Analysis of BRCA1 and BRCA2 Mutations in Eastern Egyptian Breast Cancer Patients

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ABSTRACT

*Mutations in breast cancer susceptibility genes 1 and 2 (BRCA1 and BRCA2) have higher frequency in breast cancer cases. Three founder mutations 185delAG and 5382insC in BRCA1 and 6174delT in BRCA2 are frequently reported in breast cancer patients from various ethnic backgrounds. The aim of the current study was to evaluate the frequency of the BRCA1 and BRCA2 mutations in Eastern Egyptian sporadic breast cancer patients and their relatives as possible diagnostic markers. One hundred women with sporadic breast cancer and one hundred healthy first-degree relatives were included in the study. Multiplex polymerase chain reaction method was used to analyze the DNA prepared from peripheral blood. Data analysis showed that 185delAG mutation in BRCA1 was expressed at low frequency (3%), whereas the 5382insC in BRCA1 and 6174delT in BRCA2 were not detected within the Eastern Egyptian population. **Conclusion:** The low percentage of BRCA1 and BRCA2 mutations in apparently sporadic early- onset cancer and relatives suggested that mutation detection is insufficient to screen Egyptian population.*

INTRODUCTION

Breast cancer is the most common malignancy in women and the major cause of cancer related-deaths among women worldwide[1,2]. Development of breast cancer involves multiple factors such as environmental, genetic or interaction between both genetic and environmental factors[3]. Cancer can develop through accumulation of mutations in proto-oncogenes and tumor suppressor genes, as a result of genetic predisposition or exposure to physical, chemical, biological and environmental factors[4]. These mutations are either inherited (germline) or acquired (somatic) during daily activities. Early detection of breast cancer has the priority in medical management of the disease because the treatment of the advanced breast cancer is futile and disfiguring.

Breast cancer susceptibility gene 1 (BRCA1) and gene 2 (BRCA2) are tumor suppressor genes that are inactivated during neoplastic development[5,6]. They code for tumor suppressor proteins that interact with regulatory elements for DNA repair, transcription and cell cycle control to stop the uncontrolled cell proliferation. Several studies show that different polymorphisms in

BRCA1 and BRCA2 genes could be associated with higher risk of breast cancer. Germline mutations of these genes can either produce truncated proteins or reduce their expressions which lead to cancer[7,8]. These mutations are transmitted in autosomal dominant fashion and may predispose to breast or ovarian cancers[9,10]. The anomaly of these proteins may contribute to 90% of the risk of development of familial cancer breast cases[7,8]. Mutation such as 185delAG and 5382insC in BRCA1 and 6174delT in BRCA2 are associated with higher risk of breast cancer in individuals of Ashkenazi Jewish background[8,11].

The three mutations accounted for 62% of Ashkenazi patients with ovarian and/or breast cancer[12]. A potential role in identifying individuals at risk was attributed to the high frequency of these mutations in BRCA genes.

The aim of our study was to analyze the frequency and evaluate the BRCA 1 and BRCA 2 gene polymorphisms as a possible early diagnostic markers in sporadic breast cancer cases and their relatives using a simple and rapid multiplex PCR method that allows the simultaneous detection of the three common mutations, 185delAG and 5382insC in BRCA1, and 6174delT in BRCA2.

MATERIAL & METHODS

Subjects:

One hundred sporadic breast cancer patients (mean age: 42 $\pm$ 9 years) and one hundred relatives with no signs or symptoms of malignancy (mean age: 43 $\pm$ 8 years) were selected. They either have no family history for breast cancer or have an early onset or bilateral breast cancer. The subjects were recruited from various surgery and Oncology Clinics at Sharkia Governorate, Egypt. The study was approved by the ethical committee of Faculty of Medicine, Zagazig University, Egypt and a written informed consent for the experimental use of specimens was obtained from all subjects. All participants were informed about benefits and importance of the genetic testing. The aim of the study was clarified and explained to all subjects as a trial for early prediction of cancer breast especially in families that did not have previous family history taking. Patients and relatives were subjected to full clinical examination and detailed family history. History of age of menarche, menopause, marital status, parity, age at 1st delivery and breast feeding were collected for statistical analysis after genotyping analysis. Cases with cancer were subjected to modified radical mastectomy procedure after keeping their records for further correlation between the incidence of mutations and the response to surgical treatment, chemotherapy and radiotherapy.

DNA Preparation:

EDTA peripheral blood samples (3 ml) were obtained from patients and relatives, coded and analyzed in a blind manner for genomic DNA extraction using Promega genomic DNA extraction kit (Promega, Madison, USA) as described in the user manual. The quality of the genomic DNA was tested using agarose gel electrophoresis.

Multiplex PCR:

To study the genotypes of 5382insC and 185delAG mutations in BRCA1 and 6174delT mutation in BRCA2, a Multiplex polymerase chain reaction was performed with allele-specific primers as described by (13). For each allelic mutation three primers (one common, one specific for the wild type allele, and one specific for the mutant allele) were used (Table 1). The PCR reactions were performed in 25 μl reaction containing 100 ng of genomic DNA, 1X PCR mix (20 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.3 mM dNTPs and 2U Taq DNA polymerase) and 2 μM for P1 and P3, 0.4 μM for P2, 0.12 μM for P4, P5 and P6, 0.3 μM for P7 and P9, and 0.24 μM for P8 primers. The reaction mixtures were heated at 94 ^{0}C for 5 min followed by 35 cycles of amplification, each consisting of 40 sec of denaturation at 94 ^{0}C, 40 sec, of annealing at 55 ^{0}C, and 60 sec of extension at 72 ^{0}C, followed by final extension for 5 min at 72 ^{0}C. The amplified products were resolved by electrophoresis in a 3% agarose gel, visualized using gel documentation system and analyzed visually for the presence or absence of the specific bands.

Table 1: Primers sequence and amplicon size for BRCA 1 and BRCA2 sites

Primer name	*Sequence of the Primers*	*Size (bp)*
BRCA1 185delAG		
Common Forward (P1)	5'GGTTGGCAGCAATATGTGAA3'	
Wild Type reverse (P2)	5'GCTGACTTACCAGATGGGACTCTC3'	335
Mutant Reverse (P3)	5'CCCAAATTAATACACTCTTGTCGTGA CTTACCAGATGGGACAGTA3	354
BRCA1 5382insC		
Common Reverse (P4)	5'GACGGGAATCCAAATTACACAG3'	
Wild Type Forward (P5)	5'AAAGCGAGCAAGAGAATCGCA3'	271
Mutant Forward (P6)	5'AATCGAAGAAACCACCAAAGTCC TTAGCGAGCAAGAGAATCACC3'	295
BRCA2 6174delT		
Common Reverse (P7)	5'AGCTGGTCTGAATGTTCGTTACT3'	
Wild Type Forward (P8)	5'GTGGGATTTTTAGCACAGCTAGT3'	151
Mutant Forward (P9)	5'CAGTCTCATCTGCAAATACTTCAG GGATTTTTAGCACAGCATGG3'	171

Serum levels of CEA and CA15.3 were measured by an enzyme-linked immunosorbent assay (ELISA) using ELISA commercial kit according to the manufacturer's instructions (DRG Diagnostics, GmbH, Germany). The assay employs the quantitative sandwich enzyme immunoassay technique.

Statistical analysis:

Statistical Analysis was performed using SPSS-11 Software

package to determine the Chi square, student unpaired (t) test and other statistical parameters.

RESULTS

Multiplex allele-specific PCR was used for simultaneous detection of 5382insC and 185delG in BRCA1 gene and 6174 in BRCA2 gene, to investigate the frequency of these mutations among Eastern Egyptian women in Sharkia Governorate, Egypt. The study involved sporadic breast cancer patients and their relatives (Table 2) and subjected for multiplex PCR analysis.

Table 2: Characterization of patients and controls included in the study

Character	*Patients (N=100)*	*Controls (N=100)*
Age at onset	42 ± 9	43 ± 8
Age of menarche	14 ± 2	13 ± 2
Family history of breast cancer	No	No
Family history for different cancer	1	1
Parity %	90	80
Age at 1st delivery	25 ± 5	26 ± 4
Breast feeding %	95	96
Menopause %	35	30
Menopause age	45 ± 5	44 ± 5

The mutant amplicons were different from that of the wild type by 20 bp that were included in the primer. For the 185delAG mutation, 5382insC and 6174delT, the mutant and wild type amplicons are 354 and 335, 295 and 271, 171 and 151, respectively. A minimum of three bands were detected in absence of any mutant alleles whereas, a maximum of six bands were observed in presence of all three mutations. Analysis of PCR products showed a low incidence for 185delAG mutation as 3% of that mutant was detected in both patients and relatives whereas mutations 6174delT and 15382insC were not detectable in the present study (figure 1).

The level of carcinoembryonic antigen (CEA) and cancer antigen 15-3 (CA15-3) tumor markers were assayed and used to correlate the mutation expression associated with their serum levels (Table 3).

Table 3: The serum levels of CEA and CA15.3 in breast cancer patients and in the control subjects.

Parameters	*Patients*	*Control*	p-*value*
CEA(ng/ml)	6.7 ± 2.2	2.8 ± 0.3	*P*<0.001
CA15.3(IU/L)	66.24 ± 23	2.8 ± 0.4	*P*<0.001

*=*P*<0.001 by student *t* test; CEA= carcinoembryonic antigen; CA15.3= Cancer antigen 15.3.

In the breast cancer patients the CEA ranged 4.5 to 8.9 ng/ml, with a mean value ± SD (6.7± 2.2 ng/ml), while in the control group, the CEA ranged 2.5-3.1 ng/ml, with a mean value of 2.8± 0.3 ng/ml (Table 3), There was significant difference in CEA levels (P<0.001) comparing patients to relatives control, whereas, no correlation between the level of CEA and the frequency of any of the three studied mutations was found. Regarding to CA15.3, there was significant difference between breast cancer cases and the control group (P<0.001), as the mean level of CA15.3 ± SD in the patients were 66.24± 23 IU/l, with a range of 43-89 IU/l, while in the control group CA15.3 ranged 2.5 to 3.2 IU/l, with a mean value ± SD of 2.8± 0.4 IU/l (Table 3). There was also no correlation between the level of CEA and the frequency of any of the three studied mutations.
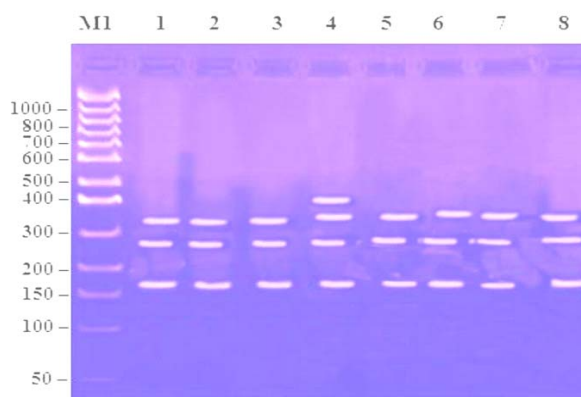
Fig. 1: Gel electrophoresis for the 185delAG mutation, 5382insC and 6174delT, the mutant and wild type amplicons are 354 and 335, 295 and 271, 171 and 151, respectively. Lane M: marker A, Lane 1,2,4,5,6,7, three bands were detected in absence of any mutant alleles .lane 4.additional mutant A allele (354bp) of 185delAG.

DISCUSSION

BRCA1 and BRCA2 genes are needed for the homologues recombination repair pathway which identifies and repairs DNA – strand breaks caused by chemical or physical agents, so mutations of these genes are often found in cases of familial breast cancer. Nearly 5-10 % of all breast cancer patients are familial, with earlier onset while others are sporadic. The variation of germline mutation in BRCA1 or BRCA2 gene in breast cancer depends on the ethnicity of studied population and familial versus sporadic definition.

A large number of different mutations in the BRCA1 and BRCA2 genes have been reported worldwide, but little is known about their frequency in Eastern Egypt. The current study was to detect the role of

these two susceptibility genes in breast cancer in Eastern Egyptians.

In the present study, we found that the 185delAG mutation in BRCA1 was present in 3% of the Eastern Egyptian population, whereas, the 5382insC of BRCA1 and the 6174delT of BRCA2 were not detected in our study groups.

A similar study was performed in Alexandria, Egypt for breast cancer patients under 40 years old with bilateral breast cancer and positive family history to find the frequency of the BRCA1, 185delAG mutation in Alexandria population[14].

They found a higher frequency of the 185delAG mutation (10% compared to 3% in our study). A second study was done in Alexandria and also show a higher expression of the 185delAG founder mutation[15]. This difference may be explained on the basis that their selection of patients with higher probability for familial breast cancer which was different from our selection criteria for patients of sporadic breast cancer and their relatives (Table 2). A similar study that show a low frequency of the 185delAG founder mutation was carried out on Iranian patients, where they found that a percent of 1.2 of 185delAG mutation in BRCA1 gene[16]. Other studies on Iranian also could not detect any of the three mutations in their population[17]. In that study, they did not identify the ethnicity of the two sisters that carry the 185delAG mutation[16].

In addition, there are several studies reporting these founder mutations in other populations[7,18,19]. The Ashkenazi Jewish breast cancer patients showed the heights

prevalence of the 185delAG mutation in BRCA1 gene[12].

There was significant difference in CEA and CA15.3 levels when patients were compared to healthy relative controls. On the other hand, there was no correlation between the level of CEA or CA15.3 and the frequency of any of the three studied mutations in our study groups.

To our knowledge no other studies were performed to determine the 5382insC in BRCA1 or the 6174delT in BRCA2 genes in Egyptian population.

Our data and the data from Alexandria collectively indicate a significant frequency of the 185delAG mutation and no detection of the 5382insC in BRCA1 or the 6174delT in BRCA2 genes in Egyptian population. Thus, there may be other sites in the BRCA genes or other genes that may be significant to development of breast cancer in Egyptian population. Special attention and further studies for the 185delAG mutation should be considered.

Hence our study involved only random sample of Eastern Egyptian Population from Sharkia, we recommend more studies in other parts of Egypt that involve sporadic cases and their relatives. We also recommend performing the study in familial breast cancer after diagnosis of such case and scanning of BRCA1 and BRCA2 genes for diagnostic mutations.

CONCLUSION

The low percentage of the 185delAG mutation in BRCA1 and the non-detection of other mutations

in apparently sporadic early- onset cancer suggested that early onset cancer alone is insufficient to justify screening in the Egyptian population.

Our study suggested that the prevalence of BRCA 1 and BRCA 2 mutations are lower in Eastern Egyptian. So, complete BRCA 1 and BRCA 2 genes sequence analysis might be required for identification of specific mutation in Egyptian. Furthermore, there might be other genes that contribute more significantly to familial breast cancer in this population than BRCA genes.

REFERENCES

1. **Marcus J.N., Watson P., Page D.L., Narod S.A., Lenoir G.M., Tonin P., Linder-Stephenson L., Salerno G., Conway T.A., Lynch H.T (1996):** Hereditary breast cancer. pathobiology, prognosis, and BRCA1 and BRCA2 gene linkage, Cancer 77 : 697-709.

2. **Parker S.L., Tong T. , Bolden S. , Wingo P.A (1997):** Cancer statistics 1997 , CA Cancer J. Clin. , 47:5-27.

3. **Shattuck-Eidens D., McClure M., Simard J., Labrie F., Narod S., Couch F. , Hoskins K., Weber B. , Castilla L., Erdos M (1995):** A collaborative survey of 80 mutations in the BRCA1 breast and ovarian cancer susceptibility gene. Implications for presymptomatic testing and screening, Jama 273 : 535-541.

4. **Omar S. , Khaled H. , Gaafar R. , Zekry A.R. , Eissa S. , el-Khatib O (2003):** Breast cancer in Egypt: a review of disease presentation and detection strategies, East Mediterr Health J., 9 : 448-463.

5. **Collins N., McManus R., Wooster R., Mangion J., Seal S., Lakhani S.R. , Ormiston W., Daly P.A. , Ford D., Easton D.F (1995):** Consistent loss of the wild type allele in breast cancers from a family linked to the BRCA2 gene on chromosome 13q12-13, Oncogene 10 : 1673-1675.

6. **Smith S.A., Easton D.F. , Evans D.G., Ponder B.A (1992):** Allele losses in the region 17q12-21 in familial breast and ovarian cancer involve the wild-type chromosome, Nat Genet 2 : 128-131.

7. **FitzGerald M.G., MacDonald D.J., Krainer M., Hoover I., 'Neil E.O., Unsal H., Silva-Arrieto S., Finkelstein D.M., Beer-Romero P. , Englert C., Sgroi D.C., Smith B.L., Younger J.W., Garber J.E., Duda R.B., Mayzel K.A., Isselbacher K.J., Friend S.H. , Haber D.A (1996):** Germ-line BRCA1 mutations in Jewish and non-Jewish women with early-

onset breast cancer, N. Engl. J. Med. , 334 : 143-149.

8. **Roa B.B., Boyd A.A., Volcik K., Richards C.S (1996):** Ashkenazi Jewish population frequencies for common mutations in BRCA1 and BRCA2, Nat Genet 14: 185-187.

9. **Ford D., Easton D.F., Peto J (1995):** Estimates of the gene frequency of BRCA1 and its contribution to breast and ovarian cancer incidence, Am. J. Hum. Genet., 57 : 1457-1462.

10. **Wooster R., Bignell G., Lancaster J., Swift S. , Seal S. , Mangion J., Collins N., Gregory S., Gumbs C., Micklem G (1995):** Identification of the breast cancer susceptibility gene BRCA2, Nature 378 : 789-792.

11. **Struewing J.P., Abeliovich D., Peretz T., Avishai N., Kaback M.M., Collins F.S., Brody L.C (1995):** The carrier frequency of the BRCA1 185delAG mutation is approximately 1 percent in Ashkenazi Jewish individuals, Nat Genet 11:198-200.

12. **Abeliovich D., Kaduri L., Lerer I., Weinberg N., Amir G., Sagi M., Zlotogora J., Heching N., Peretz T (1997):** The founder mutations 185delAG and 5382insC in BRCA1 and 6174delT in BRCA2 appear in 60% of ovarian cancer and 30% of early-onset breast cancer patients among Ashkenazi women, Am. J. Hum. Genet. , 60 : 505-514.

13. **13-Chan P.C., Wong B.Y., Ozcelik H., Cole D.E (1999):** Simple and rapid detection of BRCA1 and BRCA2 mutations by multiplex mutagenically separated PCR, Clin. Chem. , 45 : 1285-1287.

14. **El Gezeery A., Mahmoud N., Moustafa A., Mahrous H., Mahmoud H., Abd El-Menam N (2008):** BRCA1 gene mutation in familial breast cancer, Turkish J. of Cancer 38: 167-174.

15. **Ibrahim S.S., Hafez E.E., Hashishe M.M (2010):** Presymptomatic breast cancer in Egypt: role of BRCA1 and BRCA2 tumor suppressor genes mutations detection, J. Exp. Clin. Cancer Res. , 25: 29- 82.

16. **Mehdipour P., Hosseini-Asl S., Savabi-E A., Habibi L., Alvandi E., Atri M (2006):** Low fequency of 185delAG founder mutation of BRAC1 gene in Iranian breast cancer patients, J. cancer Molecules 2 : 123-127.

17. **Fattahi M.J., Mojtahedi Z., Karimaghaee N., Talei A.R. , Banani S.J. , Ghaderi A (2009):** Analysis of BRCA1 and BRCA2 mutations in southern Iranian Breast cancer patients, Arch. Iran. Med., 12 : 584-587.

18. **Peelen T. , van Vliet M. , Petrij-Bosch A. , Mieremet R. , Szabo C., van den Ouweland A.M., Hogervorst F., Brohet R., Ligtenberg M.J., Teugels E., van der Luijt R. , van der Hout A.H., Gille J.J., Pals G., Jedema I., Olmer R., van Leeuwen I. , Newman B. , Plandsoen M., van der Est M. , Brink G. , Hageman S. , Arts P.J. , Bakker M.M. , Devilee P (1997):** A high proportion of novel mutations in BRCA1 with strong founder effects among Dutch and Belgian

hereditary breast and ovarian cancer families, Am. J. Hum. Genet., 60:1041-1049.

19. **Sokolenko A.P. , Mitiushkina N.V. , Buslov K.G. , Bit-Sava E.M., Iyevleva A.G., Chekmariova E.V., Kuligina E., Ulibina Y.M., Rozanov M.E., Suspitsin E.N., Matsko D.E. ,** **Chagunava O.L., Trofimov D.Y. , Devilee P. , Cornelisse C. , Togo A.V. , Semiglazov V.F. , Imyanitov E.N (2006):** High frequency of BRCA1 5382insC mutation in Russian breast cancer patients, Eur. J. Cancer 42 : 1380-1384.

تحليل طفرات BRCA1 و BRCA2 فى مرضى سرطان الثدى فى المصريين

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إن مولد الطفرات الجينيه لقابليه حدوث سرطان الثدى (BRCA1) و (BRCA2)توجد بنسبه عاليه فى مرضى سرطان الثدى .لقد وجد ثلاث طفرات 185delAG و 5382insC فى BRCA1 وفى 6174delT BRCA2بنسبه متكررة فى مرضى سرطان الثدى من عرقيات مختلفه.

يهدف هذا البحث لتقييم نسبة حدوث طفرات BRCA1 و BRCA2 فى مرضى سرطان الثدى المصريين وفى أقاربهم وفى إمكانية إستخدام هذه الطفرات كدلالات محتمله لتشخيص المرض.

إشتملت هذه الدراسه على مائة مريضه بسرطان الثدى ومائة أخرين من أقاربهم من الدرجه الأولى .وقد أستخدم التفاعل المتسلسل البوليمرى المركب فى تحليل ال دي إن ايه من الدم لهؤلاء المريضات وأقاربهن.

بتحليل النتائج وجد ان طفرة 185delAG فى جين BRCA1 كانت قليلة الحدوث (3%) فى حين ان طفرة 5382insCفى BRCA1 و 6174delT فىBRCA2 لم يتم إكتشافهما فى المرضى المصريين.

من هذه الدراسة وجد أن النسبه الضئيلة لحدوث الطفرات فى جينات BRCA1 و BRCA2 فى مرضى سرطان الثدى وأقاربهن غير كافية لمسح وإكتشاف المرض فى المرضى المصريين.