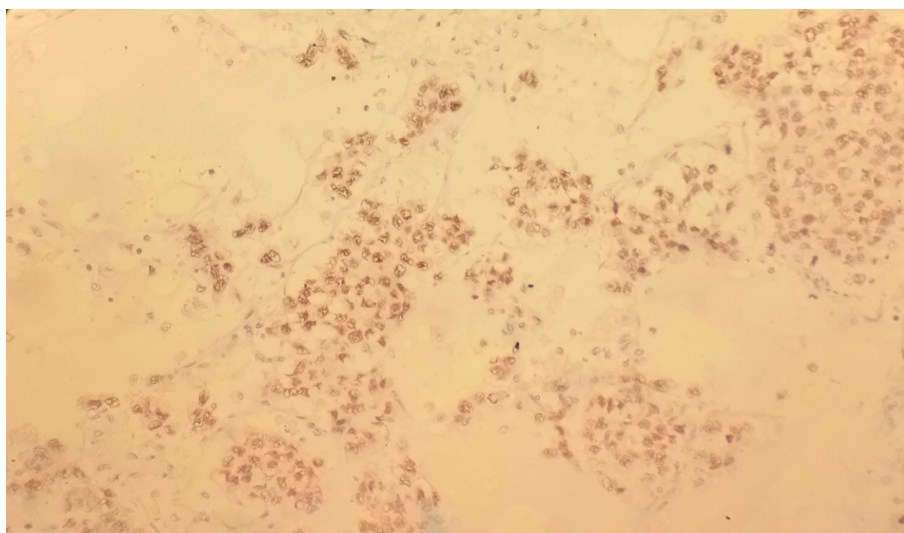


Figure 11: Immunohistochemical Expression of Oct3/4 in Tumour Cells (Ihc, $\times 200$). Strong Nuclear Staining in Most Tumour Cells (Grade 9).

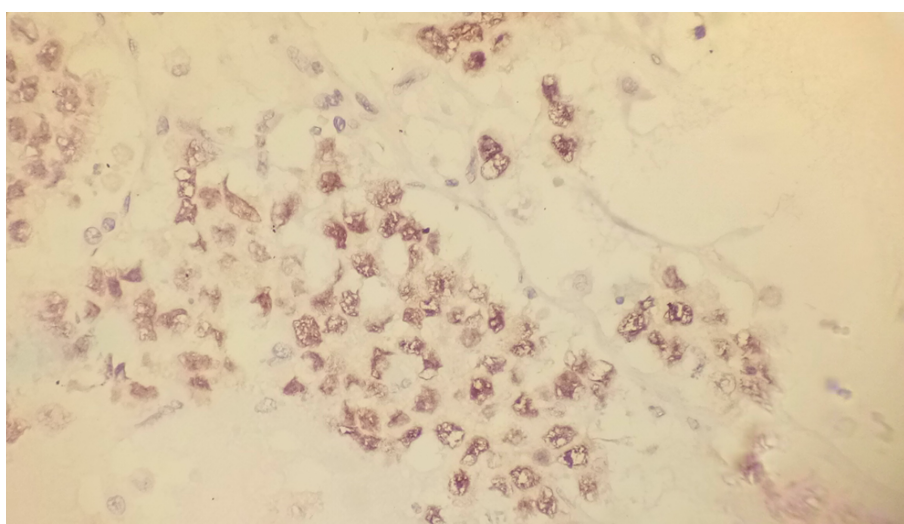


Figure 12: Immunohistochemical Expression of Oct3/4 in Tumour Cells (Ihc, $\times 400$). Moderate Nuclear Staining in Most Tumour Cells (Grade 6).

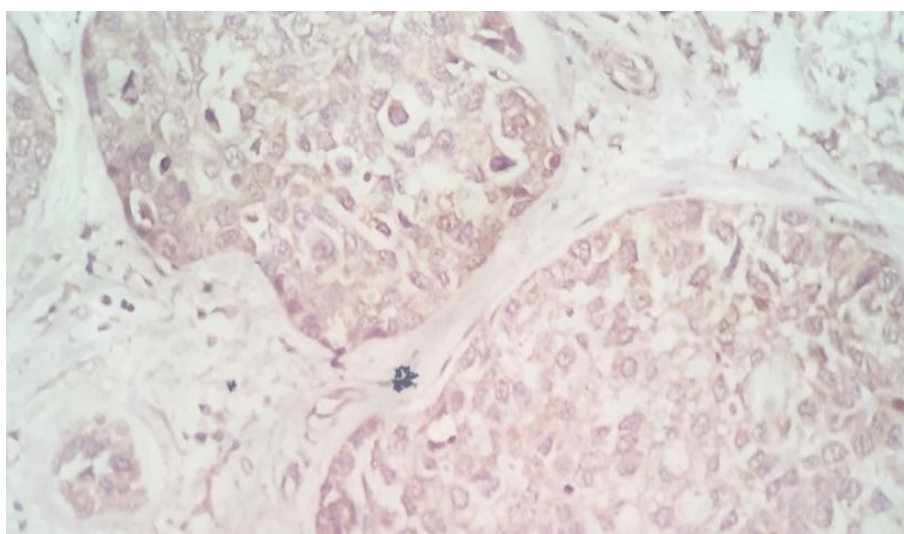


Figure 13: Immunohistochemical Expression of Sox-2 in Tumour Cells (Ihc, $\times 400$). Weak Nuclear Staining In $<10\%$ Of Tumour Cells (Grade 1).

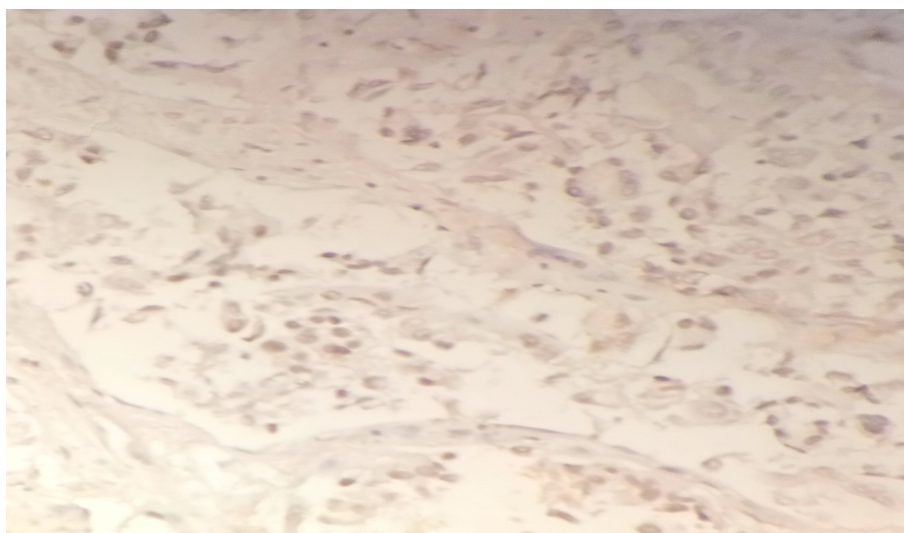


Figure 14: Immunohistochemical Expression of Sox-2 in Tumour Cells (Ihc, ×400).Intermediate Nuclear Staining In >50% Of Tumour Cells (Grade 6).

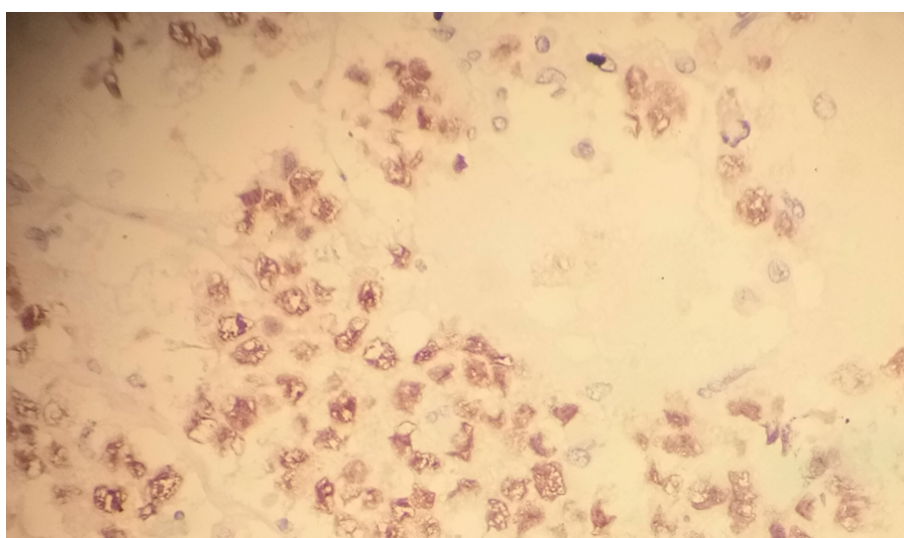


Figure 15: Immunohistochemical Expression of Sox-2 in Tumour Cells (Ihc, ×400).Strong Nuclear Staining In >50% Of Tumour Cells (Grade 9).

Discussion

The current research aimed to determine the intensity of SOX-2 and OCT3/4 immunohistochemical levels in the invasive breast carcinoma and investigate the clinicopathological correlations. In our results, we have shown that only minority of tumors expressed such markers but expression was strongly related to poor pathologic phenotypes.

SOX-2 expression (14%) aligns with previous reports ranging from 10% to 43% in invasive ductal carcinoma [15,16]. SOX-2 is a transcriptional regulator implicated in maintaining pluripotency and tumorigenic potential [17]. Its association with higher grade and lymph node metastasis in our study supports the hypothesis that SOX-2 expression contributes to aggressive tumor behavior. Leis et al. [8] observed similar findings, noting SOX-2 expression in basal-like breast

carcinomas with poorer differentiation. Moreover, its significant association with PR negativity and triple-negative phenotype indicates enrichment within more undifferentiated subtypes. OCT3/4 expression (8%) was lower than SOX-2, consistent with observations by Jin et al. [18] and Tai et al. [10], who demonstrated variable OCT3/4 expression in breast and germ cell tumors. OCT3/4's function in sustaining self-renewal and suppressing differentiation may endow tumor cells with stem-like features, promoting invasiveness. Our data showed OCT3/4 expression correlated with lymph node metastasis ($p=0.042$), reinforcing its role in progression. Notably, both markers demonstrated higher prevalence among triple-negative breast carcinomas (TNBC), which are typically characterized by poor prognosis and lack of targeted therapy options [19]. This finding supports the concept that CSC-associated transcription factors contribute to the molecular

signature of TNBCs, potentially explaining their chemoresistance. Previous studies have indicated functional synergy between SOX-2 and OCT3/4, maintaining an undifferentiated transcriptional program [20]. Although concurrent expression was infrequent, their combined presence may demarcate a subset of tumors with particularly aggressive potential.

The study's limitations include a modest sample size and lack of follow-up data to correlate marker expression with survival outcomes. Nonetheless, the consistent pattern of association with adverse features underscores the clinical relevance of SOX-2 and OCT3/4 as potential biomarkers. Their inclusion in extended immunohistochemical panels could refine prognostic stratification and guide future therapeutic research.

Conclusion

This paper raises the issue that SOX-2 and OCT3/4 are transcription factors of stem cells that are expressed in some breast carcinomas and are associated with high-grade histology, lymph node involvement, PR negativity, and triple-negative molecular subtype. Such results confirm the hypothesis that tumor aggressiveness is observed as a result of re-expression of stemness-related genes. Having these markers histochemically expressed on an immunohistochemical level may be a supplement to the usual prognostic predictors and provide insight into CSCs-mediated pathogenesis. Their use as prognostic markers and treatments in breast carcinoma should be accepted in larger prospective studies.

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