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Detection of BRCAII genetic variations in Iraqi breast cancer patients

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Abstract: Presently breast cancer is the most common cancers in females. As BRCA1 and BRCA2 genes play a critical role in the DNA repair of double-strand breaks, the preponderance of the hereditary forms of breast cancer are caused by mutations in the these genes , therefore females with a family history of breast cancer should make frequent genetic follow-up . Genetic variants with unclear clinical significance may appear a diagnostic challenge when performing a targeted risk. In this study, exon 14 of BRCA2 gene was targeted for genetic variables identification in the group of some female Iraqi breast cancer patients using High Resolution Melting (HRM). For our knowing, this is the first time to use this technique for this purpose on Iraqi DNA breast cancer samples. DNA samples were obtained from 36 female Iraqi breast cancer patients (mean age 50.5±9.85) and 10 age-matched apparently health controls. Pre-approval was obtained from all subjects. Thirty five (97.22%) of breast cancer patients were harbouring gene mutations on exon 14 of BRCA2 gene, all were missense. The thirty five (97.22%) of patients were with (t.7397T>C.V2466A) mutation, while a novel missense mutation (a, 7387 A>g.N2463D) was recorded in 22(61.11%) of patients. This study recommend to include larger sampling aiming to determine the prevalence of the mutations in the general Iraqi population.

Key word: Breast cancer, BRCA2 gene, Exon 14, Mutation.

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Introduction:

Breast cancer (B.C.) 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 B.C. is the first of the commonest ten 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 an 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 damag repair pathway associated with the activation of homologous recombination and double-strand break repair (10). The risk of developing breast cancer in women who carry *BRCA1* or *BRCA2* mutations increases with the number of relatives who have a breast cancer diagnosis (11).

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 (12).

Materials and Methods:

Patients Stud:

Thirty-six blood samples of Iraqi breast cancer (B.C.) patients (mean age 50.5±9.85) were diagnosed by their physician recruited to Baghdad Medical city and Nuclear Medicine Hospital /Baghdad, Iraq and ten blood samples of apparently healthy control females were collected between the age (45-55)years. 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 venipuncture 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 200 µl of peripheral blood using QIAmp DNA mini kit (50) from Qiagen. DNA yield was measured using NanoDrop ND-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. 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 14 was divided into three amplicons by using designed primers (Table 1).

Polymerase chain reaction (PCR) was done by using HotStarTag®Master Mix Kit under the amplification program (Table 2).

Table (1): Size of primer sequence for BRCA2 gene exon 14

Amplicon No	Primer	Product size	GC%	Tm/°C
A1	F-ATGAGGGTCTGCAACAAAGG-3' R- GGGGAAAACCATCAGGACAT-5'	595	50 50	60.1 61.0

A= Amplicon

Table (2): amplification program

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

The PCR product was cleaned up using Charge Switch PCR Clean-Up Kit (Invitrogen, USA) and then running on the gel. Sequencing reaction was done using dye terminator cycle sequencing (ABI) for the PCR products, the component of sequencing reaction mix preparation listed in (Table3). The reaction program was stored in thermo cycler for this reaction (Table 4). Clean-up of sequencing reaction products by using Agencourt CleanSEQ kit (Agencourt®CLEANSEQ® Dye Terminater Removal from Beckman Coulter/USA). Thirty five µl of the clear sample was transferred 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 ^{o}C	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 of patients with breast cancer from whom blood samples was taken (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 present results on Iraqi patients revealed that a high age frequency of cancer occurred between (51-60), years of age (38.89%), while other demographic Iraqi studies revealed that the age range (40-49) years old accounting for 67 out of 216 breast cancer patients (31%) was most frequent (13). The study result comes with finding of increasing risk of breast cancer with age (14), also due to using of menopausal hormone therapy (15). 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 (16). This may be attributed to many factors, such as environmental factors, the nutrition, low exercise, 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.

Thirty five breast cancer patients (97.22%) were harboring BRCA2 gene mutations exon 14 (Table 5)**.** Exon 14 was amplified with one set of primers to give one amplicons (17)**.** The primer designing was done by primer3plus website. All the mutations were missense (Table 5). A first missense mutation (t, 7397T > C.V 2466A) appeared in 35 (97.22%) patients, while the (a,7387 A>G.N2463D) mutation appeared in 22 (61.11%) of patients (Figure 1 and 2). Both mutations did not recorded in controls. The first one as a matter of face is a common missense single nucleotide polymorphism (SNPs) that have been previously reported in literatures (18, 19) as well as in the Breast Cancer Information Core (BIC) data base. Another one was novel missense mutation (a,7387 A>G.N2463D). The difference in the frequencies of the mutations between the patients and controls was significant (P<0.01) and among the patients were significant difference P<0.01 also.

Table (5): Frequency of BRCA2 mutation exon 14

Mutation	Freq. in patients		Freq. in control		Chi-square value
	no.	%	no.	%	
t,7397T>C.V2466A	35	97.22	0	0	11.68 **
a,7387 A>G.N2463D	22	61.11	0	0	9.70 **
Chi-square value	---	9.44 **	----	8.00 **	
** (P<0.01), ns: non-significant.					

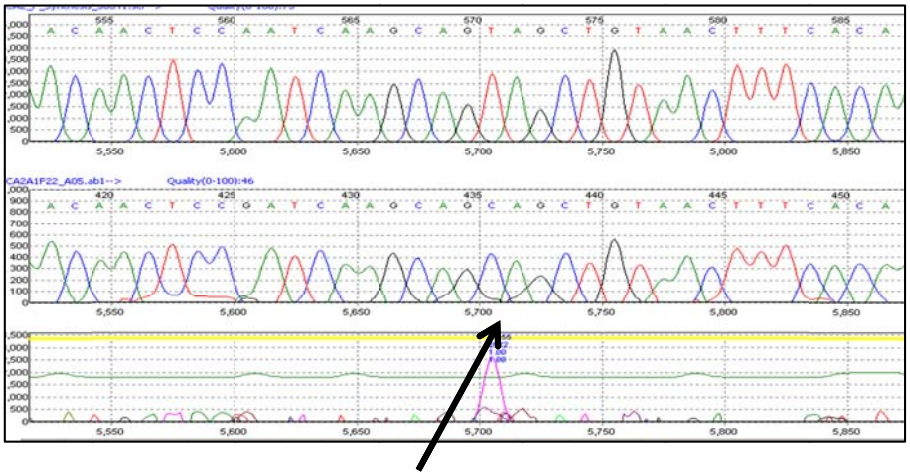

Figure (1) BRCA2 exon 14 mutation (t, 7397T>C.V2466A)

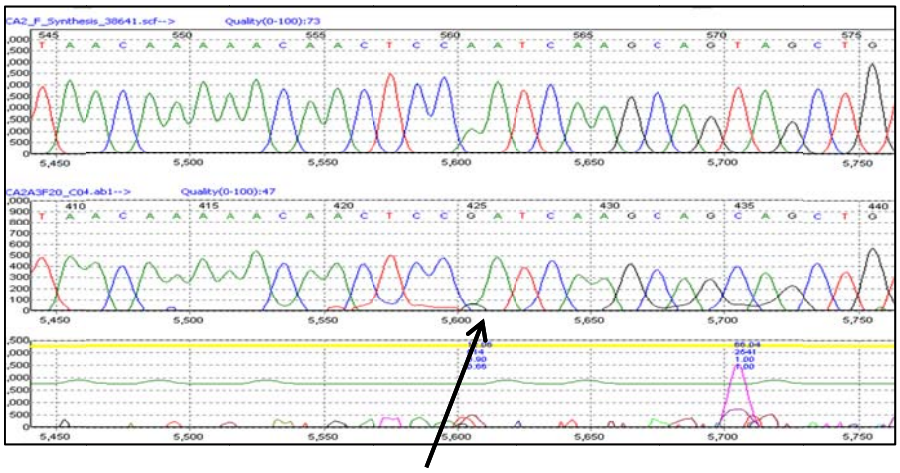

Figure (2): BRCA2 *exon 14* mutation (a,7387 A>G.N2463D)

Conclusion:

High percentage of Iraqi breast cancer women harboring mutations in BRCA2 gene exon 14 in reach to (97.22.44%) (35 cases out of 36). A novel missense mutation (a,7387 A>G.N2463D) (Figure 2) was recorded in 22 (61.11 %) of the study cohort from the breast cancer patients, none of the controls were harboring any of mutation in BRCA2 exon 14 .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.

Reference:

1- Sariego, J. (2010). Breast cancer in the young patient. ***The American surgeon***, 76(12): 1397–1400.

2- Anderson, B.O.; Yip, C.H.; Smith, R.A.; Shyyan, R.; Sener, S.F.; Eniu, A. and Harford, J. (2008). Guideline implementation for breast healthcare in low-income and middle-income countries: overview of the Breast Health Global

Initiative Global Summit 2007. ***Cancer***; 113(8): 2221–2243.

3- Iraq Ministry of Health, Cancer Board, (2012).

4- Wooster, R.; Bignell, G.; Lancaster, J.; Swift, S.; Seal, S.; Mangion, J.; Collins, N.; Gregory, S.; Gumbs, C. and Micklem, G. (1995). Identification of the breast cancer susceptibility gene BRCA2. ***Nature***; 378(6559):789-792.

5- Norbury, C.J. and Hickson, I.D. (2001). Cellular Responses to DNA Damage. ***Annual review of pharmacology and toxicology***; 41: 367–401.

6- Duncan, J.A.; Reeves, J.R. and Cooke, T.G. (1998). BRCA1 and BRCA2 Proteins: Roles in Health and Disease. ***Molecular pathology***; MP 51(5): 237–247.

7- Yoshida, K. and Yoshio, M. (2004). Role of BRCA1 and BRCA2 as Regulators of DNA Repair, Transcription, and Cell Cycle in Response to DNA Damage. ***Cancer science***; 95(11): 866–871.

8- Wong, A.K.; Pero, R.; Ormonde, P.A.; Tavtigian, S.V. and Bartel, P.L. (1997). RAD51 Interacts with the Evolutionarily Conserved BRC Motifs in the Human Breast Cancer Susceptibility Gene Brca2. ***The Journal of biological chemistry***; 272(51): 31941–31944.

9- Chen, P.L.; Chen, C.F.; Chen, Y.; Xiao, J.; Sharp, Z.D. and Lee, W.H. (1998). "The BRC Repeats in BRCA2 Are Critical for RAD51 Binding and Resistance to Methyl Methanesulfonate Treatment." ***Proceedings of the National Academy of Sciences of the United States of America***; 95(9): 5287–5292.

10- Chen, J.J.; Silver, D.; Cantor, S.; Livingston, D.M. and Scully, R. (1999). BRCA1, BRCA2, and Rad51 operate in a common DNA damage response pathway. ***Cancer Res***.; 59(7):1752-1756.

11- Wise, J. *et al*. (2017). Family history is strong risk factor in BRCA1 and BRCA2 carriers, study finds. ***BMJ***;357(3020).

12- Easton, D.F.; Steele, L.; Fields, P.; Ormiston, W.; Averill, D.; Daly, P. A. and Goldgar, D. E. (1997). Cancer risks in two large breast cancer families linked to BRCA2 on chromosome 13q12-13. ***American journal of human genetics***; 61(1): 120–128.

13- Alwan, N.A.S. (2010). Breast cancer: demographic characteristics and clinico-pathological presentation of patients in Iraq. ***Eastern Mediterranean health journal***; 16(11); 1159–1164.

14- Olson, J.S. (2002). Bathsheba's breast: women, cancer, and history. Johns Hopkins University Press, Baltimore.

15- Beral, V.; Reeves, G.; Bull, D. and Green, J. (2011). Breast cancer in relation to interval between menopause and starting the hormone therapy. ***Journal of international cancer institute***; 4(103): 296-305.

16- Mortality and global health estimates. (2013). Adult mortality by country. http://apps.who.int/gho/data/node.main.11 ?lang=en

17- Jalkh, N.; Nassar-Slaba, J.; Chouery, E.; Salem, N.; Uhrchammer, N.; Golmard, L. and Mégarbané, A. (2012). Prevalance of BRCA1 and BRCA2 mutations in familial breast cancer patients in Lebanon. ***Hereditary cancer in clinical practice***; 10(1): 7.

18- Ishitobi, M.; Miyoshi, Y.; Ando, A.; Hasegawa, S.; Egawa, C.; Tamaki, Y.; Monden, M. and Noguchi, S. (2003) Association of BRCA2 polymorphism at codon 784 (Met/Val) with breast cancer risk and prognosis. ***Clin. Cancer Res***.; 9: 1376–1380.

19- Freedman, M.L.; Penney, K.L.; Stram, D.O.; Le Marchand, L.; Hirschhorn, J.N., Kolonel, L.N. and Haiman, C.A. (2004). Common variation in BRCA2 and breast cancer risk:a haplotype-based analysis Multiethnic Cohort. ***Human molecular genetics***; 13(20):2431–2441.