Comparison of the clinical and pathological features between patients with recurrent metastatic breast carcinoma and patients with initially metastatic breast carcinoma

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ABSTRACT

الأهداف: نظراً لكون سرطان الثدي المنتشر الأولي أقل انتشارا من سرطان الثدي المنتشر المعاود، تم الإبلاغ عن بيانات قليلة تقود الى الملامح الحيوية والسريرية لسرطان الثدي المنتشر الأولي. في الدراسة الحالية تم مقارنة الانتشار الأولي لسرطان الثدي مع الانتشار المتكرر لسرطان الثدي وتقييم أنماطهم الظاهرية وسلوكهم السريري.

الطريقة: تم تقييم مقارنة الخواص السريرية والحيوية ومتوسط وقت البقاء على قيد الحياة الكلي في ٢٥١ مريضة مصابة بسرطان الثدي المنتشر بجامعة أيج الطبية، شعبة الأورام ومستشفى تيبيسك الحكومي، في الفترة ما بين ١٩٩٥م و ٢٠٠٤م. أجريت طريقة إظهار مستقبلات الهرمون، سي–إي أر بي بي–٢، وكي أي–٦٧، وتعابير بي ٥٣ بواسطة الكيمياء النسيجية المناعية.

النتائج: تعاني٢٠٦ مريضة من تكرار الإصابة بسرطان الثدي المنتشر من بين ٢٥١ مريضة تعاني من سرطان الثدي، ٤٥ مريضة مصابة بسرطان الثدي المنتشر الأولي. وفقاً إلى النجاة لم يكن هنالك فرقاً بين المجموعة المصابة بتكرار الإصابة بسرطان الثدي المنتشر والمجموعة المصابة بسرطان الثدي المنتشر الأولي. كان لدى المجموعة المصابة بسرطان الثدي المنتشر الأولي أورام تي ٤ أعلى تناسباً (٤٦٪ مقابل ٢٧٪) وأورام تي١–٢ أقل تناسباً (٣١٪ مقابل ٥٥٪، نسبة الخطأ= ٠١,٠). نسبة أعلى من المريضات اللواتي لديهن ظهور عالي لهرمون ني أي–٦٧ (٦٤٪ مقابل٤٩٪ : نسبة الخطأ= ٠٥,٠). أظهر التحليل المتغير المتعدد أن مرحلة تي كانت عامل تكهني مستقل (نسبة الخطأ= ٠٢,٠).

خاتمة: تميل المريضات المصابات بسرطان الثدي المنتشر الأولي إلى الحضور مع أورام أكبر حجماً. يمكن شرح العلاقة بواسطة التشخيص المفصل. إن احتمالية تخفيض الوفاة من سرطان الثدي ممكنة بالتطور التعليمي ومعدلات الكشف المستمر.

Objective: To compare initial metastatic breast carcinoma (MBC) with recurrent MBC and assess their biologic phenotypes and clinical behaviors.

Methods: A comparison of clinical and biological characteristics and median overall survival times were assessed in the 251 patients with MBC at the Division of Medical Oncology, Ege University School of Medicine, and the Division of Radiation Oncology, Tepecik Government Hospital, Izmir, Turkey between 1995 and 2004. Hormone receptors, c-erbB-2, Ki-67, and p53 expressions were performed by immunohistochemistry.

Results: Out of 251 MBC patients, 206 patients had recurrent MBC, and 45 had initial MBC. Regarding survival, there was no difference between the recurrent MBC group and the initial MBC group. The initial MBC group had a higher proportion of T4 tumors (46% versus 27%), a lower proportion of T1-2 tumors (31% versus 55%; p=0.01), and a higher percentage of patients with high Ki-67 expression (64% versus 49%; p=0.05). Multivariate analysis showed that T stage was an independent prognostic factor (p=0.02).

Conclusion: Patients with initial MBC tended to present with larger tumors. This relationship can be explained by delayed diagnosis. The potential for reducing death rates from breast cancer is contingent on educational improvement and increased screening rates.

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Breast carcinoma is one of the main cause of carcinoma death for women in the Western World.[1] The American Cancer Society (ACS) estimates that by the end of 2007, approximately 178,480 new cases will have been

diagnosed, and 40,460 people will have died from breast carcinoma.[2] Although a larger proportion of patients are being diagnosed with breast carcinoma when treatment with curative aim is possible, 24-30% of patients with node-negative disease, and at least 50-60% of those with node-positive disease at diagnosis will be recurrent metastatic breast carcinoma (RMBC).[3] Additionally, approximately 6-10% of patients are supposed to present with initial metastatic breast carcinoma (IMBC).[3,4] The main goal of therapy in patients with metastatic breast carcinoma (MBC) includes improving quality of life and survival times. However, the clinical course of these patients varies usually; some patients die shortly after they develop metastatic disease, whereas others have long survival. The reason for the difference in survival could be explained by the difference in clinical and biological characteristics of the patients. The prognostic factors in patients with early-stage breast carcinoma have been clearly documented. However, there have been only few trials on prognostic factors in patients with metastatic disease. Biologic markers of the primary breast tumor, such as estrogen receptor (ER) status,[5,6] progesterone receptor (PgR) status,[7] S-phase fraction,[7] Bcl-2 status,[7] and clinic factors such as prior disease free interval,[7,8] sites of involvement,[8,9] and performance status,[9,10] can predict survival after patients develop metastatic disease. To date, there has been only one study comparing clinical characteristics and therapeutic outcomes of RMBC and IMBC,[11] however, there has been no study comparing primary tumor characteristics in these patient groups. In this study, we compared the clinical and biological characteristics, and overall survival of patients with IMBC and patients with RMBC.

Methods. ***Patient eligibility***. Patients with MBC were selected from the clinic database. It included 271 patients from the Division of Medical Oncology, Ege University School of Medicine, and Division of Radiation Oncology, Tepecik Government Hospital, between 1995 and 2004. This study was performed as a retrospective analysis based on the patients files. Subjects with insufficient data or lacking follow-up (n=20) were excluded, and 251 patients with MBC were enrolled into this retrospective analysis. Patients were considered to have RMBC if metastases had developed during follow-up after curative therapy for localized breast carcinoma. Patients were considered to have IMBC in case of presence of metastatic disease at the diagnosis of breast carcinoma. The clinical files of these 251 patients were reviewed in March 2005 in order to determine the differences in patients and tumor characteristics between the 2 groups. Median overall survival (OS) time was defined as the time from diagnosis of metastatic disease to death or last contact. All consecutive patients with the diagnosis of IMBC and RMBC as described previously were enrolled into study irrespective of their treatment protocols. Pre-treatment tumor specimens had been obtained from patients by surgical biopsy. Tumor size was obtained at the time of initial diagnosis of breast carcinoma. Recurrences or metastases were defined as visceral (lung, liver, and other organs) or nonvisceral (lymph node, bone, and cutaneous involvement). Physical examination and radiologic imaging documented the site of recurrences. Axillary node status in 24/45 of IMBC patients was not known since they did not have any axillary lymph node dissection, and because of this, it was not included in the comparative assessment. The examined biological and clinical variables included patient's age at the time of diagnosis of MBC (<50 versus ≥50), performance status (0-1 versus 2-3), histological type, tumor size (≤T2, T3, versus T4), tumor grade (≤2 versus 3), ER and/or PgR status (positive versus negative), c-erbB-2 expression (positive versus negative), Ki-67 expression (high versus low), p53 expression (high versus low), and dominant site of metastasis (visceral versus nonvisceral). This study was approved by the Local Ethics Committee.

Immunohistochemical studies. All tissues were fixed in 4% buffered formalin, processed, and embedded in paraffin. From each block, 5 μm thick sections were cut on coated slides and dried overnight at 37°C. The sections were deparaffinized in xylene, dehydrated through graded concentrations of ethanol to distilled water, and boiled in citrate buffer (pH=6.0) in a microwave oven for 20 minutes. Immunohistochemical staining was performed using a commercial ABC Kit (Lab Vision; Ultra vision Large Volume Detection System Anti-Mouse, HRP, CA) directed against mouse IgG. Blocking serum was applied for 15 minutes followed by overnight incubation with the diluted monoclonal primary antibody c-erbB-2 (Dako, code-no: A0485, Denmark), ER (Dako, IDS code-no: M: 7047, Denmark), PgR (Dako, PgR 636 code-no: M3569, CA), Ki-67 (Dako, code-no: A0047, Denmark), and p53 (Dako, clone D0-7, code-no: M7001, Denmark). The sections were then incubated with the biotinylated second antibody and the peroxidase-labeled ABC for 30 minutes each. All dilutions were made in phosphate buffer solution (PBS) (pH=7.2), and all incubations were performed in humid chambers at room temperature. Between each step in the staining procedures (except before incubation with the antibody), the slides were rinsed 3 times in PBS-bound peroxidase and then were visualized all slides with a 3-amino-9-ethylcarbazole solution (Sigma: 0.2 mg/ml in 0.05 M acetate buffer containing 0.03% perhydrol, pH=5.0) at room temperature for 15 minutes. Finally, the sections were lightly counterstained in Mayer's hematoxylin and mounted in Surgipath Micromount (Surgipath Medical Industries, Grayslake, IL). Cells

were considered positive for p53, Ki-67, and HR when distinct nuclear staining was identified. For depiction of the material and phenotype analysis, a 10% cut off point for low and high expression for HR, p53,[12] and Ki-67,[13] was chosen. Tumor specimens were considered HR negative for ER and PgR if staining for both receptors was negative; specimens were considered HR positive for ER/PgR if staining for either or both receptors was positive. The membrane staining for c-erbB-2 was graded from no staining (0) to weak staining (1+), moderate (2+), and intense membrane staining (3+), in the presenceof more than >10% of tumor cells in the tissue section.[14] Score of 3+ was used as the cut off point for analysis.

Table 1 - Comparison of clinical and biological characteristics between patients with RBMC and patients with IMBC.

Variables	RMBC	IMBC	*P*-value
	n (%)		
Age			
<50	110/206 (53)	26/45 (58)	0.3
≥50	96/206 (47)	19/45 (42)	
Performance status			0.4
0-1	189/204 (93)	40/44 (91)	
2-3	15/204 (7)	4/44 (9)	
Histologic type			
Ductal	150/204 (74)	35/45 (78)	0.1
Lobular	20/204 (10)	3/45 (7)	
Ductal/lobular	25/204 (12)	2/45 (4)	
Other	9/204 (4)	5/45 (11)	
T stage			
≤T2	105/190 (55)	12/39 (31)	0.01
T3	34/190 (18)	9/39 (23)	
T4	51/190 (27)	18/39 (46)	
Tumor grade			
≤2	65/180 (36)	13/40 (32)	0.4
3	115/180 (64)	27/40 (68)	
Hormone receptor status	113/204 (55)	22/44 (50)	0.3
Positive	91/204 (45)	22/44 (50)	
Negative			
C-erbB-2 expression			
Positive (3+)	75/204 (37)	13/44 (29)	0.2
Negative (0/1+/2+)	129/204 (63)	31/44 (71)	
Ki-67 expression			
High (≥10%)	89/181 (49)	28/44 (64)	0.05
Low (<10%)	92/181 (51)	16/44 (36)	
Ki-67 expression			
High (≥10%)	89/181 (49)	28/44 (64)	0.05
Low (<10%)	92/181 (51)	16/44 (36)	
p53 expression			
High (≥10%)	59/175 (34)	13/43 (30)	0.4
Low (<10%)	116/175 (66)	30/43 (70)	
Dominant site metastatic disease	132/206 (64)	26/45 (58)	0.2
Viscera	74/206 (36)	19/45 (42)	
Nonviscera			

RMBC - recurrent metastatic breast carcinoma,
IMBC - initially metastatic breast carcinoma.

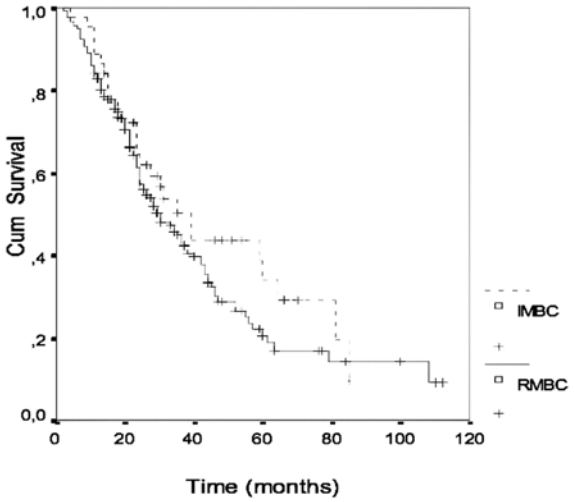

Figure 1 - Overall survival of patients with recurrent metastatic breast carcinoma (RMBC, lower curve) and initially metastatic breast carcinoma (IMBC, upper curve) were 30 and 39 months (p=0.2).

Statistical analysis. Data analysis was performed using SPSS statistical package (Version 11.5; SPSS Inc., Chicago, IL). Analysis of overall survival was performed by Kaplan-Meier survival curves and comparing subsets of patients using log-rank tests. Chi-square test was used to assess measures of association in frequency tables. Only those variables that achieved statistical significance (p≤0.05) in the univariate approach were subsequently evaluated in the multivariate analysis using a logistic regression analysis. Differences were considered statistically significant when p ≤0.05.

Results. Two hundred and fifty-one patients with MBC were included in this retrospective study. Two hundred six patients with MBC had RMBC, and 45 patients had IMBC. The characteristics of these 2 groups were summarized in **Table 1**. The median follow-up was 24 months (95% confidence interval [CI] 21.5-26.5 months). There was no difference between the RMBC group and IMBC group regarding survival. The median OS of patients with RMBC was 30 months (95% CI 23-37 months), and for patients with IMBC was 39 months (95% CI 27.6-50.4 months), (p=0.2, **Figure 1**). The median age at the time of metastatic disease of patients with RMBC was 49 (range 24-82), and 50 (range 31-75) in patients with IMBC. The IMBC group had a higher proportion of T4 tumors, a lower proportion of T1-2 tumors, and a higher percentage of patients with high Ki-67 expression (**Table 1**). There was no difference between the 2 groups in terms of patient

age distribution, performance status, histological characteristics, tumor grade, HR status, c-erbB-2 expression, p53 expression, and presence of visceral metastases. Logistic regression analysis was used to examine the independent prognostic value of significant variables in univariate analysis. Multivariate analysis showed that T stage was an independent prognostic factor (odds ratio: 0.38; CI 95%: 0.17-0.85; p=0.02).

Discussion. In our study, the rate of IMBC to MBC is 18%. Several recent studies have reported this rate between 6-10%.[3,4] However, in another study comparing similar patients, this rate was reported as 32%, which is higher than others.[11] The reason for this difference could be explained by the definition of IMBC as the inclusion criteria. While in our study, patients were considered to have IMBC if there were metastatic disease at presentation of breast carcinoma, in another study,[11] all patients in whom metastases was detected within the first 3 months after surgery were classified as IMBC. In the same study, median ages of IMBC patients were higher compared to RMBC patients. However, in our study the age distribution was similar in both patient groups. The median OS rates were similar in our groups as reported in the previous study.[11] In the present study, comparison of the clinical and biological characteristics of patients with IMBC and patients with RMBC revealed some differences between the 2 groups. Patients with IMBC have tended to present with T4 tumors and high Ki-67 expression. A higher rate of T4 and lower rate of T1-2 tumors in IMBC compared to RMBC patients have been concluded as important findings. In a recent study by Jimeno et al,[11] T1-2 versus T3-4 tumors were evaluated and it was found that the T3-4 tumor rate was higher in IMBC patients. However, in our study, while T3 tumor rates were similar in both groups, there was a significant difference in T1-2 and T4 tumor rates. In IMBC patients, 17 T4 tumors out of 18 have inflammatory breast carcinoma, which signifies that this is a special group. A higher frequency of the most aggressive form of the disease found in this patient group, and can explain the higher rate of metastasis.[15] Delay in diagnosis can also be the cause of metastasis at the time of diagnosis. For many years, it has been known that larger lesions are more likely than smaller lesions to be associated with metastatic disease.[16]

Stage at diagnosis is one of the most important prognostic factors for most cancers. For many cancers, early stage disease can be effectively treated with a good chance for cure, whereas late stage disease is generally incurable. Stage at diagnosis is associated with a number of factors, including race and ethnicity,[17] socio-economic status,[18,19] age,[20] and marital status.[21] With regard to mortality and survival, socio-economic factors are related to breast carcinoma survival and mortality rates. Lower socio-economic status is related to poorer health outcomes, including higher breast carcinoma mortality, higher stage at diagnosis, and poorer breast carcinoma survival.[18,19] The reasons these groups have less favorable cancer outcomes are not certain, but has been attributed to lower rates of screening.[22-25] Additionally, persons lacking health insurance are more likely diagnosed with late stage cancer.[26] The efforts to improve access to cancer-screening programs are warranted for these groups. Screening mammography, breast self-examination, and physician examination have resulted in more frequent detection of early-stage breast carcinoma. These data provide additional evidence that women of low socio-economic status, or lacking health insurance could benefit from targeted screening. In our study, high Ki-67 expression was significantly higher in the IMBC group. The monoclonal antibody Ki-67 reacts with a nuclear antigen that is present throughout the cell cycle of proliferating cells.[27] Direct correlation between Ki-67 staining and adverse prognostic factors,[28-31] or inverse correlations with hormone receptors status,[31] have been reported in patients with early-stage breast carcinoma. Additionally, Ki-67 seems to be an effective indicator of prognosis for disease-free survival and overall survival.[32-34] Increased Ki-67 expression has been reported in lung carcinoma metastatic to brain and in breast carcinoma metastatic to lymph node and was an indicator of aggressive biological features of tumor.[35,36] Additionally, an inverse correlation was observed between E-cadherin expression on primary breast tumors and MIB-1 expression, which is another proliferation index.[37] The cadherins are an invasion suppressor system in tumor cells,[38] and E-cadherin is the most important cell-cell adhesion molecule in epithelial cells.[39] It is thought that in MIB-1 immunostaining tumors, loss of E-cadherin expression could trigger metastasis. Another study has documented the relationship between Ki-67 expression and vascular endothelial growth factor (VEGF) expression in breast carcinoma cells.[40] The prognostic importance and role of VEGF in metastasis has been shown in recent studies.[41] These findings could remind the potential predictive role of high Ki-67 expression for the aggressiveness of primary tumor. However, further studies are still needed to clarify this finding, as our study had a relatively low number of patients, borderline p-value, which lost its importance in multivariate analysis in our study. Although our study has provided some important points related to the differential aspects of IMBC and RMBC, the designation of our study as retrospective and the potential effects of treatment modalities could limit the wide range application of our results and we encourage prospective large randomized studies.

As a result of our study, IMBC patients differ from RMBC in terms of T stage and Ki-67 expression. Higher rates of larger primary tumor or high Ki-67 expression may have a role in metastasis at time of diagnosis. Delaying the diagnosis has been found to be among the most important factor in the development of IMBC. Therefore, effective screening programs would provide the early diagnosis of breast cancers and the increase in the potential cure chance.

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