ORIGINAL RESEARCH

Evaluation of tumor infiltrating lymphocytes (TILs) with hormonal status in breast cancer

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ABSTRACT

Background: Tumour infiltrating lymphocytes (TILs) play a major role in many solid tumours. Expression of Oestrogen Receptor (ER), Progesterone Receptor (PR) and Human Epidermal Growth factor Receptor 2 (HER2) in breast cancer plays strong association with treatment outcome. Treatment options for Triple negative breast cancer (TNBC) is limited. Recent days, Tumour infiltrating lymphocytes (TILs) play a major role in Triple negative breast cancer (TNBC). **Methods:** 100 cases of Modified radical mastectomy specimen of breast cancer were chosen from surgical pathology records .formalin fixed paraffin embedded tissue samples. Patient's age, sex, Grade and status of Estrogen Receptor, Progesterone Receptor, and HER2 were assessed and evaluated for Tumor Infiltrating Lymphocytes with Immunohistochemical stains CD45 and CD3. **Results:** In this study, 31 % cases Showed 50-90 % Stromal TILs 51% of cases showed 20-40% TILs and rest 16 patients had less than 10% TILs.In our study coming to hormonal status 13% showed ER+ /PR-, 22% showed ER+ /PR+ ,18% showed HER2NEU positive , 29% showed triple negative and 18% showed triple positive. Triple negative breast cancer and HER2 positivetumours showed more number of stromal TILs.Stromal Tumor Infiltrating Lymphocytes have strong association with triple negative breast cancer. **Conclusion:** Tumour-infiltrating lymphocytes are white blood cells that have left the bloodstream and migrated into a tumour. They are mononuclear immune cells, a mix of different types of cells (i.e., T cells, B cells, NK cells, macrophages) in variable proportions, T cells being the most abundant cells. They can often be found in the stroma and within the tumour itself. Tumour infiltrating lymphocytes emerging as host antitumor immune response in triple-negative breast cancer.Among the ER, PR, HER2neu status, most of the TILs were expressed in triple negative tumors in this study.

Keywords: Breast cancer, Tumor Infiltrating Lymphocytes, Hormonal status

INTRODUCTION

Breast cancer is the most common malignant neoplasm and also the most common cause for cancer deaths in females with more than 1 million cases being newly diagnosed annually[1]. Triple receptor positive tumours with expression of ER, PR & HER2neu are higher grade tumours with increased proliferating potential and frequent metastasis to lymph nodes is seen.These tumours respond well to chemotherapy. Triple negative breast tumours are high grade tumours with increased proliferating potential and aggressive clinical behaviour. [2,3]

Cancer cell causing alteration in the immune response leads to death of transforming tumour cells. However,escape from immune system occurs when there is an incomplete elimination of transformed cell. Intact immune system prevent the transforming cells from developing cancer with immune responses[4]. Infiltration of immune cells, particularly infiltration of anti-tumour type 1 lymphocytes, has predicted improved prognosis in many different tumour types including colon, ovarian, lung and breast cancer. Historically breast cancer was not thought to be immunologically active, particularly when compared to tumours such as melanoma. However recent evidence has emerged that tumour infiltrating lymphocytes (TILs) present in breast cancer prior to

treatment can predict response to therapy and improved prognosis.

Not only does the amount of lymphocytic infiltration but also the phenotype of that infiltrate determine clinical outcome. Type 1 T-cells are associated with favourable prognosis. $CD4^+$ T-helper 1 (Th1) cells facilitate antigen presentation through cytokine secretion and activation of antigen presenting cells. $CD8^+$ cytotoxic T-cells (CTL) are essential for tumour destruction. On the other hand, type 2 $CD4^+$ T-helper cells (Th2), including Forkhead box P3 (FOXP3) $CD4^+$ regulatory T-cells, inhibit CTL function, support proliferation of B-lymphocytes, and may promote an anti-inflammatory immune response that could enhance tumour growth.

Most antigens present in breast cancer are self-proteins that stimulate T cells and induce a regulatory immune response[5]. The interaction of the immune system with tumour cells in breast cancer appears to be associated with triple negative breast cancer (TNBC) and HER2-positive breast cancer, and they are thought to be more immunogenic[5].

According to Denkertet al[6], tumour infiltrating lymphocytes to be counted from only the stromal areas and areas occupied by carcinoma cells is not taken into the count. Percentage of TILs is stromal area and not the stromal nuclei.An International TILs Group 2014 has recommended to classify TIL percentage into 0-10%, 20-40% and 50-90%.Based on this aim of our study is to correlate ER, PR, and HER2 status of the tumour with percentage of Tumour Infiltrating Lymphocytes present in the stroma.

MATERIALS AND METHODS

This is a descriptive retrospective study of Primary breast carcinomas conducted in the tertiary care hospital. Cases diagnosed as primary carcinoma breast in mastectomy specimens was included and benign tumours,benign and malignant phyllodes, non-neoplastic lesions of breast and small biopsies were excluded. 100 cases of Invasive breast carcinoma with ER, PR and HER2 status was studied. 93 cases from Invasive ductal carcinoma NOS and 07 cases from special types such as metaplastic, mucinous, medullary, IDC- NST with papillary features were randomly selected and their representative formalin fixed paraffin embedded tissue samples were subjected for analysis of Tumor Infiltrating Lymphocytes with H&E slides and with CD 45 And CD 3 expression using Rabbit monoclonal and mouse polyclonalantibody respectively. Slides were evaluated for percentage of tumor infiltrating lymphocytes.

Immunohistochemistry

ER, progesterone receptor (PR) and HER2 status was confirmed by pathology reports. Briefly, buffered formalin-fixed paraffin-embedded surgical specimens were cut into 4-µm sections and prepared for immunohistochemistry for ER, PR and HER2. Immunostaining was performed using antibodies against ER (clone 1D5); PR (clone PgR636); and HER2 (clone cerbB-2). ER- and PR-positive status was determined by a nuclear staining rate ≥1%. HER2 immunostaining expression was scored as 0, 1+, 2+ and 3+, stratified according to staining intensity of the cell membrane. In cases with a HER2 score of 2+, a fluorescence *in situ* hybridization (FISH) assay was also performed. HER2-positive status was determined as a HER2 score 3+ or HER2 score 2+ plus FISH-positive assay.

RESULTS

Tumor infiltrating lymphocytes were assessed initially and compared with the hormonal status of breast cancer. The following results were obtained.In this study, 31 % cases Showed 50-90 % Stromal TILs 51% of cases showed 20-40% TILs and rest 16 patients had less than 10% TILs.

Table 1: Distribution of TILs in Breast Cancer

TILs %	No. of patients	%
0-10	16	16.0%
20-40	53	53.0%
50-90	31	31.0%
Total	100	100.0%

In our study coming to hormonal status we evaluated all important receptors pertaining to cancer breast – oestrogen receptor, progesterone receptor and HER2NEU receptor, 13% showed ER+ /PR-, 22%showed ER+ /PR+ ,18% showed HER2NEU positive , 29% showed triple negative and 18% showed triple positive

Table 2: Hormonal Status

Receptors	No. of patients	%
ER+/PR-	13	13%
ER+PR+	22	22%
HER2NEU2+	18	18%
Triple Negative	29	29%
Triple Positive	18	18%

Figure 1: CD3 Expression in 50-90% of Stromal Cells and in 20-40% of Stromal Cells (100x)

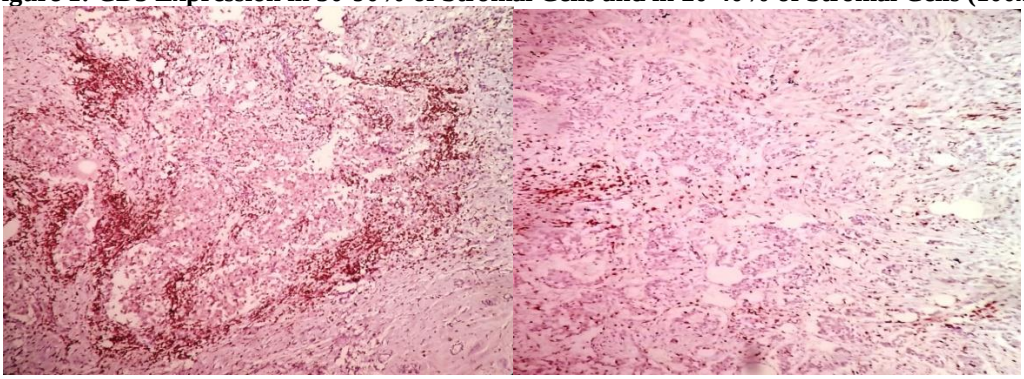

Table 3: Correlation of TILs with ER/PR/HER2neu breast tumors

Hormonal status			TILs			Total
			0-10	20-40	50-90	
	ER+ /PR-	Count	2	6	5	13
		% within TILs	12.5%	11.3%	16.1%	13.0%
	ER+/PR+	Count	4	14	4	22
		% within TILs	25.0%	26.4%	12.9%	22.0%
	HER2NEU 2+	Count	4	10	4	18
		% within TILs	25.0%	18.9%	12.9%	18.0%
	TRIPLE NEGATIVE	Count	4	15	10	29
		% withinTILs	25.0%	28.3%	32.3%	29.0%
	TRIPLE POSITIVE	Count	2	8	8	18
		% within TILs	12.5%	15.1%	25.8%	18.0%
Total		Count	16	53	31	100
		% within TILs	100.0%	100.0%	100.0%	100.0%

There is strong association with triple negative and ER+/PR+ tumours with tumour infiltrating lymphocytes in this study.

DISCUSSION

Breast carcinoma is the most common cancer in the women in urban areas and the second most common cancer in the rural areas.

Carcinoma breast is a heterogeneous disease both clinically and pathologically. Mortality of breast cancer can be reduced by early detection, appropriate management and targeted therapies. Apart from the prognostic markers like stage,grade, lymphnode status, ER, PR, HER2, there are many new theories studied for prognosis. One such attempt was tumor infiltrating lymphocytes.

Therefore evaluation of TILs might provide information regarding the biological profile and may help in evaluation of patient's immune response to the tumor.

In the present study, tumor infiltrating lymphocytes in the breast cancer was assessed by counting stromal TILs microscopically and IHC evaluation done by using CD45 and CD3 and an attempt was made to correlate the TILs with ER,PR, HER2 status.

Molecular study done in this study showed 13% of ER+ /PR- cases, 22% of ER+ /PR+ cases, 18% of HER2 neu positive cases , 29% of triple negative cases and 18% of triple positive cases.

Several retrospective studies have suggested the potential use of TIL count as a prognostic factor in ER-negative breast cancer. Moreover, Denkert *et al*[6] confirmed that a high TIL grade was a powerful prognostic factor in a large number of patients with early-stage triple-negative breast cancer. In our previous study, ~30% of ER-negative patients had a high TIL count, which was a powerful prognostic marker.Previous studies suggested that ER potentially activated tumour immunosuppression. However, for the treatment of ER-positive breast cancer, the relationship between TIL count and response to endocrine therapy remains unclear. Tumour mutational burden is an important factor associated with tumour immunity and response to immune therapy

Many studies showed that Triple negative tumours and HER2neu positive tumours have increase in stromal TILs both in and around the tumour. Sasha E. Stanton et al showed that there is incremental increase in and around the tumour in triple negative and HER2neu positive tumors.[8]. According to H. Othani et al, if there is decrease in tumourinfiltrating lymphocytes in triple negative breast cancer and HER2neu positive tumours is considered as high grade.[9].

To confirm the potential role of TIL count in breast cancer prognosis, the characteristics of immune cells constituting TILs will have to be examined in more detail. Cytotoxic T cells, which attack cancer cells, are positive for CD8, and T-reg cells, which inhibit

immune responses to cancer cells, are positive for CD4, CD25 and FOXP3. How TIL count impacts patient response to treatment should also be determined, as CD8-positive T lymphocytes are associated with the therapeutic efficacy of chemotherapy.

CONCLUSION

Tumour-infiltrating lymphocytes are white blood cells that have left the bloodstream and migrated into a tumour. They are mononuclear immune cells, a mix of different types of cells (i.e., T cells, B cells, NK cells, macrophages) in variable proportions, T cells being the most abundant cells. They can often be found in the stroma and within the tumour itself. Tumour infiltrating lymphocytes emerging as host antitumor immune response in triple-negative breast cancer.Among the ER, PR, HER2neu status, most of the TILs were expressed in triple negative tumors in this study.

REFERENCES

1. Parkin DM, Bray F, Ferlay J, PisaniP.Estimating the world cancer burden.Globocan 2000. Int J Cancer 2001, 94:153–156.
2. Constantinidou A, Jones RL, Reis-Filho JS. Beyond triple-negative breast cancer the need to define new subtypes. Expert Rev Anticancer Ther 2010, 10: 1197–1213
3. Schnitt SJ. Will molecular classification replace traditional breast pathology? Int J SurgPathol 2010, 18: 162S–166S
4. Mittal D, Gubin MM, Schreiber RD, Smyth MJ. New insights into cancer immunoediting and its three component phases–elimination, equilibrium and escape. CurrOpinImmunol. 2014; 27:16–25.
5. Disis ML, Stanton SE. Triple-Negative Breast Cancer: Immune Modulation as the New Treatment Paradigm. 2015 ASCO Educational Book. Asco.org/edbook.
6. Denkert C, Loibl S, Noske A et al. Tumor-associated lymphocytes as an independent predictor of response to neoadjuvant chemotherapy in breast cancer. J ClinOncol 2010; 28: 105–113.
7. Stanton and Disis Clinical significance of tumor-infiltrating lymphocytes in breast cancer,Journal for ImmunoTherapy of Cancer (2016) 4:59 DOI 10.1186/s40425-016-0165-6
8. Salgado et al The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: recommendations by an International TILsWorking Group 2014
9. H. Ohtani et al. defining lymphocyte-predominant breast cancer by the proportion of lymphocyte-rich stroma and its significance in routine histopathological diagnosis. Pathology International 2015