DOI: https://doi.org/10.53350/pjmhs02024181280

ORIGINAL ARTICLE

The Association of Immune Infiltrate of Primary Luminal B-Like Breast Cancer with Age

HUSSAIN FAROOQ[1], MADIHA EHSAN-UL-HAQ[2], RAFIQ AHMED SHAHID[3], ATTIA LATEEF[4], USMAN NASIR[5], SAMEEN HASSAN[6]
[1]Senior Demonstrator, Department of Hematology, Shaikh Zayed Hospital, Lahore
[2,5]Assistant Professors, [3]Associate Professor, [4]Demonstrator, [6]Demonstrator, Department of Pathology, Sharif Medical & Dental College, Lahore
Correspondence to: Madiha Ehsan-ul-Haq, Email: mdiha_ehsan@hotmail.com, Cell: 0322-8519085

ABSTRACT

Background: The effect of aging on immune response of luminal breast cancer is not effectively characterized leading to lack of substantial data which can be generated from age associated dynamics of immunity in luminal B cells.
Objective: To assess the association of immune infiltrate of primary luminal B like breast cancer with age.
Study Design: Prospective study.
Place and Duration of Study: Department of Pathology, Sharif Medical & Dental College, Lahore from 1st April 2019 to 31st October 2023.
Methodology: Seventy breast carcinoma patients were enrolled. The patients were diagnosed with grade I, II or grade III invasive breast carcinoma (identified through core needle biopsy) and had upfront surgery. The patient included in the study has clinical tumor size of ≥1.5 cm, receptor-status HR+/HER2-, and were within the age group of 25-70 years. Those patients who were between 35-50 years were categorized as younger patients while those between 51-65 years were categorized as middle patients and those above 65 years were old patients. To characterize the immunogenic tumor immune microenvironment (TIME) the tissue microarrays (TMA) were made by applying the Gran master for TMA. An overall three tissue microarrays were formulated which contained 250 tissues from 70 patients. These were used for orientation within tissue microarrays during multiple labeling by antibody neo deposition (MILAN) workflow. Staining and scanning of TMA slides was performed. Overall methodology included tissue dissection as well as analytical cell composition, functional and spatial analysis.
Results: Majority within the age of 66-70 years followed by 51-65 years. The deserted tumor pattern was highest in elderly patients. The histological subtypes present invasive breast carcinoma-no special subtype (IBC NST) in all the breast carcinoma cases within the age group of 35-50 years. The tumor grading showed that majority of the breast carcinoma patients had grade II tumor with 53.3% seen in younger age while 60% of the breast carcinoma patients each from middle and older age also presented grade II tumor. The grade III tumor was observed in 46.6%, 40% and 34.28% breast carcinoma cases young to adult respectively. The nodal status presented with pNO cases at highest frequency. The B cell infiltration observed in the invasive front (IF) and tumor centre (TC) were significantly increased in the inflamed tumor than in desert tumors. The cell fraction presented increased number of T cytotoxic (Tcy), as well as T follicular helper (Tfh) cells in inflamed tumors while compared with deserted tumors in TC and IF. In the old age category it was observed that there was consistent negative correlation of OX-40+ Tcy cells with percentage.
Conclusion: Aging is related to the immune profile alterations at cellular level inside the microenvironment of luminal B-like breast carcinoma tumor cells. The expression of OX-40 has no association with age signifying persisted capacity of inducing an anti-tumor immune-response.
Keywords: Association, Immune infiltrate, Luminal B like breast cancer

INTRODUCTION

Breast cancer has a worldwide high prevalence with women as most effected population through this lethal disease. Early diagnosis and treatment are the key to survival. Within the various breast carcinoma (BC) receptors the most crucial are the hormone receptor (HR) positive and the human epidermal growth factor-receptor 2 negative (HER2-). It is presented in more than 2/3rd of the Breast carcinoma cases heterogenous-subtypes.[1,2] The two subgroups can be distinguished through immunochemistry using tumor discrimination and protein (K167) identification protocol. These subgroups overlaps luminal B related or luminal A related BC having a raised as well as reduced proliferative activity respectively.[3,4]

Luminal B-like breast cancer is a subtype characterized by estrogen receptor (ER) positivity, high Ki-67 expression (proliferation marker), low/absent HER2 expression as well as high genomic grade. The immune infiltrate in luminal B like breast cancer tumor microenvironment: consist of immune cells infiltrate the tumor, which includes T cells (CD4+, CD8+), B cells, macrophages and the dendritic cells. Immune cell-density and constitution differ between patients. The immune cell subsets includes tumor-infiltrating lymphocytes (TILs) such as CD8+T cells which are associated with better prognosis, CD4+ T cells constituting of mixed roles, depending on subset (Th1, Th2, Treg) and the tumor associated macrophages (TAMs) consisting of MI-like (anti-tumor) and M2 like pro tumor which produce pro-inflammatory cytokines and immune suppression respectively.[1-5]

With advancement in the research the stromal tumor infiltrating lymphocytes in breast carcinoma has achieved great popularity with endorsement from breast tumor classification methodology.[5]Guidelines available internationally recommends examination of hematoxylin as well as eosin-stained sections of tumor for determining the infiltration of lymphocyte. This is recommended through a scoring system using percentage measures of peri-tumoral stroma infiltrates.[6,7]

It has presented the association of aging with decreased immunogenic, tumor-immune microenvironment (TIME) with reduced subsets tumor-infiltrating lymphocytes, T and B lymphocytes.[8,9] There is limited research on the association of the immune infiltrate of primary luminal b-like breast cells with age.[10] Furthermore, there is a lack in the in-depth investigation of IC molecule expression in accordance with age. The present study is focused to provide evidence-based data on association of the age with immune infiltrate of primary luminal B-like breast cells.

MATERIALS AND METHODS

This was a biomarker based prospective study enrolling 70 patients at Department of Pathology, Sharif Medical & Dental College Lahore from 1st April 2019 to 31st October 2023. The patients were diagnosed with grade I, II or grade III invasive breast carcinoma (identified through core needle biopsy) and had upfront surgery. The study was approved by the ethical committee and a written informed consent was obtained from each patient prior to enrolment. The sample size was calculated keeping the prevalence of breast cancer as 11.1%[11] within regional population.

Received on 03-11-2023
Accepted on 27-12-2023

The calculations of sample size were done through already WHO approved sample size available software using 80% power of test, 5% margin of error and 95% confidence of interval. The patient included in the study has clinical tumor size of ≥1.5 cm, receptor-status HR+/HER2-, and were within the age group of 25-70 years. Those patients who were between 35-50 years were categorized as younger patients while those between 51-65 years were categorized as middle patients and those above 65 years were old patients. To characterize the immunogenic tumor immune microenvironment (TIME) the tissue microarrays (TMA) were made by applying the Gran master for TMA. Certified histopathologists selected 4 regions in each tumor which was already tumor block which was fixed in formalin and embedded in paraffin. Out of the four region two were sampled from invasive front (IF) while the other two were sampled from the tumor center (TC) in a 2mm diameter through block formation. An overall three tissue microarrays were formulated which contained 250 tissue from 70 patients. The control tissue was also used for comparison containing 30 cores from 10 control tissues and as blank. The control tissue was made from volunteered tonsils. These were used for core-orientation within tissue microarrays during multiple labelling by antibody neode position (MILAN) workflow. MILAN wet lab workflow included rounds of three colors using indirect immunofluorescence. The tumors were subdivided into desert, excluded as well as inflamed types. Fingerprinting of cell types were done through cluster technique and mapping was performed on uMAP blank for blood vessel, conventional dendritic cell type 1 and type 2, dendritic cells, follicular dendritic cells, natural killer cells, plasmacytoid dendritic cell, T-cytotoxic, T follicular helper, T helper as well as T regulatory. QuPath cytometric values were applied for assessing the density of relevant inflammatory cell populations keeping the geographical tumor region into account. Primarily the tumor was manual outlined by QuPath Polygon tool while software was used. The region covering 500 µm outwards and 500 µm inward within the tumor border was specified as the IF. The TC was labeled as the region surrounded within the IF.[9] Staining and scanning of TMA slides was performed. Overall methodology included tissue microdissection as well as analytical cell composition, functional and spatial analysis. Data was analyzed through SPSS version 26.0 using frequency and percentages for the qualitative data while mean±SD was applied for quantitative variables. The imaging results were interpreted in terms of differentiation observed with aging. Chi square tool was applied for comparing the aging groups with variables. P value <0.05 was considered as significant.

RESULTS

The mean age of the patients was 64.5±2.83 years. There were only 21.42 BC cases within the age group of 35-50 years. The inflammatory patterns showed 42.85% BC cases to have deserted patterns followed by excluded pattern in 25.71% BC cases. The deserted pattern was highest in elderly patients (Table 1).

The histological subtypes present IBC NST subtype in all the BC cases within the age group of 35-50 years. While the similar subtype was observed as in majority of the middle age and older age groups as well. The other histological subtypes were presented in a very low number than the IBC NST subtype. The tumor grading showed that majority of the BC patients had grade II tumor with 53.3% seen in younger age while 60% of the BC patients each from middle and older age also presented grade II tumor. The grade III tumor was observed in 46.6%, 40% and 34.28%BC cases young to adult respectively. The mean tumor size was observed as 29.8±29.2 mm with largest size observed in older cases as 30.0±44.2 mm (Table 2).

The nodal status presented with pNO cases at highest frequency followed by the pN1 and least p N3. There was a significant increase of nodal status with the age p value 0.023. The TILs median value was observed as 7.1% among all BC cases. The Magee equation interpreted <18 score in older adults with 54.28% cases presented (Table 3).

The B cell infiltration observed in the IF and TC were significantly increased in the inflamed tumor inflamed were significantly higher in inflamed tumors than in desert tumors. The cell fraction presented increased number of Tcy, Th, as well as Tfh cells in inflames and excluded while compared with deserted tumors in TC and IF (Fig. 1).

After the deserted tumors the excluded tumor type additionally presented high concentration in the middle and old age groups of BC cases. These were significantly presented in the Th cells in IF than TC. Within the young age group there was highest count of inflamed cell while deserted were presented at highest in middle and older age BC cases (Fig. 2).

In the old age category it was observed that there was consistent negative correlation of OX-40+ Tcy cells with percentage. These OX -40 + cells were far distant from the epithelial tumor cells presenting increased density of the OX -40 + in activated form. Stronger Tcy and Th cells were observed in old age when in close proximity of malignant cells. The spatial-gradient of OX-40$^+$ T- cell density was not noticeable in young age BC cases (Fig. 3).

Table 1: Age and inflammatory pattern distribution within breast carcinoma cases

Variable	Total Patients	Younger (35–50 years) n=15	Middle (51–65 years) n=20	Older (66-70 years) n=35	P value
Total	70 (100%)	15 (21.42%)	20 (28.57%)	35 (50%)	0.045
Age (years)	64.5±2.83	39.0±2.5	58.0±3.1	66.5±2.9	0.543
Inflammatory patterns					
Desert	30 (42.8%)	-	12 (60%)	18 (51.4%)	0.551
Excluded	25 (35.8%)	7 (46.6%)	4 (20%)	14 (40%)	0.032
Inflamed	15 (21.4%)	8 (53.4%)	4 (20%)	3 (8.6%)	0.121

Table 2: The distribution of histological subtypes and tumor grades in various age groups of breast carcinoma cases

Variable	Total Patients	Younger (35–50 years) n=15	Middle (51–65 years) n=20	Older (66-70 years) n=35	P value
Histological subtype					
IBCNST	56 (80%)	15 (100%)	13 (65%)	28 (80%)	0.011
ILC	7 (10%)	-	4 (20%)	3 (8.6%)	0.038
Mixed NST or ILC	4 (5.7%)	-	2 (10%)	2 (5.8%)	1.875
Invasive solid papillary	2 (2.8%)	-	1 (5%)	1 (2.8%)	1.861
Micropapillary	1 (1.5%)	-	-	1 (2.8%)	--
Tumor grading					
I	2 (2.8%)	-	-	2 (5.7%)	--
II	41 (58.6%)	8 (53.4%)	12 (60%)	21 (60%)	1.325
III	27 (38.6%)	7 (46.6%)	8 (40%)	12 (34.3%)	0.673
Mean Tumor size	29.8±29.2	29.5±27.8	30.0±15.6	30.0±44.2	0.987

Table 3: The distribution of nodal status and Magee equation within various ages of breast carcinoma cases

Variable	Total Patients	Younger (35-50 years) n=15	Middle (51-65 years) n=20	Older (66-70 years) n=35	P value
Node status					
pN0	34 (48.6%)	3 (20%)	11 (55%)	20 (57.1%)	0.049
pN1	32 (45.8%)	10 (66.6%)	8 (40%)	14 (40%)	0.023
pN2	3 (4.2%)	2 (13.4%)	1 (5%)	-	--
pN3	1 (1.4%)	-	-	1 (2.9%)	--
TILs -Median	7.1%	16.3%	3.5%	5.7%	0.067
Magee Equation 2					
<18	37 (52.8%)	8 (53.4%)	10 (50%)	19 (54.2%)	0.992
18–25	22 (31.4%)	4 (26.6%)	8 (40%)	10 (28.7%)	0.756
>25	11 (15.8%)	3 (20%)	2(10%)	6 (17.1%)	0.074

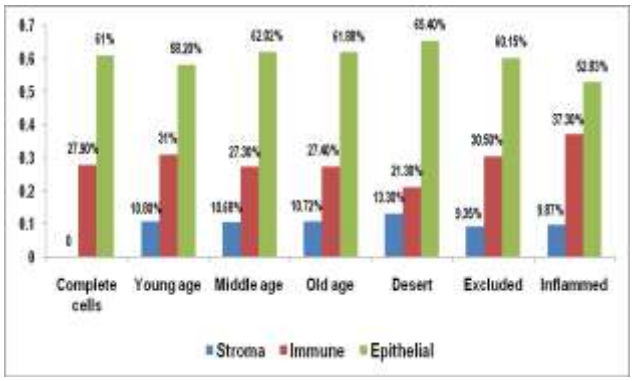

Fig. 1: The cell fraction distribution among various ages and tumor categorization

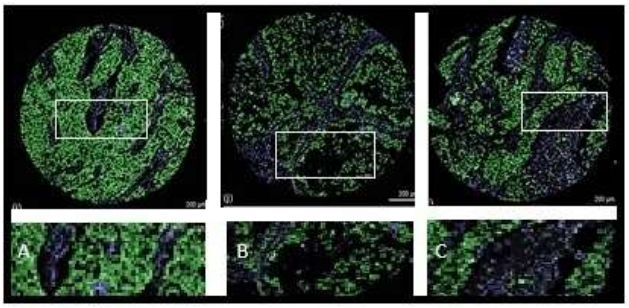

Fig. 2: Th cells illustration of expression OX-40 central tumor location. Scale=150 µm with mean fluorescence intensity and programmed cell death-ligand kept under consideration. A, B, C= young, middle and old age categories respectively

Fig. 3: Concentration count of tumor types among various age groups

DISCUSSION

It has elaborated the fact that the composition of the TIME may assist in indicating the beneficial role of immunotherapy in BC patients.[12,13] Scientist has yet not been able to assess the integration of IC therapy with the luminal like BC. Although there are a few recent studied highlighting the pCR increased rated in the luminal BC suggesting the anti-tumor response of immune system in BC cases.[14]

There is a strong association of age with remodeling of immune system as mentioned in the current study as well as previously reported studies.[15,16] There is a variance in the immune response of the older breast cancer patients than younger BC patients.[17] The present study presented data that T and B lymphocytes relative frequencies in the luminal B like breast tumor reduces with age. This is interpreted by 3 distribution patterns such as inflamed, excluded and deserted as reported in this study and elsewhere.[18]

In this study more inflamed tumor cells patterns were observed in younger BC patients while deserted and excluded tumor patterns were observed in middle and older ages BC patients. Inflamed tumors type was linked with raised TIL levels showing increased number of patients with grade 3 tumors with positive lymph nodes formation.[19]

This relates to the decreased exhaust profile of T&B cells in older BC cases. The immune capability of the cells to identify and counter to an evolving tumor may be affected in the aforesaid scenario leading to poor immune response. The present study results show the presence of OX-40+ Th cells in older BC patients.[20]

CONCLUSION

Aging is related to the immune profile alterations at cellular level inside the microenvironment of luminal B-like BC tumor cells. The expression of OX-40 has no association with age signifying persisted capacity of inducing an anti-tumor immune-response.

REFERENCES

1. Howlader N, Altekruse SF, Li CI, Chen VW, Clarke CA, Ries LA, et al. US incidence of breast cancer subtypes defined by joint hormone receptor and HER2 status. J Natl Cancer Inst 2014; 106(5): dju055.
2. Institute NC. SEER cancer statistics review, [Updated 4 September 2020]. Available from: https://seer.cancer.gov/csr/1975_2016/.
3. Ades F, Zardavas D, Bozovic-Spasojevic I, Pugliano L, Fumagalli D, de Azambuja E, et al. Luminal B breast cancer: molecular characterization, clinical management, and future perspectives. J Clin Oncol 2014; 32(25): 2794-2803.
4. Bediaga NG, Beristain E, Calvo B, Viguri MA, Gutierrez-Corres B, et al. Luminal B breast cancer subtype displays a dicotomic epigenetic pattern. Springerplus 2016; 5: 623.
5. Dushyanthen S, Beavis PA, Savas P, Teo ZL, Zhou C, Mansour M, et al. Relevance of tumor-infiltrating lymphocytes in breast cancer. BMC Med 2015; 24: 202.
6. Hendry S, Salgado R, Gevaert T, Russell PA, John T, Thapa B, et al. Assessing tumor-infiltrating lymphocytes in solid tumors: a practical review for pathologists and proposal for a standardized method from the International Immunooncology Biomarkers Working Group: part 1: assessing the host immune response, TILs in invasive breast carcinoma and ductal carcinoma in situ, metastatic tumor deposits and areas for further research. Adv Anat Pathol 2017; 24(5): 235-51.
7. Salgado R, Denkert C, Demaria S, Sirtaine N, Klauschen F, Pruneri G, et al. The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: recommendations by an International TILs Working Group 2014. Ann Oncol 2015; 26(2): 259-71.
8. Denkert C, von Minckwitz G, Brase JC, Sinn BV, Gade S, Kronenwett R, et al. Tumor-infiltrating lymphocytes and response to

neoadjuvant chemotherapy with or without carboplatin in human epidermal growth factor receptor 2-positive and triple-negative primary breast cancers. J Clin Oncol 2015; 33(9): 983-91.
9. Berben L, Wildiers H, Marcelis L, Antoranz A, Bosisio F, Hatse S, et al. Computerised scoring protocol for identification and quantification of different immune cell populations in breast tumour regions by the use of QuPath software. Histopathology 2020; 77(1): 79-91.
10. Nagarajan D, McArdle SEB. Immune landscape of breast cancers. Biomedicine 2018; 6: 20.
11. World Health Organization. The global cancer observatory: all cancers Source. Geneva: WHO, 2019.
12. Denkert C, von Minckwitz G, Darb-Esfahani S, Lederer B, Heppner BI, Weber KE, et al. Tumour-infiltrating lymphocytes and prognosis in different subtypes of breast cancer: a pooled analysis of 3771 patients treated with neoadjuvant therapy. Lancet Oncol 2018; 19(1): 40-50.
13. Gruosso T, Gigoux M, Manem VSK, Bertos N, Zuo D, Perlitch I, et al. Spatially distinct tumor immune microenvironments stratify triple-negative breast cancers. J Clin Invest 2019; 129(4): 1785-1800.
14. Chen DS, Mellman I. Elements of cancer immunity and the cancer-immune set point. Nature 2017; 541: 321-30.
15. Nederlof I, Horlings HM, Curtis C, Kok M. A high-dimensional window into the micro-environment of triple negative breast cancer. Cancers (Basel) 2021; 13(2): 316.
16. Thommen DS, Schumacher TN. T cell dysfunction in cancer. Cancer Cell 2018; 33: 547-62.
17. Keenan TE, Tolaney SM. Role of immunotherapy in triple-negative breast cancer. J Natl Compr Canc Netw 2020; 18: 479-89.
18. Cardoso F, McArthur HL, Schmid P, Karantza VV, Tryfonidis KE, Bardia A, et al. LBA21 KEYNOTE-756: phase III study of neoadjuvant pembrolizumab (pembro) or placebo (pbo) + chemotherapy (chemo), followed by adjuvant pembro or pbo + endocrine therapy (ET) for early-stage high-risk ER+/HER2– breast cancer. Ann Oncol 2023; 34(2): S1260-61.
19. Loi S, Curigliano G, Salgado RF, Pacius M, Wu JQ, McArthur HL, et al. LBA20 a randomized, double-blind trial of nivolumab (NIVO) vs placebo (PBO) with neoadjuvant chemotherapy (NACT) followed by adjuvant endocrine therapy (ET) ± NIVO in patients (pts) with high-risk, ER+ HER2− primary breast cancer (BC). Ann Oncol 2023; 34(2): S1259-60.
20. White MC, Holman DM, Boehm JE, Peipins LA, Grossman M, Henley SJ. Age and cancer risk: a potentially modifiable relationship. Am J Prev Med 2014; 46(3 Suppl 1): S7-15.

This article may be cited as: Farooq H, Haq MEU, Shahid RA, Lateef A, Nasir U, Hassan S: The Association of Immune Infiltrate of Primary Luminal B-Like Breast Cancer with Age. Pak J Med Health Sci, 2023; 18(1): 280-283.