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Molecular Subtyping: status of the molecular factors in the locally advanced breast cancer and its correlation with risk factors.

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Article Info

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Abstract

Molecular factors play an important role in the management and treatment outcome of breast cancer. Molecular subtyping has been developed depending upon estrogen and progesterone receptors, human epidermal growth factor receptor-2 and ki76 level. This cross-sectional study was done to assess the molecular subtypes of locally advanced breast cancer and its associated risk factors. Total 94 patients with locally advanced breast cancer were included in the study. The mean age was 42.6 years with a standard deviation of 9.56. In total, 91.5 percent of respondents had menarche at or after the age of 12, and 26.6 % had used hormonal contraception in the past. Tobacco users and positive family history were found in 21.2% and 5.35% of the cases. Among the patients, 3.4 % of cases had their first childbirth after the age of 30 and 95.5% of patients feed their babies from their both breasts. Among 94 cases 5 did not have any child. Estrogen receptor was found positive in 35% of cases, progesterone receptor-positive patient was 33% and HER-2 was found positive in 39.4% of cases. Ki-67 level was found high in 66% of cases. Among the 94 cases, the Luminal A subtype was found in 18% and the Luminal B subtype was found in 27.7% cases. The human epidermal growth factor receptor-2 subtype was found relatively less frequent than Luminal type B (24.5% vs. 27.7%). Triple-negative breast cancer was most commonly diagnosed among the patients (almost 30%). The increased number of triple-negative variants signifies poor prognostic outcomes. The risk factor of breast cancer did not show any statistical correlation with molecular subtypes.

Introduction

Breast cancer is one of the major concerns in the realm of oncology as it is the most commonly occurring malignant disease in female with 23% of all new cases and 14% of all cancer-related deaths.[1] With the advancement of management protocol early detection, overall survival duration is increasing without any doubt. One notable phenomenon among the patients with breast cancer is that histologically similar type of diagnosis in the same stage of disease does not show the same prognosis with the similar treatment modality. It is largely due to the difference in its molecular factors. To date, several molecular factors have been identified that have shown different impacts on treatment and prognosis. Among those factors Estrogen and Progesterone Receptors (ER and PR), Human Epidermal Growth Factor Receptor (HER-2), TP53, Ki67, BRCA 1, BRCA 2, P 14 ARF (a locus with the multifunctional activity of tumour suppression), Cyclin D1 (cell cycle progression regulatory protein), Cycline E, different cytokeratins (5/6), B Myb-a proto-oncogene, Twist (transcriptional repressor that effects E-Cadherin), DMP1β, VEGF, TBX2/3 were found critical in various aspect of breast cancer management.[2,3] Different molecular factors have been adopted to develop a surrogate definition of molecular subtypes in the St Gallen International Breast Cancer Conference in 2011 as Luminal A(ER and/or PR positive HER-2 negative and low ki67, Luminal B (ER and/or PR positive HER-2 positive and/ low ki67 or ER and/or PR positive HER-2 negative and high ki67, HER2 type(ER and PR negative, HER-2 positive) and tipple negative(TNBC) (ER, PR and HER-2negetive). [4,5] These subtypes have shown a remarkable difference in treatment outcome. As this part of the globe still encountering a substantial number of locally advanced breast cancer (LABC) patients in comparison with the western world, 52.5% versus 7%, pretreatment molecular subtyping

of LABC will play an important role in overall management.[6] The subtyping has enlightened the heterogeneity of ER-positive tumours in treatment outcomes. The luminal A subtype has a favourable prognosis compared to the luminal B subtype and the systemic therapy advocated for the patients with luminal A tumour is generally restricted to endocrine therapy. The luminal B subtype has a high proliferation rate and/or a high histological grade and systemic treatment with chemotherapy followed by endocrine therapy is recommended.[4, 5.] Human Epidermal Growth Factor Receptor Type 2 (HER2) has been used as predictive markers for identifying a high-risk phenotype and for the selection of the most efficient therapies.[7] The prognostic and predictive value of Ki-67 was evaluated in a review developed by Luporsi et al, 2012 and they concluded that this biomarker could be considered as a prognostic factor for the therapeutic decision. [8]

This study was performed to observe molecular subtyping in locally advanced breast cancer and its association with known risk factors of breast cancer like age, the onset of menarche, hormonal contraceptive use, menopause, tobacco consumption, and family history, age of 1st childbirth, and breastfeeding practice.

Materials and Methods

This cross-sectional study was conducted from July 2014 to June 2016. According to inclusion and exclusion criteria, patients were enrolled in the study after physical examination and investigation. A total of 94 patients with locally advanced breast cancer (T3N0/T2N2/T3N1-2/T4Nx/TxN3) attending the outpatient and inpatient departments of the National Institute of Cancer Research and Hospital (NICRH), Mohakhali, Dhaka were included. Ethical clearance was obtained from the Ethical Committee of NICRH. A structured pre-tested questionnaire containing patient profiles was prepared. Informed written consent was obtained from each patient. As gene expression array data is not routinely available immunohistochemical (IHC) marker, expression data was used for molecular subtyping i.e. luminal A (ER + and/or PR+, Ki67 low and HER2-), luminal B (ER + and/or PR+, Ki-67 high and/ or HER2+), HER2-positive (ER-, PR- and HER2+) and triple-negative (ER-, PR-, and HER2-).[4] The collected paraffin blocks prepared from core cut biopsy and mastectomy specimen from Histopathology department of NICRH were sent for immunohistochemistry for ER, PR, HER-2 and Ki67. HRP (Horseradish Peroxidase) polymer-based detection system was used for all immunohistochemistry. Allred scoring method was used for ER, PR expression in breast cancers, scores 0 - 2 are deemed negative while scores 3–8 denote positive expression (Hammond ME et al 2010). HER-2 scoring was done as per the ASCO/CAP reconciled guidelines for HER-2/neu expression in breast cancer.[9] Ki-67 values were acquired as the percentage of positively marking malignant cells using the anti-human Ki-67 monoclonal antibody MIB1 which is one of the most commonly used antibodies and is considered as the gold standard.[10] Reports were collected and recorded in the data collection sheet. A purposive sampling technique was applied. For analysis of the data, Statistical Package for Social Sciences (SPSS) for Windows (IBM SPSS Statistics for Windows, version 23.0) was used. To see the association between categorical variables Chi-Square test (and Fisher's exact test where applicable) was performed. A *p*-value ≤ .05 was considered statistically significant.

Results

In this study, 94 patients with diagnosed locally advanced breast cancer were included. The mean (SD) age of the patients was 42.6 (9.56) years (Figure-1). More than 50% patient was in the 40 to 60 years age group. Only 2 patients were found in the 61 to 80 years age group (Table - I).

Most of the patients had menarche ≥12 years of age (91.5%) and 26.6 % had a history of using hormonal contraceptives. Tobacco users and positive family history were found in 21.2% and 5.35% of the cases. Menopause was found in only 10 (10.6%) of cases

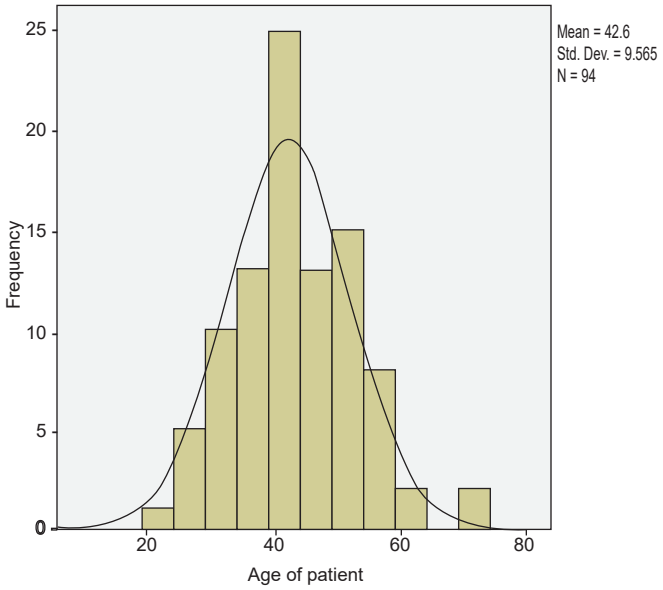

Figure - 1: Histogram showing age distribution of the patients

Table-I

Distribution of patients by age group (n=94)

Variable	Data	Frequency	Percentage
Age group (years)	20-40	44	46.8
	41-60	48	51.1
	61-80	2	2.1

(Table - II). Among the patients, 3.4% had their first childbirth after the age of 30 and 95.5% fed their babies from both breasts (Table - III). Total 5 cases did not have any child, 2 were unmarried.

ER was found positive in 35% of cases, PR positive patient was 31 in number and HER2 was found positive in 39.4% of cases. In 66% of the cases were with Ki-67 level >14% in the highest proliferative area (Table - IV).

Among the 94 cases, the Luminal A subtype was found in 15(18%) and the Luminal B subtype was found in 26 (27.7%) cases. HER-2 subtype was found relatively less frequent than Luminal type B (24.5% vs. 27.7%). The important phenomenon is that TNBC (triple-negative), which is the clinically most aggressive variant was diagnosed in maximum (almost 30%) patients (Table-V). This information plays a definitive concern in the management plan. No statistical association was found between the molecular subtyping and the risk factors of breast cancer (Table -VI).

Table-II

Distribution of patients by risk factors (n=94)

Variable	Data	Frequency	Percentage
Onset of Menarche	<12years	8	8.5
	≥12 years	86	91.5
Hormonal contraceptive use	Yes	25	26.6
	No	69	73.4
Menopause	Yes	15	16.0
	No	79	84.0
Tobacco consumption	Yes	20	21.3
	No	74	78.7
Family history	Positive	5	5.3
	Negative	89	94.7

Table-III

Distribution of patients by Maternity and Breastfeeding (n=89)

Variable	Data	Frequency	Percentage
Age of 1st childbirth	≤30years	86	96.6
	>30 years	3	3.4
Breast Feeding	Yes	85	95.5
	No	4	4.5

Table-IV

Distribution of patients by molecular factors (n=94)

Variable	Data	Frequency	Percentage
Molecular factors	Positive	33	35.1
Estrogen Receptor (ER)	Negative	61	64.9
Progesterone	Positive	31	33.0
Receptor (PR)	Negative	63	67.0
HER-2	Positive	37	39.4
	Negative	57	60.6
Ki-67 percentage	≤14%	32	34.0
	>14%	62	66.0

Table-V

Distribution of patients by molecular subtypes

Variable	Data	Frequency	Percentage
Molecular subtypes	Luminal A	17	18.1
	Luminal B	26	27.7
	HER-2	23	24.5
	TNBC	28	29.8

Table-VI

Relationship between molecular subtypes and risk factors

Variables	Data	Luminal n (%) A	Luminal B n (%)	HER2 n (%)	TNBC n (%)	Total n (%)	P-value
Age (years)	≤40	11 (11.7)	11 (11.7)	13 (13.8)	9 (9.2)	44 (46.8)	.53
	>40	10 (10.6)	15 (15.9)	10 (10.6)	15 (15.9)	50 (53.2)	
BMI	≤ 24.9	13 (13.8)	23 (24.4)	21 (22.3)	23 (24.4)	80 (85.1)	.60
	>25.0	4 (4.2)	3 (3.2)	2 (2.1)	5 (5.3)	14 (14.9)	
Menarche (years)	<12	1 (1.1)	2 (2.1)	4 (4.2)	1 (1.1)	8 (8.5)	.39*
	≥12	16 (17.0)	24 (25.5)	19 (20.2)	27 (28.7)	86 (91.4)	
Breast feeding	Yes	17 (18.0)	24 (25.5)	22 (23.4)	27 (28.7)	90 (95.7)	.83*
	No	0 (0)	2 (2.1)	1 (1.1)	1 (1.1)	4 (4.2)	
Hormonal contraceptive use	Yes	5 (5.3)	5 (5.3)	7 (7.4)	8 (8.5)	25 (26.6)	.79
	No	12 (12.7)	21 (22.3)	16 (17)	20 (21.2)	69 (73.4)	
Menopause	Yes	2 (2.1)	4 (4.2)	2(2.1)	7 (7.5)	15 (15.9)	.48*
	No	15 (15.9)	22 (23.4)	21 (22.3)	21(22.3)	79 (84.1)	

*Fisher's Exact Test

Discussion

In this study, most of the patients were in the 41- 60 years age group followed by 20 - 40 years age group. No patient was found below 20 or above 80 years of age. The mean age of the patients was 42.6 years. A study by Rahman M 2015 showed a mean age of 44.7 years which was similar to this study.[6]

The present study showed that most of the patients (91.5%) had menarche at or above 12 years of age and 26.6 % had a history of using hormonal contraceptives. Jabeen S et al observed that 54% of patients had menarche at eleven or below the age and 42% of patients used oral contraceptives.[11] In an analysis of data from a multicenter, population-based case-control study, Marchbanks PA et al found that breast cancer risk did not vary by oral contraceptive use.[12]

Among the patients, tobacco users and positive family history were found in 21.2% and 5.35% of the cases. Jabeen S et al, 2013 reported in her study that majorities (97%) of the respondents were nonsmoker and only 3% was a smoker.[11] Epidemiological investigations of the relations between smoking and breast cancer have yielded conflicting results. Several studies have suggested that smoking may decrease the risk of breast cancer. Others have reported no evident association, while a few have suggested that smoking may increase the risk of breast cancer, especially in pre-menopausal women.[13] A study by Anderson TI 1996 showed that about 20 % of breast cancer patients have a family history of the disease in a first-degree relative.[14] In this study, menopause was found in only 15 (16%) of cases which were 57.2% in a study conducted by Rahman M 2015.[6] This discrepancy might be due to the inclusion of all stages of breast cancer in their study.

The study finding showed that about 97% of cases had their first childbirth below the age of 30 and 95.5% of patients feed their babies from both breasts. Most of the cases in this study were were housewives (86.2%) and the office staff was foundto be 5.3%. This was consistent with other studies.[11,15] Palmer JR et al showed that a longer duration of breastfeeding has been associated with a greater reduction in breast cancer risk.[16]

Estrogen Receptor (ER) was found positive in 35% of cases. Progesterone Receptor (PR) positive patient was 31 in number and HER-2 was found positive in 39.4% of cases. In 66% of the cases were with Ki-67 level >14% in the highest proliferative area. Miglietta L et al, 2009 observed that ER receptors were low or absent in 29% and high in 71% of the tumors, PR receptors low or absent in 46% and positive in 54%.[17]

TNBC subtype was found in approximately 28 (30%) cases followed by Luminal B subtype 26 (27.7%). Luminal A was found in 17 (18.1%) cases. Wang J et al, 2016 reported in their study that there were 61(25.4%) with luminal A, 127(52.9%) with luminal B, 31 (12.6%) with HER-2 overexpression and 21(8.8%) were triple-negative type.[18] In another study by Prat A et al, 2015 observed that Luminal A, Luminal B and HER-2 types represented 30.6%, 18.2% and 10.3% respectively.[19] As all the recorded cases were selected with locally advanced breast cancer it can be assumed that aggressive variants will be common among the cases.

Regarding correlation among various risk factors and molecular subtypes, no statistical significance was found. Wang et al showed that menopausal status had an association between molecular subtypes.[16] Turkoz et al found a significant association of age (≥40years), first baby at ≥30 years, postmenopausal status and obesity with molecular subtypes; and no correlation with early menarche, late menopause, family history, oral contraceptive and smoking.[20]

Conclusion

The triple-negative subtype which is the most aggressive variant concerning prognosis was found as the most common subtype and the known risk factors of breast cancer did not show any association with a particular molecular subtype.

Conflict of Interest

The author declares no conflict of interest.

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