ORIGINAL RESEARCH PAPER

Oncology

THE PREVALENCE OF BREAST CANCER DIFFERENT MOLECULAR SUBTYPES IN GEORGIAN WOMEN.

KEY WORDS: Breast Cancer, Molecular subtypes, Histology, Grade, Metastasis.

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ABSTRACT

Several studies have shown that the different biological subtypes of breast cancer are associated with variations in treatment response and disease-specific outcomes. Treatment of breast cancer patients is based on several factors including tumor morphology and expression of ER, PR and HER2/neu. Therefore, the aim of our investigation was to evaluate the prevalence of the BC molecular subtypes, its 5-year survival and recurrence rates in Georgia. **Methods:** This is a retrospective and descriptive study of 1985 patients with a confirmed diagnosis of BC referred in the "Institute of Clinical Oncology" and treated there from January 1st 2012 to December 31st 2022. All statistical analyses were performed using SPSS ver. 22.0 (SPSS, Chicago, IL). The data were reported as frequencies for histological type, tumors grade, breast cancer subtypes and as means for patient's age at presentation. Categorical variables were analyzed using the chi-squared test in univariate analysis. P-value<0.05 was considered to be statistically significant. Oncologic outcomes were assessed using Kaplan–Meier analysis to identify factors affecting local recurrence, distant metastasis, or death. A P value<0.05 was considered. **Results:** The most prevalent subtypes in patients with BC were Luminal A (36.0%) and Luminal B (41.8%). The distribution of molecular subtypes is different than reported in developed countries asian and african regions . This study is based on the data of a single clinic and it is impossible to provide nation-wide programs for patients with BC for gene expression profiling. Therefore, IHC expression of ER, PR, and HER2 was carried out in the frames of some municipal programs and self-financing mode. Therefore in 9.2% we got unknown subtype patients with BC.

INTRODUCTION

The breast cancer (BC) is one of the leading cause of cancer-related morbidity and mortality. Based on GLOBOCAN 2018 incidence and mortality data BC was the second most common malignancy, accounting for more than 11.6% of all diagnosed cancers in women [1]. It ranks fifth in cancer-related deaths in women, accounting for 6.6% of all cancer deaths in the world. The BC causes a significant public health burden, resulting in a loss of 14.8 million Disability Adjusted Life Years (DALYs) [2–4]. In developed countries, the incidence of breast cancer is significantly higher; Its age-standardized incidence rate (ASR) was 54.5 per 100,000 women in countries with a high or very high development index (HDI), compared to 31.3 in low and medium HDI countries [1]. The lower life expectancy in low-income countries (LICs) does not support the common perception that BC is more frequent in younger age groups. However, the incidence of BC in young women in LICs is actually not higher than in developed countries [5-8]. When life expectancy is less than 60 years, fewer women are at risk of developing BC, leading to a reduction in breast cancer incidence rate. And in countries where the life expectancy is higher, the incidence of breast cancer is also higher due to the elevated levels in the older age groups of patients [7,9].

To date, several studies have shown that the different biological subtypes are associated with variations in treatment response and disease-specific outcomes [10-16]. Currently, decision-making for individual patients is based on several factors including tumor morphology and grade classification, tumor size, presence of lymph node metastases, and expression of ER, PR and HER2/neu. There is a clear need to enhance the understanding of both prognostic and predictive markers that will facilitate customized treatment. The advent of novel technologies to aid in the identification of new markers will also be critical.

According the data of recent studies, it is well-known about the impact of different molecular subtypes on disease-specific survival rates; more recent studies have also investigated their role in the development of the local-regional recurrence. Through molecular analysis of BC with gene expression profiling, both Perou [17] and Sorlie [18] showed that BC could be sub-classified into different subtypes. These subtypes include: luminal A, luminal B, HER2 enriched, and Triple negative TNBC. Nevertheless, the American Society of Clinical Oncology (ASCO) and the St. Gallen Group have issued guidelines and recommendations for the implementation of molecular analysis as useful tool for risk stratification and for treatment planning [19,20]. However, gene expression profiling can be costly and time consuming [21,22]. Therefore, gene expression profiling can be difficult to implement on a wide-scale. Moreover, it is very difficult to implement this technique in the low- and middle-income countries. But it should be noted that gene expression profiling improves significantly the treatment outcomes – survival and recurrence rates. Through the advocacy of BC patients there are a lot of opportunities to reach the implementation of this technique through some municipal and supporting projects.

Therefore, the aim of our investigation was to evaluate the prevalence of the BC molecular subtypes, its 5-year survival and recurrence rates in Georgia. It should be noted that identification of the molecular subtypes wasn't implemented in Georgian National BC screening program.

PATIENTS AND METHODS

Patients

This is a retrospective and descriptive study of patients with a confirmed diagnosis of BC referred the centers of clinical oncology and treated there from January 1st 2012 to December 31st 2022 (11 years).

Data were extracted from the records of 1985 patients (Table #1). BC has been classified according to the World Health Organization 2012 (WHO 2012). Histological classification was performed using the Nottingham classification system and staging according to the 8th edition of the AJCC classification of 2017.

BC subtypes were defined according to the IHC expression of ER/PR/HER2/neu and Ki-67 count:

- Luminal A (ER+/PR+, HER2/neu , Ki 67≤14% or grade 1

or 2 tumor grading);
- Luminal B (ER+/PR+, HER2/neu+ or ER+/PR+, HER2–, Ki–67>14% or grade 3);
- HER2 enriched (ER–, PR–, HER2/neu+) – HER2 positive
- Triple negative (ER–, PR–, HER2/neu–) – TNBC;

Table #1. Clinical Characteristics Of Study Participants.

#	Parameter	Mean (SD) or n (%)
1	Age	56.9 (11.6)
	0-39 years	169 (8.4%)
	40-59 years	967 (48.3%)
	60+ years	967 (42.4%)
2	Tumor Localization by ICD code	
	C50.0	23 (1.2%)
	C50.1	198 (10.0%)
	C50.2	288 (14.5%)
	C50.3	112 (5.6%)
	C50.4	1001 (50.4%)
	C50.5	200 (10.1%)
	C50.8	3 (0.2%)
	C50.9	156 (7.9%)
	D05.1	4 (0.2%)
3	Grade classification	
	Grade 1	12 (0.6%)
	Grade 2	490 (24.7%)
	Grade 3	1483 (74.7%)
4	Clinical Stage	
	Stage 0	13 (0.7%)
	Stage 1	424 (21.4%)
	Stage 2	807 (40.7%)
	Stage 3	655 (33.0%)
	Stage 4	86 (4.3%)

According to the obtain data study cohort was divided by 5 groups:
Group 1 – patients with breast cancer of Luminal A subtype;
Group 2 – patients with breast cancer of Luminal B subtype;
Group 3 – patients with breast cancer of HER2+ subtype;
Group 4 – patients with breast cancer of TNBC subtype;
Group 5 – patients with breast cancer of unknown subtype (in case of the unavailability of the patients to perform the subtype evaluation).

Statistic

All statistical analyses were performed using SPSS ver. 22.0 (SPSS, Chicago, IL). The data were reported as frequencies for histological type, tumors grade, breast cancer subtypes and as means for patient's age at presentation. Categorical variables were analyzed using the chi-squared test in univariate analysis. P-value < 0.05 was considered to be statistically significant. Oncologic outcomes were assessed using Kaplan–Meier analysis to identify factors affecting local recurrence, distant metastasis, or death. A P value < .05 was considered

RESULTS

The distribution of study participants by the groups (molecular subtypes) are given in the Table #2.

Table #2. Distribution Of Study Participants By The Groups (Molecular Subtypes).

#	Molecular Subtype	n=	%
1	**Luminal A**	**714**	**36.0%**
2	**Luminal-B**	**830**	**41.8%**
	Luminal-B + HER2-negative	673	33.9%
	Luminal-B + HER2-positive	157	7.9%
3	**HER2-positive**	**112**	**5.6%**
4	**Triple negative**	**137**	**6.9%**
5	**Unknown**	**192**	**9.7%**

Overall 5-year survival rate of study participants was 80.8%. The results of 5-year survival of study participants in total and each group are given on diagram #1. Survival analysis clearly showed that the survival rate in group 1 (Luminal A, 92.0%) was significantly higher than in other groups. Hazard Ratio (HR) between group 5 (Unknown subtype) and group 1 was 3.1 (95%CI – 1.9-5.1, p<0.001); HR between group 4 (Triple Negative) and group 1 was 2.6 (95%CI – 1.4-4.7, p<0.001); HR between group 3 (HER2-positive) and group 1 was 2.9 (95%CI – 1.5-5.5, p<0.001); HR between group 2 (Luminal B) and group 1 was 2.0 (95%CI – 1.5-2.7, p<0.001). It was obtained significant HR between group 5 (Unknown subtype) and group 2 (Luminal B) - 1.5 (95%CI – 1.1-2.3, p=0.012). HRs between other groups was not significant.

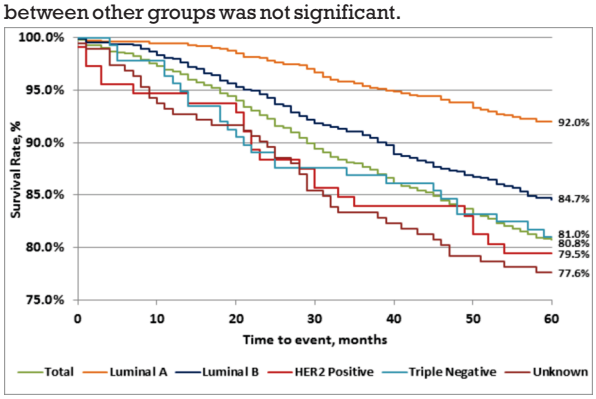

Diagram #1. 5-year survival rates in the study groups.

There were 427 cases of reccurences in the study groups. 96 of them had local character, and 350 were distant. The distribution of recurrences by BC molecular subtypes are given in table 3. Survival analysis clearly showed that the recurrence rate in group 1 (Luminal A, 92.0%) was significantly lower than in other groups. Hazard Ratio (HR) between group 5 (Unknown subtype) and group 1 was 1.6 (95%CI – 1.04-2.4, p=0.015); HR between group 4 (Triple Negative) and group 1 was 2.1 (95%CI – 1.3-3.3, p<0.001); HR between group 3 (HER2-positive) and group 1 was 1.8 (95%CI – 1.1-3.1, p=0.002); HR between group 2 (Luminal B) and group 1 was 2.0 (95%CI – 1.5-2.7, p<0.001). It was obtained significant HR between group 5 (Unknown subtype) and group 2 (Luminal B) - 1.3 (95%CI – 1.01-1.8, p=0.044). HRs between other groups was not significant.

Table #3. Distribution Of Recurrences By The Groups (Molecular Subtypes).

#	Molecular Subtype	Total	Local Recurrence	
		n=	n=	%
1	Luminal A	714	22	3.1%
2	Luminal-B	830	43	5.2%
	Luminal-B + HER2-negative	673	34	5.1%
	Luminal-B + HER2-positive	157	9	5.7%
3	HER2-positive	112	6	5.4%
4	Triple negative	137	13	9.5%
5	Unknown	192	13	6.8%
#	**Molecular Subtype**	**Total**	**Distant Metastasis**	
		n=	n=	%
1	Luminal A	714	89	12.5%
2	Luminal-B	830	170	20.5%
	Luminal-B + HER2-negative	673	134	19.9%
	Luminal-B + HER2-positive	157	36	22.9%
3	HER2-positive	112	27	24.1%
4	Triple negative	137	27	19.7%
5	Unknown	192	35	18.2%

Most prevalent distant metastasis in group 1 (Luminal A) was occurred in the bones - 64 cases out of 89 (71.9%); in the liver - 19 cases (21.3%); in the lungs - 23 cases (25.8%); in the brain - 1 case (1.1%). Most prevalent distant metastasis in group 2 (Luminal B) was occurred in the bones - 118 cases out of 170 (69.4%); in the liver - 45 cases (26.5%); in the lungs - 55 cases (32.4%); in the brain - 10 cases (5.9%). Most prevalent distant

metastasis in group 3 (HER2-positive) was occurred in the bones- 9 cases out of 27 (33.3%); in the liver - 11 cases (40.7%); in the lungs - 9 cases (33.3%); in the brain - 3 cases (11.1%). Most prevalent distant metastasis in group 4 (Triple Negative) was occurred in the bones - 6 cases out of 27 (22.2%); in the liver - 12 cases (44.4%); in the lungs - 11 cases (40.7%); in the brain - 6 cases (22.2%). Most prevalent distant metastasis in group 5 (unknown subtype) was occurred in the bones - 8 cases out of 35 (22.9%); in the liver - 19 cases (54.3%); in the lungs - 9 cases (25.7%).

DISCUSSION

It is very important to define the role of determination of the IHC expression of ER, PR, and HER2/new in the patients with BC for the effective treatment and overall survival. Our study is first attempt to clarify the importance of the statement mentioned above and confirmed by many RCTs.

Our study revealed that most prevalent subtypes in patients with BC were Luminal A (36.0%) and Luminal B (41.8%). The distribution of molecular subtypes is different than reported in developed countries [23,24], asian [25,26] and african regions [27]. It should be noted that our study is based on the data of a single clinic. Unfortunately, it is impossible to provide nation-wide programs for patients with BC for gene expression profiling. Therefore, IHC expression of ER, PR, and HER2 was carried out in the frames of some municipal programs and self-financing mode. Unknown subtype occurs in only 9.2% of patients with BC.

5-year overall survival rate (5-OSR) of study participants was 80.8%. For Luminal A subtype 5-OSR was significantly higher (92.0%), followed by Luminal B subtype (84.7%). Worst 5-OSR had Triple Negative (81.0%)., HER2 positive (79.5%) and Unknown subtypes (77.6%). These results are in agreement with rates reported by National Cancer Institute [28]. It should be noted that we have no official results of National Cancer Registry of Georgia. This institution was formed in 2015 and is on the development stage. Therefore, we have no information about survival rates of patients with BC and compare the results of our study with population ones. It is obvious that targeted treatment regimens will be useful to achieve best outcomes. Only data of researcher T.Gvazava based National Cancer Register about 3-year survival rate is available in Georgia [29]. She reported that 3-year OSR in Georgian patients with BC in 2020 was 81.1%. If we compare our 5-OSR (80.8%) with this rate, it is obvious that identification of hormone-receptor status is useful tool in the management of BC.

The results of our study showed that the prevalent locations of distant metastasis were bones liver and lungs. Among molecular subtypes prominent places occupied HER2 positive and Triple Negative. But, it should be noted high percentage of metastasis in the bones in the case of Luminal A subtype. Similar results (except Luminal A and bones) were reported by Xiao et al. [30].

Limitations

The present study has some limitations. 1) Our cohort consisted of data of a single clinic, so it may not represent a general patient population with BC. Nonetheless, most of our results are in agreement with some previous publications with some respect for the frequency differences and outcomes of BC molecular subtypes. 2) An unclear definition of cause of death (especially during COVID-19 pandemic) may have impact on the study of the survival and the cancer recurrence. 3) socio-economic and environmental factors were not available in the study, so their contribution could not be assessed.

CONCLUSION

For the first time, we report the distribution of molecular subtypes and the rates of some outcomes – 5-year survival, local recurrence and distant metastasis associated with these subtypes in Georgian women with BC. Most prevalent subtypes in study group were Luminal A and Luminal B subtypes. This study emphasizes the importance of implementation of the molecular testing in the routine clinical practice to offer the best options for breast cancer management.

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