Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/0ydzr015

IMMUNOHISTOCHEMICAL STUDY ON THE ROLE OF CD8+T LYMPHOCYTES IN THE PROGNOSIS OF INFILTRATING DUCTAL CARCINOMA BREAST

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ABSTRACT

Background: Breast carcinomas are often infiltrated by inflammatory cells, particularly T lymphocytes and macrophages, but significance of these cells remain unclear. One possible role of these inflammatory cells is that they produce a cell mediated immune response against the malignant cells. CD8+T lymphocytes are a known crucial component of cell mediated immunity. Cytotoxic CD8+T lymphocytes attach to tumor cells through major histocompatibility complex class-I on their surface. This leads to production of interferon gamma after interaction with tumor target and produces apoptosis, cell cycle inhibition, angiostasis and induction of macrophages which suppress the tumor growth.The accepted parameters for predicting the prognosis of breast carcinoma are grading and staging of tumor.

Objectives: To study the correlation between the CD8+ T lymphocyte density and histological grading of breast carcinoma. It also aims to compare the frequency of CD8+ T lymphocytes in breast carcinoma and benign proliferative breast lesions.

Method: Tissue blocks from 40 cases diagnosed as Invasive breast carcinoma NST(No special type) along with 20 benign proliferative breast lesions were included in the study. Histological grading was done based on Modified Bloom and Richardson grading system for invasive ductal carcinoma. All the 60 cases were immunostained with CD8 antibody and density of CD8+ T lymphocytes were semi-quantitatively graded according to Kreik et al[11].

Conclusion: Among the 40 cases of Invasive breast carcinoma NST, 90% cases showed CD8+T lymphocytes. 60% cases have moderate, 17.5% have minimal and 12.5% cases have extensive lymphocytic infiltrate. In the benign lesions, 50% cases showed the presence of CD8+T lymphocytic infiltrate, in which 30% showed minimal infiltrate and 20% showed moderate infiltrate. The densities of CD8+T lymphocytes were high in higher histological grades of the tumor. On comparing the density of lymphocytes with the stage of tumor, extensive infiltrates were noted in early stages of tumor and the distribution of these infiltrate were in peritumoral pattern.

Keywords*:* Breast carcinoma, CD8+T lymphocytes, immunosurviellance.

INTRODUCTION

Invasive breast carcinoma NST is a group of malignant epithelial tumors characterized by invasion of adjacent tissues and a marked tendency to metastasize to distant sites.[1]Breast cancer is the leading cause of cancer incidence and mortality in India, accounting for 13.5% of new cancer cases and 10.6% of all cancer death.[2]Majority of the breast carcinomas are detected during the reproductive age.[3]TNM staging and histologic grading which is Nottingham Modification of the

Bloom Richardson scale has become widely accepted as a powerful indicator of prognosis of the tumor.[4]Immunity in older people tends to be less effective than in young and are highly susceptible to infection and cancer. This is known as 'Immunosenescence.[5] The immune system not only suppress tumor growth by destroying cancer cells or inhibiting their outgrowth but also promotes tumor progression either by selecting tumor cells that are more fit to survive in an immunocompetent host or by establishing conditions within the tumor micro- environment that facilitate tumor outgrowth. Evading immune destruction is an emerging hallmark of cancer.[6] At the site of immune attack, the CD8+ T cells release cytokines to mediate local inflammation[7]and deposit of cytotoxic granules at the vicinity of target-cell membranes to induce target-cell apoptosis.[8-10]

OBJECTIVES

1) To study the correlation between the CD8+ T lymphocyte density and histological grading of breast carcinoma.
2) To compare the frequency of CD8+ T lymphocytes in breast carcinoma and benign proliferative breast lesions.

MATERIALS AND METHODS

A retrospective study was conducted for a period of 3 years at a tertiary care hospital in Kerala.Representative paraffin blocks and hematoxylin and eosin stained slides of patients who were diagnosed as Invasive breast carcinoma NST and underwent Modified Radical Mastectomy and lumpectomy were collected from the Department of Pathology. The files of the patients were reviewed and patient's age, clinical history and examination findings were collected.In addition, cases of benign proliferative lesions of breast were collected to compare the distribution of CD8+ T lymphocytes in benign and malignant breast lesions. Patients with history of pre-operative chemotherapy, radiotherapy and hormonal therapy and other histological types were excluded. The H & E stained sections were examined for

1. Histological grading of tumors according to Nottingham Modification of Bloom Richardson criteria and classified as grade-I (well-differentiated), grade-II (moderately differentiated) and grade-III (poorly differentiated).
2. The density and pattern of lymphocytic infiltrate in benign and malignant breast lesions.
3. Assessment of nodal status for staging of tumor.

Immunohistochemistry was done using PathnSitu PolyExel clone 144B mouse CD8 antibody according to the standard protocol. The density of CD8+ T lymphocytes in the immunostained slides are semi-quantitatively graded according to Kreike *et al*[11](Table.1).Lymph nodes were taken as positive control for CD8+ T lymphocytes.

• None = No lymphocytes • Minimal = Scattered lymphocytes [<10 lymphocytes per high power field (40x)]. • Moderate = Lymphocytes easily identified, but no large aggregates. • Extensive = Large aggregates of lymphocytes in >50% of the tumor
Table 1. Semi-quantitative grading of lymphocytic infiltrate by Kreik et al[11]

RESULTS

The study sample is constituted of 60 cases in which 40 are diagnosed cases of Invasive breast carcinoma NST and 20 are benign proliferative breast diseases fulfilling the inclusion criteria. The hematoxylin-eosin stained slides were reviewed and graded according to Nottingham Modification of Bloom - Richardson system.

Histological Grading

When the Invasive ductal carcinomas were graded according to Nottingham Modification of Bloom Richardson system, 60% of cases were histological grade II tumors followed by 22.5% cases being grade I tumors and 17.5% cases being grade III tumors.

Benign Breast Lesions

Among the 20 benign proliferative breast diseases, 85% cases were fibroadenoma and 15% cases were fibrocystic disease of the breast.

Tumor Infiltrating Lymphocytes

The density of CD8+ T lymphocytes was significantly higher in Invasive breast carcinoma NST than benign proliferative breast lesions. In Invasive breast carcinoma NST, 60% of the cases showed moderate amount of CD8+ T lymphocytic infiltrate followed by 17.5% cases with minimal infiltrate. 12.5% of cases showed extensive and only 10% cases showed none or absent infiltrate. Among the benign proliferative breast lesions 50% of cases showed none or absent CD8+T lymphocytic infiltrate followed by 30% cases showing minimal and 20% cases with moderate infiltrate.

Grading of lymphocytic infiltrate according to Kreik et al[11]	**No .of Cases of invasive breast carcinoma NST (%)**	**No .of. Cases of benign proliferative lesions of breast (%)**
None	4(10%)	10(50%)
Minimal	7(17.5%)	6(30%)
Moderate	24(60%)	4(20%)
Extensive	5(12.5%)	0(0%)
TOTAL	40(100%)	20(100%)

Table 2. Semiquantitative assessment of density of CD 8+ lymphocytes in Invasive carcinoma breast NST

Tumor infiltrating lymphocytes in different tumor grades

In histological grade I tumors, 22.2% of cases showed none or absent CD8+ T lymphocytes and none of the cases showed extensive infiltrate. 66% of grade I tumors showed moderate infiltrate and 11.1% showed minimal infiltrate. .

62.5% cases of grade II tumors showed moderate amount of CD8+T lymphocytic infiltrate followed by 25% cases showing minimal, 8.3% cases showing extensive and 4.2% cases showing none or absent CD8+ T lymphocytic infiltrate.

In histological grade III tumors 42.8% cases showed extensive and moderate amount of CD8+T lymphocytic infiltrate. Only 14.2% cases of grade III tumors showed none or absent lymphocytic infiltrate.

Bloom Richardson grading	**Density of CD 8 + T lymphocytes**					**Statistical significance**
	None	**Minimal**	**Moderate**	**Extensive**	**TOTAL**	
Grade I	2 (22.2%)	1 (11.1%)	6 (66.6%)	0 (0%)	9	
Grade II	1 (4.2%)	6 (25%)	15 (62.5%)	2 (8.3%)	24	p value – 0.04 (significant)
Grade III	1 (14.2%)	0 (0%)	3 (42.8%)	3 (42.8%)	7	
Total	4	7	24	5	40	

Table 3. Association between Bloom Richardson grading and density of CD 8+ T lymphocytes

Tumor infiltrating lymphocytes and tumor staging

In the 40 cases of Invasive breast carcinoma NST, 33 were Modified radical mastectomy specimens and 7 were lumpectomy specimens. The 33 cases were staged according to TNM. On comparing the stages of the tumor with the density of CD8+ T lymphocytes, as the stage progressed the density of lymphocytes reduced.

In the 33 cases which were staged according to TNM, there were total of 6 cases in stage I, 16 cases in stage II and 11 cases in stage III tumor. 50% of stage I tumors showed extensive and 50% showed moderate CD 8+ T lymphocytic infiltrate. In stage II tumors 62.5% cases show moderate infiltrate followed by 18.7% showed minimal, 12.5% showed none and 6.25% showed marked CD8+ T lymphocytes. Among the stage III tumors, 45.5% showed moderate, 36.3% showed minimal and 18.1% showed none or absent CD8+ T lymphocytic infiltrate. The P value is significant with a value of 0.04.

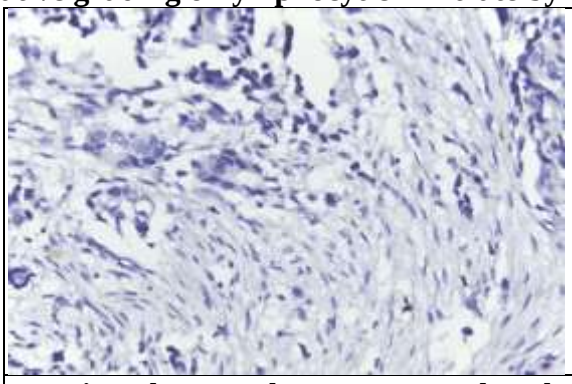
Stage of tumor	**Density of CD8+ T lymphocytes**					
	None	**Minimal**	**Moderate**	**Extensive**	**Total**	**Statistics**
Stage I	0	0	3(50%)	3(50%)	6	P value – 0.04
Stage II	2(12.5%)	3(18.7%)	10(62.5%)	1(6.25%)	16	
Stage III	2(18.1%)	4(36.3%)	5(45.5%)	0	11	
Total	4	7	18	4	33	
Table 4. Relationship between density of CD8+ T lymphocytes and stage of tumor						
P value – 0.04(significant)						

Distribution pattern of CD8+ T lymphocytes

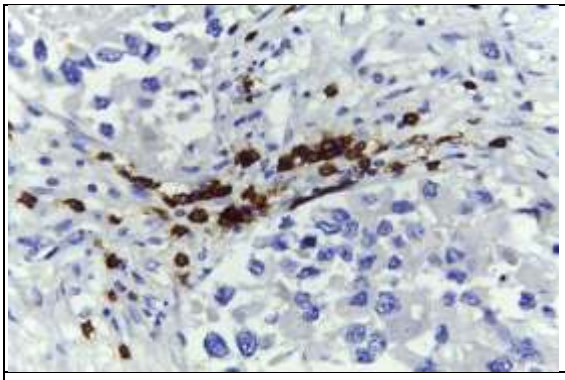
In 40 cases of Invasive breast carcinoma NST, 35 cases showed the presence of CD8+ T lymphocytes and 5 cases showed none or absent CD8+ T lymphocytes. Among the 35 cases 62.8% cases showed intratumoral infiltrate and 34.2% showed peritumoral infiltrate. 4 cases show extensive infiltrate and 3 out of them have peritumoral pattern of arrangement.

CD8+T lymphocyte Density grading	**Patterns of distribution of CD8+ T lymphocytes**			
	Peritumoral	**Intratumoral**	**Both**	**Total**
None	0	0	0	0
Minimal	0	7	0	7
Moderate	9	15	0	24
Extensive	3	0	1	4
Total	12(34.2%)	22(62.8%)	1(2.8%)	35(100%)
Table 5. Patterns of CD8+ T lymphocyte distribution in the tumor tissue				

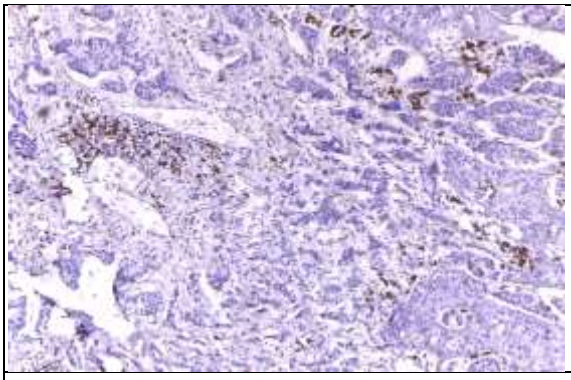
I. Semi-quantitative grading of lymphocytic infiltrate by Kreik et al[11]



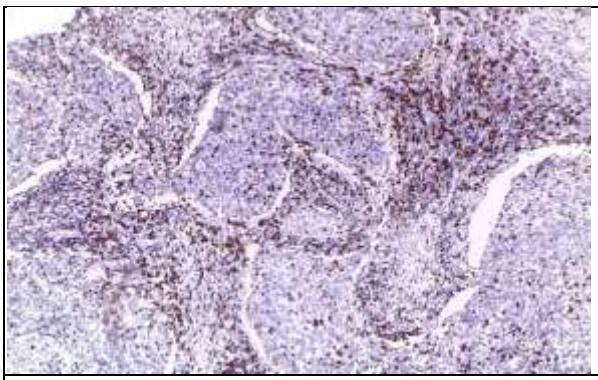
Microphotograph 1. None = No lymphocytes present (10X)



Microphotograph 2. Minimal - Scattered lymphocytes [<10 lymphocytes per high power field (40X)]



Microphotograph 3. Moderate – Lymphocytes easily identified, but no large aggregates. (10X)



Microphotograph 4. Extensive – Large aggregates of lymphocytes in >50% of the tumor (10X)

DISCUSSION

The prognostic significance of tumor infiltrating lymphocytes has been a longstanding topic of debate and many studies have evidenced that the immune system participates both in the tumor development through chronic inflammation and tumor elimination and control via the action of the adaptive immune system. The analysis of tumor infiltrating lymphocyte density in the tumor microenvironment helps to estimate the immune balance and prognosis of the disease. Many studies have been conducted on colonic and gastric carcinomas in demonstrating the effect of tumor infiltrating lymphocytes in the progress of the tumor. There are only few studies demonstrating the correlation between the density of CD8+ T lymphocytes and the different prognostic parameters of invasive carcinoma breast NST.

Density of CD8+ T lymphocytic infiltrate

In the present study, out of 40 cases of Invasive breast carcinoma NST, 90% of cases showed presence of CD8+ T lymphocytes, in which 60% cases with moderate infiltrate, 17.5% with minimal infiltrate and 12.5% with extensive lymphocytic infiltrate. 10% of cases show absence of CD 8+ T lymphocytes. In the study conducted by Helal TE et al[12] on 48 cases of invasive carcinoma NST, 56.2% of cases showed the presence of intermediate or moderate amount of CD8+ T lymphocytes and 43.8% cases showed absence of the lymphocytes. None of the cases showed extensive lymphocytic infiltrate. According to A.S. Rathore et al[16] in his study, 95% of invasive breast carcinoma NST cases showed CD8+ T lymphocytes. Among the 95% cases, 64% cases showed extensive and moderate amount and 36% of cases showed low or minimal amount of lymphocytic infiltrate.

In benign proliferative lesions of breast, our study showed absence of CD8+ T lymphocyte in 50% of cases and 30% cases showed minimal and 20% cases showed moderate grades of density. In a study conducted by Helal TE et al[12] 20% cases of benign breast lesions showed CD8+ T lymphocytic infiltrate. Sood et al[13] and Ben Hur et al[14] also noted high densities of CD8+ T lymphocytes in invasive breast carcinoma NST compared to the benign proliferative breast lesions. These observations probably indicate that cytotoxic T lymphocytes are emerging hallmark of carcinoma and they produce interferon gamma through interaction with tumor related antigen and potentiate it for the tumoricidal activity by induction of apoptosis. Certain tumor specific antigens like p53 and beta-actin are common targets of cytotoxic T lymphocyte and can induce immunological reaction.

Association with Bloom Richardson Grade

In the present study, on comparing the histological Modified Bloom Richardson grading of the tumor with the density of CD8+ T lymphocyte, 22% of the grade I tumors showed absent infiltrate, 11% minimal, 66.6% moderate and none of them showed extensive infiltrate. In grade II tumors, 4.2% showed absent infiltrate, 25% minimal, 62.5% moderate and 8.3% showed extensive infiltrate. Among the grade III tumors, 14.2% showed absent infiltrate, 42.8% with moderate and 42.8% with extensive infiltrate. Since 42% of grade III tumors showed extensive CD8+ T lymphocytic infiltrate and none of the grade I tumors showed extensive infiltrate, our study was showing a direct correlation between the tumor grade and the CD8+ T lymphocytic infiltrate.

Our results are in concordance with the studies conducted by Helal TE et al[12], Mahmoud SM et al[15] and A.S. Rathore et al.[16] In the study by Helal TE et al[12], 50% of grade II tumors showed absent and 50% showed intermediate CD8+ T lymphocytic infiltrate. In grade III tumors 41.6% showed absent and 58.4% showed intermediate infiltrate. None of the cases had extensive infiltrate but in the intermediate group 22.2% cases were grade II and 77.7% were grade III. This indicates direct correlation of grade with density of lymphocyte.

According to the study by Mahmoud SM et al[15], 72% grade I, 80% grade II and 81% grade III tumors showed CD8+ T lymphocytic infiltrate, which also evidenced direct correlation between tumor grade and lymphocyte density. This phenomenon was explained by ability of T lymphocytes to produce vascular endothelial growth factor. In high histological grades the tumor is highly vascular. The vascularity is increased by the neoangiogenesis induced by vascular endothelial growth factor (VGEF) and proliferating fibroblasts that helps tumor growth and vascular and lymphatic metastasis. According to a study conducted by Mor F et al[17], angiogenesis and inflammation are interconnected. When there is neovascularisation there is increase in blood supply to that site which will further recruit more number of inflammatory cells to the site. VEGF plays a critical role in angiogenesis. Inflammation at tumor site is a process of tumor cell destruction as well as a healing process. The role of T lymphocytes includes molecular signaling that can enhance tissue healing and also tumor cell distruction. The homing T cell at the site of inflammation provides a source for VEGF and it is enhanced by the degree of T cell activation. Hypoxia associated with

tissue damage leads to production angiogenic factors including VEGF at the site of damage that would convert T cells into a proinflammatoryTH1 cell and further amplify inflammatory process.

Association with stage of tumor

In stage I tumors 50% of cases show moderate and 50% cases show extensive infiltrate. Regarding the stage II tumors, 62.5% of cases show moderate, 18.7% show minimal, 12.5% show absent or none and 6.25% show extensive infiltrate. In stage III, 45% cases show moderate, 36.3% cases show minimal and 18.1% cases show none or absent CD8+ T lymphocytic infiltrate. None of the stage III cases show extensive infiltrate. Hence the stage of the tumor showed positive correlation with the density of CD8+ T lymphocytes. In a study conducted by Gu X et al[18] he noted that the concentration of IFN-gamma produced by the CD8+T lymphocytes in late stages tumor was remarkably lower than the early stage of the tumor, which indicate that density of CD8+ T lymphocyte density was low in late stages compared to the early stage.Another study was undertaken by Gisterek I et al[19] where he found that high density of CD8+ T lymphocytic infiltration was associated with less number of nodal metastasis which corresponds to early stage of the tumor. Both these studies have similar results as that of our study, that is high density of CD8+ T lymphocytes are associated with early stage of tumor and hence it can be used as a parameter for assessing the stage or indirectly the prognosis of the patient.

Pattern of distribution

In the present study two different patterns were noted in the distribution of CD8+ T lymphocytes. One was peritumoral and the other intratumoral. 61.1% of the cases showed intratumoral infiltrate and 36% showed peritumoral infiltrate. 1n the 4 cases which showed extensive infiltrate by CD8+ T lymphocytes, 3 of them had peritumoral distribution pattern. According to the study conducted by Mohamoud SM et al[15], 77% of cases showed peritumoral pattern of distribution.

According to B.Melichar et al[20], an increase in both intratumoral and peritumoral lymphocytes showed increase in survival rate and disease free survival even in ER negative and HER-2 negative cases. S.Lui et al[21] in his study noted that CD8+ lymphocytes had peritumoral pattern of arrangement and found that this pattern was seen in healthy young patients.

In our study, majority of cases showed intratumoral pattern of arrangement. But the cases with extensive CD8+ T lymphocytic infiltration were stage I tumors and showed peritumoral pattern of arrangement. Hence the peritumoral pattern may be considered as a parameter for better immune response against the tumor.

CONCLUSION

This study comprise of 60 cases in which 40 cases are Invasive breast carcinoma NST and 20 are benign proliferative breast diseases diagnosed in the Department of Pathology at a tertiary care hospital in Kerala for a period of 3 years. This study was done to find the correlation between density of CD8+ T lymphocytes in invasive carcinoma breast and benign proliferative lesions of breast, and its significance in prognosis of the patient.

The following conclusions were derived from the study –

- Density of CD8+T lymphocytes was significantly high in Invasive breast carcinoma compared to the benign proliferative lesions of breast which indicate that the there is strong antitumoral response produced by the CD8+ T lymphocytes against the neoplastic cells.
- The density of CD8+T lymphocytes was high in higher Bloom Richardson histologic grades of the tumor. This may be due to the neoangiogenesis and high vascularity in higher grades of tumor.
- Early stages of tumor showed extensive CD8+ T lymphocytic infiltrate compared to late stages.
- Peritumoral pattern of distribution of CD8+T lymphocytes were seen in stages I tumors compared to the late stages.
- The above findings can be considered as an attempt by the immune cells to contain the tumor.

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