Journal of Pathology of Nepal (2022) Vol. **12**, 1909 - 1912

Journal of
PATHOLOGY
of Nepal

www.acpnepal.com

Original Article

Evaluation of tumor infiltrating lymphocytes in breast carcinomas

Shovana Karki[1], Sansar Babu Tiwari[1], Alina Basnet[1]

[1]*Department of Pathology, Institute of Medicine, Tribhuvan University Teaching Hospital, Kathmandu, Nepal*

Keywords:

Breast cancers; Lymphocyte predominant breast cancers; Tumor-infiltrating lymphocytes

ABSTRACT

Background: Among the parameters that have a prognostic and/or predictive significance to therapeutic response, in the case of breast carcinoma, the study of tumor-infiltrating lymphocytes in breast carcinomas is emerging. The present study aimed to access the clinicopathological profiles of tumor-infiltrating lymphocytes in patients with primary breast carcinoma.

Materials and Methods: Information was collected from the archive of the Department of Pathology, about invasive breast cancers diagnosed between April 2019 –March 2020. Hormone receptor status and Ki-67 index were assessed. For tumor-infiltrating lymphocytes, hematoxylin and eosin sections were evaluated following the guidelines of the "International Working Group for tumor-infiltrating lymphocytes in Breast Cancer—2014".

Results: High tumor-infiltrating lymphocytes were seen in 64.2% and 43.8% in ages < 50 and > 50 respectively. All cases of invasive lobular carcinoma had low tumor-infiltrating lymphocytes. 62.5% of T1 tumors were associated with low TILs whereas 80% of low tumor-infiltrating lymphocytes were T3. 71.4% of lymph node-positive tumors had low tumor-infiltrating lymphocytes. All breast cancers showed lymphocytic infiltrate. 40% of the tumors showed intermediate to high tumor-infiltrating lymphocytes. Lymphocyte-predominant breast cancers were 30%. Among the LPBC, 55.5% were triple-negative breast cancers, 33.3% were HR+, and 11.2% were HER2 positive.

Conclusions: Low tumor-infiltrating lymphocytes presented at an advanced stage of the disease at the time of diagnosis. tumor-infiltrating lymphocytes in breasts decreased with increasing age. LPBCs were predominantly TNBCs. All breast cancers were immunogenic, stating the necessity to report tumor-infiltrating lymphocytes in all breast carcinomas.

Correspondence:

Dr. Shovana Karki, MD

Department of Pathology, Institute of Medicine, Tribhuvan University Teaching Hospital, Kathmandu, Nepal

ORCID ID: 0000-0001-5429-2422

Email: shovana_karki@hotmail.com

Received : June 24, 2022 ; **Accepted :** September 18, 2022

Citation: Karki S, Tiwari SB, Basnet A. Evaluation of tumor-infiltrating lymphocytes in breast carcinomas. J Pathol Nep 2022;12(2):1909-12. DOI: 10.3126/jpn.v12i2.47200

INTRODUCTION

Breast cancer (BC) is the most frequent form of malignancy in females worldwide.[1] BC represents a spectrum of neoplasm characterized by differences in terms of its clinical behavior, prognosis, and treatment response.[1] Studies have been carried out in recent years to identify morphological or molecular parameters that are related to the patient's outcome. Among the parameters that have a prognostic and/or predictive significance to therapeutic response, the study of tumor-infiltrating lymphocytes (TILs) in breast carcinomas is emerging.

The presence of TILs, within the tumor and/or in the peritumoral sites, is considered an important immunological biomarker that reflects the antitumor immune response in BC, as in other malignancies which include ovarian and endometrial carcinomas.[2] Elevated TIL levels have been associated with better response to neoadjuvant chemotherapy and with improved survival for patients with triple-negative (TNBC) and HER2-positive BC. However, the prognostic and predictive value of TILs in luminal breast cancer is poorly understood.[3]

TILs are classified as stromal (sTILs) and intratumoral (iTILs). sTILs are lymphocytes in the tumor stroma, up to and including the invasive front of the tumor while iTILs are lymphocytes within the tumor nests and are in direct contact with tumor cells.[4-5] Extensive studies about the composition of TILs have shown that 75% of TIL is T lymphocytes, B lymphocytes less than 20%, monocytes less than 10%, and natural killer (NK) and NK- T cells less than 5%.[6] sTILs represent a more reproducible parameter because they are easily detected in hematoxylin and eosin (H&E)-stained sections with no need of immunohistochemical methods. The present study was carried out to access the clinicopathological profiles of TILs in patients with primary breast carcinoma.

MATERIAL AND METHODS

Information was collected retrospectively from the archive of the Department of Pathology about histologically confirmed invasive breast cancers diagnosed between April 2019 –March 2020. Among these diagnosed cases, those cases with available information regarding hormonal receptor status were included in the study. Estrogen receptor (ER), progesterone receptor (PR), HER2, and Ki 67 index were assessed as reported in the immunohistochemistry report. For the evaluation of TILs, H&E stained slides were retrieved from the department and TILs scored.

The guidelines of the "International Working Group for TILs in Breast Cancer—2014" were followed for the evaluation of TILs.[5] TILs were evaluated as a percentage of the stromal area occupied by lymphocytes and plasma cells within the border of the invasive tumor. According to the International Working Group for TILs in Breast Cancer 2014, TILs were categorized as low (0–10%), intermediate (11–40%), and high TILs. Statistical Analysis was performed using SPPS Statistics 26 software (Statistical Package for Social Science, SPSS Inc, Armonk, New York, NY, USA). Statistical significance was defined when $p < 0.05$.

RESULTS

During the study period from April 2019-March 2020, forty-two mastectomy specimens were received in the department. Thirty patients among them, who met the inclusion criteria, were included in the study. Age ranged from 30-73 years with a median age of 50 years. All cases were females. The characteristic of patients with breast carcinoma is presented in Table 1.

Table 1. Patient characteristics

	Characteristics	Low TIL	Intermediate-High TIL	p Value
Age	<50 (n=14)	5(35.8%)	9 (64.2%)	<0.05
	≥50(n=16)	9 (56.2%)	7(43.8%)	
Histopathological type	Invasive carcinoma, NST(n=24)	13(54.1%)	11(45.9%)	>0.05
	Invasive lobular carcinoma(n=4)	4(100%)	0	
	Mixed carcinoma(n=2)	1(50%)	1(50%)	
Grade	G1(n=15)	11(73.3%)	4(26.7%)	>0.05
	G2(n=11)	5(45.4%)	6(54.6%)	
	G3(n=4)	2 (50%)	2(50%)	
T status	T1(n=8)	5(62.5%)	3(37.5%)	<0.05
	T2(n=17)	9(52.9%)	8(47.1%)	
	T3(n=5)	4(80%)	1(20%)	
Lymphnodal status	Positive(n=14)	10(71.4%)	4(28.6%)	>0.05
	Negative(n=16)	8(50%)	8(50%)	
Margins	Negative (n=30)	18(60%)	12(40%)	>0.05
	Positive (n=0)	0	0	
Ki 67	<20(n=27)	17(62.9%)	10(37.1%)	>0.05
	≥20(n=3)	1(33.3%)	2(66.7%)	
Receptor status	HR+(n=14)	12 (85.7%)	2(14.3%)	<0.05
	HER2 +(n=4)	1(25%)	3 (75%)	
	TN (n=12)	5(41.6%)	7(58.4%)	

DOI : 10.3126/jpn.v12i2.47200

Though a larger number of cases, 10/18 (71.4%) of low TILs (fig. 1) presented with lymph nodal metastasis, and only 4/12 cases (28.6%) of intermediate-high TILs (fig. 2) presented with positive lymph node, it was not statistically significant.

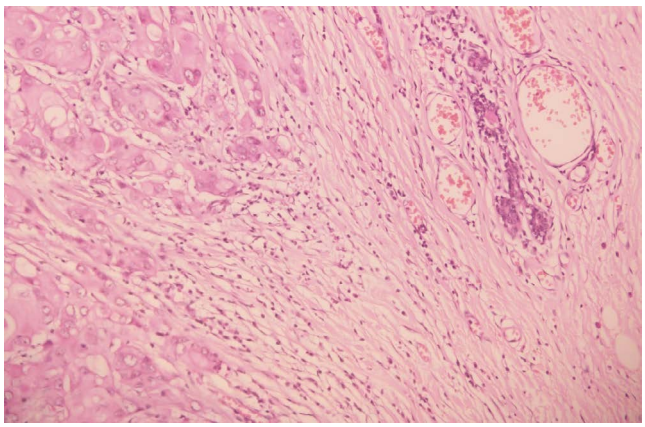

Figure 1: *Invasive carcinoma, NST with low tumor-infiltrating lymphocytes at the invasive front of the tumor (HE stain, X100).*

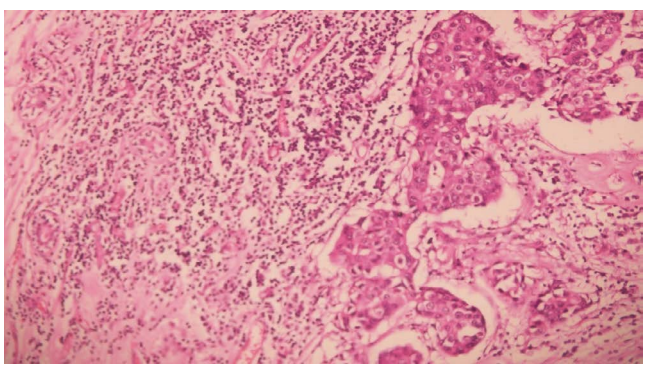

Figure 2: *High tumor-infiltrating lymphocytes in Grade 3, Invasive carcinoma, NST (HE stain, 200X)).*

Low TILs were associated with HR+ tumors and intermediate -high TILs with triple-negative (TN) BC. All breast cancers showed lymphocytic infiltrate. 40 % (12/30 cases) of the tumors showed intermediate to high TILs. Lymphocyte-predominant breast cancers (LPBC) were 30% (9/30 cases). Among the LPBC, 55.5% (5/9 cases) were triple-negative breast cancers, 33.3% (3/9 cases) were HR+ and 11.2% (1/9 case) were HER2 +.

DISCUSSION

Earlier, breast cancers were not taken to be immunologically active as tumors like melanoma. But now, with the defined prognostic role of TILs in breast cancers, quantitating TILs and associating their levels with a prognosis of breast cancers is being undertaken.[3] In 2014, recommendations for a standardized assessment of TILs in breast cancer was proposed by an international TILs working group,[5] following which many studies depicted the prognostic and predictive value of TILs in various cohorts of patients; especially among triple-negative BCs (TNBCs). Since TILs positively correlated with the response to adjuvant chemotherapy, they can be used as a prognostic marker.

This study showed that all breast cancers displayed some degree of the adaptive immune response as evidenced by the presence of TILs. Intermediate to high-grade TILs was seen in HER 2+ and TNBCs. Moreover, LPBCs defined as >50% of lymphocytes were predominantly TN breast cancers. This supports the findings of other studies stating that TN breast cancers are the subtype frequently associated with LPBC.[7]

It is stated that patients with HER2+ breast cancers have a similarity of LPBC to TN breast cancers.[8] But in this study, due to the paucity of HER 2+ cases this similarity could not be demonstrated.

As a morphological sign of host defense, breast cancers are surrounded by inflammatory cells. T- lymphocytes comprise most of these inflammatory cells indicating a cytotoxic response.[9]

In TN and HER2+ breast cancers, even a slight increase in TILs within and surrounding the tumor has shown to predict response to chemotherapy as well as improved survival in patients.[10]

A study including 256 TNBC demonstrated that every 10% increase in TIL correlated with a 17% decrease in risk of recurrence and a 27% decreased risk of death.[11] This shows that though the best response is seen in LPBCs, even a slight increase in TIL can lead to improved survival.

The majority of HR-positive tumors were associated with low TILs in this study which is similar to other studies performed on breast cancers.[12] The magnitude of TIL has been proposed to reflect the tumor mutational burden (TMB), which is lower in HR-positive tumors as compared to TN and HER-positive tumors.[12]

The TN status of the tumor, histological grade, and type, nuclear morphometric variables, sex steroid receptor content, and S-phase fraction oncoproteins signify prognostic information in breast cancers.[13] In this analysis we sought a correlation between TILs and the clinical and histopathological parameters.

The tumor stage, according to The American Joint Committee on Cancer (AJCC) staging, high stage, T3, showed a significantly high number of cases with low TILs. This is in concordance with other TILs studies in breast cancers.[14] Our study found that a high Ki 67 index was also associated with high TILs as with other studies performed.[14] So, high TILs may be indicative of a subset of the tumor with a high proliferative index.

In our study through a larger number of cases with low TILs showed lymph node metastasis it was not statistically significant. A study evaluating TILs in breast cancers in 76 patients who had surgery first and 96 patients who had pre-operative chemotherapy showed a correlation between TILs and lymph node metastasis in both these groups.[15]

DOI : 10.3126/jpn.v12i2.47200

No statistical significance between TILs and histological subtypes and histological grades of tumors was observed in this study. This is similar to the finding of a study carried out by Takada et al.[16] In this study all cases of Invasive lobular carcinomas (ILC) showed low TILs. Other studies have also stated that ILC harbors a lower level of TIL as compared to IDC,[17-18] although gene expression profiling has shown that the transcriptomic immune response signatures are upregulated in ILC.[18] ILC with high TIL has been associated with poor prognostic factors and less favorable clinical outcomes.[12] In this study with increasing age TILs in breast cancers decreased. This is in concordance with a study conducted on breast cancers in Japan in 2020.[16] This decrease in TILs with increasing age is probably due to a decrease in immunity with an increase in age.

This study has some limitations. The role of TILs in breast cancers is more clearly defined in HER2-positive and TN breast cancers. This study includes fewer of these cases and more HR-positive cases. So, a study including a larger number of HER 2 positive and TN cases is recommended.

CONCLUSIONS

We found that patients with low TILs presented at an advanced stage of the disease at the time of diagnosis. TILs in breasts decreased with increasing age. LPBCs were predominantly TNBCs. All breast cancers were immunogenic, stating the necessity to report TILs in all breast carcinomas in hematoxylin and eosin-stained sections.

Conflicts of interest: None

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