Original Article

Prevalence of molecular subtypes of breast cancer: experience in a tertiary care cancer centre in Sri Lanka

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Abstract

Breast cancer is the commonest cancer among females. Patient outcome and treatment options are largely influenced by its molecular subtypes. This study aims to evaluate molecular subtypes of breast cancers among patients treated at a tertiary cancer centre in Sri Lanka.

Clinical and demographic data were collected retrospectively from electronic medical records available at the cancer institute, Maharagama, Sri Lanka. Histology reports of 200 patients who had undergone mastectomy or wide local excision during an 18-month period starting from January 2018 were analyzed. Patients' age, histological subtype of cancer, receptor status of estrogen receptor (ER) progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2), Ki-67 index, and tumour grading were considered.

The age range of the patients was 29 to 84 years (mean=57.7, SD=11.76). The 50-59 and 60-69 age groups represented the most patients (28% each). Females represented 99% (n=199) of the total while three patients had bilateral breast cancer. Histologically, invasive carcinoma of no special type (NST) was the commonest cancer type (n=178, 89%), with the majority being grade 2 cancers (52.5%) according to the Nottingham cancer grading (grade 1=15.1% and grade 3=32%). Luminal A was the most common (64%) molecular subtype, followed by basal-like (21%), HER2-enriched (11.5%), and luminal B (4.5%). Data on the Ki-67 index was available for only 98 patients, and 8.1% (8) of them had a Ki-67 index less than 10, while 22.5% (n=22) between 10 and 20. All others (69%) had a higher Ki-67 index (>20). There is a significant association between the molecular subtype of breast cancer and Nottingham grading ($p<0.0001$). 48% of tripple negative cancers were grade 3 and only 24 % of luminal A cancers fell into the grade 3 category.

Majority of Sri Lankan women with breast cancers present in the 5th and 6th decades of their lives. Luminal A molecular subtype was the predominant, followed by basal-like, HER2- enriched, and luminal B. Aggressive HER2-enriched and basal subtypes are more common in younger age groups. We recommend breast cancer subtyping before surgery so that more people will benefit from neoadjuvant chemotherapy.

Keywords: Breast cancer, Estrogen receptor, Progesterone receptor, Human epidermal growth factor receptor 2, Tripple negative breast cancer

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Background

Breast cancer is the commonest cancer worldwide in females [1]. It accounts for 30% of all cancers diagnosed in females [2]. The global incidence of breast cancer is rising, and the greatest increase is seen in developing countries. Although the incidence of breast cancer remains high in developed countries, there has been a significant increase in breast cancer cases among women

in South America, Africa, and Asia, leading to a shift in the global distribution of cases [3].

Breast cancer incidence in Sri Lanka is on the rise, and as per the most updated information, the breast cancer incidence rate is 33.2 for 100,000 populations [4]. Breast cancer-specific survival rates are low in developing countries like Sri Lanka, compared to the western world [5]. This is partly due to the late presentation as a result of not having a national breast cancer screening program and partly due to the absence of uniformity in the treatment [6].

Breast cancer is a heterogeneous disease. It comprises multiple entities with different histological, genetic, and morphological features. Clinical presentations and treatment options, and overall outcome depend on the specific subtype. Apart from the tumour size and the nodal stage, histological type, histological grade, estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2) status, and Ki-67 index are key prognostic factors for breast cancer [7]. A higher Nottingham grading indicates an aggressive tumour with poor outcomes. ER, and PR receptor-positive breast cancers are generally considered as good prognostic cancers, whereas HER2 receptor-positive cancers are aggressive. The current trend is to give upfront chemotherapy and immunotherapy for a patient with HER2-positive tumours, so identifying the receptor status before surgery is important.

Neoadjuvant chemotherapy to HER2-positive disease is not widely practised in Sri Lanka yet. One of the main reasons for this is the lack of availability of receptor status of the breast cancers at the time of decision making. Currently, there is a lag of few weeks for immunohistochemistry (IHC) results after histological confirmation of breast cancer. The Cancer Genome Atlas (TCGA) Network has helped to establish refined subtypes of breast cancer through extensive profiling of protein levels, microRNA, and DNA. The molecular subtypes include "luminal A," "luminal B," "HER2-enriched," and "basal-like," each of which has changed the paradigm of breast cancer treatment [8].

Pathology plays a crucial role in understanding complex diseases such as breast cancer, and refined pathological subtyping allows clinicians to offer individualized, targeted therapy, which improves the outcome of breast cancer [9]. However, in Sri Lanka, there is a paucity of data on the key epidemiological findings, especially with regard to the distribution of various subtypes. We aimed at describing the distribution of different subtypes of breast cancer in the Sri Lankan community.

Methodology

The study was conducted as a retrospective cross-sectional study by extracting epidemiological and clinical data from the national cancer institute, Maharagama, Sri Lanka. National cancer institute, which is also known as the Apeksha hospital, Maharagama is the largest tertiary cancer care centre on the island. Patients from all parts of the country are referred to this center for specialized management of different types of cancers. Apart from cancer treatment, it is one of the main centers involved in postgraduate and undergraduate medical training.

Patient population

A total of 200 histopathologically confirmed breast cancer patients who underwent surgeries at the national cancer institute, Maharagama, were selected for the analysis. All the selected patients had undergone either mastectomy or wide local excision during the 18 months starting from January 2018.

Exclusion criteria

Patients who were not operated on due to metastatic disease or comorbidities.

Data collection

All records were collected retrospectively from the hospital's electronic medical records. Data related to key prognostic factors, including age, ER, PR, and HER2 status, tumor histology, grading, and Ki-67 index, were considered. Basic demographic details, age, and gender were also extracted.

Details of the routine records maintained

As per the routine practice in the cancer institute, Maharagama, all the histopathological and IHC examinations were performed in accordance with the College of American Pathologists/American Society of Clinical Oncology (CAP/ASCO) guidelines [10].

Data recording and analysis

Data recording and analysis were done using Microsoft Office Excel (version 15.13.3). Summary measures for continuous variables were expressed in terms of measures of central tendencies and the categorical

variables in proportions. Age-wise distribution of all the breast cancer patients was analyzed first. ER, PR and HER2 receptor status were noted separately, and the percentages of different molecular subtypes were calculated. The age-specific distribution of each subtype was also taken into consideration. Associations were tested with Chi-square analysis. The distribution of Nottingham grading and Ki-67 index was analyzed separately, and the relationship of Ki-67 index to each tumour subtype was stated. ER and PR scoring for all cases was done using Allred scoring [11].

Definitions and standard classifications

Definitions and standard classifications followed are in tables 1 and 2.

Table 1: Allred scoring system for ER &PR (11)

Proportion of Staining (PS) (Nuclear staining)	Score	Staining Intensity (IS)	Score
No nuclear staining	**0**	No staining	**0**
< 1%	**1**	Weak	**1**
1- 10%	**2**	Moderate	**2**
11 - 33%	**3**	Strong	**3**
34-66%	**4**		
67-100%	**5**		

Total Score =Proportion of staining (PS) +Staining intensity (IS)

Adding the two scores together gives a maximum score of 8.

ER and PR were considered positive for cases, which scored three or more on the Allred score.

HER2 scoring was done according to the ASCO/CAP guidelines [12]

Table 2 - Scoring method for HER2

Result category	Score to report	Staining pattern
Negative	0	No staining is seen.
Negative	1+	Incomplete membrane staining in any percentage of cells
Equivocal	2+	Strong, complete membrane staining in <30% of cells

We classified all breast cancers into four subtypes based on the hormonal receptor (ER, PR) and HER2 status. These were luminal A (ER+ and/or PR+/HER2−), luminal B (ER+ and/or PR+/HER2+), HER2-enriched (ER− and PR−/HER2+), and basal-like (ER− and PR−/HER2−).

Results

The age range of the included patients was 29 to 84 years, with a mean of 57.7 years. The 50-59 and 60-69 age groups represented most of the patients (28% each) followed by the 40-49 age group (19.5%), 18.5% of patients were above 70 years, and only one patient (29years) belonged to < 30-year category **(Figure 1)**.

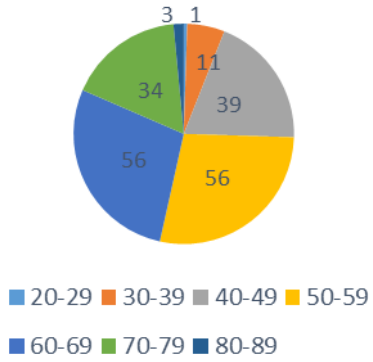

Figure 1: Age (years) wise distribution of the study cohort (The respective colour code of the age groups is illustrated below the chart)

Of the studied patients, 198 (99%) were females. Three patients had bilateral breast cancer. Both male patients were above 70 years and had ER, PR positive HER2 negative breast cancers. One of the bilateral cases was triple-negative, and the other two were luminal A tumours. Histologically, invasive carcinoma of no special type (NST) is the commonest cancer type in our study. It represents 178 (89%) of all cancers and 9 (4.5%) patients had invasive lobular carcinoma, 4 (2%) had papillary carcinoma, with the remaining 9 (4.5%) having medullary, metaplastic, or mucinous carcinomas **(Figure 2a).**

According to the Nottingham cancer grading, 31 (15.1%) had grade 1 cancers, 105(52.5%) had grade 2 cancers, and 64 (32%) had grade 3 cancers **(Figure 2b)**. Out of 200 patients, 128 (64%) had hormone receptor positivity (either ER or PR). HER2 receptors were present in 30 (15%) cancers. Among the molecular subtypes, luminal A was the most common 122 (64%), followed by basal-

like 48 (21%), HER2 enriched 21 (11.5%), and luminal B 9 (4.5%) **(Figure 2c)**.

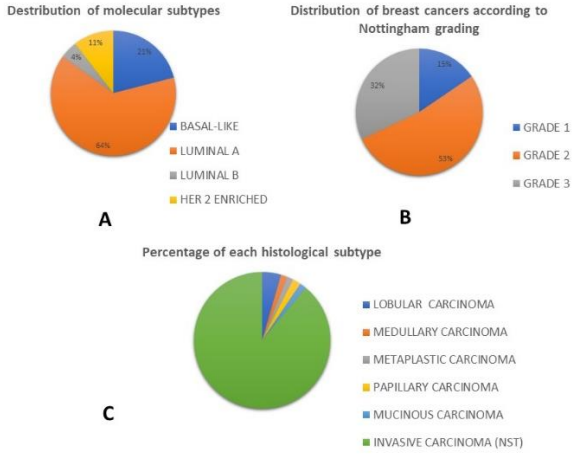

Figure 2: (A) Distribution of molecular subtypes of breast cancer; (B) Distribution of breast cancers according to Nottingham grading; (C) percentage of each histological subtype

The Ki 67 index was calculated in only 98 patients. Among these patients, 8 (8.1%) had Ki-67 index less than 10, 22 (22.5%) had a Ki-67 index between 10 and 20, and all other patients (69%) had a Ki-67 index above 20. All the patients who had a Ki-67 index less than 10 were either luminal A or luminal B, and 87.5% of basal cancers had a Ki-67 index of more than 20. Incidence of Luminal A cancers increased with age, and the highest number of cases (71%) were seen in the 60-69 age group. The basal subtype is more common in the 40-49 (31%) age group. The highest frequency of HER2- enriched tumours was found in the 30-39 age group, which is 37% **(Table 3)**.

Table 3: Age-wise distribution of molecular subtypes of breast cancer

Age group (in years)	Total cases	Luminal A n(%)	Luminal B n(%)	Her two enriched n(%)	Basal n(%)
20-29	1	1(100)	0	0	0
30-39	11	5(45)	0	4(37)	2(18)
40-49	39	20(54)	4(10)	2(5)	13(31)
50-59	56	35(62.5)	1(1.5)	6(11)	14(25)
60-69	56	41(71)	1(2)	6(11)	8(16)
70-79	34	19(56%)	3(9)	2(6)	10(30)
80-89	3	1(33.3)	0	1(33.3)	1(33.3)

Over 90% of both triple-negative (44/47) and HER2-positive (28/30) subtypes had advanced histological grading, either II or III. Of the grade 1 breast cancers, the vast majority (25 out of 30, 83%) had luminal A molecular subtype **(Table 4)**.

Table 4: Associations between breast cancer molecular subtypes and the histological grading

Molecular sub-type		Histological grading [n (%)]		
		Grade 1 (n=30)	Grade 2 (n=104)	Grade 3 (n=65)
Triple negative / Basal like (n= 48)		4 (8.3)	21 (43.7)	23 (47.9)
Non triple negative (n = 152)	Luminal A (n=122)	25 (20.5)	73 (59.8)	24 (19.7)
	Luminal B+ HER2e* (n=30)	2 (6.7)	10 (33.3)	18 (60.00)

*Luminal B + HER2e category represents all HER2 positive cancers. Chi-square P value, <0.0001

Twenty-three (49%) of the triple-negative breast cancers were grade 3 cancers, whereas only 28% of non-triple-negative cancers were grade 3.

Discussion

The ages of the patients in our study ranged from 29 to 84 years. More than half of them belonged to the 50-59 and 60-69 age categories. Invasive carcinoma of no special type is the commonest cancer type in our population. The number of patients who had Nottingham grade 2 cancers was more than the number of patients who had grade 1 and grade 2 cancers in combination. Luminal A was the most frequent (64%) molecular subtype, followed by basal-like (21%), HER2-enriched (11.5%), and luminal B (4.5%). Out of all who had the Ki-67 index checked, up to about two-thirds had a Ki-67 index of more than 20.

Globally, breast cancer remains a leading cause of cancer-related mortality. The histological subtype and receptor state is one of the main determinants of the prognosis. Breast cancer treatment is frequently updated, and most of the novel treatments target these receptors. When introducing these new treatments, it is essential to identify each subtype's distribution in the population. Although sufficient data is available from the western world, there is a paucity of data from the South Asian region, including Sri Lanka.

In this study, we primarily explored the distribution of different breast cancer subtypes. The age distribution of the patients in the current study is compatible with previous findings in south Asia, denoting the regional similarities of related demographic factors for breast cancer [13].

In our study, luminal A was the most frequent subtype, and luminal B was the least common. This subtype distribution is similar to subtype distribution around the

world in general [14]. However, in some studies, the luminal B subtype is more frequent than the basal subtype [15,16]. This itself does not affect the overall management of patients; however, luminal B cancers have a better prognosis than the basal subtype.

As per literature, HER2- enriched molecular subtype is observed in about 15% to 20% of breast cancers [17], and in the current study, the HER2-rich subtype was observed in 11.5% of patients. A total of 16% of our patients were HER2-positive, which suggests aggressive tumours. According to the current evidence, these patients will benefit from neoadjuvant chemotherapy combined with Tratsuzumab [18]. Failing to identify the receptor status before surgery means at least one out of 6 of our patients will receive sub-standard treatment. So we would like to highlight the importance of core biopsy and determinants of receptor status before embarking on surgery. Furthermore, 21% of our breast cancers were triple-negative, and of them, 61% had Ki-67 index above 20. This indicates the higher prevalence of aggressive subtypes of breast cancer in our community.

Our study confirms a significant association between the histological subtype and the grading of the tumour. More aggressive grade 2 and grade 3 tumours were more common in triple-negative and HER2-positive cancers. Similar to the present study, a higher proportion of grade 3 tumours was found in luminal B, HER2-enriched, and triple-negative breast cancers in studies done in Indonesia, India, and America [19].

As per the guidelines, triple-negative breast cancers will also require upfront chemotherapy, so in total, 37 % of our patients require neoadjuvant chemotherapy. If receptor subtype is ignored before surgery, all these patients will receive sub-standard treatment. This ultimately results in more patients getting recurrences which adds to our health care burden, and overall disease-free survival of the patients will also come down.

In our setting, it may be difficult to fast-track IHC results of each and every patient. Considering the multiple delays, we encounter in conducting mammography, USS-guided biopsy, it is also unfair to keep the patients in a long wait for the IHC results before the surgery.

This problem can be overcome to a certain degree by identifying special patient groups who are more likely to get HER2 positivity and triple negativity (e.g., young patients). We could attempt to fast-track the IHC results of these patient groups and identify a larger portion of patients who need neo-adjuvant chemotherapy.

Conclusion

Our study concludes that breast cancer in Sri Lankans is most common in the 5th and 6th decades. Luminal A was the predominant subtype, followed by basal-like, HER2 enrich, and luminal B. Presence of the most virulent basal subtype in a high frequency indicates the aggressive nature of breast cancer in our community. We also highlight the importance of identifying these aggressive subtypes to plan targeted therapies to improve overall outcomes. Moreover, aggressive tumour types are more common in younger age groups. These patients are more likely to benefit from neoadjuvant chemotherapy. So we would like to point out the importance of identifying the molecular subtype before surgery, at least for younger patients.

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