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Detection of BRCA1and BRCA2 mutation for Breast Cancer in Sample of Iraqi Women above 40 Years

*Amina N. AL-Thaweni** *Waleed H. Yousif ***
*Sarah Salih Hassan***

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Abstract

Breast cancer is the commonest cancer affecting women worldwide. Different studies have dealt with the etiological factors of that cancer aiming to find a way for early diagnosis and satisfactory therapy. The present study clarified the relationship between genetic polymorphisms of BRCA1 & BRCA2 genes and some etiological risk factors among breast cancer patients in Iraq. This investigation was carried out on 25 patients (all were females) who were diagnosed as breast cancer patients attended AL-Kadhemya Teaching Hospital in Baghdad and 10 apparently healthy women were used as a control, all women (patients and control) aged above 40 years. The Wizard Promega kit was used for DNA isolation from breast patients and normal individuals. By this method suitable quantities of DNA approximately (50 µl) with purity ranged from (1.7-1.9) were obtained from 100-200µg of fresh biopsy which had been taken from women breast patients. The extracted DNA was successfully used in amplification of BRCA1 & BRCA2 genes by PCR and some mutation were detected. The outcome of genetic analysis indicated that the percentage of 185delAG mutation was 16 (4 patients) whereas, the percentage of 5382insC mutation was 32 (8patients) in BRCA1 gene and the third mutation 6174delT in BRCA2 present in 3 patients only (12%). The study demonstrated that the frequency of BRCA1 mutation (48%) was higher than BRCA2 (12%) in this sample of Iraqi women with breast cancer.

Key words: BRC, BRCA2 , Breast Cancer

Introduction

Breast cancer is any abnormal growth within breast tissue that is inconsistent beside age and sex of the individual. Similar to the development of other cancer types, breast tumor genesis is a multistep process; it starts with ductal hyper proliferation and progresses into *in situ*, then invasive, and finally metastatic carcinoma (Polyak, 2002). The identification of breast cancer genes is a major scientific and social bring, therefore the identification of such genes will not only enable the identification of individuals at high risk, but also aid in the design of more-effective control. Two breast cancer susceptibility genes have been isolated, BRCA1 and BRCA2, with germline mutations in these genes accounting for the majority of hereditary breast cancer. The presence of a mutation in either BRCA1 or BRCA2 will increase an individual's lifetime risk of developing breast cancer to 60-85% (Rebbeck *et al*., 2001).

In Iraq, where the population was exposed to high levels of depleted uranium following the first and second Gulf Wars, breast cancer is the most common tumor type in females. Over the last ten years, there has been a three-fold increase in the incidence of breast cancer (Al-Azzawi, 2006). No

* Genetic Engineering Ins. Baghdad University
** College of Science , AL-Nahrain University

really attempt was done to study this problem on the molecular basis while some studies concerning breast cancer were accomplished using cytogenetic techniques & Random polymorphic DNA amplification (Jaffer, 1999; Jasim, 2004). Up to our knowledge, no study in Iraq have dealt with BRCA1 & BRCA2 mutation and there relation with breast carcinogenesis.

Aim of study

Investigate the presence of (185del AG,5382ins C and 6174delT) in BRCA1 & BRCA2 genes in Iraqi women above 40 years in addition to evaluation the relationship of developing breast cancer with estrogen hormone level & lipid profile.

Materials and Methods :

Samples Collection

One g fresh tissues were collected in container tubes with normal saline. All samples were obtained after informed consent of the participants prior to their inclusion in the study. A structured questionnaire was used to elicit detailed information on age, age at menarche, blood group and family history of breast cancer and another cancer

Molecular Study of Tissue Samples

Isolation of genomic DNA by Promega kit.

Mutagenically separated Polymerase Chain Reaction (PCR) amplification for__BRCA1 and BRCA2. The following chemicals were used for MS-PCR amplification (pak *et al*., 2008).

Primers:- (pak *et al*., 2008).

Table (1) Primers sequences and their size of amplicon

Primer		Sequences	Size of amplicon
1*BRCA1 185del AG	P1	5'GGTTGGCAGCAATATGTGAA'3	
	P2	5'GCTGACTTACCAGATGGGACTCTC '3	335bp
	P3	5'CCCAAATTAATCACTCTTGTCGTGACTTACCAGATGGGACAGTA'3	354bp
2*BRCA1 538 insC	P4	5'GACGGGAATCCAAATTACACAG'3	
	P5	5'AAAGCGAGCAAGAGAATCGCA'3	271bp
	P6	5'AATCGAAGAAACCACCAAAGTCCTTAGCGAGCAAGAGAATCACC'3	295bp
3*BRCA2 6174del T	P7	5'AGCTGGTCTGAATGTTCGTTACT '3	
	P8	5'GTGGGATTTTTAGCACAGCTAGT '3	151bp
	P9	5'CAGTCTCATCTGCAAATACTTCAGGGATTTTTAGCACAGCATGG'3	171bp

P1=common forward p4=common reverse p7=common reverse
P2=wild-type reverse p5=wild-type forward p8=wild-type forward
P3=mutant reverse p6=mutant forward p9=mutant forward

PCR Reaction :- (pak *et al*., 2008). The reaction mixture of MS-PCR was prepared according to the addition

order shown in table (2-2, 2-3, 2-4).The reaction mixture samples were mixed genetly by vortex and centerifuge at 1300 rpm for few seconds to collect all drops to the bottom of tubes. The tubes were then placed in appollo thermal cycler (with heating lid) to carry out amplification. The amplifications were run according to the program shown in table (2, 3, 4). Twenty microliter of amplified DNA was drawn into another tube and analyzed by agarose electrophoresis.

Reagents were used in MS-PCR (25µl) at final concentration

- To a 25 µl PCR tube, 4µl DNA was utilized.
- A 30 picomoles of primers forward and reverse (2µl for each), were added to the tubes, then 2.5 D.W was added (for each tube).
- Finally, a 12.5µl master mix (Promega Co.) were added. The BRCA1 and BRCA2 genotypes were analysed by PCR, Genomic DNA was amplified by using 9 sets of primers (Table 2-4,2-5,2-6)

Table (2) The reaction mix (25µl) for BRCA1 185delAG mutation

Addition order	Chemical	Volume	Concentration
1-	PCR Master Mix	12.5µl	2x
2-	P1	2 µl	30 pmol/ml
3-	P2	2 µl	30 pmol/ml
4-	P3	2 µl	30 pmol/ml
5-	DNA	4 µl	50 ng/µl
6-	D.W	2.5 µl	-
Not :- final volum =25 µl			

P1=common forward P2=wild-type reverse P3=mutant reverse

Table (3) The reaction mix (25µl) for BRCA1 5382insC mutation

Addition order	Chemical	Volume	Concentration
1-	PCR Master Mix	2.5µl	2x
2-	P4	µl	30 pmol/ml
3-	P5	µl	30 pmol/ml
4-	P6	µl	30 pmol/ml
5-	DNA	µl	50 ng/µl
6-	D.W	.5 µl	-
Not :- final volum =25 µl			

p4=common reverse p5=wild-type forward p6=mutant forward

Table (4) The reaction mix (25µl) for BRCA26174delT mutation

Addition order	Chemical	Volume	Concentration
1-	PCR Master Mix	12.5µl	2x
2-	P7	2 µl	30 pmol/ml
3-	P8	2 µl	30 pmol/ml
4-	P9	2 µl	30 pmol/ml
5-	DNA	4 µl	50 ng/µl
6-	D.W	2 .5 µl	-
Not :- final volum =25 µl			

p7=common reverse p8=wild-type forward p9=mutant forward

Primer and PCR conditions:

The PCR products were subjected to electrophoresis on 2% agarose gel stained with ethidium bromide. The presence of bands of 354, 295 bps and 171 was indicative of the BRCA1 and BRCA2 genotypes whereas the absence indicated 335bp, 271bp and 151bp for these genes. A negative control without template DNA was used in each run as shown in table (5).

Table (5): PCR conditions for BRCA1 and BRCA2 genes.

No.	Steps	Temperature	Time	No. of cycles
I	Denaturation	94ºC	15s	35
II	Annealing	57 ºC	15s	1
III	Extension	7 2ºC	30s	1
IV	Extension	72 ºC	15min	1

Because of the low quality and the low size of the products, another low melting agarose gel with the same concentrations (2%) was utilized. Furthermore, a new electrophoresis chamber containing 16 wells was used to improve visibility for larger number of samples at one time. Control cases were 10, and they were derived either from normal tissue samples of the same patients confirmed by histopathological examination or from other cases of benign breast conditions because of limitations of getting normal tissue from normal individuals

Results and discussion :

Genomic DNA isolation from tissue samples

In this study, the quantities of DNA obtained from biopsy of normal women and breast cancer patients were equal or less than 20 µg, and the purity of prepared DNA was 1.7- 1.9. The PCR technique does not required large quantities of DNA (Rafalski, 1997), but it requires highly purified DNA (Strauss, 2002). Hence, the genomic DNA obtained by DNA extraction kit was found to be suitable for the purpose of experimental work designed in this research.

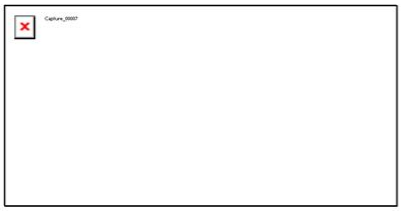

Figure (1): 0.8% agarose gel electrophoresis of DNA samples. (100V., 30 min.)

The PCR-amplified exons 2 and 20 of the BRCA1 gene and exon 11 of the BRCA2 gene together with the adjacent regions of the boundary introns obtained from DNA of the patients with breast cancer were annealed with amplified control DNAs figure (1). The amplified DNA of patients that do not carry these mutations does not form heteroduplexes when annealed with amplified fragments of normal DNA; however, it forms heteroduplexes when annealed with amplified cloned DNA carrying the mutation (Mansukhani *et al*., 1997). In breast cancer women, age-specific indicator refers to that breast cancer will develop in a carrier of mutation by a certain age. Table 6 Presented that 8(32%) of 16(64%) women of breast cancer less than 50 ages have genetic mutation, 6 of them have BRCA1 whereas 2 have BRCA2 mutation while 7(28%) of 9 (36%) women above 50 years have these mutation that clarified that the risk of mutation increased with age which lead to increase the breast cancer. Miki, (1997) recorded that Breast cancer is occur approximately 20 percent by the age of 40 and 80 percent by the age after 50 years. Women with harmful BRCA1 or BRCA2 mutations often develop breast cancer after age 50 (Lynch *et al*., 2008),and women carrying the BRCA1 mutation have an 85% risk of developing breast cancer whereas
women with the BRCA2 gene mutation have an a 27% risk of developing breast cancer above 40 years of age (Llort *et al*., 2007).

Table (6) association between age and BRC A mutations

Age of patient	No of cases	No of mutation	BRCA1 185delAG	BRCA1 5382insC	BRCA2 6174delT	Normal genotype
≤50	16	8	3	3	2	8
≥50	9	7	1	5	1	2
total	25	15	4	8	3	10

All patients were analyzed for constitute BRCA mutation. The genomic DNA was used to detect the mutations by using 9 primers. The gremlin BRCA1 185del AG mutation was detected in 4 patients (16%), in this mutation the mutant and wild type amplicons showed the bands at 354bp and 335bp fig (2).

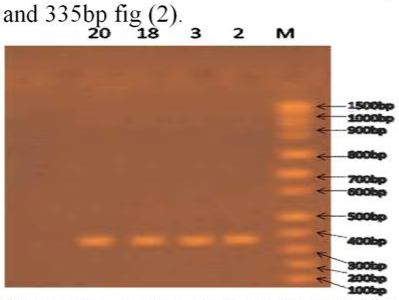

Figure (2):- Agarose gel electrophoresis (2%) showing BRCA1 185delAG detected by (100 V., 45 min) PCR Amplification, lane 2, 3, 18, 20 show mutant type. M: DNA marker (100 -1500bp).

Whereas, the patients with BRCA1 5382insC appeared in 8 patients (32%) in this mutation the mutant and wild type amplicons showed band at 295bp and 271bp as shown in the fig (3).

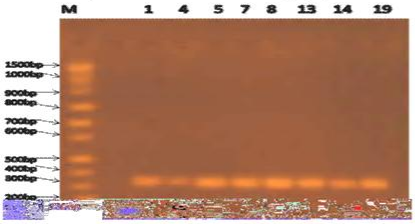

Figure (3-3):- Agarose gel electrophoresis (2%) showing BRCA1 5382insC detected by (100 V., 45 min) PCR Amplification, Lane 1, 4,5,7,8,13,14,19 show mutant type, M: DNA marker (100 -1500bp).

In the present study it is clearly appear that BRCA1 5382insC was more frequency (32%) than BRCA1 185delAG (16%).This result was in agreement with (Struewing *et al* 1997) concluded that the breast cancer risk was highest for the 5382insC mutation in BRCA1 and Brose (2002) found comparable results, with a higher breast cancer risk in BRCA1 5382insC carriers.

The third mutation was BRCA2 6174delT, present in 3 patients (12%), BRCA2 6174delT showed two bands of mutant and wild type at 171bp and 151bp as fig (4)

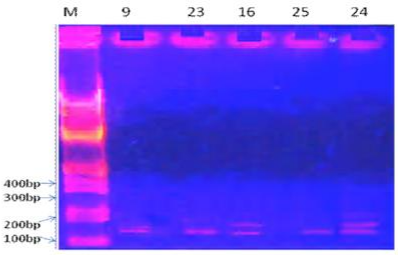

Figure (3-4):- Agarose gel electrophoresis (2%) was showing BRCA2 6174del T detected by (100 V., 45 min) PCR Amplification, lane 9, 16, 24 show mutant type, but 23, 25 show wield type. M: DNA marker (100 -1500bp).

The results of the present study agree with Levy-Lahad *et al*., (1997) who found the mutations 185del AG and 5382ins C in BRCA1were about 60% and 6174del T BRCA2 were about 30% of breast cancer incidence in Ashkenazi Jewish population. It was found that the penetrance of BRCA1 185del AG and 5382insC were found to be significantly higher than that of BRAC2 6174T and the frequency of BRCA1 mutation 48% was higher than BRCA2 (12%) and this study by Easton *et al*., (1994) found that the incidence of BRCA1 mutation was 87% and incidence of BRCA2 was 26% .

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عند عينه من النساء العراقيات BRAC2وBRCA1 تشخيص طفرات المصابات بسرطان الثدي لعمر فوق الاربعين

امنه نعمه الثويني ***وليد حميد يوسف ***** ***ساره صالح حسن* ****

*معهد الهندسه الوراثيه ، جامعة بغداد
** كليه العلوم /قسم التقانه الاحيائيه ، جامعة النهرين

الخلاصة:

يعد سرطان الثدي السرطان الاكثر شيوعا الذي يصيب النساء في مختلف انحاء العالم . وقد تناولت الدراسات والبحوث مختلفه العوامل المسببه لهذا المرض من اجل التوصل للتشخيص المبكر والعلاج الناجح له. تناولت الدراسه الحاليه العلاقه بين التغايرات الوراثه للجينين BRCA1 و BRCA2 والتي تعد من العوامل الخطرة المسببه لسرطان الثدي في النساء .وشملت الدراسه 25 مريضة بسرطان الثدي من مراجعي مستشفى الكاظمية التعليمي و10 نساء طبيعيات ظاهريا تمثل مجموعه السيطرة . بينت النتائج ان كميات الدنا المعزوله من النساء الطبيعيات والمصابات بسرطان الثدي تراوحت 50 مايكروليتر لكل 100-200مايكروغرام من النسيج وبنقاوه تراوح من(1,7-1,9) عند استعمال العده المستخدمه من شركه بروميكا .وقد خضعت المريضات لدراسه وراثيه جزيئيه تم من خلالها استخلاص الدنا من العينات النسيجيه المأخوذه من ثدي المريضات و استخدم هذا الدنا المعزول لتضخيم جيني BRCA2 و BRCA1 بواسطه تفاعل البلمره المتسلسل (PCR) وتم تحديد بعض الطفرات في جيني BRCA2 و BRCA1 . اظهر التحليل الوراثي ان نسبه الحذف في مريضات سرطان الثدي كانت في4 حالات (16%) للطفره الموجوده في جين BRCA1 185del AG, اما نسبه الاضافه للطفره الموجوده في جين BRCA1 5382insC للمريضات المصابات بسرطان الثدي(8 حالات) (32 %) , اما الطفره الاخيره BRCA2 6174Tموجوده في ثلاث حالات بنسبه (12%) و قد استنتج من الدراسه ان نسبه حدوث الطفره في جين BRCA1 48% هي اعلى من نسبه حدوث الطفره في جين BRCA2 12% في العيّنة من النساء العراقيات المصابات بسرطان الثدي.