



STUDY OF BREAST CARCINOMA-HISTOPATHOLOGICAL CORRELATION WITH ER, PR & HER 2 NEU RECEPTOR STATUS-2 YEAR EXPERIENCE OF A TERTIARY CARE CENTRE IN CENTRAL UTTAR PRADESH

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ABSTRACT A total of 64 cases were included in this study. 43.75% cases belonged to 41-50 years age with mean age of 48.90 years. 56(87.5%) cases were IDC, 3 cases lobular and 2 each of medullary and mucinous type respectively and 1 case of apocrine carcinoma. In BRG 54.68% cases belongs to grade II, 15.64% grade III and 17.18% to grade I. 54.68% cases were ER +, 42.18% were PR + and 29.68% were Her2 neu +. Most of the grade 1 and 2 tumors were ER positive and grade 3 were ER negative. Out of 64 cases 21 cases were belongs to luminal A, 14 cases were luminal B, 11 cases were her2 neu enriched and 18 cases were triple negative phenotype. After applying chi square test, significant association between histological grade and molecular subtypes was seen & no significant association between age and molecular subtypes was seen.

KEYWORDS : breast carcinoma, ER, PR, Her 2 neu

INTRODUCTION

India is a sub-continent with wide ethnic, cultural, religious, and economic diversity and variation in the health care infrastructure. Owing to the lack of awareness of this disease and in absence of a breast cancer screening program, the majority of breast cancers are diagnosed at a relatively advanced stage (Gaurav Aggrawal et al 2008) Breast cancer is the most common female cancer in the world with an estimated 1.67 million new cancer cases diagnosed in 2012. While the age adjusted incidence rates of breast cancer in India is lower than the western countries, because of the large population the burden of breast cancer is high.

Breast cancer is a multifaceted disease having distinct biological subtypes with diverse natural history, presenting a varied spectrum of clinical, pathologic and molecular features with different prognostic and therapeutic implications Only about one half of patients with early breast cancer are treated and cured by local surgical excision alone. Therefore, it is important to identify the set of patients in whom the disease is destined to recur and which patients are likely to benefit from systemic chemotherapy. The choice between hormonal therapy which has minimal side effects and chemotherapy with well-known morbidity and risks is a major responsibility.

MATERIALS AND METHODS:

Methodology:

- Specimen received in 10% formalin. Put the specimen in 10% formalin after loafing for 6-8 hr. Perform grossing. Paraffin sections of 5µm thickness will be stained by haematoxylin and eosin (H & E) for histopathological study.
- For immunohistochemistry- Sections of 3µm taken on charged/coated slides with poly-L-lysine. Slides are put on the hot plate for dewaxing for 1 hr. As dewaxing is completed dip the slides into xylene directly from hot plate. Keep the slides in the xylene for 10 min. Transfer the slides into second xylene solution for 10 min. After it pass the slides into downgrading concentration of alcohol in order of 100%, 70%, and 50% for 5 min in each. Wash the slides with distilled water for 5-10 min. Next step is antigen retrieval. For ER PR her 2 retrieval done by the citrate buffer (pH-6) at high temperature and high pressure When retrieval is complete put the slides in moist chamber and allow it to cool down to room temperature for app. 30 min. Wash the slides with TRISS buffer (pH-7.5)(2-3 times). Add the peroxidase blocker to each section for 15 min. 3 washing with TRISS buffer. Label the slides ER, PR, Her 2 and incubate the slides with respective primary antibody for 1 hr. 3 washing with TRISS buffer. Incubate the slides with secondary antibody HRP (horse radish peroxidase) for 30 min. 3 washing with TRISS buffer. Mix the DAB chromogen in

DAB buffer in the ratio of 1:50 and apply it for 30 mins. Give 3 washing of distilled water. Counterstain the slides with hematoxylin for appropriate time (3-5min)

OBSERVATIONS

A total of 64 cases of infiltrating breast carcinoma were included in this study. 43.75% cases belonged to age-group of 41-50 years with mean age of 48.90 years (Table1). 56(87.50%) cases were diagnosed as Invasive Ductal carcinoma, 3 cases of lobular and 2 each of medullary and mucinous type respectively and 1 case of apocrine carcinoma (Table2). In Bloom Richardson grading 54.68% cases belongs to grade II, 15.64% grade III and 17.18% to grade I. About 60% cases have metastatic lymph node half of them are having >4 metastatic lymph node.

Table1 - Distribution of cases based on age group (n=64)

AGE GROUP	NO. OF CASES	PERCENTAGE
0-30 Yrs	3	4.68%
31-40 Yrs	7	10.93%
41-50 Yrs	28	43.75%
51-60 Yr	12	18.75%
61-70 Yrs	13	20.31%
>70 Yrs	1	1.56%
total	64	100

Table 2- Distribution of cases based on histological types (n=64)

HISTOLOGICAL TYPES	NO. OF CASES	PERCENTAGE
Invasive duct carcinoma NOS	56	87.50%
Invasive lobular carcinoma	3	4.68%
Medullary carcinoma	2	3.12%
Mucinous carcinoma	2	3.12%
Others	1	1.56%

- 35 cases are ER positive and 29 cases are ER negative. 27 cases are PR positive and 37 cases are PR negative. 19 cases are Her2 neu positive and 45 cases are Her 2 neu negative (Table3). Most of the grade 1 and 2 tumors are ER, PR positive and most of the cases of grade 3 are ER, PR negative. Overall lesser no. of Her2neu positive cases are seen. 18 (28.12%) cases are triple negative, of which most cases belong to grade 3. (Table4)

Table-3- Distribution of cases based on Receptors (n=64)

	no. of cases ER positive	no. of cases PR positive	No. of cases Her2 neu +
Positive	35	27	19
Negative	29	37	45

Table- 4- Distribution of cases based on ER positive cases acc. to grade of tumor (n=64)

Grade of tumor	No. of cases	ER positive cases	PR positive Cases	Her 2 neu positive Cases	Triple negative cases
I	11	9	7	4	0
II	35	23	19	11	4
III	18	3	1	4	14

Out of 64 cases 21 were belongs to luminal A, 14 cases were luminal B, 11 cases were her2 neu enriched and 18 cases were triple negative phenotype. Acc to histological grades max triple negative belongs to grade 3 and most grades 1 are luminal A type. (Table5). These cases are also divided according age group and there molecular classes considering average age of 45 years.

Table-5- Relation between histological and molecular classes

Histological grade	Luminal A	Luminal B	Her 2 neu overexpression	Triple negative
Grade 1	9	1	1	0
Grade 2	9	13	9	4
Grade3	3	0	1	14

Table – 6- Relation between patient's age and molecular classes

Age of patient	Luminal A	Luminal B	Her 2 neu overexpression	Triple negative
<45 yrs	8	7	6	10
>45 yrs	13	7	5	8

After applying chi square test, significant association between histological grade and molecular subtypes is seen (Table5). ($X^2=44.16$) ($p<0.05$). After applying chi square test no significant association between age and molecular subtypes is seen ($p>0.05$) (Table6).

DISCUSSION AGE GROUP

- This study comprised 64 cases of invasive breast carcinoma with mean age at the time of diagnosis was 48.90 years. Maximum incidence of breast cancer (43.75%) was observed in the age-group of 41- 50 years which is comparable to **Vedshree et al** (Mean age-50.18 years), **P. urmila et al** (mean age-51.5), and less than **Rashmi et al** (mean age-56 yrs). In India, the average age of developing breast cancer has shifted over the last few decades and younger women (40-50 yr) are being affected. The lifestyle factors such as late age at marriage, reduced breast feeding, and westernization of diet may be associated with occurrence of breast cancer in younger population in India. **Sofi et al** reported 59.1% cases ≤ 50 years of age, whereas **Pallavi Shrigondekar et al** reported 57.65% cases between 31 to 50 years age group very similar to the study of **Jain et al**, **Nikhra et al**, and emphasizing the predominant presentation in younger age group

HISTOLOGICAL TYPES AND GRADES

- Most common histological type is invasive ductal carcinoma (NOS) comprises 87.50% of total cases, which is similar to other Indian studies. 54.68% cases belongs to grade II of Bloom Richardson grading, while 28.14% and 17.18% cases belonged to grade III and grade I respectively, which is comparable to study done by **Geethamala et al**. Similar findings were recorded by **Nikhra et al**, **Ghosh et al** and **Bhagat et al**, **Bushra et al** showing infiltrating ductal carcinoma (NOS) as the most common histological type and lobular carcinoma as the second most common type. In **Nikhra et al** study, 95.34% of the tumor was infiltrating ductal carcinoma. **Mushood et al** study shows that Infiltrating Ductal carcinoma (IDC) was the predominant histopathology with IDC NOS (not otherwise specified) in 126 (90.6%) cases. Infiltrating Duct carcinoma NOS was also the predominant histopathology in studies by, **Sofi et al** (80.30%), **Pallavi Shrigondekar et al** (93.2%), **Suvarchala S B et al** (93.7%) and **Bhagat Vasudha M et al** (94.8%).
- Studies by **Suvarchala S B et al**, **Sofi et al**, **Bhagat Vasudha M et al**, also recorded Grade II as the predominant Grade in 42.1%, 52.1%, 43.1% and 59% cases respectively. **Pallavi Shrigondekar et al** reported Grade III tumour as the commonest type.

HORMONAL RECEPTOR STATUS

- 35 (54.68%) cases are ER positive and 29 (45.32%) cases are ER negative. 27 (42.18%) cases are PR positive and 37 (57.82%) cases are PR negative. 19 (29.68%) cases are Her2 neu positive and 45 (70.32%) cases are Her 2 neu negative. Most of the grade 1 and 2 tumors are ER positive and most of the cases of grade 3 are ER negative. 18 (28.12%) cases are triple negative. Most of the studies noted relatively higher percentage of estrogen and progesterone positivity like **Jain et al** reported ER, PR positivity in 37% and 34% cases respectively while **Nikhara et al** noted ER positivity in 39% cases and PR positivity in 42% cases. Similarly **Munjal et al** demonstrated ER, PR positivity in 41% of their cases. This might be contributed to the inclusion of older or post-menopausal females in their study. **Jain et al**, **Nikhra et al**, **Ambroise et al** demonstrated 35%, 32%, 27% her2neu positivity respectively. **Ambroise et al** got 25% triple negative cases. Similarly, various other studies have reported triple negative cases ranging from 13% to 47% amounting to regional and geographical variation and comparatively lesser prevalence in non-African American females.
- Most of the grade 1 and 2 tumors are PR positive and most of the cases of grade 3 are PR negative It was observed in this study that Grade III tumors, in most cases have unfavorable hormone receptor status, in contrast to Grade I and Grade II tumors which show association with unfavorable hormone receptor status .In study of **Puvitha et al**, ER and PR were positive in 51.6% cases, and both the receptors were negative in 48.4% cases. Her2 neu overexpression was observed in 42.7% cases. This is in correlation with **Kumar et al** study [Her2 neu-46.3%], **Shet et al** study (receptor expression range from 52 to 57%), and **Mudduwa** study (ER - 45.7%, PR -48.3%). **Munjal K et al** was 28%. We observed a higher proportion of Her-2/neu subtype (25.7%) than previously reported in literature, same were observed by **Munjal K et al** (29.0%).
- There was no correlation found between molecular type of breast cancer and age of the patients. (p value >0.05), However study done by **Munjal K et al** signifies that, luminal A subtype found in slightly older women.

CONCLUSION

Carcinoma of breast is a common clinical problem in our society. Patients usually present in late stage mainly due to lack of awareness leading to late diagnosis. There is an immediate need for breast cancer screening, health education and public awareness programs (including self breast examination) to detect the disease in early stages. Present study showed Invasive ductal carcinoma is most common histological type prevalent in Indian population but at an early age compared to western countries. The patients presenting at early age are associated with higher grades of tumour along with over expression of Her 2 neu and triple negative cases. So it is of concern that there is not only increase in incidence in early age group but also these cases are also associated with poor prognostic factors. So, it is of utmost importance to investigate the breast lumps, in all age groups, histologically, immunohistochemically and biologically to diagnose, treat and eventually improve the survival and quality of life.

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