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CLINICAL STUDY

Clinicopathologic characteristics and BRCA-1/BRCA-2 mutations of Turkish patients with breast cancer

Aydin F[1], Akagun T[2], Yildiz B[1], Fidan E[1], Ozdemir F[1], Kavgaci H[1]

Department of Medical Oncology, School of Medicine, Karadeniz Technical University, Trabzon, Turkey.
drbulentyildiz@hotmail.com

Abstract: *Background:* BRCA-1 and BRCA-2 are two major susceptibility genes for breast cancer. Between 30–70 % of hereditary breast cancers are thought to be attributed to mutations in BRCA-1 or BRCA-2. The aim of our study was to investigate the clinicopathologic characteristics and BRCA mutations of patients with breast cancer living in the northeast region of Turkey.
Materials and methods: Two hundred eleven patients diagnosed with breast cancer were enrolled in the present study. All patients provided their demographic, environmental, family, and reproductive traits. Patients were screened for mutations of BRCA-1 and BRCA-2 with multiple ligation-dependent probe amplification (MLPA) test.
Results: In the present study, the mean age of breast cancer patients was 50.7 years and the mean age at first delivery was 21.8 years. Large genomic rearrangements in BRCA-1 were detected in 4 (1.9 %) patients; 2 of them had exon 21 deletions and the remaining 2 patients had exon 18 and 19 deletions. No BRCA-2 rearrangements were detected in the study population.
Conclusions: Different mutation analyses results were detected in different countries by using different testing techniques. The characteristics and sensitivities of patients as determined by all methods were different in these studies. Due to these factors, BRCA mutation rates were different. This is the first study where MLPA was used to screen BRCA-1 and BRCA-2 mutations in Turkish population *(Tab. 1, Ref. 10)*. Full Text in free PDF *www.bmj.sk.*
Key words: BRCA-1, BRCA-2, breast cancer.

Breast cancer is the most frequently encountered cancer among females and accounts for approximately 26% of newly diagnosed cancers. In the United States, 184,450 new cases occur per year. Among females, deaths from breast cancer were the second most frequent cause after lung cancer, in 2008 (1). Many factors, such as environmental and hormonal factors, and genetic susceptibility play a role in the etiology of breast cancer. BRCA-1 and BRCA-2 are the two major genes responsible for genetic susceptibility. BRCA-1 and BRCA-2 account for 30 % to 70 % of hereditary breast cancers. Mutations in the BRCA-1 and BRCA-2 genes lead to susceptibility against breast and ovarian cancers. When compared to the normal population, the lifetime risk of breast cancer for females with a BRCA mutation is 87%. Many studies have been carried out concerning factors that play a role in sporadic breast cancer, such as diet and physical activity. The effect of other risk factors in subjects with positive BRCA mutations is not completely understood (2). Certain BRCA mutations have been determined in specific ethnic groups (Icelanders, Jews, Russians, and Israelis). The prevalence of BRCA-1 and BRCA-2 mutations is variable among different populations (3). In the present study, the prevalence of BRCA gene mutation positivity was aimed to determine among females with breast cancer in Turkey.

[1]Department of Medical Oncology, School of Medicine, Karadeniz Technical University, Trabzon, Turkey, and [2]Department of Internal Medicine, School of Medicine, Karadeniz Technical University, Trabzon, Turkey

Address for correspondence: B. Yildiz, MD, Karadeniz Technical University Faculty of Medicine, Medical Oncology Department, 61080, Trabzon, Turkey.
Phone: +90.462.3775023, Fax: +90.462.3250518

Materials and methods

Patients

Two hundred eleven patients who had been diagnosed with breast cancer and sought evaluation in the Oncology Outpatient Clinic of Farabi Hospital at Karadeniz Technical University, Faculty of Medicine, Internal Medicine Department between May 2007 and June 2008 were included in the present study. All the clinical characteristics of the patients included in the study were examined. Blood samples were obtained from each patient for the determination of BRCA-1 and BRCA-2 mutations. DNA isolation from the blood samples were performed and stored at -80 °C until the study day. The multiple ligation-dependent probe amplification (MLPA) method was carried out for the analysis of BRCA-1 and BRCA-2 mutations (4, 5).

DNA isolation

Genomic DNA was isolated from leucocytes of affected patients with an Invisorb Spin Blood Mini kit (Invitek, Berlin, Germany) according to the instructions of the manufacturer (6).

MLPA analysis

The MLPA test for BRCA-1 and BRCA-2 ensures a sensitive, comprehensive, and high-throughput screening test for genomic rearrangement. MLPA analysis was performed according to the instructions of the manufacturer using SALSA P002 BRCA-1 and SALSA P045 BRCA-2 test kits (MRC-Holland, Amsterdam, Netherlands). Five mcL of genomic DNA was denatured at 98 şC and hybridized with the BRCA-MLPA probe mix at 60 şC for 16 hours. This was followed by ligation of the annealed probe with Ligase-65 mix at 54 şC for 15 minutes. The appropriate PCR primers, SALSA PCR buffer, and SALSA polymerase were added and the subsequent PCR reactions were carried out with 33 cycles (30 seconds at 95 şC, 30 seconds at 60 şC, 60 seconds at 72 şC), followed by a final extension at 72 şC for 20 minutes. Fragment analysis was carried out on ABI Prism model capillary sequencer (GeneScan, Perkin-Elmer Applied Biosystems, Foster City, CA, USA). Size standard peaks were checked. MLPA data were analyzed by a MRC Coffalyser (MRC-Holland, Amsterdam, Netherlands) analysis sheet, as recommended by the company or research institution (4, 6).

Statistical analysis

Numeric data were presented as numbers and percentages, whereas qualitative data were presented as the mean±standard deviation. Student's t-test was used for the comparison of numerical variables, and a chi-square test was used for the comparison of quantitative data. The significance level was considered as $p < 0.05$.

Results

Clinicopathologic characteristics of the patients

The mean age of the 211 patients included in the study was 50.7±10.7 years. Of the patients, 29 had no history of a completed pregnancy and the mean age at the first delivery of the remaining 182 patients was 21.8±4.2 years. The mean age at menarche of the 211 patients was 14±1.4 years. The mean age at menopause of the 159 patients who were postmenopausal at the time of the interview was 45.5±5.4 years. The mean BMI of the patients, which was calculated at the time of the diagnosis, was 28.2±5.3 kg/m¸.

Of the 182 patients with a history of pregnancy, 27 % had 1–2, 31.2 % had 3–4, and 28.1 % had =5 pregnancies. One hundred eighty one patients had a history of breastfeeding. The number of patients with a history of oral contraceptive pill use and hormone replacement therapy was 46 (21.8 %) and 14 (6.8 %), respectively.

The distribution of the patients according to their hormone receptor and Her-2/neu (c-erbB2) oncogene status

Estrogen receptor (ER), progesterone receptor (PR), and c-erbB2 were positive in 45.2 %, 37.9 %, and 33.3 % of the patients, respectively. When the patients were divided into groups as ER-, PR-, and c-erbB2-negative (triple negative), 26.5 % of the patients were triple negative.

Tab. 1. The results of BRCA-1/BRCA-2 analyses of the patients.

	BRCA-1 (n / %)		BRCA-2 (n / %)	
Positive	4	1.9	0	0
Negative	204	98.1	185	100
Total	208	100.0	185	100

The results of BRCA-1 and BRCA-2 gene mutation analysis

BRCA-1 and BRCA-2 mutation analysis of the patients was performed by the MLPA technique (5). BRCA-1 and BRCA-2 could not be analyzed in 3 and 26 patients, respectively, due to technical problems. The results of BRCA-1 and BRCA-2 analyses of the patients are presented in Table 1.

By using the MLPA method, 24 and 27 exons were screened for BRCA-1 and BRCA-2, respectively. A BRCA-1 mutation was positive in 4 (1.9 %) of 208 patients. There was a deletion in 4 of the patients with positive BRCA-1 mutation. In the 1st patient was noted that the deletion was in the 21st exon of the BRCA-1 gene on the red-marked area. In the 2nd and 3rd patients, a deletion in the 18th and 19th exons and in the 4th patient, a deletion was determined on the 21st exon (Data not shown). No duplication was determined in the patients. Neither a deletion nor a duplication was determined in 185 patients in whom BRCA-2 mutation analyses had been performed.

Discussion

In the previous studies, it was demonstrated that genomic rearrangements account for 40 % of all BRCA-1 mutations. It is known that the majority of the prevalence studies in BRCA gene mutations originated from Europe and the United States (4). The aim of the present study was to evaluate the prevalence of a genomic rearrangement among Turkish patient population and to determine the type as well. MLPA is a sensitive, relatively low-cost, comprehensive, and highly productive method with proven efficacy for the screening of genomic rearrangements (4, 7). In the present study, a genomic rearrangement in the BRCA-1 genes was determined in 4 (1.9 %) patients by using the MLPA method. However, no BRCA-2 gene mutations were shown. A deletion was determined in the 21st exon in 2 patients and in the 18th and 19th exons in the other 2 patients. No duplications were demonstrated. As a result of the analyses that Lim et al (4) conducted on 100 high risk patients (those who had been diagnosed with breast cancer at early ages and those with a family history of ovarian and breast cancers), in whom BRCA mutation analysis had previously been performed by conventional methods and found to be negative; a new BRCA-1 mutation in 2 (2 %) patients was determined by the MLPA method. They found duplication in the 13th exon of the BRCA-1 gene in 1 patient, and deletions in the 13–15th exons in the other patient. They also showed duplication in the 4–11th exons of the BRCA-2 gene in 1 (1 %) patient (4). This prevalence value is consistent with the present study in terms of BRCA-1. In the present study, 1 of our

patients in whom a deletion was determined, had been diagnosed at an early age (24 years), whereas there was a history of concomitant ovarian cancer in the other patient. These two patients are in agreement with the literature in terms of being in the high-risk patient group for BRCA gene mutation (8). In a large study performed by Engert et al (6) in Germany, 1506 families who had been tested before by means of PCR-based techniques and in whom no mutations were found, were screened for BRCA-1 mutation by the MLPA method, and a positivity rate of 2.1 % was determined. This rate is similar with that of the present study; however, the location of the deletion is different from our patients. The most frequently encountered deletion among German patients is in exon 17 (6). In the study carried out in Italy by Montagna et al (9), deletions were determined in the 18th and 19th exons of the BRCA-1 gene, similar with the present study. In Denmark, Thomassen et al (10) screened 154 families via the MLPA method in whom BRCA-1 mutations had not been determined by conventional methods and detected large genomic rearrangements in 1.3 %; however, found no BRCA-2 mutations. These results are consistent with the results of the present study in terms of BRCA-2 mutation not being determined. In the studies performed previously, the rates of BRCA-1 and BRCA-2 gene mutations were different from each other. This may be due to the genetic and ethnic divergence between the countries.

In conclusion, there are many studies in the literature performed by different methods in different countries, and the characteristics of patient groups and the sensitivity of the methods used in those studies are different. Therefore, the positivity rates of BRCA-1 and BRCA-2 gene mutations may vary according to the characteristics of the patient groups as well as the ethnic and genetic features of the screened population. The characteristic of the present study, of the 208 patients, 45 have a family history of breast cancer, 1 have a family history of ovarian cancer, and the remaining are sporadic breast cancer cases. Furthermore, the other characteristic of the present study is the MLPA analysis, which is a new method first used for BRCA-1 and BRCA-2 gene mutations in Turkish patients. More comprehensive studies are needed.

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