Frontiers in Health Informatics www.healthinformaticsjournal.com
ISSN-Online: 2676-7104
2024; Vol 13: Issue 4 Open Access

Study of CD4 biomarker in Breast Carcinoma and Its Correlation with Clinical Parameters

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Cite this paper as: Dr Rishav Chauhan Dr Vijayalaxmi S Patil , (2024) Study of CD4 biomarker in Breast Carcinoma and Its Correlation with Clinical Parameters. *Frontiers in Health Informatics, 13* (4), 416-427

Abstract

Background

Breast carcinoma is one of the biggest causes of cancer worldwide. Breast carcinoma is one of the highest cases of morbidity and death in women in India. Despite the existence of this immune response, many breast cancers progress and spread, questioning the function of tumor infiltrating lymphocytes in the tumor micro environment. There is a controversy around the precise role of TILs in breast carcinoma i.e whether they are present as nonspecific inflammatory reaction or a specific immunologic response. The purpose of this study was to assess the role of CD4+ TILs in breast cancer tissues. Breast cancer tumor type, histological grading, clinical stage, lymph node status, and hormone receptor status are among the traditional prognostic factors. Correlation between these factors and tumor infiltrating lymphocytes is studied to know their future role in breast carcinoma.

METHODS

45 cases of breast carcinoma patients were studied and various clinicopathological parameters were noted. IHC staining for ER, PR, Her2neu and CD4 marker in tissue sections was done and CD4 intratumoral and stromal TILs were counted and correlated with clinicopathological prognostic factors.

RESULTS

Intratumoral and stromal CD4 expression was detected in all 45 cases of breast carcinoma studied. CD4 stromal expression and intra-tumoral expression did not show any correlation with age, histological grade, tumor size, lymph node status, ER, PR, or HER2Neu status. In the triple-negative breast cancers, high stromal CD4 expression was noted.

CONCLUSION

The recruitment and deposition of large numbers of lymphocytes in tumor tissue acts as an important local barrier to neoplastic progression. The presence of substantial TILs in the tumor promotes improved clinical outcomes. Tumor infiltrating lymphocytes should be routinely evaluated as a prognostic marker for aggressive breast cancers, including triple negative breast cancers. We identified a higher concentration of stromal CD4 TILs in triple negative breast tumors.

KEYWORDS

Breast carcinoma, Tumor infiltrating lymphocytes, Intratumoral lymphocytes, stromal lymphocytes, Immunohistochemistry, CD4.

Introduction

Breast carcinoma is the most common cancer in women worldwide, and its incidence continues to rise, making it a leading cause of cancer-related morbidity and mortality. According to the World Health Organization (WHO), it is estimated that over 2 million women are diagnosed with breast cancer each year, and the disease is responsible for approximately 15% of all cancer-related deaths [1,2].

In India, breast carcinoma has become a significant public health issue, with increasing numbers of cases being reported every year. It is the most prevalent cancer among Indian women, surpassing cervical cancer in incidence. The age of onset has also been shifting, with a growing number of younger women being diagnosed with the disease, which further underscores the need for early detection and effective treatment strategies. Factors such as genetic predisposition, lifestyle changes, and delayed childbearing are contributing to the rising incidence. Despite improvements in healthcare infrastructure and awareness, breast carcinoma continues to pose a major challenge in both urban and rural regions [3,4]

CD4 are t-cell proteins that encourage other immune cells to combat infection, including macrophages, B lymphocytes (B cells), and CD8 T lymphocytes (CD8 cells), in order to help coordinate the immunological response. CD4+ T helper cells, are a vital component of the human immune system. They go by the names CD4 cells, T helper cells, or T4 cells frequently. Because one of their primary functions is to communicate with other immune cell types, such as CD8 killer cells, which subsequently eliminate the infectious particle, they are known as helper cells [5,6].

Materials & Methods

A total 45 histologically proven cases of breast cancer were included in the study. Their clinical history, clinical stage, tumor grade, lymph node status, oestrogen receptor, progesterone receptor and HER2 status were recorded. All patients had pre-operative diagnosis of breast cancer either by core biopsy of the breast or by fine needle aspiration cytology. All patients underwent surgery with axillary lymph node dissection and no patient had received pre-operative anti tumor therapy. Histological grading was done by Bloom and Richardson grading. No patient had evidence of an active infection or inflammatory disease. Detailed histopathological examination was done in the department of pathology.

All mastectomy specimens received at the histopathology section of the department of Pathology at BLDE Vijayapura, were studied. The specimen was preserved in 10 percent formalin and processed routinely. Five sections of four micron thickness were prepared from the most suitable tissue block. Sections were mounted on poly Lysine-coated slides, which were subjected to ER/PR, HER2-neu and CD4 immunohistochemical staining according to standard protocol. Immunohistochemical expression of CD4 was correlated with prognostic factors such as tumor size, histological grade, lymph node status, ER, PR, and HER-2neu status.

Scoring of TILs was done by the following procedure: 1) A tumor area was defined in which TILs are to be evaluated. 2) Stromal area was segmented. 3) CD4+ TILs were counted in randomly selected five high power fields in 40x magnification and the counts were averaged. 4) TIL counts were recorded as 1+ (1-25 cells), 2+ (26-50 cells) and 3+ (> 51 cells) in the tumor and stroma separately.

5) Percentage of TILs was assessed as the fraction of stromal tissue covered by them. Positive TILs up to 25 cells were considered as low TIL count and more than 25 cells (++, +++) were considered as high TIL count. Mastectomy specimens of patients who received neoadjuvant chemotherapy and radiotherapy were excluded from the study

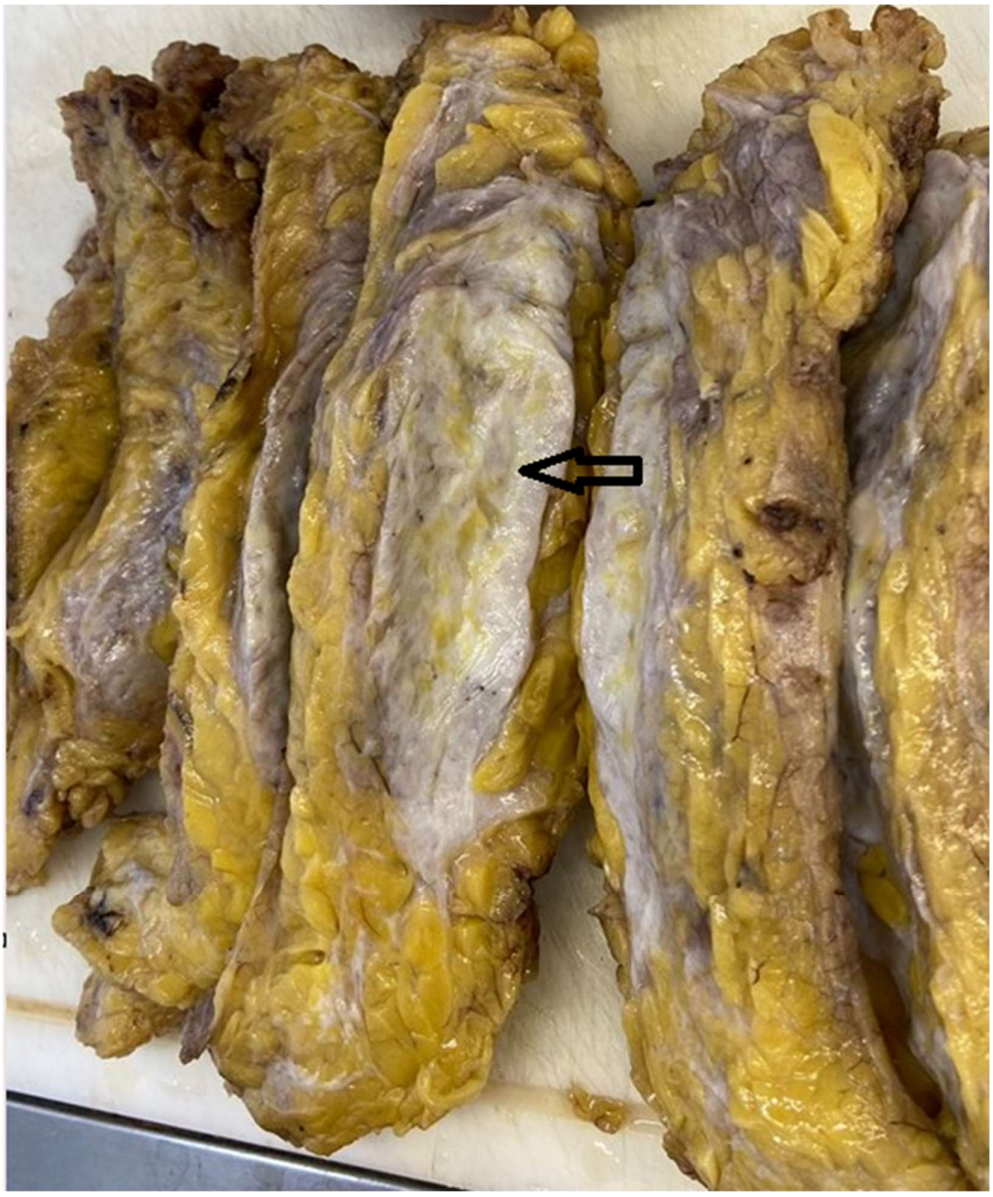


Figure 1: Gross photograph of the cut section of tumor. Arrow showing the tumor part

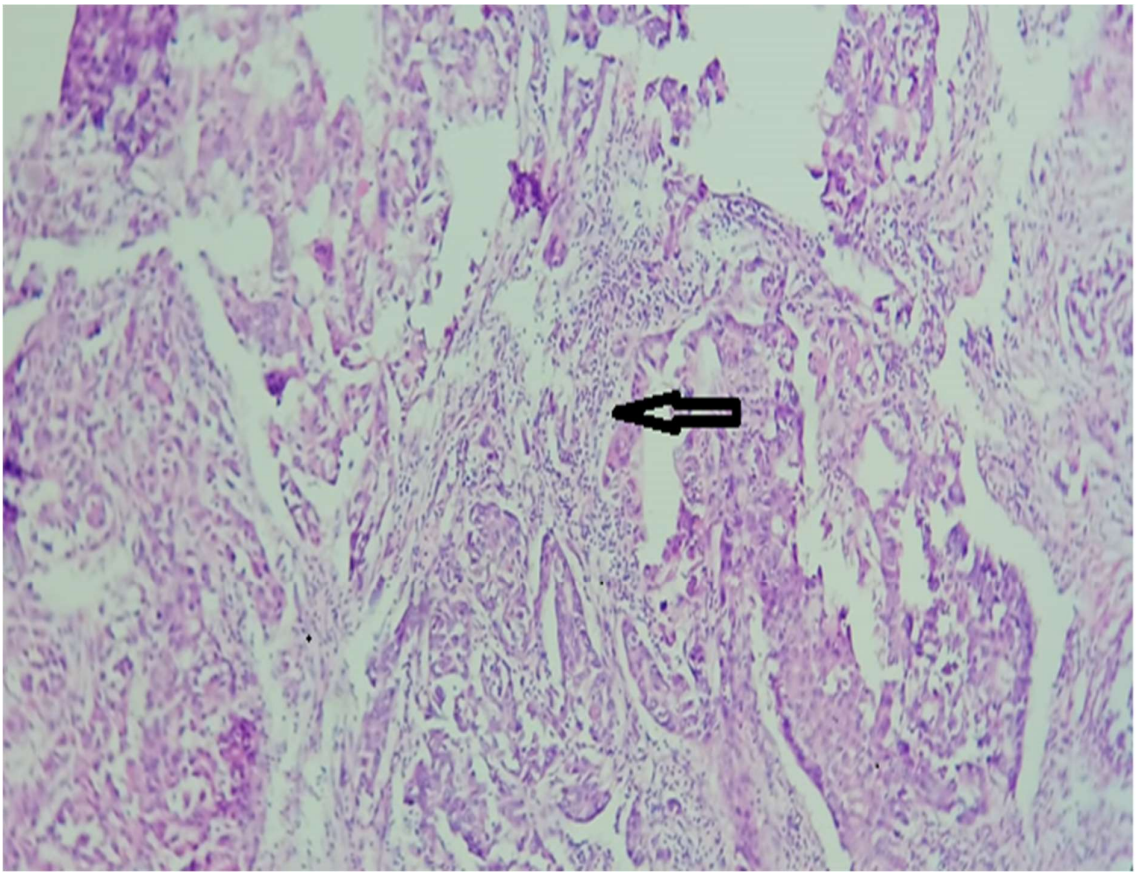


Figure 2: Photomicrograph of invasive breast carcinoma showing stromal tumor infiltrating lymphocytes. (H&E, 100x)

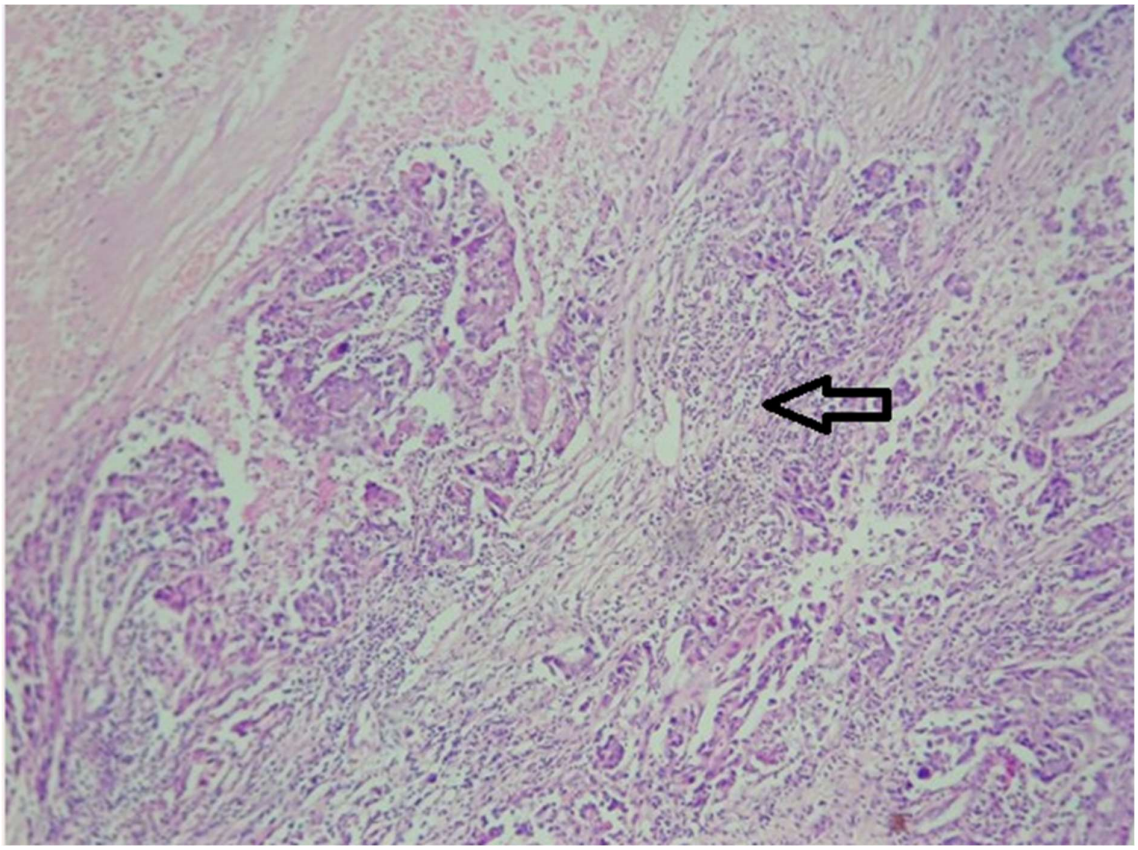


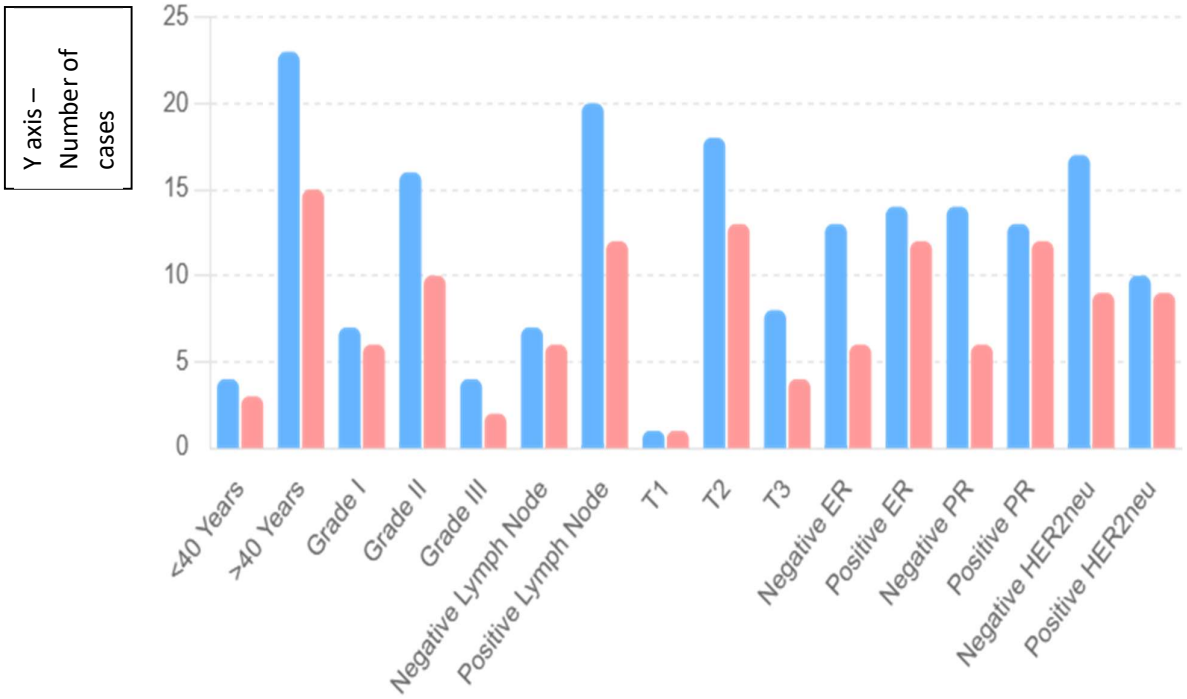
Figure 3: Photomicrograph of invasive breast carcinoma showing intratumoral tumor infiltrating lymphocytes (H&E, 100X)

Correlation of CD4 expression with various prognostic parameters in breast carcinoma

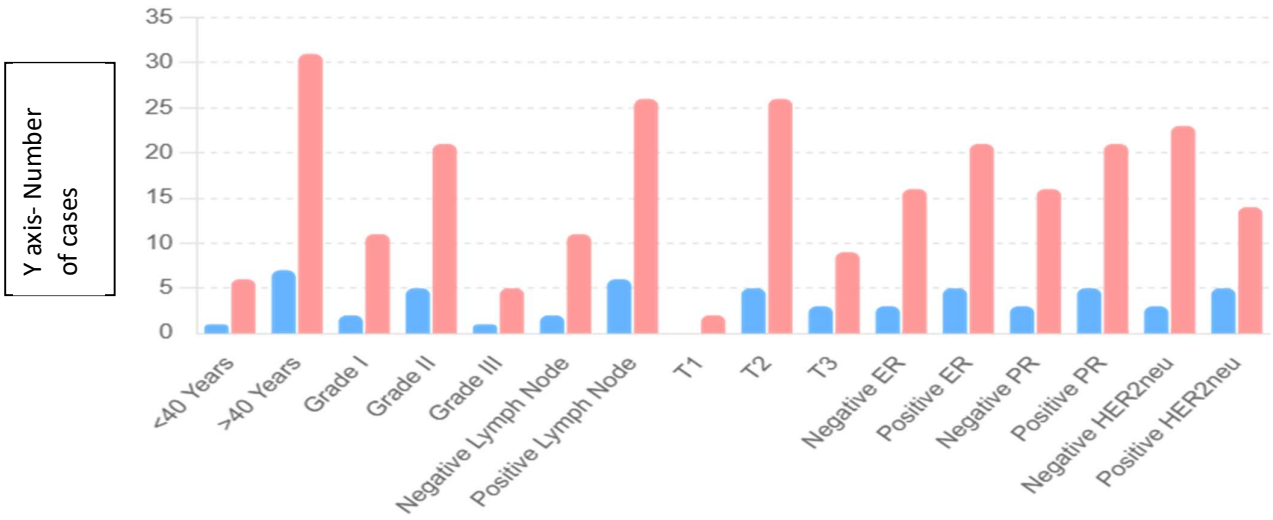
PARAMETERS	CD4					
	STROMAL			INTRATUMORAL		
	HIGH	LOW		HIGH	LOW	
AGE						
<40 YEARS	4(57.1%)	3(42.9%)	Chi-square value-0.028 P value-0.867	1(14.3%)	6(85.7%)	Chi-square value-0.069 p value-0.793
>40 YEARS	23(60.5%)	15(39.5%)		7(18.4%)	31(81.6%)	
HISTOLOGIC GRADE						
I	7(53.8%)	6(46.2%)	Chi-square value-0.342 p value-0.843	2(15.4%)	11(84.6%)	Chi-square value-0.094 p value-0.954
II	16(61.5%)	10(38.5%)		5(19.2%)	21(80.8%)	

III	4(66.7%)	2(33.3%)		1(16.7%)	5(83.3%)	
LYMPH NODE						
NEGATIVE	7(53.8%)	6(46.2%)	Chi-square value-0.288 P value-0.591	2(15.4%)	11(84.6%)	Chi-square value-0.072 p value-0.789
POSITIVE	20(62.5%)	12(37.5%)		6(18.8%)	26(81.3%)	
TUMOR SIZE						
T1	1(50%)	1(50.0%)	Chi-square value-0.354 p value-0.838	0(0.0%)	2(100.0%)	Chi-square value-0.918 p value-0.632
T2	18(58.1%)	13(41.9%)		5(16.1%)	26(83.9%)	
T3	8(66.7%)	4(33.3%)		3(25.0%)	9(75.0%)	
ER STATUS						
NEGATIVE	13(68.4%)	6(31.6%)	Chi-square value-0.97 p value-0.324	3(15.8%)	16(84.2%)	Chi-square value-0.089 p value-0.766
POSITIVE	14(53.8%)	12(46.2%)		5(19.2%)	21(80.8%)	
PR STATUS						
NEGATIVE	14(70%)	6(30%)	Chi-square value-1.50 p value-0.221	3(15.8%)	16(84.2%)	Chi-square value-0.089 p value-0.766
POSITIVE	13(52%)	12(48%)		5(19.2%)	21(80.8%)	
HER2 neu STATUS						
NEGATIVE	17(65.4%)	9(34.6%)	Chi-square value-0.744 p value-0.388	3(11.5%)	23(88.5%)	Chi-square value- 1.64 p value-0.20
POSITIVE	10(52.6%)	9(47.4%)		5(26.3%)	14(73.7%)	

STROMAL CD4 EXPRESSION



X Axis - Parameters for evaluation



X Axis - Parameters for evaluation

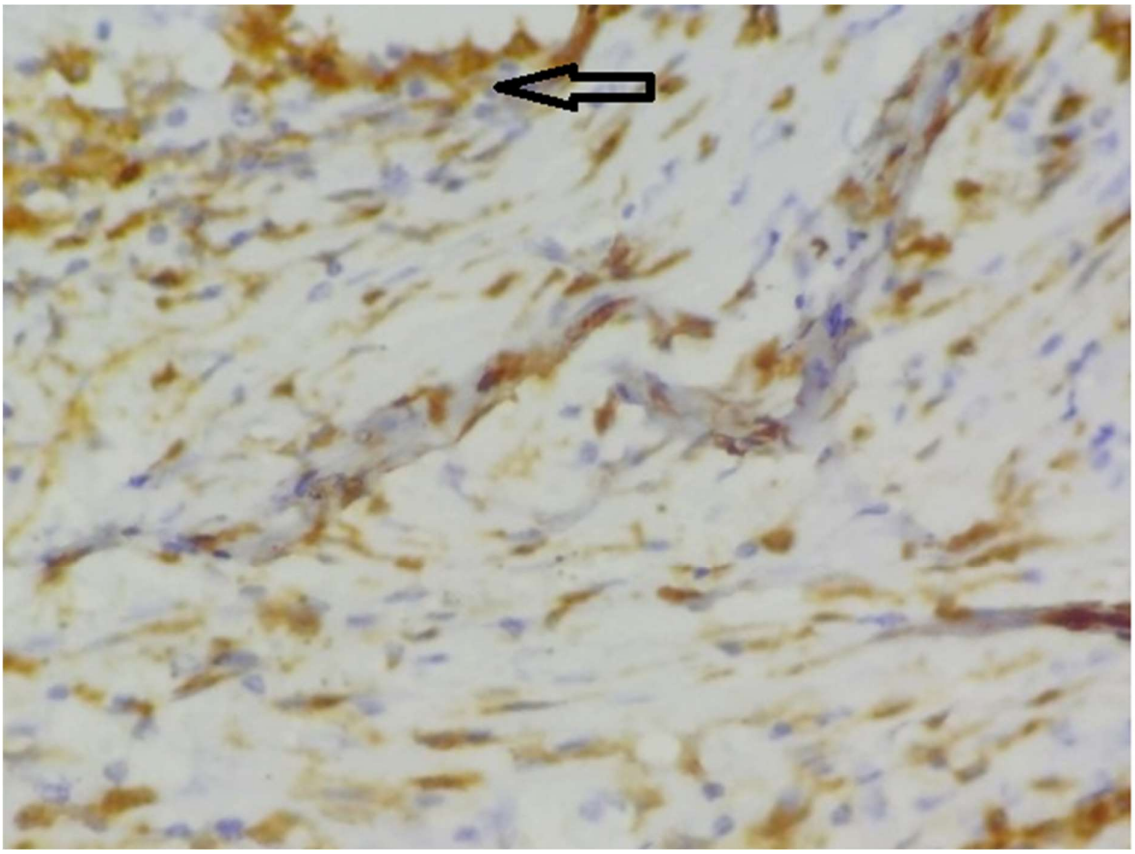


Figure 4: Photomicrograph of high CD4 positivity in invasive breast carcinoma (20x view)

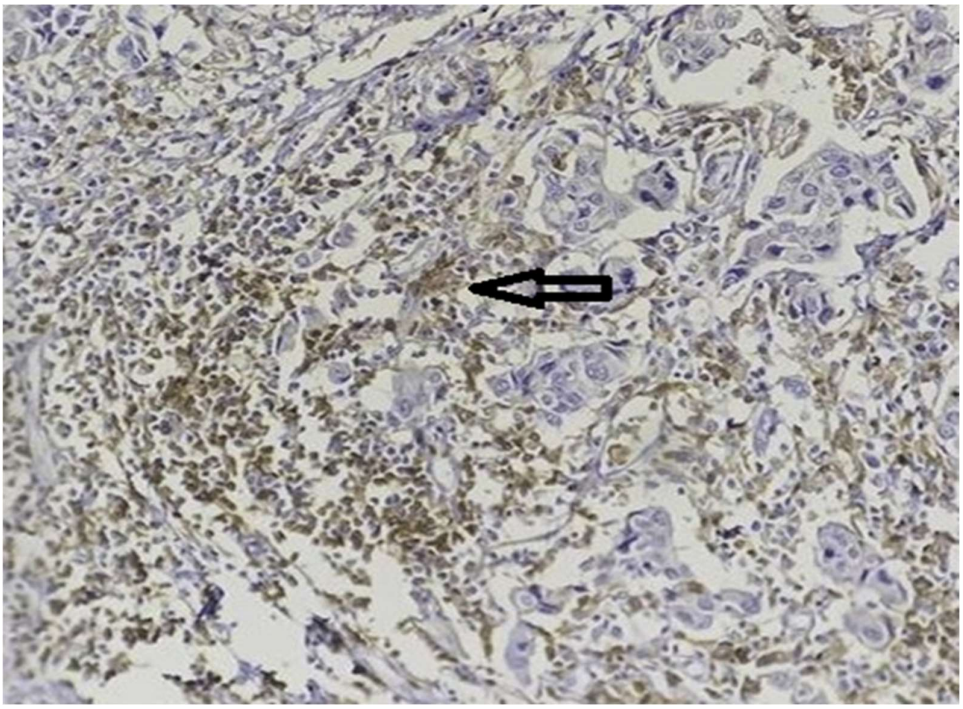


Figure 5: Photomicrograph of high CD4 positivity in invasive breast carcinoma (20x view)

RESULTS

In the present study, high CD4 stromal expression was observed in 27 cases, comprising 60% of all cases, while low stromal expression was observed in 18 cases (40%). For CD4 intratumoral expression, high levels were seen in 8 cases (17.7%), with low expression noted in 37 cases (82.3%).

The comparison of CD4 stromal expression in breast carcinoma across different age groups showed that among 38 cases in patients above 40 years, 23 cases (60.5%) exhibited high CD4 stromal expression, and 15 cases (39.5%) showed low expression. For the 7 cases in patients below 40 years, high CD4 stromal expression was observed in 4 cases (57.1%), while low expression was seen in 3 cases (42.9%). For CD4 intratumoral expression, 7 cases (18.4%) of patients above 40 years had high expression, while 31 cases (81.6%) had low expression. Among patients below 40, 1 case (14.3%) showed high intratumoral expression, while 6 cases (85.7%) had low expression. The p-values for stromal and intratumoral expression were 0.867 and 0.793, respectively, indicating no statistically significant association between CD4 expression and age.

Regarding histological grade, high CD4 stromal expression was present in 7 of 13 grade I cases (53.8%), 16 of 26 grade II cases (61.5%), and 4 of 6 grade III cases (66.7%). For CD4 intratumoral expression, high expression was found in 2 grade I cases (15.4%), 5 grade II cases (19.2%), and 1 grade III case (16.7%). The p-values for stromal and intratumoral expression were 0.843 and 0.954, showing no significant association between CD4 expression and histological grade.

Lymph node involvement was noted in 32 cases. Of these, high CD4 stromal expression was found in 20 cases (62.5%) and low expression in 12 cases (37.5%). High CD4 intratumoral expression was seen in 6 cases (18.8%), and low expression in 26 cases (81.3%). Among 13 cases without lymph node involvement, high CD4 stromal expression was observed in 7 cases (53.8%), with low expression in 6 cases (46.2%). High CD4 intratumoral expression was noted in 2 cases (15.4%), and low expression in 11 cases (84.6%). The p-values for stromal and intratumoral CD4 expression were 0.591 and 0.789, respectively, indicating no statistically significant association with lymph node involvement.

In tumor stages, for T1 cases, high and low CD4 stromal expression were each observed in 1 case (50%). Among T2 cases, high stromal expression was seen in 18 cases (58.1%), and low expression in 13 cases (41.9%). For T3 cases, 8 cases (66.7%) showed high stromal expression, and 4 cases (33.3%) showed low

expression. Regarding CD4 intratumoral expression, high expression was observed in 1 of the T1 cases (100%), 5 of the T2 cases (16.1%), and 3 of the T3 cases (25%). The p-values for stromal and intratumoral expression were 0.838 and 0.632, respectively, showing no significant association with tumor size.

In ER-positive cases, high stromal CD4 expression was observed in 14 cases (53.8%) and low expression in 12 cases (46.2%). For intratumoral expression, high CD4 was observed in 5 ER-positive cases (19.2%) and low CD4 expression in 21 cases (80.8%). In ER-negative cases, high stromal CD4 expression was observed in 13 cases (68.4%), and low expression in 6 cases (31.6%). High CD4 intratumoral expression was seen in 3 cases (15.8%) and low expression in 16 cases (84.2%). The p-values for stromal and intratumoral expression were 0.324 and 0.766, respectively, showing no significant association between CD4 expression and ER status.

In PR-positive cases, high stromal CD4 expression was noted in 13 cases (52%) and low expression in 12 cases (48%). For intratumoral CD4 expression, high levels were found in 5 PR-positive cases (19.2%) and low levels in 21 cases (80.8%). In PR-negative cases, high CD4 stromal expression was noted in 14 cases (70%), with low expression in 6 cases (30%). High CD4 intratumoral expression was observed in 3 cases (15.8%), while low expression was found in 16 cases (84.2%). The p-values for stromal and intratumoral expression were 0.221 and 0.766, respectively, indicating no statistically significant association with PR status.

In HER2-positive cases, high CD4 stromal expression was noted in 10 cases (52.6%) and low expression in 9 cases (47.4%). For intratumoral CD4 expression, high expression was observed in 5 HER2-positive cases (26.3%) and low expression in 14 cases (73.7%). In HER2-negative cases, high stromal CD4 expression was seen in 17 cases (65.4%) and low expression in 9 cases (34.6%). High intratumoral expression was observed in 3 cases (11.5%) and low expression in 23 cases (88.5%). The p-values for stromal and intratumoral expression were 0.388 and 0.20, respectively, indicating no statistically significant association with HER2 status.

DISCUSSION

CORRELATION OF CD4 MARKERS WITH VARIOUS CLINICOPATHOLOGICAL PARAMETERS

In this study, we objectively measured TILs in immunohistochemically stained sections of invasive breast carcinoma and compared them to patient outcomes. We investigated TIL marker CD4 in several locations of the tumor and its surrounding environment. Our findings revealed a broad correlation between TIL quantity and overall survival [4,5].

The scientific community has paid close attention to the characterization of tumor-associated immune infiltrates in breast carcinoma throughout the previous decade. Many studies found that TILs have prognostic relevance for breast carcinoma. A standardized and well-established approach for assessing TILs has been published. The International TIL Working Group (ITWG) has published recommendations for assessing TILs in breast carcinoma [6,7].

Their proposed method consists of visually estimating the percentage of mononuclear inflammatory cells in the stromal and intra-tumoral area in an H&E section. TILs in tumour stroma are counted and thus by using IHC markers we are able to count tumor infiltrating lymphocytes in intra-tumoral areas also [7].

This study was done to measure the impact of CD4 on the prognosis of breast carcinoma and it's correlation with other clinical parameters. The p-values for stromal and intratumoral CD4 expressions in relation to age were 0.867 and 0.793, respectively, indicating no significant association. This result aligns with findings from a study by Jafarian et al., which examined 85 cases of invasive breast carcinoma and found no significant association with age [8].

In terms of lymph node involvement, the p-values for stromal and intratumoral CD4 expressions were 0.591 and 0.789, respectively, showing no significant association. This result is similar to the study by Koenig et al., which reported no significant association between CD4 expression and lymph node involvement in 87 cases of invasive breast carcinoma [9].

Regarding histologic grade, the p-values for stromal and intratumoral CD4 expressions were 0.843 and 0.587, respectively, indicating no significant association. This finding is consistent with the study by Sun et al., who

reported no significant association between CD4 expression and grade in 25 cases of invasive breast carcinoma [10].

The p-values for stromal and intratumoral CD4 expressions in relation to tumor size were 0.838 and 0.632, respectively, indicating no significant association. This finding aligns with Helal et al., who observed no significant association between CD4 expression and tumor size in 48 cases of invasive breast carcinoma [11].

The p-values for stromal and intratumoral CD4 expressions in relation to ER status were 0.324 and 0.766, respectively, showing no significant association. Helal et al. similarly reported no significant association between CD4 expression and ER status [11]. For PR status, the p-values were again 0.324 and 0.766 for stromal and intratumoral expressions, respectively, indicating no significant association, consistent with the findings of Sun et al. The p value for stromal and intratumoral CD4 expressions in relation to HER2was 0.388 and 0.20 respectively showing no significant association, a finding similar to study done by Helal et al [10,11].

Study comprised of 45 patients, 10 were diagnosed with triple-negative breast cancer, and 7 of these cases exhibited high levels of CD4 stromal expression. Increased TILs were significantly associated with favourable outcomes in TNBC patients [12,13].

TILs produce varying degrees of effective immunity in different tumors, and the relative absence of tumor-killing cytotoxic TILs in invasive breast carcinoma may explain, in part, the negative correlation between TILs and invasive breast carcinoma biology [14].

TILs are a key component of the host's defense against the growth of solid neoplasms. It is widely believed that the recruitment and deposition of high concentrations of lymphocytes in tumor tissue serve as an essential local barrier to neoplastic propagation. It has been claimed that the presence of significant TILs in the tumor contributes to the better clinical outcomes [15].

CONCLUSION

Tumor infiltrating lymphocytes have a complex connection with many prognostic variables in invasive breast cancer. In the current work, we used immunohistochemistry with CD4 to examine the distribution of T cells inside the tumor and stroma. Greater numbers of stromal CD4 TILs were observed in triple negative breast tumours. Tumor infiltrating lymphocytes should be frequently analysed for their potential as novel prognostic and predictive indicators, especially in cases of aggressive breast cancer, such as triple negative breast cancers. More research is needed to better understand the molecular processes that link intratumoral lymphocytes with prognosis.

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