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Correlation of Tumor-infiltrating Lymphocytes with Tumor Staging and Grading in Breast Carcinomas: A Retrospective Study

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ABSTRACT

Background and aim: The global breast cancer mortality rate has increased by 0.23% per year globally, with a statistically significant increase in the age group under 50 and 70+. The present study evaluated Tumor-infiltrating lymphocytes (TIL) in breast carcinoma and correlated TIL with tumour stage, grade, and lymph node metastasis.

Material and methods: A retrospective study was conducted on 66 cases of modified radical mastectomy specimens of invasive breast carcinoma. The corresponding patients' clinicopathological details like age, location, laterality, tumour size, and lymph node involvement were retrieved from case sheets. The histopathological reports and H&E slides were reviewed again. The representative H&E stained section of each case was selected, and TIL assessment was done based on the International TIL Working Group (ITILWG) recommendations.

Results: A total of sixty-six cases were included in our study. Out of 66 cases, the majority were seen in the age group between 51-60 years, which is 36.4%. Forty-one cases (62.1%) out of 66 were in size groups of 2- 5 cm. 86.4% (57 cases) were invasive ductal breast carcinoma. On invasive tumour lymphocyte (TIL) evaluation, grade I was noted in 22 cases, grade II in 33 cases, and 11 in grade III.

Conclusions: Our study validates the method of histomorphological evaluation of TILs proposed by the International TIL Working Group (ITWG) 2014 in the Indian population and explores its role as a novel prognostic immune biomarker. Our analysis significantly correlated with tumour size, lymph node status, SBR grading, and histological types.

1. Introduction

Breast cancer is the most common cause of death among women globally and in India. The global breast cancer mortality rate has increased by 0.23% per year globally, with a statistically significant increase in the age group under 50 and +70.^[1] Indian women have breast cancer at a rate of 25.8 per 100,000 women, with a mortality rate of 12.7 per 100,000.^[2] The immune system helps maintain tissue homeostasis through continuous surveillance and activation of the adaptive and innate immune systems. This immune system has a role in tumour elimination. However, if the neoplastic cells escape, it can lead to cancer progression.^[3] Breast tumours are usually infiltrated by lymphocytes, neutrophils, and macrophages.^[4] The presence of tumor-infiltrating lymphocytes (TILs) has been recognized as a biomarker of an anti-tumor immune response in a wide range of tumours.^[5] Tumor immune response has prognostic and predictive significance in many solid malignancies, including breast cancer.^[6] There are two types of TILs - stromal and intratumoral. The stromal TILs (sTILs) are dispersed throughout the stroma and do not directly contact the carcinoma cells. There are lymphocytes within the tumour nest and in direct contact with the tumour cells, referred to as intratumoral TILs (iTILs).^[7] The role of the immune response in breast

cancer has not been fully defined. However, interest in tumor-infiltrating lymphocytes (TILs) as an immune biomarker of breast cancer has quickly gained momentum through various studies.^[8] Several studies have demonstrated that TIL is an inexpensive, readily available, reliable, and reproducible marker of immunity to tumours that already exist in patients with breast cancer based on hematoxylin and eosin (H&E) slides.^[9] Very few studies have evaluated the predictive importance of tumor-infiltrating lymphocytes (TILs) in breast cancer (BC).^[10] Including planned TIL assessment in current and future clinical studies and diagnostic evaluations requires a detailed description of a standardized method. The role of TIL in breast cancer as a risk and prognostic biomarker to guide and improve therapies for better clinical outcomes are well established.^[11] However, the assessment of TILs is subjective and not reproducible. Therefore, to ensure consistency, standardization, and objectivity in TIL assessment, the International TIL Working Group (ITILWG) has published recommendations and guidelines. However, studies evaluating this approach are limited, and the literature is sparse, especially on the Indian subcontinent.^[11] Therefore, the

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present study evaluated TIL in breast carcinoma and determined the correlation between TIL and tumour stage, stage, and lymph node metastasis.

2. Material and methods

This retrospective cohort study was conducted on modified radical mastectomy specimens of invasive breast carcinoma from 2019 to 2021 at Hassan Institute of Medical Sciences, Hassan. Ethical approval for the study was obtained by the Ethics Committee of the Hassan Institute of Medical Sciences, Hassan (approval code HIMS/IRC/18/22). Sixty-six cases were included in the sample size with respective inclusion and exclusion criteria. Inclusion criteria: All Invasive breast Carcinoma cases were included in the study. Exclusion criteria: carcinoma in situ cases were excluded. The corresponding patients' clinicopathological details like age, location, laterality, tumour size, and lymph node involvement were retrieved from case

sheets. The histopathological reports and H&E slides were reviewed again. Then, each case's representative H&E stained section was selected, and TIL assessment was done based on the recommendations from the International TIL Working Group (ITILWG). The percentage of sTIL was determined within the borders of the invasive tumour. The grade of TIL ranged from 0 to 3 [Fig. 1], where;

- 0= Virtually no lymphocytes,
- 1= Sparse intra-tumoral lymphocyte seen only under 40× magnification,
- 2=Frequent easily recognized lymphocytes seen under 20× magnification.
- 3= Lymphocytes obscure tumour.

Data were expressed as numbers, percentages, and mean \pm SD as applicable. Analytic and descriptive data will be analyzed along with the p-value.

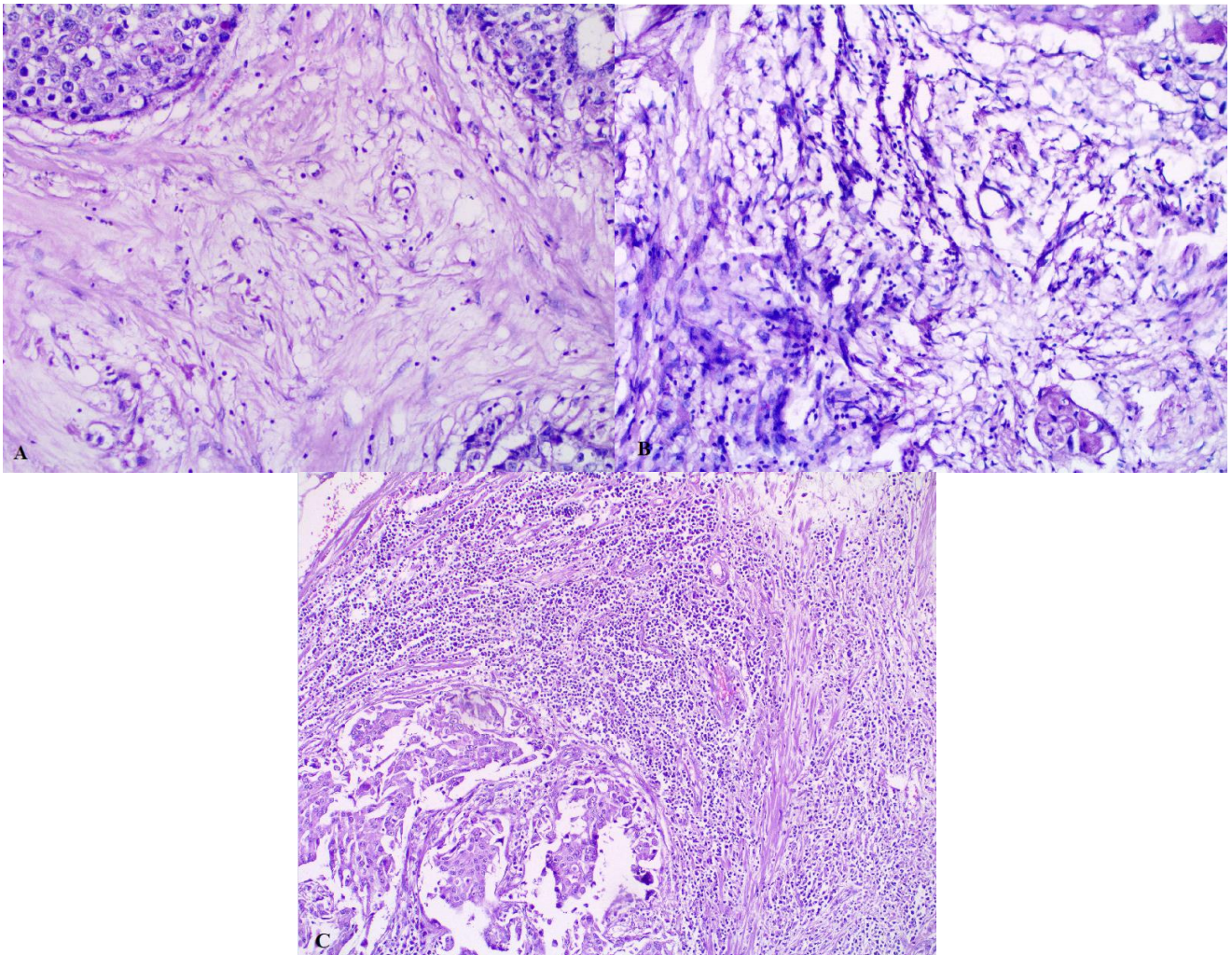


Fig. 1. Photomicrograph showing tumor-infiltrating lymphocytes grading on H&E slides grades I, II, III.

Part A shows TIL Grade I with sparsely distributed lymphocytes. Part B shows TIL Grade II with frequent, easily recognized lymphocytes at 20x magnification. Part C shows TIL Grade III with lymphocytes obscuring tumour.

3. Results

Out of 66 cases, the majority were seen in the age group between 51-60 years which is 36.4%. Forty-one cases (62.1%) out of 66 were 2- 5 cm in size

group. 86.4% (57 cases) were invasive ductal breast carcinoma. Thirty-six cases (54.5%) were seen in the left breast. On revised histopathological

examination, we noted 39 cases (59.1%) in SBR grade II with IDC-NOS as the most common histological subtype. Lymphovascular invasions were absent in 49 cases (74.2%). Forty-two cases were negative for lymph node involvement by invasive carcinoma. On tumor invasive lymphocyte

evaluation, grade I was noted in 22 cases, grade II in 33 cases, and 11 cases were in grade III. A total of 25 cases showed central quadrant involvement, with 4 cases of the lower inner and outer quadrant, 12 cases of the upper inner quadrant, and 21 cases with the upper outer quadrant. [Table 1]

Table 1. Tumor distribution according to size, laterality, histological type, SBR Grade, and quadrant.

Category	Characteristics	Frequency	Percentage
Tumor size (cm)	<2	11	16.7
	2-5	41	62.1
	>5	14	21.2
Laterality	Left	36	54.5
	Right	30	45.5
Histological type	IDC – NOS	57	86.4
	Medullary carcinoma	4	6.06
	Lobular carcinoma	2	3.03
	Mucinous carcinoma	3	4.54
SBR Grade	Grade I	21	31.8
	Grade II	39	59.1
	Grade III	6	9.09
Quadrant	Central quadrant	25	37.9
	Lower inner quadrant	4	6.06
	Lower outer quadrant	4	6.06
	Upper inner quadrant	12	18.18
	Upper outer quadrant	21	31.8

Pearson chi-square test was done between TILs Scoring and various parameters. We found a linear correlation between the size, SBR grade, histopathological type of invasive breast carcinoma, lymphovascular

invasion, and TIL score, with a p-value of 0.001, which is statistically significant. [Table 2].

Table 2. TIL Score distribution.

TIL	TIL I	TIL II	TIL III	Total
Frequency	22	33	11	66
Percentage	33.3%	50%	16.7%	100%

Tumor size <2 cm had 11 cases, tumor size 2-4 cm had 41 cases, and tumor size >4 cm had 14 cases, respectively. P-value was 0.001; Hence there is a statistically significant correlation between TIL score and size. [Table 3]. Pearson's coefficient p-value is 0.001. Hence there is a statistically significant

correlation between TIL score and histological type. [Table 4]. Pearson's coefficient p-value is 0.001. Hence there is a statistically significant correlation between TIL score and SBR grading. [Table 5].

Table 3. TIL Score correlation with tumor size.

Tumor Size(cm)	TIL I	TIL II	TIL III	Total
< 2	3	5	3	11
2- 4	12	23	6	41
>4	7	5	2	14

Table 4. TIL Score correlation with the histological type of breast carcinoma.

Histological Type	TIL I	TIL II	TIL III	Total
IDC – NOS	20	30	7	57
Medullary carcinoma	0	0	4	4
Lobular carcinoma	1	1	0	2
Mucinous carcinoma	1	2	0	3

Table 5. TIL Score correlation with SBR grading.

SBR Grading	TIL I	TIL II	TIL III	Total
I	8	10	3	21
II	10	23	6	39
III	4	0	2	6

The p-value is 0.001; there is a statistically significant correlation between TIL score and Lymph node status. In our study, we analyzed the comparison between the tumor staging, including tumor size, lymph node status, and TIL score. We found a statistical significance between the two.

Metastasis was not analyzed in the current study due to inadequate information. This study also showed a significant correlation between the SBR grading of breast carcinoma and TIL score.[Table 6].

Table 6. TIL Score correlation with Lymph node status.

Lymphnode Status	TIL I	TIL II	TIL III	Total
Positive	20	3	1	24
Negative	2	30	10	42

4. Discussion

Breast cancer is the most common cause of death among women globally and in India. The worldwide breast cancer mortality rate has increased by 0.23% per year. For breast cancer, many prognostic indices are used, including stage, tumour grade, lymph node metastasis, distant metastasis, and, more recently, estrogen-based molecular subtype (ER), progesterone (PR), and human epidermal growth factor receptor.^[12] Tumours are infiltrated by tumor-infiltrating lymphocytes (TILs) with a local immune response against tumour growth and metastasis. Over the past decade, many researchers have emphasized the importance of TIL assessment to assess immune response in various malignancies, including colon, ovary, lung, bladder optic, and breast.^[13, 14] Moreover, the extent of lymphocyte infiltration has emerged as an independent marker of good prognosis in many tumours.^[15] There are two types of TIL - stromal and intratumoral. Salgado et al. proposed that TIL should be included in all the histopathological reports and a standardized approach for measuring the percentage of stromal TILs in primary tumour

specimens using visual assessment of standard H&E stained sections.^[11] In a study conducted by pujani et al. to evaluate tumour infiltrating lymphocytes in breast carcinoma and its correlation with tumour staging, grading, distant metastasis, and lymph node metastasis, they concluded that TIL has a significant correlation with tumour staging, grading, and distant and lymph node metastasis.^[7] A study by Loe et al. concluded that both stromal and intratumoral TIL have statistically significant correlations with major factors of breast carcinoma, i.e., tumour size and lymph node metastasis.^[6] A study by Marchetti et al. concluded that TIL correlates with tumour size and lymph node metastasis.^[17] In a study conducted by Kolarov-Bjelobrck et al., a positive correlation was found between TIL and tumour staging.^[10] Tsang J et al. and Thanna E et al. also found a significant correlation between TIL with tumour staging.^[8, 9] In the current study, we concluded TIL correlation with tumour staging and grading, similar to the above mentioned studies.

5. Conclusion

Our study validates the method of histomorphological evaluation of TILs proposed by the International TIL Working Group (ITILWG) 2014 in the Indian population and explores its role as novel prognostic immune biomarkers. Out of 66 cases studied in this study, TIL grade II was the most common. TIL score of the higher grade was correlated with cases with negative lymph node metastasis. We concluded that higher tumour size correlated with lower TIL Grading. Compared to other histological types, medullary carcinoma was found to have a higher TIL score. Our analysis significantly correlated with tumour size, lymph node status, SBR grading, and histological types.

Conflict of Interest

The authors declared that there is no conflict of interest.

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