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Low Prevalence of BRCA1 and BRCA2 Common Founder Mutations among Iraqi Breast Cancer Cases and At Risk Families

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Abstract

Introduction

Breast cancer is the most common malignancy affecting women worldwide and in Iraq. Most of the cases are sporadic, however, familial cases constitute 5-10%. A strong family history of breast and/or ovarian cancer can often be explained by mutations in BRCA1 or BRCA2. Common founder BRCA1/BRCA2 genetic mutations have not yet been studied on a large scale in Iraqi cohort.

Objective: To evaluate the frequency of common founder mutations in BRCA1 and BRCA2 (namely 185delAG and 5382insC in BRCA1 and 6174delT in BRCA2) in a clinic based cohort of mixed phenotype (affecting breast cancer patients and at risk individuals).

Methods and Methods: Multiplex PCR was used to screen the most common founder mutations in BRCA1 and BRCA2 in 80 index cases and 20 controls.

Results: The data of this investigation indicated that the aforementioned founder mutations were not detected in the tested groups.

Conclusion: Our results indicate that 185delAG and 5382insC mutations in BRCA1 and 6174delT in BRCA2 are infrequent among Iraqi breast cancer families. However, further testing (e.g. by MLPA) are recommended to consolidate these findings and identify other relevant mutations.

Keywords: Breast Cancer; Genetics; BRCA1 &BRCA2 Founder Mutations; Iraq

Introduction

Breast cancer is the most common malignancy among women worldwide and in Iraq, it comprises about one third of the registered female malignancies. The number of new cases reported in 2009 was 2906, with an incidence rate of 18.45/100 000 in female population. Within the last two decades, there has been an obvious increase in the incidence rates of breast cancer, which became one of the major threats to Iraqi female health [1]. The mortality rate is considerably high as most of the cases are still diagnosed at advanced stages with more aggressive forms [2,3].

A family history of breast or ovarian cancer is one of the main risk factors for the development of these diseases [4]. It is estimated that about 5–10% of breast cancers may be due to

inherited predisposition. Epidemiological studies have provided an evidence of at least two genes conferring inherited susceptibility to breast and ovarian cancer, i.e. BRCA1 (17q21) [5] and BRCA2 (13q12) [6]. BRCA1 accounts for nearly all families with multiple cases of both early onset breast and ovarian cancer and for about 45% of families with breast cancer only [6]. BRCA2, on the other hand, is responsible for about 35% of site-specific breast cancer families and the majority of male breast cancer families. More than 200 germline mutations have been described to date distributed along the gene in BRCA1 [7]. BRCA1/and BRCA2 have a wide range of functions as they are involved in pathways important for checkpoint control, DNA damage recognition, transcription regulation, chromatin remodeling, and double-strand break repair.

Women with a germline mutation in *BRCA1* or B*RCA2* carries a 50 - 80% lifetime risk of breast cancer and 20% to 40% lifetime risk of ovarian cancer [8]. It has been estimated that 80-90% of patients with a strong family history carry a defective BRCA1 and/ or BRCA2 gene in their germlines [9]. Furthermore, BRCA1 gene mutations are involved in 45% of hereditary breast cancer [10], and 2-30% of sporadic breast cancer [11].

The identification of the ethnic group of families undergoing genetic counselling enables the geneticist and oncologist to make more specific choices as the incidence of specific mutations was suggested to be relevant to the geographic and ethnic origin of the target population [17]. Three founder mutations (185delAG, 5382insCin BRCA1 and 6174delT in BRCA2) were frequently observed in Ashkenazi Jewish breast cancer patients. The 185delAG was also observed in non-Ashkenazi Jews, including Iranian-born Jews, [18] and in non-Jewish individuals from several ethnic backgrounds [19]. The 5382insC, on the other hand, could also be found in non-Jewish as Russian [20] and Turkish [21] populations.

In Iraq, familial breast cancer seems to constitute a large proportion of breast cancer cases. In one comprehensive survey, 16% of breast cancer cases have shown a family history of the disease [2]. However, no large scale study has been conducted so far. This study could be considered as the first step in the establishment of breast cancer genetics service in Iraq. The detection of patients with targeted gene mutations (BRCA1/2 founder mutations) is significant in:

1) Targeted gene mutation testing offered for the first degree relatives
2) Exclusion from gene scanning tests
3) Provision of an estimated prevalence of these mutations.

The overall aim of this study was to evaluate the incidence of those founder mutations among patients affected by breast cancer in Iraq compared to a risky group.

Subjects and Methods

Clinical cases

In this study, we have examined 40 affected patients (diagnosed with Invasive Ductal Carcinoma) and 40 unaffected indexes with different risk categories including "high and moderate risk" cases. An additional 20 "at population risk" individuals were considered as controls. All cases have been selected based on referral guidelines recommended NICE guidelines. A risk assessment was done for the unaffected cases using different models like BRCA mutation Carrier Probability Model (BRCAPRO) and International Breast Intervention Study (IBIS). Manchester score has been utilized to identify mutation carrier probability. The work was guided by the West Midland Regional Genetics Service (WMRGS)/ UK. All index cases have signed an informed consent (derived mainly from the WMRGS consent form and translated to Arabic language and ethically approved by the Iraqi Cancer Board).

Two groups of cases were investigated in this study, 40 affected cases diagnosed as invasive ductal carcinoma and 40 unaffected individuals. The unaffected group was composed of individuals that were referred due to having a positive family history. For this particular group, risk assessment was done and, accordingly, then classified into high an moderate risk subgroups. An additional Twenty normal individuals were selected as the "Control Group" as shown in Table 1.

Affected		Non affected		Control
Female	Male	High risk	Moderate risk	20
37	3	5	35	
40		40		

Table 1. The clinical cases involved in this study. 40 breast cancer patients, including 3 male cases), 40 unaffected individuals, but classified as "at risk" and 20 normal individuals.

DNA Extraction and multiplex PCR

5ml whole blood were drawn from all cases and DNA extraction was carried out using the Wizard *Genomic DNA extraction Kit (Promega, Madison, USA)* as recommended by the manufacturer. Briefly, 300 µl of blood were lysed, followed by lysis of the lymphocytes and their nuclei. DNA was precipitated by isopropanol and eluted in DNA rehydration solution.

PCR was performed using Ampli- set BRCA1-2 Multiplex kit *(Dia-chem srl, Napoli, Italy)* for detection of the BRCA1& BRCA2 mutations (185delAG, 188 del-11bp and 5382insC mutations in BRCA1, 6174delT in BRCA2). The reaction mix was composed of 20 µl of sample DNA, 0.5 µl of Taq Polymerase, 28 µl of Mixture A, and a 2 µl of RNase free water forming a 50 µl total volume.

The whole mix was then amplified using the following conditions, 95 C° for 5 minutes, followed by 35 cycles of 94 C° for 15 sec., 60 C° for 15 sec. and 72 C° for 30 sec. followed by a final extension at 72 C° for 10 minutes. The PCR products were mixed with loading dye (Bromophenol Blue) and visualized on 2% agarose gel electrophoresis by Gel Documentation System (DNR Bio-imaging system, China). The product sizes are as shown in Table 2 and an internal positive control (male control) was provided within the kit.

	Allele Specific Primer	**Product size** (Normal)	**Product size** (Mutant)
PCR Mix A	BRCA1 185 del AG	335	354
	BRCA1 5382 insC	271	295
	BRCA2 6174 delT	151	171
PCR Mix B	BRCA1 188del-11bp	112	101

Table 2. The sizes of the PCR product for both mutant and normal alleles.

Results and Discussion

It has been reported that BRCA1-185delAG mutation was found in 1% of Ashkenazi Jews and that contributed to 16%–20% of breast cancer diagnosed before age 50 [22]. Other investigators observed 5382insC mutation in BRCA1 among 0.13% of this population [20]. On the other hand, the third founder mutation, 6174delT in the BRCA2 gene, was observed in 1.52% in Ashkenazi [22].

All cases and controls tested in our study have shown the wildtype bands in the gel electrophoresis as shown in Figure 1, indicative of having no germline mutation in the target sites.

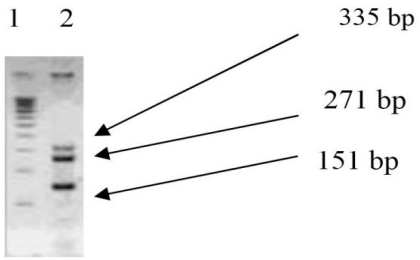

Figure 4. Gel electrophoresis analysis of the PCR products of genomic DNA isolated from primary breast cancer patients. The presence of the bands with sizes equivalent to 335 bp, 271 bp and 151 bp indicates the presence of wild type BRCA1 and BRCA2 genes. Lane 1 reveals DNA size markers while Lane 2 shows an example of the PCR results extracted from a breast cancer patient

The results from this study seem broadly consistent with other population specific studies in the Middle East. It has been reported earlier that a very low frequency of one founder mutation in BRCA1 (185delAG) was displayed among breast cancer patients in Iran (about 0.5%) [23].

An Egyptian study, using Single strand conformation polymorphism assay and heteroduplex analysis, was conducted on selected cases with similar referral criteria to ours, showed that delAG was observed in 10% of the target sample, accounting for the least detected mutation in that study [24].

The absence of founder mutations in our, in comparison to others, could be explained by the possibility that other mutations might be more common among the Iraqi population than the tested ones. However, the lower number of cases included in the study should be also taken in consideration. Consanguinity might be an equivocal risk-modifying factor as well. Indeed, the Iraqi population has a relatively high rate of consanguineous marriages and studies on the frequency of BRCA1 and BRCA2 in highly consanguineous populations pointed that consanguinity might lead to a decreased incidence of breast cancer caused by these mutations [25].

Up to our knowledge, the is the first study that focused on the prevalence of founder mutations among the Iraqi population based on clinical background. It has been conducted as a first step towards the implementation of an Iraqi Familial Breast Cancer Strategy with the intention to use a more comprehensive BRCA1/BRCA2 genetic testing in the future. A regional comparative breast cancer research projet has already been initiated by the Iraqi researchers to compare the demographic characteristics, clinicopathological presentation and treatment outcomes of patients complaining of breast cancer in the Middle East [26].

Conclusion

Due to the lack of any previous report on the prevalence of founder mutations in BRCA1 and BRCA2 among the Iraqi breast cancer patients, the results of this study can be considered as a preliminary data on the low prevalence of the common founder mutations in the Iraqi population. However, further studies using gene sequencing and MLPA, on larger cohorts, are recommended to explore the BRCA status in Iraqi breast cancer cases and at risk individuals.

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