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Original Research

Role Of Natural Killer Cells In Clinico-Histological Presentation Of Breast Cancer

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ABSTRACT
BACKGROUND: Globally, breast cancer is the most common cancer in women. Natural Killer cells are one of the major components of the antitumor immune response. There is development of tumor specific T- cell responses to the parental tumour cells after the NK cell mediated elimination of tumor cells, thus serving as a link between innateand adaptive immune responses. Aim of the study role of natural killer cells in clinico-histological presentation of breast cancer.
MATERIAL AND METHODS: 47 diagnosed cases of invasive duct carcinoma were selected as study cases that were diagnosed 24-48 months back from the initiating point of the study. By using immunohistochemistry, an anti- human CD56/NCAM-1 rabbit monoclonal antibody for CD56 Clone was used as a primary antibody, NK cells were counted and then compared with histological stage and grades of the tumor.
RESULTS: Out of 47 cases, CD 56+ NK cell count by IHC was negative in majority of cases (27/47), followed by low count (12/47) and moderate count of IDC cases (8/47).Though negative and low NK cell expression could not correlate, however, moderate NK cell count was associated significantly with high histological grade (p=0.003) and stage(p=0.019). An inverse relationship was seen between low NK cell expression and axillary nodal status, but not so with moderate NK cell count.
CONCLUSION: Knowledge about the role of NK cells, they home-less in breast carcinoma and the multifaceted immunosuppressive microenvironment blunts NK cell cytotoxicity, thus responsible for tumour progression. Study concentrates towards a possible immunotherapeutic solution of breast carcinoma in the form of reactivation of NK cells.
KEYWORDS Breast cancer, CD56, NK cells, Immunohistochemistry.
Key words: Breast feeding, knowledge, practices, observation, attachment and positioning

INTRODUCTION

Worldwide, breast cancer is the most common cancer affecting women. In 2022, WHO reported a total of 2.3 million newly diagnosed cases worldwide[1]. A proposed theory by Stephen Paget in 1889 called as the “seed and soil” in relation to cancer metastasis led to extensive study of tumour microenvironment.[2] Metastasis occurs when tumour cells acquire invasive features3 and the ability to escape from antitumor immunity.[4,5]

Natural killer (NK) cells are one of the major components of the antitumor immune response[6]. CD56, a well-known marker for NK cells, is an integral membrane protein. Apart from breast cancer, its over expression is seen in other malignant tumours like acute lymphoblastic and myeloid leukaemia, malignant melanoma and is associated with an aggressive tumour type, inadequate therapeutic response, and a reduced survival time.[7]We studied expression of CD 56+ NK TIL (Tumor infiltrating lymphocytes) by immunohistochemistry using CD 56 monoclonal antibody in breast carcinoma cases and their counts were correlated with various clinico-histological parameters of the patient.

MATERIAL AND METHODS

The present study was a cross-sectional observational study conducted in Histopathology section of the Department of Pathology Mahatma Gandhi Institute of Medical Sciences, Sewagram from October 2016 to September 2019. 47 cases of invasive duct carcinoma with reported ER, PR and Her2 neu were selected as

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study cases that were diagnosed 24-48 months back from the initiating point. Patients who refused to give consent or patients who were died soon after initial diagnosis were excluded from the study.The clinical history and relevant information was collected from primary and follow up records.

METHOD OF CD56 IHC staining

After initial removal of paraffin and rehydration of 3-5 µ tissue sections, antigen retrieval was done using a microwave for 5 minutes on high power (~750 watts) and 15 minutes. at low power (200 watts). Endogenous peroxidase was inactivated by using super block provided in Ultratek HRP. 0.1 ml concentrate anti human CD56/NCAM-1 rabbit monoclonal antibody for CD56 Clone MRQ-42. Isotype-IgG1 (Cell Marque) was used and dilution made in 1:300 with antibody diluent (Tris buffer pH 7.3-7.7, with 1 % BSA and < 0.1% Sodium azide). Slides were incubated overnight at 2-8°C kept in humidified chamber. Ultratek Antipolyvalent and Ultratek HRP polymer was applied for 10-20 minutes each at room temperature. After completion of every step, slides were washed in PBS, three changes were given, each for 3 minutes. Thereafter, slides were covered with DAB (3, 3 DiaminoBenzidine), counterstained with hematoxylin, washed under running water, dehydrated, cleared and mounted.

Evaluation of immunohistochemical staining[7]:

Cytoplasmic positivity of CD 56 staining was given as score of - Negative-0 cells/HPF; Low- 1-25 cells/HPF; Moderate- 26-50 cells/HPF; High- > 51 cells/HPF

The patients were followed up for 2-4 years by checking the records of the patient's outdoor and indoor visits to the hospital after primary management.

Statistical analysis was done by using descriptive and inferential statistics using Chi square test, paired t test and One Way Anova test. Disease free Survival (DFS) was calculated using log rank test. Software used was SPSS 17.0 and $p<0.05$ was considered as significant.

RESULTS

Perimenopausal age group (41-60 years) was the commonest age group for clinical presentation of breast carcinoma in our study area (66% cases), 49 years being the mean age at clinical presentation. 70% (33/47) of our cases belonged to two categories i.e. Luminal without HER-2 and HEBC. Ki-67 immune marker was not studied in the present study due to financial restraint, so we could not classify our cases in further molecular categories. For patients ≤ 2 children, history of use of OC pills/HRT was present in 14/21 cases (67%) History of OCP or HRT and low parity act in conjunction and was significantly associated with risk of carcinoma breast. (p=0.0140)

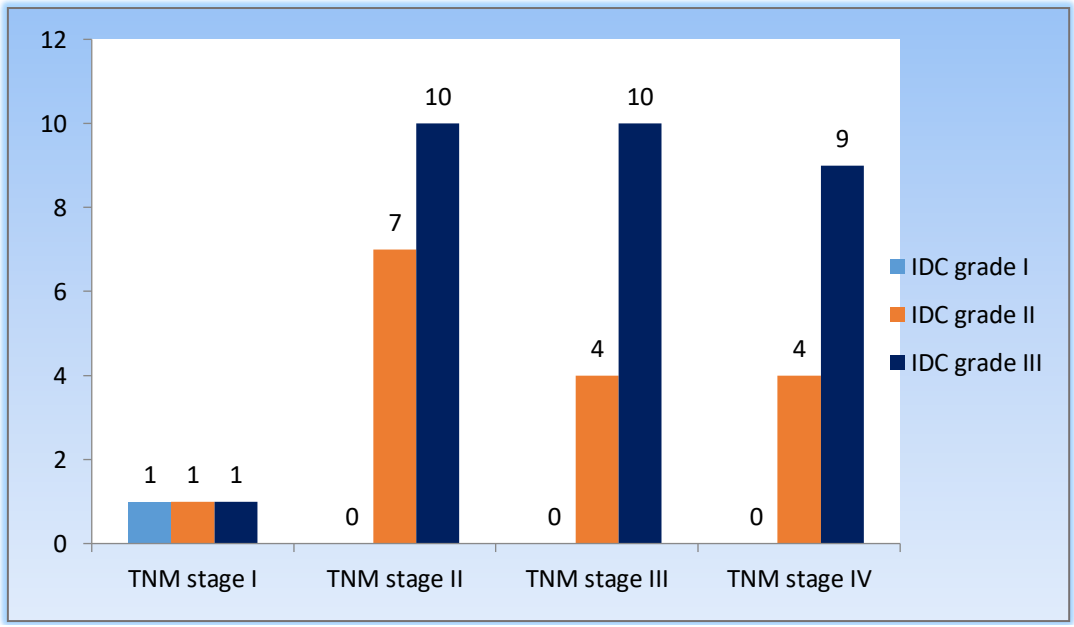

Figure 1 -Histological grading and clinical staging in study cases

Most of the cases in our series presented in TNM stage II, III and IV (17, 14 and 13 respectively) with more than half of the cases in our study, showed histological grade III tumours (n=30, 64%)

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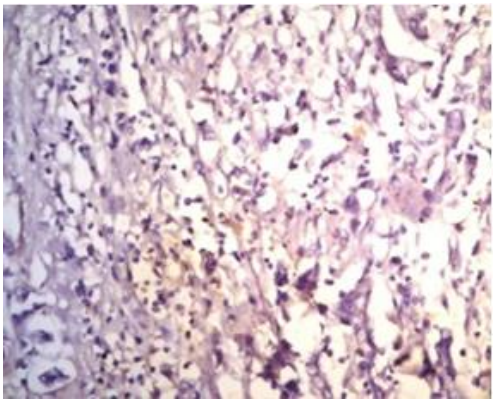
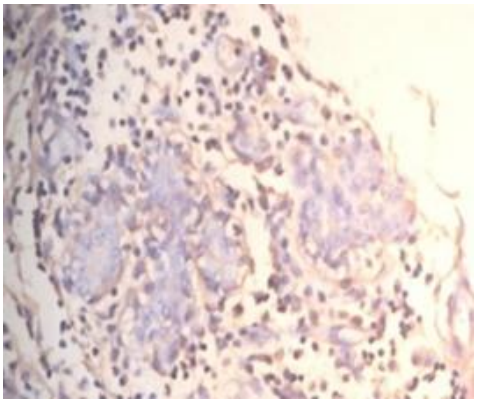
Table 1- NK cell (CD56) expression in breast cancer cases

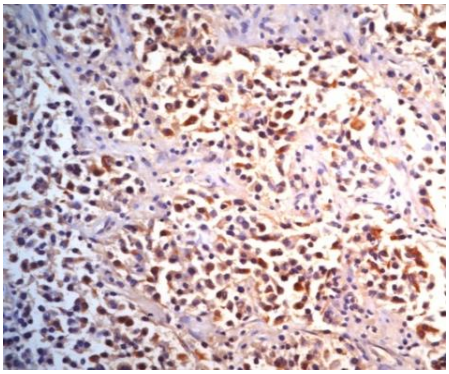
NK Cell expression	Negative (No cells/HPF)	Low expression (1-25cells/HPF)	Moderate expression (25-50cells/HPF)	High expression (>50 cells/HPF)	TOTAL
No. of cases	**27**(57%)	12(25%)	8(18%)	0	47





Photomicrograph shows CD 56 negativity with absence of CD 56 expression in Natural killer cells CD 56 staining score: 0(X400, IHC)

Photomicrograph shows CD 56 positivity with low expression of Natural killer cells, CD 56 staining score: 1(X400, IHC)



Photomicrograph shows CD 56 positivity with moderate expression of Natural killer cells, CD 56 staining score: 2(X400, IHC)

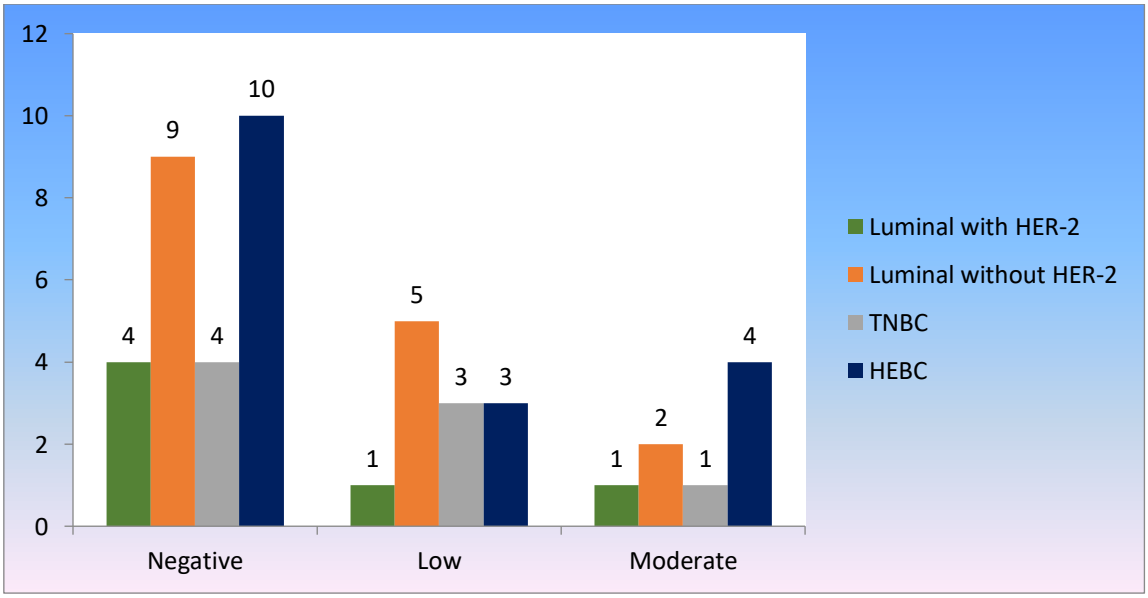


FIGURE 2- NK cell expression in molecular types of breast cancer.

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As shown in figure 2, all four molecular types showed similar degree of absent CD 56 NK cells in most of the cases [Luminal with HER-2 (4/6 cases), Luminal without HER-2 (9/16 cases), TNBC (4/8 cases) and HEBC (10/17 cases)].

TABLE 2- NK cell expression Vs Histological grading

NK cell expression	Histological Grading		Total	One way annova test
	LOW(I-II)	HIGH(III)		
Negative	10(37%)	17(63%)	27	F=4.760 p= 0.068
Low	6(50%)	6(50%)	12	F=1.745 p= 0.193
Moderate	**1**(12%)	**7**(88%)	**8**	F=10.785 **p= 0.003**
Total	17	30	47	

TABLE 3-NK cell expression Vs TNM staging

NK cell expression	TNM staging		Total	One way annova test
	Stages I and II	Stages III and IV		
Negative	14(52%)	13(48%)	27	F=3.235 p= 0.062
Low	4(33%)	8(67%)	12	F=2.135 p= 0.082
Moderate	**2**(25%)	**6**(75%)	**8**	F=6.222 **p= 0.019**
Total	20	27	47	

Table 2 and 3 shows that though no significant relationship was found between histological grade and TNM stage with both negative and low NK cell expression, however moderate expression of NK cells was significantly found with high grade tumours showing an inverse relationship with histological grade (7 of 8;p=0.003) and in cases with higher TNM stages(6 of 8;p=0.019)

Table 4- NK cell (CD56) Expression vs. Axillary Nodal Status

NK cell expression	Axillary Nodal Status		Total	One way annova test
	Negative	Positive		
Negative	9(33%)	18(67%)	27	F=6.825 **p= 0.003**
Low	3(25%)	9(75%)	12	F=6.535 **p= 0.001**
Moderate	4(50%)	4(50%)	8	F=2.235 p= 0.182
Total	16	31	47	

Table 4 shows a statistically significant and inverse relationship of axillary nodal status with negative and low NK cell expression cases. However, this relationship started fading with moderate expression of NK cells.

Table 5- NK cell (CD56) Expression Vs ER/PR/Her2 neu status

NK cell expression	ER status		PR status		Her-2 neu status	
	Negative	Positive	Negative	Positive	Negative	Positive
Negative	14(56%)	13(59%)	14(56%)	13(59%)	13(54%)	14(60%)
Low	6(24%)	6(27%)	6(24%)	6(27%)	8(33%)	4(17%)
Moderate	5(20%)	3(14%)	5(20%)	3(14%)	3(13%)	5(21%)
Total	25	22	25	22	24	23

Table 5 shows that positivity with all the three basic immunohistochemistry markers were found to have similar distribution of expression of NK cells, highest in negative NK cells expression and lowest in moderate expression, irrespective of their positivity or negativity. Thus, NK cells expression could not relate with basic immunohistochemistry markers expression.

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Table 6 NK cell expressions and disease free survival (DFS) in study cases

NK cell expression	No. of cases	Mean DFS (in days)	Number of deaths	Number of cases lost to follow up	P value by log rank test
Negative	27	884	3	4	0.41
Low	12	1077	1	1	
Moderate	8	776	2	3	
Total	47				

Mean disease free survival (DFS) could not show any definite relation with negative, low or moderate CD 56+ NK cell expression in primary tumour. P value by log rank test is 0.41. (Table 6)

DISCUSSION

Our study showed the peak incidence of breast carcinoma in the study area (Central India) is in the perimenopausal age group 41-60 years.i.e. 66% of the total cases with mean age being 49 years. This finding is well correlated with other Indian and Asian studies.[8,9,10,11,12,13] Leong SP et al[14] in their review paper stated that there is a striking difference in the peak age of incidence in the western countries (60-70 years) and in Asian countries (40-50 years).As shown in figure 1, most of the cases in our series presented in TNM stage II, III and IV (17, 14 and 13 respectively). As far as histological grade is concerned, it was seen that 64% (n=30) of study cases were diagnosed to be of histological grade III and most of them (n=20) were at TNM stage II and III at the time of clinical presentation. These findings are consistent with several other studies in India and western countries [8,9,10] CD 56+ NK cells as tumour infiltrating lymphocyte (TIL) in our study cases of breast carcinoma were generally present in low numbers. 57%Cases (27/47) no NK cells were present at all followed by low count in 12 cases (25%) and moderate count in further 8 cases (18%). We could not find high NK cell count (Table 2) These findings correlate well with Georgiannos SN et al[16] who stated that there is paucity of NK cells: CD 56 + score of 0 (21/60 cases) or 1 (31/60 cases) and Garner WL et al[15] also found significantly less level of NK activity of all patients with breast cancer. Park MH et al[19] studied NK cell using CD 57 as a immunohistochemistry marker found out that very few cells show positive staining. Macchetti AH el al[18] too found that tumours are poorly infiltrated by NK cells using flow cytometry. de Kruijf EM et al[20] also found that NK cells using PEN 5 as immunoshistochemical marker, are rarely found in 822 study cases of primary breast cancer.

We could not find any significant relationship of NK cell count and other basic IHC marker positivity. These findings correlate well with other studies done by Rathore AS et al[7] , Georgiannos SN et al[16]and Garner WL et al[15] stating no significant co-relation with NK cell expression and the ER, PR and HER2/neu status of the patient.

Present study showed that though negative and low NK cell expression could not correlate with the histological grading of tumours, however, in moderate NK cell expression 7 out of 8 cases (88%) belong to high histological grade which was proven to be statistically significant.This suggests that presence of CD 56+NK cell is associated more with high grade tumours rather than low grade tumours. This finding correlate well with study done by Rathore AS et al[7] who too found that high NK cell count was found in high grade tumours with p value <0.0001, thus having a significant and direct association. Also, Vgenepoulou Set al[17] studied CD 57+NK cell in breast cancer cases found an increased number of NK cells in grade III tumor (p value 0.003). However, Georgiannos SN et al[16] found an insignificant relation of NK cell expression and histological grading.

The present study showed a statistically significant and inverse relationship of axillary lymph node status with NK cell expression. (Table 6). Our finding correlate well with Garner WL et al[15] who found decreased NK activity with the presence of palpable axillary metastases.Our findings did not correlate with Rathore AS et al[7] who found direct relationship of intratumoral CD 56 cell count and lymph node status as 51/85 cases with high NK cell count show positive lymph nodes and 56/90 cases with low NK cell count show no metastases to lymph nodes with a p value of 0.003 and Vgenopoulou S et al[17] found an increase number of NK cells in cases with more than 3 involved lymph nodes.However, Georgiannos SN et al[16] found an insignificant association between NK cell count and lymph node status.

We also evaluated mean DFS in our study cases in overall cases(Table 8), where we could not find any relationship at all, with NK cell expression in the tumor and prognosis.

Rathore AS et al[7] stated that cases with a low intratumoral CD 56+ NK cell count had a better prognosis with 0.52 fold lower death rate as compared to patients with high CD 56+ NK cell count. Same findings are reported by Vgenopoulou S et al[17] i.e. NK cell expression is associated with comparatively poor prognosis than other types of TILs.However, Park MH et al[19] in their study determined that high expression of CX3CL1 (chemoattractant for T-cells and NK cells) by tumour cells cause increase in stromal CD 57+ NK cells and number of tumour-infiltrating CD8T cells and Dendritic Cells which correlates with a good prognosis in breast carcinoma cases.This may point towards the suggestion that besides NK cells some other factors too, like cytokines, other tumour infiltrating cells and stromal factors are also responsible for prognosis and need to

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be evaluated, especially the nature of the NK cells infiltrating the tumour, their phenotype and subset distribution, potential interaction with other immune cells, and presence of various cytokines in the tumour milieu.Poli A et al[22] suggested that $CD56^{bright}$ $CD16^{dim}$NK cells perform antibody dependent cellular cytotoxicity only weakly. $CD56^{bright}$ NK cells are very likely precursor cells of the $CD56^{dim}$NK cells and are less mature as they display shorter telomeres.Levy EM et al[23] stated in his review that in metastatic melanoma patients, an increase was observed in noncytotoxic $CD16^{dim}CD56^{bright}$.. The tumour cells shed soluble NK cell receptor ligands in the serum in large quantities which downregulates the NK cell receptors and might contribute to the decreased levels of NK cell activity. Mamessier E et al[6] stated that NK cells cytotoxicity is blunted and their final maturation processis prevented because of many causes creating a multifaceted immunosuppressive microenvironment in the tumour, thus leading to breast tumour progression. Wang B et al[21] in his study concluded that due to reduced expression of MICA and MICB, two ligands for the stimulatory NK cell receptor NKG2D, primary breast cancer stem cells(BCSC) were resistant to cytotoxicity medicated by autologous/allogeneic NK cell. The use of all-trans-retinoic acid (ATRA), a breast cancer cell differentiation inducing agent, restored miR-20a-MICA/MICB axis and sensitized BCSC to NK cell-mediated killing.

CONCLUSION

Whatever little is known about role of NK cells, it is beyond doubt that these cells home less in breast carcinoma cases. However, other findings are mixed and confusing at present. Few studies found less NK cell with advanced disease[15] while few others[7,17] found more in similar conditions. The multifaceted immunosuppressive microenvironment should be studied in detail as to find the reason behind NK cell inactivity when present. Study concentrates towards a possible immunotherapeutic solution of breast carcinoma in the form of reactivation of NK cells. Data from the present study is still incomplete and not well balanced for all molecular types of invasive ductal carcinoma, owing to less number of cases due to financial constraints of a small study.

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REFERENCES

1. Bray F, Laversanne M, Sung H, Ferlay J, , Siegel RL, Soerjomataram I, Jemal A. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries CA: a cancer journal for clinicians. 2024 May;74(3):205-313.
2. Fidler IJ. The pathogenesis of cancer metastasis: the'seed and soil'hypothesis revisited. Nature reviews cancer. 2003 Jun;3(6):1-6.
3. Joyce JA, Pollard JW. Microenvironmental regulation of metastasis. Nature reviews cancer. 2009 Apr;9(4):239-252.
4. Kute TE, Savage L, Stehle JR, Kim-Shapiro JW, Blanks MJ, Wood J, Vaughn JP. Breast tumor cells isolated from in vitro resistance to trastuzumab remain sensitive to trastuzumab anti-tumor effects in vivo and to ADCC killing. Cancer immunology, immunotherapy. 2009 Nov 1;58(11):1887-96.
5. Smyth MJ, Dunn GP, Schreiber RD. Cancer immunosurveillance and immunoediting: the roles of immunity in suppressing tumor development and shaping tumor immunogenicity. Advances in immunology. 2006 Jan 1;90:1-50.
6. Mamessier E, Sylvain A, Thibult ML, Houvenaeghel G, Jacquemier J, Castellano R, Gonçalves A, André P, Romagné F, Thibault G, Viens P. Human breast cancer cells enhance self tolerance by promoting evasion from NK cell antitumor immunity. The Journal of clinical investigation. 2011 Sep 1;121 (9):3609-22.
7. Rathore AS, Goel MM, Makker A, Kumar S, Srivastava AN. Is the tumor infiltrating natural killer cell (NK-TILs) count in infiltrating ductal carcinoma of breast prognostically significant. Asian Pac J Cancer Prev. 2014 Jan 1;15(8):3757-61
8. Saxena S, Rekhi B, Bansal A, Bagga A, Murthy NS. Clinicomorphological patterns of breast cancer including family history in a New Delhi hospital, India-A cross-sectional study. World journal of surgical oncology. 2005 Dec;3(1):67-73.
9. Acharya SC, Jha AK, Manandhar T. Clinical profile of patients presenting with breast cancer in Nepal. Kathmandu University Medical Journal. 2012;10(3):3-7.
10. Khokher S, Qureshi MU, Riaz M, Akhtar N, Saleem A. Clinicopathologic profile of breast cancer patients in Pakistan: ten years data of a local cancer hospital. Asian Pacific Journal of Cancer Prevention. 2012;13(2):693-98.
11. Wei JT, Huang WH, Du CW, Qiu SQ, Wei XL, Liu J, Zhang GJ. Clinicopathological features and prognostic factors of young breast cancers in Eastern Guangdong of China. Scientific reports. 2014 Jun 19;4:5360.
12. Khokhar A. Breast cancer in India: where do we stand and where do we go?. Asian Pacific Journal of Cancer Prevention. 2012;13(10):4861-66.
13. Adnan M, Saldanha E, Palatty PL, Naik T, Monteiro AD. Incidence of Breast Cancer in Women from Agriculture based Areas of Costal Karnataka, India: A Twenty Year Observations from a Tertiary Care Hospital. Clin Oncol. 2018;3:1437.
14. Leong SP, Shen ZZ, Liu TJ, Agarwal G, Tajima T, Paik NS, Sandelin K, Derossis A, Cody H, Foulkes WD. Is breast cancer the same disease in Asian and Western countries?. World journal of surgery. 2010 Oct 1;34(10):2308-24.
15. Garner WL, Minton JP, James AG, Hoffmann CC. Human breast cancer and impaired NK cell function. Journal of surgical oncology. 1983 Sep;24(1):64-66.
16. Georgiannos SN, Renaut A, Goode AW, Sheaff M. The immunophenotype and activation status of the lymphocytic infiltrate in human breast cancers, the role of the major histocompatibility complex in cell-

DOI: 10.69605/ijlbpr_14.2.2025.135

mediated immune mechanisms, and their association with prognostic indicators. Surgery. 2003 Nov 1;134(5):827-34.

17. Vgenopoulou S, Lazaris AC, Markopoulos C, Boltetsou E, Kyriakou V, Kavantzas N, Patsouris E, Davaris PS. Immunohistochemical evaluation of immune response in invasive ductal breast cancer of nototherwise-specified type. The Breast. 2003 Jun 1;12(3):172-78.
18. Macchetti AH, Marana HR, Silva JS, Andrade JM, Ribeiro-Silva A, Bighetti S. Tumor-infiltrating CD4+ T lymphocytes in early breast cancer reflect lymph node involvement. Clinics. 2006 Jun;61(3):203-8.
19. Park MH, Lee JS, Yoon JH. High expression of CX3CL1 by tumor cells correlates with a good prognosis and increased tumor-infiltrating CD8+ T cells, natural killer cells, and dendritic cells in breast carcinoma. Journal of surgical oncology. 2012 Sep 15;106(4):386-92.
20. de Kruijf EM, Engels CC, van de Water W, Bastiaannet E, Smit VT, van de Velde CJ, Liefers GJ, Kuppen PJ. Tumor immune subtypes distinguish tumor subclasses with clinical implications in breast cancer patients. Breast cancer research and treatment. 2013 Nov 1;142(2):355-64.
21. Wang B, Wang Q, Wang Z, Jiang J, Yu SC, Ping YF, Yang J, Xu SL, Ye XZ, Xu C, Yang L. Metastatic consequences of immune escape from NK cell cytotoxicity by human breast cancer stem cells. Cancer research. 2014 Oct 15;74(20):5746-57
22. Poli A, Michel T, Thérésine M, Andrès E, Hentges F, Zimmer J. CD56bright natural killer (NK) cells: an important NK cell subset. Immunology. 2009 Apr;126(4):458-65.
23. Levy EM, Roberti MP, Mordoh J. Natural killer cells in human cancer: from biological functions to clinical applications. BioMed Research International. 2011Apr26;2011:1-10.