Original Article
Middle East Journal of Cancer; April 2021; 12(2): 269-275

Age-related Variation in Expression of Breast Cancer Tumour Markers in Iranian Patients

Fatemeh Homaei Shandiz*#, MD, Fahimeh Afzaljavan#, PhD, Amir Tajbakhsh**, PhD, Maryam Rivadeh*, MD, Nourieh Sharifi***, MD, Mohammad Taghi Shakeri****, PhD, Alireza Pasdar**,*****♦, MD, PhD**

**Cancer Research Center, Mashhad University of Medical Sciences, Mashhad, Iran*
***Department of Medical Genetics and Molecular Medicine, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*
****Department of Pathology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*
*****Department of Biostatistics, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*
******Division of Applied Medicine, Medical School, University of Aberdeen, Foresterhill, Aberdeen, U.K.*
#These authors contributed equally in the study

Please cite this article as: Homaei Shandiz F, Afzaljavan F, Tajbakhsh A, Rivadeh M, Sharifi N, Shakeri M, et al. Age-related variation in expression of breast cancer tumour markers in iranian patients. Middle East J Cancer. 2021;12(2): 269-75. doi: 10.30476/mejc.2020.83033.1127.

♦**Corresponding Author:**
Alireza Pasdar, MD, PhD
Department of Medical Genetics and Molecular Medicine, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
Email: Pasdara@mums.ac.ir
a.pasdar@abdn.ac.uk

Abstract

Background: There are believed to be several risk factors affecting the prognosis of breast cancer through their effect on the growth rate of tumour. In the present study, we investigated estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), Ki-67, and tumor protein P53 (TP53) as well-known biomarkers, particularly in breast cancer prognosis, associated with age.

Method: In a case-control study, 406 breast cancer patients were considered retrospectively. In order to extract the clinical and pathologic data, we employed the patients' records. The extracted information was compared between two groups: for patients under 40 (group I) and above 40 years of age (group II). Herein, the researchers performed statistical analysis using SPSS Ver16.

Results: The most prevalent type of cancer in both groups was found to be invasive ductal carcinoma. The major method of treatment was modified radical mastectomy. According to our observations, grade 3 breast cancer was more common in group I. Lymph node involvement significantly increased in group I, while oestrogen and progesterone receptor expressions were less in this group. HER2, TP53, and Ki-67 oncogenes were overexpressed in group I compared with group II.

Conclusion: Expression of HER2, TP53, and Ki-67 biomarkers and a reduction in the number of hormonal receptors in younger patients (<40YO) indicated that breast cancer might be more invasive in younger women with breast cancer and therefore, they might have poorer prognosis and less favourable outcomes.

Keywords: HER2, Breast cancer, Biomarkers, Ki-67, TP53, Triple negative

Received: August 24, 2019; Accepted: October 10, 2020

Introduction

Breast cancer is known as one of the most fatal diseases worldwide, which is the cause of the majority of female tumours all over the world.[1] Global statistics demonstrate that the annual incidence of breast cancer is growing further in those countries where there have not been many cases of breast cancer, Iran for instance.[2] Based on the reports from the Ministry of Health and Medical Education (MOHME), cancer is a major public health problem in Iran. Additionally, this type is the most common cancer among Iranian women with an age-standard incidence rate of 28.1 per 100000 population.[3] Despite the moderately low incidence of breast cancer in Iran, its cause-specific mortality is far higher compared with developed countries.[4] Results of certain studies suggested that breast cancer affects Iranian women at least one decade earlier compared to women from other countries.[1, 5]

Many researchers have indicated that breast cancer in younger age groups have more aggressive behaviours with a higher rate of mortality, lower specific surveillance, and worse prognosis.[6, 7] There are several risk factors that influence the prognosis and outcome of breast cancer in patients such as: hormonal receptors level, oncogenes, and tumour suppressor genes expression that could affect the growth rate of tumour. One of the important markers related to receptors in breast cancer is human epidermal growth factor receptor 2 (HER2). HER2 is a known cancer oncogene, particularly in breast cancer, with its positivity being critical to determine the appropriate treatment. The overexpression of HER2 as a tumour marker influences the biological behaviours of cancer including: cell proliferation, prognosis, response to treatment, and the risk of recurrence.[8] Ki-67, another marker, is an oncogene mostly utilized as a diagnostic marker in various cancers; its expression reveals the cellular proliferation rate and is often associated with the clinical course of cancer.[9] The final marker is tumor protein P53 (TP53) that is recognized as a tumour suppressor key gene in the cell cycle regulation, genomic stabilization, DNA repair, apoptosis facilitation, cellular senescence, and telomere attrition.[10, 11] Previous studies have indicated that the TP53 mutation has a pivotal role in primary stages of breast cancer due to the loss of normal tumour suppression role, which may contribute to more aggression, poorer prognosis, and mortality.[10, 12] The presence of estrogen receptor (ER) is another main prognostic indicator for surveillance. ER negative tumours are non-responsive to antioestrogen therapy and account for a more aggressive clinical course.[13] Studies demonstrated that the combination of these risk factors is more critical than one factor.[14]

Thus, in this study, we examined all these risk factors at the same time to investigate the prognosis and progression aspect of tumour based on genetic risk factors in under and above 40-year-old patients in an Iranian population.

Material and Methods

Patients and samples

The study protocol was approved by the Ethics Committee of Mashhad University of Medical Sciences. In a case- control study, 406 breast cancer patients referred to the oncology department of teaching hospitals affiliated with Mashhad University of Medical Sciences, Mashhad, Iran, between 2001 and 2012. The patients were screened retrospectively for prognostic factors including HER2, TP53, Ki-67, ER, progesterone receptor (PR) and classified in two categories, including the groups of patients under and above the age of 40 years.

We extracted the clinical parameters including age and sex, and pathological information comprised of type of the tumour, ER, PR, HER2 receptor, Ki-67, and TP53 status and the stage of the disease from medical records for all the subjects. We employed immunohistochemistry method to characterize the tumor markers, according to the standard protocols. The values of zero and +1 were considered negative and +3 was considered positive for HER2 evaluation. In addition, we analysed +2 samples again with FISH method to define the status of the marker.

Table 1. Comparison of tumour properties in two different age groups (<40 & ≥40 years)

	Characteristics	Age <40 N=110		Age ≥40 N=296		*P*-value
		N	%	N	%	
Stage	Early stage (I & II)	61	55.5	151	51	0.968
	Late stage (III & IV)	36	32.7	90	30.4	
	Unstaged	13	11.8	55	18.6	
Tumor subtype	Ductal	86	78.2	233	78.7	0.891
	Lobular	5	4.5	15	5.1	
	Medullary	6	5.5	11	3.7	
	Other	5	4.5	13	4.4	
	Missing	8	7.3	24	8.1	
ER	Positive	48	43.6	177	59.8	0.004
	Negative	62	56.4	119	40.2	
PR	Positive	48	43.6	156	52.7	0.104
	Negative	62	56.4	140	47.3	
HER2	Positive	44	40	111	37.5	0.645
	Negative	66	60	185	62.5	
Triple negative (ER-/PR-/HER2-)	Positive	35	31.8	58	19.6	0.009
	Negative	75	68.2	238	80.4	
TP53	Positive	52	47.3	145	49	0.759
	Negative	58	52.7	151	51	
Ki-67	Positive	42	38.2	114	38.5	0.063
	Negative	8	7.3	47	15.9	
	Unknown/not done	60	54.5	135	45.6	

ER: Estrogen receptor, PR: Progesterone receptor, HER2: Human epidermal growth factor receptor 2, TP53: Tumor protein p53

In addition, hormone receptors with expression above 1% were reported as positive and otherwise negative. Ki-67 was considered to be positive provided that the percentage of stained tumour cells was more than 20%.

Statistical analysis

We carried out the analysis of all the data using SPSS software (version 13). Chi-square was utilized to compare the prognostic factors and age categories. A *P* value less than 0.05 were considered to be statistically significant. Additionally, we performed a statistical test (Chi-Square Test) in order to discover the relationship between HER2 and Ki-67 expression and results.

Result

In the current work, the authors considered 406 breast cancer patients for prognostic factors, including HER2, TP53, Ki-67, ER, and PR in this study (Table 1). The mean age at diagnosis of patients was 47.7±11.6 (SD) years. All the patients were classified based on their age; 110 patients were under 40 years old (group I) and 296 patients were over 40 (group II). The most common type of tumour was found to be invasive ductal carcinoma (78.6%) and the most common surgery method was modified radical mastectomy (84.0%). The most prevalent stage in both groups was stage II with percentages of 49.1% and 43.9% nodes in groups I and II, respectively. The mean number of involved lymph nodes in groups I and II was 3.57 and 3.42, respectively, with no statistically significant difference (*P*=0.817).

ER expression was 43.6% in group I and 59.8%

Table 2. Expression of different biomarkers in patients with different age groups: HER2 vs. TP53; TP53 vs. Ki-67 and HER2 vs. Ki-67

		TP53			
	Group status	Age <40 years old		Age ≥ 40 years old	
		Positive	Negative	Positive	Negative
	Positive N (%)	18 (40.9%)	26 (59.1%)	57 (51.4%)	54 (48.6%)
HER2	Negative N (%)	34 (51.5%)	32 (48.5%)	88 (47.6%)	97 (52.4%)
	Total N (%)	52 (47.3%)	58 (52.7%)	145 (49.0%)	151 (51.0%)
P value		0.05		>0.05	
		Ki-67			
	Group status	Age <40 years old		Age ≥ 40 years old	
		Positive	Negative	Positive	Negative
	Positive N (%)	28 (87.5%)	4 (12.5%)	71 (78.9%)	19 (21.1%)
TP53	Negative N (%)	14 (77.8%)	4 (22.2%)	43 (60.6%)	28 (39.4%)
	Total N (%)	42 (84.0%)	8 (16.0%)	114 (70.8%)	47 (29.2%)
P value		>0.05		0.011	
		Ki-67			
	Group status	Age <40 years old		Age ≥ 40 years old	
		Positive	Negative	Positive	Negative
	Positive N (%)	18 (85.7%)	3 (14.3%)	48 (80.0%)	12 (20.0%)
HER2	Negative N (%)	24 (82.8%)	5 (17.2%)	66 (65.3%)	35 (34.7%)
	Total N (%)	42 (84.0%)	8 (16.0%)	114 (70.8%)	47 (29.2%)
P value		>0.05		0.048	

HER2: Human epidermal growth factor receptor 2, TP53: Tumor protein p53.

in group II, which was significantly different (*P*=0.004). PR positive patients were 43.6% and 52.7%, respectively in group I and II, and the difference was not statistically significant (*P*=0.104). HER2 expression in group I (40%) was more than group II (37.5%), yet not significantly different (*P*=0.645). We also assessed the triple negative (TNBC) form of ER, PR and HER2 combination, the results of which are represented in table 1. The frequencies of triple negative patients were different in group I (31.8%) compared with group II (19.6%) (*P*=0.009).

The level of TP53 expression in patients under 40 years old was 47.3% and 49% in 40 or above 40-year-old patients (*P*=0.759). Unfortunately, only 211 patients had Ki-67 test results, among whom 38.2% were reported to be positive in group I and 38.5% in group II, with no significant difference between the groups (*P*=0.063).

Categorization using HER2 and Ki-67 revealed a significant difference in patients who were 40 or over 40 (*P*=0.048) but not in group I (*P*>0.5). This difference was also significant (*P*=0.049) in all the patients, regardless of their age. We came to a similar result concerning mutation of TP53 and Ki-67 (*P*=0.368, for group I and *P*=0.011, for group II). Regardless of age classification, the difference of these two recent factors was significant among all the patients (*P*=0.005). The results are represented in table 2.

Discussion

According to our findings, the evaluation of the major breast cancer biomarkers in this research indicated that ER and PR expressions were less and HER2, TP53, and Ki-67 oncogenes were

more in breast cancer patients younger than 40 years of age.

The number of lymph nodes is an important factor to determine the recurrence and outcome of malignancy.[15] Lymph node status and stage at the time of diagnosis are major factors to determine breast cancer survival. Our results showed that the mean number of involved lymph nodes is higher in group I compared with group II. Furthermore, younger patients were more likely to be of higher stages. However, there was no significant difference between the two groups. These findings could be reasons for a worse prognosis in younger patients. It is also worth mentioning that in Iran, there is no national screening program for breast cancer. Therefore, most of the patients are diagnosed at more advanced stages of breast cancer.

The results of our work indicated that the frequency of TNBC is about 23%. The incidence of TNBC varies in racial or ethnic origin groups at all ages. A study reported that the prevalence of TNBC is 83.3% in Ghanaian women compared with 41.9% in African- American and 15.4% in white American women.[16] In our study triple negative cases were found to be more common in the younger group (less than 40 years of age; P=0.009). Previously, we mentioned that an increased risk of TNBC is attributed to young age at diagnosis [OR for 20-39 years: 1.77, 95% CI (1.18-2.64)].[17] This could reflect a poorer prognosis, aggressive behaviour and lack of targeted therapy effectiveness.

While HER2/neu overexpression commonly occurs in 25-30% of breast carcinomas, our outcome indicated that the rate of HER2 positive cases is about 38%. Moreover, we found that HER2 positive status is more likely to be observed in a younger group, which was consistent with previous studies.[18, 19] Based on more aggressive behaviour of tumour in younger patients,[20] it is deducted that HER2 could improve the biological course of breast cancer. Recent studies also point out that HER2 overexpression in young breast cancer patients demonstrates direct correlation with the grade of the tumour. This is also due to ER absence and wider necrosis areas in histological samples.[21] It is similarly demonstrated that HER2 expression is an indicator for extension of lymph nodes involvement and a higher risk of recurrence, as well as the more aggressive behaviour of tumour.[22]

TP53 mutation distribution is varied from 17 to 88% depending on molecular subtypes.[23] There are certain data implying that TP53 nuclear accumulation augments the probability of higher metastasis and bad prognosis. In our study, TP53 nuclear accumulation was 47.3% in young patients compared to 49% in older patients with no significant difference. Other researchers reported different rates of TP53 mutation based on age categories. Results of these studies expressed that TP53 mutation is not varied in different age categories.[24] There are; however, few studies on the incidence of TP53 positive that is slightly higher in younger patients due to higher grads of tumor.[25]

As indicated previously, there was a significant association between HER2 overexpression and the accumulation of nuclear TP53.[26] The coexistence of TP53 and HER2 accumulation seems to be a strong prognostic marker for breast cancer.[27] This phenotype can trigger poor prognosis. This phenotype was higher in the patients younger than 40 (Table 2). On top of that, the results showed that 18% of cases are HER2 and TP53 positive, which is in accordance with the similar reports in Egyptian breast cancer patients.[26]

The frequency of high Ki-67 has been reported 55.6% in an Iranian population.[28] In this work, the frequency of Ki-67 positive was 74%, which was similar in young and old patients. According to the results, we could observe a high level of Ki-67 in younger patients demonstrating more aggressive behaviour of tumour in younger patients. This finding is consistent with other published reports of higher Ki-67 overexpression in young breast cancer patients.[29] There is a report expressing that tumour proliferation is lower in primary breast cancer patients over 65 years old; however, no difference has yet been seen between 36-50 and 50-65 years age categories in the Ki-67 index values.[30]

In this study, we found that TP53/Ki-67 positive samples were mostly in group II (>40 years old) but there was no significant difference between the two groups (Table 2). HER2/Ki-67 positive status was also similar in the two groups of age (Table 2). According to certain studies, the existence of these tumour markers might be attributed to a more critical condition. Reports also show that these conditions are mostly observed in young patients.[29] Limited sample size, methods of laboratory analysis, different types of breast cancer, genetic background, and other involved risk factors that affect a patient's condition are amongst factors that may cause controversial results.

We acknowledge that there were also certain limitations in our study. All the patients were those who referred to the hospital on a case series basis. On the other hand, the retrospective design of the study, missing data with no chance to get them completed, and possible recall bias might also be regarded as other possible limitations. In addition, HER2/neo information was based on IHC not FISH/CICH assays. However, there is a prevalent notion that indicates overall survival and progression free survival may help better understand the value of different prognostic factors in breast cancer patients. This study sought the age related changes in tumour marker expressions in our population.

In conclusion, due to lack of a national screening program, it is more likely that breast cancer patients present at an advanced stage. Breast cancer in younger women may be more invasive. Higher rate of TNBC, as well as higher expression of HER2, TP53, and Ki-67 biomarkers might be indicative of poorer prognosis and less favourable outcomes.

Acknowledgement

We would like to thank Mashhad University of Medical Sciences, Omid and Ghaem hospitals who supported the project.

Conflict of Interest

None declared.

Reference

1. Mousavi SM, Zheng T, Dastgiri S, Miller AB. Age distribution of breast cancer in the Middle East, implications for screening. *Breast J*. 2009;15(6):677-9. doi:10.1111/j.1524-4741.2009.00843.x.
2. Wilson CM, Tobin S, Young RC. The exploding worldwide cancer burden: the impact of cancer on women. *Int J Gynecol Cancer*. 2004;14(1):1-11. doi:10.1111/j.1048-891x.2004.14178.x.
3. Almasi Z, Mohammadian-Hafshejani A, Salehiniya H. Incidence, mortality, and epidemiological aspects of cancers in Iran; differences with the world data. *J BUON*. 2016;21(4):994-1004.
4. Harirchi I, Kolahdoozan S, Karbakhsh M, Chegini N, Mohseni SM, Montazeri A, et al. Twenty years of breast cancer in Iran: downstaging without a formal screening program. *Ann Oncol*. 2011;22(1):93-7.
5. Harirchi I, Ebrahimi M, Zamani N, Jarvandi S, Montazeri A. Breast cancer in Iran: a review of 903 case records. *Public Health*. 2000;114(2):143-5.
6. Chung M, Chang HR, Bland KI, Wanebo HJ. Younger women with breast carcinoma have a poorer prognosis than older women. *Cancer.* 1996;77(1):97-103.
7. El Saghir NS, Seoud M, Khalil MK, Charafeddine M, Salem ZK, Geara FB, et al. Effects of young age at presentation on survival in breast cancer. *BMC Cancer.* 2006;6:194.
8. Ferretti G, Felici A, Papaldo P, Fabi A, Cognetti F. HER2 role in breast cancer: from a prognostic foe to a predictive friend. *Curr Opin Obstet Gynecol*. 2007;19(1):56-62.
9. Yerushalmi R, Woods R, Ravdin PM, Hayes MM, Gelmon KA. Ki-67 in breast cancer: prognostic and predictive potential. *Lancet Oncol*. 2010;11(2):174-83.
10. Runnebaum IB, Nagarajan M, Bowman M, Soto D, Sukumar S. Mutations in p53 as potential molecular markers for human breast cancer. *Proc Natl Acad Sci USA.* 1991;88(23):10657-61.
11. Chen Z, Trotman LC, Shaffer D, Lin HK, Dotan ZA, Niki M, et al. Crucial role of p53-dependent cellular senescence in suppression of Pten-deficient tumorigenesis. *Nature*. 2005;436(7051):725-30.
12. Simpson JF, Page DL. The p53 tumor suppressor gene in ductal carcinoma in situ of the breast. *Am J Pathol*. 2000;156(1):5-6.
13. Bauer KR, Brown M, Cress RD, Parise CA, Caggiano V. Descriptive analysis of estrogen receptor (ER)-negative, progesterone receptor (PR)-negative, and HER2-negative invasive breast cancer, the so-called triple-negative phenotype: a population-based study from the California cancer Registry. *Cancer*. 2007;109(9):1721-8.
14. Dworkin AM, Huang TH, Toland AE. Epigenetic alterations in the breast: Implications for breast cancer detection, prognosis and treatment. *Semin Cancer*

Biol. 2009;19(3):165-71. doi:10.1016/j.semcancer.2009.02.007.
15. Fisher B, Bauer M, Wickerham DL, Redmond CK, Fisher ER, Cruz AB, et al. Relation of number of positive axillary nodes to the prognosis of patients with primary breast cancer. An NSABP update. *Cancer.* 1983;52(9):1551-7.
16. Stark A, Kleer CG, Martin I, Awuah B, Nsiah-Asare A, Takyi V, et al. African ancestry and higher prevalence of triple-negative breast cancer: findings from an international study. *Cancer.* 2010;116(21):4926-32.
17. Dolle JM, Daling JR, White E, Brinton LA, Doody DR, Porter PL, et al. Risk factors for triple-negative breast cancer in women under the age of 45 years. *Cancer Epidemiol Biomarkers Prev.* 2009;18(4):1157-66. doi:10.1158/1055-9965.EPI-08-1005.
18. Wang B, Wang X, Zou Y. Association between hormone receptors and HER-2/neu is age-related. *Int J Clin Exp Pathol*. 2015;8(7):8472-9.
19. AlZaman AS, Mughal SA, AlZaman YS, AlZaman ES. Correlation between hormone receptor status and age, and its prognostic implications in breast cancer patients in Bahrain. *Saudi Med J*. 2016;37(1):37-42.
20. Cancello G, Maisonneuve P, Rotmensz N, Viale G, Mastropasqua MG, Pruneri G, et al. Prognosis and adjuvant treatment effects in selected breast cancer subtypes of very young women (<35 years) with operable breast cancer. *Ann Oncol*. 2010;21(10):1974-81.
21. Rodrigues NA, Dillon D, Carter D, Parisot N, Haffty BG. Differences in the pathologic and molecular features of intraductal breast carcinoma between younger and older women. *Cancer*. 2003;97(6):1393-403.
22. Maru D, Middleton LP, Wang S, Valero V, Sahin A. HER-2/neu and p53 overexpression as biomarkers of breast carcinoma in women age 30 years and younger. *Cancer.* 2005;103(5):900-5.
23. Bertheau P, Lehmann-Che J, Varna M, Dumay A, Poirot B, Porcher R, et al. p53 in breast cancer subtypes and new insights into response to chemotherapy. *Breast.* 2013;22 Suppl 2:S27-9.
24. Eerola H, Heikkilä P, Tamminen A, Aittomäki K, Blomqvist C, Nevanlinna H. Histopathological features of breast tumours in BRCA1, BRCA2 and mutation-negative breast cancer families. *Breast Cancer Res.* 2005;7(1):R93-R100. doi:10.1186/bcr953
25. Pratap R, Shousha S. Breast carcinoma in women under the age of 50: Relationship between p53 immunostaining, tumour grade, and axillary lymph node status. *Breast Cancer Res Treat.* 1998;49(1):35-9.
26. Rashed MM, Ragab NM, Galal MK. The association of HER-2/neu over-expression in relation to p53 nuclear accumulation, hormonal recceptor status and common clinico-pathological prognostic parameters in a series of Egyptian women with invasive ductal carcinoma. *Eur J Gen Med.* 2007;4(2):73-9.
27. Yamashita H, Nishio M, Toyama T, Sugiura H, Zhang Z, Kobayashi S, et al. Coexistence of HER2 over-expression and p53 protein accumulation is a strong prognostic molecular marker in breast cancer. *Breast Cancer Res.* 2004;6(1):R24-30.
28. Hosseini S, Shahbaziyan H, Razmjoo S, Jasemizade N. Evaluation the correlation between Ki-67 and 5 years disease free survival of breast cancer patients. *Biosci Biotech Res Asia.* 2015;12(3):2221-5.
29. Shokouh TZ, Ezatollah A, Barand P. Interrelationships between Ki-67, HER2, p53, ER, and PR status and their associations with tumor grade and lymph node involvement in breast carcinoma subtypes: Retrospective-observational analytical study. *Medicine (Baltimore)*. 2015;94(32):e1359.
30. Nishimura R, Osako T, Okumura Y, Hayashi M, Toyozumi Y, Arima N. Ki-67 as a prognostic marker according to breast cancer subtype and a predictor of recurrence time in primary breast cancer. *Exp Ther Med.* 2010;1(5):747-54.