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BRCA1 gene mutation in familial breast cancer

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ABSTRACT

This study was conducted to estimate the frequency of BRCA1 (185delAG) mutation in Egyptian female patients with breast cancer. Forty selected female patients with breast cancer, 80 of their female relatives and 10 healthy females as a control group were included in this study. The age of onset of breast cancer was below 40 years in 25 (62.5%) patients and above 40 years in 15 (37.5%) patients. There were significant differences among the patients regarding the age at menarche before 13 years (p=0.011), onset of breast cancer (p<0.001), parity (p<0.001), first delivery before 30 years of age (p=0.04), breast feeding (p=0.002), and positive family history (p<0.001). The frequency of BRCA1 (185delAG) mutation was 10% in the patients. Eight percent of patients with early onset below 40 years and 13.5% of patients with onset after 40 years were heterozygotes for the mutation. Three percent of patients with unilateral breast cancer, 40% of patients with bilateral breast cancer and 50% of patients with breast-ovarian cancer were carrying the mutation. Our results indicated that breast-ovarian cancer and bilateral breast cancer patients were likely to have BRCA1 (185delAG) mutation than in unilateral breast cancer. [Turk J Cancer 2008;38(4):167-174]

KEY WORDS:

BRCA1, breast cancer, Egypt

INTRODUCTION

Breast cancer (BC) is the most common malignancy in women, accounting for 31% of all female cancers, and responsible for 15% of cancer deaths in women (1). One million females world wide are diagnosed with BC every year. Treatment of advanced BC is futile and disfiguring, making early detection have a high priority in medical management of the disease (2).

The risk factors of BC include genetic, environmental and hormonal. Genetic risk factors contribute to about 5%-10% of all cases, 90%-95% of them result from somatic mutation and about 5%-10% are inherited as the result of germ line mutation in autosomal dominant BC susceptibility genes (3,4).

Many genes have been found to increase susceptibility to cancer and are also associated with familial breast cancer. These genes include Breast Cancer Anti-estrogen resistance-1 (BRCA1), Breast Cancer Anti-estrogen resistance-2 (BRCA2), Ataxia Telangiectasia mutant gene (ATM), Phosphate and Tensin homology (PTEN) and Tumor Protein (P53). Several other less frequently predisposing genes are also involved but to lesser extent (3,4).

BRCA1 gene (chr 17q21) is a tumor suppressor gene that encodes tumor suppressor protein which acts as a negative regulator for tumor growth. It accounts for 45% of inherited BC and 90% of inherited breast-ovarian can-

cer (BOC) in highly affected families (5). Different types of mutation have been found in the BRCA1 gene and predispose to development of cancer. The most common of them is the frame shift mutation at position 185 in exon 2, involving deletion of adenine and guanine (185 del AG mutation) (6-8). The identification and study of this mutation could facilitate early diagnosis and proper counseling for BC. Therefore, the present study aimed to identify the 185 del AG mutation (exon 2) of BRCA1 gene in Egyptian females with early BC and BOC and their female relatives. This will allow early diagnosis and proper genetic counseling for susceptible cases in the families.

PATIENTS AND METHODS

The present study included 40 female patients with BC collected from the Oncology Unit, Medical Research Institute, Alexandria University. Two close female relatives for each patient (80 females) and ten healthy age matched females without any family history of any cancer, as a control group, were also included.

Female patients participated in the study were selected according to having one of the following:

1- Early onset breast cancer (below 40 years).
2- Bilateral breast cancer.
3- Breast-ovarian cancer.
4- Positive family history of breast cancer.

The patients and control group were subjected to the following:

1- Detailed history:
   a- Personal history including age, age of menarche, marriage, age of first full term pregnancy, lactation and menopause.
   b- Family history of breast cancer or any other cancer (for patients and relatives).
2- Molecular studies to detect BRCA1 (185 del AG) mutation as follows:
   a- DNA isolation and purification from peripheral blood using standard method (9)
   b- Polymerase chain reaction (PCR) amplification: according to Lahad et al. (10) The sequence of the primers used was as follows:

   Forward: 5'-GAAGTTGTCATTTTATAAACCTTT-3'

   Reverse: 5'-TGTCTTTTCTTCCCTAGTATGT-3'

   The PCR mix was performed in a final volume of 25 µL containing 100 ng (3µl) DNA, 10 pmole (3µl) of each primer, 12.5 µl of the ready made PCR master mix (Promega Chemical Co.) and 3.5 µl of nuclease free water was added to adjust the volume to 25 µl.
   c- PCR conditions as follows: 1 cycle at 95°C for 5 minutes followed by 33 cycles at 94°C for 1 minute, 58°C for 1 minute and 72°C for 1 minute, 1 cycle at 72°C for 10 minutes.
   d- Agarose gel electrophoresis: was carried out on a 3% agarose gel and visualized with ethidium bromide (11)
   e- Single Strand Conformational Polymorphism (SSCP) Analysis (12,13): Ten µl of PCR product was diluted 1:1 in formamide buffer (95% formamide, 20 mM EDTA pH 8.0, 0.05 xylene cyanol and 0.05% bromophenol blue), kept at 85°C for 5 minutes and then cooled quickly in ice. The samples were loaded onto a non-denaturating polyacrylamide and run at room temperature for 4 hours using constant voltage (150V) and 1x TBE buffer. Detection was carried out using silver stain and ethidium bromide.

Statistical analysis

Statistical differences between patients and controls were determined with the Fisher's Exact Test.

RESULTS

This study included 40 female patients with BC or BOC, 80 of their close female relatives and 10 healthy adult females as a control group.

Characteristics of cases

The age of menarche was below 13 years in 28 patients (70%) and over 13 years in 12 patients (30%) with a mean age of 13.20±1.3 years. The age of menopause was more than 45 years in 8 patients (20%) with a mean age of 49.10±3.4 years. The remaining 32 patients (80%) did not experience menopause as they developed cancer at young

age. The majority of the patients, 37 cases (92.5%), were married. Nullipara constituted 6 patients (15%). Among 34 patients (85%); 23 (67.6%) had their 1st child below 30 years of age while 11 (32.4%) were over 30 years. Breast feeding was practiced in 30 patients (75%).

Table 1 shows the characteristics of the patients and the controls. There was no significant differences between patients and controls in age at menarche (p=0.138), marital status (p=1.00), parity (p=0.653), age at first delivery (p=0.402) and breast feeding (p=0.707).

Classification of cases

The female patients were classified into 2 groups according to the age of onset of the disease. The first group included 25 (62.5%) patients with early onset BC before 40 years. Among this group 2 patients (8%) were with bilateral BC, 22 patients (88%) with unilateral BC and one patient (4%) with BOC. The second group included 15 patients (37.5%) with late onset BC above 40 years of age. Three of them (20%) were with bilateral BC, 11 patients (73.3%) with unilateral BC and one patient (6.7%) with BOC. Positive family history with BC was detected in 2 patients (8%) in the first group and in 13 patients (86.6%) in the second group. Positive family history was significantly higher in the second group patients (p<0.001) (Table 2).

Molecular study

DNA was amplified using intronic specific primers spanning exon 2 (262 bp). Similar amplified PCR prod-

Table 1
Characteristics of patients with breast cancer and controls

	Patients		Control		
	N	%	N	%	p value
Age at menarche					
<13 y	28	70	4	40	p* = 0.138
>13 y	12	30	6	60	
Onset of cancer					
Premenopausal	32	80	-	-	-
Postmenopausal	8	20	-	-	
Marital status					
Married	37	92.5	9	90	p* = 1.000
Not married	3	7.5	1	10	
Parity					
Parous women	34	85	8	80	p* = 0.653
Multiparous women	6	15	2	20	
Age at 1st delivery	(n = 34)		(n = 8)		
< 30 y	23	67.6	7	87.5	p* = 0.402
> 30 y	11	32.4	1	12.5	
Breast feeding					
Yes	30	75	7	70	p* = 0.707
No	10	25	3	30	

p*: p value between patients and controls

ucts for all samples subjected to the present study was detected (Figure 1).

The SSCP analysis revealed 4 patients (10%) carried 185 del AG mutation in BRCA1 gene (Figures 2&3). No mutation was detected in the patient's relatives or in control groups.

The main findings of the four patients with BRCA1 mutation are summarized in (Table 3).

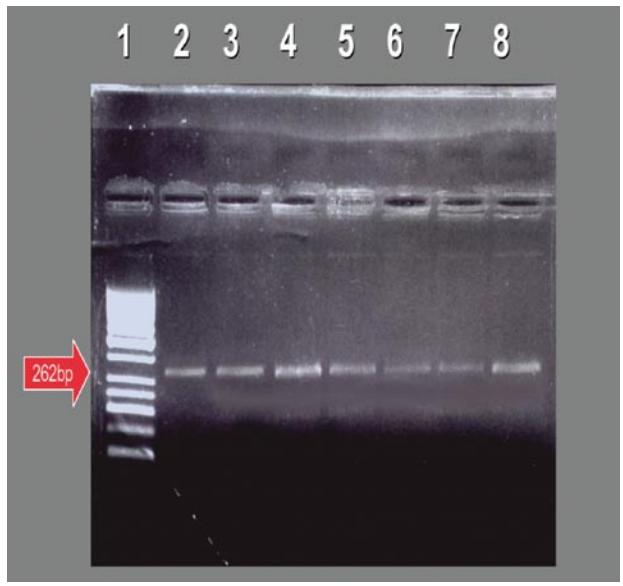

Fig 1. Agarose gel (3%) electrophoresis of PCR products for BRCA1 exon 2 gene (lanes 2-8). Lane 1 represents 50 bp ladder molecular weight marker

DISCUSSION

Multiple menstrual and reproductive events; menarche, pregnancy, breast feeding may alter BC risk. In the present study there was a significant increase in the patients with menarche before 13 years of age ($p<0.05$) and the mean age of menarche of patients was 13.2 years and menopause at 49 years. These results were similar to those reported by Knudson et al. (14) who found that both early age at menarche (before 13 years) and late age at menopause (more than 45 years) were found to be associated with increased risk of BC especially in susceptible women. This may be related to a higher life time exposure to the hormones estrogen and progesterone.

No association between BC and marital status of the patients was detected in the present study as there was no significant difference between patients and controls ($p=1.000$). This is consistent with other studies that confirmed the absence of significant association between BC and marital status which may indicate that marital status alone without parity has no role in hormone level change in the body (15-17). However, the married women were significantly higher in the patients group ($p<0.001$).

Single and nulliparous women were reported to have an increased risk of BC, about 1.4 times the risk of parous women. This low risk of BC in parous women might result from the protective effect of age at first pregnancy and child birth (16-18). The finding of the present study

Table 2
Distribution of patients according to age of onset of the disease

		Age of onset	
	Cases	**≤40 y**	**>40 y**
	(n=40)	**N (%)**	**N (%)**
	N (%)	**25 (62.5%)**	**15 (37.5%)**
Positive family history for BC*	15 (37.5%)	2 (8%)	13 (86.6%)
Positive family history for another cancer	1 (2.5%)	-	1 (6.7%)
Unilateral BC	33 (82.5%)	22 (88%)	11 (73.3%)
Bilateral BC	5 (12.5%)	2 (8%)	3 (20%)
Breast ovarian cance	2 (5%)	1 (4%)	1 (6.7%)

*Significant difference in positive family history of BC between the 2 groups of patients ($p <0.001$)

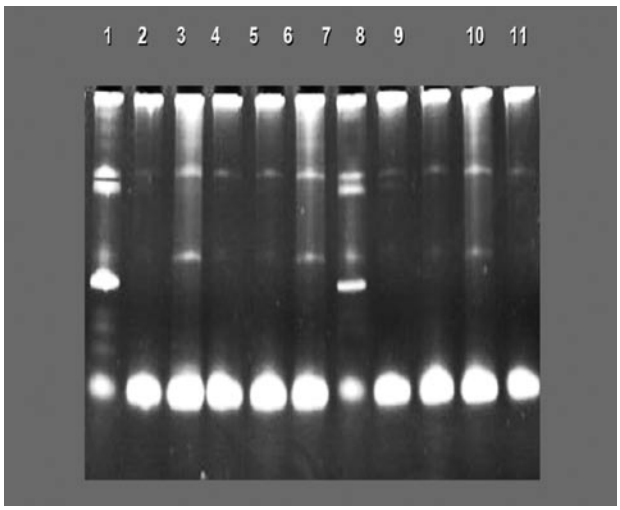

Fig 2. Single strand conformational polymorphism (SSCP) analysis of PCR products for BRCA1 exon 2 gene (using ethidium bromide stain). Lanes (1,7) show band variation: upper bands represent the single stranded DNA; the lower bands represent the reannealed double strand DNA. Lanes (10,11) control PCR product (negative for the mutation). Lanes (2,3,4,5,6,8,9) represent normal PCR products for patients without the mutation

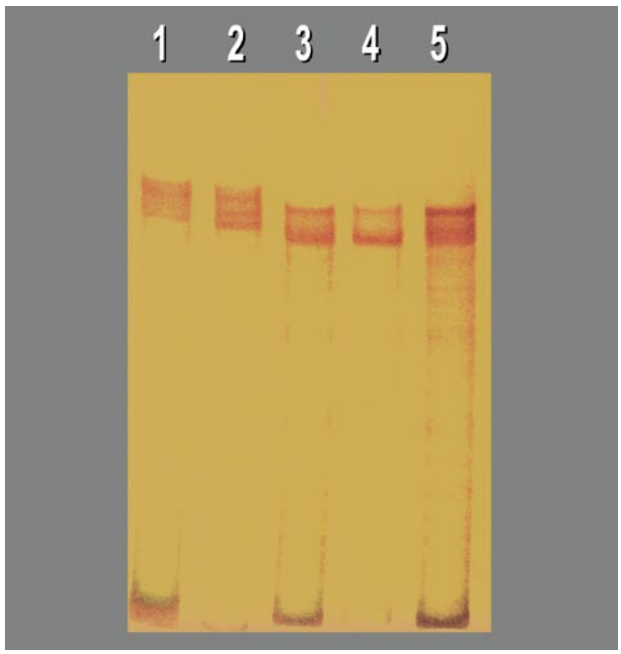

Fig 3. Single strand conformational polymorphism (SSCP) analysis of PCR product for BRCA1 exon 2 gene (using silver staining). Lanes (1,2) show band variation: upper bands represent the single stranded DNA and lower bands represent the reannealed double stranded DNA. Lane 5 control PCR product (negative for the mutation). Lanes (3,4) represent PCR products for patients without the mutation

was contrary to that as parous women were significantly higher in the patients group ($p<0.001$). These parous women may be exposed to strong risk factors that lead to the BC in spite of the protective effect of parity.

Late age at first child birth increases the life time incidence of BC (19,20). It was reported that being older than 30 years at first delivery is a risk factor for BC (21). The highest risk was in those who have a first child after the age of 35 years, they appear to be at even a higher risk than that of nulliparous women (22). Moreover, epidemiological studies have consistently shown that women who undergo an early first full term pregnancy have a significantly reduced life time risk of BC, this association is independent of parity, i.e. number of live birth (19,20). In the present study there was no significant difference in age at first delivery between parous patients and controls, moreover the parous patients who gave birth to their first child

Table 3
Characteristics of the patients with BRCA1 (185 del AG) mutation

	Unilateral breast cancer (one case)	Bilateral breast cancer (2 cases)		Breast-ovarian cancer (one case)
Age of onset	32 y	35 y	47 y	47 y
Age of menarche	<13 y	<13 y	<13 y	>13 y
Parity	Yes	Yes	Yes	Yes
Age at 1st delivery	<30 y	<30 y	>30 y	>30 y
Lactation	Yes	Yes	Yes	Yes
Family history of BC	+	-	-e	+
Family history of another cancer	-	-	-	+

before 30 years of age were significantly higher (p=0.040) than those who gave birth after 30 years of age.

It has been suggested that breast feeding may protect against BC and increasing years of nursing experience may decrease the BC risk (23). In the present study no significant difference (p=0.707) between patients and controls was detected but the patients who had experienced breast feeding were significantly higher (p=0.002) among patients group.

Our results indicated that parity, age at first delivery and breast feeding had no effect on the BC risk.

In the present study, the frequency of BRCA1 (185delAG) mutation was 10% in the patients. This is similar to that reported by Santarosa et al. (24) who found the mutation in 10% of Italian females with familial BC, but lower than 19% reported by Lahad et al. (10) in Ashkenazi Jewish. However our result was higher than 1.2% reported by Peelen et al. (25). This variation in the frequency may be attributed to the ethnic differences.

The age is an important risk factor for breast cancer as the risk increases steadily with age, so occurrence of breast cancer in young age group gives strong implication for the presence of inherited genetic predisposition for breast cancer (10). In the present study, two patients with 185delAG mutation were below 40 years (2/25; 8%). Several studies found frequencies of 3.5%, 6.7%, 9% and 20% in the patients below 40 years of age (26-29). In the present study the other 2 patients carrying the mutation were above 40 years (2/15; 13.5%) at the age of onset of the disease. Other studies reported frequencies of 1.9% and 2% in patients above 40 years of age (26,27). Although the differences between the results of many studies conducted in different populations, most of these studies concluded that BRCA1 185delAG mutation is a strong candidate for early onset breast cancer than in late onset breast cancer. This was inconsistent with our results as the frequency of the mutation was higher in the old age group. This may be attributed to a possible impact of gene-environment interaction which delays the onset of the BC in the old age group.

The occurrence of multilocular cancer is usually associated with inherited mutation of one of cancer predisposing genes, so occurrence of bilateral BC or BOV is suggestive for the presence of dominantly inherited mutation of one of the breast cancer susceptibility genes (30). In the present study 2 out of 5 (40%) patients with bilateral breast cancer, 1 out of 2 (50%) patients with BOC and 1 out of 33 (3.0%) patients with unilateral breast cancer were found to have the mutation. Steinmann et al. (31) found that the frequency of the mutation was not different between the unilateral and bilateral BC patients and explained the development of bilateral BC to familial aggregation of additional susceptibility factors modifying the penetrance of BRCA1 mutation. Other studies reported a mutation frequency of 20% to 100% in BOC (32,33). In the present study, although unequal number of patients with unilateral, bilateral BC and BOC, our results indicate that BRCA1 (185delAG) frequency is higher in bilateral BC and BOC than in unilateral BC Egyptian female patients.

A positive family history of breast cancer usually reflects genetic susceptibility and it can be considered as one of the strongest risk factors for the disease (34,35). In the present study, 2 out of 15 (13.5%) patients with positive family history of BC had BRCA1 gene mutation while 2 out of 25 (8%) patients with negative family history had the mutation. Our finding is higher than 7.4% reported by Friedman et al. (36) and 5.8% reported by Guran et al. (37) in patients with positive family history of BC. However it was lower than the 21% reported by Kumer et al. (38) and 40% by Schubert et al. (39). Our results indicated that a considerable proportion of the familial risk of BC is attributable to genes other than BRCA1 (185delAG) mutation.

In conclusion, our results indicated that BRCA1 (185delAG) mutation has a role in breast cancer but a considerable proportion of the early breast cancer and familial breast cancer may be due to genes other than BRCA1 (185delAG) mutation. Also, bilateral breast cancer and breast ovarian cancer patients were likely to have the mutation than unilateral breast cancer patients.

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