Evaluation of BRCA1 Gene Mutations Frequency in Breast Cancer Patients

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Abstract
Breast cancer is the most frequent carcinoma in females. The present study aimed to shed light on the association between the breast cancer incidence and *BRAC1* gene mutations. This study was carried out on two groups of 30breast cancer patients and 10 of healthy control. Polymerase chain reaction (PCR) for exon 2 of *BRCA1* was performed to detect the gene mutations.The results showed 21(70%) samples were negative to *BRCA1*gene mutation while 9 (30%) samples were positive to it, which was a significant difference (p value=0.0984 <0.05). The statistical analysis showed no significant differences in the frequency of *BRCA1* gene mutation with age and family history. In correlation with the tumor stage, the highest percentage of *BRCA1*gene mutation positive samples 7(77.77%) were stage II that statistically no significant differences from percentage of stage III.In conclusion, the BRCA1 gene mutation may play a key role in breast cancer incidence and have a relatively high frequency in tumor stage II .
Keywords: Breast cancer, *BRCA1* Gene ,mutation

1- Introduction

Breast cancer is the most frequent carcinoma in females and the second most common cause of cancer related mortality in women (Bombonati and Dennis , 2011) . It is the most commonly diagnosed malignancy in women around the world, especially in the Western countries. It accounts for almost one fifth of deaths caused by cancer (Winer *et al.*, 2001). Every year, one million new cases are reported worldwide, representing 18% of the total number of cancer in women. It has been established that one out of eight women (in USA) and one out of 10 women (in UK) (Evans and Lalloo , 2002) will develop breast cancer at some point in her life. An Iraqi studies shown that the number of breast cancer cases are steadily rising since the 1991 war (Berchuck , 1999 ; Jasim , 2004). The genetic contribution to breast cancer risk is indicated by the increased incidence of breast cancer among women with a family history of the disease and by the observation of families in which multiple members have breast cancer in a pattern compatible with autosomal dominant inheritance of cancer susceptibility. Linkage analysis studies of families have led to the identification of highly penetrant genes as the possible cause of inherited cancer risk in many cancer-prone families (Hall *et al*., 1990). However, mutations in these genes are rare, and account for no more than 5–10% of breast cancer cases. It is probably that other background genetic factors contribute to the etiology of breast malignancies, Hopper(2001). Autosomal dominant inherited predisposition to breast cancer is characterized by early age at onset, bilaterally, vertical transmission through both maternal and paternal lines, and familial association with tumors of other organs. Identification of the *BRCA1* and *BRCA2* genes marked an important step in understanding the molecular bases of the hereditary susceptibility to cancer (Wooster *et al.*,1995). *BRCA1*gene appears to be responsible for disease in 45% of families with multiple cases of breast cancer only and up to 90% of families with both breast and ovarian cancer. BRCA1 is mapped to chromosome 17q21 (Hall *et al*., 1990) ; it contains 24 exons and encodes a protein of 220 KDa, composed of 1863 amino acids. BRCA1 contains only two recognizable protein motifs, a RING finger domain near the N-terminus and a BRCT domain at the C-terminus. The phenotype for BRCA-related tumors appears to be heterogeneous, and to date is better characterized in BRCA1 than in BRCA2 (Phillips,2000). 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) (Bonatti *et al.,* 2006). The identification and study of this mutation could facilitate early diagnosis and proper counseling for breast cancer. In this study, we analyzed the frequency of the 185 del AG mutation (exon 2) of BRCA1 gene *BRCA1* gene mutation in 30 breast cancer samples using conventional polymerase chain reaction and estimate the hypothesis that there is an association between risk for developing breast cancer and *BRCA1* gene mutation.

2-Materials and Methods

Patients and clinical samples: the blood samples from 30 patients with different stages of newly diagnosed breast cancer were provided by Tumor center/ Alsader Hospital /Missan during the period from December 2015 to March 2016. 20 blood samples from healthy donors were used as a control in this study. From each sample, 2 mL of peripheral blood was collected into an EDTA-containing tube. The samples were stored at -20C° until further processing.

2-1 DNA extraction and Polymerase chain reaction

DNA was extracted from frozen blood samples by the gSYNCDNA Mini method (Geneaid/ Taiwan) according to the "frozen blood protocol".

Polymerase chain reaction (PCR) of exon 2 *BRCA1* was performed using an ABI thermal cycler (Applied Biosystems / Korea). The primer sequences used are shown in Table 1, (Alpha DNA/Canada). PCR amplification was performed in a total volume of 25 µl containing, 5 µl dNTPs, 1 µl of each primer, master mix (Promega/USA),12.5 µl, and nuclease free water 5.5 µl. The following program was used : 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. The PCR amplification products were separated by 2% agarose gel electrophoresis and visualized by exposure to ultraviolet light after ethidium bromide staining.

Table 1: Primers used in polymerase chain reaction.

Primer	Sequence	Size/bp
BRCA1-F	5'-GAAGTTGTCATTTTATAAACCTTT-3'	
BRCA1-R	5'-TGTCTTTCTTCCCTAGTATGT-3'	262

2-2 Statistical analysis

The overall frequency of exon 2 *BRCA1* mutations were computed for all 30 cases with respect to age at diagnosis, family history of breast cancer, and tumor stages. Differences in proportions were evaluated using the Z- test for proportions comparing.

3- Results

In the present study the patients' age range was 25-70 years and the median is 49 years with high frequency of patients in the range of 40-49 years. According to the family history, 29(96.6%) of patients had negative family history which statistically high significance differences (p value=0.0151 <0.01) in comparison with patients that have positive family history1(3.33%). The distribution of patients according to the tumor stages showed that 5(16.6%) out of total patients were stage I, 14(46.6%) were stage II, 10(33.33%) were stage III, and 1 (3.33%) were stage IV. Relation between *BRCA1* gene mutations and clinicopathologic parameters, are listed in Table 2. The results of the present study showed that, out of the 30 samples with breast cancer and ten samples of Healthy controls examined for exon2 of *BRCA1* gene mutation,9(30%) samples were positive, which was significantly higher (p value=0.0984 <0.05) in compare with healthy controls that showed no mutation for *BRCA1* gene and 21(70%) of samples were negative for *BRCA1* gene mutation (Fig. 1) .

In correlation with age groups, the present study showed statistically significant differences in the frequency of *BRCA1* gene mutation with age while no significant differences with family history since all mutant samples were negative for family history. In correlation with tumor stage the results of the present study showed that the highest percentage of *BRCA1* gene mutation positive patients 7(77.77%) were stage II that statistically no significant differences from percentage of stage III *BRCA1* gene mutation positive patients 2(22.22%) (p value= 0. 192),while both stage I and IV showed no mutation.
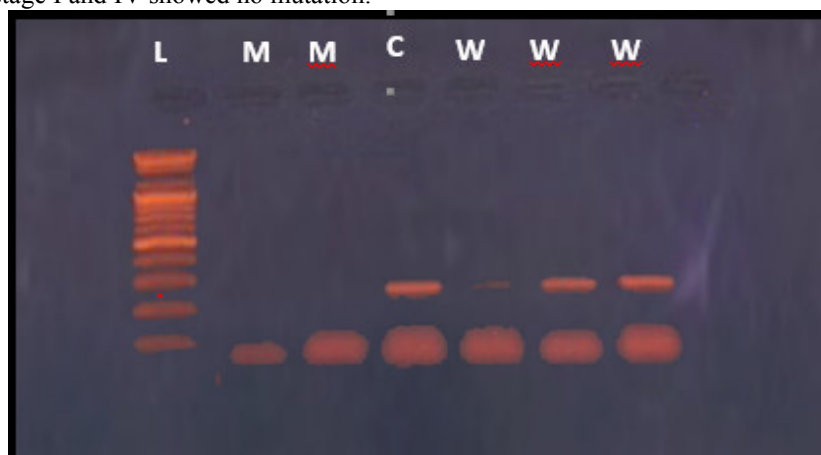

Figure 1. PCR products analysis for *BRCA1* gene mutation in breast cancer samples. L: 100 bp DNA ladder, M: mutant tumor sample, C: control, W: wild sample **.**

Table 2**.** Patient characteristics, type of tumor, stage of tumor, and frequency of mutations

Total Number Patient 30	BRCA1 mutation number 9 of 30	
Characteristics	All cases (N 30)	BRCA1 mutation (N 9 of 30)
Age groups		
20-29	1 (3%)	0
30-39	6(20%)	3(33.33%)
40-49	15(27.27%)	3(33.33%)
50-59	9(30%)	1(11.11%)
60-69	7(23%)	2(22.22%)
70-79	1(3%)	0
Family history		
Positive	1(3.33%)	0
Negative	29(96.6%)	9(100%)
Tumor stage		
Stage I	5(16.6%)	0
Stage II	14(46.6%)	7(77.77%)
Stage III	10(33.33%)	2(22.22%)
Stage IV	1(3.33%)	0

4-Discussion

Multiple menstrual and reproductive events; menarche, pregnancy, breast feeding may alter breast cancer risk. Studies have also consistently identified an elevated risk of contra lateral breast cancer in *BRCA* mutation carriers.

In the present study, the frequency of *BRCA1* mutation was 30% in the patients. These results have some similarity to that reported by Lahad *et al*. (1997) who found the mutation in 19% of breast cancer patients in Ashkenazi Jewish. However our result was higher than 1.2% reported by Peelen *et al*. (1997), Santarosa *et al*. (1999) who found the mutation in 10% of Italian females with familial breast cancer, and El gezeery *et al*.(2008) who found the mutation in 4(10%) in Egyptian female patients with breast cancer. This variation in the frequency may be attributed to the ethnic differences, on the other hand the results were much lower than that of Al-mashdani who detected *BRCA1* mutations in 30 cases (83.33%) of Iraqi patients(Jumaa , 2013), and lower than the frequency of *BRCA*1 exon 2 mutation which found in 55% (11/20) patients of breast cancer and only 20% (2/10) healthy control subjects carried this mutation by Mohammad *et al*.(2014). The age is an important risk factor for breast cancer as the risk increases with age, so occurrence of breast cancer in young age group gives strong implication for the presence of inherited genetic predisposition for breast cancer (Lahad *et al.,* 1997). In the present study, two patients with *BRCA1* mutation were below 40 years (22.22%). Several studies found frequencies of 3.5%, 6.7%, 8%, 9% and 20% in the patients below 40 years of age (El gezeery *et al*., 2008 ; Sue Richards *et al*., 1997 ; Peto *et al.,* 1999). In the present study the other 7 patients carrying the mutation were above 40 years (77.77%). Other studies reported frequencies of 1.9% 2% and 13% in patients above 40 years of age (El gezeery *et al*., 2008 ; Peto *et al.,* 1999 ; Abeliovich *et al* ., 1997). Although the differences between the results of many studies conducted in different populations, most of these studies concluded that BRCA1 gene 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.

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 (Margolin & Lindblom , 2006). In the present study, all the 9 mutant patients (100%) had negative family history. Our finding is lower than 13.5 % reported by El gezeery *et al*.(2008) 7.4% reported by Friedman *et al*. (1995) and 5.8% reported by Gura*n et al*. (2005) in patients with positive family history. In conclusion, we noticed that the result of present study different from that reported previously which indicated that BRCA1 gene mutation account for no more than 5–10% of breast cancer cases in compare with our results 30%. These differences may be due to multiple reasons including choosing of random samples instead of particular samples with definite family history as followed in other studies, the other reason is may be related with environmental factors and geographical disruption of the samples which showed that the frequent of this gene mutation in Iraqi patients is differs from that obtained from the previous studies, we also noticed that sometimes there is either omission from hospital side or secrecy from patients side in getting or providing real information about family history which leading in result to get inaccurate family history. Until recently, the management of BRCA mutation-associated breast cancer did not necessarily differ from the management of sporadic breast cancer. However, consideration of the future cancer risks faced by this population, new data regarding unique sensitivity to systemic therapies and the availability of BRCA mutation

testing at the time of breast cancer diagnosis are changing this treatment regimen.

5-Conclusions

In conclusion, There was a surprising high degree of *BRCA1* gene mutation carriage rate, *BRCA1* gene mutations were found to have a relatively high frequency in tumor stage II in the study.

Acknowledgments

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