Original article

Molecular Analysis of Breast Cancer 1 (BRCA1) Gene Mutations (5382insc and 185delag) and its Detection in Libyan Women with Breast Cancer

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Abstract
Breast cancer remains a common cancer-related mortality in females aged 20 to59 years worldwide and is estimated that more than one million women have been diagnosed every year BRCA1and BRCA2 are two important tumor suppressor genes. Breast cancer is associated with mutations in the coding exons in the BRCA1 gene like 185delAG and 5382insC mutations. Our aim of study is to identify new mutations in BRCA1 gene in Libyan population and may contribute to the knowledge on the risk factors of tumor suppressor gene mutations which allows Libyan clinicians to assess whether an individual patient is at high risk. A total of 77 Libyan females were genetically analyzed by simple and rapid detection of 185delAG and 5382insC mutations in BRCA1 gene by multiplex mutagenically separated by polymerase chain reaction (MS-PCR) in exons 2 and 20. Whereas is heterozygous alleles of germ line mutation in the BRCA1 gene is at high risk for the development of breast cancer. Blood samples were collected from 64 Libyan Women attending the breast clinic, Surgery Department, Central Hospital, Tripoli-Libya and 13 normal females were used. The blood samples were transported to DNA Laboratory in Biotechnology Research Center, Twesha, Libya. Of the Studied 6 patients had a family history of breast cancer, 26 don't have a family history of breast cancer, 4 had a family history of Benign Breast Disease, 4 don't have a family history of familial of Benign Breast Disease and 24 had a relative with breast cancer. The results of this study showed the presence of 185delAG mutation as high as 81.25% of the subjects and The5382insC mutation is absent in Arab Libyan women.The conclusion of this study is the detection of 185delAG mutation and the absence of 5382insC mutation in BRCA1 gene.

Key words: BRCA1 gene; 185delAG; 5382 insC mutation; breast cancer; Molecular analysis

Introduction

Cancer is a multifactorial disease involving interaction of environmental, hormonal and dietary risks in addition to genetic predispositions. Breast cancer is the second leading cause of cancer death among women and the breast cancer (BRCA) is an extremely common malignancy, affecting 1 in 8 women during their lifetime (Kenneth *et al*.1998; Piril Welch *et al*. 2001; Rosen *et al*. 2003) genetic susceptibility has been estimated to contribute to 5-10% of all BRCA cases.

Mutations in BRCA1 are inherited in an autosomal dominant pattern but it is a recessive disease (Roy *et al*. 2012). BRCA1gene was identified in 1990, is located on the long arm of chromosome 17. The gene was mapped to chromosome band 17q12-21) and cloned in 1994. More than 300 different germ line mutations in BRCA1 gene have been identified. BRCA1 contains 24 exons, this large gene has 22 coding exons and 2 non-coding exons distributed over 100kb of genomic DNA. BRCA1gene is composed of 5592 nucleotides and codes for 1863 amino acids (Atoum *et al*.2004; Ghaderi *et al*.2001; Ganguly *et al*. 1997). Specific mutations in BRCA1 are common among certain geographic or ethnic groups. For example, two BRCA1 mutations (185delAG and 5382insC) are most common in people of Ashkenazi (central or eastern European) Jewish heritage. Other mutations are more common among other ethnic groups (Struewing *et al*. 1995). The purpose of this study is to identify 185delAG and 5382 mutations of Breast Cancer 1 (BRCA1) gene in Libyan females with breast cancer attending the breast clinic, Surgery Department, Central Hospital, Tripoli- Libya.

Materials and methods

Blood sample collection

Blood samples were collected from 64 attending the breast clinic; Surgery Department, Central Hospital, Tripoli-Libya and 13 normal females were used as a normal control within the period of two years. Five ml of blood were drawn from each woman and collected in tubes containing EDTA anticoagulant and kept in freezer (-20 ºC). The blood samples were transported to DNA Laboratory, Human's Tissues and the Genetic

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DNA Extraction

DNA was extracted from whole blood by a QIAamp® (QIAamp® DNA Mini Kit 2004), a DNA mini Kit for DNA extraction and purification (QIAgen). DNA concentration was measured by spectrophotometer at wavelength of 260nm. DNA was visualized by 3% agarose gel electrophoresis.

Detection of mutations by Mutagenically Separated Polymerase Chain Reaction (MS-PCR) and Agarose gel electrophoresis

PCR amplification was carried out using thermocycler in 25µl PCR reaction, 2 µl of genomic DNA (100ng) was added to 23 µl of reaction mixture consisting of 1× PCR reaction buffer (10mmol/L Tris-HCl, pH 8.3, 50mmol/L KCl, 0.01 g/L gelatin), 3.25 mmol/L MgCl2, 0.2 mmol/L dNTPs, and 0.5 U/ µlTaq polymerase. Allele specific primers were added at 2.0 µmol/L for P1 and P3, 0.4 µmol/L for P2 (Table 1).

Each PCR reaction consisted of an initial 12 min of Taq polymerase activation at 95 ºC followed by 35 cycles of 15 Sec of denaturation at 94 ºC , 15 Sec of annealing at 57ºC and 30 Sec of extension at 72 ºC and final extension step of 5 min at 72 ºC. At the conclusion of the reaction, the PCR product was mixed with 2 µl of loading dye (bromophenol blue) and separated on 3% agarose gel electrophoresis (120V, 45min). The resolved amplicons were then stained with 0.5mg/L ethidium bromide and visualized under ultraviolet illumination (Westermeier, 1997).

Allele specific oligonucleotides primers for MS-PCR assay designed on the basis of published BRCA1 sequence (Serena *et al* 2012) and checked against the NIH database for possible false priming. For each mutation, three primers (one common, one specific for the mutant allele and one specific for the wild type allele) were used. The competing mutant and wild type primers were designed to differ by ~ 320 bp in size, allowing easy detection of PCR products by routine electrophoresis and ultraviolet illumination after ethidium bromide staining. The mutant (long) and wild-type (short) primes both contain a mismatched sequence base sequence near the 3' end. In the early cycles of amplification, the mismatched sequences generate mutagenized PCR products that are refractory to cross-amplification by the competing primer, thereby ensuring specificity of the reaction. The long (mutant) primer also incorporates two additional mismatched bases at two contiguous positions corresponding to the 5' end of the short (wild-type) primer. During the final cycle- of the PCR reaction, heteroduplexes may be formed from the short and long products, but the contiguous mutagenized sequences in the long product prevent filling up of the short product by using the long strand as template. As a result, the mutant and wild type products were separated mutagenically (Pak Cheung *et al.*, 1999). The primer sequences and sizes of corresponding amplicons are shown (Table1).

Table 1: Primers used to amplify exons of BRCA1 gene (Pak Cheung *et al.* 1999).

Exon	Mutation	Primer	Primer sequence	Size of Amplicon
2	185delAG	Common forward (P1)	5'-GGTTGGCAGCAATATGTGAA-3'	
		Wild-type reverse (P2)	5'-GCTGACTTACCAGATGGGACTCTC-3'	335bp
		Mutant reverse (P3)	5'CCCAAATTAATACACTCTTGTCGTGACTTACCAGATGGGACAGTA-3'	654bp
20	5382insC	Common forward (P4)	5'-GACGGGAATCCAAATTACACAG-3'	
		Wild-type reverse (P5)	5'-AAAGCGAGCAAGAGAATCGCA-3'	271bp
		Mutant reverse (P6)	5'AATCGAAGAAACCACCAAAGTCCTTAGCGAGCAAGAGAATCACC-3'	595bp

Results

In this study 64 Libyan females investigated for BRCA1 gene mutations we found that 6 had a family history of breast cancer, 26 without a family history of breast cancer, 4 had a family history of Benign Breast Disease and four did not have a family history of familial of Benign Breast Disease, 24 had a relative with breast cancer and 13 healthy females were used as control. All patients were genetically analyzed by simple and rapid detection of 185delA and 5382insC mutations in BRCA1 gene by multiplex mutagenically separated polymerase chain reaction (MS-PCR), if a mutation is present in one of the alleles, two bands will be present by agarose gel electrophoresis for mutation detection in exon 2 and 20. and sequencing by DNA Sequencing Capillary Electrophoresis from Applied Biosystem and analyzed by SeqScape Software, this software demonstrated that 185delAG mutation is present in 52/64 of the patients (Figure 1, and Table 2) and 5382insC mutation was absent in all studied samples (Table2). At the same time all 77 patients were run by mutagenically separated PCR (MS-PCR) and all results for the 5382C mutation shown negative (Figure 2).

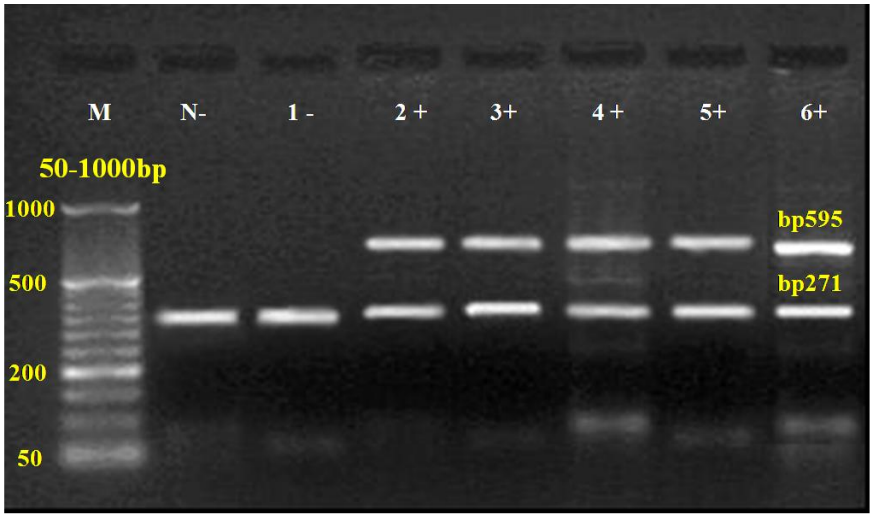

Figure 1: Electrophoretgram of MS-PCR products. Lane M, Maker; Lanes N and 1, wild type samples, lanes 2-6 patient samples with 185delAG;(N) normal case; (+) case with mutation; (−) case without mutation.

Table 2: Number of both controls and cases with BRCA1 gene mutations patients and healthy women with 185delAG and 5382insC in Libyan breast cancer women.

Groups	Ages	Total No. of cases	No. of cases with BRCA1 gene mutations	
			185delAG	5382insC
Group A	17-26	16	12	0
Group B	27-36	19	13	0
Group C	37-46	29	20	0
Group D	47-56	9	5	0
Group E	57-66	1	0	0
Group F	67-76	0	0	0
Group G	77-86	3	2	0
	Total	77 (patients and controls)	52	0

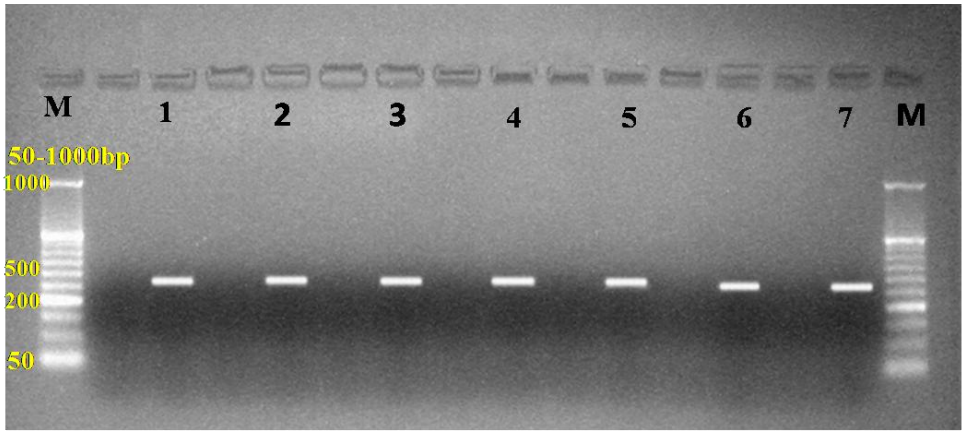

Figure 2: Electrophototogram MS-PCR products. Lane M, DNA maker, Lanes 1,2,3,4,5,6,7 wild type samples for the 5382 insC mutation.

Discussion

Breast cancer is the leading cause of cancer deaths in females worldwide occurring in both hereditary and sporadic forms. Breast cancer remains the second most common cause of cancer mortality in the western world (after lung cancer), despite ongoing efforts to understand its etiology and improve its outcomes (Ferlay *et al*. 2012). Women with inherited pathogenic mutations in the BRCA1 or BRCA2 genes have up to an 85% risk of developing breast cancer in their lifetime (Aloraifi *et al*. 2015). The important of role of BRCA1 in DNA double-strand breaks repair pathways *in vitro* has been well characterized and the double-strand breaks are the most cytotoxic forms of DNA damage (Pfeiffer *et al*.2004).

Literature on genetic predisposition of breast cancer showed that 185delAG mutation is limited in Arab women. The literatures reported that The 185delAG mutation is absent in Brazilian, Canadian, Tunisian, Iranian and Greek women (Ganguly *et al*. 1997; Gayther *et al*. 1997; Gorski *et al*. 2000; Kenneth *et al*. 1998; Rosen *et al*. 2003), whereas in Jordanian, Ashkenazi and Czech women is present (Atoum *et al*. 2004; Simard *et al*. 1994; Westermeier *et al*. 1997). The results of this study have showed that 185delAG mutation among Libyan women who has breast cancer without family history is present in 52/64 (81.25%) and absence of 5382insC mutation. So, screening of other exons than 2 and 20 among these breast cancer females may detect more mutations within Libyan women with breast cancer. Further DNA sequencing could be useful for pinpointing nucleotide changes among this breast cancer. This is an expected finding as the BRCA1 gene has a large coding sequence and screening whole gene may show other mutations. Previous reports had reported that more than 300 heritable mutations in the BRCA1 gene have been spread throughout the whole gene within different ethnic populations (Table 3). Arg841Trp, Phe486Leu and Asn550His were reported in Saudi Arabia (Denic *et al*. 2003; De Los Rios *et al*. 2001).

It has been found that 5382insC mutation present in Ashkenazi, Brazilian, Canadian, Polish, Czech, Russian, and Greek women (Gorski *et al*. 2000; Juliano *et al*. 2004;Zikan *et al*. 2005; Ladopoulou *et al*. 2002) (Table 4); whereas in Jordanian women is absent (Atoum *et al*. 2004). In this study 5382insC mutation was absent. So, farther screening of other exons and within intronic boundaries among these breast cancer females may detect more mutations within Libyan with breast cancer than 185delAG mutation. 1294del40 mutation and mutations in exon 11 of BRCA1 gene were detected in Arab Tunisian women, but 185delAG mutation was absent in Arab Tunisian women (Mestiri *et al*. 2000; Monastiri *et al*. 2002). The 185delAG mutation and other mutations in exon 11 were present in Arab Jordanian women (Atoum *et al*. 2004).Whereas Arab Palestine detect have 2482delGACT mutation in BRCA2 gene (Denic *et al*. 2003; El-Harith *et al*. 2003). Therefore more breast cancer families need to be screened in order to determine the types of mutations or polymorphism within the Libyan population, whereas divided to geographic or ethnic groups.

The identification of founder and recurrent mutation is an extremely important step towards the improvement of genetic counseling since molecular testing can be targeted to the founder and recurrent mutations allowing for a more rapid and less expensive test (Mahfoudh *et al*. 2012). The high frequency of founder mutations, allowing for analyzing a large number of cases, might provide accurate information regarding their penetrance. Furthermore, the evidence of differences in susceptibility and in age onset of cancer among carriers of a specific mutation could make it possible to define the role and importance of risk-modifying factors with the resulting improved disease management (Ferla *et al*.2007).

Table 3: Mutations in BRCA1 gene among different ethnic populations

Origin	5382insC	References
Arab Libyan Women	Absent	This Study
Arab Jordan Women	Absent	Atoum *et al*.(2004)
Brazilian Women	Present	Juliano *et al*.(2004)
Canadian Women	Present	Simard *et al*.(1994)
Czech Women	Present	Janatová *et al*.(2003)
Greek Women	Present	Ladopoulou *et al*.(2002)
Polish Women	Present	Gorski *et al*.(2000)
Russian Women	Present	Gayther *et al*.(1997)

Table 4: 5382 insC mutations in BRCA1 gene among different ethnic population.

Origin	Others Mutations in BRCA1 gene	538 2insC	185delAG	References
Arab Libyan Women	-	Absent	-	This Study
Arab Tunisian Women	1294del40 and exon11	-	Absent	Mestiri *et al.*(2000) and Monastiri *et al.* (2002)
Arab Jordan Women	Exon11	Absent	Present	Atoum *et al.* (2004)
Arab Egypt Women	Arg841Trp	-	-	El-Harith *et al.*(2003)
Iranian Women	Mutations in Other exons	-	Absent	Ghaderi *et al.*(2001)
Jewish Libyan Women	1100delAT	-	-	Gal *et al.*(2004)
Brazilian Women	Mutations in Other exons	Present	Absent	Juliano *et al.*(2004)
Canadian Women	Mutations in Other exons	Present	Absent	Simard *et al.*(1994)
Czech Women	1135delA in exon11	Present	Present	Janatová *et al.*(2003)
Greek Women	Mutations in Other exons	Present	Absent	Ladopoulou *et al.*(2002)
Arab Palestinian Women	2482delGACT in BRCA2	-	-	El-Harith *et al.*(2003)

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