Immunohistochemistry-Based Molecular Subtyping of Breast Carcinoma in Industrial Population in India, Haryana: A Correlation with Clinicopathological Parameters

Sujata Raychaudhuri*, MD, Akanksha Bajaj*♦, MD, Charu Agarwal**, MD, Mukta Pujani*, MD, Kanika Singh*, MD, Varsha Chauhan*, MD, Ekta Gupta***, MD

*Department of Pathology, ESIC Medical College, Faridabad, Haryana, India
**Department of Pathology, Alfalah Medical College, Faridabad, Haryana, India
***Department of Community Medicine, ESIC Medical College, Faridabad, Haryana, India

Abstract

Background: Breast carcinoma is the most prevalent malignancy in females globally and also the leading cause of cancer-related mortality. The immunohisto-chemistry (IHC)-based molecular subtyping has put newer insights into the biological behaviour and clinical management of breast carcinoma.

We conducted the present study to correlate the four IHC-based molecular subtypes: Luminal A, Luminal B, Human epidermal growth factor receptor 2 positive, and triple negative breast carcinoma with various clinicopathological parameters amongst the industrial population of Haryana.

Method: This cross-sectional study was conducted on 92 cases of invasive breast carcinoma, who underwent modified radical mastectomy over a period of 2.5 years with the prior approval of Institutional Ethical Committee at ESIC Medical College and Hospital, Faridabad. We performed routine histopathological examination along with IHC (Estrogen receptor, Progesterone receptor and Human epidermal growth factor receptor 2) study. The correlation of the four molecular subtypes with various clinicopathological parameters were also studied. We analysed the data using SPSS software.

Results: The mean age of the patients in this study was 47 years with a maximum number of cases in the 3rd and 4th decade of their life. The most common subtype was luminal B (40.9%) with the maximum number of cases presenting in stage II (53.26%) and with grade II (51.1%). Triple-negative breast cancer was found to be associated with brisk mitosis, lymphovascular invasion (66.67%), necrosis (77.78%), and ductal carcinoma in situ (66.67%). These findings were clinically significant. ($P<0.05$)

Conclusion: The early age of presentation of breast carcinoma in the industrial population would warrant the need to focus on various molecular subtypes and clini-copathological parameters that may have different prognostic implications in this population.

Keywords: Breast, Immunohistochemistry, Molecular subtying, Industrial population

♦**Corresponding Author:**
Akanksha Bajaj, MD
Department of Pathology, ESIC
Medical College, Faridabad,
Haryana, India
Email: bajaj.akanksha29@gmail.com

MEJC
Middle East Journal of Cancer

Introduction

Breast cancer is known as the most prevalent malignancy among women all over the world (22%) and the leading cause of cancer-related mortality.[1,2] It ranks as the leading cause of cancer in Indian females.[3] The burden of breast cancer is on an increasing trend in both the developed and developing countries with more than 1 million women being diagnosed with breast carcinoma each year.[4] In India, the average age of developing breast cancer has shifted over the last few decades and younger women in the age group of 40 to 50 years are being affected. The life-style changes, such as late age of marriage, reduced breast feeding, and westernization of diet, may attribute to the occurrence of breast cancer in younger age groups in Indian population.[5] The majority of the breast carcinoma are invasive ductal type (IDC), constituting up to 90% of cases out of which 60 to 80% are of no special type or not otherwise specified (NOS).[6]

The factors affecting the prognosis of the breast cancer are tumour size, tumour grade, histological subtype, and lymph node metastasis.[7,8,9] The evaluation of these factors and their correlation with the biomarkers, such as estrogen receptors (ER), progesterone receptors (PR), and human epidermal growth factor receptor 2 (Her- 2), are highly recommended for making therapeutic and management-associated decisions regarding breast cancer cases.[10] The hormone receptors expression and response to hormone therapy along with chemotherapy is a crucial development in the treatment of breast carcinoma.[11] Tumours with overexpression of ER and PR have a favourable prognosis and respond well to adjuvant hormone therapy, tamoxifen, while those with over expression of Her-2 decrease the survival and respond well to trastuzumab, a monoclonal antibody that targets the Her-2 receptors as previously indicated by several studies.[9,10,12,13]

In the present research, the breast cancers were subdivided into four molecular subtypes based upon their IHC profile (ER, PR, and Her-2 expression). ER/PR+, Her-2- correlates with subtype luminal A (Figure 1), ER/PR+, Her-2+ correlates with subtype luminal B (Figure 2), ER/PR-, Her-2+ (Figure 3) with Her-2 positive subtype, and ER/PR-, Her-2- with triple-negative/ basal-like subtype (Figure 4) (triple-negative breast carcinoma (TNBC)).

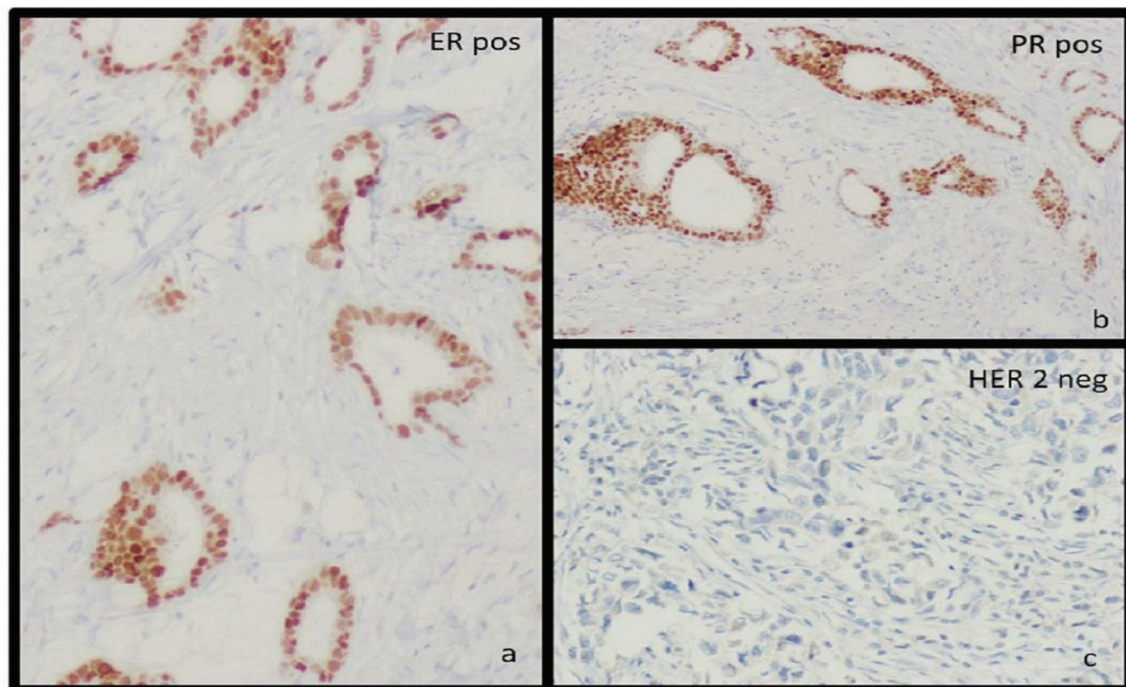Luminal A tumour cells look like the cells of

Figure 1. Luminal A subtype showing estrogen receptor (ER) and progesterone receptor (PR) positivity and human epidermal growth factor receptor 2 (Her-2) negative staining.

Table 1. Molecular subtypes and the associated clinicopathological parameters

Clinico pathological parameters	Molecular Subtypes (n=92)				
	Luminal A (ER/PR+, Her2/neu-) (n=19)	Luminal B (ER/PR+, Her2/neu+) (n=37)	Her2/neu positive (ER/PR-, Her2/neu+) (n=18)	Triple negative (ER/PR-, Her2/neu-) (n=18)	Total (n=92)
Age					
<40 yrs	09(47.37%)	25(67.57%)	10(55.56%)	14(77.78%)	58(63.05%)
>40 yrs	10(52.63%)	12(32.43%)	08(44.44%)	04(22.22%)	34(36.95%)
BRS Grade					
I	12(63.16%)	09(24.32%)	01(05.56%)	00(00.00%)	22(23.91%)
II	04(21.05%)	25(67.67%)	08(44.44%)	02(11.11&)	39(42.39%)
III	03(15.79%)	03(08.10%)	09(50.00%)	16(88.89%)	31(33.70%)
TNM Stage					
I	05(26.32%)	08(21.63%)	02(11.11%)	02(11.11%)	17(18.47%)
IIA	03(15.80%)	15(40.54%)	05(27.78%)	01(05.56%)	24(26.09%)
IIB	07(36.84%)	09(24.33%)	04(22.22%)	05(27.78%)	25(27.17%)
IIIA	02(10.52%)	04(10.80%)	04(22.22%)	05(27.78%)	15(16.31%)
IIIB	00(00.00%)	00(00.00%)	01(05.56%)	00(00.00%)	01(01.09%)
IIIC	2(10.52%)	01(02.70%)	02(11.11%)	05(27.78%)	10(10.87%)
Tumor Size					
<2cm	05(26.32%)	10(27.02%)	05(27.77%)	02(11.11%)	22(23.91%)
2-5cm	12(63.16%)	23(62.17%)	10(55.56%)	07(38.89%)	52(56.52%)
>5cm	02(10.52%)	04(10.81%)	03(16.67%)	09(50.00%)	18(19.57%)
Subtype					
IDC	18 (94.74%)	33(89.19%)	14(77.78%)	15(83.33%)	80(86.95%)
ILC	00(00.00%)	01(02.70%)	01(05.55%)	00(00.00%)	02(02.17%)
Others	01(05.26%)	03(08.11%)	03(16.67%)	03(16.67%)	10(10.87%)
Mitosis					
<5/10hpf	04(21.05%)	07(18.93%)	00(00.00%)	00(00.00%)	11(11.96%)
6-10/10hpf	08(42.11%)	24(64.86%)	06(33.33%)	03(16.79%)	41(44.56%)
>10/10hpf	07(36.84%)	06(16.21%)	12(66.67%)	15(83.21%)	40(43.48%)
LN metastasis					
Absent	12(63.16%)	23(62.17%)	08(44.44%)	05(27.78%)	48(52.18%)
Present	07(36.84%)	14(37.83%)	10(55.56%)	13(72.22%)	44(47.82%)
LVI					
Absent	13(68.42%)	23(62.16%)	07(38.89%)	06(31.58%)	49(53.26%)
Present	06(31.58%)	14(37.84%)	11(61.11%)	12(68.42%)	43(46.74%)
DCIS					
Absent	14(73.68%)	28(75.68%)	11(61.11%)	06(33.33%)	59(64.13%)
Present	05(26.32%)	09(24.32%)	07(38.89%)	12(66.67%)	33(35.87%)
Necrosis					
Absent	13(68.42%)	31(83.79%)	10(55.56%)	04(22.22%)	58(63.04%)
Present	06(31.58%)	06(16.21%)	08(44.44%)	14(77.78%)	34(36.96%)
Perineural Invasion					
Absent	17(89.47%)	35(94.60%)	14(77.78%)	13(72.22%)	79(85.87%)
Present	02(10.53%)	02(05.40%)	04(22.22%)	05(27.78%)	13(14.13%)

ER: Estrogen receptors, PR : Progesterone receptors, Her2/neu: Human epidermal growth factor receptor,BRS: Bloom Richardson score, TNM: Tumor node metastases, IDC: Intraductal carcinoma, ILC : Intraluminal carcinoma; LN: Lymph node; LVI: Lymphovascular invasion; DCIS: Dental carcinoma in situ

breast cancer present in the inner (luminal) cells lining of the mammary ducts. Most of them are of tumour grade 1 or 2. About 30-70% of breast cancers are luminal A type.[14,15,16,17] Out of all the four subtypes, this one has the best prognosis with fairly high survival rates and very low recurrence rates.[15,16,18,19,20] Luminal B tumour cells look like breast cancer cells originated from the inner (luminal) cell lining of the mammary ducts. Women with luminal B type are often diagnosed at a younger age as compared with luminal A.[19, 20, 21] Compared with luminal A, they have poorer prognosis, larger tumour size, and higher number of positive lymph nodes. About

10-20% of breast cancers are of luminal B subtype and patients have fairly high survival rates, yet it is not as high as those of luminal A.[14, 15, 16, 19, 22]

Her-2 positive subtype tends to have a poorer grade with a higher number of positive lymph nodes. Approximately 15%-20% of the breast carcinomas are categorised in this subtype.[15, 16] These women are usually diagnosed at an earlier age as compared to the luminal subtypes A and B.[19, 21] Triple-negative/ basal like subtype has cells similar to those of the outer (basal) cells surrounding the mammary ducts. About 15-20% of the breast cancers belong to this group.[14, 15, 23] These tumours often tend to occur in younger age groups and are BRCA-1 positive.[18,24,25,26] They are very aggressive with a very poor prognosis.[15,16,20,27]

Studies have suggested that luminal subtypes have better prognosis than non-luminal subtypes. Amongst non-luminal subtypes, Her-2 positive cases have been shown to be of better prognosis than the TNBC. Thus, molecular subtyping not only aids in the clinical practice and research, but is also essential for breast cancer management as different molecular subtypes have different prognosis and therapeutic options.[9,10,12,13] Even though molecular and genetic testing are very precise and accurate and has high prognostic and predictive value, these are very costly and not easily available. In a country like India, with limited availability of resources, molecular subtyping based on immunohistochemistry is a surrogate for recent molecular classification of breast cancer.

The present study aimed to correlate different IHC-based molecular subtypes with the various clinicopathological parameters among the patients attending a tertiary care centre in the industrial belt of Haryana, India.

Material and Methods

This cross-sectional study included all the cases presenting to the Department of Pathology, ESIC Medical College and Hospital Faridabad, Haryana, who underwent MRM for breast carcinoma over a period of 2.5 years. The
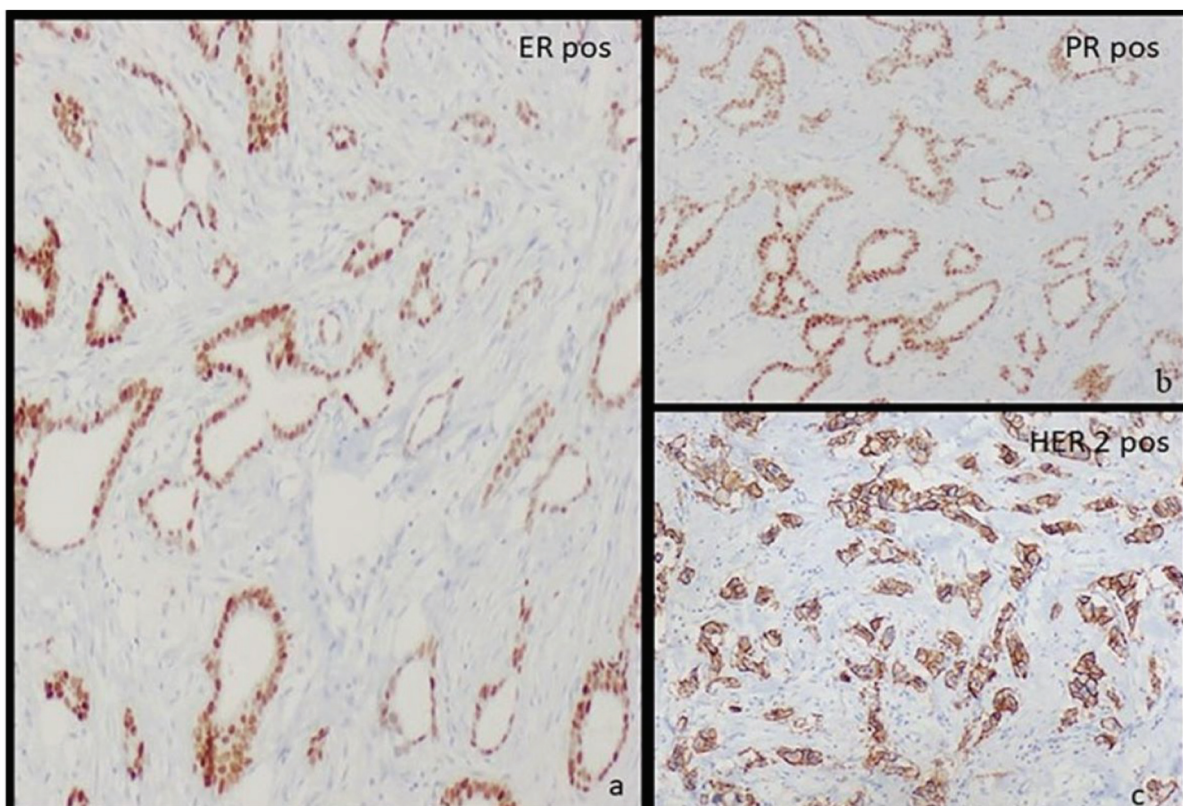

Figure 2. Luminal B subtype showing estrogen receptor (ER) and progesterone receptor (PR) positivity along with human epidermal growth factor receptor 2 (Her-2) positive staining.

Institutional Ethical Committee of ESIC Medical College and Hospital, Faridabad approved this study. We obtained signed written informed consent from all the patients. The clinical parameters of all the cases were retrieved from the hospital Cancer Registry. MRM specimens were fixed in 10% buffered formalin immediately after the surgical resection. All the specimens were grossly examined. Representative sections from the tumour and lymph nodes were taken and submitted for processing followed by Haematoxylin and Eosin (H&E) staining. All the cases were graded and staged according to the 7th edition of AJCC Cancer Staging manual. The morphological characteristics reported are as per the College of American Pathologist Protocol (CAP).

We performed IHC for ER, PR, and Her-2 on the representative paraffin-embedded tumour tissue sections. ER/PR was considered positive once over 1% of tumour cell nuclei were immunoreactive. For Her-2 staining interpretation,

the following method (DAKO scoring system) was used:

DAKO scoring system for Her-2

* Score 0 (Negative): No staining or membrane staining in <10% tumour cells

* Score 1+ (Negative): Faint/ barely perceptible staining in >10% tumour cells

* Score 2+ (Weakly Positive): Faint/ barely perceptible staining in >10% tumour cells

* Score 3+ (Strongly Positive): Strong complete membranous staining in >30% tumour cells

In this study, the breast cancers were subdivided into four molecular subtypes depending upon IHC profile (ER/PR and Her-2 expression). The groups included:

* ER/PR+, Her-2 : ER/PR+, Her-2, ER-/PR+, Her-2+, ER+/PR-, Her-2 +

* ER/PR+, Her-2 : ER/PR+, Her-2-, ER-/PR+, Her-2-, ER+/PR- , Her-2 -

* ER/PR-, Her-2+

* ER/PR- , Her-2-

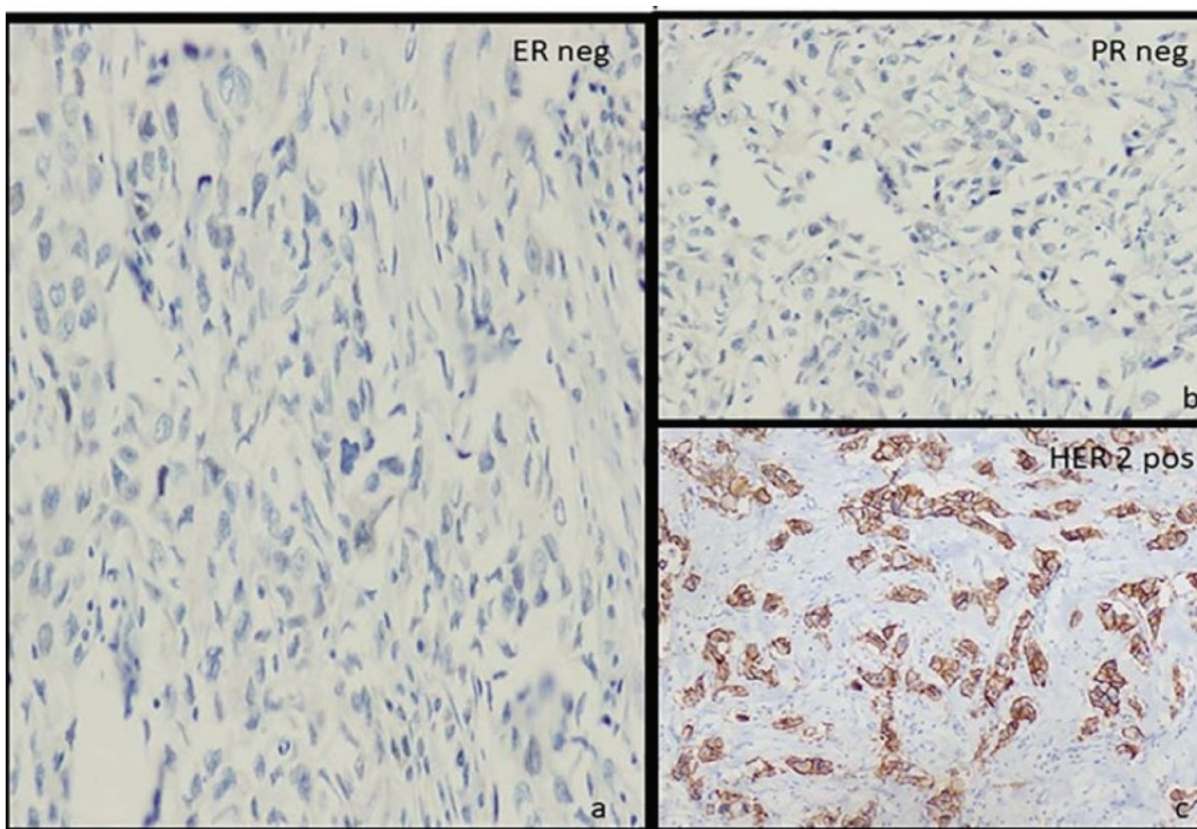Chi-square tests (Pearson chi-square) were

Figure 3. Her-2 neu positive subtype showing estrogen receptor (ER) and progesterone receptor (PR) negative and human epidermal growth factor receptor 2 (Her-2) positive staining.

employed to determine the statistical analysis with the SPSS software version 20 for windows. A *P* value of <0.05 was considered as significant.

Results

The study was conducted on 92 patients with invasive breast carcinoma that underwent MRM over a period of 2.5 years at ESIC Medical College and Hospital, Faridabad. (Please refer to table 1 for tabulated results). The cases were categorized into four molecular subtypes based on the IHC profile: Luminal A (20.65%, 19/92), luminal B (40.21% cases, 37/92), Her-2 positive (19.57%, 18/92), and triple-negative (19.57%, 18/92). The majority of our cases were of luminal B subtype.

Age

The mean age was observed to be 47 years, but the maximum number of the cases (53.26%) was in the 3rd and 4th decade of their life. In TNBC (77.77%, 14/18) and Luminal B (67.56 %, 25/37) subtypes, most of the patients were below 40 years of age.

Bloom Richardson Scoring (BRS) Grade

Grade II was the most common histological grade in our study (42.39%,) followed by Grade III (33.70%) and Grade I (23.91%)), respectively, in TNBC and Her-2 positive subtypes; most of the subjects were categorized as grade III, while in luminal subtypes A and B, most of them were categorised as grade I and grade II (*P*=0.016).

TNM staging

The majority of the cases were in stage II category, (53.26%) followed by stage III (28.27%) and stage I (18.47%). In TNBC subtype, most of the cases belonged to higher stage III; whereas, in Her-2, luminal A, and liminal B subtypes, most of the cases were lower stage II (*P*=0.048).

Tumour size

Most of our subjects had a tumour size in the range of 2 to 5cm (56.52%). In triple negative subtype, most of the cases had a tumour size of above 5 cm (50.00%). In Her-2 positive and luminal B and A subtypes, they mostly had a tumour size in the range of 2 to 5cm (55.56%,
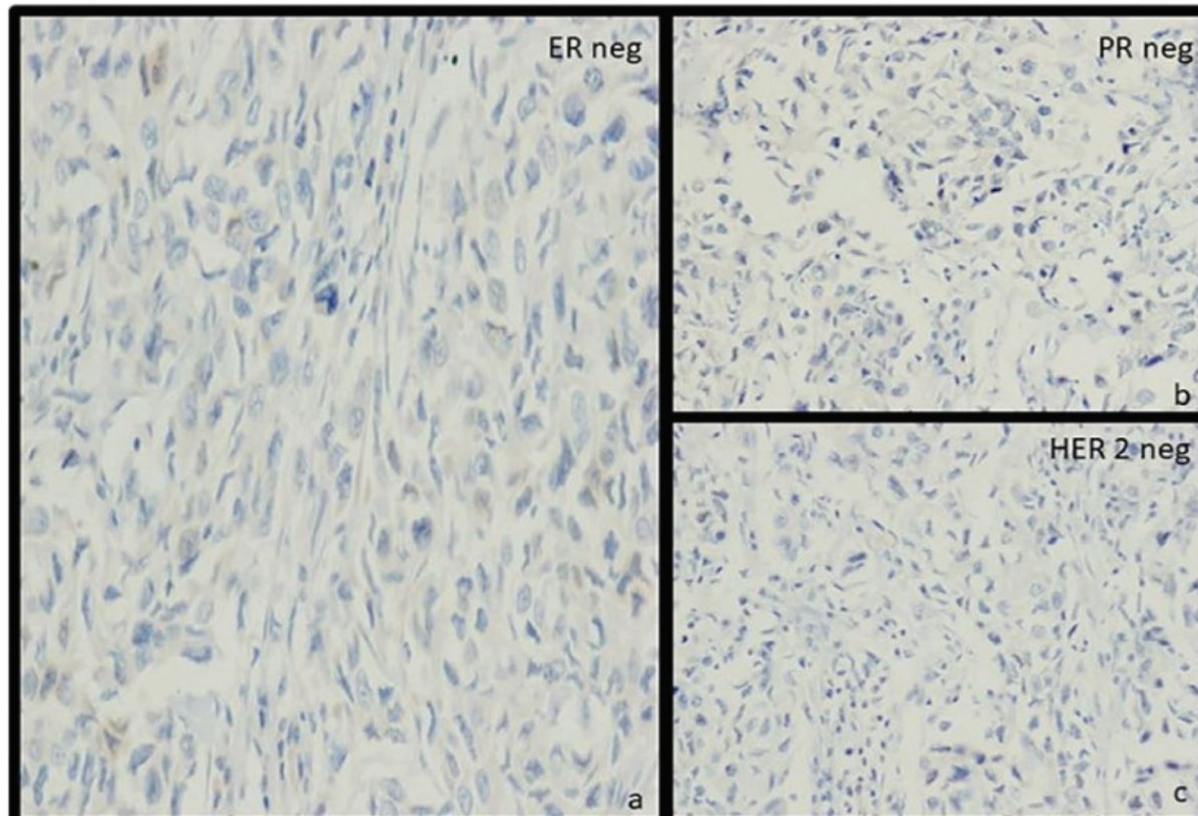

Figure 4. Triple-negative subtypes showing estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (Her-2) negative staining.

62.17% and 63.16%, respectively). Thus, triple-negative subtype was associated with larger tumour sizes in most of the cases; whereas, the other three subtypes were associated mostly with a comparatively smaller tumour size (*P*= 0.038).

Pathological Subtype

86.96% of the cases were IDC and NOS. Meanwhile, the other 13.04% of the cases were included in invasive lobular carcinoma (ILC), Pleomorphic variant of ILC, medullary carcinoma, apocrine carcinoma, and metaplastic carcinoma.

Mitosis

83.21% of the cases were in TNBC subtype and 66.67% of them were categorised in Her-2 positive subtypes. These percentages were associated with brisk mitotic activity (>10/10hpf); whereas, the high percentage of the cases in luminal B (83.79%) and luminal A subtype (63.16%) was associated with a comparatively lower mitotic activity (<10/10hpf). Hence, non-luminal subtypes were associated with poor prognosis compared to the luminal ones (*P*<0.001).

Lymph Node metastasis

The highest rate of nodal metastasis belonged to TNBC subtype (72.22%) followed by Her-2 neu positive (55.56%, luminal B (37.83%), and the lowest rate was observed in luminal A (36.84%). Thus, TNBC subtype was seen to be associated with the worst prognosis, while luminal A subtype had the best prognosis (*P*=0.04).

Lymphovascular Invasion (LVI)

TNBC had the most association with LVI (68.42%) followed by Her-2 positive (61.11%) and luminal B (37.84%). The least association was seen with luminal A subtype (31.68%). Thus, TNBC subtype showed more aggressive behaviour and luminal A subtype had the best prognosis, in terms of LVI, yet it was not statistically significant (*P*=0.063).

Ductal Carcinoma in Situ (DCIS)

Foci of DCIS were seen in 35.87% of the cases. They were most commonly seen in TNBC subtype (66.67 %), followed by Her-2 positive (38.89 %), and luminal A (26.32 %), least frequently observed in luminal B (24.32%) subtype (*P*=0.016).

Necrosis

Foci of necrosis were seen in 36.96% of the cases. They were most commonly seen in TNBC subtype (77.78%) followed by Her-2 positive subtype (44.44%) and luminal A subtype (31.58%) and least frequently observed in luminal B subtype (16.21%) (*P*<0.001).

Perineural Invasion

Perineural invasion was found in 14.13% of our subjects. It was most commonly seen in TNBC subtype (27.78%) followed by Her-2 positive subtype (22.22%) and luminal A subtype (10.53 %,). Meanwhile, it was least frequently observed in luminal B subtype (05.40%). However, this was not statistically significant (*P*=0.100).

Discussion

We conducted the current study on 92 patients in the industrial belt of Haryana, India on the patients, who underwent MRM for breast carcinoma. This study is first of its kind in the Indian subcontinent, as it correlated the molecular subtype status with clinicopathological parameters in the industrial population of Haryana. We aimed to investigate if any variations are noted in the above-mentioned parameters amongst the factory workers.

According to the obtained results herein, breast cancer is a multivariable disease having several features to consider. The distinct biological subtypes were found to have variations in the presentation of clinical spectrum and molecular presentations. All these lead to different therapeutic and prognostic connotations.[28]

There are eight possible subtypes of breast cancer as previously quoted by several authors.[29] However, the study conducted by Ontilio classified breast cancer into four subtypes, including luminal A, luminal B, Her-2 , and TNBC.[28] This classification is practical, informative, and clinically useful. The present study also categorised breast cancer into the same four subtypes.

Age

In our study, the mean age was 47 years, which was similar to the mean age of 47.76 years

reported by Smriti et al. and 44.64 years as reported by Gayatri et al.[5, 30] In the western world, the mean age reported by several studies was 53 years.[31] The lower mean age in Indian population compared with the western world could be attributed with receptor negative status of a large percentage of cases.[30, 32]

In the present study, the highest incidence of breast cancer was observed in the 3rd and 4th decade of a subject's life, which is a decade earlier than that observed by Smriti et al. (41 to 50 years).[5] This might be due to the early exposure of the industrial population to carcinogenic agents.

77.78% of the TNBC cases were below 40 years of age, which is comparable to other studies indicating the association of TNBC cases with lower age groups. The study conducted by Gayatri et al. reported that the median age of TNBC subtype was 39 years and the mean age was 35 years.[30]

Category

Herein, the majority of the patients were in the category of luminal B (40.21%). This is in contrast to the study conducted by Brig et al. and that by Jeihua et al., in which luminal A subtype was the most common subtype (34% and 35.5%, respectively).[31,32] This fact could be attributed to the involvement of the lower age group, which is a decade earlier in the industrial population.

It was also observed that in the age group of less than 40 years, luminal B was the most common subtype (43.10%) followed by TNBC (24.14%), Her-2 positive (17.24%), and luminal A subtype (15.51%). This finding was similar to that of a study on young women in Turkey (less than 35 years), where luminal B (36.5%) was reported to be the most frequent subtype followed by luminal A (30.8%), TNBC (23.2%), and Her-2 positive (9.5%), respectively.[33]

TNBC comprised 19.5% cases of our study, which is comparable to the study conducted by Cherry et al. (23.6%) and that by Goksu et al. (23.2%).[33,34] These findings were also analogous to other studies conducted by Ayadi et al., Ahmed et al., and Vasudha et al.[35,36,37]

BRS Grade

42.39% of the cases were categorised as grade

II, while 33.70% and 23.91% of them were categorised as grade III and I, respectively. Moreover, Gokku et al. had the similar findings with 51.2% of the cases as grade II, 31.8 % and 9.5% of them as grade III and I, respectively.[33] This is also comparable to the findings of Smriti et al., which categorised 60% of the cases as grade II, while 22.9% and 17.1% of them were categorised as grade III and I, respectively.[5] Jiehua et al. also suggested grade II (39.6%) as the most common grade whereas Gayatri et al. found 41.5% of them cases to be as grade II.[30,32] Most of the grade I cases were ER positive (92.85%). This finding is similar to that of Brig et al., who also reported 67% of the grade I cases to be ER positive.[31]

TNBC cases were observed to belong to grade II or higher in this study. This result is concordant with that of a study by Jiehua and Gayatri et al., who classified 94.4% and 89.3% TNBC cases as grade II or worse, respectively.[30, 32] In our study, 67.67% of luminal B cases were categorized as grade II. This finding is in accordance with that of the study by Jiehua et al., who categorised 62% of luminal B cases as grade II.[32]

TNM Stage

This study revealed that luminal subtypes were associated with a lower stage compared with non-luminal subtypes. This finding is concordant with that of Jiehua et al.[32]

Tumour size

In our study, the tumour size at presentation was bigger than 2 cm in 76.09% of the cases; however, in a study conducted by Smriti et al., 98.65% of the cases had a tumour size of over 2 cm.[5] 88.89% of the TNBC cases had a tumour size of over 2 cm, which is in agreement with the study conducted by Brig et al., who reported a tumour size of bigger than 2 cm in all the TNBC cases.[31]

Pathological subtypes

The predominant subtype in our study was IDC (86.95%) followed by ILC (02.17%), as also shown in most of the studies conducted worldwide.[38,39] Gayatri et al. reported 86.9% of IDC cases and 08.13% of ILC cases in their study.[30] Similar findings were observed in the

study by Smriti et al., which reported 90% IDC and 04% ILC cases.[5]

Lymph node metastasis

In our study, 47.82% of the cases had positive lymph nodes at presentation. Meanwhile, in the study conducted by Smriti et al., 74.3% of the cases had positive lymph nodes at presentation.[5] Most of the Indian studies have reported higher numbers of lymph nodes at presentation compared with the developed countries.[40] The large percentage of the TNBC (72.22%) and Her-2 positive (55.56%) cases was attributed to positive lymph nodes, whereas the lower number of luminal A cases (36.84%) and luminal B cases (37.83%) was assigned to positive lymph nodes. This finding is in concordance with that of the studies conducted by Kim et al., Spitale et al., and Munjal et al.[38, 41, 42]

Conclusion

According to the obtained results, we could conclude that luminal B is the most prevalent subtype in the industrial population, which presents at a younger age with larger size and more lymph node metastasis at presentation. However, our investigation had certain limitations, including the fact that it was a cross-sectional study and only a limited number of cases could be analysed.

The current work could be utilized as a pilot study and extended to private and corporate hospitals where the population under study is from different sections of the society. It would help to study the presentation of breast carcinoma in Indian population, compare the clinicopathological parameters with various molecular subtypes, and analyse if any variations are noted in the general population of the industrial group. Furthermore, this would help to reflect the role of industrial carcinogens in the expression of different molecular subtypes.

In a country like India with lack of resources, molecular subtyping based on IHC could be a cost-effective way of classifying breast carcinomas as per the recent genetic classification.

Conflict of Interest

None declared.

References

1. Harirchi I, Kolahdoozan S, Karbakhsh M, Chegini S, Mohseni SM, Montazeri A, et al. Twenty years of breast cancer in Iran: downstaging without a formal screening program. *Ann Oncol*. 2011;22(1):93-7. doi: 10.1093/annonc/mdq303.
2. Siadati S, Sharbatdaran M, Nikbakhsh N, Ghaemian N. Correlation of ER, PR and HER-2/Neu with other prognostic factors in infiltrating ductal carcinoma of breast. *Iran J Pathol.* 2015;10(3):221-6.
3. Ghoncheh M, Momenimovahed Z, Salehiniya H. Epidemiology, incidence and mortality of breast cancer in Asia. *Asian Pac J Cancer Prev.* 2016;17:47-52. doi: 10.4103/2278-330X.187552.
4. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA Cancer J Clin.* 2005;55:74-108. doi.org/10.3322/canjclin.55.2.74.
5. Pandey ST, Malik R, Trichal, Nigam, RK, Arvind R, Balani Sh, et al. Breast cancer: Correlation of molecular classification with clinicohistopathology. *Sch J Appl Med Sci*. 2015;3:1018-26. doi: 10.17511/jopm. 2019.i03.08.
6. Rosai J. Rosai and Ackerman's surgical pathology. 10th ed. Mosby: Edinburg, London, New York, Oxford, Philadelphia, St. Loius, Sydney, Toronto; Elsevier; 2011.p.1660 -771.
7. Harigopal M, Berger AJ, Camp RL, Rimm DL, Kluger HM. Automated quantitative analysis of E-cadherin expression in lymph node metastases is predictive of survival in invasive ductal breast cancer. *Clin Cancer Res.* 2005;11(11):4083-9. doi: 10.1158/1078-0432. CCR-04-2191.
8. Azizun-Nisa , Bhurgri Y, Reza F, Kayani N. Comparision of ER,PR and HER-2/neu (C-erb B2) reactivity pattern with histologic grade, tumor size and lymph node status in breast cancer. *Asian Pac J Cancer Prev.* 2008;9(4):553-6.
9. Chen XS, Ma CD, Wu JY, Yang WT, Lu HF, Wu J, et al. Molecular subtype approximated by quantitative estrogen receptor, progesterone receptor and Her2 can predict the prognosis of breast cancer. *Tumori*. 2010;96(1):103-10.
10. Onoda T, Yamauchi H, Yagata H, Tsugawa K, Hayashi N, Yoshida A, et al. The value of progesterone receptor expression in predicting the recurrence score for hormone-receptor positive invasive breast cancer patients. *Breast Cancer*. 2013;22(4):406-12. doi:10.1007/s12282-013-0495-x.
11. Ariga R, Zarif A, Korasick J, Reddy V, Siziopikou K, Gattuso P. Correlation of Her-2/neu gene amplification with other prognostic and predictive factors in female breast carcinoma. *Breast J.* 2005;11(4):278–80. doi:

10.1111/j.1075-122x.2005.21463.x.

12. Huang HJ, Neven P, Drijkoningen M, Paridaens R, Wildiers H, Van Limbergen E, et al. Association between tumor characteristics and HER-2/neu by immunohistochemistry in 1362 women with primary operable breast cancer. *J Clin Pathol.* 2005;58:611-6. doi: 10.1136/jcp.2004.022772.

13. Ambroise M, Ghosh M, Mallikarjuna VS, Kurian A. Immunohistochemical profile of breast cancer patients at a tertiary care hospital in South India. *Asian Pac J Cancer Prev.* 2011;12(3):625-9.

14. Fan C, Oh DS, Wessels L, Weigelt B, Nuyten DSA, Nobel AB, et al Concordance among gene-expression-based predictors for breast cancer. *N Engl J Med.* 2006;355(6):560-9. doi: 10.1056/NEJMoa052933.

15. Voduc KD, Cheang MC, Tyldesley S, Gelmon K, Nielsen TO, Kennecke H. Breast cancer subtypes and the risk of local and regional relapse. *J Clin Oncol.* 28(10):1684-91, 2010. doi: 10.1200/JCO.2009.24.9284.

16. Carey LA, Cheang MCU, Perou CM. Chapter 29: Genomics, prognosis, and therapeutic interventions. In: Harris JR, Lippman ME, Morrow M, Osborne CK, editors. Diseases of the breast. 5th ed. Lippincott Williams & Wilkins: Philidelphia; 2014.

17. Howlader N, Altekruse SF, Li CI, Chen VW, Clarke CA ,Ries LAG, et al. US incidence of breast cancer subtypes defined by joint hormone receptor and HER2 status. *J Natl Cancer Inst.* 2014;106(5): dju055. doi: 10.1093/jnci/dju055.

18. Arvold ND, Taghian AG, Niemierko A, Raad R A F, Sreedhara M, Nguyen PL, et al. Age, breast cancer subtype approximation, and local recurrence after breast-conserving therapy. *J Clin Oncol.* 2011;29(29): 3885-91. doi10.1200/JCO.2011.36.1105.

19. Metzger-Filho O, Sun Z, Viale G, Price KN, Crivellari D, Snyder RD, et al. Patterns of recurrence and outcome according to breast cancer subtypes in lymph node-negative disease: Results from international breast cancer study group trials VIII and IX. *J Clin Oncol.* 2013; 31(25):3083-90. doi:10.1200/JCO.2012.46.1574.

20. McGuire A, Lowery AJ, Kell MR, Kerin MJ, Sweeney KJ. Locoregional recurrence following breast cancer surgery in the trastuzumab era: a systematic review by subtype. *Ann Surg Oncol.* 2017;24(11):3124-32. doi: 10.1245/s10434-017-6021-1.

21. Partridge AH, Hughes ME, Warner ET, Ottesen RA, Wong Y N, Edge SB, et al Subtype-dependent relationship between young age at diagnosis and breast cancer survival. *J Clin Oncol.* 2016;34(27):3308-14. doi: 10.1200/JCO.2015.65.8013.

22. Haque R, Ahmed SA, Inzhakova G, Shi J, Avila C, Polikoff J, et al. Impact of breast cancer subtypes and treatment on survival: an analysis spanning two decades. *Cancer Epidemiol Biomarkers Prev.* 2012;21(10):1848-55. doi: 10.1158/1055-9965.EPI-12-0474.

23. Kohler BA, Sherman RL, Howlader N, Jemal A, Ryerson AB, Henry KA, et al. Annual report to the nation on the status of cancer, 1975-2011, featuring incidence of breast cancer subtypes by race/ethnicity, poverty, and state. *J Natl Cancer Inst.* 2015;107(6): djv048. doi: 10.1093/jnci/djv048.

24. Atchley DP, Albarracin CT, Lopez A, Valero V, Amos CI, Gonzalez-Angulo AM, et al. Clinical and pathologic characteristics of patients with BRCA-positive and BRCA-negative breast cancer. *J Clin Oncol.* 2008;26(26):4282-8. doi: 10.1200/JCO.2008.16.6231.

25. Hartman AR, Kaldate RR, Sailer LM, Painter L, Grier CE, Endsley RR, et al. Prevalence of BRCA mutations in an unselected population of triple-negative breast cancer. *Cancer.* 118(11):2787-95, 2012. doi: 10.1002/cncr. 26576.

26. Couch FJ, Hart SN, Sharma P, Toland AE, Wang X, Miron P, et al. Inherited mutations in 17 breast cancer susceptibility genes among a large triple-negative breast cancer cohort unselected for family history of breast cancer. *J Clin Oncol.* 2015;33(4):304-11. doi: 10.1200/JCO.2014.57.1414.

27. Millar EK, Graham PH, O'Toole SA, McNeil CM, Browne L, Morey AL, et al. Prediction of local recurrence, distant metastases, and death after breast-conserving therapy in early-stage invasive breast cancer using a five-biomarker panel. *J Clin Oncol.* 2009; 27(28):4701-8.

28. Ontilio AA, Greenlee RT, Mukesh BN. Breast cancer subtypes based on ER/PR and Her2 expression: comparison of clinicopathologic features and survival. *J Clin Med Res.* 2008;7:4-13.

29. Nguyen PL, Taghian AG, Katz MS, Niemierko A, Abi Raad RF, Boon WL, et al. Breast cancer subtype approximated by estrogen receptor, progesterone receptor, and Her-2 is associated with local and distant recurrence after breast-conserving therapy. *J Clin Oncol.* 2008;26:2373-8.

30. Gogoi G, Borgohain M, Saikia P, Fazal SA. Profile of molecular subtypes of breast cancer with special reference to triple negative: A study from Northeast India. *Clin Cancer Investig J.* 2016;5:374-83

31. 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):254-8. doi: 10.1016/j.mjafi. 2015.04.006.

32. Li J, Chen Z, Su K, Zeng J. Clinicopathological classification and traditional prognostic indicators of breast cancer. *Int J Clin Exp Pathol.* 2015;8(7):8500-5.

33. Goksu SS, Tastekin D, Arslan D, Gunduz S , Murat A , Tatli DU, et al. Clinicopathologic features and molecular subtypes of breast cancer in young women (Age <= 35). *Asian Pac J Cancer Prev.* 2014;15(16):6665-8.doi: 10.7314/APJCP.2014.15.16.

6665.

34. Bansal C, Sharma A, Pujani M, Pujani M, Sharma KL, Srivastava AN, et al. Correlation of hormone receptor and human epidermal growth factor receptor-2/neu expression in breast cancer with various clinicopathologic factors. *Indian J Med Paediatr Oncol.* 2017;38(4):483-9. doi: 10.4103/ijmpo.ijmpo_98_16.

35 Ayadi L, Khabir A, Amouri H, Karray S, Dammak A, Guermazi M, et al. Correlation of HER-2 over-expression with clinico-pathological parameters in tunisian breast carcinoma. *World J Surg Oncol.* 2008;6:112.

36. Ahmed HG, Al-Adhraei MA, Al-Thobhani AK. Correlations of hormone receptors (ER and PR), Her2/neu and p53 expression in breast ductal carcinoma among Yemeni women. *Open Cancer Immunol J.* 2011;4:1-9.

37. Vasudha M, Bharti M, Prashant R. Correlation of hormone receptor & Her 2/neu expression in breast cancer: A study at tertiary care hospital in South Gujarat. *Natl J Med Res.* 2012;2:295-8.

38. Munjal K, Ambaye A, Evans MF, Mitchell J, Nandedkar S, Cooper K. Immunohistochemical analysis of ER, PR, Her2 and CK5/6 in infiltrative breast carcinomas in Indian patients. *Asian Pac J Cancer Prev.* 2009; 10(5). 773-8.

39. Ambroise M, Ghosh M, Mallikarjuna VS, Kurian A. Immunohistochemical profile of breast cancer patients at a tertiary care hospital in South India. *Asian Pac J Cancer Prev.* 2011;12(3): 625-9.

40. Vaidyanathan K, Kumar P, Reddy CO, Deshmane V, Somasundaram K, Mukherjee G. ErbB-2 expression and its association with other biological parameter of breast cancer among Indian women. *Indian J Cancer.* 2010;47(1):8-15. doi: 10.4103/0019-509X.58852.

41. Spitale A, Mazzola P, Soldini D, Mazzucchelli L, Bordoni A. Breast cancer classification according to immunohistochemical markers clinicopathologic features and short-term survival analysis in a population-based study from the South of Switzerland. *Annal Oncol.* 2009;20(4): 628-35. doi: 10.1093/annonc/mdn675.

42. Kim MJ, Ro JY, Ahn SH, Kim HH, Kim SB, Gong G. Clinicopathologic significance of the basal-like subtype of breast cancer: A comparison with hormone receptor and HER2neu-overexpressing phenotypes. *Hum Pathol.* 2006; 37(9):1217-26. doi: 10.1016/j.humpath.2006.04.015.