Prevalence of BRCA1/2 Gene Mutation Carriage Rate among Local Iraqi Population

Mohammad Sabri A. Razzak¹ Mohammed Abbod Muhsin¹
Ifad Kerim Alshibly¹ Wala’a Nori Bearem²
¹ College of Medicine, University of Babylon, Hilla, IRAQ.
² Breast Cancer Center, Hilla General Teaching Hospital, IRAQ.

Received 26 January 2014 Accepted 9 February 2014

Abstract

Background: Cancer is one of the leading causes of death in the general population. It is proposed that, one-fourth of women will be affected with cancer at some point in their lives. Breast cancer is the most prevalent malignancy in women. Mutations in breast cancer susceptibility genes BRCA1 and BRCA2 account for the majority of breast cancer cases. BRCA1 and BRCA2 are tumor suppressor genes that repair and correct errors in DNA, act as sensors of DNA damage and participate in the DNA repair processes.

Aim: to illustrate the role of and analyzing the types and frequencies of the most common BRCA1, BRCA2 mutations in different groups of local Iraqi women.

Materials and methods: This is a case-control study design. A total of forty women were chosen for the genetic study which was performed to detect BRCA1 and BRCA2 mutations, DNA was amplified by polymerase chain reaction (PCR), exon 2 and 20 of BRCA1 and exon 11 of BRCA2 genes, using a specific primer pairs and PCR conditions in four groups of Babylonian populations, including patients with breast cancer, healthy control people, first degree relatives, and patients with benign breast tumors.

Results: The overall frequency of BRCA genes mutation was more often detected among breast cancer patients than other groups in the study (P=0.05).

Conclusions: There was a surprising high degree of BRCA gene mutation carriage rate, BRCA gene mutations were found to have a relatively high frequency (up to 75%) among breast cancer patients in the study.

Introduction

Breast cancer is the most common malignancy affecting women and its incidence is increasing worldwide, making it the most common type of non-skin cancer in women and the fifth most common cause of death due to cancer [1]. The majority of breast and ovarian cancers are sporadic or not inherited [2]. In addition to specific genetic changes, many personal and environmental factors have been identified that may influence a person's risk of developing
breast cancer[3]. Mutation in some genes such as the breast cancer susceptibility genes *BRCA1* and *BRCA2* predispose to familial risk of breast cancer [4]. *BRCA1* and *BRCA2* are human genes that belong to a class of genes known as tumor suppressors. In normal cells, *BRCA1* and *BRCA2* help ensure the stability of the cell’s genetic material (DNA) and help prevent uncontrolled cell growth. Mutation in these genes has been linked to the development of hereditary breast and ovarian cancer[5]. Over 200 individual *BRCA* mutations have been described, they are found throughout the length of the gene, some areas appear to be "hot spots" for mutation[6]. A mutated *BRCA* gene usually makes a protein that does not function properly because it is abnormally short[7]. Mutations in the *BRCA* genes are inherited in an autosomal dominant fashion[8]. A number of screening test have been employed for breast cancer including: clinical and self breast exams, mammography, genetic screening, ultrasound, and magnetic resonance imaging[9]. A breast cancer (*BRCA*) gene test is a blood test to check for specific mutations *BRCA* genes. This can help determine the chance of developing breast cancer and ovarian cancer. This test is only done for people with a strong family history of breast cancer or ovarian cancer[10]. Early diagnosis and screening, especially when combined with adequate therapy, offer the most immediate hope for a reduction in breast cancer mortality. This was the basis of the Iraqi national program for early detection of breast cancer, which was initiated in 2000 in an attempt to downstage this disease at the time of presentation. Since then specialized centers and clinics for early detection of breast cancer have been established in the major hospitals in all Iraqi provinces[11]. Genetic assays to test patients for specific mutations are commercially available, but their use remains somewhat controversial and they should not be applied to the population at large. When carriers are identified, it is appropriate to offer radiological studies at an increased frequency and beginning at an earlier age, as well as to discuss the option of prophylactic surgery and counseling/testing other family [6].

The prevalence of *BRCA1* or *BRCA2* mutations varies considerably among ethnic groups and geographical areas. In this study we tried to identify the carriage rate of some selected mutations among *BRCA1/2* genes in a specified local iraqi population.

**Patients and Methods**

**Study design:** This is a case-control study design.

**Study population:**
A total of forty subjects were chosen for the genetic study, the primary set of subjects were drown from those attending the breast cancer center in Hilla General Teaching Hospital from March through October 2013. Four groups were recruited; 20 breast cancer patients, 10 healthy controls, 5 benign breast tumor patients and 5 first degree relatives to a known case of breast cancer. Both of the breast cancer and the benign breast tumor patients were clinically, radiologically and histopathologically confirmed as having breast cancer and benign breast tumor, respectively, while healthy control and first degree relatives to the breast cancer patients were clinically and histopathologically confirmed as having no breast problems. All cases and controls were of Iraqi origin resident in Hilla city, and its peripheries. All subjects were screened for the presence of mutation in *BRCA1* exon2, *BRCA1* exon20, and *BRCA2* exon11.
DNA extraction:
The screening of mutation in BRCA genes were carried out on genomic DNA. DNA was extracted from blood samples using the conventional methodologies supplied by the Promega company (www.promega.com), DNA was extracted from fresh whole blood collected in EDTA, heparin and citrate anticoagulant tubes. The extracted DNA was stored in 2-8°C.

Screening for BRCA1 and BRCA2 mutations:
To detect BRCA1 and BRCA2 mutations, DNA was amplified by polymerase chain reaction (PCR), exon 2 and 20 of BRCA1 and exon 11 of BRCA2 genes, using the primer pairs and PCR conditions in table (1)[12].

Ethical Issues:
All patients and family members were counseled then they signed a written informed consent before testing. This study was performed with permissions from Babylon University; College of Medicine, Hilla General Teaching Hospital and from the Ministry of Health in Iraq.

Results
In the present study, the prevalence of BRCA1/2 gene mutation carriage rate was investigated among local Iraqi women, using conventional PCR technique and gel electrophoresis with required technical information illustrated in table(1).

Table 1 Primers sequences, product size and PCR conditions for BRCA genes mutation.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer Sequence</th>
<th>Size /bp</th>
<th>PCR conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Temp, time, cycles</td>
</tr>
<tr>
<td>BRCA1 185delAG (exon 2)</td>
<td>F-5'-GAA GTT GTC ATT TTA TAA ACC TTT-3' R-5'-TGT CTT TTC CCT AGT ATG T-3'</td>
<td>275</td>
<td>95°C 5min 1x, 94°C 1min</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>58°C 1min 33x</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>72°C 1min</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>72°C 10min</td>
</tr>
<tr>
<td>BRCA1 5382insC (exon 20)</td>
<td>F-5'-ATA TGA CGT GTC TGC TCC AC-3' R-5'-GGG AAT CCA AAT TAC ACA GC-3'</td>
<td>425</td>
<td>95°C 5min 1x, 94°C 1min</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>58°C 1min 33x</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>72°C 1min</td>
</tr>
<tr>
<td>BRCA2 6174delT (exon 11)</td>
<td>F-5'-AAC GAA AAT TAT GGC AGG TTG TTA C-3' R-5'-GCT TTC CAC TTG CTG TAC TAA ATC C-3'</td>
<td>534</td>
<td>95°C 5min 1x, 94°C 1min</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>55°C 1min 33x</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>72°C 1min</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>72°C 10min</td>
</tr>
</tbody>
</table>

In order to measure the frequency of BRCA mutation among local Iraqi population, a genetic analysis about the most common BRCA mutation was carried out, using conventional PCR technique and a highly specific primer sets. It was observed that there was a surprising high degree of BRCA gene mutation carriage rate. Figures (1) shows the electrophoretic imaging of BRCA1 exon-2, BRCA1 exon-20, and BRCA2 exon-11 gene mutation respectively. Figure (2) illustrates the results about the frequency of BRCA gene mutation among the study groups.
**Figure 1** The electrophoretic imaging of BRCA1 exon-2, BRCA1 exon-20, and BRCA2 exon-11 gene mutation.

**Figure 2** Frequency of BRCA gene mutations

**Discussion**
According to the Iraqi health ministry registrations of breast cancer, the incidence rate of breast cancer have been doubled from 3.26/100,000 in 1990 to 6.33/100,000 in 2000, with annual incidence rate of about 50,000/year.

The overall frequency of BRCA genes mutation was more often
detected among breast cancer patients than others in the study (figure 2). Eight patients with breast cancer carried all of the three analyzed mutations, and seven had two mutations. Otherwise, BRCA genes frequency showed low prevalence among first degree relatives and benign breast tumor patients, and to lesser extent among healthy controls, with only few, had all of the mutations combined.

The frequency of BRCA1 exon 2 mutation was found in 55% (11/20) patients of breast cancer, while only 20% (2/10) healthy control subjects carried this mutation. On the other side, both the first degree relatives and patients with benign tumors had the same frequency of this type of BRCA mutation giving a value of 40% (2/5).

In this study, BRCA1 exon 20 mutation was the most frequently recorded mutation, it was detected in (15/20) patient of breast cancer, forming 75% of this group. The healthy controls had the lowest frequency of this gene mutation, with 30% carriage rate (3/10). Likewise, 40% (2/5) of the first degree relative had the mutation, while 60% (3/5) of patients with benign tumors carried it.

BRCA2 gene mutation was also studied, the results found that 40% of breast cancer and benign tumor patients were carrier of mutation in the exon 11 of BRCA2 gene, 20% of first degree relatives had the mutation, and only 10% of the control were mutated.

Coming with accordance with these results, a study of BRCA mutations by Couch et al., (2007)[13], showed that the most commonly detected BRCA1 mutations are a deletion of adenine and guanine [BRCA1 185delAG (exon 2)], and insertion of cytosine [BRCA1 5382insC (exon 20)], and BRCA2 mutation is the deletion of thymine [BRCA2 6174delT (exon 11)], which have a high frequency in the general population.

Likewise, Easton et al., (2009) [14] pointed that mutations in the breast cancer susceptibility genes BRCA1 and BRCA2 confer a high risk of developing breast cancer. Breast cancer is about twice as common in the first-degree relatives of women with the disease as in the general population, consistent with variation in genetic susceptibility to the disease.

There is an evidence that specific mutations in BRCA1 and BRCA2 more common in certain populations, for example, Friedenson, (2007)[15] had reported that three specific mutations, were the most common mutations found in these genes in the Ashkenazi Jewish population. Other ethnic and geographic populations around the world, such as the Norwegian, Dutch, and Icelandic peoples, also have higher frequencies of specific BRCA1 and BRCA2 mutations. In addition, limited data indicate that the frequencies of specific BRCA mutations may vary among individual racial and ethnic groups.

Ravi, (2010)[16] has established that according to the cancer registries of almost all countries within the Eastern Mediterranean Region, as in Iraq, the breast cancer continues to rise. The Iraqi National Cancer Research Program has to better understand the underlying molecular and environmental causes in an effort to curb the incidence of cancer.

The results presented in this study illustrate the high rate of BRCA gene mutation carriers, this come in parallel with that of Al-Dujaily et al., (2008)[17] and his colleagues who demonstrate that there were three-fold increase of all types of breast cancer in Iraq due to long-term exposure to radiation. Radiation levels were estimated to be around 320–800 tons in
the aftermath of the first Gulf war in 1991 with further comparable levels occurring in 2003. Since the targets were always in heavily populated areas in the middle and south of Iraq, the extent of exposure on individuals was extensive but has been very hard to be documented accurately. The significance of this risk factor in women with a genetic susceptibility to breast cancer is unclear. The possibility that genetic susceptibility to breast cancer occurs via a mechanism of radiation sensitivity raises questions about radiation exposure. However, they propose that despite the three-fold increase of all types of breast cancer in Iraq due to long-term exposure to depleted uranium, the increment in the incidence may not become evident with time until mutations are passed to future generations.

The proportion of the high risk breast cancer attributable to BRCA1 and BRCA2 has been shown to vary considerably between different studies. The observed frequency is not always the same in any two countries surveyed so far. The mutation frequencies reported in the present study came in parallel with another study undertaken in the north of Iraq by Majid et al.,(2009)[18], who found that the age specific annual incidence rates of breast cancer were higher among Iraqi Kurds than for Israeli Arabs and Jordanians and were similar to Egyptians. Age specific breast cancer incidence rates for Jordanian and Israeli Arab women lag behind those in the United States . The traditional culture of the Middle-East encouraged large families. The comparatively lower rates of breast cancer among older Middle-Eastern Arabs and Kurds could be a cohort effect due to increased childbearing having a protective effect on earlier generations of women.

The frequency of the BRCA1/BRCA2 mutations in the Iraqi population has been compared with other populations including those of Ashkenazi. Lahad et al.,(2007)[12] have studied the frequency of BRCA1/2 mutations in Ashkenazi Jews in Israel including cases of Iraqi and Iranian origin, they found that the frequency of BRCA1 185delAG and BRCA1 5382insC mutations was significantly higher than that of BRCA2 6174delT mutation. Analysis of these three markers in four Iranian/Iraqi BRCA gene mutation carriers revealed the same genotype that previous reports have described in Ashkenazi Jews, suggesting a common origin for the mutations in Ashkenazi and Iraqi/Iranian Jews. These results confirmed the present study reports, in which the three mutations accounted for most of high-risk breast cancer cases.

Identification of BRCA mutations in a substantial proportion of our Iraqi patients indicates that these genes play an important role in the incidence of breast cancer in the general population. These results were supported by Turkish study accomplished by Yazıcı et al.,(2000)[19], who demonstrated the presence of BRCA mutations in patients with a personal and family history of breast cancer below age 50. Their results suggest that BRCA1 and BRCA2 mutations are observed in a significant proportion of Turkish families with breast cancer, and those with early onset of disease. This study demonstrates the importance of the consideration of inherited predisposition to breast cancer in the clinical management of breast cancer risk.

The data considering the frequency of BRCA gene mutation in the current study came in line with the fact that there is a significant association between the presence of
BRCA1 or BRCA2 mutations and the occurrence of cancer. Al-Mulla et al. (2009) [2] have studied different mutations in the BRCA gene in Britain population, they reveal that more than half of individuals carried a mutated BRCA1 gene, and less had a mutated BRCA2 gene, while about one-third of the screened individuals carried wild-type genes. Exon 2 mutations were the most frequent with 185delAG being the most common mutation within exon 2, followed by mutation of exon 20 (5382insC). For BRCA2 gene, mutations in exon 11 (6174delT) was the most frequent. Mutations in the BRCA1 gene accounted for the majority of breast cancer. It was noted that carriers of the exon 2 of BRCA1 gene mutation had significantly lower cancer incidence compared with carriers of other exons mutation. These findings are consistent with the present work, suggesting that either the 185delAG mutation is of low penetrance or, more likely, carriers of this mutation would develop cancer at an older age compared with other BRCA1 gene mutations.

It is well accepted that mutation frequencies of the BRCA1 and BRCA2 genes in high-risk people vary widely among different populations, the present study is consistent with this fact. The contributions of BRCA1 and BRCA2 to breast cancer in Italian patients appear to be less significant than in patients from Iraq and other communities with these mutations. In one Italian study, Ottini et al. (2000)[20] have found that BRCA1 mutations were detected in only one out of 10 cases from breast cancer families. This is a low proportion compared with other studies which suggested that mutation in BRCA1 and BRCA2 are responsible for the large majority of breast cancer families, with the greater proportion due to BRCA1. In this respect, the limits of mutation detection techniques and the small number of breast cancer cases tested should be taken into account.

Finally, it became clear that ethnicity affects frequency of BRCA gene mutation among populations. Showing that particular mutations in genes associated with breast cancer are more common among certain geographic or ethnic groups. The genetic changes occur more frequently in these groups because they have a shared ancestry over many generations. Different ethnic groups may have different mutations. The prevalence of these genes varies depending on ethnicity.

Conclusions
In conclusion, There was a surprising high degree of BRCA gene mutation carriage rate, BRCA gene mutations were found to have a relatively high frequency (up to 75%) among breast cancer patients in the study.

References