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Original Research Article

A Study of Ki-67 Expression in Cases of Breast Carcinoma- A Retroprospective Study

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Abstract

Background: Most common malignancy in women in western world is breast cancer. Ki-67 is a prognostic and proliferative marker distinguishing luminal type A from type B breast cancer.

Aims: The aim of the present study was to detect Ki-67 expression in breast carcinoma cases by IHC and to study Ki-67 expression with some histopathological features such as histological type of tumor, grade of tumor & involvement of lymph node (LN).

Study Design: Retroprospective

Material and Methods: The retroprospective study of 36 cases was conducted in the Department of Pathology at tertiary care hospital (U.P. West) during a period of 5 years.

Statistical Analysis used: It was done by a descriptive statistics and chi square test to look for any correlation between Ki-67 expression with histological type of tumor, grade of tumor and lymph node involvement. It was considered significant when p value was <0.05.

Result: Invasive carcinoma of no special type were the most common histological malignancy followed by invasive lobular carcinoma, carcinoma of neuroendocrine differentiation and metaplastic carcinoma. Majority of breast carcinoma cases showed T2 stage, Nottingham Modified Bloom Richardson System grade II and lymph node positivity. 63.89% cases of breast cancer were under group 1 of Ki-67 positivity, 25% were under group 2 Ki-67 positivity and 11.11% cases showed group 3 Ki-67 positivity.

Conclusion: Invasive carcinoma of no special type was the commonest histological type. No statistically significant association of Ki-67 positivity was found with grade, histological type, TNM stage & lymph node positivity.

Keywords: Breast; Ki-67; Cancer; Positivity; Grade.

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Introduction

Most common malignancy in women in western world is breast cancer affecting one in eight [1]. In India, cervical cancer is the most common cancer followed by breast cancer in women. Annually, approximately 80,000 cases occur; the age adjusted incidence rate (AAR) range is 16 to 25 per 100,000 population [2]. Annually, global breast cancer incidence increased by 3.1% from 1980 to 2010 [3].

Breast cancer is no longer seen as a single disease but rather a multifaceted disease comprising of distinct biological subtypes with diverse natural history, presenting a varied spectrum of clinical, pathological and molecular features with different prognostic and predictive implications. Age of the patient, menopausal status, family history, tumor size, grade, lymph node status and distant metastasis are among the numerous factors affecting the prognosis. At the molecular level, prognostic factors

include ER, PR, HER-2/neu, Ki-67, p53, cyclin D1 and VEGF. Ki-67 protein is present during all active phases of the cell cycle (G_1, S, G_2, and mitosis), but is absent in resting/ quiescent cells (G_0) [4]. Ki67 labelling index is defined as percentage of cancer cell nuclei that are positive for Ki67 immunostaining over total cancer cell nuclei on histopathological slide. The Ki-67 index, proliferation marker is a prognostic factor in primary breast cancer and also distinguishes between luminal type A and type B breast cancer. High Ki-67 index values are associated with poorly differentiated breast carcinomas, invasive carcinoma of no special type (NST) with necrosis and in triple negative breast carcinomas. Carcinomas with high proliferation rates have a poorer prognosis.

Aims

The aim of the present study was to detect Ki-67 expression in breast carcinoma cases by IHC and to study Ki-67 expression with some histopathological features such as histological type of tumor, grade of tumor & involvement of lymph node (LN).

Materials and Methods

The retroprospective study was conducted in the Department of Pathology at Tertiary care hospital, West Uttar Pradesh retrospectively for 4 years (July 2012 to June 2016) and prospectively for one year (July 2016 to June 2017). The study included 36 cases (out of 36 cases, 29 cases were mastectomy and 7 lumpectomy cases) observed during a period of 5 years (July 2012 to June 2017). Complete anonymity of the patients was maintained in retrospective cases and written informed consent, an institutional ethical clearance was obtained in all prospective cases.

The breast tissue was fixed in 10% buffered formal saline and was routinely processed and embedded in paraffin wax. 3-4 µm thick sections were taken from each block for Haematoxylin and Eosin staining (H & E). Sections stained with H&E were used to identify the histological type & grade of breast cancer. For Ki-67 detection, sections were taken on PLL (Poly L- Lysine) coated slides for immuno-histochemistry. On IHC, brown colour of the nucleus was taken as Ki-67 positivity.

For the purpose of this study- Ki-67 values were divided into 3 groups (Nishimura R et. al., 2014)

- Group 0 - Non -stained.
- Group 1- The fraction of proliferating cells are <20%.
- Group 2- The fraction of proliferating cells are 20-49%.
- Group 3- The fraction of proliferating cells are ≥50%.

Observations and Results

Out of 36 cases of breast carcinoma, majority were of invasive carcinoma of no special type (29 cases, 80.55%) followed by invasive lobular carcinoma (3 cases, 8.35%) and carcinoma of neuroendocrine differentiation, metaplastic carcinoma (2 cases, 5.55% each).

23/36 (63.89%) cases of breast cancer were under group 1 of Ki-67 positivity followed by 9/36 were under group 2 Ki-67 positivity (25%) and 4 cases showed group 3 Ki-67 positivity (11.11%).

Table 1: Ki-67 status according to histological type (n= 36)

Histological Type	Total No. of Cases	Ki-67 Group 1		Group 2		Group 3	
		n	%	n	%	n	%
Invasive Carcinoma of No Special Type	29	19	65.52	8	27.58	2	6.90
Invasive Lobular Carcinoma	3	2	66.67	1	33.33	0	0
Carcinoma with neuroendocrine differentiation	2	2	100	0	0	0	0
Metaplastic Carcinoma	2	0	0	0	0	2	100

Table 2: Ki-67 status according to nottingham modified bloom richardson system grade of tumor (n= 36)

Grade	Total No. of Cases (%age)	Ki-67 Group 1		Group 2		Group 3	
		n	%	n	%	n	%
I (3-5 score)	10 (27.78)	9	90	1	10	0	0
II (6-7 score)	18 (50.00)	12	66.67	4	22.22	2	11.11
III (8-9 score)	8 (22.72)	2	25	4	50	2	25

Table 3: Ki-67 status according to TNM stage (n=29)

TNM Stage	Total No. of Cases	Ki-67					
		Group 1		Group 2		Group 3	
		n	%	n	%	n	%
Stage 0	0	0	0	0	0	0	0
Stage IA	2	1	50	1	50	0	0
Stage IB	0	0	0	0	0	0	0
Stage IIA	5	3	60	2	40	0	0
Stage IIB	5	2	40	2	40	1	20
Stage IIIA	11	7	63.64	3	27.27	1	9.09
Stage IIIB	0	0	0	0	0	0	0
Stage IIIC	6	4	66.67	0	0	2	33.33

Table 4: Ki-67 status according to lymph node positivity (n= 29)

Lymph node	Total No. of Cases (% age)	Ki-67					
		Group 1		Group 2		Group 3	
		n	%	n	%	n	%
Lymph Node Positive	20 (68.97)	12	60	5	25	3	15
Lymph Node Negative	9 (31.03)	5	55.56	3	33.33	1	11.11

[Out of total 36 breast carcinoma surgical specimens, lymph nodes recovered in 29 cases only (n= 29)]

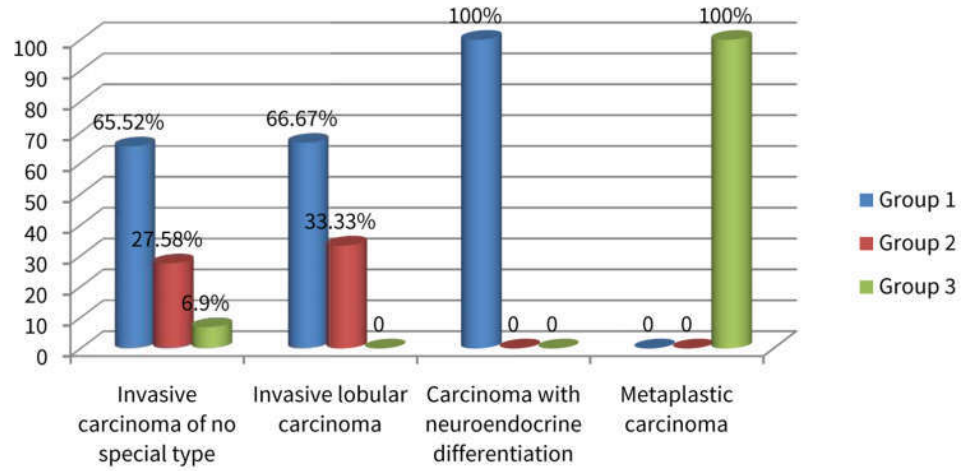


Fig. 1: Ki-67 Status According To Histological Type (N= 36)

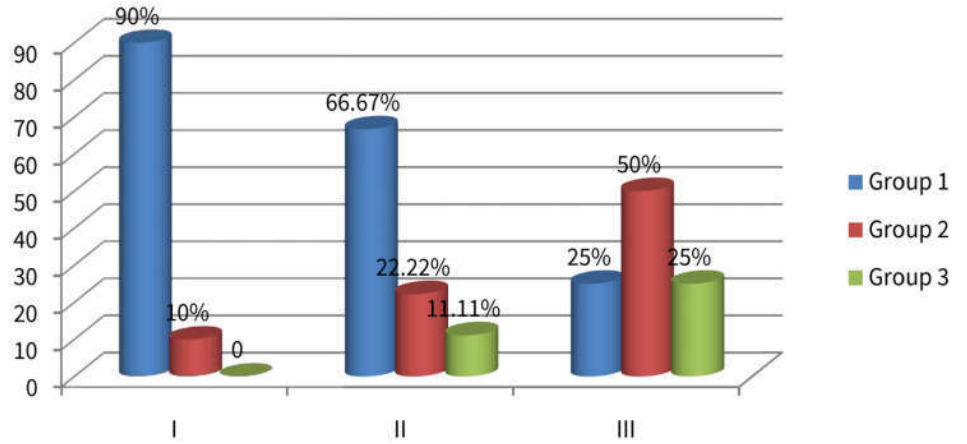


Fig. 2: Ki-67 Status According To Nottingham Modified Bloom Richardson System Grade Of Tumor (n= 36)

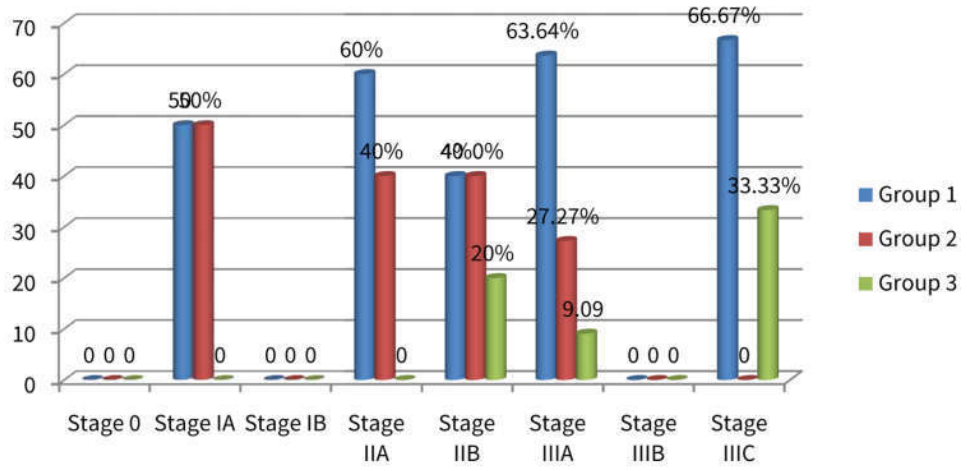


Fig. 3: Ki-67 status according to t staging as per ajcc 2013 classification in breast carcinoma (n= 36)

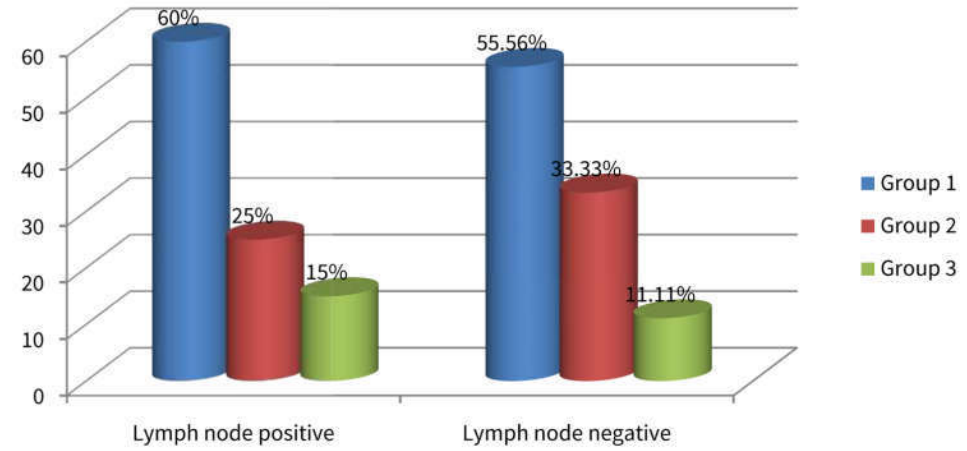


Fig. 4: Ki-67 status according to lymph node positivity (n= 29)

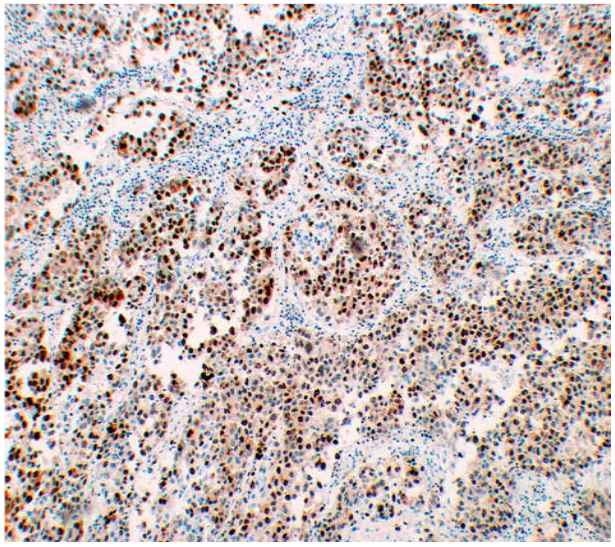
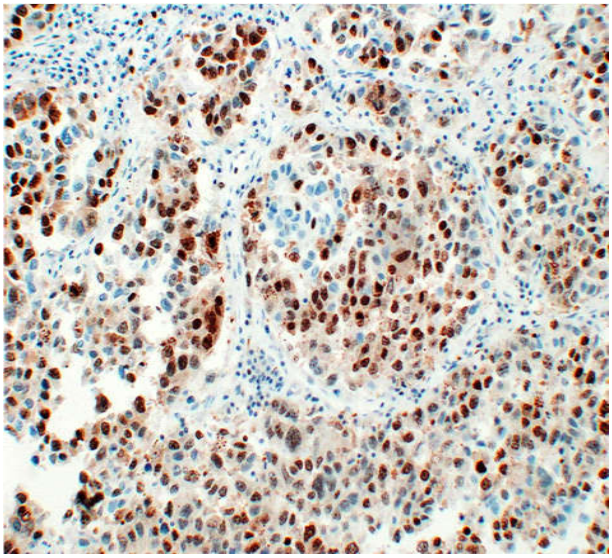


Fig. 1: Expression of Ki-67: photomicrograph showing nuclear staining within/ >50% of invasive tumor cells (group 3). (IHC, 100X)



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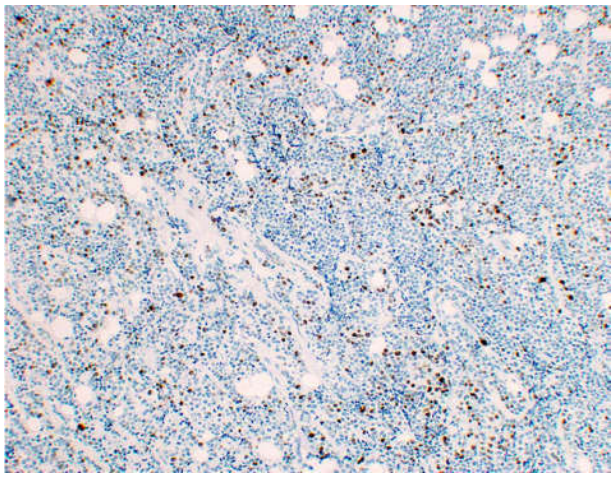

Fig. 3: Expression of Ki-67: photomicrograph showing nuclear staining between 20 to 49% of invasive tumor cells (group 2). (IHC, 100X)

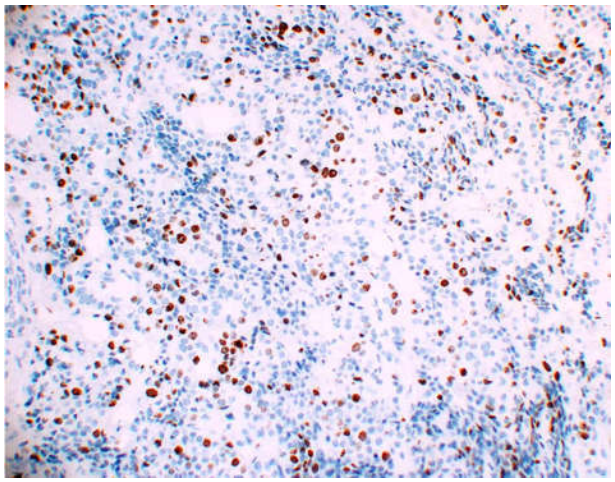

Fig. 4: Expression of Ki-67: photomicrograph showing nuclear staining between 20 to 49% of invasive tumor cells (group 2). (IHC, 200X)

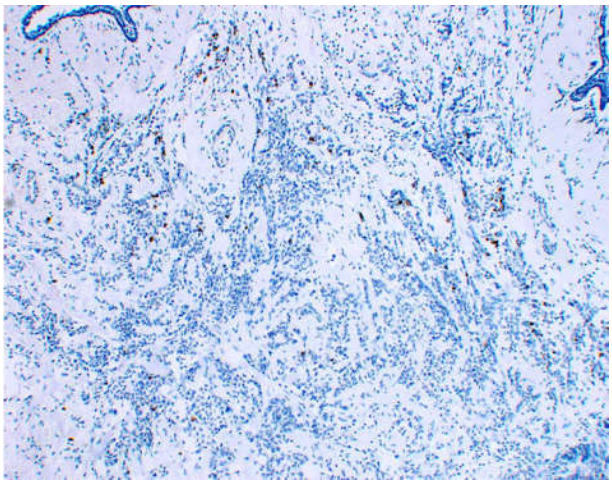

Fig. 5: Expression of Ki-67: photomicrograph showing nuclear staining in <20% of invasive tumor cells (group1). (IHC, 100X)

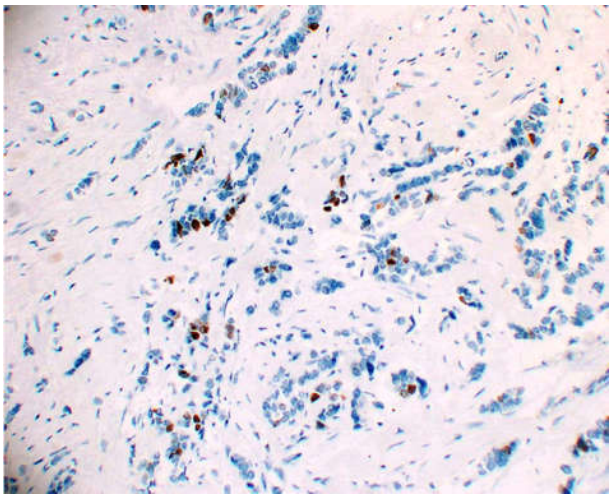

Fig. 6: Expression of Ki-67: photomicrograph showing nuclear staining in <20% of invasive tumor cells (group1). (IHC, 200X)

Discussion

The terminology for the most common histological type of breast carcinoma has changed from invasive ductal carcinoma, not otherwise specified (NOS) 2003 to invasive carcinoma of no special type (NST) 2012 [5]. Special subtypes of invasive breast carcinoma include invasive lobular, tubular, cribriform, metaplastic, apocrine, mucinous, papillary and micropapillary carcinoma. In the present study, invasive carcinoma of no special type was the commonest histological type (80.55%) followed by invasive lobular carcinoma (8.35%), carcinoma with neuro-endocrine differentiation and metaplastic carcinoma (5.55% each). Almost similar results have been reported in the past by various workers [6,7,8].

Comparative analysis of distribution of histological variants of the study with others

Histological Type	Hameed 2008 (%)	Ayadi et al 2008 (%)	Puvitha & Shifa 2016 (%)	Present study (%)
IBC- Nos type	>70	83.3	79	80.55
Invasive lobular carcinoma	5-15	3.8	3	8.35
Solid neuroendocrine carcinoma	Rare	1.9	3	5.55
Metaplastic carcinoma	2-5	0.6	3	5.55

Results of the present study were in close proximity to Ayadi et al, 2008. Shailza & Mohan, 2015 also reported invasive carcinoma of no special type as the commonest morphological type accounting for 88.9%.

Ki-67 and Histologic Type

Higher Ki-67 expression (group 3) was seen in all metaplastic breast carcinoma cases (100%). Higher Ki-67 expression (group 3) was seen in invasive carcinoma of no special type in comparison to invasive lobular carcinoma. Invasive lobular carcinoma and carcinoma with neuroendocrine differentiation showed group 2 Ki-67 positivity. There was no statistically significant relation between Ki-67 and histological type in this study. Thor et al, 2008 found that MIB1 labelling in invasive ductal carcinoma was significantly higher (mean, 32.2%; median, 28.6%; range, 0% to 99%) than in invasive lobular carcinomas (median, 20.8%; mean, 17.8%; range, 2% to 52%). High MIB1 labelling was also associated with diagnosis before age 50, ER negativity, PR negativity, high tumor grade, larger tumor size and lymph node metastases.

Association of Ki-67 with Grade

All the cases were graded according to Nottingham modification of Bloom Richardson method. Majority of the cases were in grade II (50.00%) followed by grade I (27.78%) and grade III (22.72%). A large number of studies have reported majority of patients in grade II [8,9.10].

In the present study, increased expression of Ki-67 (group 3) was associated with grade III breast tumors (25%) followed by grade II breast tumors (11.11%). The results were found to be statistically insignificant.

MdPaiman et al (2014) [11] considered Ki-67 (MIB-1) as an independent proliferative marker and correlated it with clinical stage, histopathological grade, ER/PR positivity and HER-2/neu receptor status. The authors concluded that increased expression of Ki-67 was associated with a higher clinical stage (Stage III), with increased histopathological grade (III).

TNM Stages

TNM stage at presentation at 4 major cancer centers in India indicated that almost 50% of patients present with locally advanced disease. Quite a few patients have large operable breast cancers (Saxena et al, 2005). Some 8-10% of patients have TNM stage IV disease at presentation, and only very few (approximately 5%) have stage I disease.

Stage of breast cancer at presentation at 4 major cancer centers.

Stage	Agarwal & Ramakant 2000 Lucknow[12]	Chopra 2001 Trivandrum[13]	Patient % Chopra 2001 Chennai[13]	Chopra 2001 Mumbai[13]	Present study
I	4	4.4	1	7.8	6.90
II	33	42.3	23	57.4	34.48
III	47	40.5	52	28.9	58.62
IV	9	12.8	24	5.9	0.00

In the present study stage III was most common breast carcinoma (58.62%) followed by stage II (34.48%) and stage I (6.90%). No case was found in stage IV. This is almost similar to study done by Chopra; 2001 Chennai. In Shrivastava et al (2016) study also most of the patient of carcinoma breast presented in stage II (45.7%) followed by stage III (32.8%), stage IV (17.1%) and stage I (4.2%).

In western countries, stage I breast carcinoma (56.4%) are the majority followed by stage II and III possibly due to increased awareness and aggressive breast screening programs [14].

Conclusion

Invasive carcinoma of no special type was the commonest histological type of breast carcinoma. In majority of breast carcinoma cases, T staging (tumor size between 2-5 cms) and showed Nottingham Modified Bloom Richardson System grade II. Majority of cases showed lymph node positivity. Out of 36 cases, 23 (63.89%) cases of breast cancer were under group 1 of Ki-67 positivity, 9 (25%) were under group 2 Ki-67 positivity and 4 (11.11%) cases showed group 3 Ki-67 positivity. No statistically significant association of Ki-67 positivity was found with grade of tumor, histological type, TNM stage & lymph node positivity ($p > 0.05$).

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Conflict of interest: None declared

Ethical approval: Not required

References

1. Carter D, Schmitt SJ and Millis RR. The breast In Sternberg's diagnostic surgical pathology. Stacey E. Millis (Editor) 5th ed. Wolters Kluwer, Lippincott, Williams Wilkins, 2010;Vol. 1.p.285.

2. Harrison PA, Srinivasan K, Binu VS, Vidyasagar MS, Nair S. Risk factors for breast cancer among women attending a tertiary care hospital in southern India. International Journal of Collaborative Research on Internal Medicine & Public Health. 2010;2(4):109-16.
3. Forouzanfar MH, Foreman KJ, Delossantos AM, Lozano R, Lopez AD, Murray CJL, et al. Breast and cervical cancer in 187 countries between 1980 and 2010: a systematic analysis. Lancet. 2011 Oct 22;378(9801):1461-84.
4. Bruno, S. and Darzynkiewicz, Z. Cell cycle dependent expression and stability of the nuclear protein detected by Ki-67 antibody in HL-60 cells. Cell Proliferation, 1992;25:31-40. doi:10.1111/j.1365-2184.1992.
5. Lakhani, S. R., I. O. Ellis, S. J. Schnitt, P. H. Tan, and M. J. Van de Vijver. "World Health Organisation classification of tumors of the breast." *Lyon: IARC* (2012).
6. Ayadi L, Khabir A, Amouri H, Karray S, Dammak A, Guermazi M, et. al. Correlation of HER-2 over-expression with clinicopathological parameters in Tunisian breast carcinoma. World J Surgical Oncol 2008;6:112-19.
7. Hammed O. Washington Manual of Surgical Pathology. 1st ed. Philadelphia: Lippincott Williams & Wilkins; 2008.p. 256-8.
8. Puvitha RD, Shifa S. Breast carcinoma, receptor status, and HER-2/neu over expression revisited. International J. of Scientific study. 2016;3:52-58.
9. Suvarchala SB, Nageshwararao R. Carcinoma breasthisto-pathological and hormone receptors correlation. J Biosci Technol 2011;2:340-8.
10. Shailza and Mohan A. correlation of HER-2 over-expression and tumor grade in ductal carcinoma breast. J. of basic and applied medical research: 2015;5:157-64.
11. MdPaiman, Noorasmaliza, Siti Aishah Md Ali, Reena MdZin, Meor Zamari Meor Kamal, Wan Anna Md Amin, Mohan Nallusamy, Pavitratha Puspanathan, Rohaizak Muhammad, Sharifa Ezat Wan Puteh, and Srijit Das. "Estrogen receptor-negative breast ductal carcinoma: clinicopathological features and Mib-1 (Ki-67) proliferative index association." *PloS one* 2014;9(2):e89172.
12. Agarwal G, Ramakant P. Breast Cancer Care in India: The Current Scenario and the Challenges for the Future. Breast Care (Basel). 2008 Mar;3(1):21-7.
13. Chopra R. The Indian scene. J Clin Oncol. 2001;19(18 Suppl): 106S-11.
14. Alvarez Goyanes RI, Escobar Pérez X, Camacho Rodríguez R, Orozco López M, Franco Odio S, Llanes Fernández L, et al. Hormone receptors and other prognostic factors in breast cancer in Cuba. MEDICC Rev. 2010;12:3640.