Breast cancer (BRCA) is an extremely common malignancy, affecting one in 8 women during their lifetime. Genetic susceptibility has been estimated to contribute to 5-10% of all BRCA cases. Studies on large families clustering many breast or ovarian cancers, have lead to the identification of 2 tumor-predisposing genes, breast cancer susceptibility genes 1 (BRCA1) and BRCA2. Breast cancer susceptibility genes 1 and BRCA2 account for approximately 80% of inherited BRCA cases. Linkage analysis performed on large sets of families have estimated that the BRCA1 gene is involved in 45% (27-65%) of inherited BRCA and more than 80% of inherited breast and ovarian cancer. Breast cancer susceptibility gene 1 is inherited in an autosomal dominant manner but it is a recessive disease. Therefore, the individual is not born with a cancer but with time, due to loss of heterozygosity, breast carcinoma will be developed. It was mapped to chromosome 17q12-21 and it is composed of 22 coding and 2 non-coding exons distributed over 100kb genomic DNA. Breast cancer susceptibility gene 1 protein may play a role in cell cycle regulation and DNA repair. Since the isolation of BRCA1 in 1994, more than 300 different germline mutations in BRCA1 gene have been identified. In Jordan, the first year of the cancer registry was 1996. During January to December 2003, 1,598 cases were diagnosed. Breast cancer was the leading cancer, and represents 28% of all female cancers. The latest Jordanian record of cancer registry was in 2000 and a breast cancer is still the leading cancer.

Objective: To screen mutations of the tumor suppressor breast cancer susceptibility gene 1 (BRCA1) within 3 exons among Jordanian breast cancer females.

Methods: A total of 135 Jordanian breast cancer females were genetically analyzed by denaturing gradient electrophoresis (DGGE) for mutation detection in 3 BRCA1 exons (2, 11 and 20) between 2000-2002 in Al-Basheer Hospital, Amman, Jordan.

Results: Of the studied patients 50 had a family history of breast cancer, 28 had a family history of cancer other than breast cancer, and 57 had no family history of any cancer. Five germline mutations were detected among breast cancer females with a family history of breast cancers (one in exon 2 and 4 mutations in exon 11). Another germline mutation (within exon 11) was detected among breast cancer females with family history of cancer other than breast cancer, and no mutation was detected among breast cancer females with no family history of any cancer or among normal control females.

Conclusion: Screening mutations within exon 2, exon 11 and exon 20 showed that most screened mutations were within BRCA1 exon 11 among breast cancer Jordanian families with a family history of breast cancer.


ABSTRACT

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cancer and represents 16.5% of all cancers.\textsuperscript{13} The purpose of this study is to screen BRCA1 mutations within 3 BRCA1 exons using denaturing gradient electrophoresis (DGGE) in a large set of 135 Jordanian breast cancer females.

**Methods.** Blood samples were collected from 135 females who were pathologically diagnosed with BRCA in Al-Basheer Hospital, Amman, Jordan between 2000-2002, and from 20 normal individuals (as a control) to study genetic polymorphism. Control groups were selected from healthy females that were relatives to the BRCA females and were age-matched to BRCA females enrolled in this study. Criteria used for classification of familial breast cancer were based on at least 2 first, or second-degree relatives of the same lineage affected with invasive cancer at any age. Tumor histology records were verified for all breast cancer females and signed permission for involvement in this study was carried out from every female enrolled. Deoxyribonucleic acid was extracted from fresh blood samples (maximum one hour after sample collection) in extremely clean conditions by a Wizard kit for DNA extraction and purification (Promega). Screening for small or point mutations was performed by polymerase chain reaction (PCR) assay from genomic DNA followed by denaturing gradient gel electrophoresis (BioRad) and ethidium bromide staining. Primers for PCR assay were constructed according to Stoppa-Lyonnet et al.\textsuperscript{14} A GC clamp sequence was added at either the 3' or 5' ends to improve the DGGE.\textsuperscript{15} Primers were constructed to screen 3 exons (2, 11, 20) in the BRCA1 gene. Screening was limited to these exons because these exons represent hot spot mutation areas in the BRCA1 gene according to Stoppa-Lyonnet et al.\textsuperscript{14} Polymerase chain reaction primer sequences, annealing temperature, expected amplicon size and denaturing gradient electrophoresis percentage are summarized in Table 1.

**Results.** Among the 135 Jordanian females investigated for breast cancer mutations, 50 had a family history of breast cancers (group 1), 28 had a family history of familial cancers other than breast (group 2) and 57 had no family history of any cancer (group 3). Twenty normal females were used as a normal control (group 4) to study any polymorphism among Jordanian females (Table 2). Denaturing gradient electrophoresis screening for BRCA1 gene mutation showed 6 different migration patterns of DNA variants. Five DNA variants were detected within group 1: one within exon 2 (Figure 1a), 2 exon 11a (Figure 1b) and 2 within 11b (Figure 1c). Another DNA mutation was detected within group 2 females within exon 11a (Table 2 and Figure 1b lane 3).

**Discussion.** Literature on genetic predisposition of breast cancer is limited in Arab women. In Jordan, and as part of Arab world, genetics of Jordanian breast cancer females is also limited. Screening of BRCA1 gene mutation among Jordanian revealed 6 different mutations. Five mutations (5/50) were found among Jordanian females (within exon 2, 11, 20) who have a familial history of breast cancer (Table 2). The literature showed that the ratio of BRCA1 mutation was the highest in Russia (79%), then Israel (47%), Italy (29%), Britain, France, Scandinavia and Hungary (20-25%).\textsuperscript{5} Mutation rates within the BRCA1 gene in Italy were 9-12.5%,\textsuperscript{16} in Taiwan 11%\textsuperscript{17} and Iceland 9%.\textsuperscript{18} The previous rate reflecting genetic predisposition of BRCA1 mutation on familial breast cancer, is strengthened by the fact that over 50% of marriages are consanguineous.\textsuperscript{19} This study is a limited preliminary study on exon 2, 11 and 20. It showed that screened mutations among familial Jordanian breast cancer females were higher within exon 11 of the BRCA1 gene compared to exon 2 and 20. The exact cancer-predisposing mutation in BRCA1 gene may be higher than previously reported (5/50). This is because only 3 exons within the BRCA1

<table>
<thead>
<tr>
<th>Amplicon</th>
<th>Forward primer</th>
<th>Reverse primer</th>
<th>Annealing temperature</th>
<th>Size (bp)</th>
<th>Gradient %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exon 2</td>
<td>F(GC)TATATATTTTCTAATGTTT</td>
<td>TAATACACTCTTGCTGAC</td>
<td>50</td>
<td>209</td>
<td>20-70</td>
</tr>
<tr>
<td>Exon 11a</td>
<td>ACAGAGGCACAAATTGAAT</td>
<td>R(GC)TTTCTGGACGCTTTTGCTAA</td>
<td>50</td>
<td>382</td>
<td>20-70</td>
</tr>
<tr>
<td>Exon 11b</td>
<td>F(GC)CTAAAGAAGAGGAGAATTT</td>
<td>TCCCCAAAGGATACATAACATTT</td>
<td>58</td>
<td>426</td>
<td>20-70</td>
</tr>
<tr>
<td>Exon 20</td>
<td>F(GC)GCTTCTCTTCTTATCC</td>
<td>CAAAAGGGAGTGGAATAC</td>
<td>50</td>
<td>250</td>
<td>30-80</td>
</tr>
</tbody>
</table>

F(GC) - CCC CGC CCG GCC GCC GCC GCC GCC GCC GCC GCC GCC GCC GCC GCC GCC GCC GCC
R(GC) - CCC CAC GCC ACC CGA CGC CCC AGC CGG ACC CCC CCG CCG CCG CCG CCG CCG CCG C
A - adenine, G - guanine, C - cytosine, T - thymine
Mutation analysis of the breast cancer gene BRCA1 ... Atoum & Al-Kayed

Table 2 - BRCA1 mutant exons among Jordanian breast cancer females.

<table>
<thead>
<tr>
<th>Family status</th>
<th>Total N of cases</th>
<th>N of cases with BRCA1 gene mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Exon 2</td>
</tr>
<tr>
<td>Group 1</td>
<td>50</td>
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<tr>
<td>Group 2</td>
<td>28</td>
<td>-</td>
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<tr>
<td>Group 3</td>
<td>57</td>
<td>-</td>
</tr>
<tr>
<td>Group 4</td>
<td>20</td>
<td>-</td>
</tr>
</tbody>
</table>

Group 1 - breast cancer females with a family history of breast/ovarian cancer, Group 2 - breast cancer females with a family history of cancer other than breast/ovarian cancer, Group 3 - breast cancer females with no family history of any cancer, Group 4 - Normal Jordanian females

gene were screened for BRCA1 mutations. At the same time, DGGE does not detect genetic gene deletions within intronic boundaries. So, farther screening of other exons and within the intronic boundaries among these breast cancer females may detect more mutations within Jordanian breast cancer females. The most frequent mutation among familial breast cancer Jordanian females was within exon 11; part 11a showed 2 mutations and part 11b showed 2 mutations (Table 2). This is an expected finding as the BRCA1 gene has a huge exon 11 and screening the whole exon may show other mutations. Previous reports recording more than 300 heritable mutations in the BRCA1 gene have been spread throughout the whole coding sequence.12 Within Arab patients, Arg841Trp, Phe486Leu and Asn550His were reported in Saudi Arabia.20 The reputability of BRCA1 mutations varies widely among different populations, from nearly no repeated mutations in Italy,16 to highly repeatable mutations in the BRCA1 gene in Iceland and Israel.21 In this study, the amplicon profile of exon 11a (Figure 2) showed that at least 2 different types of mutations. Further DNA sequencing could be useful for pinpointing nucleotide changes among these breast cancer mutations. One mutation was detected within exon 11a among group 2 females with a family history of cancers other than breast cancer. This may be due to the effect of other genes that modify BRCA1 gene and increase the risk for breast cancer,22 or may be due to a rare polymorphism in the BRCA1 gene that is not detected within group 4 normal controls. Therefore, more breast cancer families need to be screened in order to determine the types of mutations or polymorphism within the Jordanian populations.

References


