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Study of BRCA1 and BRCA2 Gene Mutations in Relation to Clinico-pathological Criteria of Breast Cancer in Basrah.

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ABSTRACT

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 study was planned to detect the probable occurrence of three founder mutations, 185del AG and 5382ins C (BRCA1) and 6174del T (BRCA2) mutations in Iraqi breast cancer female patients diagnosed in Basrah by the use of Polymerase Chain Reaction (PCR) technique and correlate them with other clinicopathological parameters like the histological type, grade and stage of breast cancer. Patients with positive family history were subdivided into high and low risk groups. Breast cancer in this study was characterized by an early age of onset with grade II and stage II at time of presentation. However, molecular analysis revealed that none of the participants in the study harbored any of the previously mentioned 3 founder mutations of BRCA1 and BRCA2 genes.

Keywords: BRCA1, BRCA2, Founder mutations, Polymerase Chain Reaction.

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INTRODUCTION

Breast cancer is a highly heterogeneous disease in terms of clincial course, gross and microscopic pathology and imaging characteristics [1]. Hereditary breast cancer contributes to about 5-10% of all cases with an earlier age of onset [2, 3]. Mutations of BRCA1 and BRCA2 genes (Breast cancer susceptibility genes 1 and 2) are the most well recognized mutations responsible for an increased risk of breast cancer [4]. Germ-line mutations of these two genes are transmitted in an autosomal dominant fashion [5]. BRCA1 is considered to be responsible for about one-half of all cases of early-onset breast cancer and for the majority of familial breast and ovarian cancers [6,7]. Female carriers of BRCA1 mutations have an 80-85% risk of developing breast cancer over their life-time[2]. The BRCA2 gene mutations are believed to account for a similar percentage of inherited breast cancer cases as BRCA1 mutations [8]. BRCA2 mutation carriers have a lifetime risk of 45–85% for breast cancer and 11–23% for ovarian cancer [9].

BRCA1-related tumors show an excess of medullary histopathological features, are of higher histologic grade and are more likely estrogen and progesterone receptors-negative, and are less likely to exhibit HER2/neu overexpression with a higher frequency of TP53 mutations than in sporadic tumors [10,11,12]. BRCA2-related tumors show similar histopathological features but to a much lower grade with a more variable degree of estrogen receptor status [12]. Cases of invasive lobular, pleomorphic lobular, tubular and cribiform forms have shown to be more frequently in this group [13]. Hereditary breast and ovarian cancer (HBOC) resulting from germ-line mutations in BRCA1 and BRCA2 is suspected and further risk evaluation is defensible, if the family shows one or more of the following features [10,11,14,15]:

- Early-age-onset breast cancer including both invasive ductal carcinoma and ductal carcinoma in situ.
- Two breast primaries or breast and ovarian/fallopian tube/primary peritoneal cancer in a single individual or in close (first- second- and third-degree) relatives(s) from the same side of the family.
- Breast or ovarian cancer in certain ethnic groups like Jewish families .
- Family member with primary cancer occurring in both breasts .
- Family member diagnosed with invasive serous ovarian cancer .
- Presence of male breast cancer in the family
- A family member with an identified BRCA1 or BRCA2 mutation and
- Multiple cases of breast or ovarian cancer.

MATERIALS AND METHODS

A total of 54 female patients diagnosed with breast cancer and whom attended either the surgical ward or oncology clinic at Al-Sadder Teaching Hospital or private histopathological labs in Basrah and 55 healthy female controls with no history of any breast lesion, were selected. Personal and family history was taken by interview or self-report questionnaire. A histopathological confirmation of the diagnosis was obtained from the Basrah cancer registry and blood samples were drawn from all the patients. DNA was extracted from the blood samples with the use of Wizard Genomic DNA purification kit, (Promega Corporation, USA) and was detected by the use of 0.8% agarose gel electrophoresis. The concentration of the extracted DNA was measured spectrophotometrically. In this study, 3 founder mutations, 185delAG and 5382insC in BRCA1, and 6174delT in BRCA2, were considered. For each mutation, three primers (one common, one specific for the mutant, and one specific for the wild-type allele) were used. The mutant and wild-type primers differed by ~20 bp in size, so the size of amplified mutant and wild-type segments differed by ~20 bp. For the 185delAG mutation, the mutant and wild-type PCR products were 354 and 335 bp, while those of 5382insC were 295 and 271 bp, and those of 6174delT were 171 and 151 bp , respectively. In the absence of any mutant allele only one band appears, while in the presence of one of the mentioned mutations, two bands appear. The allele-specific oligonucleotide primers sequences and sizes of corresponding amplicons are shown in Table 1.

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Table 1: Nucleotide sequences of the primer sets.

Primer	Primer sequence	Size of amplicon
BRCA1 185delAG		
Common forward (P1)	5′-ggttggcagcaatatgtgaa	
Wild-type reverse (P2)	5′-gctgacttaccagatgggactctc	335 bp
Mutant reverse (P3)	5′-cccaaattaatacactcttgtcgtgacttaccagatgggacagta	354 bp
BRCA1 5382insC		
Common reverse (P4)	5′-gacgggaatccaaattacacag	
Wild-type forward (P5)	5′-aaagcgagcaagagaatcgca	271 bp
Mutant forward (P6)	5′- aatcgaagaaaccaccaaagtccttagcgagcaagagaatcacc	295 bp
BRCA2 6174delT		
Common reverse (P7)	5′-agctggtctgaatgttcgttact	
Wild-type forward (P8)	5′-gtgggatttttagcacagctagt	151 bp
Mutant forward (P9)	5′-cagtctcatctgcaaatacttcagggatttttagcacagcatgg	171 bp

In case of 185del AG mutation of BRCA1, the PCR mix was performed in a final volume of 50 µL containing 50 ng DNA, 25 µl of the GoTaq Hot Start PCR master mix (Promega Chemical Co.), 2 µmole of each of P1 and P3, and 0.4 µmole of P2. Nuclease free water was added to adjust the volume to 50 µl. A similar reaction mixture was used for 5382ins C mutation of BRCA1 except for the primers, in which 0.12 µmole of each of P4,P5 and P6 was used. Each PCR reaction consisted of initial denaturation at 95 ^{o}C for 3 minutes, 35 cycles of denaturation at 95 ^{o}C for 1 minute , annealing for 1 minute at (50 ^{o}C in case of 185del AG and 48^{o}C in case of 5382ins C), extension at 72 ºC for 2 minutes , followed by final extension at 72 ^{o}C for 10 minutes then hold at 4ºC [2].

In case of 6174del T mutation of BRCA2, the PCR mix was performed in a final volume of 50 µL containing 50 ng DNA, 25 µl of the GoTaq Hot Start PCR master mix, 0.31 µmole of each of P7 and P9, and 0.24 µmole of P8. Nuclease free water was added to adjust the volume to 50 µl. Each PCR reaction consisted of an initial 12 minute of AmpliTaq Gold activation at 95 °C , followed by 35 cycles of 15 seconds of denaturation at 94 °C , 15 seconds of annealing at 52 ^{o}C , and 30 seconds of extension (with an increment of 1 second for each subsequent cycle) at 72 ^{o}C , and a final extension step of 5 minute at 72 °C [8]. Finally, agarose gel electrophoresis was carried out on a 2% agarose gel and visualized with ethidium bromide.

RESULTS

The age of the breast cancer patients ranged from 20 to 71 years, with a mean age of 46.66 years ± 11.179 SD. More than half the patients (62.96%) were younger than or equal to 50 years. Regarding the control group, the age of the participants ranged from 20 to 71 years, with a mean age of 37.76 years ± 10.415 SD.The majority of controls (87.27%) were younger than or equal to 50 years (figure 1). About 37.04% of patients had family history of cancers like breast, colonic, hepatic, bronchogenic carcinomas and leukemia, in addition to male breast, prostate and bladder cancers while, the majority (62.96%) had no family history of any type of cancer. Sixty five percent of patients had one family member with cancer, 30% had two and 5% had 3 or more family members with cancer. Forty percent of patients had history of a 1st degree relative with cancer, 45% had history of a 2nd degree relative and 15% had history of a 3rd degree relative with cancer. Patients with positive family history were further subdivided into high risk and low risk groups according to the previously mentioned criteria of HBOC.

Regarding the histological type of breast cancer, most patients (85.19%) had invasive ductal carcinoma (NOS), with few patients with invasive lobular carcinoma, mucoepidermoid carcinoma and Paget′s disease. The majority of patients (79.63%) had grade II breast cancer and more than two- third of the patients (78.43%) presented with stage II . A significant association between positive family history (high and low risk groups) and patient's age was observed, in which 72.73% of patients of the high risk group were less than or equal to 50 years in comparism to 22.22% of patients of the low risk group. Neither patients nor controls harbored any of the three founder mutations (185delAG and 5382ins C (BRCA1) and 6174del T (BRCA2)), as shown in figures 1,2and 3. For the 185delAG mutation, the mutant and wild-type PCR products were 354 and 335 bp, while those of 5382insC were 295 and 271 bp, and those of 6174delT were 171 and 151 bp , respectively. The appearance of only one band, indicates the absence of any mutant allele.

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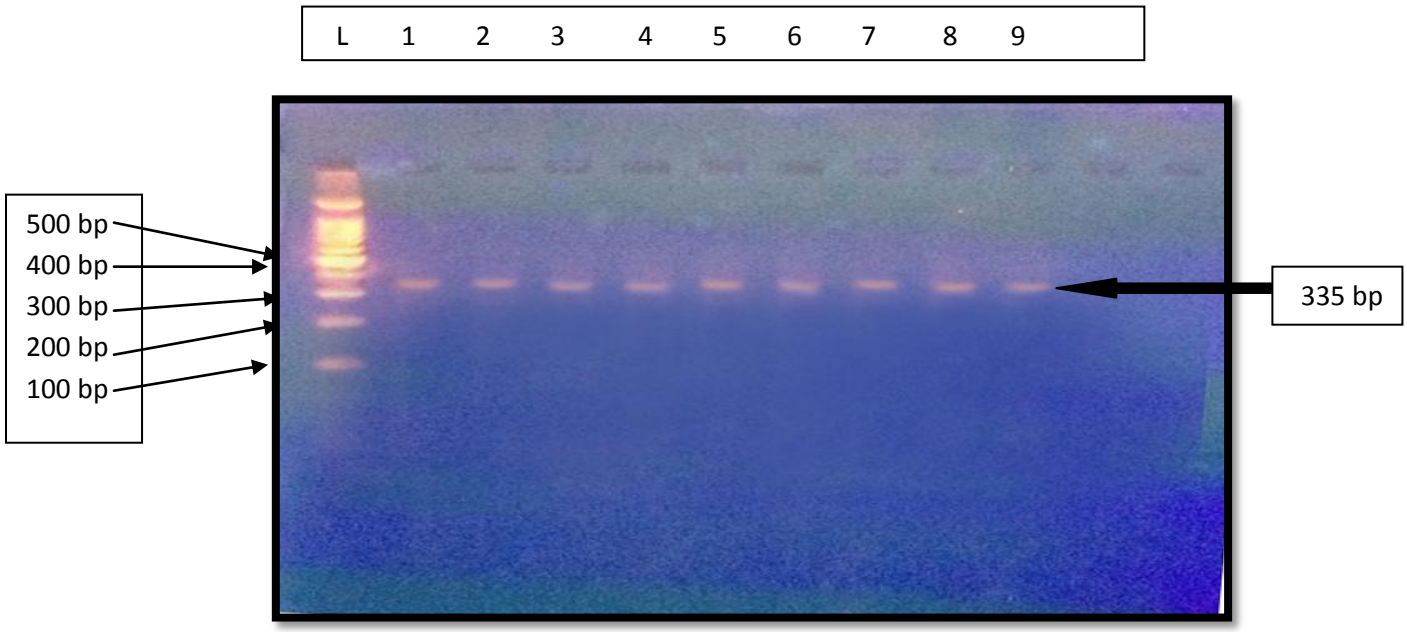

Figure 1: Agarose Gel Electrophoresis showing PCR band products of BRCA1 gene (lanes 1-9)
The size of the wild type amplicon is 335 bp
Lane L represents the 100 bp DNA step ladder

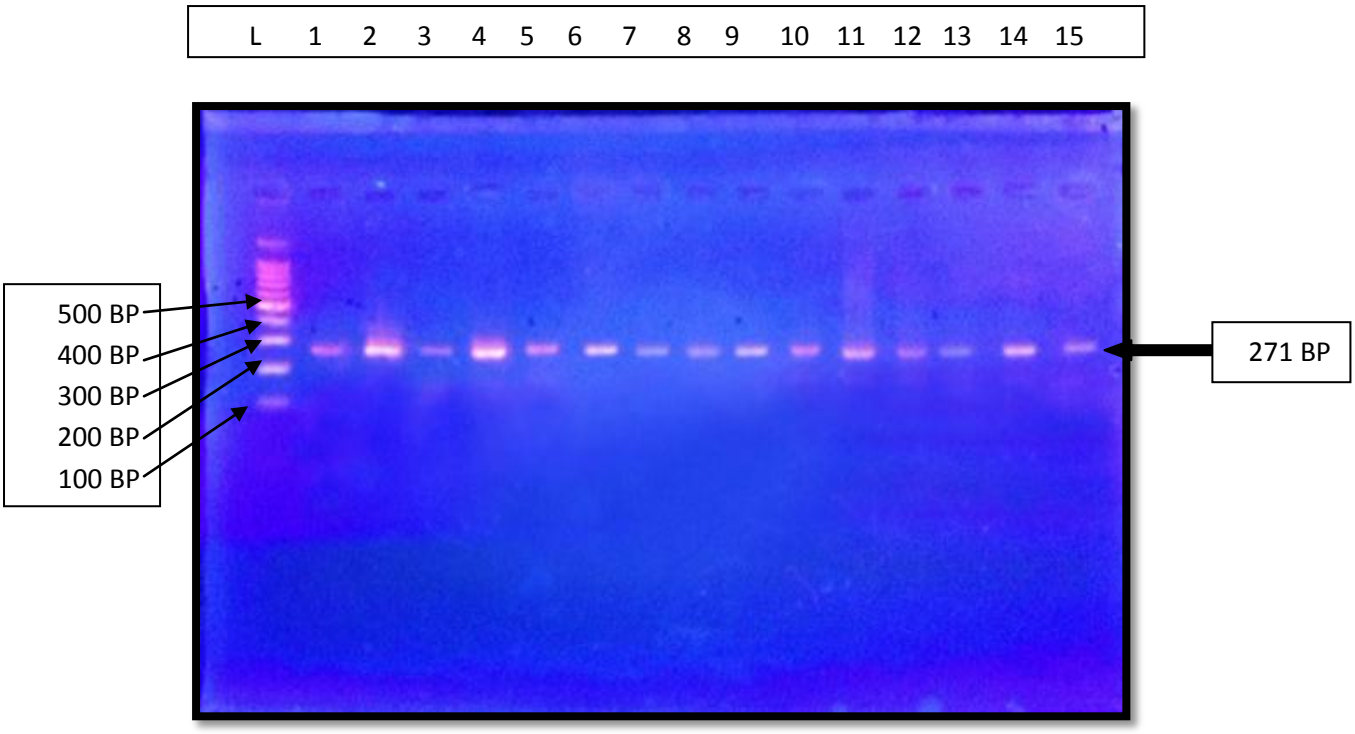

Figure 2: Agarose Gel Electrophoresis showing PCR band products of BRCA1 gene (lanes 1-15)
The size of the wild type amplicon is 271 bp
Lane L represents 100 bp DNA step ladder

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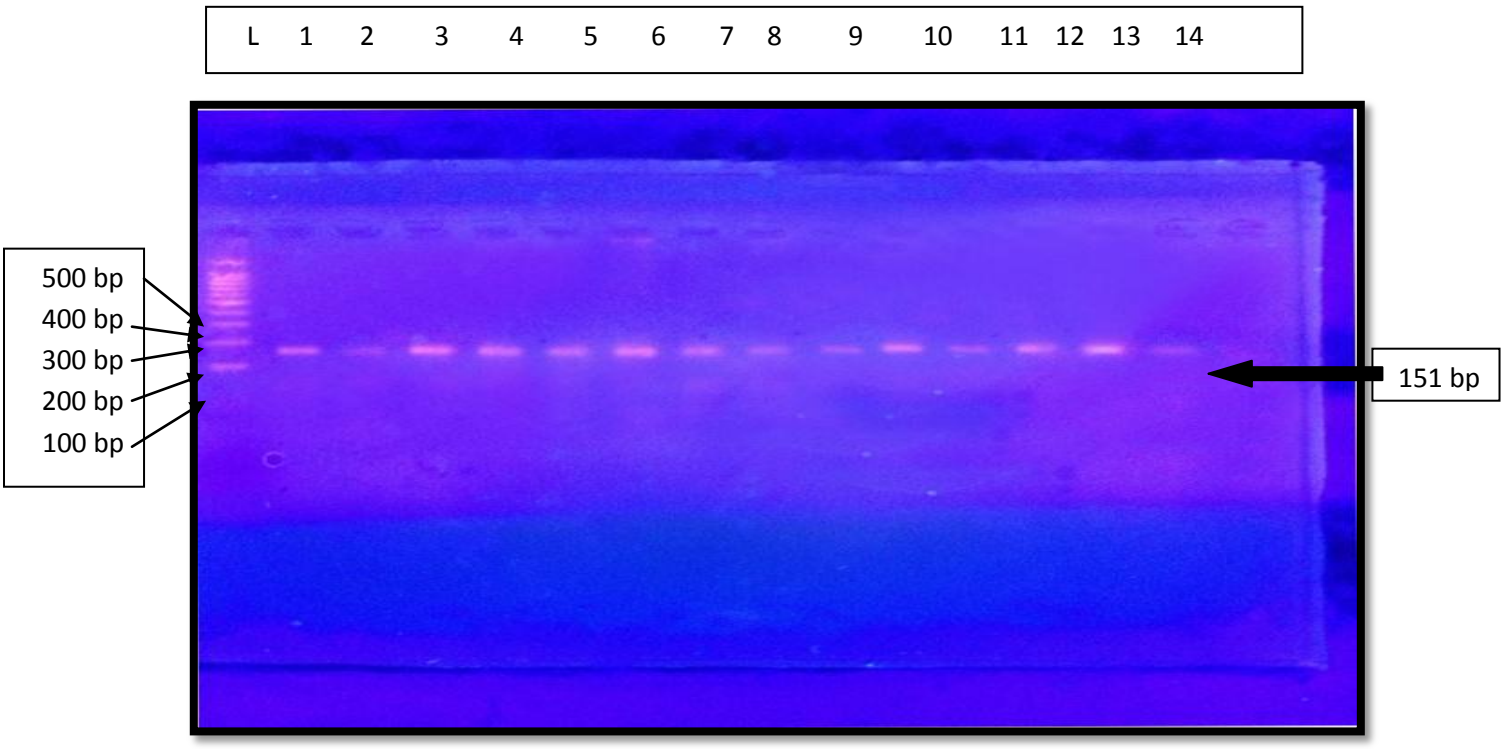

Figure 3: Agarose Gel Electrophoresis showing PCR band products of BRCA2 gene (lanes 1-14)
The size of the wild type amplicon is 151 bp
Lane L represents 100 bp DNA ladder

DISCUSSION

This is the first study carried out to detect the occurrence of familial breast cancer in Basrah. In this study, breast cancer patients presented with an early age of onset i.e. in premenopausal women that is most alarming since young age at the time of diagnosis of breast cancer is an independent factor of poor prognosis. Similar results were obtained in Baghdad [16,17], Kurdistan [18], some Arabic [19,20,21]and other developing countries [21,22,23,24]. On the other hand, studies in developed countries revealed higher age at time of diagnosis (> 60 years) i.e. in post menopausal women [20], which is probably due to the different life style, habits and a longer life expectancy.

In the present study, most patients presented with grade II and stage II breast cancer with invasive ductal carcinoma being the commonest histological type. Such findings have also been documented in some Arabic and other developing countries [16,17, 21-25], while in developed countries, most patients present with an early stage [23]. Thus, most patients in developing countries present at a moderate or advanced grade and stage of breast cancer. This is mainly due to the lack of health education, delay medical consultation, absence of screening programmes, rejection of management strategy and defects in follow-up.

Half of the cases with positive family history (who fulfill one or more of the criteria of HBOC mentioned previously) were regarded as a high risk group for HBOC. A strong association has been observed between high and low risk groups and patient's age, in which about three-fourths of patients of the high risk group were less than or equal to 50 years of age. This finding may suggest that the high risk group patients are possible candidates of hereditary breast and ovarian cancer that tends to occur at an early age of onset [3].

The current study showed that neither patients nor controls showed any of the three founder mutations of BRCA1 and BRCA2 genes, which may reflect the genuine picture of the incidence of such mutations in Basrah, particularly since there was no data base and no previous study in this field. In addition, other factors may explain the absence of these mutations like the small sample size; the mutations might be of somatic nature rather than germ-line mutations. Also, breast cancer may be due to mutations in other coding regions of BRCA1 and BRCA2, or may be due to mutations of other breast cancer genes like p53 [26]. A similar result was obtained by Al-Sanati and Al-Aubaidy in Baghdad regarding *BRCA2* mutation, however,*BRCA1*

mutations were detected in 6.2% of cases (185delAG and 5382insC) [17]. Another study carried out by Fattahi MJ et al. in 2009 in Iran, was in agreement with the current study, in which none of the three founder mutations were detected [3]. Similar results were obtained in studies carried out in Turkey and India [27,28].

CONCLUSION

Breast cancer was characterized by an early age of onset (younger than the ages estimated in the developed countries), a moderate grade (grade II) and an intermediate stage (stage II), with positive family history of breast and/or other tumors in more than one third of the cases, with invasive ductal carcinoma (NOS) being the commonest type of breast cancer among the participants. Neither patients nor controls, harbored any of the 3 founder mutations (185del AG and 5382ins C mutations of BRCA1 and 6174del T mutation of BRCA2). Nevertheless, half of the cases with positive family history, were regarded as a high risk group for hereditary breast and ovarian cancer (HBOC) (who fulfill one or more of the criteria of HBOC) and most of them were at an early age at time of presentation, thus, increasing the possibility of familial breast cancer (due to probably other BRCA1 and BRCA2 mutations or other gene mutations).

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