Bcl-2 and Ki-67 Expression in Young Women with Breast Cancer

Genç Yaş Meme Kanserli Kadın Hastalarda Bcl-2 ve Ki-67 Ekspresyonu

Çok Genç Yaş Meme Kanseri / Breast Cancer in Very Young Women

Arsenal Sezgin Alikanoğlu[1], Mustafa Yıldırım[2], Dinç Süren[1], Ayşegül Kargı[2], Mustafa Yıldız[2], Cem Sezer[1], Birsel Tutuş[1]
[1]Department of Pathology, [2]Department of Medical Oncology, Antalya Education and Research Hospital, Antalya, Turkey

Özet

Amaç: Meme kanseri insidensi yaşla birlikte artar ve hastaların yaklaşık %85'i 50 yaşından sonra tanı alır. Genç hastalarda meme kanserinin klinik seyri ileri yaştaki hastalara göre daha kötüdür. Çalışmamızın amacı çok erken yaş meme kanserli hastalar ile ileri yaş meme kanserli hastalar arasında Bcl-2 ekspresyonu ve Ki-67 proliferasyon indeksi açısından fark olup olmadığının belirlenmesi ve eğer belirlenebilirse bu farkın klinik öneminin araştırılmasıdır. Gereç ve Yöntem: Antalya Eğitim ve Araştırma Hastanesi'nde 2008-2010 yılları arasında meme kanseri tanısı almış 35 yaş ve altı 15 hasta (Grup A) ile 35 yaş üzeri meme kanserli 30 hasta (Grup B) çalışmaya alındı. Bulgular: Bcl-2 ekspresyonu 33 hastada (73.3%) pozitif, 12 hastada (26.7%) negatif olarak değerlendirildi. Bcl-2 ekspresyonu açısından Grup A ve B hastalar arasında fark saptanmadı. (p:0.475). Bcl-2 ile ER ekspresyonu arasında daha belirgin olmak üzere hormon reseptör ekspresyonu arasında anlamlı ilişki saptandı. (p<0.001, p:0.001). Ki-67 proliferasyon indeksi 8 hastada (17.8%) negatif, 10 hastada (22.2%) düşük ve 27 hastada (60%) yüksek olarak bulundu. Grup A ve Grup B hastalar arasında Ki-67 proliferasyon indeksi arasında ilişki saptanmadı. (p:0.555) Ki-67 proliferasyon indeksi ve bcl-2 ekspresyonu arasında anlamlı ilişki saptanmadı. (p:0.736). Grup A hastalarda daha ileri evre hastalık saptandı. Tartışma: DNA mikroarray çalışmaları meme kanserinde farklı moleküler subtiplerin farklı klinik sonuçlarla ilişkili olduğunu göstermiştir. Çalışmamızda çok genç yaş hastalar (Grup A) ile diğer hastalarda (Grup B) immunohistokimyasal yöntemle araştırılan bcl-2 ve Ki-67 ekspresyonu arasında fark saptanmadı.

Anahtar Kelimeler

Meme Kanseri; Bcl-2; Ki-67; Genç Yaş

Abstract

Aim: Incidence of breast cancer increases with age and nearly 85% of them are diagnosed after the age of 50. Clinical outcome of breast cancer in young patients is worse than older patients. The aim of this study is to search the difference in expression of bcl-2 and Ki-67 between young and older women with breast cancer, and if there is any; its clinical importance. Material and Method: This study includes 15 patients under the age of 35 years old (Group A) and 30 patients over the age of 35 years (Group B) all of whom were diagnosed with breast carcinoma at Antalya Education and Research Hospital between 2008-2011. Results: Bcl-2 expression was found positive in 33 (73.3%) and negative in 12 (26.7%) patients. There were no difference in expression of bcl-2 between Group A and Group B patients (p:0.475). A meaningful relationship was observed between expression of bcl-2 and hormon receptors , being more significant in ER (p:0.001,p<0.001). Ki-67 proliferation index was found negative in 8 patients (17.8%), low in 10 patients (22.2%) and high in 27 patients (60%). No relationship was found in Ki-67 proliferation index between Group A and Group B patients (p:0.555). There were no meaningful relationship between Ki-67 proliferation index and bcl-2 expression (p:0.736). In Group A patients a more advanced stage disease was observed. Discussion: DNA microarray studies established that different molecular subtypes in breast cancer are related with different clinical outcome. In our study, no difference was found in bcl-2 and Ki-67 expression searched by immunohistochemical method , between very young (Group A) and other (Group B) patients.

Keywords

Breast Cancer; Bcl-2; Ki-67; Young Age

DOI: 10.4328/JCAM.1110 Received: 27.05.2012 Accepted: 18.06.2012 Printed: 01.09.2013 J Clin Anal Med 2013;4(5): 380-383
Corresponding Author: Mustafa Yıldırım, Antalya Education and Research Hospital, Department of Medical Oncology, Varlik Mah. Kazim Karabekir Cad. Soğuksu, 07050 Antalya, Turkey. T.: +905333948252 F.: +90 2422494402 E-Mail: mustafayildirim7@yahoo.com

Introduction

Breast cancer is the neoplasia with the highest incidence in the female population worldwide. Incidence of breast cancer increases with age and nearly 85% of them are diagnosed after the age of 50 [1]. Very young patients (≤35 years) with breast cancer constitute 2.7% of all patients in western countries [2]. This ratio is between 10-25 % in Asian countries whereas it is established as 17% in our country [3;4].

Clinical outcome of breast cancer in young patients is worse than older patients. Histologic grade and lymph node involvement rate are found to be high and a lower hormone receptor expression is found in these patients at the time of diagnosis [5] . Genetic inheritance and BRCA1, BRCA2 mutations are observed with a higher rate in young patients [6] .

Bcl-2 gene is firstly identified in patients with B cell follicular lymphoma establishing t(14;18) translocation but its expression is not related to this translocation. Bcl-2 increases the life cycle of the cell by inhibiting apopitosis and therefore causes the cell to come accross mutagenic factors more. In most of the human tumours a relation between bcl-2 expression and survival has been shown [7]. In patients with breast cancer, variable expression rates of Bcl-2 have been determined [8]. Besides, response to adjuvant endocrine therapy is found to be better in patients with bcl-2 expression [9;10].

Ki-67 is a marker that is widely used to determine the proliferating cells . Results of the studies that searched the relationship between Ki-67 expression and prognosis in patients with breast cancer are not clear. While Ki-67 proliferation index was found in association with bad prognosis in the majority of the studies, this association was not shown in other studies [11-13].

The aim of our study is to search the difference in expression of bcl-2 and Ki-67 between young and older women with breast cancer, and if there is any; its clinical importance.

Material and Method

Choosing the patients

This study includes the 15 female patients under the age of 35 years, who were histopathologically diagnosed with invasive breast carcinoma, and followed up at the Medical Oncology Clinic of Education and Research Hospital between 2008 and 2010. Thirty patients with the same diagnosis who were over the age of 35 years were chosen as the control group. Patients whose screening and clinical staging studies were completed were staged according to the 7th Staging System of the American Joint Committee on Cancer (AJCC). Patients' files were analysed and information on age, gender, disease stage and other clinicopathological characteristics were obtained. Patients without histopathological diagnosis and patients whose initial treatments were started at another center and continued at our center were excluded from the study.

Immunohistochemistry

Tumor samples obtained right after the surgery were fixed in 10% formaldehyde. After fixation, tumor samples were embeded in paraffin. Then, histologic sections with a 4 µm thickness were obtained from paraffin blocks and were initially stained with hemotoxylin- eosine for assessment.

The histologic sections were de-paraffinized in incubators at 60°C for one hour. Afterwards, they were kept in xylene for 10 minutes and in 100% alcohol for 5 minutes and then washed in water. Slides were kept in solution buffered with 10% citrate solution in microwave at maximum power (800 watts) for 15 minutes. Then the power was decreased to the half and they were kept in the microwave for another 20 minutes. Slides taken out of the microwave were kept in room temperature for 20 minutes. Endogenous peroxidase activity was removed by being kept in 3% hydrogen peroxide for 20 minutes. Slides washed in distilled water were treated with 3x5 PBS and protein blockage was dripped on them. Five minutes later, Ki-67 and Bcl-2 antibodies were dripped on the slides without washing off the blockage. After being kept in primer antibody for 30 minutes, they were taken into PBS and washed for 5 minutes. Afterwards, they were treated with biotinylated secondary antibody for 20 minutes and washed in PBS for 5 minutes. They were kept with peroxidase conjugate antibody for 20 minutes. Then they were washed in PBS for 5 minutes. They were kept in chromogenous (DAB) for 5 minutes. Slides washed under tap water were adversely stained with haematoxylene. They were dehydrated, dried and mounted with entellane.

For the staining of the samples Ki-67 protein, lyophilized monoclonal mouse antibody (clone B P53;12, 1:100, Invitrogen, Carmennillo, Canada) and Bcl-2 onco-protein, lyophilized mouse monoclonal antibody (clone100/D5, 1:50, Thermo Scientific, Fremont, USA) were used. After the staining, slides were inspected under Nicon Eclipse 80 microscope.

Immunohistochemistry scoring

Slides were evaluated by two pathologists (ASA, DS) who did not know the clinical features of the patients. Each section that was stained by immunohistochemical method was examined under optical microscope. Bcl-2 expressions were assessed according to the cytoplasmic staining of the cells. Interval value for Bcl-2 staining was accepted as 10%. If Bcl-2 staining was more than 10%, it was considered as positive expression, 10% or less staining was accepted as negative expression [7]. The areas with the highest nuclear staining were selected for Ki-67 and 1000 tumor cells were counted. The ratio of tumor cells were calculated and their staining indices were identified as percentage. The interval value for Ki-67 was determined as 10%, and a staining index ≥ 10% was accepted as high, 1% to 10% staining was accepted as low, no staining was accepted as nil [14].

Statistical Analysis

Statistical analyses were performed using the SPSS software version 15. Differences between groups were inspected by using Chi-square and Man Whitney U tests. A p value of <0.05 was considered significant.

Results

A total of 45 patients; 15 (33.3%) of whom were under the age of 35 years (Group A) and 30 (71.7%) of whom were over the age of 35 years (Group B) were enrolled in this study.

Median age was 32.4 (range 25-35 years) in Group A and 52.6 (range 38-71 years) in Group B patients. Thirteen of the Group A patients had a diagnosis of invasive ductal carcinoma whereas 2 of them had a diagnosis of invasive lobular carcinoma and

all of the Group B patients had a diagnosis of invasive ductal carcinoma. Expression of estrogen receptor (ER) and progesterone receptor (PR) showed no difference between Group A and B patients (p:0.612, p:0.642). There were also no difference in expression of Her2 between the two groups (p:0.245). In Group A patients a more advanced stage disease was observed. (Table 1)

Table 1. Patient groups according to age

	Grup A	Grup B	P value
Age	32.4 (range 25-35)	52.6 (range 38-71)	
Histology			
Invasive ductal	13	30	
Invasive lobular	2	0	
Hormon Receptor			
ER positive	11 (73.3%)	24 (80%)	0.612
PR positive	10 (66.7%)	22 (73.3%)	0.642
HER 2 positive	6 (%40)	7 (23%)	0.245
Sigara	4 (26.7%)	5 (16.7%)	0.429
Oks kullanımı	1 (6.7%)	1 (3.4)	0.627
T evresi			0.043
T1	0	1 (3.3%)	
T2	9 (60%)	26 (%86.7)	
T3	6 (40%)	2 (6.7%)	
T4	0	1 (3.3%)	
Nodal status			0.277
Positive	11 (73.3%)	17 (56.7%)	
Negative	4 (26.7%)	13 (43.3%)	
Histologic grade			0.499
Grade 1	0	2 (6.7%)	
Grade 2	10 (66.7%)	31 (68.9%)	
Grade 3	3 (33.3%)	7 (23.3%)	
Lymphovascular invasion	10 (66.7%)	15 (53.6%)	0.407
Perineural invasion	4 (28.6%	4 (15.4%)	0.320

Bcl-2 expression was found positive in 33 (73.3%) and negative in 12 (26.7%) patients. There were no difference in expression of bcl-2 between Group A and Group B patients (p:0.475). No significant relationship was found between Bcl-2 expression and T stage, lymph node status (p:0.849, p:0.746). A meaningful relationship was observed between expression of bcl-2 and hormon receptors , being more significant in ER (p:0.001,p<0.001). ER expression was found as 90.9% in in Bcl-2 positive group and 40.7% in Bcl-2 negative group. Her-2 expression was not different in Bcl-2 positive and negative groups (p:0.06).

Ki-67 proliferation index was found negative in 8 patients (17.8%), low in 10 patients (22.2%) and high in 27 patients (60%). The relationship of Ki-67 proliferation index between Group A and Group B patients was analysed by Man Whitney U test and no relation was found between the two groups (p:0.555). There were no meaningful relationship between Ki-67 proliferation index and Bcl-2 expression (p:0.736).

Discussion

Breast cancer is a heterogoneous disease in terms of clinical and pathological features and response to therapy. Conventional histologic classification of breast cancer does not suggest adequate information about the clinical outcome. Histologic type, grade and size of the tumour, expression of ER, PR and HER2 receptors, leymph node and metastasis status are considered as the important prognostic factors. Age of the patient is also considered as another prognostic factor. Age is related to prognosis independent of the performance score of the patient [15;16]. Breast cancer in very young premenopousal patients behave more agressively than postmenopousal patients with an older age. As in our study, many other studies established that these yuong patients have a disease of advanced stage at the time of diagnosis [17;21].

DNA microarray studies established that different molecular subtypes in breast cancer are related with different clinical outcome [22]. In our study, the role of the apopitotic marker Bcl-2 and the cell proliferative marker Ki67 in clinical progress between very young patients (Group A) and others (Group B) was searcehed and no difference was observed between the two groups.

Although we found no difference in expression of ER, PR and HER2 between Group A and Group B patients, some studies suggest that there is a difference in expression of these receptors between the two groups. In their large series Colleoni et al.. [23] found that ER and PR negativity were significant in patients with the age of 35 years when compared to patients over the age of 35. Also, rate of Ki-67 expression was found higher in these patients. Expression of HER2 was not found different. In their study, similiar to our study, the incidence of grade 3 tumours was found higher in young patients.

Bcl-2 expression had been searched in many types of cancer and in many of the studies it had been shown as a good prognostic factor. Varaible expression of Bcl-2 in normal duct epithelium, intraductal carcinoma and invasive ductal carcinoma was established in different studies. In breast tissue, Bcl-2 expression was found as 96% in normal duct epithelium, 79% in intraductal carcinoma and 45% in invasive carcinoma. It was also shown that Bcl-2 expression has decreased in the development of carcinoma from the normal duct epithelium [24]. It was determined that Bcl-2 expression is related to well differantiation and ER expression, in breast cancer [25]. There are studies establishing the prognostic importance of Bcl-2 expression whereas some

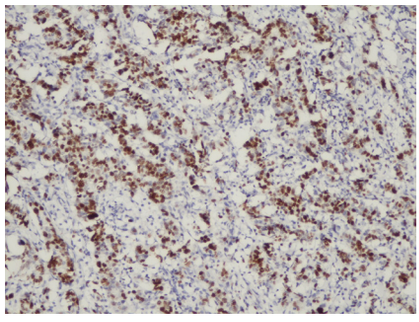


Figure 1. Ki-67 staining in >10% of the tumour cells (Ki-67, immunohistochemistry, method, X100)

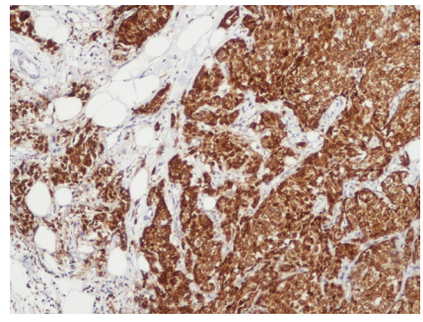


Figure 2. Bcl-2 staining in >10% of the tumour cells (Bcl-2 , immunohistochemistry, X100)

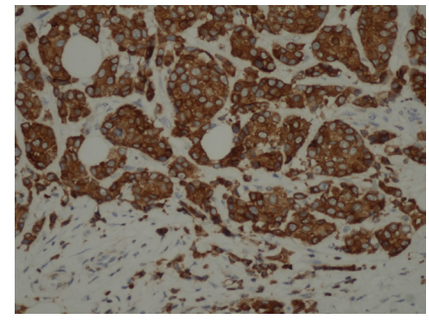


Figure 3. Bcl-2 staining in >10% of the tumour cells (Bcl-2 , immunohistochemistry, X200)

studies show vice-versa [26].

Yu et al. [27] published a study in which they evaluated 40 patients under the age of 35 and 40 postmenopousal patients over the age of 60 and found Bcl-2 expression as 19/40 in the group of young patients and as 30/40 in the group of older patients and showed a significant difference between the two groups. In their study, different from our study; e negative correlation was found between Bcl-2 expression and histologic grade and lymph node metastasis. A positive correlation was established between Bcl-2 expression and ER, PR expression, similiar to our study. In similiar studies concerning ductal carcinoma in situ (DCIS) cases, contrary to invasive ductal carcinoma, no differnce was determined between age groups and Bcl-2 expression [28]. Although many studies establish that Ki-67 proliferation index is higher in very young patients, we were not able to determine a similiar result in our study [29]. We think that this is due to the low number of patients under the age of 35 years , in our study. In a study in which Bcl-2 expression in breast cancer was searched, Bcl-2 expression was found as 4.1% and ve Bcl-2 expression was found in correlation with a lower Ki-67 proliferation index. Besides, Bcl-2 expression was found in a lower rate in premenopousal patients , in the same study [30].

There is a few number of studies in which Bcl-2 expression and Ki-67 proliferation index have been evaluated together in very young patients with breast cancer. Choi et al. [34] searched 103 patients between 25-45 years old with breast cancer and found Bcl-2 expression as 35.6% and Ki-67 proliferation index as 39.7% in their study.

In conclusion, the bad progress in very young patients suggest that this subtype of breast cancer acquires a different tumour biology. Therefore, we think that it is important to demonstrate prognostic factors spesific to this group in order to plan proper treatment fort this group.

References

1. Hankey BF, Ries LA, Edwards BK. The surveillance, epidemiology, and and results program: a national resource. Cancer Epidemiol Biomarkers Prev 1999 Dec; (12):1117-21.
2. Anders CK, Johnson R, Litton J, Phillips M, Bleyer A. Breast cancer before age 40 years. Semin Oncol 2009;36(3):237-49.
3. Garwal G, Pradeep PV, Aggarwal V, Yip CH, Cheung PS. Spectrum of breast cancer in Asian women. World J Surg 2007;31(5):1031-40.
4. Tuncer .M editor. Türkiye'de kanser kontrolü. Ankara: T.C. Sağlık Bakanlığı, ;2009. p. 385-91
5. Han W, Kim SW, Park IA, Kang D, Youn YK, Oh SK, et al. Young age: an independent risk factor for disease-free survival in women with operable breastcancer. BMC Cancer 2004;4:82.
6. Malone KE, Daling JR, Doody DR, O'Brien C, Resler A, Ostrander EA, et al.Family history of breast cancer in relation to tumor characteristics and mortality in a population-based study of young women with invasive breast cancer. Cancer Epidemiol Biomarkers Prev. 2011;20(12):2560-71.
7. Yildirim M, Suren D, Goktas S, Dilli UD, Kaya C, Copuroglu R. The predictive role of Bcl-2 expression in operable locally advanced or metastatic gastric carcinoma. J BUON. 2012;17(1):106-9.
8. Won KY, Kim GY, Kim YW, Song JY, Lim SJ. Clinicopathologic correlation of beclin-1 and bcl-2 expression in human breast cancer. Hum Pathol. 2010;41(1):107-12.
9. Gasparini G, Barbareschi M, Doglioni C, Palma PD, Mauri FA, Boracchi P. et al. Expression of bcl-2 protein predicts efficacy of adjuvant treatments in operable node-positive breast cancer, Clin Cancer Res, 1995;1(2):189–198.
10. Hellemans P, van Dam PA, Weyler J, van Oosterom AT, Buytaert P, Van Marck E, Prognostic value of bcl-2 expression in invasive breast cancer, Br J Cancer, 1995;72(2):354–360.
11. Brown RW, Allred CD, Clark GM, Osborne CK, Hilsenbeck SG. Prognostic value of Ki-67 compared to S-phase fraction in axillary node-negative breast cancer. Clin Cancer Res. 1996;2(3):585-92.
12. Wintzer HO, Zipfel I, Schulte-Mönting J, Hellerich U, von Kleist S. Ki-67 immunostaining in human breast tumors and its relationship to prognosis. Cancer. 1991;67(2):421-8.
13. Bottini A, Berruti A, Bersiga A, Brizzi MP, Bruzzi P, Aguggini S. et al.Relationship between tumor shrinkage and reduction in Ki-67 expression after primary chemotherapy in human breast cancer. Br J Cancer 2001; 85: 1106-1112.
14. Tan PH, Bay BH, Yip G, Selvarajan S, Tan P, et al. Mod Pathol. Immunohistochemical detection of Ki67 in breast cancer correlates with transcriptional regulation of genes related to apoptosis and cell death. 2005;18(3):374-81.
15. Rudas M, Gnant MF, Mittlböck M, Neumayer R, Kummer A, Jakesz R, et al. Thymidine labeling index and Ki-67 growth fraction in breast cancer: comparison and correlation with prognosis. Breast Cancer Res Treat 1994; 32: 165-175.
16. Goldhirsch A, Gelber RD, Yothers G, Gray RJ, Green S, Bryant J, et al. Adjuvant therapy for very young women with breast cancer: need for tailored treatments. J Natl Cancer Inst Monogr. 2001;(30):44-51.
17. de la Rochefordiere A, Asselain B, Campana F, Scholl SM, Fenton J, Vilcoq JR, et al. Age as prognostic factor in premenopausal breast carcinoma. Lancet 1993, 341, 1039–1043.
18. Walker RA, Lees E, Webb MB, Dearing SJ. Breast carcinomas occurring in young women (<35 years) are different. Br J Cancer 1996;74(11):1796–800.
19. Adami HO, Malker B, Holmberg L, Persson I, Stone B. The relation between survival and age at diagnosis in breast cancer. N Engl J Med 1986;315:559–63.
20. 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.
21. Kollias J, Elston CW, Ellis IO, Robertson JF, Blamey RW. Early-onset breast cancer histopathological and prognostic considerations. Br J Cancer 1997;75(9):1318–23.
22. Sørlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications, Proc Natl Acad Sci, 2001; 98(19):10869–10874.
23. Colleoni M, Rotmensz N, Robertson C, Orlando L, Viale G, Renne G, et al. Very young women (<35 years) with operable breast cancer: features of disease at presentation. Ann Oncol. 2002 Feb;13(2):273-9.
24. Zhang GJ, Kimijima I, Abe R, Kanno M, Katagata N, Hara K, et al. Correlation between the expression of apoptosis-related bcl-2 and p53 oncoproteins and the carcinogenesis and progression of breast carcinomas Clin Cancer Res. 1997;3(12 Pt 1):2329-35.
25. Silvestrini R, Veneroni S, Daidone MG, Benini E, Boracchi P, Mezzetti M, et al. The Bcl-2 protein: a prognostic indicator strongly related to p53 protein in lymph node-negative breast cancer patients, J Natl Cancer Inst, 1994, 86(7):499–504.
26. Ihemelandu CU, Dewitty RL Jr, Leffall LD Jr, Suryanarayana SM, Frederick WA. Clinical significance of p53 and bcl-2 protein coexpression phenotypes in molecular breast cancer subtypes of pre-menopausal and post-menopausal African-American women. Am Surg. 2009;75(9):776-84.
27. Yu B, Sun X, Shen HY, Gao F, Fan YM, Sun ZJ. Expression of the apoptosis-related genes BCL-2 and BAD in human breast carcinoma and their associated relationship with chemosensitivity. J Exp Clin Cancer Res. 2010;29:107.
28. 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.
29. Goldhirsch A, Gelber RD, Yothers G, Gray RJ, Green S, Bryant J, et al. Adjuvant therapy for very young women with breast cancer: need for tailored treatments. J Natl Cancer Inst Monogr. 2001;(30):44-51.
30. Zaha DC, Lazăr E. Molecular characterization of apoptosis by the immunohistochemical evaluation of Bcl-2 in breast cancer. Rom J Morphol Embryol. 2012;53(1):155-60.
31. Choi DH, Kim S, Rimm DL, Carter D, Haffty BG. Immunohistochemical biomarkers in patients with early-onset breast carcinoma by tissue microarray. Cancer J. 2005;11(5):404-11.