Detection of BRCA2 exon 10 genetic variations in Iraqi breast cancer patients

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Abstract: Breast cancer (BC) is the most prevalent malignancy in women in Western countries currently accounting for one third of all female cancers, and in Iraq rank the first among all cancers. Germline mutations in BRCA2 genes have been demonstrated to increase the risk of developing breast cancer. Conversely, the impact of BRCA mutations on prognosis and survival of breast cancer patients is still debated.Familial aggregation is thought to account for 5–10 % of all BC cases and germline mutations in BRCA1 and BRCA2 account for less of the half of these inherited cases. In Iraq breast cancer represents the principal death-causing malignancy among women, with (44.44 %) of the cases diagnosed before the age of 50 years. In order to study BRCA2 mutation spectra in the Iraqi population, direct sequencing of the entire coding region and intronicsequences flanking the exon was performed. A total of three BRCA2 sequence variants (a, 865A>C, N289H a, 1114 A>C. N372H a, 1153 A>G. K385E)were found. One of them a, 1153 A>G. K385E was novel. In conclusion, this study represents the evaluation of the deleterious and unclassified genetic variants in the BRCA2 gene exon 10 found in Iraqi population harboring of breast cancer.

Key words: Breast cancer, BRCA2 gene mutation exon 10, Gene, Iraq, Mutation.

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التحري عن التغايرات الجينية في المحور 10 لجين الـ BRCA2 في مرضى سرطان الثدي العراقيين

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الخلاصة: سرطان الثدي من اكثر السرطانات شيوعا بين النساء في دول العالم الغربي، ويمثل ثلث انواع السرطانات التي تصيب النساء في العالم. في العراق يحتل سرطان الثدي الصداره بين انواع السرطانات. وتظهر التغايرات الجينيه الموروثه في BRCA2 احتماليه كبيره للتعرض الى سرطان الثدي بالاضافه لذلك فان دور التغايرات الجينيه في BRCA في تشخيص سرطان الثدي وتاثيره في معدل الوفيات

في المرضى مازال قيد البحث والدراسة. حيث يشكل سرطان الثدي نسبه 5- 10% بين السرطانات الموروثه ضمن العوائل، ومن ضمن هذا تشكل التغايرات في BRCA1/2 نسبه اقل من النصف ، ويعتبر في العراق سبب مهم من اسباب الوفيات بين النساء حيث ان معظم الحالات في هذه الدراسه ظهرت قبل عمر ال 50 عاما وهذا يشكل نسبه (44.44%) من الحالات. ولدراسة هذه التغايرات في هذا الجين بين المرضى العراقيين تم استخدام بادئات خاصه تضمن لنا تغطيه المنطقه بالكامل في تفاعل البلمره التسلسلي ليتسنى لنا بعدها دراسه تتابعات القواعد النتروجينيه لكل حاله ومقارنتها مع التسلسل المصدر ضمن برامج خاصه وبالتالي تحديد التغايرات ومواقعها، وواحدة من هذه التغايرات التي تم تحديدها لم تسجل مسبقا عالميا. وبالنتيجه فان الهدف من هذه الدراسه التحري عن التغايرات الوراثيه الخطره وغير المصنفه مسبقا في جين الـ BRCA2 المنطقه المشفره 10 بين مرضى سرطان الثدي في المجتمع العراقي.

Introduction

Breast cancer (BC) is a type of cancer originating from breast tissue most commonly from the inner lining of milk ducts or the lobules that supply the ducts with milk (1). Globally breast cancer is the most common among women comprising 23% of the female cancers. It is also the leading cause of cancer-related deaths in low-resource countries (2). Women BC is the first of the ten commonest cancers in Iraq according to the latest of Iraq Cancer Registry and the commonest type of malignancy in females and there is a general trend towards the increase in the frequency and incidence of breast cancer in younger age group. The most common histo-pathological types were invasive ductal carcinoma (IDC) (77.2%) and invasive lobular carcinoma (ILC) (9.8%). Patients less than 30 years old age formed about 5% of cases whereas about 75% of the cases occurred in women older than 40 years. The highest number of cases is between 40-50 years old age groups (Iraqi Cancer Board 2000) also it is ranked the 1st among the ten common cancers in 2009 (3).In 1995 BRCA2 (Breast cancer susceptibility gene type 2) a second gene termed after BRCA1(Breast cancer susceptibility gene type 2) was found related to hereditary breast cancer (4). BRCA2 (breast cancer type 2 susceptibility protein) is a protein found inside cells.

In humans the commands to make this protein are carried by a gene also called BRCA2 (5). BRCA2 belongs to the tumor suppressor gene family (6, 7). It covers about 70 kb of genomic sequence in 13q12 encoding a protein of 3418-amino-acid-long protein (8,9). BRCA2 can bind with BRCA1 contributing in DNA damage repair pathway associated with the activation of homologous recombination and double-strand break repair (10). In addition to the risks of breast and ovarian cancers several reports have suggested that BRCA2 mutations may be associated with an increased risk of other cancers (11).

Materials and Methods
Patients Studies
Thirty-six blood samples of Iraqi breast cancer (BC) patients were diagnosed by their physician recruited to Baghdad Medical city and Nuclear Medicine Hospital –Baghdad. Iraqi ten blood samples of healthy control were collected. All the entire patients have infiltrative ductal carcinoma NOS (Non Otherwise Specified). The consent of patients was taken.

Blood sampling
Five ml of human peripheral blood from all patients and control subjects were collected by veni puncture into heparinized tubes during the period October 2010 to August 2011. The blood was placed in a cool box under

aseptic conditions and transfer to the laboratory.

BRCA2 analysis

DNA was extracted from 200μl of peripheral blood usingQIAmp DNA mini kit (50) from Qiagen. In the fingerprint lab./ Institute of genetic engineering and biotechnology for higher studies/University of Baghdad. DNA yield was measured using NanoDropND-1000 spectrophotometer in which 1μl of nuclease free water is used first as blank, then 1μl of the patient genomic DNA is loaded in order to be measured, stored and then can be used for genetic tests. Polymerase chain reaction (PCR) was done by using HotStarTag®Master Mix Kit from Qiagen under the amplification program table (1) this step and the primer designing were done in the Molecular Oncology Unit lab. In GSTS Pathology Guy's and St. Thomas' NHS Foundation Trust/ London, and the primers were designed by using primer 3 plus website (12) each PCR reaction was carried out in a total volume of 25μl containing 12.5μl of PCR master mix (Qiagen, Germany), 8.5 μl of nuclease free water, 1 μl (5-10 pmol/ μl) of each primer and 2μl of DNA. Exon 10 was divided into three amplicons by using designed primers table (2).

Table (1) amplification program

Stage	Temp/Time	Cycles
Initial denaturation	95ºC/10min	
Denaturation	95ºC/30 sec	51 Times
Annealing	54ºC/45sec	
Extension	72ºC/45sec	
Final extension	72ºC/5min	
End	4ºC/forever	

Table (2) Size of primer sequence for BRCA2 gene exon 10

Amplicon No.	Primer	Product size	GC%	Tm/°C
A1	F-AAACTGTTTCTATGAGAAAGGTTGTG-3' R-CTTGGAGATTTTGTCACTTCCA-5'	450	34.6 40.9	59.2 59.2
A2	F- CCAAGTGAAAGAAAAATACTCATTTG-3' R-TGCATTGAAAGTCTCTTTAGGTGA-5'	517	30.8 37.5	59.5 60.3
A3	F-TCTTGCAGTAAAGCAGGCAAT-3' R- TCATGTATACAGATGATGCCTAAGA-5'	597	42.9 36	60.0 57.9

A= Amplicon

This step and the primer designing were done in the Molecular Oncology Unit lab. In GSTS Pathology Guy's and St. Thomas' NHS Foundation Trust/ London.The PCR product was cleaned up by using Charge Switch PCR Clean-Up Kit (Invitrogen) and then running on the gel. Sequencing reaction was done by using Dye terminator cycle sequencing (ABI)for the PCR products, and the component of sequencing reaction mix preparation table (3).

The reaction program was stored in thermo cycler for this reaction table (4). Clean-up of sequencing reaction products by using AgencourtCleanSEQ kit (Agencourt®CLEANSEQ® Dye Terminater Removal from Beckman Coulter/USA). Transferred 35µl of the clear sample into a 96 well plate for loading on the 3730 sequencer. The data were examined by using the Mutation Surveyor software.

Table (3) Sequencing master mix components

REAGENT	Ready Mix (from kit)	5X Buffer (from kit)	10µM primer	molecular grade water	Total	DNA template (cleaned PCR product)
Volume (µl) (X1)	0.7	2	1	2.8	6.5	3.5

Table (4) Sequencing reaction Program

Temperature ᵒC	Time (seconds)	Cycles
96	1min	X1
96	10s	X15
60	1min 20s	
96	10 s	X5
60	1min 35s	
96	10s	X5
60	2min5s	

Results and Discussions

The mean age for the patients with breast cancer was (50.5±9.85) years ranging from (30-68) years old; eight cases were under 40 years old (22.22%). Eight cases were between 41-50 years old (22.22%); fourteen cases were between 51-60 years old (38.89%) and six cases were above 60 years old (16.67%). The mean age of the control was (48.8±7.83) years, ranging from 36-55 years old. One case was under 40 years old (10%); six cases were between (41-50) years old (60%) and three cases were between (51-60) years old (30%).

The study result comes with finding of increasing risk of breast cancer with age (13)also due to using of menopausal hormone therapy (14). In this study the age group over 60 years was not appeared high risk of breast cancer due to the decreasing of age group averages in Iraqis (15). This may be attributed to many factors such as environmental factors, the nutrition, low exercise and poor health education. The exposure to a high dose of depleted uranium may be one of the reasons for the increased breast cancer risk in the Iraqi community. Furthermore, there are no national screening programs for the breast cancer patients in all the provinces of the country.

Twenty five breast cancer patients (69.44%) were harboring BRCAII gene mutations including exon10 (9). Exon 10 was amplified with three sets of primers to give three amplicons (16). The primer designing were done by primer3plus website. All the mutations were missense table (5). A first missense mutation (a, 865A>C, N289H) (figure 1) appeared in 4 (11.11%) patients and 6 (60%) of controls. While the (a, 1114 A>C. N372H) mutation (figure 2) appeared in 10 (24.78%) of patients but not in controls. These two as a matter of face are common missense single nucleotide polymorphism (SNPs) that have been previously reported in literature (17,18) as well as in the Breast Cancer Information Core (BIC) data base . Another novel missense mutation (a, 1153 A>G. K385E) (figure 3) was found in 11 (30.56 %) of breast cancer patients and none of the controls.

The difference in the frequencies of the first mutation between the patients and controls was not significant (P>0.01) and those between patients and controls in the second and third mutations were significant P<0.01.

Table (5) Frequency of BRCA2 mutation exon 10

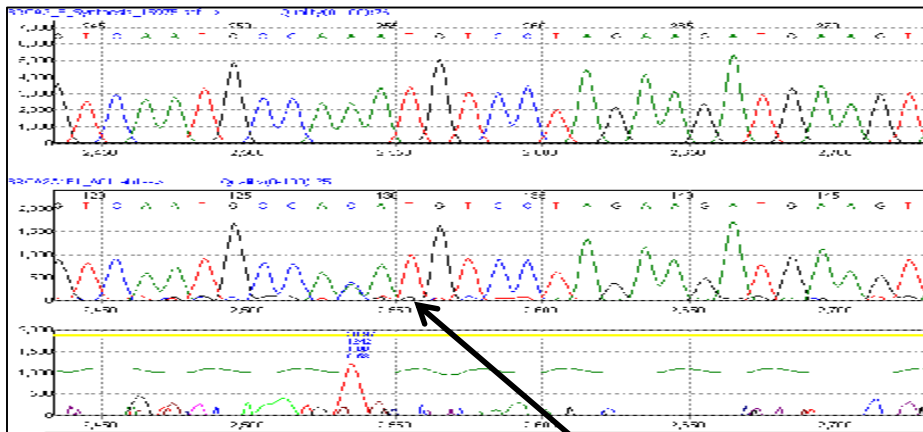| Mutation	Freq. in patients		Freq. in control		Chi-squarevalue
	no.	%	no.	%	
A1a, 865A>C, N289H	4	11.11	6	60	5.08 *
a,1114 A>C. N372H	10	27.78	0	0	7.29 **
A3a,1153 A>G. K385E	11	30.56	0	0	7.84 **
** (P<0.01), ns: non-significant					

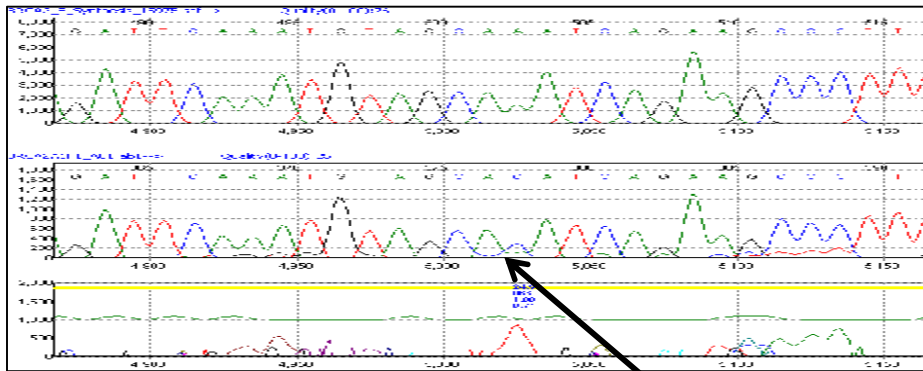**Figure (1) BRCA2 exon 10 mutation (a, 865A>C, N289H)**

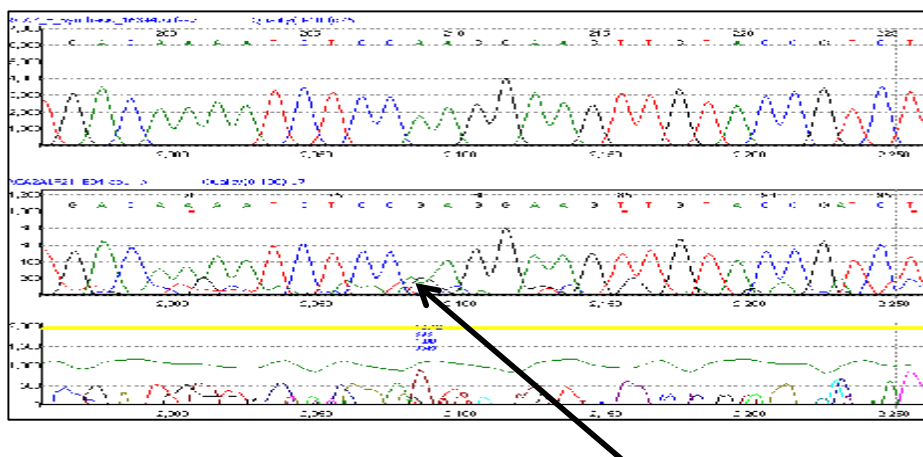**Figure (2) BRCA2 exon 10 mutation (a, 1114 A>C. N372H)**

Figure (3) BRCA2 exon 10 mutation (a, 1153 A>G. K385E)

Conclusion

High percentage of Iraqi breast cancer women harboring mutations in BRCA2 gene exon 10 inreach to (69.44%) 25 cases out of 36. A novel missense mutation (a, 1153 A>G. K385E) (figure 3) was recorded in 11 (30.56 %) of the study cohort from the breast cancer patients and none of the controls. 60% of apparently healthy control cases were harboring the mutation (a, 865A>C, N289H). To date, no other mutational analysis on breast cancer has been conducted in Iraq. This report helps determining the spectrum of *BRCA2* point mutations in our country and must be the beginning of other studies with a larger cohort in order to determine the prevalence of the mutations in the general population.

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