Hormone receptor expression and Her/2neu amplification in breast carcinoma in a cohort of Sri Lankans

N Ratnatunga¹ and L V C Liyanapathirana²

Abstract

Objective 1) To determine the profile of estrogen and progesterone receptors (ER, PR) expression and Her/2neu amplification in carcinoma of breast of Sri Lankan women. 2) To determine their inter-relationships, and associations with age at diagnosis and histological grade.

Design Retrospective analysis of data.

Setting Department of Pathology, Faculty of Medicine, University of Peradeniya.

Materials 124 samples of invasive ductal carcinoma of breast, stained for steroid receptors and Her/2neu amplification with immunohistochemistry.

Measurements 1) Semiquantitative scores of steroid receptors and Her/2neu amplification. 2) Correlations of ER, PR, Her/2neu amplification, age at diagnosis and histological grade.

Results The prevalence of ER, PR and Her/2neu amplification were 53.2%, 50% and 14.6% respectively. The expression of ER and PR were significantly correlated ($p < 0.001$), and had a significant negative correlation with Her/2neu amplification ($p = 0.003$ each). Lower grade of the tumour was significantly related to the expression of ER ($p = 0.000$) and PR ($p = 0.000$) but not to Her/2neu amplification ($p = 0.331$). Age at diagnosis was significantly correlated to the expression of ER ($p = 0.004$), but not to PR ($p = 0.365$) or Her/2neu amplification ($p = 0.456$).

Conclusion Prevalence of ER, PR and Her/2neu amplification in carcinoma of breast among Sri Lankans is similar to that described internationally. The correlations of ER, PR, Her/2neu amplification, to each other, age at diagnosis and grade of tumour is as reported in other countries.

Introduction

The expression of estrogen (ER), progesterone (PR) receptors and amplification of Her/2neu, in breast carcinomas determine modern treatment modalities and help in prognostication [1,2,3]. No published data is available on these profiles in the Sri Lankan context to the best of our knowledge, at the time of writing. As an ethnic variability in the expression of these immunohistological markers is documented [2,4], extrapolation of the results of foreign studies to Sri Lankan population is not possible.

The aims of this study are, to determine the prevalence of ER, and PR expression and Her/2neu amplification in carcinoma of the breast in Sri Lankans, to determine the inter-relationships between ER, PR and Her/2neu, and to determine the relationships these markers have with the age at diagnosis and histological grade of the tumour.

Methods

Sample

The sample consisted of 124 specimens of invasive breast carcinoma, referred to the principal investigator for assessment of steroid receptors and Her/2neu amplification.

Hormone receptor (ER and PR) values were available in all 124 and Her/2neu score was available in 123.

Tumour grade was available on 112 of the specimens. The age at diagnosis was available in 103 patients.

Ethical clearance

Approval was obtained from the Ethical Review Committee, Faculty of Medicine, University of Peradeniya.

Method

Formalin fixed, paraffin embedded blocks were used for both immunohistochemical analysis and for histological grading of the tumours.

Tumours were graded according to the Nottingham's grading system as quoted by Rosai [1].

Two groups, those aged 45 years and below, and above 45 years, were considered for the analysis of correlation between age at diagnosis and hormone receptors.

Three staged Strept ABC immunohistochemistry systems using DAKO antibodies (M7047, M3569, A0485) were used to visualise the hormone receptors and Her/2neu amplification. Semiquantitative scoring of ER and PR was done using the quick Allred score (0-8) and a total score of more than 2 was taken as being positive for both (maximum 8) [1].

¹Professor and ²Lecturer, Department of Pathology, Faculty of Medicine, University of Peradeniya, Sri Lanka.

Correspondence: LVCL, e-mail <veranjacl@yahoo.com>. Received 20 March and accepted 23 November 2007.

Conflicts of interest: none declared.

Her/2neu amplification was scored according to the guidelines given in Pathology Reporting of Breast Diseases, NHSBSP Publication No 58, January 2005 [5]. A score of +3 was taken as being positive, and +2 was taken as borderline.

Statistical analysis

Statistical analysis was carried out using SPSS version 10.5 (SPSS Inc. Chicago, IL). Associations between variables were calculated by using Spearman's rho correlation and chi square test. All tests of statistical significance were two tailed and a p value of < 0.05 was taken as being significant.

Results

Hormone receptor and Her/2neu profile

Of the 124 invasive breast carcinomas, 66 (53.2%) were positive for ER and 62 (50%) were positive for PR. Eighteen (18/123) were Her/2neu +3 positive and 7 (5.9%) were +2 positive. Thirty seven (56%) of the 66 ER positive tumours and 33 (43.3%) of the 62 PR positive tumours had scores of +7 or +8.

The combined profile of hormone receptors and Her/2neu is given in table 1.

Table 1. Profile of hormone receptors and Her/2neu status

Immunohistochemical type	Number	Percentage %
ER(+)/PR(+)/Her2neu(-)	50	40.32
ER(-)/PR(-)/Her2neu(-)	35	28.23
ER(-)/PR(-)/Her2neu(+3)	13	10.48
ER(+)/PR(-)/Her2neu(-)	8	6.45
ER(-)/PR(+)/Her2neu(-)	5	4.03
ER(-)/PR(-)/Her2neu(+2)	3	2.42
ER(+)/PR(+)/Her2neu(+2)	3	2.42
ER(+)/PR(-)/Her2neu(+3)	2	1.61
ER(-)/PR(+)/Her2neu(+3)	2	1.61
ER(+)/PR(+)/Her2neu(Not done)	1	0.81
ER(+)/PR(+)/Her2neu(+3)	1	0.81
ER(+)/PR(-)/Her2neu(+2)	1	0.81
Total	124	100.00

(ER= estrogen receptors, PR= progesterone receptors)

Relationship between ER and PR

Fifty five (44.5%) tumours were positive for both receptors and 51 tumours (41.1%) were negative for both receptors. Eleven tumours (8.9%) were ER(+)/PR(-) and 7 (5.6%) were ER(-)/PR(+)(table 2).

Table 2. Relationship between ER and PR

	PR (+)		PR (-)		Total
	N	%	N	%	
ER(+)	55	88.7%	11	17.7%	66
ER(-)	7	11.3%	51	82.3%	58
Total	62	100	62	100	124

A statistically significant correlation was found between the expression of ER and PR (correlation coefficient 0.711, p value < 0.001).

Relationship between hormone receptor expression and Her/2neu amplification

Her/2neu amplification was found more among the hormone receptor negative tumours, and absence of Her/2neu amplification was more among the hormone receptor positive tumours (table 3). Thus, there was a statistically significant negative correlation between the expression of hormone receptors and Her/2neu amplification. (correlation coefficient - 0.267, and Pearson's chi square - 0.268 with p values of 0.003 each).

Table 3. Relationship between hormone receptor expression and Her/2neu amplification

	Her 2 neu (+)3	Her 2 neu (+)2	Her 2 neu (-)	Total
*ER(+)/PR(+)	1	3	50	54
ER(+)/PR(-)	2	1	8	11
PR(-)/PR(+)	2	0	5	7
ER(-)/PR(-)	13	3	35	51
Total	18	7	98	123

*Her/2neu not done on one specimen

Relationship of tumour grade with hormone receptor expression and Her/2neu amplification

The grade of the tumour was significantly correlated to the expression of both ER and PR (correlation coefficients 0.459 and 0.397 with p values < 0.000 and 0.000 respectively). More grade I and II tumours were found to be expressing the hormone receptors than grade III tumours. The reverse was also found to be true with hormone receptor negative tumours being found more among grade III tumours than grade I and II tumours. (figure 1).

No statistically significant relationship was found between Her/2neu amplification and tumour grade (figure 1) (correlation coefficient of - 0.093 and p value of 0.331).

Figure 1. Relationship of tumour grade to hormone receptor expression and Her/2neu amplification.

Figure 2. Relationship of age at diagnosis to hormone receptor expression and Her/2neu amplification.

Relationship of age at diagnosis with hormone receptor expression and Her/2neu amplification

A statistically significant relationship was found between the expression of ER and age at diagnosis (correlation coefficient - 0.281, p value 0.004 and Pearson's chi square 8.116, p value 0.004). Thirty eight (71.7%) ER+ tumours were in > 45 years old group. Twenty eight (56%) ER- tumours were in the age group ≤ 45 years old i.e. estrogen receptor positive tumours were found more among the older group of patients than the younger group of patients and vice versa for the ER negative tumours (figure 2).

There were no statistically significant relationships

between the age at diagnosis and the expression of PR (p values of 0.365 and 0.360) and Her/2neu amplification (p values of 0.456 and 0.163). However, there was a trend for the PR positive tumours to be found more among the older (> 45 years) group and the negative tumours to be found more among the younger (≤ 45 years) group. A trend was also noted in Her/2neu amplification; Her/2neu was found to be amplified more among the younger (≤ 45 years) group than the older (> 45 years) group (figure 2).

Discussion

In the cohort studied, the prevalence of ER and PR expression and Her/2neu amplification were 53.2%, 50.0%

and 14.4% respectively. These fall within the ranges given in international literature [3,6,7].

Our cohort had 44.4 % ER+PR+, 41.1 % ER-PR-, 8.9 % ER+PR- and 5.6 % ER-PR+ tumours and these are also similar to those reported in the literature [2,4].

A clear positive correlation was shown between the expression of ER and PR, as reported in other studies [2,8]. This also abides by the theory of ER dependant PR synthesis [8].

The expression of ER and PR correlated inversely with Her/2neu amplification. Similar results were shown by previous studies [3,6,9,10]. The generally accepted inverse correlation between the expression of hormone receptors and Her/2neu amplification is explained by estrogen dependant down regulation of Her/2neu involving complex molecular interactions, as reviewed by Ciocca et al, and as shown by in vitro studies [3,9].

The lower grade tumours showed a high ER and PR positivity, being similar to other studies [7].

Contrary to many previous reports no statistically significant relationship was found between the tumour grade and Her/2neu amplification [6,10]. Similar results were also reported in some of the earlier work [11].

In most of the studies showing the correlation, fluorescence in situ hybridization (FISH) has been performed on Her/2neu borderline positive cases to determine whether they are actually positive or not whereas others have taken borderline positivity as being Her/2neu positive. These may explain the discrepancy in our result.

Our study revealed a significant relationship between the patients' age at diagnosis and the presence of ER, which was found more among the older patients. This has been more or less a constant finding in other countries too [4,6,12].

Previous studies give contradictory reports on the relationships PR and Her/2neu amplification has with age [4,6,12]. Since it is accepted both by clinical data and in vitro studies that ER and PR is correlated to each other, an age dependant increase of ER should theoretically be associated with an age dependant increase in PR. However, it has been shown that there is an age and oxidant stress related uncoupling of the ER-PR axis which may explain the lack of relationship between age and PR [12].

For Her/2 amplification, equivocal results are given in the literature, with some indicating a relationship where Her/2neu is more prevalent among younger patients and others showing no relationship between age and Her/2neu amplification [6,10,11].

In conclusion, the prevalence of ER, PR and Her/2neu amplification among Sri Lankan breast cancer patients

fall within the ranges given in literature. Also, ER and PR are positively correlated and hormone receptors and Her/2neu amplification are negatively correlated. The grade of the tumour is related to expression of both ER and PR but not to Her/2neu amplification. The age at diagnosis is related only to the presence of ER.

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