Expression of Breast Cancer Subtypes Based on the Most Important Biomarkers: Comparison of Clinicopathological Factors and Survival

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Abstract

Background: Breast cancer (BC) is the most common cancer among women worldwide. It can be categorized into at least 5 main groups, based on antibody markers, such as estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2), which are dissimilar in terms of risk factors, distribution, prognosis, treatment, and clinical outcomes. In this study, we evaluated the survival and therapeutic outcomes of BC using immunohistochemical biomarkers in order to improve disease prognosis and eliminate concerns among women.

Methods: The subjects included 1772 women with a diagnosis of BC from January 1999 to January 2014, admitted to Shohada educational hospital and Azar clinic, Tehran, Iran. In this analytical cross sectional study, we selected a simple classification, based on the expression of ER, PR, and HER2. Then, we classified BC patients into 4 subgroups: luminal A (ER+ and/or PR+, HER2-); luminal B (ER+ and/or PR+, HER2+); basal-like (BCL) (ER-, PR-, HER2-); and HER2/neu (ER-, PR-, HER2+) subtypes. In addition, we integrated lymphovascular invasion (LVI) and tumor grade for identifying the groups in terms of HER2 (1+ or 2+ if LVI- was attributed to luminal A or if LVI+ was attributed to luminal B, respectively). P value ≤ 0.05 was considered statistically significant.

Results: The majority of tumors were luminal A (37.16%), luminal B (15.14%), and BCL (13.12%) subtypes, whereas only 6.82% were related to HER2/neu and others were missing. There was a significant difference between immunohistochemical subgroups with respect to tumor grade ($P < 0.001$). Grade 2 was more frequent among luminal A and B subtypes, while grade 3 was more common among BCL and HER2-like subtypes, respectively. In the comparison of hazard ratio (HR) in each group, luminal B subtype was significantly more frequent than luminal A in HER2/neu. Therefore, HR of luminal A, HER2/neu, and luminal B subtypes was 1, 3.5, and nearly 2, respectively ($P < 0.001$); in fact, risk of HER2/neu was 3.5 times higher than that of luminal A.

Conclusions: These findings indicate that risk of mortality in each subgroup can be reduced by adjusting for tumor grade and stage.

Keywords: Breast Neoplasms, Estrogen Receptor, Progesterone Receptor, HER2, Survival

1. Background

Breast cancer (BC) is one of the most commonly diagnosed cancers among women worldwide (1-3). The prevalence of BC is increasing in both developed and developing countries (4), accounting for 19% - 34% of all cancer cases among women around the world (5). This disease can affect people worldwide and result in high rates of mortality (6). Therefore, early diagnosis and treatment can decrease the overall rate of mortality. In fact, it is necessary to identify important factors and predict biological markers for improving the patients' prognosis and reducing mortality (7, 8).

Genetic factors and molecular biomarkers, grade and stage of tumor, and receptor status are important in the

diagnosis of patients with an uncertain prognosis (4). Although histopathological and molecular features are very important, the gold standard for comprehensive classification of treatment depends on gene expression for prediction and prognosis of complications (9, 10).

In recent years, the association between BC prognosis and biological factors has been indicated (11, 12). Molecular profiling distinguishes major BC subtypes, which are often determined via immunohistochemical (IHC) analysis of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) (10, 12, 13). These biomarkers are used to classify BC into at least 5 main groups, based on their molecular characteristics: luminal cell-like tumors (subdivided into luminal A and B), basal cell-like (BCL) or triple-negative phenotypic (TNP), Erb-B2,

HER2/neu gene amplification, and normal breast-like subtypes (3, 14).

The mentioned groups have distinct clinical, survival, pathological, and prognostic features. We can control and obtain satisfying results by identifying and categorizing patients, based on these criteria (5). Approximately 75% of all BC patients are ER positive, 65% are PR positive, while HER2 overexpression may occur in 18% to 20% of BC cases (15). Therefore, it is vital to identify the patient's subtype and the relationship between molecular phenotypes and survival.

Gene expression based on molecular phenotypes is one of the most important prognostic factors, which can affect early diagnosis and prediction of disease outcomes and burden (16-19). Also, determination of gene expression is useful in making decisions about BC treatment (16, 20-22). In previous studies on DNA microarray in BC patients, biomolecular subtypes were assessed separately with different clinical outcomes (23). With this background in mind, in the present study, we aimed to compare and evaluate the survival and therapeutic outcomes of BC patients, based on IHC biomarkers for proper selection of treatment and increasing their life expectancy.

2. Methods

2.1. Study Population

The study population consisted of 1772 new cases of BC, diagnosed via core needle biopsy. This analytical cross sectional study was performed from January 1999 to January 2014 at Shohada educational hospital and Azar clinic, Tehran, Iran. The patients were from several provinces of Iran. The sample size was calculated, based on Cochrane stratified sampling (Cochrane.w.1979), and only 1 observer was involved in our research. Informed consent forms were first obtained from all patients at the time of recruitment and hospital admission.

Subjects with missing IHC data and unknown birth were excluded from the study. Data were extracted by reviewing the medical records and pathological reports. Information on HER2, ER, and PR status of the population, age at diagnosis, stage of disease, tumor grade, and death was also collected. Considering some changes in the address or telephone number of some patients, we did not have access to 487 (21%) patients. Trained individuals contacted and interviewed the patients or their families and collected data on their current status. The review board of cancer research center of Shahid Beheshti University of Medical Sciences approved this study (predicted power, 0.80).

2.2. Definition of Subgroups

The present study was designed with a simple classification, based on ER, PR, and HER2 expression. We classified BC patients into 4 subgroups: luminal A (ER+ and/or PR+, HER2-); luminal B (ER+ and/or PR+, HER2+); BCL (ER-, PR-, HER2-); and HER2/neu (ER-, PR-, HER2+). In addition, we integrated lymphovascular invasion (LVI) and tumor grade for identifying the groups as HER2 (1+ or 2+ if LVI- was attributed to luminal A or if LVI+ was attributed to luminal B, respectively). In grade-specific IHC subgroups, if LVI was unknown, grades 1 and 2 were attributed to luminal A, and grade 3 was attributed to luminal B. All equipments for IHC analysis and flow cytometry were calibrated in the pathological analysis.

2.2.1. Criteria for ER and PR Measurements

The criteria for ER and PR measurements were as follows: 0, 0% nuclear staining; 1+ < 10% nuclear staining; 2+, 10% - 75% nuclear staining; and 3+ > 75% nuclear staining.

2.2.2. Criteria for the Analysis of ER and PR Variables

The following criteria were used for ER and PR measurements: 0, no staining is observed or membrane staining is < 10% of tumor cells; 1+, negative, faint or barely perceptible membrane staining is detected in > 10% of tumor cells (cells are only stained in part of the membrane); 2+, weak positive, poor to moderate complete membrane staining is observed in > 10% of tumor cells; and 3+, strong and complete membrane staining is observed in > 10% of tumor cells.

2.2.3. Histological Grade Criteria

The histological grade was classified as follows: well differentiated, moderately differentiated, and poorly differentiated.

2.3. Statistical Analysis

This descriptive, analytical, cross sectional study was conducted among patients with BC. We compared the characteristics of patients according to the IHC subgroup, using Chi square and Bonferroni correction tests. Mortality rate per 1000 people was calculated for each IHC subgroup. We used age as a time scale for setting the data; time was measured as age at follow-up until either event or censoring age. If time is defined as 0 at birth, an individual's survival time can be represented by age. In this study, survival time was measured since age at diagnosis until age at death or end of follow-up (whichever occurs first).

For calculation of survival rate, the Kaplan-Meier method was used. We calculated 5-, 10-, and > 10- year survival rates for the whole cohort and IHC subgroups.

The log-rank test indicated significant differences in survival among IHC subgroups. We calculated the hazard ratios (HRs) for tumor grade and stage via Cox proportional hazard model with 95% confidence interval (CI). The log-minus-log plot against survival time for each covariate did not show any deviation from the proportionality assumption. Two variables of tumor grade and stage were integrated in the Cox model for univariate and multivariate analyses. The data were analyzed using Stata version 12.

This study was approved by the cancer research center of Shahid Beheshti University of Medical Sciences, Tehran, Iran, and the study protocol was approved by the institutional review board and institutional oncologist board of this research center.

3. Results

A total of 1772 BC patients were diagnosed between 1999 and 2014, and 160 missing cases were identified due to unknown birth. Overall, 1612 patients were included in the primary analysis. About 86.4% (n, 1393) of cases were alive during the follow-up. The mean age at diagnosis was 582.02 ± 140.7 months in 1612 patients. The mean age at diagnosis and mean tumor size in each group are shown in [Table 1](#). The shortest and longest follow-ups were 198.8 and 210 months, respectively.

3.1. Patient Characteristics

The clinicopathological characteristics of tumors in IHC subgroups are presented in [Table 1](#). The majority of tumors were luminal A (37.16%), luminal B (15.14%), and BCL (13.12%) subtypes, respectively, whereas only 6.82% of tumors were related to HER2/neu and others were missing. The IHC information was not available in 446 out of 1612 women. There was a significant relationship between IHC subgroup and mean age at diagnosis and tumor size ($P = 0.03$ and $P < 0.001$, respectively). Based on the Bonferroni post hoc test, this significant relationship was attributed to differences in the mean age of patients with the luminal A subtype.

The most common tumor grades in IHC subgroups are shown in [Table 1](#). There was a significant relationship between IHC subgroup and tumor grade ($P < 0.001$). Grade 2 was more frequent in luminal A and B subtypes, whereas grade 3 was more common in BCC and HER2-like subtypes, respectively. Patients with the HER2-like subtype, followed by luminal B subtype, showed the highest mortality rate ([Table 1](#)).

3.2. Survival Analysis

At the end of the follow-up, 219 (13.6%) women died. The final event was not reported in 30% of patients (loss to follow-up). Survival in patients with luminal A subtype was significantly longer than those with other subtypes. The log-rank test for equality of survivor functions showed a significant difference between the IHC subgroups ($P < 0.001$). [Figure 1](#) presents the unadjusted survival rates for the IHC subgroups over 100 months. Moreover, the 5-year Kaplan-Meier survival rate was found to be 85% (CI, 0.83 - 0.87). The 5-, 10-, and > 10-year survival rates for different IHC subgroups are shown in [Table 2](#). The lowest 5- and 10-year survival rates were reported in the HER2/neu group.

3.3. Association of IHC Subgroups with Prognostic Variables

According to Cox regression analysis for comparison of HR in each group, the prevalence of luminal B subtype was significantly higher than luminal A in HER2/neu; therefore, HR was 1 for luminal A subtype and about 2 for luminal B ($P < 0.001$). Indeed, the risk of HER2/neu was about 3.5 times higher than that of luminal A (based on the univariate analysis). Moreover, Cox regression model for multivariate analysis was performed in order to determine which IHC subgroup is significantly different after adjusting for tumor grade and stage.

The HR of HER2/neu subtype decreased after controlling for tumor grade (HR, 2.38; $P < 0.001$), while the HR of luminal B subtype was nearly 2 in the models. Therefore, tumor grade played a positive confounding role in model 2 for HER2/neu subtype, and HR for HER2/neu subtype increased by discarding the tumor grade effect. Based on the findings, the HR for HER2/neu subtype, compared with luminal A, was 2.7 with respect to tumor stage ($P < 0.001$).

In the model integrating tumor stage, only HER2 showed significant effects, while in the model of tumor grade, HER2/neu and luminal B subtypes had significant effects, and stage was a more effective variable. On the other hand, in model 3 integrating both tumor stage and grade, HR was only higher in HER2/neu subtype, compared to luminal A subtype (HR, 2.4; $P = 0.004$). Tumor grade and stage (especially stage) decreased the risk of HER2/neu, and there was no interaction between grade and stage ([Table 3](#)).

4. Discussion

In the present study, we described 5- and 10-year survival in BC patients via IHC analysis. We aimed to estimate HR for each subgroup via Cox regression analysis. Based on the results, the total 5- and 10-year survival rates were estimated at 0.85 (95% CI, 0.83 - 0.87) and 0.75 (95% CI, 0.71 - 0.78), respectively. Furthermore, HR for luminal B, BCL, and

Table 1. Clinicopathological Characteristics of Molecular BC Subtypes

IHC Subgroups	ER+, PR+, HER2-	ER+, PR+, HER2+	ER-, PR-, HER2-	ER-, PR-, HER2+
Age at diagnosis (months)	591.6	568.07	576.64	559.17
Frequency (%) ^a	599 (37.16)	244 (15.14)	213 (13.21)	110 (6.82)
Tumor size ($\pm$ SD)	2.99 (1.43)	3.62 (1.58)	3.44 (1.52)	3.64 (1.58)
Grade, (%)				
1	81 (14.86)	24 (10.96)	15 (8.02)	8 (9.20)
2	338 (62.02)	121 (55.25)	75 (40.11)	33 (37.93)
3	126 (23.12)	74 (33.79)	97 (51.87)	46 (52.87)
Total	545 (100)	219 (100)	187 (100)	87 (100)
Mortality per 1000 (95% CI)	19.33 (14.18 - 26.35)	35.92 (25.54 - 50.53)	28.35 (19.16 - 41.96)	58.19 (39.32 - 86.12)

^a 446 (27%) missing.**Table 2.** The 5-, 10- and > 10-Year Survival Rates of BC in IHC Subgroups

IHC Subgroups	Survival, y	Total (Baseline)	Death	Lost	Survival	Error	95% CI
ER+, PR+, HER2-	5	599	29	433	0.92	0.01	0.89 - 0.95
	10	137	9	95	0.83	0.03	0.76 - 0.88
	> 10	33	2	31	0.74	0.07	0.57 - 0.85
ER+, PR+, HER2+	5	244	24	160	0.85	0.03	0.79 - 0.9
	10	60	9	34	0.68	0.06	0.55 - 0.77
	> 10	17	0	17	0.68	0.06	0.55 - 0.77
ER-, PR-, HER2-	5	213	24	130	0.84	0.03	0.77 - 0.89
	10	59	1	42	0.82	0.04	0.73 - 0.88
	> 10	16	0	16	0.82	0.04	0.73 - 0.88
ER-, PR-, HER2+	5	110	20	62	0.75	0.05	0.64 - 0.83
	10	28	3	18	0.63	0.07	0.46 - 0.76
	> 10	7	2	5	0.35	0.15	0.09 - 0.63
Total	5	1612	168	988	0.85	0.01	0.83 - 0.87
	10	456	40	256	0.75	0.02	0.71 - 0.78
	> 10	160	11	149	0.65	0.03	0.59 - 0.71

HER2/neu subtypes, compared to luminal A, was calculated to be 1.51, 1.32, and 2.38, respectively.

In this regard, Najafi et al. (2013) examined the 5-year disease-free survival rate in 4 molecular subtypes, including luminal A, luminal B, HER-2 enriched, and basal (triple negative) subtypes. They also estimated survival in patients, who were in the early stages of BC between 2001 and 2010. The results showed that the maximum and minimum disease-free survival was 55.4 and 43 months in luminal A and HER-2 enriched subtypes, respectively (24). In the present study, the maximum and minimum survival ratios were also estimated in luminal A and HER-2 enriched sub-

types in the same order.

In this regard, Asadzadeh Vostakolaei et al. (2012) assessed the 5-year survival rate among patients in Iran. They studied 1500 patients at 6 hospitals in 5 provinces of Iran. The results indicated that the 5-year survival rate was 71% (25). Furthermore, Vahdaninia and Montazeri (2004) measured the total 5-year survival rate to be 62%, while in our study, it was reported to be 85% (26). Movahedi et al. (2012) also calculated the 5-year survival rate to be 72% in BC patients during 2001 - 2006 (27); differences in the time of these studies could explain this gap.

Some subnational studies have also estimated the 5-

Figure 1. The Kaplan-Meier Survival in IHC Subgroups Over 100 Months

Table 3. The HRs (95% CI) Estimated by Univariate and Multivariate Survival Analyses

IHC Subgroups	HR (95% CI) Univariate Analysis	HR (95% CI) Multivariate Analysis
ER+, PR+, HER2-	1	1
ER+, PR+, HER2+	1.97 (1.23 - 3.16) ^a	1.71 (0.9 - 2.54)
ER-, PR-, HER2-	1.58 (0.95 - 2.63)	1.32 (0.76 - 2.29)
ER-, PR-, HER2+	3.49 (2.07 - 5.86) ^b	2.38 (1.32 - 4.27) ^a
Tumor grade	2.8 (2.2 - 3.8) ^b	3.05 (2.29 - 4.06) ^a
Tumor stage	3.4 (2.8 - 4.1) ^b	1.55 (1.06 - 2.24) ^b

^aP < 0.05.

^bP < 0.001.

year survival of BC patients, while they have not estimated the survival rates in molecular subgroups. In a study by Rezaianzadeh et al. (2009) in Fars province, the survival rate was estimated at 58% during 2000 - 2005; this study was the first research evaluating BC survival in Southern Iran (28). In another study, Karimi et al. (2014) reported the 5-year survival rate to be 75% during 2006 - 2014 in Kurdistan province (29).

Fouladi et al. (2011) conducted a study to estimate the 5-year survival rate of BC. The results indicated a survival rate of 51% during 2003 - 2008 in Northwest of Iran (30). Additionally, in another study by Ziaei et al. in Northwest of Iran (2013), the 5-year survival rates in patients below 40 and over 40 years were estimated at 65% and 83% during 1997 - 2008, respectively (31). These disparities between the re-

sults can be justified by geographical variations in socioeconomic factors, quality of health services (27), and time differences between the studies.

Additionally, Fernandez et al. (2013) evaluated the survival rate of BC in different molecular subgroups. The results indicated that patients with the luminal A subtype had a significantly longer survival, compared to others. They also estimated the adjusted HR for the subgroups. The maximum and minimum HR were estimated at 1.7 and 1.2 for the triple negative and luminal B subtypes, respectively by defining luminal A as the reference (32). Meanwhile, our study showed maximum HR for HER/neu and minimum HR for the triple negative subtype; this discrepancy might be due to different adjustments for HR.

Parise and Caggiano (2014) studied BC-specific survival for 8 ER/PR/HER2 subtypes. According to the results, the risk of mortality in luminal B/HER2- subtype was higher than the risk of mortality for the luminal B/HER2+ group. Moreover, the risk of mortality for the triple negative subgroup was at the maximum level in all stages. Inversely, the risk of luminal B/HER2+ was minimum in all stages (33). Overall, there has been increasing attention to new molecular classifications of BC, based on the genetic map (3, 34-43), which can improve the prognostic power with low cost and high accessibility. In fact, IHC classification can improve the prognostic and therapeutic approaches (3, 44).

The first shortcoming of this study is the missing data, which might have affected the results; sensitivity analysis is an appropriate method for estimating the range of changes. Another limitation of this study is the classifica-

tion of subtypes, which was found to be controversial. The present study is of great importance, as in the survival analysis, age was considered as the time scale for setting the data; therefore, effect of age was correctly adjusted.

Moreover, this study had some limitations due to lack of relevant literature in Iranian communities on molecular gene expression in BC, since the majority of resources and documents in the present research are specific to other populations, and it is impossible to achieve great consistency in the status of ER, PR, and HER2 in Iran. Another important limitation of this study is the incomplete medical information of patients and those who did not answer our calls.

In conclusion, our findings highlighted disparities in the survival of patients with different molecular subtypes. Based on the results, it is not rational to study BC as only a single condition. Moreover, the findings showed that risk of mortality in each subgroup could be modified by adjusting for tumor grade and stage. There is also an urgent need to conduct further studies on the status of ER, PR, and HER2 expression in Iranian BC patients in order to determine proper therapeutic strategies.

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Footnotes

Authors' Contribution: Study concept and design, Mohammad Hadizadeh; collection, analysis, and interpretation of data, Maedeh Olya; drafting of the manuscript, Hamid Zaferani Arani; critical revision of the manuscript for important intellectual content: Hamid Zaferani Arani; statistical analysis and administrative, technical, and material support, Hamid Zaferani Arani and Maedeh Olya; study supervision, Mohammad Hadizadeh.

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Implications for Health Policymakers/Practice/Research/Medical Education: We aimed to compare and evaluate the survival and therapeutic outcomes of BC in different BC patients, based on immunohistochemical biomarkers for a better selection of treatment and improvement of patients' life expectancy.

Considering the high prevalence of BC among women, this study can present new important information about the prognosis and treatment of BC.

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