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ARAŞTIRMA / RESEARCH

Prognostic significance of PRAME (preferentially expressed antigen of melanoma) expression in breast cancer

Meme kanserinde PRAME (preferentially expressed antigen of melanoma) ekspresyonunun prognostik önemi

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Abstract

Purpose: The aim of this study is to detect the PRAME (Preferentially Expressed Antigen of Melanoma) in 54 patients with breast cancer and 37 patients with benign breast lesions.

Materials and Methods: PRAME expressions in 54 breast cancer, 20 benign breast lesions and 10 normal breast tissue samples were studied with RT-PCR. Expression of PRAME was studied with IHC in 37 benign breast lesions, in 54 breast cancer patients from both tumor and normal breast tissue. RT-PCR and IHC results for PRAME were compared in this study.

Results: PRAME was found to be expressed in 50 % of the breast cancer and 25 % of the benign breast lesions. Using IHC method, (+), (++) and (+++) staining for PRAME expression were found in 29,6%, 31,5% and 3,7% of the cases, respectively in invasive component of the breast cancer. PRAME expression detected by both IHC and RT-PCR was compared with prognostic parameters. PRAME expression in breast cancer was found to be associated with high tumor grade and negative hormone receptor. We found an important association between PRAME RT-PCR and of PRAME IHC.

Conclusion: Both RT-PCR in fresh tissues and IHC method in paraffin embedded tissues can be used to identify PRAME expression and the predictive role of PRAME expression.

Keywords: Breast cancer, PRAME, prognostic factors, predictive factors, RT-PCR, IHC, immunotherapy, Cancer-testis antigen.

Öz

Amaç: Bu çalışmanın amacı 54 meme kanseri ve 37 iyi huylu meme lezyonu olan hastada PRAME (Preferentially Expressed Antigen of Melanoma) ekspresyonunu tespit etmektir.

Gereç ve Yöntem: Reverse transkripsiyon polimeraz zincir reaksiyonu (RT-PCR) ile 54 meme kanseri, 20 benign meme lezyonu ve 10 normal meme dokusu örneğindeki PRAM Eekspresyonu çalışıldı. İmmunohistokimyasal (IHC) yöntem ile PRAME ekspresyonu, 54 meme kanserili hastanın hem tümör hem de normal meme dokusundan ve 37 benign meme lezyonunda çalışıldı. PRAME için RT-PCR ve IHC sonuçları bu çalışmada karşılaştırıldı.

Bulgular: Meme kanserinin %50'sinde, benign meme lezyonlarının ise %25'inde PRAME ekspresyonu olduğu saptandı. İnvaziv meme kanseri olan dokularda IHC yöntemi kullanılarak bakılan PRAME ekspresyonunda; (+), (++) ve (+++) boyanma oranları sırasıyla %29,6, %31,5 ve %3,7 oranında bulundu. Hem IHC hem de RT-PCR ile saptanan PRAME ekspresyonu prognostik parametrelerle karşılaştırıldı. Meme kanserinde PRAME ekspresyonunun yüksek grade ve negatif hormon reseptörü ile ilişkili olduğu tespit edildi. RT-PCR ile IHC ile bakılan PRAME sonuçları arasında anlamlı ilişki bulundu.

Sonuç: Hem taze dokularda RT-PCR yöntemi, hem de parafin bloklarda IHC yöntemi, meme kanserinde PRAME ekspresyonunu ve PRAME'in prediktif önemini belirlemede kullanılabilir.

Anahtar kelimeler: Meme kanseri, PRAME, Prognostik faktörler, Prediktif faktörler, RT-PCR, IHC, İmmünoterapi, Kanser-testis antijeni.

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INTRODUCTION

Cancer testis antigens are gene family generally considered as limited to tumor cells and not expressed in normal tissues except testis and fetal tissues. MAGE, GAGE/PAGE, BAGE, LAGE/NYESO-1, and (Preferentially Expressed Antigen of Melanoma) (PRAME) are the major members of the family of cancer-testis antigens. It is known that cancer testis antigens have considerable roles in cancer immunotherapy. It has been shown that, in some tumors, cancer testis antigens have prognostic significance[1].

The gene coding PRAME has been firstly found in a patient with recurrent melanoma by Ikeda et al[1]. PRAME expression has been studied through Reverse transcription polymerase chain reaction (RT-PCR) in various tumors and expression has been found in 97% of malignant melanomas, 93% of neuroblastomas, 80% of sarcomas, 70% of lung cancers, 40,5% of renal cell cancers and 29% of head and neck cancers. PRAME expression in Wilm's tumor and acute leukemia has been found to be highly variable rate. When the normal tissues are studied, it was found that surrenal, ovarian, and endometrial tissues had very low PRAME expression[1-7].

Preferentially Expressed Antigen of Melanoma gene takes place in the 22nd chromosome (22q11.22) and codes a protein of 509 amino acid and its function is not known exactly[1]. PRAME alerts the cytotoxic T-cell mediated immune response by autologous lymphocytes and it promises tumor immunotherapy. PRAME inhibits the differentiation stimulated by retinoic acid, apoptosis and it is the dominant suppressor of the retinoic acid receptor signal[8,9]. On the other hand, it has been shown that temporary excessive expression triggers caspase-independent cell death in cell culture series and leukemia's that had a good prognosis expressing high PRAME[10]. In another study, it has been reported that leukemic cells expressing high PRAME decreases the expression of the genes related with apoptosis and causes the survival of the leukemic cells and the formation of a multi-drug resistance[11]. Briefly, the importance of PRAME expression in tumor biology changes according to the tumor type. PRAME expression is generally considered as a negative prognostic determiner, however, it was found that as a positive determiner for some leukemia types such as Acute promyelocytic leukemia[12].

PRAME expression has been studied in breast cancer, was found 16%, 27%, and 53% [13,14,15]. In the cases in which adjuvant chemotherapy was administrated, an association existed between PRAME expression and shortened relapse-free survival. Epping et al. found that PRAME expression is a prognostic marker for metastasis-free interval and overall survival in primary breast cancer and PRAME expression predicts benefit of chemotherapy. These data differed from each other. These studies raise the possibility that PRAME may have different roles in tumor development dependent on different tumor type[13,14]. The protein PRAME plays a role in preventing the proliferation and metastasis of breast cancer cells. The knocking down of PRAME promotes breast cancer cell proliferation and inhibits apoptosis. In addition, inhibition of PRAME promotes the invasion of breast cancer cells. The PRAME expression has been found as a negative prognostic determinant in breast cancer[16].

PRAME gene generally has been studied using RT-PCR method in fresh tissues, immunohistochemistry (IHC) method has been used in a few studies but it has not been studied in breast cancer until 2010. In this study, PRAME gene expression was analyzed both in fresh tissue and paraffin-embedded samples by RT-PCR and IHC, respectively. İn order to find out whether the gene expression in breast cancer will give compatible results with these two methods.

In this study, PRAME mRNA expression was determined quantitatively in fresh tissue samples taken from 54 breast cancer patients and 20 patients' benign breast lesions with RT-PCR. Qualitative PRAME staining patterns in IHC method were compared with RT-PCR results and also PRAME expression was compared with known prognostic factors in breast cancer.

MATERIALS AND METHODS

This prospective study was carried out in 212 patients with breast mass who applied to Cukurova University, Faculty of Medicine, General Surgery Department between September 2009 and May 2010. Ethics committee approval was received from the Cukurova University Ethics Committee (30.06.2009/7-8). Study has been performed in accordance with the ethical standards of the 1975 Declaration of Helsinki which was revised in 2000. In addition, written consent form was obtained from all patients included in the study.

PRAME expressions in 54 breast cancer, 20 benign breast lesions and 10 normal breast tissue samples were studied with RT-PCR. Expression of PRAME was studied with IHC in 37 benign breast lesions, in 54 breast cancer patients from both tumor and normal breast tissue. During the study, the information of 140 patients diagnosed with invasive breast cancer has been reviewed. Breast cancers in which tumor diameter less than 1 cm and who were diagnosed with tru-cut biopsy were excluded. Also, breast cancers diagnosed with excisional biopsy were excluded. Tumors smaller than 1 cm in diameter were excluded from the study to provide sufficient tissue for pathological examination. The reason we excluded patients with excisional biopsy was the absence of cancer diagnosis during the operation. Fresh tumor tissue samples were collected from 54 breast cancer patients with these criteria.

Of the remaining 72 breast mass patients, 35 were not included in the study because they had infectious breast disease or cysts. All of 37 benign breast lesions were fibroadenomas which diagnosed with tru-cut biopsy or clinicoradiologically. Fresh benign tissue samples were taken from 20 of 37 patients. During the operation, fresh normal breast tissue samples were taken from all patients with breast cancer away from the tumor.

Tissue samples

Fresh tissue samples were taken by the pathology department within 1 hour after the operation is completed. All fresh tissue samples are stored at -80 ^{o}C and were used for RT-PCR. Paraffin-embedded blocks of the same patients were used for IHC.

PRAME RT-PCR method

RNA Isolation from fresh tissue: Samples isolated from tissue samples by using High Pure RNA Isolation Kit (Roche Applied Science).

cDNA Reaction**:** Transcriptor First Strand cDNA kit was used. cDNA reaction condition was 10 minutes at 25 ^{o}C, 60 minutes at 50 ^{o}C, 5 minutes at 85 ^{o}C.

Real-Time PCR Analysis: cDNA samples analyzed by using Light Cycler FastStart DNA Master Sybergreen kit Roche: 3003230 on a LightCycler 480 (Roche Applied Science) system.

PCR Primers for PRAME was Forward prime; AF, 50-CCA TGA CAA AGA AGC GAA AA-30.

Reverse primary: AR, 50-CAT CTG GCC CAG GTA AGG AG-30.

The standard group was prepared together with the sample to calculate at the end of the quantitative values to the PRAME samples according to the lowest and the highest standards. The amount of PRAME was calculated quantitatively.

PRAME IHC method

For the IHC; 0.5 mm thick tissue sections from paraffin-embedded breast tumor samples were used. After deparaffinization, the sections were heated in a 600 w household microwave oven for 50 minutes in EDTA buffer (PH: 8) and washed with phosphate-buffered saline (PBS; PH 7.2). After an additional PBS wash, the sections were incubated for 20 minutes with 1:10 diluted normal rabbit sera (DAKOX902) at room temperature in a humidified chamber to prevent non-specific immunoglobulin binding. The sections were treated with primary antibody (Rabbit polyclonal Ab to PRAME cat no: 32185) for 3 hours at room temperature. A streptavidin-biotinylated horseradish peroxidase-based detection system (DAKO K 0690) was used to reveal specific binding. Testicular tissue with intact spermatogenesis was used as positive control. Non-neoplastic ductal epithelial cells were indeed present in all specimens, as internal controls. The staining of invasive, in-situ component, and normal breast tissue were evaluated separately. IHC staining of PRAME was visible as cytoplasmic/nuclear staining limited to tumor cells. Immunoreactivity of tumor cells was graded as follows: 0 (no positive tumor cells), + (<25% positive tumor cells), ++ (25-50% positive tumor cells), and +++ (>50% positive tumor cells).

Statistical analysis

Categorical measurements were summarized as numbers and percentages and continuous measurements were summarized as mean and standard deviation (median, and minimum-maximum when necessary). Student-t test or one-way ANOVA were used to compare PRAME expression results with age, tumor diameter, the number of reactionary lymph node, the number of metastatic lymph node between the groups. Chi-square test or Fisher's Exact test were used to compare PRAME expression results with menopausal status, tumor grade, tumor stage, LVI, ER status, PR status, and Cerb-B2 receptor status. Correlation test was used to compare the results of PRAME expression with RT-PCR and IHC methods. In all tests, the statistical significance level was taken as ≤0,05.

SPSS 17.0 packet program was used in the statistical analysis of the data.

RESULTS

The age range of 54 patients with breast cancer was between 30 and 69; 24 of the patients were in the premenopausal period and 30 of them were in the postmenopausal period. The location of tumors was on the right breast in 24 patients and on the left breast in 30 patients. Histological subtype was invasive ductal carcinoma in 49 patients, invasive lobular carcinoma in 3 patients, medullary carcinoma in 1 patient, and ductal in-situ carcinoma in 1 patient. Tumors were uni-focal in 48 patients and multi-focal in 6 patients. The family history was positive in 6 patients. Tumor diameter was Tis in 1 patient, T1 in 27 patients, T2 in 25 patients, and T3 in 1 patient. According to the breast cancer classification system (AJCC 2003), 1 patient was classified as stage 0, 10 patients as stage 1, 24 patients as stage 2, 17 patients as stage 3 and 2 patients as stage 4. As an operation method, modified radical mastectomy was performed to 44 patients, breast-conserving surgery and axillary dissection to 6 patients, breast-conserving surgery and sentinel lymph node biopsy to 2 patients, toilet mastectomy to 1 patient, simple mastectomy, sentinel lymph node biopsy and tissue expander to 1 patient. The tumor was grade 2 in 31 patients and grade 3 in 23 patients, histologically. Lymphovascular invasion (LVI) was found in 32 patients. Estrogen receptor (ER) was positive in 36 patients, progesterone receptor (PR) was positive in 31 patients, cerb-B2 receptor was positive in 28 patients. The maximum number of the reactionary lymph node which was removed was 48 and the maximum number of the metastatic lymph node was 44.

Table 1. PRAME results by RT-PCR according to lesion type

PRAME results by RT-PCR	**Lesion n(%)**	
	Malign	Benign
0 - 2 μ/L	27 (50.0)	18(90.0)
2 – 100 μ/L	11(20.4)	2(10.0)
100 – 1000 μ/L	9(16.6)	0(-)
>1000 μ/L	7(13.0)	0(-)

Table 2. PRAME expression in breast cancer and benign breast lesions by IHC method

PRAME expression	**Lesion Subtype n(%)**		
	Invasive Component	In Situ Component	Benign Breast Lesions
No staining	19 (35.2)	29 (53.7)	5 (13.0)
(+) staining	16 (29.6)	11 (20.4)	3 (8.0)
(++) staining	17 (31.5)	14 (25.9)	19(51.0)
(++++) staining	2 (3.7)	0 (0)	10 (27.1)

Table 3. Important findings of the study

PRAME expression by IHC in invasive breast cancer									
		(-)		(+)		(++)		(+++)		
		n	%	n	%	n	%	n	%	P
Grade	2	15	78.9	10	62.5	6	35,3	0	0	0.020*
	3	4	21.1	6	37.5	11	64,7	2	100	
PRAME (RT-PCR)	(-) < 2 μ/L	13	68.4	4	25	8	47,1	2	100	0.035**
	(+) >2 μ/L	6	31.6	12	75	9	52,9	0	0	
		(-) and (+)				(++) and (+++)				
		n		%		n		%		P
Menopausal status	Premenopausal	19		54.3		5		26.3		0.044*
	Postmenopausal	16		45.7		14		73.7		
Grade	2	25		71.4		6		31.6		0.005*
	3	10		28.6		13		68.4		
ER status	(-)	8		22.9		10		52.6		0.029*
	(+)	27		77.1		9		47.4		

*Chi square test was used. ** Correlation test was used. P ≤0.05 is statistically significant.

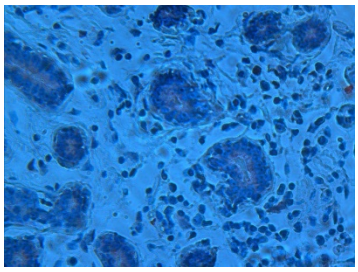

Figure 1. PRAME painting (+) in normal breast tissue (IHCx40)

PRAME expression by IHC

PRAME expression by IHC was evaluated both in invasive and in in-situ component by using IHC method in 54 patients with breast cancer. PRAME staining in invasive component was not detected in 19 patients (35,2 %). There was (+) staining in 16 patients (29,6%), (++) staining in 17 patients (31,5%) and (+++) staining in only 2 patients (3,7%). PRAME staining in "in situ" component was not found in 29 patients (53,7%), (+) and (++) staining were detected in 11 (20,4%) and 14 (25,9%) patients, respectively. There was no (+++) staining in in-situ component. PRAME staining in benign breast lesions was found (+++) in 10 (27, 07%), (++) in 19 (51,03%) and (+) in 3 (8%) patients and there was no PRAME staining in 5 (13%) benign lesions (Figures 1-3) (Table 2)

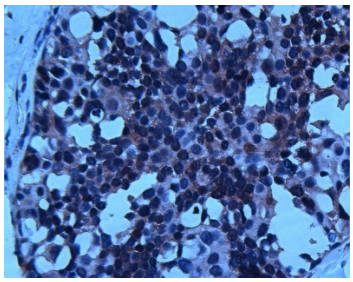

Figure 2. PRAME staining in invasive ductal carcinoma Cytoplasmic ++, nuclear ++) (IHCx40

The association between PRAME in invasive component and clinical variables

PRAME IHC staining was classified 2 groups; (-) and (+) staining patients called as group I (35 cases: PRAME (-) group) and (++) or (+++) staining as group II (19 cases: PRAME (+) group) was accepted. There was an important association between PRAME expression and high tumor grade (p=0.005), negative ER status (p=0.029), postmenopausal status (p=0.044).

There was no association between PRAME IHC and age, tumor diameter, the number of reactionary lymph node, the number of metastatic lymph node, menopausal status, LVI, ER status, PR status, Cerb-B2 receptor expression, and tumor stage. There was no association between IHC PRAME expression in in-situ component and age, tumor diameter, the number of reactionary lymph node, the number of metastatic lymph node, menopausal status, LVI, ER status, PR status, Cerb-B2 receptor status, and tumor stage.

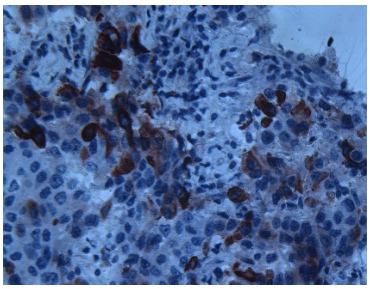

Figure 3. PRAME staining (+++) of metastatic lymph node in invasive ductal carcinoma (IHCx40)

There was an important association between PRAME IHC and PRAME RT-PCR (P=0.035) and PRAME RT-PCR and high tumor grade (P=0.020). There was no important association between PRAME RT-PCR and age, tumor diameter, the number of reactionary lymph node, the number of metastatic lymph node, menopausal status, tumor grade, tumor stage, LVI, ER status, PR status, and Cerb-B2 receptor status. In the invasive component, there was an important association between PRAME IHC and high tumor grade (P=0.005) (Table 3).

DISCUSSION

Female breast cancer is the most common cancer, represents 15.3% of all new cancer cases and 7 % of all cancer deaths in the United States[17,18]. The diagnosis of breast cancer brings many questions to mind. What kind of surgery should be chosen for the local control? Which patients are candidates to receive adjuvant radiotherapy and/or systemic therapy? As other malignant tumors, biology of the breast cancer is one of the most important factor in the outcome of the patients. Due to the very high incidence of breast cancer among the woman cancers, tumor biology has been investigated very well and there are many factors predicting response

to therapy such as ER, PR, cerb-B2 and Ki67 expression and prognostic factors such as histological type, age, menopausal status, axillary lymph node involvement, metastasis, tumor diameter, tumor grade, stage, LVI [19]. However, there are still a lot of unknown factors in breast cancer and most of the cases die due to their disease progression. For these reasons, we need additional factors predicting the biology of the disease.

Cancer testis antigens are one of the most attractive areas in tumor immunology. PRAME is an important member of cancer testis antigens. It encodes a protein consisting of 509 amino acids and localized in the 22nd chromosome (22q11.22). Its function is not known exactly. Very low expression, except testis, has been reported in some normal tissues including endometrium, adrenal glands, and in the brain. It has been found to be expressed in 97% of malignant melanomas, 93% of neuroblastomas, 80% of sarcomas, 70% of lung cancers and in variable rates in Wilms' tumor, and acute leukemia's (AML M3 75%, ALL 64%, KML BK 50%, AML M2 45%)[1,3,4,6,7,11,12,20]. PRAME has been studied in a relatively limited number of the series in breast cancer and has been shown as a negative prognostic factor[13,14]. We studied PRAME mRNA in 54 cases with invasive breast cancer, in 20 cases with benign breast lesions and in 10 normal breast tissues with RT-PCR. In addition, we studied PRAME by IHC in 54 patients with invasive breast cancer and 37 benign breast lesions. Our aim was to find the prognostic significance of PRAME in breast cancer and also compare these two techniques. So, if there is an association between RT PCR and IHC, we planned to show the probability of the detection of PRAME expression for the prognostic and/or predictive value of PRAME expression using IHC in paraffin-embedded tissue sections of the patients followed for a long time period.

PRAME mRNA has been studied by Epping and Doolan[13,14]. High PRAME expression in one third (98/295) and low PRAME expression in two thirds of the patients (197/295) have been found by Epping et al. PRAME expression has been found in 53 % of the cases with breast cancer and 37% of the normal breast tissue by Doolan et al [13.14]. The prognostic and predictive value of the PRAME expression in breast cancer has been looked for by both authors. Doolan et al. did not find statistically significant association between PRAME expression and clinicopathologic factors including age, tumor diameter, axillary metastases, tumor grade, histological type, and ER status in breast cancer. But they showed an important association between PRAME expression and shorter disease-free survival and overall survival and shorter time for disease recurrence [13]. Epping et al. found statistically significant association between high PRAME expression and high tumor grade, negative ER status, and poorly differentiated tumors. Epping et al. also found an association between high PRAME expression and shorter disease-free survival and overall survival[14]. These studies may suggest that PRAME is a negative prognostic indicator in breast cancer independent from other clinical and pathological factors. On the other hand, these two authors analyzed the association between PRAME expression and adjuvant chemotherapy. Epping et al. found earlier recurrence and decreased overall survival cases with high PRAME expression and not receiving adjuvant chemotherapy as compared with treated by adjuvant treatment. With these results, they suggested that PRAME expression is an independent predictive factor to determine the subgroup of the cases with breast cancer that will benefit from adjuvant systemic chemotherapy. However, Doolan et al. did not find a correlation with PRAME expression and requirement of adjuvant treatment[14]. Al-Khadairi et al. demonstrated that PRAME facilitates the transition to a mesenchymal phenotype through the reprogramming of several epithelial to mesenchymal transition genes, resulting in enhanced migration and invasion of triple negative breast cancer cells. Moreover, increased PRAME expression was correlated with a worse survival, further supporting its clinical value as a prognostic biomarker and/or therapeutic target in cancer[21]. In our study, we found PRAME expression in 27 cases with breast cancer (50%), in 5 cases with benign breast lesions (25%) and did not find PRAME expression in normal breast tissue. Our results were compatible with reported PRAME expression ratios. By using IHC, we found PRAME expression in 35 cases with invasive breast cancer and 25 cases with in-situ cancer. There was no staining in 19 cases with invasive cancer and 29 cases with in-situ cancer. We found a highly significant association for PRAME expression detected by IHC and RT-PCR (P=0.035). This is very important because this result suggests that paraffinized tissue samples may be used to detect the PRAME expression, so it is possible to determine the prognostic and/or predictive value of the PRAME expression in cases followed for a long time with various treatment strategies. We could not

compare these IHC results with literature due to the lack of report about PRAME expression detected by IHC in breast cancer.

We did not find an association between PRAME expression and all known prognostic parameters. We can define these results with 3 ways: 1- Our study group includes a limited number of the patients. It is difficult to find a huge number of the cases in a limited time period and it is difficult to give informed consent from all the patients, 2- Although Doolan et al. and Epping et al. studied relatively large number of the cases, they did not find an important association between PRAME and well known prognostic indicators. In fact, other studies evaluating PRAME expression in other cancers have highly variable results. This may be due to the limited information about PRAME function and/or due to the possibility of its independent risk factor. 3- The studies evaluating PRAME in breast cancer are very limited and are not informative for the decision about the prognostic and/or predictive value of PRAME in breast cancer. It is very well known that breast cancer is very heterogeneous disease and all the prognostic/predictive factors determined in very large study groups have not been confirmed by all studies and authors. With limited study results, we cannot argue the independent prognostic/predictive value of PRAME in breast cancer but we need prospective and retrospective evaluations using different methods for PRAME to determine its biologic significance in breast cancer. The other important property of PRAME is the immunogenicity and the possibility of using anti-PRAME technologies in PRAME expressers. Cancer testis antigens of NY-ESO-1, PRAME and WT-1 antigen expressions were studied in breast cancer samples by IHC. A significantly higher expression of NY-ESO-1 and WT-1 antigen was detected in triple negative breast cancers compared with ER positive tumors. PRAME over-expression was detected 16% of HER2 positive tumor samples as compared to no triple negative and ER positive cancers. Limited therapeutic options for triple negative breast cancer, cancer testis antigen-based vaccines or immunotherapic agents might be useful for patients with this phenotype of breast cancer [15].

The limitations of our study are as follows: Firstly, this study contains low case volume. Secondly, this prospective study was completed in one-year and does not reflect long-term oncological outcomes associated with PRAME gene.

In conclusion, PRAME was studied using two different methods, IHC and RT-PCR, so it becomes distinct from the existing studies until 2010 which could not allow the comparative evaluation for PRAME expression with fresh samples and paraffinized samples. We found a statistically significant relationship between PRAME results that were determined by IHC. The most important point of our study is giving the chance to compare the RT-PCR and IHC methods for the detection of PRAME expression. So it is possible to determine the IHC method in the detection of PRAME in archived tissues taken from patients with breast cancer and treated with or without adjuvant chemotherapy. Of course, it is necessary to confirm this finding with further studies carried out with larger patient populations.

Yazar Katkıları: Çalışma konsepti/Tasarımı: KD, YK, SP, SZ, ME, KT, GS, GU; Veri toplama: KD, YK, SP, SZ, ME, KT, GS, GU; Veri analizi ve yorumlama: KD, YK, SP, SZ, ME, KT, GS, GU; Yazı taslağı: KD, YK, SP, SZ, ME, KT, GS, GU; İçeriğin eleştirel incelenmesi: KD, YK, SP, SZ, ME, KT, GS, GU; Son onay ve sorumluluk: KD, YK, SP, SZ, ME, KT, GS, GU; Teknik ve malzeme desteği:KD, YK, SP; Süpervizyon: KD, YK, SP, SZ; Fon sağlama (mevcut ise): yok.
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