

Hormone receptor status, Her 2- neu amplification and their correlation with prognostic indices in patients with invasive ductal carcinoma of breast in Jaffna.

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Abstract

Knowing the tumour biology can help to stratify patients for prognostic and therapeutic purposes. Expression of estrogen and progesterone receptors (ER, PR), amplification of human epidermal growth factor receptor 2 (Her 2 neu), size and histological grading of tumour, axillary lymph node state and NPI (Nottingham Prognostic Index) are well established parameters that have influence on prognosis and treatment of breast carcinoma

It is an institutional based retrospective cross sectional analytic study. 181 female patients who underwent primary mastectomy and axillary clearance for invasive ductal carcinoma of breast in Teaching Hospital Jaffna from 2014 to 2018 were included in this study. ER/PR status and amplification of Her 2 neu in the infiltrating ductal carcinoma of breast and the prognostic indices were recorded. SPSS was used for statistical analysis and correlation between the receptor status and prognostic indices were calculated using Spearman's correlation, chi-square and Kruskal-Wallis tests.

Of the 181 patients with invasive ductal carcinoma of breast, 132 patients (72.9%) were above 45 years old. A total of 87 patients had ER+ (48.1%), 76 had PR+ (41.9%) and, 62 had Her 2-neu+ (34.3%). The mean ages of patients with expression of ER, PR and Her 2-neu amplification were 56.43, 54.36 and 52.95 years respectively. Triple negative tumour was found in 38 patients (21%). NPI 1

indicating good prognosis, NPI 2 with moderate and NPI 3 with poor prognostic categories were seen in 57 (31.5%), 74 (40.9%) and 50 (27.6%) patients respectively. Stage T2 sized tumour was identified in 88 patients (48.6%). A total of 110 patients (60.8%) had grade 2 tumours. 89 patients (49.1%) had axillary lymph node involvement.

Statistically significant correlation was found between the expression of ER and PR (Spearman's correlation coefficient $(r)=0.136$, p value <0.0001). Statistically significant inverse correlation existed between the expression of hormone receptors (ER&PR) and Her 2-neu amplification (chi square=12.93, p value <0.0001). Kruskal-Wallis test revealed statistically significant association between the expression of ER and the favourable NPI groups and also with low grade tumours (p value 0.046 and 0.006) and a statistically significant association between Her 2-neu amplification and axillary lymph node involvement (p value = 0.0001).

Keywords

Breast carcinoma, hormone receptors, Her 2-neu amplification, prognostic indices

Introduction

Carcinoma of breast accounts for 12.6% of all cancers and 27% of female cancers in Sri Lanka. It is the most common cancer among females in our country (1). The incidence of breast cancer

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in females in Sri Lanka has increased from 17.3 per 100,000 in 2001 to 24.7 per 100,000 in 2010. Highest incidence was noticed among female patients in the 60 to 64-year age group. Among the different histological types of breast cancer, 79.3 % are the invasive ductal carcinoma in Sri Lanka (2).

Breast cancer has diverse characteristics in oncological behaviour and treatment responsiveness. Apart from early identification of breast cancer it is also important to distinguish those breast cancers that are likely to cause morbidity and mortality. So stratifying the patients based on outcome prediction and tumour biology could aid to institute more personalized and responsive treatment (3, 4).

Several prognostic indices for breast carcinoma have been described in literature. Expression of estrogen and progesterone receptors (ER, PR), amplification of human epidermal growth factor receptor 2 (Her 2-neu), size and histological grading of tumour, axillary lymph node status are well established parameters that have influence on prognosis and treatment of breast carcinoma. Apart from these prognostic indices there are traditional prognostic indices such as the TNM (Tumour, Node, Metastasis) staging and NPI (Nottingham Prognostic Index) (5). The hormone receptor status, tumour grading and prognostic importance varies according to the histological type of tumour (6, 7). Analyzing prognostic indices on one particular histological type of breast carcinoma could nullify these variations.

The aim of this study is to assess the expression of hormone receptors (ER/PR) and amplification of Her 2-neu receptors in the infiltrating ductal carcinoma of breast and to analyse the correlation between these receptor status and prognostic indices among a cohort of female patients primarily treated by mastectomy in Jaffna.

Methodology

It is an institutional based retrospective cross sectional analytic study. Approval for the study was obtained from Ethics Review Committee, Faculty of Medicine, and University of Jaffna. Female patients who underwent primary mastectomy and axillary clearance for invasive ductal carcinoma of breast in Teaching Hospital Jaffna from 2014 to 2018 were included in this study. Patients who received neo-adjuvant therapy were excluded from this study. The expression of ER & PR and Her 2-neu amplification were identified by immunohistochemistry based on American Society of Clinical Oncology guidelines. Nuclear stain appearing in more than 1% of tumour cells would be considered positive for ER & PR. Her 2-neu amplification was scored from 0 to 3+ based on the pattern of staining. 0 and 1+ would be negative for Her 2-neu amplification. 3+ would be considered Her 2-neu +. There were five patients with equivocal Her 2 neu expression (2+) and they were excluded from this study. Informed written consent was obtained from patients. Patient's age at diagnosis, histopathological features (nodal status, tumour size and histological grade) and immunohistochemical marker expression (ER/PR/ Her 2) were recorded.

The NPI was calculated by using the formula : $(0.2 \times \text{tumour size in cm}) + \text{lymph node stage}$ (1 for node – negative, 2 for 1-3 positive lymph nodes and 3 for ≥ 4 positive lymph nodes) + histological grade (1 for good, 2 for moderate , 3 for poorly differentiated). This identified three

prognostic groups such as $\text{NPI} < 3.4$ (good prognostic group), $3.4 < \text{NPI} < 5.4$ (moderate prognostic group) and $\text{NPI} > 5.4$ (poor prognostic group).

Statistical analysis was carried out using SPSS 21.0(SPSS Inc). Correlation between the receptor status and prognostic indices were calculated using

Spearman's correlation, chi-square and Kruskal-Wallis tests. The p value < 0.05 was considered as being significant.

Results

Table 1: Association between ER and PR

Receptor status	PR + tumours	PR - tumours	Total
ER+ tumours	57 (31.5%)	30 (16.6%)	87 (48.1%)
ER- tumours	19 (10.5%)	75 (41.4%)	94 (51.9%)
Total	76 (42.0%)	105 (58.0%)	181 (100%)

57 patients (31.5%) had ER + and PR + tumours. 75 patients (41.4%) had ER – and PR – tumours [Table (1)]. The correlation between expression of ER and PR was statistically significant (Spearman's correlation coefficient (r) = 0.136, p value < 0.0001).

Table 2 : ER/PR receptor status and Her 2 neu amplification

Receptor status	Her 2 neu + tumours	Her 2 neu – Tumours	Total
ER+/PR+ tumours	9 (5%)	48 (26.5%)	57 (31.5%)
ER+/PR- tumours	9 (5%)	21 (11.6%)	30 (16.6%)
ER-/PR+ tumours	7 (3.9%)	12 (6.6%)	19 (10.5%)
ER-/PR- tumours	37 (20.4%)	38 (21%)	75 (41.4%)
Total	62 (34.3%)	119 (65.7%)	181 (100%)

Out of the 62 Her 2-neu + tumours, 37 (59.7%) were ER- & PR-. Out of the 119 Her 2-neu – tumours 48 (26.5%) were ER+ & PR+, 21 (11.6%) had ER+&PR- and 12 (6.6%) had ER- & PR+ [Table

(2)]. There is statistically significant inverse correlation between the Her 2-neu amplification and expression of either one or both of ER and PR (chi square=12.93, p value < 0.0001).

Table 3: NPI groups and the age at diagnosis of patients

NPI	Number of patients	
	< 45 years	> 45 years
Good	21 (11.6%)	36 (19.9%)
Moderate	15 (8.3%)	59 (32.6%)
Poor	13 (7.2%)	37 (20.5%)

132 patients (72.9%) were above 45 years old. Mean age of patients with invasive ductal carcinoma of breast in Jaffna is 54.5 years (Median age is 53 years). There was no statistically significant correlation observed between these different types of NPI and the age of patients at diagnosis of invasive breast cancer in Jaffna (p=0.106)

33% of invasive ductal carcinoma with expression of ER in this study was grouped under either NPI 1 or NPI 2. Another 16% of invasive ductal carcinoma with expression of ER was grouped under NPI 3. ER expression is more frequent in NPI types of favourable prognosis. This correlation was statistically significant (p=0.047). 89 patients (49.1%) had axillary lymph node involvement. 42 patients (25.2%) with axillary lymph node involvement had tumours with Her 2-neu amplification. 72 patients (39.8%) with no axillary lymph node involvement had tumors without Her 2-neu amplification. The correlation between the Her 2 –neu amplification and the axillary lymph node involvement was statistically significant (p =0.0001).

Table 4: Receptor status, age group of patients at diagnosis and prognostic indices

	ER		PR		HER 2 NEU		Total
	+	-	+	-	+	-	
Age (years)							
< 45	23 (12.7%)	26 (14.4%)	24 (13.3%)	25 (13.8%)	16 (8.8%)	33 (18.3%)	49 (27.1%)
> 45	64 (35.6%)	68 (37.6%)	52 (28.7%)	80 (44.2%)	46 (25.4%)	86 (47.5%)	132 (72.9%)
Kruskal - Wallis p value	0.853		0.246		0.782		
NPI group							
Good (1)	20 (11.1%)	37 (20.4%)	17 (9.4%)	40 (22.1%)	20 (11.1%)	37 (20.4%)	57 (31.5%)
Moderate (2)	38 (21%)	36 (19.9%)	35 (19.3%)	39 (21.5%)	24 (13.3%)	50 (27.6%)	74 (40.9%)
Poor (3)	29 (16%)	21 (11.6%)	24 (13.1%)	26 (14.4%)	18 (9.9%)	32 (17.7%)	50 (27.6%)
Kruskal-Wallis p value	0.046		0.080		0.907		
T stage							
T1	15 (8.3%)	20 (11.1%)	17 (9.5%)	18 (9.9%)	10 (5.6%)	25 (13.8%)	35 (19.4%)
T2	41 (22.6%)	47 (26%)	39 (21.5%)	49 (27.1%)	30 (16.6%)	58 (32%)	88 (48.6%)
T3	16 (8.9%)	17 (9.3%)	9 (5.0%)	24 (13.2%)	9 (5.0%)	24 (13.2%)	33 (18.2%)
T4	15 (8.2%)	10 (5.6%)	11 (6.1%)	14 (7.7%)	13 (7.2%)	12 (6.6%)	25 (13.8%)
Kruskal-Wallis p value	0.596		0.285		0.194		
Tumour grade							
1	16 (8.8%)	32 (17.7%)	14 (7.7%)	34 (18.8%)	17 (9.4%)	31 (17.1%)	48 (26.5%)
2	54 (29.9%)	56 (30.9%)	50 (27.6%)	60 (33.2%)	39 (21.6%)	71 (39.2%)	110 (60.8%)
3	17 (9.4%)	6 (3.3%)	12 (6.6%)	11 (6.1%)	6 (3.3%)	17 (9.4%)	23 (12.7%)
Kruskal-Wallis p value	0.006		0.094		0.678		
Axillary lymph node							
Involved (+)	38 (21%)	51 (28.2%)	38 (21%)	51 (28.2%)	42 (23.2%)	47 (26%)	89 (49.2%)
Not involved (-)	49 (27%)	43 (23.8%)	38 (21%)	54 (29.8%)	20 (11%)	72 (39.8%)	92 (50.8%)
Kruskal-Wallis p value	0.156		0.850		0.0001		

21% of patients with invasive ductal carcinoma of breast in Jaffna had triple negative tumour. 11%, 7.2% and 2.8% of triple negative invasive ductal carcinomas were found to be in NPI 1, NPI 2 and NPI 3 groups respectively.

Table 5 : ER/PR/Her neu subtypes and NPI groups

ER/PR/ Her 2 neu subtypes	NPI					
	Good		Moderate		Poor	
	n	(%)	n	(%)	n	(%)
ER+/PR+, Her 2+	2	(3.5%)	2	(3.5%)	5	(2.8%)
ER+/PR-, Her 2+	4	(2.2%)	4	(2.2%)	1	(0.6%)
ER-/PR+, Her 2 +	3	(1.7%)	4	(2.2%)	0	(0.0%)
ER-/PR-, Her 2 +	11	(6.1%)	14	(7.7%)	12	(6.6%)
ER+/ PR+,Her 2 -	9	(5%)	24	(13.3%)	15	(8.3%)
ER+/PR- ,Her 2 -	5	(2.8%)	8	(4.4%)	8	(4.4%)
ER-/ PR+,Her 2 -	3	(1.7%)	5	(2.8%)	4	(2.2%)
ER-/PR- Her 2 -	20	(11%)	13	(7.2%)	5	(2.8%)

Discussion

Hormone receptor status (ER/PR) in invasive breast cancer has more predictive strength than prognostic strength. Expression of these hormone receptors can predict benefit from adjuvant endocrine therapy. Over expression of Her 2 neu is associated with increased risk of recurrence and poor prognosis and it is considered as a prognostic factor. Her 2 neu expression can predict treatment responsiveness to anthracycline based chemotherapy and not to endocrine therapy. So it is a strong predictive factor as well (4,7,8).

Predictive factors are complementary to prognostic factors. Prognostic indices when used in combination with predictive factors could help to correctly identify patients with high risk and low risk after primary surgery. This will guide to tailor adjuvant therapy in order to improve patient outcome (9, 10).

Table 6 : ER, PR & Her 2-neu status in different regions (11, 7, 12, 13, 14)

Region	ER+	PR+	Her 2-neu +	Mean age (years)
Current study (Jaffna, n=181)	48.1%	41.9%	34.3%	54.5
Ratnatunga et al (Kandy, n=123)	53.2%	50.4%	14.6%	Not mentioned
Bimalka et al (Colombo, n=92)	82.1%	65.4%	6.4%	53.9
Bhaskar et al (India, n=288)	56%	41%	40%	Not mentioned
Dodson et al (UK, n=199,000)	82.7%	64.9%	13.1%	61
Onitilo et al (West indies, n=1134)	77.9%	59.1%	17.7%	62.7

When compared with similar studies carried out in different provinces in Sri Lanka, the prevalence of invasive ductal carcinoma of breast with HER 2-neu amplification (HER 2-neu +) is relatively high in Jaffna while the prevalence of tumours with expression of ER (ER +) status is relatively low. Other histological types of breast carcinoma were also taken into account in studies from different provinces and could have contributed to the differences observed.

A positive correlation between ER and PR expression was observed in this study population as well as in other studies in Sri Lanka by Ratnatunga et al and Bimalka et al (11, 7). The negative correlation between Her 2-neu and ER/PR was observed in this study as well as in other studies from Sri Lanka and India (7, 11,12).

Correlation between various demographic factors were analysed and the existence of a statistically significant relationship between ER expression and age of patients has been reported by Ratnatunga et al from Kandy, Sri Lanka (11). Faheem et al from Pakistan have identified higher incidence of ER and PR expression among post-menopausal patients with breast cancer (15). ER + tumours tend to occur in slightly older patients in Jaffna. But this correlation was not statistically significant (p =0.853) No statistically significant correlation between the Her 2-neu amplification and age of breast cancer patients was observed in studies from

Sri Lanka and India (11, 16, 12). Faheem et al from Pakistan found that Her 2–neu amplification was significantly higher in breast carcinomas occurring in premenopausal patients ($p < 0.001$) (15).

The Nottingham Prognostic Index (NPI) is a numerical value calculated from histopathological parameters such as the nodal status, tumour size and histological grade. Thus, NPI stratify the breast cancers based on their metastatic potential, rate of growth and extent of genetic instability. NPI distinguishes good, moderate, and poor prognostic groups (NPI 1, 2 & 3 respectively) in breast cancer. It is a well recognized method to predict survival of operable breast cancer (17). The 10 year survival rates of these prognostic groups, namely NPI 1, NPI 2 and NPI 3, are 83%, 52% and 13 % respectively (18).

ER expression is more frequent in NPI types of favourable prognosis. This correlation was statistically significant ($p = 0.047$). There was no statistically significant correlation observed between NPI groups and expression of PR, Her 2–neu amplification or the age of patient at diagnosis of invasive breast cancer in Jaffna (p values 0.081, 0.908 and 0.106 respectively). Peiris et al from Galle found that more NPI 1 tumours occurring with increasing age and the correlation between age of patients at presentation and NPI was statistically significant ($p = 0.011$) (16). Kurshumliu et al has demonstrated that the NPI 1 and NPI 2 groups had tumours expressing more ER and PR ($p < 0.01$) whereas the NPI 3 group had tumours expressing more Her 2-neu ($p = 0.01$) (19).

When ER/PR status and Her 2 neu status are used together in invasive breast cancer they have more prognostic and therapeutic yield. For example, triple negative (ER -, PR -, Her 2 neu -) invasive breast cancers have the worst overall and disease free survival (14).

21% of patients with invasive ductal carcinoma of breast in Jaffna had triple negative tumour [Table (5)]. Triple negative invasive breast carcinoma was found in 28.23% patients in Sri Lanka and 30% of patients India (11, 12). This included all

other histological types of breast carcinomas as well. Bimalka et al demonstrated that in breast carcinomas with histological types other than ductal and lobular carcinomas, 33.3% had triple negative tumours (7). Considering these facts, the prevalence of triple negative breast cancer in Jaffna is comparable to other regions in Sri Lanka.

Interesting finding in this study is that relatively more (11%) of triple negative invasive ductal carcinomas were found to be in NPI 1 and less (2.8%) of triple negative invasive ductal carcinomas were found in NPI 3 groups [Table (5)]. Statistical analysis for this correlation is difficult because of limited number of patients in certain sub types. Albergaria et al have demonstrated that most of the triple negative tumours were grouped under NPI 3 group (20).

T staging of invasive ductal carcinoma of breast in Jaffna did not have statistically significant correlation either with expression of ER/PR or with Her 2 –neu amplification (p values 0.599, 0.287 and 0.196 respectively). Bhaskar et al from India have observed significant association of Her 2 neu amplification with large tumours ($p = 0.008$) (12). Faheem et al from Pakistan identified significant association of expression of ER in smaller tumours ($p = 0.002$) and her 2-nue amplification in larger tumours ($p < 0.0001$) (15).

Expression of ER was more frequently seen in grade I and II tumors. The correlation between the expression of ER and grade of tumour in this study was statistically significant ($p = 0.006$). Either the expression of PR or the Her 2-neu amplification did not show significant correlation with grade of tumour. Ratnatunga et al from Kandy reported significant correlation existing between expression of ER and PR with tumour grade ($p < 0.001$) but there was no significant correlation between Her 2-neu amplification and grade of tumour (11). Balawardena et al from Colombo found that expression of ER and PR was more in low grade tumours and Her 2-neu amplification was more in high grade tumours. This correlation was statistically significant ($p = 0.001$) (21)

The correlation between the Her 2–neu amplification and the axillary lymph node involvement was

statistically significant ($p=0.0001$). There was no statistically significant correlation between expression of ER or PR and associated axillary lymph node involvement in this study. Bimalka et al from Colombo found axillary lymph node involvement in 31.52% of patients and there was no significant correlation existed between tumours with Her 2-neu amplification and associated axillary lymph node involvement (7). Peiris et al from Galle identified axillary lymph node involvement in 55.2% of patients and the frequency of lymph node involvement was reducing with increasing age ($p=0.012$). They have also described that lymph node staging was the most important independent predictor of survival in breast cancer irrespective of the age of patient at diagnosis (16).

Conclusion

There is positive correlation between the expression of ER and PR and an inverse correlation between the expression of hormone receptors and the Her 2 –neu amplification among invasive ductal carcinoma patients in Jaffna. The expression of ER is significantly associated with favourable NPI groups and also with low grade tumours. The prevalence of invasive ductal carcinoma with Her 2-neu amplification is relatively high in Jaffna and the tumours with Her 2-nue amplification are significantly associated with axillary lymph node involvement.

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