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Original Article

Pathology Section

Postchemotherapy Associated Histopathological Findings in Invasive Breast Carcinoma: A Camouflaged Signature

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ABSTRACT

Introduction: Breast cancer is the most common cancer diagnosed in women world-wide. Application of preoperative Neoadjuvant Chemotherapy (NACT) has become a frequently employed therapeutic approach to treat breast cancer patients. Such chemotherapy alters the morphology of both malignant tumour tissue and adjacent benign breast tissue.

Aim: To compare the histopathological features in mastectomies done for invasive breast carcinoma among those who received NACT with those who had not received NACT.

Materials and Methods: It was a retrospective observational study of invasive breast carcinoma cases conducted at a rural tertiary care referral institute, PES Institute of Medical Sciences and Research (PESIMSR), Kuppam, Andhra Pradesh, India, from January 2013 to April 2015. The histopathological features in mastectomies were evaluated using a modified scoring system and compared between those who received NACT (study group) and those who had not received NACT (control group). Postchemotherapy associated histopathological features were analysed for statistical significance. Frequencies, Chi-square test and crosstabs were the statistical tools used to analyse the data. All statistical calculations were done through Statistical Software Data (STATA) version 14.1.

Results: Out of 24 cases analysed, the study group (mean age=53.67 years) and control group (mean age=45.92 years) constituted 12 cases each. In postchemotherapy cases, fibroelastosis (p-value= 0.027) was a significant feature in the malignant tumour tissue. Stromal fibrosis (p-value=0.036), epithelial atypia (p-value <0.0001) and calcification (p-value=0.002) were significant features in the benign breast tissue.

Conclusion: In postchemotherapy cases, fibroelastosis was significant histopathological feature in malignant tumour tissue. Stromal fibrosis, epithelial atypia and calcification were significant histopathological features in the benign breast tissue. Such findings may be considered as camouflaged signature of chemotherapy. It may be hypothesised that calcification in the benign breast tissue may indicate a tell-tale sign of cell injury secondary to systemic chemotherapeutic agents.

Keywords: Cancer, Mastectomy, Treatment, Tumour

INTRODUCTION

Breast cancer is the most common cancer diagnosed in females world-wide. It constitutes second leading cause of death in women globally [1]. Application of NACT prior to surgical excision of the tumour has become a frequently employed therapeutic approach to treat patients with breast cancer. Recently, it has been the standard of care for the treatment of locally advanced breast cancer [2,3]. Such chemotherapy alters the morphology of both the malignant breast tissue and the adjacent benign breast tissue. The assessment of therapeutic response to chemotherapy and measurement of residual disease in the breast tissue and lymph node is important because it may predict the survival and provide guidelines for further management [2].

Pathological assessment of response to chemotherapy has been found to be superior to clinical assessment for the purpose of further management [4]. Pathological evaluation of tumour response has been considered as gold standard because the clinical and radiological responses do not correlate well with residual tumour after treatment [3]. Pathological examination of these postchemotherapy specimens can be quite challenging [5]. Most of the studies have emphasised on pathological assessment of response to chemotherapy [1,3,4,6,7]. Only an exceptional study has focused on comparison of histopathological features between those cases treated with NACT and those not treated with NACT [8].

Hence, the present study was undertaken to compare the histopathological features in mastectomies done for invasive breast carcinoma among those who received NACT with those who had not received NACT. The present study also highlights the importance of quantification of the histopathological features to know the significance of each parameter.

MATERIALS AND METHODS

The study was conducted at Histopathology section of Department of Pathology. It was an observational study done at a rural tertiary care referral institute, PES Institute of Medical Sciences and Research (PESIMSR), Kuppam, Andhra Pradesh, India. It was a retrospective study of invasive breast cancer cases from January 2013 to April 2015. Ethical clearance was obtained from the Institutional Ethics Committee (number PESIMSR/IHEC/43).

Histopathology slides of invasive breast carcinoma cases who had received NACT (study group) were retrieved. Histopathology slides of an equal number of invasive breast carcinoma cases who had not received NACT (control group) were retrieved. The cases were reviewed from January 2017 to March 2017 for a total period of three months. The histopathological features of the malignant tumour tissue, benign breast tissue and lymph node tissue were documented.

Sample size calculation: The sample size was calculated by using following formula:

$$n=\frac{\{Z_{1-\alpha/2}+Z_{1-\beta}\}^2\times[p_1(1-p_1)+p_2(1-p_2)]}{(p_1-p_2)^2}$$

n is the sample size in each group

p_1 is the expected proportion of control samples

p_2 is the expected proportion of study samples

α=0.05 (two-sided)

β=0.20

$Z_{1-\alpha/2}$ is the value of the standard distribution corresponding to level of significance at 5% that is 95% confidence interval

$Z_{1-\beta}$ is the value of the standard distribution corresponding to the desired level of power (80%)

Among the 24 cases analysed, the study group and the control group constituted 12 cases each.

Inclusion criteria: All invasive breast carcinoma cases confirmed by histopathology during the study period were included.

Exclusion criteria: Those cases in which there was no residual tumour in the specimen were excluded from the study.

The breast lesions were classified according to World Health Organisation (WHO) classification of tumours of breast 4th edition (2012) [9]. Pathological assessment of response to chemotherapy and calculation of Residual Cancer Burden (RCB) was not under the scope of the present study.

Treatment regimen constituted six cycle of chemotherapy for each case. First three cycles of chemotherapeutic drugs were given to the patients preoperatively and the other three cycles were given postoperatively. The chemotherapeutic drugs included cyclophosphamide (600 mg/m^2), adriamycin (60 mg/m^2) and 5-fluorouracil (600 mg/m^2) during each cycle. Histopathological evaluation was done following preoperative chemotherapy (neoadjuvant therapy).

Histopathological parameters of malignant tumour tissue and benign breast tissue were assessed by a single pathologist and scored according to a modified scoring system given by Muhury M et al., [10]. Originally, Muhury M et al., had conducted a study on thrombocytopenia for quantification of megakaryocytes in bone marrow aspirates [6]. In the present study, the principle of quantification proposed by Muhury M et al., was adopted with modifications to quantify the histopathological parameters of invasive breast cancer [10].

All histopathological parameters were evaluated in low power fields (LPF) (area=2.54 mm^2) and were documented in terms of number of LPF. The scoring system was used to know the extent of chemotherapy induced changes. The parameters were assigned score 0 (when the finding was absent), score 1 (when the finding was observed in 1-3 LPF), score 2 (when the finding was observed in 4-7 LPF) and score 3 (when the finding was observed in 8-10 LPF). Ten LPFs were examined for each of the parameters. The histopathological finding such as epithelial cell atypia was confirmed by observing in high power field (area=0.159 mm^2). However, the histopathological features of lymph node were just documented and were not scored.

STATISTICAL ANALYSIS

The sociodemographic variables were represented using frequencies and percentages. The data analysis of comparison between the study group and the control group was done by Chi-square test. All statistical calculations were done through statistical software STATA version 14.1.

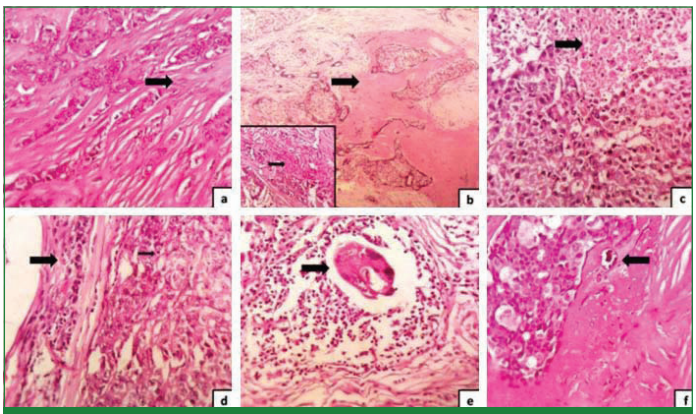
RESULTS

In the present study, 24 cases of invasive breast carcinoma were analysed. The lesions were seen in females in the range of 24-77 years. Clustering of cases was seen in fifth decade (mean=49.79 years). The study group constituted 12 cases. The lesions were seen in females in the range of 35-77 years. Clustering of cases was seen in fifth and seventh decade (mean=53.67 years). The control group constituted 12 cases. The lesions were seen in the age range of 24-59 years. Clustering of cases was seen in fifth and sixth decade (mean=45.92 years). Invasive breast carcinomas more commonly involved left breast than right breast in both the groups, nine cases (75%) in study group and eight cases (66.67%) in control group. The most common site of involvement was central area in both the study {4 cases (33.33%)} and control {6 cases (50%)} groups.

The study group constituted 12 cases (100%) of invasive carcinoma of No Special Type (NST). The control group constituted 11 cases (91.67%) of invasive carcinoma of NST and 1 case (8.33%) of invasive mucinous carcinoma.

In the study group, the most common primary tumour category was ypT2 constituting 6 cases (50%). The greatest dimension of the tumour ranged from 8 mm-80 mm (mean=39.92 mm). Lymph node metastasis was seen in 7 cases (58.33%). The common nodal categories were ypN0 and ypN1 constituting 4 cases (33.33) each. Most common grade was grade II constituting 11 cases (91.67%). In the control group, the most common primary tumour category was pT3 constituting 5 cases (41.67%). The greatest dimension of the tumour ranged from 30 mm-150 mm (mean=60.83 mm). Lymph node metastasis was seen in 7 cases (58.33%). Most common nodal category was pN0 constituting 5 cases (41.67%) each. Most common grade was grade II constituting 10 cases (83.33%). The distribution of cases between the study group and control group was not statistically significant for the primary tumour category (p-value=0.359) and nodal category (p-value=0.596).

Malignant tumour tissue: Histopathological features observed in the control group included fibrosis, necrosis, fibroelastosis, lymphocytic infiltration (peri-tumoural and intra-tumoural) and calcification. Histopathological features observed in the study group were similar to that of control group with additional findings of foci of haemorrhage and multinucleated giant cells [Table/Fig-1a-f].



[Table/Fig-1]: Postchemotherapy histopathological features in malignant tumour tissue: (a) Fibrosis (arrow) (H&E, X400); (b) Fibroelastosis (arrow) (H&E, X100). Inset: Elastic fibers (thin arrow) (H&E, X400); (c) Necrosis (arrow) (H&E, X400); (d) Peritumoural lymphocytic infiltration (arrow) and intra-tumoural lymphocytic infiltration (thin arrow) (H&E, X400); (e) Multinucleated giant cell (arrow) (H&E, X400); (f) Calcification (arrow) (H&E, X400).

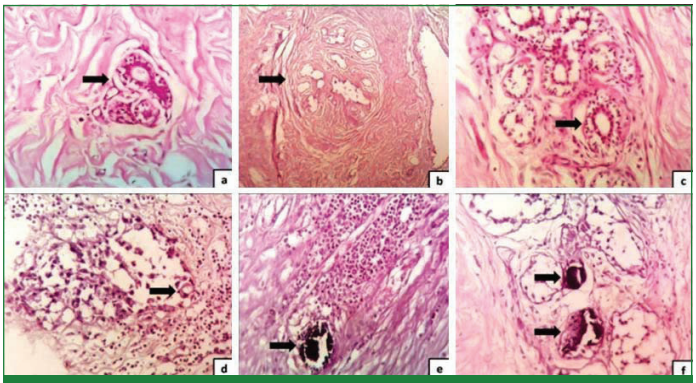
Fibrosis, necrosis, lymphocytic infiltration and calcification were marginally more in the control group than the study group, but were not statistically significant. Haemorrhage and multinucleated giant cells was seen only in the study group, but was not statistically significant. Fibroelastosis was more extensively seen in the study group and was statistically significant [Table/Fig-2].

Benign breast tissue: Histopathological features observed in the control group included lobular atrophy, lobular fibrosis, stromal fibrosis and myoepithelial cell prominence. Histopathological features observed in the study group were similar to that of control group with additional findings of epithelial cell atypia and foci of calcification [Table/Fig-3a-f].

Lobular fibrosis was marginally more in the study group than the control group, but was not statistically significant. Myoepithelial cell prominence was appreciated to a greater extent in the study group, but was not statistically significant. Stromal fibrosis was more extensive in the study group and was statistically significant. Epithelial atypia and calcification were seen only in the study group and was statistically highly significant [Table/Fig-2].

Parameters	Present	Score 0 (Absent)	Score 1 (1-3 LPF‡)	Score 2 (4-7 LPF‡)	Score 3 (8-10 LPF‡)	p-value*
Malignant breast tissue						
Fibrosis						
Study group	12 (100%)	0 (0%)	1 (8.33%)	9 (75%)	2 (16.67%)	0.824
Control group	12 (100%)	0 (0%)	1 (8.33%)	10 (83.33%)	1 (8.33%)	
Necrosis						
Study group	12 (100%)	0 (0%)	8 (66.67%)	4 (33.33%)	0 (0%)	0.673
Control group	12 (100%)	0 (0%)	7 (58.33%)	5 (41.67%)	0 (0%)	
Fibroelastosis						
Study group	8 (66.67%)	4 (33.33%)	4 (33.33%)	4 (33.33%)	0 (0%)	**0.02**
Control group	2 (16.67%)	10 (83.33%)	0 (0%)	2 (16.67%)	0 (0%)	
Lymphocytic infiltration						
Study group	12 (100%)	0 (0%)	4 (33.33%)	7 (58.33%)	1 (8.33%)	0.341
Control group	12 (100%)	0 (0%)	7 (58.33%)	5 (41.67%)	0 (0%)	
Haemorrhage						
Study group	1 (8.33%)	11 (91.67%)	1 (8.33%)	0 (0%)	0 (0%)	0.307
Control group	0 (0%)	12 (100%)	0 (0%)	0 (0%)	0 (0%)	
Calcification						
Study group	8 (66.67%)	4 (33.33%)	8 (66.67%)	0 (0%)	0 (0%)	0.219
Control group	5 (41.67%)	7 (58.33%)	5 (41.67%)	0 (0%)	0 (0%)	
Multinucleated giant cells						
Study group	1 (8.33%)	11 (91.67%)	1 (8.33%)	0 (0%)	0 (0%)	0.307
Control group	0 (0%)	12 (100%)	0 (0%)	0 (0%)	0 (0%)	
Benign breast tissue						
Lobular atrophy						
Study group	12 (100%)	0 (0%)	9 (75%)	3 (25%)	0 (0%)	0.098
Control group	12 (100%)	0 (0%)	5 (41.67%)	7 (58.33%)	0 (0%)	
Lobular fibrosis						
Study group	12 (100%)	0 (0%)	8 (66.67%)	4 (33.33%)	0 (0%)	0.587
Control group	11 (91.67)	1 (8.33%)	7 (58.33%)	4 (33.33%)	0 (0%)	
Stromal fibrosis						
Study group	12 (100%)	0 (0%)	2 (16.67%)	5 (41.67%)	5 (41.67%)	**0.036**
Control group	12 (100%)	0 (0%)	2 (16.67%)	10 (83.33%)	0 (0%)	
Myoepithelial cell prominence						
Study group	12 (100%)	0 (0%)	2 (16.67%)	3 (25%)	7 (58.33%)	0.348
Control group	11 (91.67%)	1 (8.33%)	3 (25%)	5 (41.67%)	3 (25%)	
Epithelial atypia						
Study group	12 (100%)	0 (0%)	12 (100%)	0 (0%)	0 (0%)	**<0.001**
Control group	0 (0%)	12 (100%)	0 (0%)	0 (0%)	0 (0%)	
Calcification						
Study group	7 (58.33%)	5 (41.67%)	7 (58.33%)	0 (0%)	0 (0%)	**0.002**
Control group	0 (0%)	12 (100%)	0 (0%)	0 (0%)	0 (0%)	

[Table/Fig-2]: Comparison of histopathological features of breast tissue in invasive breast carcinoma.
*Chi-square test was the statistical tool used to perform the statistical analysis; ‡LPF: Low power field; p<0.05 was considered significant and p<0.01 was considered highly significant (n=12 in each group, N-24)

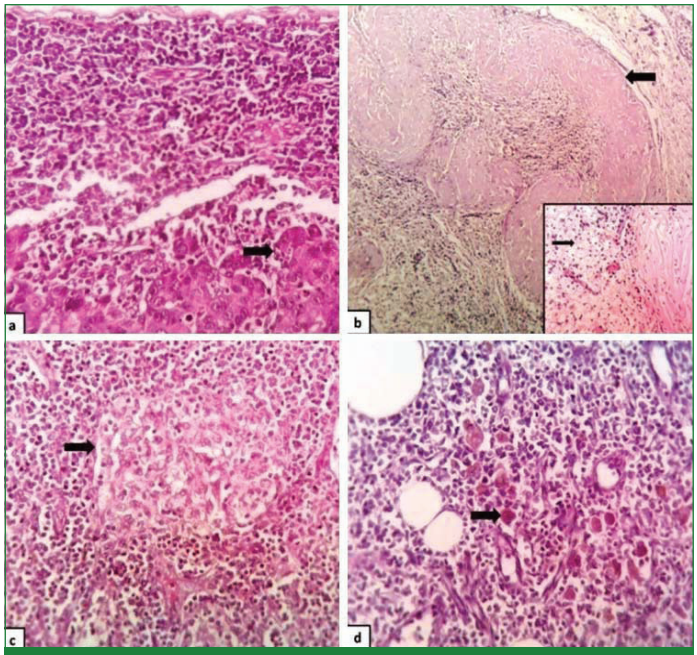


[Table/Fig-3]: Postchemotherapy histopathological features in benign breast tissue: (a) Lobular atrophy (arrow) with stromal fibrosis (H&E, X400); (b) Lobular fibrosis (arrow) (H&E, X100); (c)Myoepithelial cell prominence (arrow) (H&E, X400); (d) Epithelial atypia (arrow) (H&E, X400); (e)Stromal calcification (arrow) (H&E, X400); (f) Calcification in glands (arrows) (H&E, X400).

Lymph node tissue: Lymph nodes were isolated and evaluated in 12 patients of the control group and 11 patients in the study group. One patient of the study group did not undergo the lymph node analysis. Histopathological features observed in both the groups included metastatic tumour deposits, focal collection of macrophages, pigmented macrophages and areas of fibrosis [Table/Fig-4a-d]. These findings were appreciated more in the study group than the control group [Table/Fig-5].

DISCUSSION

Breast cancer is the most prevalent cancer among women globally [11,12]. Nevertheless, increased survival of the patients may be ascribed to the dramatic advances in the screening methods, early diagnosis and recent advances in the treatment [11]. The treatment typically includes NACT, surgery, radiation therapy with or without endocrine therapy [4]. NACT administered preoperatively,



[Table/Fig-4]: Postchemotherapy histopathological features in lymph node tissue: (a) Metastatic tumor deposit (arrow) [H&E, X400]. (b) Fibrosis (arrow) [H&E, X40]. Inset: Fibrosis with sheets of tissue macrophages (small arrow) [H&E, X100]. (c) Focal collection of tissue macrophages (arrow) [H&E, X400]. (d) Pigmented macrophages (arrow) [H&E, X400].

Lymph node tissue	Present	Absent	Total (n=23)
Metastasis			
Study group	7 (63.64%)	4 (36.36%)	11
Control group	7 (58.33%)	5 (41.67%)	12
Collection of macrophages			
Study group	11 (100%)	0 (0%)	11
Control group	8 (66.67%)	4 (33.33%)	12
Pigmented macrophages			
Study group	5 (45.45%)	6 (54.55%)	11
Control group	3 (25%)	9 (75%)	12
Fibrosis			
Study group	9 (81.82%)	2 (18.18%)	11
Control group	4 (33.33%)	8 (66.67%)	12

[Table/Fig-5]: Comparison of histopathological features of lymph node tissue in invasive breast carcinoma.
Lymph nodes were not isolated in one case of study group

is now considered as the standard of care for locally advanced breast cancers [3]. The pathological assessment of response to induction chemotherapy will help in decision making whether there is likelihood of additional benefit by continuing the same drugs in the postoperative adjuvant therapy or different drugs could be administered in case of unsatisfactory response [4]. While, most of the studies have betoned on pathological assessment of response to chemotherapy, the current study endeavors to figure out significant histopathological changes associated with chemotherapy by comparing the histopathological features between those cases treated with NACT and those not treated with NACT.

Total number of cases analysed was highest in a study conducted by Vasudevan D et al., [6]. In contrast to the other studies, the present study had less number of invasive breast carcinoma cases treated with chemotherapy. In the present study, clustering of cases was seen in fifth and seventh decade. Pasam RK et al., also documented majority of cases in fifth decade in their study [3]. The mean age of presentation was 53.67 years in the present study. Sethi D et al., Vasudevan D et al., and Philipose CS et al., documented lower mean age in their study [4,6,7]. Invasive breast carcinoma (NST) was the commonest histological type in most of the studies, including the present study [1,3,4,6,7]. The tumours more commonly involved, the left breast in the present study. In contrast, right breast was more frequently involved in a study conducted by Sheereen S et al., [1]. Philipose CS et al., documented equal frequency with respect to laterality of breast lesion in their study [7]. In the present study, the most common site of involvement was central area. In contrast, Sheereen S et al., and Philipose CS et al., documented upper and outer quadrant as the most common site of involvement [1,7]. The other studies had not specified clearly about the laterality or the site of involvement of the tumour. In the present study, the greatest dimension of the tumour ranged from 8 mm-80 mm (mean=39.92 mm). Pasam RK et al., documented greatest dimension of the tumour in the similar range in their study [3]. Vasudevan D et al., documented greatest dimension of 20-50 mm with a mean of 37.5 mm [6]. Sheereen S et al., documented greatest dimension of the tumour of lower mean value [1]. Similar to the present study, Sethi D et al., had employed cyclophosphamide, adriamycin and 5-fluorouracil (CAF regimen) as neoadjuvant therapy [4]. However, for some of the cases, Sethi D et al., used epirubicin instead of adriamycin [4]. Sheereen S et al., and Vasudevan D et al., documented the use of paclitaxel instead of 5-flurouracil in their study [1,6]. Three cycles of NACT were given to each patient in the present study. Vasudevan D et al., had also mentioned administration of three cycles of NACT in their study [6]. Sheereen S et al., mentioned that four to six cycles of NACT was employed in their study [1]. Sethi D et al., documented that two to six cycles of NACT were employed in their study [4]. Pasam RK et al., and Philipose CS et al., had not specified about the treatment regimen employed in their study [3,7]. Different studies have followed different treatment regimen. This may be reason for variation in the histopathological findings [Table/Fig-6] [1,3,4,6,7].

Malignant tumour tissue: Sheereen S et al., Pasam RK et al., Sethi D et al., Philipose CS et al., and the present study observed areas of necrosis and lymphocytic infiltration in postchemotherapy cases [1,3,4,7]. Sheereen S et al., Sethi D et al., and the present study observed areas of fibrosis, fibroelastosis, calcification and multinucleated giant cells in postchemotherapy cases [1,4]. In contrast, Pasam RK et al., had not documented areas of fibrosis, fibroelastosis, calcification or multinucleated giant cells in their study [3]. Philipose CS et al., had observed fibrosis, and multinucleated giant cells in their study, but had not documented calcification [7]. Vasudevan D et al., had documented inflammatory infiltrate, histiocytes and multinucleated giant cells in their study, but had not documented other histological features [6]. Foci of haemorrhage were observed only in the present study. In contrast, other studies had not documented haemorrhage in the postchemotherapy cases [1,3,4,6,7].

In the present study, fibrosis, necrosis, lymphocytic infiltration and calcification were marginally more in the control group than the study group, but were not statistically significant. Haemorrhage and multinucleated giant cells were seen only in the study group, but were not statistically significant. Sethi D et al., observed that collagenization and giant cells were significantly associated with better overall response to chemotherapy [4]. Aktepe F et al., found no difference between tumors treated with chemotherapy and untreated tumors with respect to elastosis, necrosis, inflammatory infiltrate [13]. In contrast, in the present study, fibroelastosis was more extensively seen in the study group and was statistically significant. The angiostatic molecule endostatin has been reported to accumulate on elastin fibres and may reduce angiogenesis and thereby limit the tumour growth and spread. The effects could be related to elastin itself, elastin receptor or elastin derived peptides. Some elastin-related effects may be mediated by elafin, an inhibitor of elastase, which has been recently shown to be positive prognostic factor in breast cancer [14].

Nuclear features of malignant cells were documented in postchemotherapy cases in the studies conducted by Sheereen S

Parameters	Present study [India (Andhra Pradesh)]	Philipose CS et al., [7] [India (Karnataka), 2019)	Sheereen S et al., [1] [India (Karnataka), 2018]	Pasam RK et al., [3] [India, (Andhra Pradesh), 2015]	Vasudevan D et al., [6] [India (Kerala), 2015]	Sethi D et al., [4] [India (Haryana), 2013]
Number of cases	12	22	39	20	48	40
Mean age (years)	Fifth and seventh decade (Mean=53.67)	Mean=49.5	-	Fifth decade	Mean=50.58	Mean=46
Common Lesion	Invasive breast carcinoma-NST (100%)	Infiltrating breast carcinoma-NOS (68.18%)	Infiltrating breast carcinoma-NOS (76.92%)	-	Infiltrating breast carcinoma-NOS (79.2%)	Infiltrating breast carcinoma-NOS (76.92%)
Laterality	Left (75%)	Equal frequency (50%)	Right (66.7%)	-	-	-
Common site	Central area (33.33%)	Upper outer quadrant (22.73%)	Upper and outer quadrant (74.4%)	-	-	-
Greatest dimension of the tumour	8-80 mm (range), 39.92 mm (mean)	-	17.5 mm (mean)	10-90 mm	20 -50 mm (range) 37.5 mm (mean)	-
Chemotherapeutic	Cyclophosphamide Adriamycin 5-Fluorouracil	Not specified	Cyclophosphamide Adriamycin Paclitaxel	Not specified	Cyclophosphamide Adriamycin Paclitaxel	Cyclophosphamide Adriamycin/ epirubicin 5-Fluorouracil
No. of chemotherapy cycles (prior to surgery)	3	3-11	4-6	-	3	2-6
Hormonal therapy	No	Not specified	Yes	No	No	No

[Table/Fig-6]: Comparison of clinicopathological parameters in invasive breast carcinoma cases in various studies [1,3,4,6,7, Present study].
NST: No special type, NOS: Not otherwise specified

et al., Sethi D et al., and Philipose CS et al., [1,4,7]. In contrast, Pasam RK et al., Vasudevan D et al., and the present study had not documented nuclear features [3,6]. The nuclear features include nuclear enlargement, hyperchromasia, increased nuclear cytoplasmic (N:C) ratio, prominent nucleoli, vacuolation, karyorrhexis, pyknosis and karyolysis [1,4]. But these features may be seen even in the breast carcinoma cases not treated with NACT. The finding may not be specific to chemotherapy cases as the statistical significance was not determined. Pasam RK et al., Sethi D et al., and Philipose CS et al., had observed cytoplasmic vacuolation in their study [3,4,7]. In contrast, Sheereen S et al., Vasudevan D et al., and the present study did not document cytoplasmic vacuolation [1,6]. The residual tumour cells may appear morphologically same without any alterations or display cytological changes suggesting treatment effect [15]. Residual tumour assessment was not emphasised as it was not under the scope of the present study.

In contrast to the present study, Sheereen S et al., Pasam RK et al., and Sethi D et al., observed hyalinised blood vessels in their study [1,3,4]. Sheereen S et al., and Sethi D et al., documented mucinous change and histiocytes in their study [1,4]. In contrast, Pasam RK et al., Vasudevan D et al., Philipose CS et al., and the present study had not documented such findings [3,6,7]. In contrast to other studies, Sheereen S et al., observed cancerisation of lobules and angiogenesis [1]. Sethi D et al., had documented dissociation, dyscohesion and loss of organisation of tumour cells in their study [Table/Fig-7] [1,3,4,6,7].

Benign breast tissue: Sheereen S et al., Sethi D et al., and the present study observed lobular atrophy in the postchemotherapy cases in their study [1,4]. In contrast, Pasam RK et al., had not documented lobular atrophy. In contrast to other studies, Pasam RK et al., and the present study observed epithelial atypia in the benign breast tissue. In contrast to other studies, Pasam RK et al., noticed sclerosed basement membrane in their study [3]. Vasudevan D et al., and Philipose CS et al., had not documented any histological changes in the benign breast tissue in their studies [6,7]. In contrast to other studies, lobular fibrosis, stromal fibrosis, myoepithelial cell prominence and foci of calcification were documented only in the present study [Table/Fig-7] [1,3,4,6,7].

Lobular fibrosis, lobular atrophy, myoepithelial cell prominence was appreciated more in the study group, but were not statistically significant. Stromal fibrosis was more extensive in the study group and was statistically significant. The exact aetiology of stromal fibrosis in the breast tissue is unknown. However, it has been speculated that it may be related to oestrogen related fibroblast proliferation [16].

Epithelial atypia and calcification were seen only in the study group and was statistically highly significant. The optimal goal NACT is to achieve complete remission of the tumour. However, there is increasing evidence suggesting that NACT has the ability to increase the risk of cancer progression [17]. Whether NACT itself could be held accountable for the small increase in local recurrence is questionable [18]. Epithelial atypia induced by chemotherapy serves as a fertile soil for local recurrence of the tumour remains conjunctural and needs to be established by molecular studies. Microcalcifications play an important role in breast cancer diagnosis. Type I calcifications are composed of calcium oxalate and were predominantly seen in benign lesions. Type II calcifications are composed of calcium phosphate (hydroxapatite) and were strictly associated with malignant lesions [19]. Calcification in the benign breast tissue was an unexpected and unique observation which had not received much attention in other studies. It may be hypothesised that calcification may indicate tell-tale sign of cell injury secondary to systemic chemotherapeutic agents.

Lymph node tissue: Pasam RK et al., Vasudevan D et al., and the present study observed fibrosis and focal collection of macrophages in postchemotherapy cases [3,6]. In contrast to other studies, Pasam RK et al., documented necrosis in their study [3]. Lymphoplasmacytic infiltration was document only in the study conducted by Vasudevan D et al., [6]. In the present study, metastatic deposits, focal collection of macrophages, pigmented macrophages and fibrosis was appreciated more in the study group than the control group [Table/Fig-5]. In contrast to the present study, Erbes T et al., documented significantly lower nodal metastasis in the primary chemotherapy group (54.9%) than the primary surgery group (84.6%) [8] but, it has been stated that NACT promotes distant metastasis of breast cancer through changes in the microenvironment [17].

Various system employed to evaluate the changes in postchemotherapy breast specimen include American Joint Committee on Cancer (AJCC) system, National Surgical Adjuvant Breast and Bowel Project (NSABP B-18) criteria system, Miller-Payne system, Chevallier system, Sataloff classification, RCB system and Clinico-Pathological Scoring (CPS) system [20].

The AJCC system includes a pretreatment clinical staging defined by radiographic and clinical findings; and postoperative pathologic stage classification based on the findings in the breast and axillary lymph nodes. NSABP B-18 criteria system includes three categories namely complete pathological response (pCR) when there is no identifiable tumour cells in the specimen, partial pathological response (pPR) when small clusters or scattered tumour cells are

Parameters	Present study [India (Andhra Pradesh)]	Philipose CS et al., [7] [India (Karnataka), 2019)	Sheereen S et al., [1] [India (Karnataka), 2018]	Pasam RK et al., [3] [India, (Andhra Pradesh), 2015]	Vasudevan D et al., [6] [India (Kerala), 2015]	Sethi D et al., [4] [India (Haryana), 2013]
Malignant breast tissue						
Fibrosis	Yes	Yes	Yes	No	No	Yes
Necrosis	Yes	Yes	Yes	Yes	No	Yes
Fibroelastosis	Yes	No	Yes	No	No	Yes
Lymphocytic infiltration	Yes	Yes	Yes	Yes	Yes	Yes
Haemorrhage	Yes	No	No	No	No	No
Calcification	Yes	No	Yes	No	No	Yes
Multinucleated giant cells	Yes	Yes	Yes	No	Yes	Yes
Nuclear features	No	Yes	Yes	No	No	Yes
Cytoplasmic vacuolation	No	Yes	No	Yes	No	Yes
Hyalinised blood vessels	No	No	Yes	Yes	No	Yes
Mucinous change	No	No	Yes	No	No	Yes
Histiocytes	No	Yes	Yes	No	Yes	Yes
Cancerisation of lobules	No	No	Yes	No	No	No
Angiogenesis	No	No	Yes	No	No	No
Dissociation, dyscohesion and loss of organisation of tumour cells	No	No	No	No	No	Yes
Benign breast tissue						
Lobular atrophy	Yes	No	Yes	No	No	Yes
Lobular fibrosis	Yes	No	No	No	No	No
Stromal fibrosis	Yes	No	No	No	No	No
Myoepithelial cell prominence	Yes	No	No	No	No	No
Epithelial atypia	Yes	No	No	Yes	No	No
Sclerosed basement membrane	No	No	No	Yes	No	No
Calcification	Yes	No	No	No	No	No
Lymph node tissue						
Collection of macrophages	Yes	No	No	Yes	Yes	No
Pigmented macrophages	Yes	No	No	No	No	No
Fibrosis	Yes	No	No	Yes	Yes	No
Necrosis	No	No	No	Yes	No	No
Lymphopl-asmacytic infiltration	No	No	No	No	Yes	No

[Table/Fig-7]: Comparison of postchemotherapy histopathological features appreciated in invasive breast cancer in various studies [1,3,4,6,7, present study].

seen in the stroma; and pathological no response (pNR) when the tumour cells does not show any changes. Miller-Payne system is composed of a five-point scale which is based on cell reduction after treatment even without a marked reduction in the size of the tumour. It does not include evaluation of axillary lymph node. Chevallier system includes a four step algorithm to grade the treatment response in breast and axillary lymph node [20]. Vasudevan D et al., and Philipose CS et al., employed Chevallier system for evaluation of pathological response in their study [6,7]. Sataloff classification is based on the response of primary tumour and lymph node to the treatment. It does not include lymphovascular infiltration. RCB is considered as a prognostic indicator for disease free survival and overall survival among the breast cancer patients treated with NACT. Residual Cancer Burder (RCB) is divided into four categories and is calculated based on formula including six parameters [20]. Pasam RK et al., used NSABP B-18 and RCB for evaluation in their study [3]. The CPS system is a Cox proportional hazard model in which factors are gradually eliminated in a backward manner. It includes clinical stage before treatment and pathological stage after treatment [20]. The treatment response was not evaluated in the present study because it was not within the scope of the study.

Most of the studies have described the histomorphological features associated with chemotherapy in invasive breast cancer cases. Most of the studies have also compared the pretreatment and posttreatment features to determine the response to treatment of invasive breast cancer cases [1,3,4,6,7]. But only occasional studies have addressed about the significance of each histopathological finding [8]. In the present study, each histopathological finding of postchemotherapy case was not only quantified but also compared with that of control group to determine the significance. This is important because some of the histopathological features may be shared by both the study group and control group. Quantification helps us to know the histopathological features that are significant, specific and unique to chemotherapy associated phenomenon.

Currently, NACT has been considered as standard treatment modality for large and locally advanced breast cancers [21]. The use of neoadjuvant therapy has also been extended to early stage breast cancer [5,21,22]. Inoperable malignant neoplasms are converted to operable ones with primary systemic chemotherapy. Furthermore, some of the patients may become candidates for breast conservation surgery [4]. Breast cancer therapy causes morphological alteration in not only the cancerous tissue but also the surrounding healthy tissue [1]. Besides drug resistance, the patients' oversensitivity to chemotherapy poses one of the serious problems in cancer treatment. The cytotoxic drugs aim the intensively proliferating tumour cells. But unfortunately, these drugs also destroy the other cells and tissues with high proliferation rates (gastro-intestinal epithelial cells, bone marrow cells and skin) resulting in chemotherapy related toxicities. The chemotherapeutic drugs used in most common breast cancer chemotherapy regimen (CAF) is responsible for genetic material damage leading to cell cycle checkpoint activation and cell death [23]. In order to overcome

the harmful systemic effects of chemotherapy, newer modalities of treatment like "Trojan Horse" approach have been devised. The approach involves intra-tumoural delivery of immunomodulator agent in order to cause immune mediated destruction of tumour cells by producing microenvironmental modifications within the tumour and concurrently avoids systemic toxicity [24].

Limitation(s)

The number of cases was relatively less in comparison with other studies. This may be attributed to poor affordability factor of breast cancer patients to avail chemotherapy in a rural setting. Preoperative biopsy details, immunohistochemistry or radiological details of individual cases could not be retrieved to assess treatment response. This is because, it was a retrospective study, focused on comparing histopathological features of postchemotherapy cases with that of breast cancer cases without history of chemotherapy.

CONCLUSION(S)

In postchemotherapy cases, fibroelastosis was significant histopathological feature in malignant tumour tissue. Stromal fibrosis, epithelial atypia and calcification were significant histopathological features in the benign breast tissue. Such findings may be considered as camouflaged signature of chemotherapy. Calcification in the benign breast tissue was an unexpected and unique observation. It may be hypothesised that calcification may indicate a tell-tale sign of cell injury, secondary to systemic chemotherapeutic agents. The other features were not specific and were appreciated in both the groups. The mechanisms underlying chemotherapy associated histopathological changes, needs to be established by molecular studies. With recent advances in cancer chemotherapy, future studies may show fewer histopathogical changes with management becoming more personalised and targeted.

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