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Molecular Detection of BRCA1 and BRCA2 Mutation in Breast Cancer Patients in Erbil Using PCR Techniques

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Abstract

Breast Cancer is one of the leading causes of death in women and It is known that one quarter of women are expected to develop breast cancer at some ages of their lives. Mutations of breast cancer susceptibility genes 1 and 2 (BRCA1 and BRCA2) are the most well recognized gene mutations responsible for an increased risk of developing breast cancer. This investigation was carried out on 50 patients (all were females) who were diagnosed as breast cancer patients attended Nanakali and Rzgari Hospital in Erbil. This study was planned to detect the probable occurrence of three founder breast cancer mutations in female patients by the use of PCR technique. The outcome of genetic analysis indicated that the percentage of 185del AG mutation was 50 (1 patients) whereas, the percentage of 5382ins C mutation was 50 (4 patients) in BRCA1 gene and the third mutation 6174del T in BRCA2 not detected in any patient from 50 samples. The study demonstrated that the frequency of BRCA1 mutation (10%) was higher than BRCA2 (0%) in this sample of Kurdistan women with breast cancer.

Introduction

Breast cancer is the most common form of cancer disease among women and the second cause of death in childbearing period. It was found that the incidence rates of breast cancer is higher in most of the developed areas (in Europe and America) than in Asia (Parkin*et al*., 2005). Breast tumor genesis is a multistep process; it starts with ductal hyper proliferation and progresses into *in situ*, then invasive, and finally metastatic carcinoma (Polyak*et al*.,2002).

Breast cancer susceptibility genes 1 (BRCA1) and breast cancer susceptibility genes 2 (BRCA2) are recorded that they play an important role in encoding for the key tumor suppressor proteins which prevent uncontrolled cell divisionby interacting witha number of important regulatory elements for DNA repair, transcription and cell cycle regulation. Germline mutations in these genes usually result in truncation or absence of the protein that confers up to a 90% lifetime risk of breast cancer in carrier females (FitzGerald *et al*., 1996; Roa*et al*., 1996). The BRCA1 gene was localized by (Hall *et al*., 1990) on chromosome 17 (locus 17q21) by a linkage analysis from breast cancer families, which was finally cloned by (Miki *et al*., 1994). BRCA1 is a large gene, consisting of 24 exons, spanning 81 Kb of DNA, and encoding a multifunctional protein that encompasses 1,863 amino acids and contains multiple domains: a RING finger domain, two

nuclear localization signals, and a tandem repeat of sequence elements near the C-terminus (Tavtigian, 1998). The *BRCA2* gene is approximately twice the size of *BRCA1* and mutations in *BRCA2* are associated with both female and male breast carcinoma susceptibility but are thought to have a lower age specific penetrance for breast and ovarian carcinoma than mutations in *BRCA1*(Struewing*et al*., 1997; Abeliovich*et al*., 1997; Roa*et al*., 1996; Levy-Lahad, 1997). Like BRCA1, the BRCA2 protein is believed to be involved in tumor suppression through its primary physiologic role in DNA repair (Chen *et al*., 1999; Wooster *et al*., 1995; Chen *et al*., 1998).Germline mutations in BRCA1 and BRCA2, account for the majority of breast and ovarian cancers in families with high-risk profiles, with carriers having a 26% to 84% lifetime risk of breast cancer and a 10% to 50% lifetime risk of ovarian cancer (Couch *et al*., 1997; Struewing*et al*., 1997; Ford *et al*., 1998; Warner *et al*., 1999; Anglian Breast Cancer Study Group; 2000; Risch*et al*., 2001; Antoniou *et al*., 2003). The distribution of BRCA mutations is considered to present significant differences between populations. One of the first reports showed that the BRCA1 185delAG, BRCA1 5382insC and BRCA2 6174delT are common in Ashkenazi Jews. Many examples of founder mutations of BRCA1 and BRCA2 genes have been reported in different populations such as in Iceland, Finnish, Dutch, and French families(Arason*et al*., 1998; Huusko*et al*., 1998; Peelen*et al*., 1997). In the east European Ashkenazi population approximately 40 and 29% of patients are reported with breast and ovarian cancer, respectively. These percentages were diagnosed before the age of 40 year (Abeliovich*et al*., 1997; Hartge*et al*., 1999; Warner *et al*., 1999).

The present study was planned to investigate at molecular level the probable occurrence of the three predominant mutant 185delAG and 5382insC (BRCA1) and 6174delT (BRCA2) ina number of Iraqi breast cancer patients.

Material and Methods

A. Sample Selection and Blood Collection

Selection of patients was mainly based on the following criteria: patients with breast cancer diagnosed under the age of 65 years; patients having a family history of breast cancer; patients having a previous personal history of breast cancer. In this experiment the 50 patients were divided into four groups depending on age as 25-35 years (Group A), 36-45 years (Group B), 46-55 years (Group C) and 56- years (Group D). Sample (5 ml) from peripheral blood was collected in EDTA tubes from patients attending in Oncology Department in Rizgari and Nanakaly Hospitals / Erbil-Iraq.

B. Molecular Analysis Experiments

DNA was extracted from the 50 blood samples representing genomic using EZHighTM DNA extraction kit according to supplier recommendations. For detection of 5382insC and 185delAG in BRCA1 and 6174delT in BRCA2, a multiplex PCR was performed with allele-specific oligonucleotide primers as described by (Chan *et al*., 1999).The primers in these experiments included one common, one specific for the mutant, and one specific for the wild-type allele (Table 1).

(Allele-Specific Polymerase Chain Reaction) ASPCR was performed in a 25 µl volume using 5x Red Load Hot Start Master (red cap) master mix of heat-activatable (Hot-Start polymerase) DNA polymerase, dATP, dCTP, dGTP, dTTP, KCl, $MgCl_2$, red dye (pH indicator dye), gel loading buffer and stablizers. The concentrations of primers used were 0.2 µM of each primer. The thermo cycler was programmed in a manner to start one initial denaturation step at 94 °C for 6 min (including polymerase activation); this initial step was followed by a 35 cycles each one consisted of denaturation for 30 sec at 94 °C, primer annealing for 30 sec at 54 Â°C, and followed by 30 sec extension at 72 °C, finally a 10 min extension step at 72 °C.

Genomic DNA and PCR Products were separated and analysis by electrophoresis on 2% agarose gel at5-8V/cm and then stained by a safe stain (Syber green).

Table -1: Oligonucleotide sequences of the primers used for detection of three founder mutations of BRCA1 and BRCA2 genes (Chan*et al*., 1999).

	Oligonucleotide primer sequence[a]	Size of amplified segment
BRCA1 185delAG		
Common forward (P1)	5'-ggttggcagcaatatgtgaa-3'	
Wild-type reverse (P2)	5'-gctgacttaccagatgggactctc-3'	335 bp
Mutant reverse (P3)	5'-cccaaattaatacactcttgtcgtgacttaccagatgggacagta-3'	354 bp
BRCA1 5382insC		
Common reverse (P4)	5'-gacgggaatccaaattacacag-3'	
Wild-type forward (P5)	5'-aaagcgagcaagagaatcgca-3'	271 bp
Mutant forward (P6)	5'-aatcgaagaaaccaccaaagtccttagcgagcaagagaatcacc-3'	295 bp
BRCA2 6174delT		
Common reverse (P7)	5'-agctggtctgaatgttcgttact-3'	
Wild-type forward (P8)	5'-gtgggatttttagcacagctagt-3'	151 bp
Mutant forward (P9)	5'-cagtctcatctgcaaatacttcagggatttttagcacagcatgg-3'	171 bp

Results and Discussions

A. Genomic DNA Analysis

The results revealed that genomic DNA was a good quality which was noticed asa single defined band with no smear (degradation). The concentration and purity were also found to be reasonable ranging between 80-110 ng/µl, 1.7-1.8 A 260/280 nm, respectively thus DNA was found to be suitable for further molecular biology experiments such as PCR amplification reactions.

B. PCR Amplification Experiments

The final PCR amplification results for all samples (1-10) tested on agarose gel electrophoresis. Like other PCR techniques, allele specific PCR or (Amplification Refractory Mutation System) ARMS technique required careful optimization of each reaction condition, including PCR master mix, magnesium concentration, dimethyl sulfoxide (DMSO), length and temperature of cycling stages. More importantly, quantity or of individual primers were also determined empirically to give optimum amplification.

The results of experiments involving the mutants under investigation in the different groups of samples produced different banding patterns; therefore, they will be described individually.

C. BRCA Mutations

I. BRCA1 Mutations

1. Mutant 185delAG

The results of these experiments involving Group A showed that all9 DNA samples to be normal homozygous (both alleles are normal) and no mutation was detected. Figure (1) shows the results of amplification with wild-type specific primers (p_1, p_2) gave 335bp bands size in the lanes 1WT, 2WT, 3WT, 4WT, 5WT, 335bp ASPCR product were detected due to the presence of wild type alleles in these samples. In the mutant type lanes (1MT, 2MT, 3MT, 4MT, 5MT) the fragment of 354bp was not detected due to absent of this mutation in these samples.

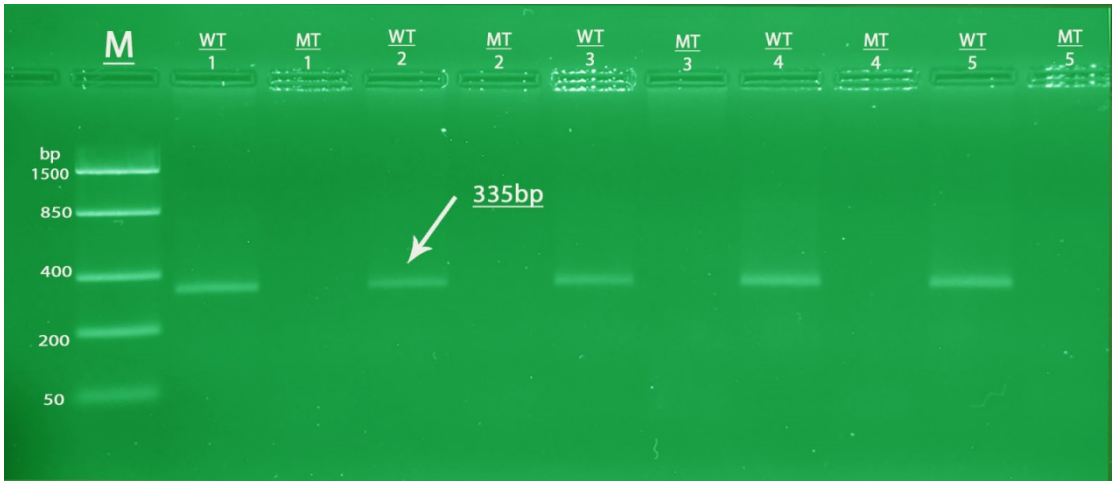

Figure -1: Represents amplification Products of BRCA1 Exon 2; wild-type alleles with 335bp by wild-type specific primers (p_1, p_2) with DNA samples in Group A. Electrophoresis was performed on 2% agarose gel and run with 5-8V/cm, for 2 hours. Lane M contained DNA molecular marker (1500-50 bp).

The mutant 185delAG was originally detected with a high frequency in Ashkenazi Jews (Friedman *et al*., 1995; Struewing*et al*., 1995). But in our study for Group A (25-35) year 185delAG mutant was not detected in this age. This result was agreed with (Trincado*et al*., 1999), who also did not detect the 185delAG mutation in 55 Chilean women affected with breast cancer, 15 of whom had a positive family history and 40 with sporadic breast cancer.In the last few years many studies have focused on screening of mutations in breast / ovarian cancer. Generally, breast cancer can occur at any age but younger women are less susceptible to ward's breast cancer (Mathew *et al*., 2004).

The results of these experiments for 185delAG in Group B samples showed again that from22 DNA samples the number of samples with normal homozygous were 22 and no mutation was found in these samples.

Figure (2) shows the wild-type fragments are in lanes (1WT, 2WT, 3WT, 4WT, 5WT). Mutant fragments are not present in lanes (1MT, 2MT, 3MT, 4MT, 5MT) due to absent of mutation or mutant fragments 354bp in these lanes.

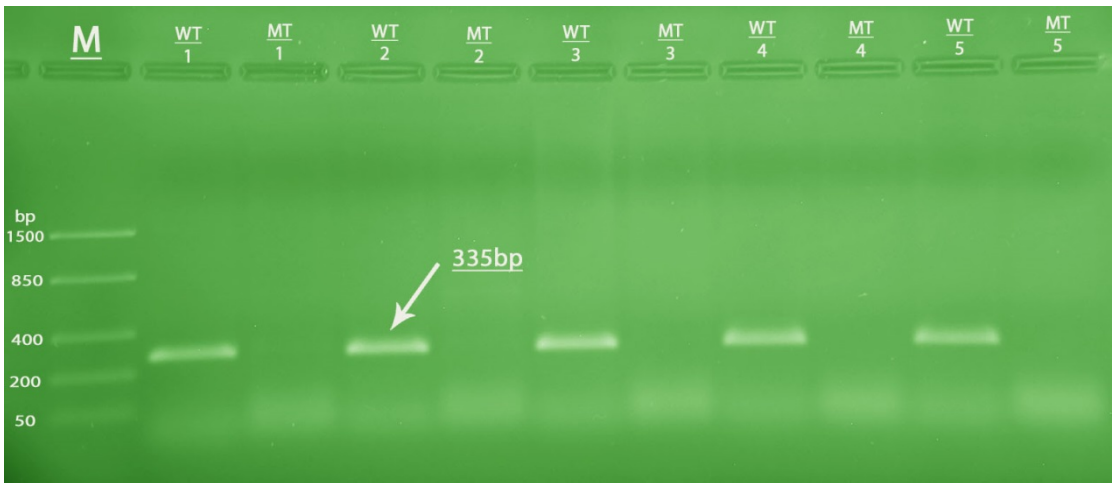

Figure -2: Represents amplification Products of BRCA1 Exon 2; wild-type alleles with 335bp by wild-type specific primers (p_1, p_2) with DNA samples in Group B. Electrophoresis was performed on 2% agarose gel and run with 5-8V/cm, for 2 hours. Lane M contained DNA molecular marker (1500-50 bp).

The results of these experiments agreed with research reported in China by (Ikeda *et al*., 2001; Zhi*et al*., 2002). These studies also proved that 185delAG mutation was not found among Chinese and Japanese families with breast cancer. However, it has been reported that 185delAG mutation occurs at different frequencies among families with breast / ovarian cancer in different populations (Mullineaux*et al*., 2003).

The results of 185delAG mutant experiments involving Group C showed that from12 DNA samples the number of normal homozygous were 11 and the number of sample with no mutation was only 1.

Amplification with wild-type specific primers (p_1, p_2) gave 335bp bands (Figure 3), according to bands size; it represents the normal exon sequence size without mutation (deletion of AG nucleotides) and amplification with mutant-type specific primers (p_1, p_3) gave 354bp bands (Figure 3), according to bands size; it represents the mutant exon sequence size with mutation (deletion of AG nucleotides). This Figure shows the lanes 1WT, 2WT, 4WT, 6WT, 335bp ASPCR product were detected due to the present of wild type alleles in these samples. In the lane 5MT the 354bp amplified PCR products were detected (presence of 185del AG mutation this sample).

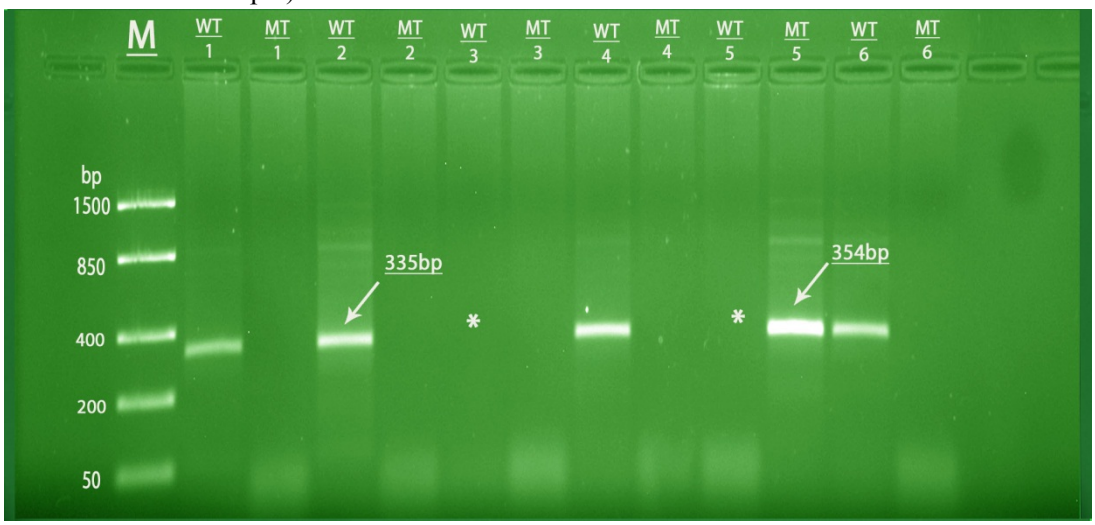

Figure -3: Represents amplification Products of BRCA1 Exon 2; including wild-type alleles with 335bp by specific primers (p_1, p_2) with DNA samples and mutant type alleles 354bp by (p_1, p_3) with DNA sample 5 in Group C. Electrophoresis was performed on 2% agarose gel and run with 5-8V/cm, for 2 hours. Lane M contained DNA molecular marker (1500-50 bp). Asterisks represent that DNA was not amplifiable.

Recent epidemiological studies have detected the 185delAG mutation in non-Jewish individuals in populations of other countries (Bar-Sade *et al*., 1998), including non-Jewish origin Spanish (Osorio *et al*., 1998; Diez*et al*., 1998; Diez*et al*., 1999). In the present study (Group C), the frequency of 185delAG mutation of exon 2 from BRCA1 was 8.33%. This result was found to be similar to previous studies reported by (Mehdipour*et al*., 2006) and (FitzGerald *et al*., 1996). They reported low frequency of the 185delAG founder mutation in BRCA1 gene in Iranian breast cancer patients.(El Gezeery*et al*., 2008) also found that the frequency of 185delAG mutation exon 2 from BRCA1 is 10% which was close to this study. The variation in the frequency may be attributed to the ethnic differences. These results also agreed with studies reported by (Grzybowsk*et al*., 2002; Shih *et al*., 2002; Gurane*t al*., 2005; Weitzel*et al*., 2005; Mehdipour*et al*., 2006; Rashid *et al*., 2006). They all proved that this mutation found to occur at a varying low frequency among white American, the Spanish from Spain, Polish Iranian, Pakistani and Turkish women.

The first report to prevail a frequency of 0.26 % for this mutation in a group of 382 healthy Chilean women having least two relatives with breast cancer was submitted by (Jara*et al*., 2002). In the previous study two families were carriers of the 185delAG mutation, and individuals of these families reported no Jewish ancestors. (Ah Mew *et al*., 2002) identified the 185delAG mutation in a non-Jewish Chilean family. Therefore, the existence of this mutation in the current admixed Chilean population may have been brought by the Spanish settlers. Nevertheless this mutation has not yet been studied in the remnants of the few Amerindians that still remain geographically isolated in some regions of the country.

In Lane 5WT 335bp fragment not detected due to Hemizygous (one allele is missing), this result agreed with previous studies which almost invariably shown LOH at BRCA1/2 loci in breast tumors from germ-line mutation carriers (Smith *et al*., 1992; Neuhausen*et al*., 1994; Collins *et al*., 1995) and frequently in sporadic breast tumors (Phelan *et al*., 1998; Rio *et al*., 1998), less is known about the mechanisms behind AI or LOH

at BRCA1/2.The relationship between AI and actual gene copy numbers of BRCA1and BRCA2 by FISH was studied in a set of hereditary breast cancers derived from both germ-line BRCA1 and BRCA2 mutation carriers (Smith *et al*., 1992; Collins *et al*., 1995).

In lanes 3WT and 3MT neither 335bp nor 354bp ASPCR product was detected this may be due to that all germline mutations in the BRCA1 and BRCA2 genes were expected to occur in heterozygotes forms since mutant homozygotes could be lethal (Gowen*et al*., 1996).

The results of this study suggest that wild-type BRCA1 and BRCA2 alleles can be inactivated not only by physical deletions, but also by alternative mechanisms such as non-disjunction or somatic recombination, since (Allelic Imbalance) AI can be detected without any reduction (absolute or relative) in the gene copy number reviewed in (Meuth, 1990; Stanbridge, 1990; Levine, 1993). These alternative mechanisms leading to AI seem to be common in breast tumors especially from BRCA1 mutation carriers and at least present in BRCA2 tumors.

The results of these experiments for 185delAG in Group D samples showed that all 7 DNA samples were also normal homozygous and no mutation was found in any sample. Figure (4) shows the lanes 1WT, 2WT, 3WT, 335bp ASPCR product were detected due to the present of wild type alleles in these samples. In 1MT, 2MT, 3MT, 354bp fragment not detected due to absence of this mutation in these samples.

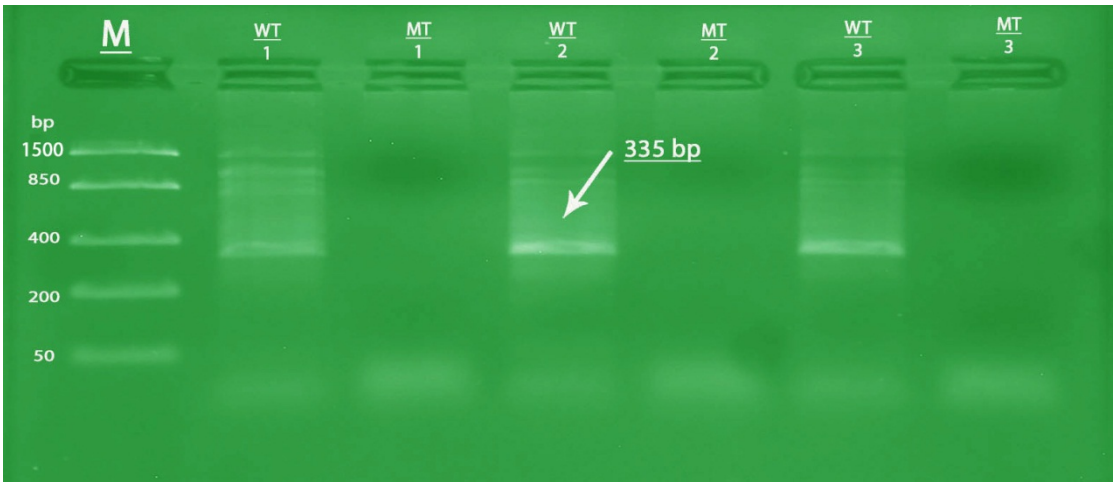

Figure -4: Represents amplification Products of BRCA1 Exon 2; wild-type alleles with 335bp by wild-type specific primers (p_1, p_2) with DNA samples in Group D. Electrophoresis was performed on 2% agarose gel and run with 5-8V/cm, for 2 hours. Lane M contained DNA molecular marker (1500-50bp).

The presence in higher frequency of mutation in the previous studies may be attributed the fact that those studies may have involved groups of very high-risk families. But in this study, the selection of the patients was randomly and these perhaps explain the absence of mutation in the samples. The lack of 185delAG mutation in BRCA 1 among the breast cancer patients may also be due to mutation in the non-coding region. Rare mutations in a number of other genes, such as CHEK2, ATM, BRIP1 and PALB1, which were considered as predispose to breast cancer (Easton *et al*., 2004; Renwick *et al*., 2006; Seal *et al*., 2006; Rahman*et al*., 2007) are more common variants in CASP8 and TGFβ1 (Cox *et al*., 2007).

2. Mutant 5382insC

The results of these experiment involving Group A showed that all 9 DNA samples to be normal homozygous (both alleles are normal) and no mutation was detected. Figure (5) shows the results of amplification with wild-type specific primers (p_4, p_5). In this figurea band of 271bp may be noticed in lanes (1WT, 2WT, 3WT, 4WT), this band was due to the presence of wild type alleles in these samples. In mutant type (1MT, 2MT, 3MT, 4MT) the fragments of 295bp was not detected due to absence of this mutation in these samples.

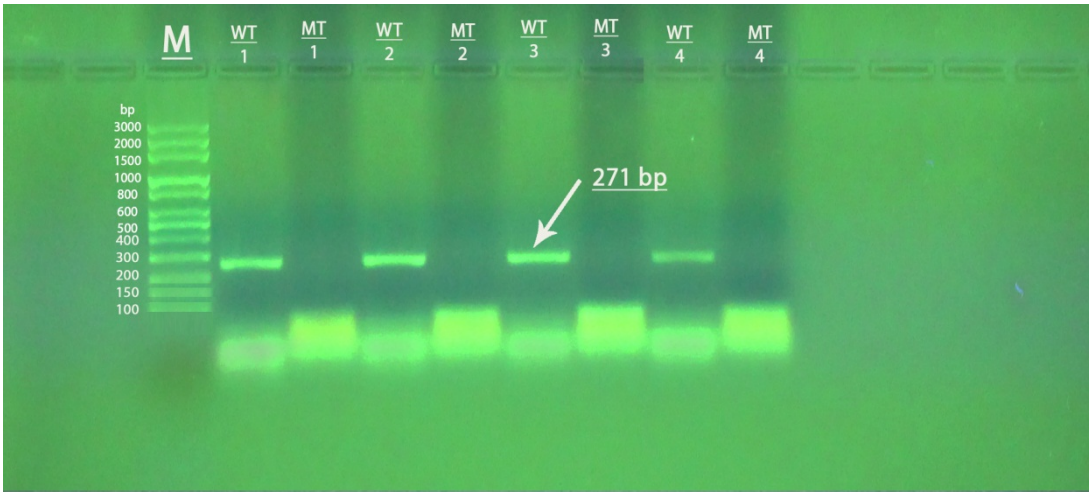

Figure -5: Represents amplification Products of BRCA1 Exon 20; wild-type alleles with 271bp by wild-type specific primers (p_4, p_5) with DNA samples in Group A. Electrophoresis was performed on 2% agarose gel and run with 5-8V/cm, for 2 hours. Lane M contained DNA molecular marker (3000-100 bp).

The result obtained here was found to be in agreement with a research studied in Southern Sweden, in which the 5382insC mutation was not identified in any of the 47 families with familial breast / ovarian cancer studied in the 106 families. Similar results were also reported in 25 families in Norway (Johannsson*et al*., 1996; Hakansson*et al*., 1996; Andersen *et al*., 1996). Scandinavian studies show a surprisingly low frequency (1%) in 100 finishes breast/ovarian cancer families (Vehmanen*et al*., 1997), this variation in the frequency may be attributed to the ethnic differences.

The results of these experiments for 5382insC in Group B samples showed that from22 DNA samples, the number of samples with normal homozygous were 21 and only one mutation was detected. Amplification with wild-type specific primers (p_4, p_5) gave a band of 271bp (Figure 6), which represented the normal exon sequence size without mutation (insertion of 5382insC nucleotide).

Figure (6) shows the wild-type fragments are in lanes (1WT, 2WT, 3WT, 4WT, 5WT). Mutant fragments are not present in lanes (1MT, 2MT, 3MT, 4MT, 5MT) due to absent of mutation or mutant fragments 295bp in these lanes.

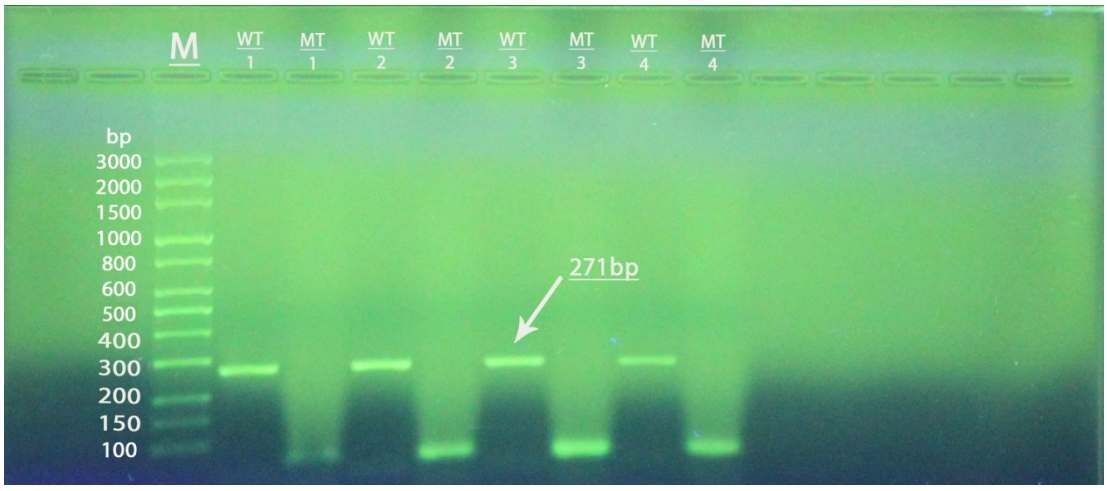

Figure -6: Represents amplification Products of BRCA1 Exon 20; wild-type alleles with 271bp by wild-type specific primers (p_4, p_5) with DNA samples in Group B. Electrophoresis was performed on 2% agarose gel and run with 5-8V/cm, for 2 hours. Lane M contained DNA molecular marker (3000-100 bp).

This result agreed with the study performed by (Atour and Al-Kayed, 2004) who revealed 6 different mutations in screened mutation with exon2, exon11 and exon 20 in 50 patients with breast cancer in Jordan. Five of these mutations were found among Jordanian females (within BRCA1 exon 2, 11 and 20) showing a

family history of breast cancer and have revealed the screened mutations were within BRCA1 exon 11 among these families.

In Figure (7) the wild-type fragments are noticed in lanes (1WT, 2WT, 3WT, 4WT, 5WT, 6WT). Mutant fragments are not present in lanes (1MT, 2MT, 3MT, 5MT, 6MT) due to absent of mutation or mutant fragments 295bp in these lanes, mutant type fragments are noticed in lane (4WT) due to presence of mutation in sample 4. This result agreed with the research studied by (Burcos*et al*., 2013) in Romania on 100 Romanian breast cancer patients among these, only 5382insC mutation in exon 20 of BRCA1 was found in three patients with breast cancer. This mutation determines a truncated BRCA1 protein that is believed to be more stable than the wild-type protein (Perrin-Vidoz*et al*., 2002).

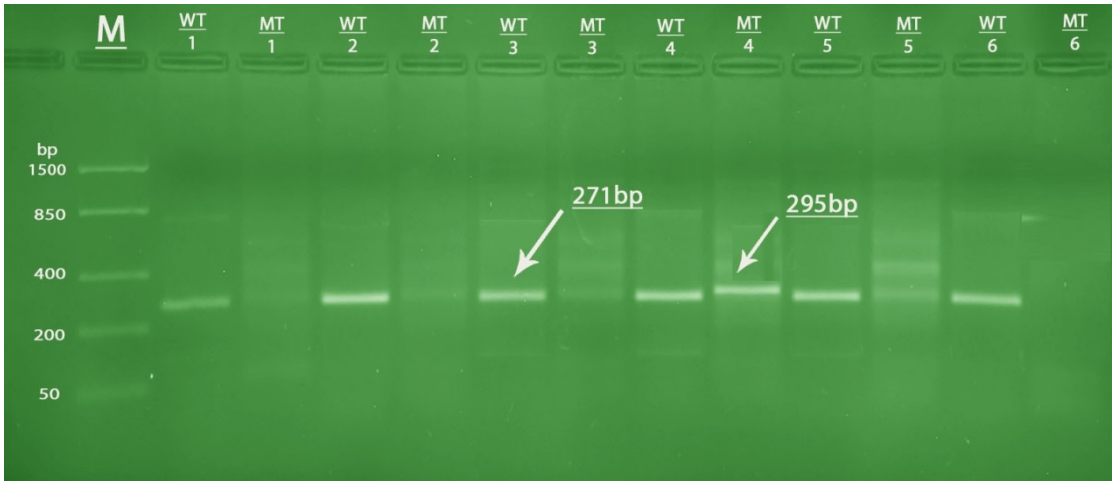

Figure -7: Represents amplification Products of BRCA1 Exon 20; including wild-type alleles with 271bp by wild-type specific primers (p_4, p_5) with DNA samples and mutant type alleles 295bp by mutant-type specific primers (p_4, p_6) with DNA samples in Group B. Electrophoresis was performed on 2% agarose gel and run with 5-8V/cm, for 2 hours. Lane M contained DNA molecular marker (1500-50 bp).

Figure (8) shows the lanes (1WT, 3WT, 4WT, 5WT), 271bp amplified ASPCR product were detected, but lane 2WT, which sign by * was negative for ASPCR reaction with wild type specific primers.

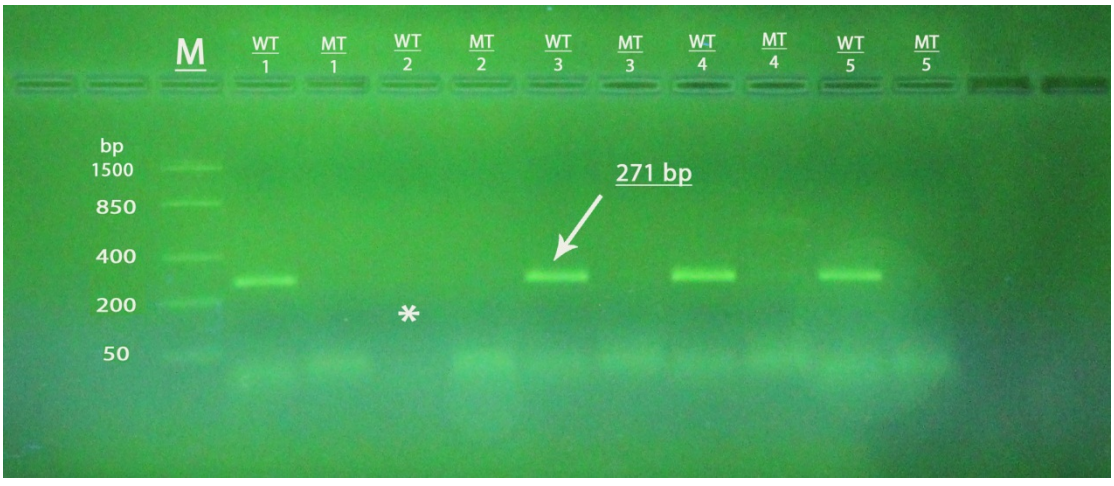

Figure -8: Represents amplification Products of BRCA1 Exon 20; wild-type alleles with 271bp by wild-type specific primers (p_4, p_5) with DNA samples in Group B. Electrophoresis was performed on 2% agarose gel and run with 5-8V/cm, for 2 hours. Lane M contained DNA molecular marker (1500-50bp). Asterisks represent that DNA was not amplifiable.

Our explanation for this case is this sample is Hemizygous (LOH), because all germline mutations in the BRCA1 and BRCA2 genes are expected to occur in heterozygotes as mutant homozygotes are lethal (Gowen*et al*., 1996) and consistent with this, none have been reported and other allele is normal. All

samples were negative for ASPCR reactions with mutant type specific primers, indicating the DNA was not amplifiable for this type of primers (Figure 8).

The results of 5382insC mutant experiments involving Group C showed that from 12 DNA samples the number of normal homozygous were 10 and the number of sample with mutation were 2.

Amplification with wild-type specific primers (p_4, p_5) gave 271bp bands (Figure 9), according to bands size; it represents the normal exon sequence size without mutation (insertion of 5382C nucleotide) and amplification with mutant-type specific primers (p_4, p_6) gave 295bp bands (Figure 9), according to bands size; it represents the mutant exon sequence size with mutation (insertion of 5382insC nucleotide).

Figure (9) wild-type fragments are noticed in lanes (1WT, 2WT, 3WT, 4WT). Mutant fragments are noticed in lanes (1MT, 2MT) due to presence of 295bp fragment in these lanes.

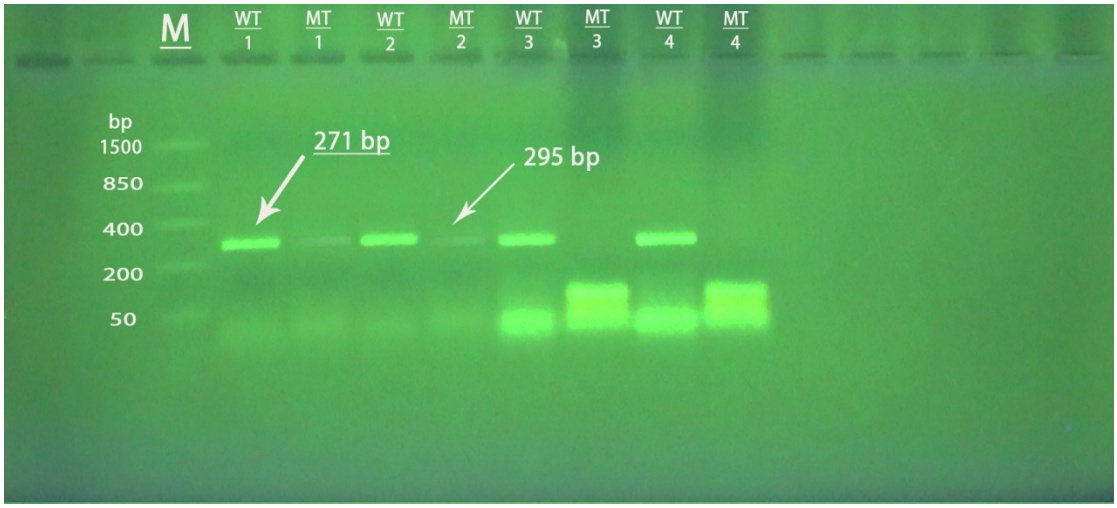

Figure -9: Represents amplification Products of BRCA1 Exon 20; including wild-type alleles with 271bp by wild-type specific primers (p_4, p_5) with DNA samples and mutant type alleles 295bp by mutant-type specific primers (p_4, p_6) with DNA samples in Group C. Electrophoresis was performed on 2% agarose gel and run with 5-8V/cm, for 2 hours. Lane M contained DNA molecular marker (1500-50bp).

In the present study it is clearly appear that BRCA1 5382insC was more frequency than BRCA1 185delAG. This result was in agreement with (Struewing*et al*., 1997) who concluded that the breast cancer risk was highest for the 5382insC mutation in BRCA1 and (Brose *et al*., 2002) found comparable results, with a higher breast cancer risk in BRCA1 5382insC carriers.

The frequency and distribution of BRCA1 5382insC is much higher in high risk families with breast / ovarian cancer and have significant differences between populations. A number of reports on analysis of BRCA1 5382insC suggested that the frequency depend on its geographical distribution (Simard*et al*., 1994; Ramus *et al*., 1997; Neuhausen, 2000) and in (1).

This mutation was thought to have been originally found in the Baltic area in the medieval period and which was the spread though migration. This may explain its prevalence in Central and Western of Asia (Pohlreich*et al*., 2005; Tikhomirova*et al*., 2005; Szabo and King, 1997; Neuhausen*et al*., 1996).

In the previous large population in Prague of Czech Republic (Pohlreich*et al*., 2005) based study to directly assess both genes and include cases over age 45 years, the Anglian Study, observeda decreased mutation frequency with increasing age, with 0.3% BRCA1 and 1.0% BRCA2 carriers in cases 45 to 54 years of age (Anglian Breast Cancer Study Group, 2000) and this result was in agreement with our result that 5382insC decrease with increasing age.

Figure (10) shows all WT lanes are positive for ASPCR reaction with wild type specific primers, the same samples show negative to mutant type specific primers.

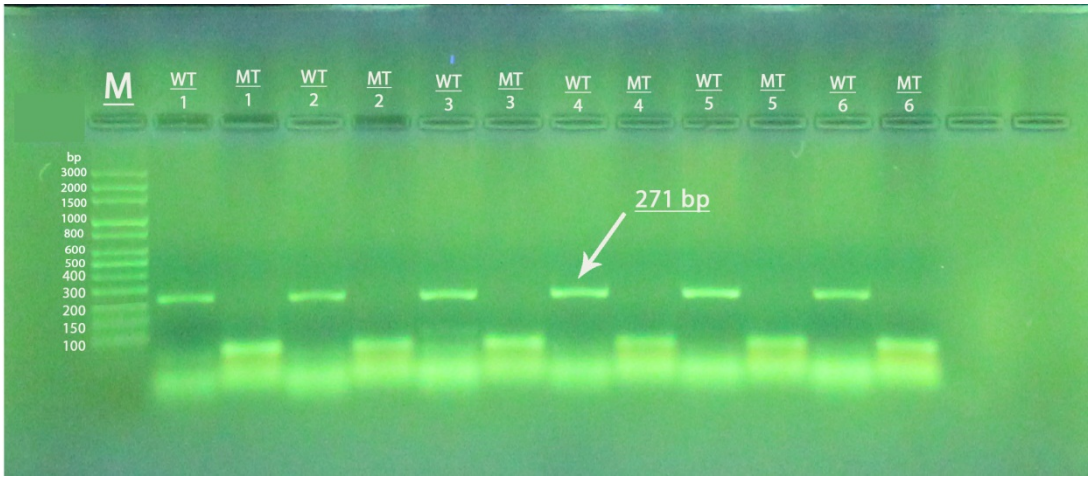

Figure -10: Represents amplification Products of BRCA1 Exon 20; wild-type alleles with 271bp by wild-type specific primers (p_4, p_5) with DNA samples in Group C. Electrophoresis was performed on 2% agarose gel and run with 5-8V/cm, for 2 hours. Lane M contained DNA molecular marker (3000-100bp).

The results of these Group D samples showed that from 7 DNA samples the number of samples with normal homozygous were 6 and the number of mutation was one.

Figure (11) the wild-type fragments 271bp are noticed in lanes (1WT, 2WT, 3WT, 4WT, 5WT). In (1MT, 2MT, 3MT, 4MT, 5MT), mutant fragment 295bp not detected due to absent of this mutation in these samples.

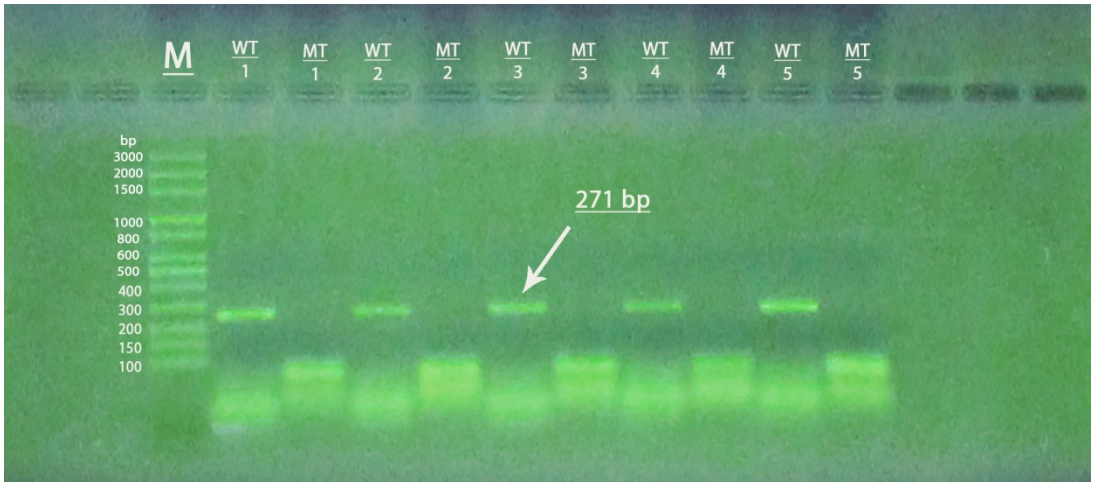

Figure -11: Represents amplification Products of BRCA1 Exon 20; wild-type alleles with 271bp by wild-type specific primers (p_4, p_5) with DNA samples in Group D. Electrophoresis was performed on 2% agarose gel and run with 5-8V/cm, for 2 hours. Lane M contained DNA molecular marker (3000-100bp).

Figure (12) shows the result of a mother and her daughter, the first 6 lanes is the mother lanes, the first two lanes for BRCA1, Exon 2, lane 3 and 4 is for BRCA1, Exon 20, lanes 5 and 6 for BRCA2, Exon 11, the same for the rest lanes about her daughter. We see 295bp fragment (mutant fragment) in both mother and her daughter.

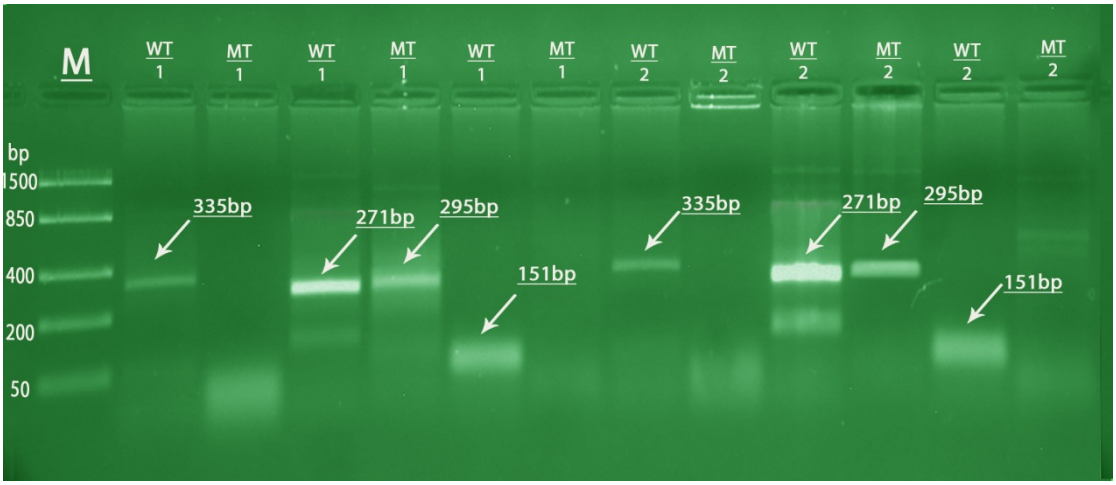

Figure -12: Represents amplification Products of two samples for a mother and her daughter for amplification of BRCA1 and BRCA2 by using wild and mutant specific primers. For three point mutation 185delAG in Exon 2 (p_1, p_2),(p_1, p_3), 5382mSc in Exon 20 (p_4, p_5), (p_4, p_6) and 6174delT in Exon 11 (p_7, p_8), (p_7, p_9) with DNA samples. Electrophoresis was performed on 2% agarose gel and run with 5-8V/cm, for 2 hours. Lane M contained DNA molecular marker (1500-50bp).

This mutation was previously detected in Ashkenazi Jews and thus found to be clearly associated with an increased risk of breast cancer (Struewing*et al*., 1997). Our result was in agreement with (Koifman and Jorge Koifman; 2001). They studied this mutation in women with breast cancer at 50 years of age with a first-degree relative with breast cancer. They concluded that, it is not surprising to find this mutation in this group of ages (50-).

Also, our result agree with (Miki *et al*., 1997), which recorded that breast cancer is occur approximately 20 percent by the age of 40 and 80 percent by the age after 50 years. Women with harmful BRCA1 or BRCA2 mutations often develop breast cancer after age 50 (Lynch *et al*., 2008) at different rates for instance women carrying the BRCA1 mutation have an 85% risk of developing breast cancer whereas women with the BRCA2 gene mutation have a 27% risk of developing breast cancer above 40 years of age (Llort*et al*., 2007).

II. BRCA2Mutations

1. Mutant 6174delT

The results for mutant 6174delT are the same for all groups, which is all 50 patients, are wild-type and no mutation detected due to that all 50 patients are normal for this mutation.

Figure (13) wild type fragments 151bp are noticed in all lanes. Mutant type are not detected due to absence of this type of mutation in all samples (50 patients).

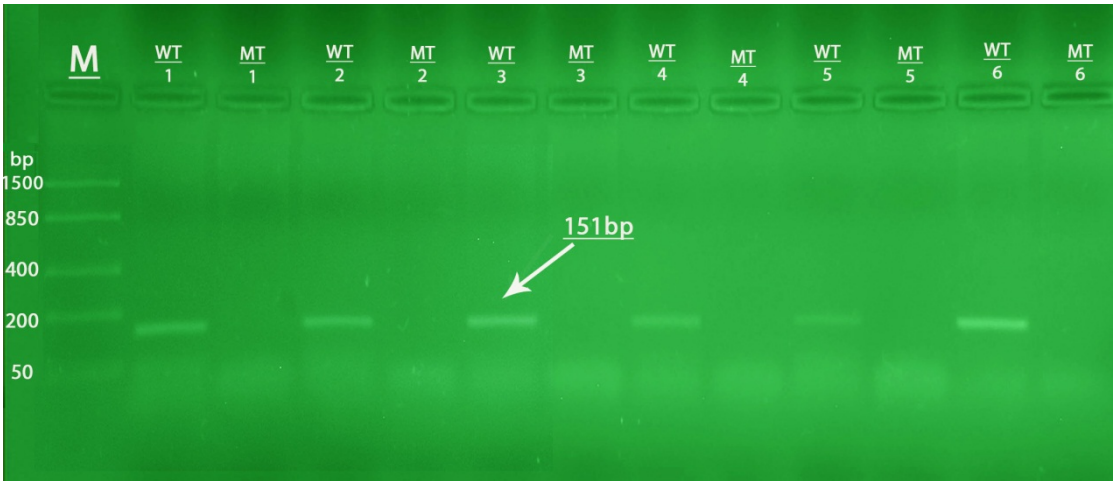

Figure -13: Represents amplification Products of BRCA2 Exon 11; wild-type alleles with 151bp by wild-type specific primers (p_7, p_8) with DNA samples in Group A. Electrophoresis was performed on 2% agarose gel and run with 5-8V/cm, for 2 hours. Lane M contained DNA molecular marker (1500-50bp).

So this study, the 6174delT mutation was not detected in 9 patients. Based on this fact, in Kurdish population with a family history of breast / ovarian cancer, these mutations in BRCA1/2 cannot be accepted as good indicators to search for this germ line mutation. So, it seems logical that Kurdish high risk individuals should first be screened for the 5382insC BRCA1 mutation before full analysis of both genes is carried out.

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