Original Article

Spectrum of Histopathological Changes in Invasive Ductal Carcinoma of Breast Following Neoadjuvant Chemotherapy: A Tertiary Care Center Study

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Abstract

Background: *Neoadjuvant chemotherapy (NACT) induces characteristic morphological changes in breast carcinoma, which are crucial for assessing treatment effect and distinguishing residual tumor from therapy-related changes.*

Objective: *To describe the spectrum and frequency of cellular and stromal histopathological changes in invasive ductal carcinoma of breast following NACT in a tertiary care setting.*

Materials and Methods: *This cross-sectional study included 60 post-NACT mastectomy specimens from patients with invasive ductal carcinoma (IDC). Pre- and post-chemotherapy H&E-stained slides were compared. Cellular changes (pyknosis, karyorrhexis, karyolysis, cytoplasmic vacuolation, multinucleation) and stromal changes (fibrosis, hyalinization, calcification, necrosis, lymphocytic infiltration, etc.) were systematically evaluated using defined criteria. Masson's Trichrome stain was used to assess fibrosis.*

Results: *Cytoplasmic vacuolation was the most prevalent cellular change, observed in 81% of cases with viable tumor. Nuclear pyknosis and karyorrhexis/karyolysis were seen in 78.3% and 75.7% of cases, respectively. Multinucleation was noted in 24.3% cases. The predominant stromal alteration was fibrosis (100%). Among this widespread fibrosis was present in 85% of all cases. Other common stromal changes included hyalinization (86.7%), minimal lymphocytic infiltration (56.7%), and degenerative changes (58.3%). Infiltration of foamy macrophages and hemosiderin-laden histiocytes was observed less frequently.*

Conclusion: *NACT induces a consistent and recognizable spectrum of histopathological changes in breast cancer. Widespread fibrosis and cytoplasmic vacuolation are the hallmark features. Recognizing these alterations is essential for accurate post-chemotherapy evaluation and avoiding diagnostic pitfalls particularly in resource-limited tertiary care settings.*

Key words: *Breast neoplasms, Neoadjuvant therapy, Histopathological changes, Fibrosis, Cytoplasmic vacuolation.*

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Introduction

Neoadjuvant chemotherapy (NAC) has become a standard of care for locally advanced and aggressive biological subtypes of breast cancer.[1] Beyond its therapeutic role, it acts as an in vivo sensitivity test.[2] The histopathological assessment of post-NACT specimens is complex, as cytotoxic agents induce a wide range of morphological alterations in both the malignant epithelial cells and the tumor stroma.[3]

These changes range from simple cell damage, such as pyknosis and vacuolation to marked stromal changes like fibrosis and hyalinization.[3] Recognizing these changes is essential. Pathologists must accurately identify these treatment effects. These helps them to distinguish viable residual carcinoma, non-viable tumor cells, and therapy-induced stromal alterations that can look like cancer.[3,4] Misinterpretation can lead to incorrect staging and prognosis.

Detailed descriptions of specific histopathological changes particularly in routine diagnostic practice, are less commonly emphasized. This study therefore aims to systematically document the spectrum of cellular and stromal histomorphological alterations in invasive ductal carcinoma following NACT. The findings are intended to serve as a practical diagnostic guide for evaluating post-chemotherapy specimens in tertiary care settings.

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Materials and Methods

This cross-sectional study was conducted in the Department of Pathology, BIRDEM General Hospital, Dhaka, from March 2022 to February 2024. Ethical approval was obtained from the Institutional Review Board of BIRDEM. Written informed consent was obtained from all participants. Sixty (60) cases of invasive ductal carcinoma (IDC) of the breast, diagnosed with core needle biopsy and subsequently treated with 4-8 cycles of NACT followed by mastectomy, were included.

Histopathological Evaluation: Formalin-fixed, paraffin-embedded tissue blocks from pre-treatment core biopsies and post-treatment mastectomy specimens were sectioned and stained with routine Hematoxylin and Eosin (H&E). For assessment of stromal fibrosis, representative sections from the tumor bed of mastectomy specimens were also stained with Masson's Trichrome (MT).

Assessment of Pathological Response and Histopathological Changes:

Pathological response to NACT was evaluated on post-treatment mastectomy specimens using the Chevallier classification system:

Pathological complete response (pCR): No residual invasive carcinoma in the breast or lymph nodes.

Pathological partial response (pPR): Residual invasive carcinoma with evidence of treatment-induced stromal changes (e.g., fibrosis, hyalinization).

Pathological no response (pNR): Viable invasive carcinoma with minimal or no morphological evidence of treatment effect. Pré- and post-chemotherapy H&E-stained slides were examined simultaneously for each case. The following parameters were recorded:

Cellular Changes (in viable tumor cells): In post-NACT specimens with residual tumor, the following changes were evaluated: 1) Pyknosis, 2) Karyorrhexis and Karyolysis, 3) Cytoplasmic Vacuolation (Figure 1), and 4) Multinucleation (Figure 2). Their presence was compared with baseline findings in the pre-NACT core biopsy.

Stromal Changes (in the tumor bed): Stromal alterations in the tumor bed were semi-quantitatively assessed as Minimal (present in <2 distinct locations), Focal (present in at least 2 distinct locations), or Widespread (present in >2 distinct locations), based on criteria adapted from Sassen et al.[5] The changes evaluated included:

1. Fibrosis (highlighted with MT stain)
2. Hyalinization
3. Calcification
4. Necrosis
5. Degenerative changes
6. Lymphocytic infiltration
7. Infiltration by foamy macrophages
8. Infiltration by hemosiderin-laden histiocytes

Statistical Analysis: Data was analyzed using SPSS software version 27. Descriptive statistics were used to present the frequencies and percentages of different histomorphological changes.

Results

All 60 post-NACT mastectomy specimens were evaluated. Residual invasive carcinoma was identified in 37 cases (61.7%), while 23 cases (38.3%) showed no residual invasive tumor (representing either pathological complete response or residual ductal carcinoma in situ only).

I. Cellular Changes in Cases with Residual Tumor (n=37):
Among the 37 cases with viable tumor cells, a consistent spectrum of chemotherapy-induced cellular changes was observed (Table I). The most frequent alteration was cytoplasmic vacuolation, present in 30 cases (81%). This was followed by nuclear pyknosis (29 cases, 78.4%) and karyorrhexis/karyolysis (28 cases, 75.7%). Multinucleation was observed in 9 cases (24.3%). These changes were significantly more pronounced and frequent compared to the pre-NACT biopsy samples.

Table I: Frequency of Cellular Changes in Post-NACT Specimens with Viable Tumor (n=37).

Cellular Change	Number of Cases	Percentage (%)
Cytoplasmic Vacuolation	30	81.0
Nuclear Pyknosis	29	78.4
Karyorrhexis/ Karyolysis	28	75.7
Multinucleation	9	24.3

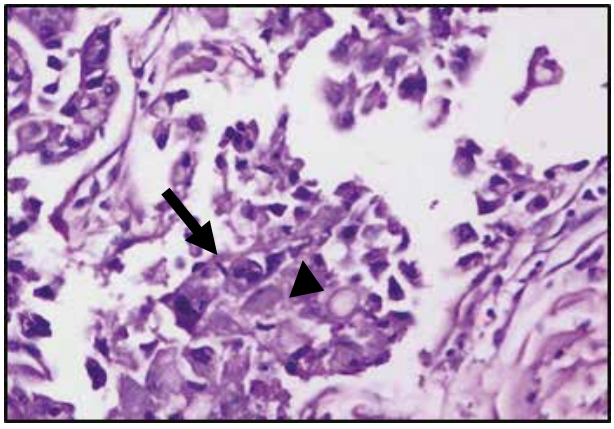

Figure 1: Post-chemotherapeutic specimen showing nuclear pyknosis (arrow) and cytoplasmic vacuolation (arrowhead) (H&E, 400X)

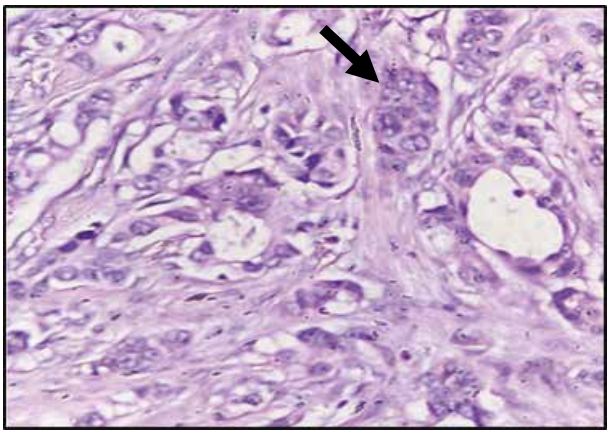

Figure 2: Multinucleated tumor giant cells (arrow) indicating aberrant mitosis following chemotherapy (H&E, 400X)

II. Stromal Changes in the Tumor Bed (All 60 cases):

Stromal remodeling was a near universal finding, present even in cases with no residual carcinoma. The distribution and extent of various stromal changes are detailed in Table II.

Fibrosis: This was the predominant stromal response. Fibrosis found in all 60 cases(100%) and widespread fibrosis was observed in 51 cases (85%), while focal and minimal fibrosis were seen in 10% and 5% of cases, respectively. MT stain confirmed the abundant deposition of blue-staining collagen fibers (Figure 3, 4, 5, 6, 7, 8).

Hyalinization: Present in 52 cases (86.7%), with a relatively even distribution between minimal (18 cases, 30%), focal (18 cases, 30%), and widespread (16 cases, 26.7%) patterns. (Figure 3)

Lymphocytic Infiltration: Present in 48 cases (80%). It was most commonly minimal (34 cases, 56.7%), with focal (12 cases, 20%) and widespread (2 cases, 3.3%) patterns being less frequent. (Figure 10)

Other Changes: Degenerative changes were noted in 35 cases (58.3%), necrosis in 27 cases (45%), and calcification in 22 cases (36.7%). Infiltration by foamy macrophages (Figure 9) and hemosiderin-laden histiocytes (Figure 9) was less common, observed in 14 (23.3%) and 13 (21.7%) cases, respectively.

Table II: Spectrum and Extent of Stromal Changes in Post-NACT Tumor Bed (n=60)

Stromal Changes	Minimal n (%)	Focal n (%)	Widespread n (%)	Total Present n (%)
Fibrosis	3 (5.0)	6 (10.0)	51 (85.0)	60 (100)
Hyalinization	18 (30.0)	18 (30.0)	16 (26.7)	52 (86.7)
Lymphocytic infiltrate	34 (56.7)	12 (20.0)	2 (3.3)	48 (80.0)
Degenerative Changes	11 (18.3)	14 (23.3)	10 (16.7)	35 (58.3)
Necrosis	8 (13.3)	11 (18.3)	8 (13.3)	27 (45.0)
Calcification	13 (21.7)	5 (8.3)	4 (6.7)	22 (36.7)
Foamy Macrophages	9 (15.0)	3 (5.0)	2(3.3)	14 (23.3)
Hemosiderin - laden Histiocytes	4 (6.7)	6 (10.0)	3 (5.0)	13 (21.7)

Note: Grading criteria as per Sassen et al.[5]

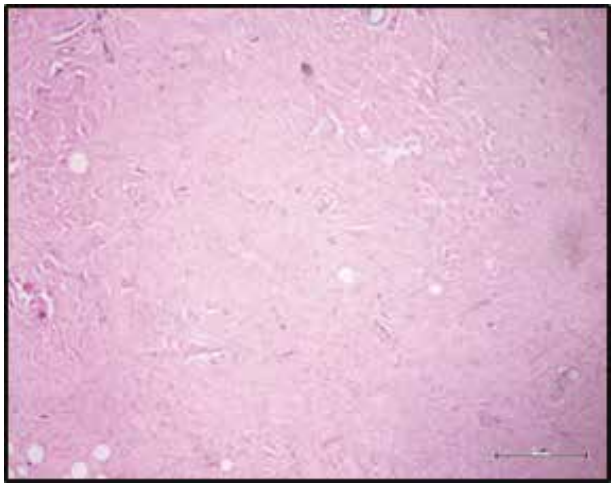

Figure 3: Post-NACT mastectomy specimen showing large areas of fibrosis and hyalinization replacing tumor bed (H&E,100X)

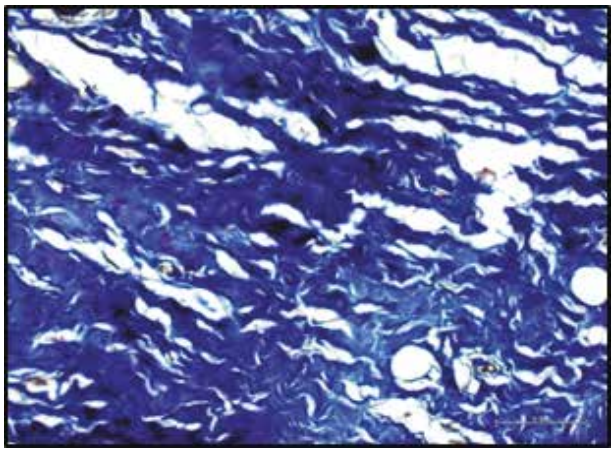

Figure 4: Post-NACT mastectomy specimen showing large areas of fibrosis and hyalinization replacing tumor bed (MT stain,100X)

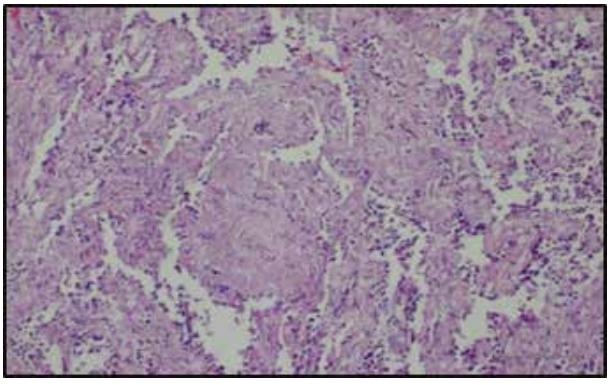

Figure 5: Photomicrograph showing stromal change with presence of tumor cells in a case of partial response (H&E, 100X)

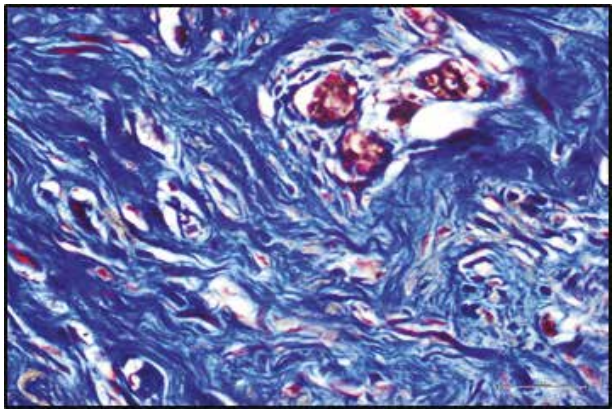

Figure 6: Photomicrograph showing large areas of fibrosis with few foci of tumor cells in a case of partial response (MT stain, 200X)

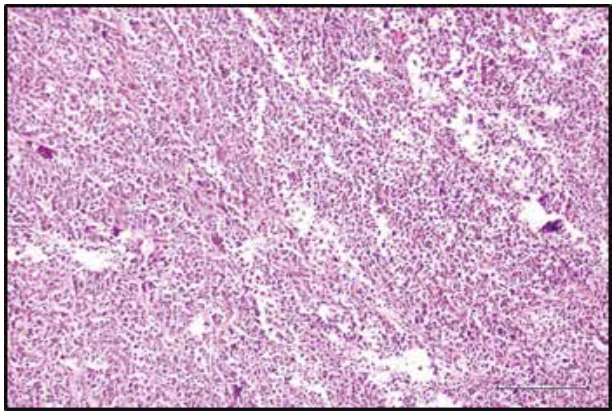

Figure 7: Photomicrograph showing no/minimal stromal change with presence of only tumor cells in a case of no response (H&E, 100X)

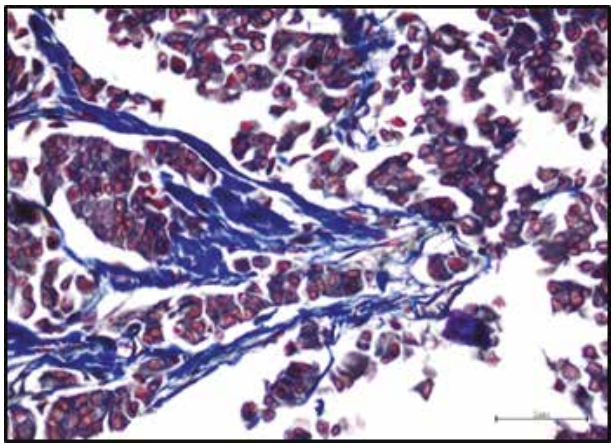

Figure 8: Photomicrograph showing minimal stromal fibrosis with mostly presence of tumor cells in a case of no response (MT stain, 100X)

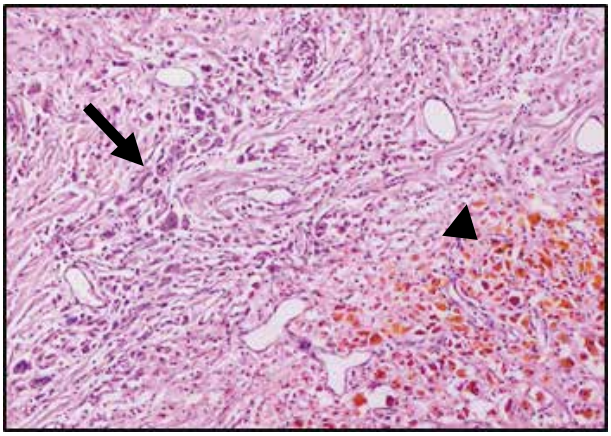

Figure 9: Photomicrograph showing infiltration of many foamy macrophages (arrow) and hemosiderin-laden histiocytes (arrowhead) in the tumor bed after chemotherapy (H&E, 200X).

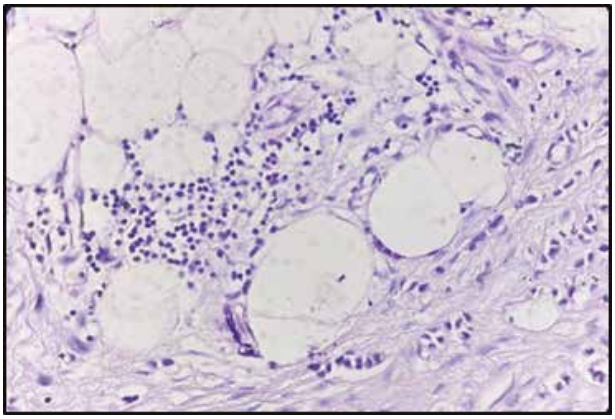

Figure 10 : Minimal infiltration of lymphocytes within the stroma, post-NACT state (H&E, 400X).

Discussion

This study provides a detailed histomorphological analysis of invasive ductal carcinoma following neoadjuvant chemotherapy (NACT) in a tertiary care setting. The findings reveal a consistent and recognizable pattern of tissue injury, characterized predominantly by stromal remodeling and specific cellular alterations that reflect both therapeutic efficacy and tumor bed regression.

The most prominent alteration observed was fibrosis and widespread fibrosis were present in 85% of all cases. This aligns with previous regional studies—Hafiz et al. in Bangladesh reported fibrosis in 63% of cases,[6] while Sheereen et al. in India also identified fibrosis as a dominant stromal response.[4] This desmoplastic reaction is considered as a hallmark of tumor microenvironment remodeling following chemotherapy, representing replacement of tumor cells with collagen-rich stroma.[3,4] In our study, the use of Masson's Trichrome stain proved particularly useful in objectively confirming and quantifying this change, which can sometimes be underappreciated on routine H&E evaluation.

At the cellular level, cytoplasmic vacuolation emerged as the most frequent alteration, affecting 81% of cases with residual viable tumor. This finding is consistent with earlier reports by Sethi et al. and Wang et al., and likely reflects severe injury to cytoplasmic organelles due to chemotherapeutic disruption of metabolic pathways.[3,7] Commonly observed nuclear changes included pyknosis (78.3%) and karyorrhexis/karyolysis (75.7%), which are established morphological markers of apoptosis and necrosis induced by cytotoxic agents.[8] The presence of multinucleation (24.3%) in a subset of cases suggests aberrant mitotic activity in cells that have undergone DNA damage but failed to complete cytokinesis.[9]

Beyond fibrosis, the tumor stroma exhibited a range of reactive changes. Hyalinization was observed in 86.7% of cases indicating stromal maturation and protein deposition providing a dense as well as glassy appearance.[10] This finding aligns with the study conducted by Sheereen and her team.[4] Lymphocytic infiltration, though mostly minimal, was noted in 80% of specimens. Tumor cell death releases tumor antigens, which activate the immune system and attract lymphocytes to the tumor bed. The role of tumor-infiltrating lymphocytes in the post-NACT setting remains an active area of investigation, with some studies suggesting a correlation between lymphocytic response and improved outcomes.[11] Less frequently, foamy macrophages (23.3%) and hemosiderin-laden histiocytes (21.7%) were observed, indicative of phagocytic clearance of lipid debris and post-treatment hemorrhage and RBC breakdown respectively.[12] These should be distinguished from other diagnostic entities and misinterpretation of the macrophages as tumor cells.

Clinical and diagnostic implications

For pathologists, recognition of this spectrum of changes is essential to avoid diagnostic pitfalls. Extensive fibrosis or hyalinization may mimic benign sclerosing lesions, potentially leading to under sampling. Conversely, reactive stromal cells, fibroblasts, or histiocytes within fibrotic areas can be mistaken for infiltrating carcinoma, especially at low magnification.[13] A systematic approach—incorporating careful high-power examination, judicious use of histochemical stains such as Masson's Trichrome, and awareness of common chemotherapy-induced alterations—is necessary to accurately distinguish between treatment effect and residual disease. This distinction is critical for correct pathological staging, prognostication, and guiding further therapeutic decisions.[14]

Limitations of this study include its single-center study and modest sample size.

Conclusion

This study systematically documents the characteristic histopathological changes in breast carcinoma following NACT. Widespread fibrosis and cytoplasmic vacuolation were identified as the hallmark alterations, accompanied by a predictable spectrum of nuclear degenerative changes and stromal remodeling. Recognizing this morphological landscape is essential for accurate pathological assessment of post-chemotherapy specimens, aiding in the distinction between treatment effect and residual disease, and ultimately supporting precise staging and clinical decision-making.

Acknowledgements

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