Paper

Pathological characteristics of triple negative breast cancer phenotype in a cohort of Sri Lankan females

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Abstract

Introduction: Breast cancer is the commonest cancer among Sri Lankan women. Triple negative is a type of breast cancer that is negative for expression of oestrogen receptors (ER), progesterone receptors (PR) and human epidermal growth factor 2 receptors (HER 2).

Objective: To identify the pathological characteristics of triple negative breast cancer phenotype in a group of Sri Lankan women.

Method: 200 cases of diagnosed breast cancers were analysed to determine the prevalence of triple negative breast cancers. All the slides were re-examined histologically to find out whether there were specific morphological features in the triple negative group when compared with the ER/PR positive HER 2 negative group, ER/PR negative HER2 positive group and ER/PR positive HER2 positive group.

Results and conclusion: The prevalence of triple negative breast cancer in the study group was 36%. The mean age affected by this tumour category was 49.5 years. The commonest age group affected was 41-50 years. The commonest histological type in all categories was invasive ductal carcinoma. A significant number of triple negative tumours were histologically Grade 3 compared to the ER/PR positive HER2 negative group. The mean tumour size was 2.73 cm. Although the percentage of triple negative tumours among the lymph node stages was similar, a statistically significant number of triple negative tumours was in stage 3 and showed tumour necrosis. The triple negativity was also associated with a lymphocytic reaction at the host tumour interface and infiltrative margins. Triple negative status showed a negative association with skin and nipple invasion, lymphovascular invasion and an in-situ component.

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Introduction

Breast cancer is the commonest cancer among Sri Lankan women.[1] Triple negative breast cancer is a specific subtype of breast cancer, so called because the tumour cells are negative for expression of oestrogen receptors (ER), progesterone receptors (PR), and human epidermal growth factor 2 receptor (HER 2) proteins. They do not respond to endocrine therapy and Herceptin as the required receptors are not expressed in them. The only treatment option available currently is chemotherapy.[2,3]

These cancers occur more commonly in young (<40years), African American women[4] and show an aggressive behaviour and poor survival rates[5]. The prevalence is about 15% in the world[6]. The range in some studies is 10-20%.6 In Sri Lanka the prevalence was 28.23% according to a study done in 2007[7]. The so called 'basal- like' breast cancers are also triple negative and they arise from the outer myoepithlial or basal cell layer of the duct epithelium and express a unique basal like (basal layer/myoepithelial-like) molecular profile and morphological characteristics.[2,8] They are positive for basal cytokeratins such as CK 5/6, CK 14, CK17 and EGFR and are more common in BRCA 1 carriers.[3,9] The morphological features include invasive ductal carcinoma like features 10 and presence of other features such as medullary carcinoma-like features and metaplastic elements such as spindle cells and squamous elements. Other features include a high grade (Grade 3), a central scar, pushing growth patterns, geographic or comedo type necrosis with two distinct growth patterns including a solid sheet like growth and a trabecular arrangement.[5,10] Many of these tumours show frequent apoptotic bodies, scanty stromal content and glomeruloid microvascular proliferations.[11] The triple negative phenotype significantly correlates with tumour size, histological grade, lymph node status, P 53 expression and EGFR according to a study done in China.[12]

It is also documented that identifying "basal like" tumours as a distinct entity has a real clinical significance because there are several associations that are opposite to those observed for luminal group of cancers[10]. More importantly, brain metastases are more common than bone metastases[10,13] in these tumours. Therefore, it is important to document the status of triple negative phenotype in Sri Lanka to decide on targeted therapy.[3]

Objectives

- To determine the prevalence of triple negative breast cancers (Group A) in

a cohort of breast cancer patients and compare it to the prevalence of ER/PR positive and HER 2 negative cancers (Group B), ER/PR negative and HER 2 positive cancer group (Group C) and ER/PR positive and HER 2 positive cancer group (Group D)

- To determine the age distribution in group A and compared to groups B C and D
- To determine the pathological characteristics of group A with special reference to basal like morphological features
- To determine the occurrence of such pathological characteristics in group A compared to the other groups (B, C and D)

Methods

The material consisted of two hundred breast carcinomas referred for receptor status analysis. The morphology of the tumour was studied with haematoxylin and eosin stained sections.

Staining for ER, PR and HER2 was done using DAKO monoclonal antibodies and a three stage system. Semi quantitative 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 in this analysis.[9] HER2 amplification was scored according to the guidelines given in pathology reporting of breast diseases NHSBSP publication no 58, January 2005. A score of +3 was taken as being positive and +2 was taken as borderline.[9] The slides were interpreted independently by the two authors, and a consensus reached if there was a discrepancy.

The pathological characteristics considered were the histological type, histological grade (Nottingham), tumour size at diagnosis, invasion of nipple and skin, lymph node stage, infiltrative or pushing tumour margins, a lymphocytic reaction at the host tumour interface, presence of lymphovascular invasion, an associated in-situ component and the presence of tumour necrosis.

Results and interpretation

The percentage prevalence of triple negative breast cancers (Group A) was 36% compared to Group B - 47.5%, Group C – 9%, and Group D – 7.5% (Table1). A 7.77% increase in the percentage of triple negative breast cancers was noted compared to 28.23% previously reported in a Sri Lankan study[7]. Moreover, the prevalence of triple negative breast cancers in our study group is more than double that of the world average prevalence.[6] This higher prevalence of triple negativity raises the possibility of technical errors or a higher

predisposition among in Sri Lankan women. The mean age of patients with triple negative breast cancers (Group A) was 49.5 years (Table1) in contrast to that seen in other countries[4]. Multiple comparisons of mean age in four groups did not show a statistically significant difference (p value > 0.05). The highest percentage of triple negative cancers was seen in 41-50 year age group. This finding contrasts with that of the findings in other countries[4]. Notably, this is also the commonest age group for the other common histological types of breast cancer (B, C, D), ((Table 2). A statistically significant difference was not seen in the histological types of tumour in group A compared to group B (p value >0.687). Statistical analysis between A and C, D was not possible due to lack of samples in one of the sub categories. Invasive ductal carcinomas were the commonest, followed by invasive lobular carcinoma in all four groups (Table 3). The finding of a high percentage of invasive ductal carcinomas among the triple negative group is in keeping with similar studies done previously.[10]

Table 1: Number and Mean age at presentation.

Category of tumour	Number (Percentage)	Mean age (years)
Triple negative breast cancers (Group A)	72 (36%)	49.5775
ER/PR positive HER2 negative breast cancers (Group B)	95 (47.5%)	52.0253
ER/PR negative HER2 positive breast cancers (Group C)	18 (9%)	49.7500
ER/PR positive HER2 also positive breast cancers (Group D)	15 (7.5%)	48.7143
Total number of samples (Groups A+B+C+D)	200 (100%)	49.5775

Table 2: Age divided into ten year intervals				
Age interval (years)	**Triple negative breast cancers (Group A)**	**RE/PR positive HER2 negative breast cancers (Group B)**	**ER/PR negative HER 2 positive breast cancers (Group C)**	**ER/PR positive HER 2 positive breast cancers (Group D)**
	Frequency (Percentage)	**Frequency (Percentage)**	**Frequency (Percentage)**	**Frequency (Percentage)**
21-30	3 (4.2%)	2 (2.1%)	0 (0%)	2 (13.3%)
31-40	11 (15.3%)	9 (9.5%)	3 (17.6%)	2 (13.3%)
41-50	24 (33.3%)	27 (28.4%)	6 (35.3%)	5 (33.3%)
51-60	22 (30.6%)	27 (28.4%)	5 (29.4%)	3 (20.0%)
61-70	9 (12.5%)	9 (9.5%)	2 (11.8%)	1 (6.7%)
71-80	2 (2.8%)	4 (4.2%)	0 (0%)	1 (6.7%)
81 and above	0 (0%)	1 (1.1%)	0 (0%)	0 (0%)
Data not available	1 (1.3%)	16 (16.8%)	1 (5.9%)	1 (6.7%)
Total	72 (100.0%)	95 (100%)	17 (100.0%)	15 (100.0%)

The least common types (listed into one category called Histological type 3 for statistical analysis) were mucinous carcinoma (1 case), cribriform carcinoma (1 case) and papillary carcinoma (3 cases) within the ER/PR positive HER 2 negative category. However, these minor categories could not be included in the statistical analysis due to insufficient sample numbers.

Grade 3 tumours were significantly higher in group A compared to group B (Table 3) (p value = 0.000). These results were in keeping with the results of the other studies and the study done in China.[10, 12] Moreover, the percentage of grade 1 tumours is considerably less within Group A (Table3). However, all four groups showed highest percentage of Grade 2 tumours (Table 3). In the Nottingham grading system, a high histological grade reflects a high mitotic count, high degree of nuclear pleomorphism and lack of tubule formation.[14,15] These are also features of the basal-like tumours.[11, 14] Some of the studied triple negative tumours may have

a molecular profile of basal-like tumours and require further molecular studies in Sri Lankan patients.

The lymph node stage at presentation was not significantly different in group A compared to Group B (p value 0.387 in Chi squared test) (Table 3).

Table 3: Histological type, histological grade and tumour stage

Category	Sub Type	Triple negative breast cancers (Group A)	ER/PR positive HER 2 negative breast cancers (Group B)	ER/PR negative HER 2 positive breast cancers (Group C)	ER/PR positive HER 2 positive breast cancers (Group D)
		Frequency (Percentage)	Frequency (Percentage)	Frequency (Percentage)	Frequency (Percentage)
Histological Type	IDC	66 91.7%	84 93.3%	17 100%	15 100%
	ILC	6 8.3%	6 6.7%	0 0%	0 0%
	TOTAL	72 100%	90 100%	17 100%	15 100%
Histological grade	1	11 15.3%	27 28.4%	0 0%	4 26.7%
	2	39 54.2%	64 67.4%	9 52.9%	9 60%
	3	22 30.6%	4 4.2%	8 47.1%	2 13.3%
	TOTAL	72 100%	95 100%	17 100%	15 100%
Lymph node stage	1	16 34%	26 47.3%	5 45.5%	2 40%
	2	15 31.9%	15 27.3%	3 27.3%	1 20%
	3	16 34%	14 25.5%	3 27.3%	2 40%
	TOTAL	47 100%	55 100%	11 100%	5 100%

The mean tumour size at diagnosis for group A was 2.73 cm. A statistically significant difference was not seen in the tumour size among the four categories when the Chi square test was applied for analysis. (Table 4). Therefore, the triple negative status does not significantly correlate with the tumour size, a finding which is contradictory to the results of the Chinese study.[12] There was no statistically significant difference in the type of tumour margin between group A and groups B or groups A and C (p values 0.115 and 0.339 respectively). Statistical analysis was not possible between groups A and D due to lack of samples in category 1 of group D. All the groups showed a high percentage of infiltrating margins (Table 4). Tumours with pushing margins are known to have a better prognosis than tumours with infiltrating margins.[14]

The presence of tumour necrosis was significant in group A compared to group B (p value = 0.001). This could be due to the fact that some tumours in group A were basal like tumours, where geographic areas or comedo type necrosis are a characteristic feature[5, 10]

However, groups C and D did not show a significant difference to group A with regard to necrosis (p values 0.431 and 0.597 respectively) (Table 4).

Table 4: Mean tumour size, type of tumour margin, tumour necrosis

Category	Sub Type	Triple negative breast cancers (Group A) Frequency (Percentage) or size	ER/PR positive HER 2 negative breast cancers (Group B) Frequency (Percentage) or size	ER/PR negative HER 2 positive breast cancers (Group C) Frequency (Percentage) or size	ER/PR positive HER 2 positive breast cancers (Group D) Frequency (Percentage) or size
Mean Tumour size		2.7353 cm	3.0049 cm	2.4909 cm	2.3600 cm
Tumour margin	Pushing	15 21.7%	11 12.4%	5 33.3%	0 0%
	Infiltrating	54 78.3%	78 87.6%	10 66.7%	14 100%
	Total	69 100%	89 100%	15 100%	14 100%
Tumour necrosis	Absent	33 45.8%	68 71.6%	6 35.3%	8 53.3%
	Present	39 54.2%	27 28.4%	11 64.7%	7 46.7%
	Total	72 100%	95 100%	17 100%	15 100%

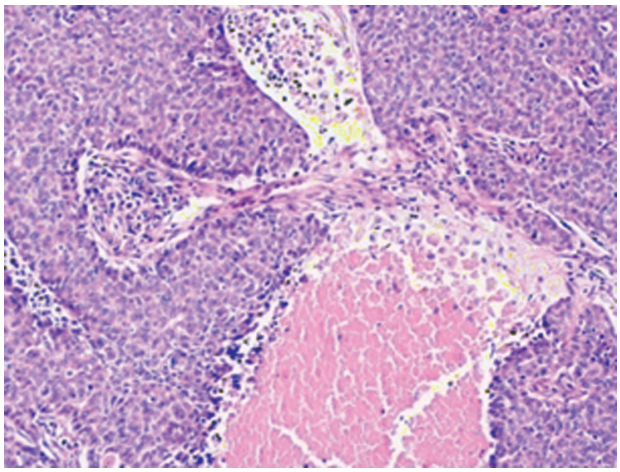

Fig.1. Basal-like breast cancer with areas of necrosis and high grade nuclei (H&Ex10)

All four groups did not have a high percentage of skin and nipple invasion (Table 5).

It has been found that tumours with an absence of an inflammatory reaction at the periphery have a lesser degree of nodal metastases and presumably a better prognosis, the only exception being medullary carcinoma[14].

In our study, all four groups showed a higher percentage of tumours with a lymphocytic reaction at the host-tumour interface (Table 5). This finding is a bad prognostic factor for triple negative and other types of cancers such as medullary carcinomas which were not included in the 200 cases analysed.

The presence of tumour emboli in lymphatic vessels within the breast is associated with an increased risk of tumour recurrence.[14] Presence of tumour emboli has shown a high correlation with tumour size, histological grade, tumour type, lymph node status, development of distant metastasis and a poor prognosis.[14] All four groups showed a higher percentage of tumours without lympho-vascular invasion (Table 5). This is a good prognostic factor observed in the triple negative group. Also the difference was not statistically significant when group A was compared with groups B, C and D (p values 0.754, 0.705 and 0.138 respectively in chi square test).

The amount of in-situ component correlates with the incidence of multicentricity and indirectly with the probability of occult invasion.[14] It is also reported that sometimes in-situ ductal malignancies of the comedo carcinoma type can be associated with metastases in the absence of detectable invasion.[14] Therefore, the presence of an in-situ component is associated with an unfavourable prognosis.

Our results showed a negative association of all four groups with the occurrence of an in-situ component and thus a better prognosis (Table 5).

Table 5: Invasion of skin and nipple, reaction at tumour margin, lymphovascular invasion, in-situ component

Category	Sub Category	Triple negative breast cancers (Group A) Frequency (Percentage)	ER/PR positive HER 2 negative breast cancers (Group B) Frequency (Percentage)	ER/PR negative HER 2 positive breast cancers (Group C) Frequency (Percentage)	ER/PR positive HER 2 positive breast cancers (Group D) Frequency (Percentage)
Invasion of skin and nipple	Absent	36 94.7%	37 92.5%	7 100%	3 75%
	Present	2 5.3%	3 7.5%	0 0%	1 25%
	Total	38 100%	40 100%	7 100%	4 100%
Reaction at tumour margin	Absent	7 10%	8 9%	0 0%	0 0%
	Present	63 90%	81 91%	15 100%	14 100%
	Total	70 100%	89 100%	15 100%	14 100%
Lympho-vascular invasion	Absent	43 59.7%	59 62.1%	11 64.7%	12 80%
	Present	29 40.3%	36 37.9%	6 35.3%	3 20%
	Total	72 100%	95 100%	17 100%	15 100%
In-situ component	Absent	53 73.6%	58 61.1%	9 52.9%	10 66.7%
	Present	19 26.4%	37 38.9%	8 47.1%	5 33.3%
	Total	72 100%	95 100%	17 100%	15 100%

Conclusions

Triple negative breast cancers showed a significantly high number of grade 3 cancers and cancers with tumour necrosis compared to ER/PR positive HER2 negative cancers.

Although statistically not significant,

triple negativity was associated with a higher number of invasive ductal type carcinoma, a high lymph node stage, infiltrative margins and presence of a lymphocytic reaction at the host tumour interface when compared with ER/PR positive HER2 negative group, ER/PR negative HER2 positive group and ER/PR negative HER2 negative groups. The triple negative group showed a negative association with tumour size skin and nipple invasion, lymphovascular invasion and an in-situ component when compared with ER/PR positive HER2 negative group, ER/PR negative HER2 positive group and ER/PR negative HER2 negative groups.

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