Original Paper

Correlation of Clinicopathological Features of Breast Cancer with Molecular Subtypes Taking Ki-67 into Consideration: Single Institution Experience Over 5 Years

CHIRAG PEREIRA[1], MANOHAR MARTIS[1], ROHAN D'SOUZA[1], LEO FRANCIS TAURO[1]

[1]Department of General Surgery, Father Muller Medical College and Hospital Mangalore, India

ABSTRACT: Background. Molecular classification of breast cancer is commonly done to determine response to therapy and cancer prognosis. Aim of the study was to compare prevalence of molecular subtypes of breast cancer in our institute using immunohistochemistry, including Ki-67, and correlate it with clinical and pathological prognostic factors. Results. 300 cases of invasive breast cancer were included in the study. Average age at time of diagnosis was 44 years and average size of tumor was 3.4cms. Invasive ductal carcinoma was the most common histological type (75.3%). The most common molecular subtype was triple negative (34.3%) followed by Luminal B (33.4%), luminal A (17%) and Her-2 positive (15.3%). Large size and poorly differentiated tumors were predominantly triple negative tumors while lymph node metastasis was most commonly seen in Her-2 positive tumors. Conclusion. Molecular subtype of breast carcinoma should routinely be done for all cases of carcinoma breast as it allows to identify aggressive tumors and target therapy accordingly.

KEYWORDS: *Breast neoplasm, immunohistochemistry, mastectomy, Ki-67.*

Introduction

According to the World Health Organization (WHO) 2018 reports, breast cancer is the second most common cancer worldwide and leading cause of cancer death among women, accounting for 15% of cancer deaths worldwide.

According to Globocan (WHO), [1] in the year 2018, breast cancer in India ranked first in newly diagnosed cancer and cancer related deaths at 162,468 and 87,090, respectively.

Molecular classification of breast cancers started about 30 years ago when hormone receptors could be marked. [2].

The objective for determining the molecular subtype of breast cancer is to predict response to different therapies and obtain prognostic information.

There are various methods of subtyping breast cancer from simple histopathological analysis to complex gene analysis.

Among the various methods available, classification of breast tumors into luminal and basal-like tumors gives the clearest picture [2].

According to St. Gallen Consensus [3], the four molecular subtypes of breast cancer are luminal A, Luminal B, triple negative and Her 2 overexpression which are classified based on Estrogen Receptor (ER), Progesterone receptor (PR), Human Epidermal Growth factor receptor 2 (Her-2) and Ki-67 molecular expression.

Luminal A tumors are ER positive and/or PR positive, Her-2 negative and show a low Ki-67 (<20%) [4].

Luminal B on the other hand includes tumors that are either ER/PR positive and Her-2 positive or ER/PR positive, Her-2 negative but shows high proliferation rate (Ki-67>20%).

Triple negative does not show any molecular expression and Her 2 overexpression is ER/PR negative and Her-2 positive.

Luminal type of breast cancers arises from epithelial cells that line the ducts of mammary glands. The have a much better prognosis as compared to non-luminal types since they show response to hormonal therapy.

The Her 2 enriched and triple negative tumors arise from the basal cells of mammary glands. They are associated with a poor prognosis. These tumors are often aggressive, have increased rates of recurrence and metastasis [5].

While studies have been done to look for correlation between clinicopathological features and molecular types of breast cancer, many studies fail to take Ki-67 into consideration which can significantly alter the type of cancer the oncologist deals with.

In the present study we aimed to analyze the prevalence of breast cancer subtypes at our institute and associated clinicopathological features, and compare our data with existing literature in India and rest of the world.

10.12865/CHSJ.47.03.03

Methods

We retrospectively reviewed the histopathological reports of all patients that had undergone modified radical mastectomy and breast conservative surgery in the departments of general surgery from January, 2014 to August, 2020.

Informed consent from patients and institutional ethics committee approval was taken prior to starting the study.

Inclusion criteria included patients with invasive breast cancer and patients with available histopathological and immunohistochemistry reports.

Patients that received chemotherapy prior to mastectomy, recurrent disease and patients that underwent lumpectomy prior to mastectomy were excluded from the study.

The following data was recorded: age at time of diagnosis, tumor size based on histopathology, Scarff Bloom Richardson (SBR) grade, histopathological subtype, lymph nodal involvement, ER, PR and Her-2 receptor status based on immunohistochemical profile and Ki-67 immunohistochemical evaluation.

Based on immunohistology staining all cases in our study was classified into 4 categories: Luminal A, Luminal B, triple negative and Her-2 positive.

Continuous data was described as average±standard deviation.

Data was analyzed using IBM SPSS Statistics for Macintosh, Version 25.0.

Categorical data were stated as percentages while continuous variables were described as mean and standard deviation.

Chi-square test was used to assess association between the different variables.

A p value of less than 0.05 were considered significant.

Results

A total of 300 cases of carcinoma breast were included in the present study.

Positive ER and PR immunostaining were seen in 52.3% and 51.7% of cases respectively. Her-2 positive immunostaining was found positive in 30.7% of cases.

Triple negative was the most common molecular subtype (34.3%) followed by luminal B (33.3%), luminal A (17%) and Her-2 overexpression (15.3%).

At the time of diagnosis, the average age of the patient was 44±8.42 years (age ranging from 28 to 86 years).

Patients aged <50 years had more number of cases showing luminal type tumors (51.1%) as compared to Her-2 positive and triple negative tumors (p=0.939) (Table 1).

The average size of the tumor was 3.4cms (standard deviation of 1.6) at time of diagnosis. 65.7% of cases presented to the hospital when tumor size was between 2 to 5cms, while only 13.6% of cases had tumor size >5cms.

Of the total cases included in the study, 55.3% of cases were positive for lymph node metastasis.

Large size tumors (>5cms) were more commonly seen in triple negative molecular subtypes (19.4%).

Her-2 positive tumors had the highest number of cases of metastatic lymph node spread (67.4%).

However, Chi-square statistics failed to show any significant variation between molecular subtypes and tumor size or nodal metastasis (Table 1).

Table 1. Clinicopathological features of breast cancer according to immunostaining of hormone receptors.

Characteristics	Luminal A (Group 1)		Luminal B		Triple negative		Her-2 positive		Total		p-value
	No	%	No	%	No	%	No	%	No	%	
Age											
<50	26	50.9	46	46	48	46.6	21	45.7	141	47	p=0.939
>=50	25	49.1	54	54	55	53.4	25	54.3	159	53	
Tumor Size											
<=2	17	33.3	19	19	17	16.5	9	19.5	62	20.7	P=0.119
>2-<=5	29	56.9	71	71	66	64.1	31	67.4	197	65.7	
>5	5	9.8	10	10	20	19.4	6	13.1	41	13.6	
LN metastasis											
Negative	23	45.1	41	41	55	53.4	15	32.6	134	44.7	P=0.092
Positive	28	54.9	59	59	48	46.6	31	67.4	166	55.3	
Total	51		100		103		46		300		

Among the various histological types of breast cancer, invasive ductal carcinoma was the most common type at 75.3%, invasive lobular carcinoma was 1.7% and 2.3% cases were mixed (invasive ductal with lobular carcinoma).

The rest of the cases (20.7%) were other histological types that included medullary, mucinous, apocrine, papillary and neuroendocrine type (Table 2).

Most of the cases of cancer that were detected were moderately differentiated (45%) followed by poorly differentiated tumors (36.3%).

Triple negative tumors had the highest percentage of poorly differentiated tumors (46.6% versus 36.3% overall), which is significant (p=0.001) (Table 2).

On assessing the clinical stage, Luminal A had the highest number of cases in Stage I (21.6%) while Her-2 positive tumors has the highest number of cases in grade III (45.7%) (p=0.016).

Most of the patients were diagnosed at stage II (59.7%) (Table 2).

Table 2. Histopathological characteristics and clinical staging of breast cancer according to immunostaining of hormone receptors.

Characteristics	Luminal A		Luminal B		Triple negative		Her-2 positive		Total		p-value
	No	%	No	%	No	%	No	%	No	%	
Histology											
Ductal	40	78.4	73	73	75	72.9	38	82.6	226	75.3	p=0.334
Lobular	2	3.9	1	1	2	1.9	0	0	5	1.7	
Mixed	3	5.9	2	2	2	1.9	0	0	7	2.3	
Others	6	11.8	24	24	24	23.3	8	17.4	62	20.7	
Grade											
1	17	33.3	21	21	15	14.6	3	6.5	56	18.7	P=0.001
2	26	51	46	46	40	38.8	23	50	135	45	
3	8	15.7	33	33	48	46.6	20	43.5	109	36.3	
Clinical Staging											
Stage I	11	21.6	8	8	9	8.7	3	6.5	31	10.3	p=0.016
Stage II	28	54.9	60	60	69	67	22	47.8	179	59.7	
Stage III	12	23.5	32	32	25	24.3	21	45.7	90	30	
Total	51		100		103		46		300		

Although patient with metastatic lymph nodes had elevated levels of Ki 67, it was not statistically significant. (p=0.956) (Table 3).

Table 3. Correlation between Ki-67 index and lymph node involvement.

		Ki 67		p-value
		<20	>20	
Involved Lymph nodes	no	32	102	P=0.965
	yes	40	126	

Discussion

Breast cancer is a heterogenous disease having a varied clinical presentation.

Histopathology alone is not sufficient in determining the behavior of the disease.

In order for us to assess the disease prognosis and outcome, we need to have an understanding of the clinical, pathological and molecular factors.

In our institute, triple negative was the most common molecular subtype followed closely by luminal B subtype.

Studies conducted by Gupta et al [6] and Kumar et al [7] in India, both had luminal A as the most common molecular subtype.

Cohort studies [8,9] conducted in the middle east also showed luminal A to be the most common molecular subtype.

All these studies did not take Ki-67 into consideration while classifying tumors and this may be the reason for higher number of luminal A cases seen in their studies.

The average age at which patients presented to our hospital was 44 years and similar finding were seen in studies done in Saudi Arabia [8] and Oman [9].

There were contrasting observations seen in USA where majority of cases were diagnosed after 55 years of age [10].

34% of patients aged less that fifty years had triple negative molecular subtype which was

10.12865/CHSJ.47.03.03

similar to findings seen in white and African-Americans in a study done by Carey et al. [11].

However, Gupta et al [6] and Lin et al [12] found that younger women presented more commonly with luminal A followed by triple negative molecular subtype.

The average tumor size was 3.4cms and 79.3% of patients presented with a tumor size larger than two centimeters.

Other studies in India [6,13] showed similar findings where tumors were greater than two centimeters at time of presentation.

In countries such as Finland, which has one of the highest participation for breast cancer screening, the tumor size at presentation is less than two centimeters in majority of cases [14].

Tumors larger than 5cms were commonly seen in triple negative subtype in our study and similar findings were seen in a Iraqi cohort study [15].

67.4% of Her-2 positive cases had lymph node metastasis on histopathology followed by luminal B tumors (59%).

Large sample studies from Saudi Arabia [8], USA [11], Iraq [15] and Indonesia [16] all showed that Her-2 positive tumors were more likely to have positive lymph nodes at presentation.

Inic et al [17] found that patients with high Ki 67 are associated with lymph nodal metastasis.

In our study, although we had similar findings it was not statistically significant.

The most common histological type of cancer was invasive ductal carcinoma (75.3%) and is also the most common cancer encountered in studies carried out in other parts of the world.

45% of cases in our study were moderately differentiated and 36.3% of cases were poorly differentiated.

46.6% of triple negative tumors were poorly differentiated and was statistically significant.

Kumar et al [7] also had similar findings. Carey et al [11] found that women with triple negative tumors were 2.5 times more likely to present with poorly differentiated tumors.

Alwan et al [15] in their study found that most clinically advanced tumors (Stage III and IV) were associated with Her 2 positive tumors which was similar to our study.

One of the limitations of our study was that we were unable to include patients with Stage IV/metastatic disease due to insufficient data in medical records.

Another limitation is that since we used histopathology tumor size as size at presentation, we had to exclude cases that had undergone neoadjuvant chemotherapy and this could have affected our findings on the most common molecular subtype of breast cancer.

Conclusion

Molecular subtype of breast carcinoma should routinely be done for all cases of carcinoma breast.

They provide valuable information not only about response to therapy but also about possible clinical outcome.

In our study we found that Her-2 positive tumors are more commonly associated with lymph node metastasis while triple negative tumors were associated with poorly differentiated tumors.

We also recommend routine use of Ki-67 during immunostaining as this can alter the luminal classification of the tumor.

Conflict of Interest

None to declare.

References

1. World Health Organization, 2018, Global Cancer Observatory [online]. Available at: https://gco.iarc.fr/ [Accessed 29.09.2021].
2. Sims AH, Howell A, Howell SJ, Clarke RB. Origins of breast cancer subtypes and therapeutic implications. Nat Clin Pract Oncol, 2007, 4(9):516-525.
3. Gnant M, Harbeck N, Thomssen C. St. Gallen 2011: Summary of the Consensus Discussion. Breast Care (Basel), 2011, 6(2):136-141.
4. Bustreo S, Osella-Abate S, Cassoni P, Donadio M, Airoldi M, Pedani F, Papotti M, Sapino A, Castellano I. Optimal Ki67 cut-off for luminal breast cancer prognostic evaluation: a large case series study with a long-term follow-up. Breast Cancer Res Treat, 2016, 157(2):363-371.
5. Chue BM, La Course BD. Case report of long-term survival with metastatic triple-negative breast carcinoma: Treatment possibilities for metastatic disease. Medicine (Baltimore), 2019, 98(16):e15302.
6. Gupta P, Rai NN, Agarwal L, Namdev S. Comparison of Molecular Subtypes of Carcinoma of the Breast in Two Different Age Groups: A Single Institution Experience. Cureus, 2018, 10(6):e2834.
7. Kumar N, Patni P, Agarwal A, Khan MA, Parashar N. Prevalence of molecular subtypes of invasive breast cancer: A retrospective study. Med J Armed Forces India, 2015, 71(3):254-258.
8. Al-Thoubaity FK. Molecular classification of breast cancer: A retrospective cohort study. Ann Med Surg (Lond), 2019, 49:44-48.
9. Mehdi I, Monem AA, Al Bahrani B, Ramadhan FA. Breast cancer molecular subtypes in oman: correlation with age, histology, and stage distribution-analysis of 542 cases. Gulf J Oncolog, 2014, 1(15):38-48.

10. National Cancer Institute, 2005, SEER cancer Statistics Review [online]. Available at: https://seer.cancer.gov/archive/csr/1975_2005 [Accessed 29.09.2021].
11. Carey LA, Perou CM, Livasy CA, Dressler LG, Cowan D, Conway K, Karaca G, Troester MA, Tse CK, Edmiston S, Deming SL, Geradts J, Cheang MC, Nielsen TO, Moorman PG, Earp HS, Millikan RC. Race, breast cancer subtypes, and survival in the Carolina Breast Cancer Study. JAMA, 2006, 295(21):2492-502.
12. Lin CH, Liau JY, Lu YS, Huang CS, Lee WC, Kuo KT, Shen YC, Kuo SH, Lan C, Liu JM, Kuo WH, Chang KJ, Cheng AL. Molecular subtypes of breast cancer emerging in young women in Taiwan: evidence for more than just westernization as a reason for the disease in Asia. Cancer Epidemiol Biomarkers Prev. 2009, 18(6):1807-1814.
13. Hariharan N, Rao TS, Naidu CK, Raju KVVN, Rajappa S, Ayyagari S, Krishnamohan MVT, Murthy S, Suryadevara A, Boleneni N. The Impact of Stage and Molecular Subtypes on Survival Outcomes in Young Women with Breast Cancer. J Adolesc Young Adult Oncol, 2019, 8(5):628-634.
14. Sihto H, Lundin J, Lundin M, Lehtimäki T, Ristimäki A, Holli K, Sailas L, Kataja V, Turpeenniemi-Hujanen T, Isola J, Heikkilä P, Joensuu H. Breast cancer biological subtypes and protein expression predict for the preferential distant metastasis sites: a nationwide cohort study. Breast Cancer Res, 2011, 13(5):R87.
15. Alwan NAS, Tawfeeq FN. Comparison of Clinico-Pathological Presentations of Triple-Negative versus Triple-Positive and HER2 Iraqi Breast Cancer Patients. Open Access Maced J Med Sci, 2019, 7(21):3534-3539.
16. Widodo I, Dwianingsih EK, Triningsih E, Utoro T, Soeripto. Clinicopathological features of indonesian breast cancers with different molecular subtypes. Asian Pac J Cancer Prev, 2014, 15(15):6109-6113.
17. Inic Z, Zegarac M, Inic M. Difference between Luminal A and Luminal B Subtypes According to Ki-67, Tumor Size, and Progesterone Receptor Negativity Providing Prognostic Information. Clin Med Insights Oncol, 2014, 8:107-111.

Corresponding Author: Chirag Pereira, Department of General Surgery, Father Muller Medical College and Hospital, Mangalore, India, e-mail: chiggy1989@gmail.com