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High resolution melting analysis of genotype BRCA 1/2 -associated with Iraqi breast cancer patients

Hayder Aial Muttar

College of Science/ University of Al-Qadisyia, Iraq

Abstract : Background: The association of BRCA1 and BRCA2 genotype with breast carcinomas (BCs) is still in controversial. **Objective:** the present study was aimed to clarify the association of BRCA1 and BRCA2 genotype and BC in Iraqi females and to assess its role as potential contributor to the development and behavioral alteration of BC. **Subjects & methods**: BRCA1 and BRCA2 was detected using PCR-HRM analysis on breast tissue from 40 female patients with 27 female breast cancer; 5 close related to breast cancer patients and 8 ages matched females as control. **Results:** BRCA1 and BRCA2 was detected in 2/32 (3.5%) BC specimens carrying c.211 dupA in exon 5 and BRCA2 was detected also in one out of 32 patients carrying c.26G> A mutation. PCR-HRM was able to detect of BRCA1 and BRCA2 variants by melting curve of wild type and mutant DNA sequence present in all patients samples .on the other hand all control specimens were negative as regards prognostic factors, no association was observed between BRCA1 and BRCA2 and patients' age. **Conclusions:** our results demonstrated the presence of the BRCA1 and BRCA2 genome in a considerable subset of BC in Iraqi patients. The mutation was more frequently associated with bad prognostic factors. This indicates that mutation may pass be passing through family generation and BRCA1 and BRCA2 play a role in the development and behavioral alteration of some aggressive BC.
Key words: BRCA1 and BRCA2, breast cancer.

Introduction

Breast cancer is the second reason of mortality in the world[1, 2] and the occurrence has increased by 2-fold over the past 30 years [2]. The identification of the causes of breast cancer is a crucial research issue for the development of effective prevention and treatment strategies. The prevalence of breast cancer is 23% among all cancers in the world [3], and its mortality rate is about 16%[1], so it is the most common and fatal cancer in women[2,4]. Risk factors of breast cancer are age, family history, menarche, delayed menopause, first pregnancy after 25 years of age, nulliparity, long-term consumption of exogenous estrogens, and obesity after menopause, and encountering ionizing ray[5].

Breast cancer 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 [6]. The size, stage, rate of growth, and other characteristics of the tumor determine the kinds of treatment. Treatment may include surgery, drugs (hormonal therapy and chemotherapy), radiation and/or immunotherapy [7].Many agents including radiation, chemicals and viruses, have been found to induce human cancer [8].

Mutations in the tumor suppressor proteins BRCA1 and BRCA2 greatly increase the susceptibility of individuals to develop breast cancer. The overall lifetime risk for a woman to develop breast cancer is about

10%, However, the lifetime risk of breast cancer for women carrying BRCA1 and BRCA2 mutations is 82%, the risk is 54% for BRCA1 mutations and 23% for BRCA2 mutations [9].

Both BRCA1 and BRCA2 appear to be involved in DNA repair pathway networks, although their mechanisms for this are not clear. It has been found that BRCA1 contains a peptide domain called BRCT that appears to be a common motif in other proteins involved in DNA repair [10]. BRCT domains bind phosphopeptides in protein-binding partners and typically occur as 80–100 amino acid sequences present as tandem repeats in BRCA1. These sequences recognize substrates phosphorylated by the DNA repair kinases ATM and ATR in response to g-irradiation. [11] .A mutation in the BRCT domain of BRCA1, which prevents binding to phosphopeptides, may explain why this mutation predisposes women to breast cancer. Yu et al. further showed that the BRCA1 BRCT domain binds a phosphorylated, BRCA-associated, DNA repair helicase. This interaction is cell cycle regulate and required for DNA damage–induced checkpoint control of the G2 to M cell cycle phase transition. These authors suggest that BRCT domain–containing proteins are a family involved in DNA repair and cell cycle checkpoint control[12].

Based on these considerations and in view of the high incidence of female BC in Iraq [1] this association required clarification as it could profoundly shape the clinical diagnosis ,disease management and, potentially, patient outcome.

Accordingly this study was carried out to clarify the association of Both BRCA1 and BRCA2 and BC in Iraqi females and to assess its role as potential contributor to the development and behavioral alteration of BC

Subjects & methods

Samples for the study:

A total of 40 blood samples were subjected for the PCR- HRM analysis from Central and hospital units of Najaf, Diawynia, Samawa and Hilla cities. There forty were divided into 27 female breast cancer patients, 5 close relative to breast cancer patients and 8 as negative cancer controls. All patients were signed the informed consent to participate. The classification of proved cases as malignancy was confirmed by clinical, histopathological and radiological diagnosis. All forty samples were ethnic Arabic Iraqi in mean age 51 years for the screening of mutations in BRCA1 and BRCA1.

DNA extraction:

The peripheral blood in EDTA tube is taken for blood DNA extraction using MagDEA DNA 200 whole blood plus kit on Magtration system 12GC plus machine. The extracted genomic DNA samples were measured by Nano Drop -1000 spectrophotometer and then stored at 2-8 °C until PCR-HRM analysis.

HRM Assay:

The reference DNA sequence of BRCA1 (GenBank NM_007294.3) and BRCA1 (GenBank NM_000059.3)(BRCA2) were used for designed the specific primers which cover of exons 5 of BRCA1 produced 278bp by applying of forward primer 5' CTCTTAAGGGCAGTTGTGAG3' and reverse primer 5' ATGGTTTTATAGGAACGCTATG 3' and exons 2 of BRCA2 produced 311bp by applying of forward primer 5' CCAGGAGATGGGACTGAATTAG 3' and reverse primer 5' CTGTGACGTACTGGGTTTTTAGC 3'. These primers were according into recorded by P. D. Murphy (1). The real-time PCR including high resolution melting HRM were performed on CFX 96 Real-time PCR System combined with precision melt analysis software (Bio Rad). The volume of PCR assay consist of 10 µl of Precision melt supermix , 2 µl of 2 µM primer mix and 5 µl of genomic DNA, then added grade water up to final reaction volume 20 µl. The real-time PCR-HRM program included one cycle of 95 ºC for 2 min and 40 cycles of 3-steps(95 ºC for 10 sec , 60 ºC for 30 sec and 72 ºC for 30 sec) , then HRM analysis included one cycle of each 95 ºC for 30 sec ,60 ºC for 1 min and 65-95 ºC for 10 sec.

DNA sequencing:

The PCR products were subjected to direct DNA sequencing by using ABI 3730 XL genetic analyzer (Applied Biosystem). The data were aligned by using false-negative rate and validation of PCR-HRM validation.

Statistical analysis:

Fisher's exact test and t-test were used to obtain statistically significant differences between two groups with $p<0.05$ being considered statistically significant.

Results and discussion

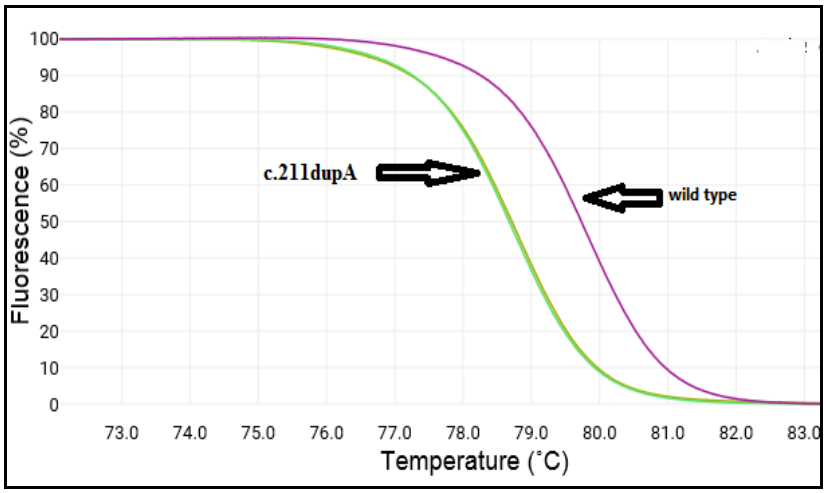
Many studies reported that breast cancer be more opportunities for more than 45 years women's (13). In this study showed 50% (16 patients) breast cancer patients were more than 60 years, 33% (11 patients) were ranging 40-60 years and 17% (5 patients) less than 40 years (table 1). Into comparison with patient group that control group was the same age categories. Several factors especially in Iraq community believed important in breast cancer risky such as unfavorable environmental factors, lifestyle, obesity and hormonal therapy addition to the age and hormonal imbalances in women (14).

In current study used the PCR-HRM assay for detection and frequency of BRCA1 and BRCA2 gene mutations in forty Iraqi breast cancer through specific primers design which were targeted for c.211 dupA (rs397508938) in exon 5 of BRCA1 gene and c.-26G>A (rs1799943) in exon 2 of BRCA2 gene. As this study record two of the 32 breast cancer patients carrying c.211 dupA and one carrying c.-26G>A mutations.

PCR-HRM was able to detection BRCA1 and BRCA2 variants by melting curve of wild type and mutant DNA sequence present in all patients' samples as shown in figure (1). This study identified a heterozygous genotype of BRCA 1 and BRCA 2 carrying c.211 dupA and c.-26G>A variations, respectively.

Table (1): Distribution of Mean age (years) Among the Studied Groups

Studied groups	N	Mean years	Std. Deviation	Mini.	Maxi.	Student (t-test)	
						P-value	Sig.
Healthy Control group	8	59.7	8.9	48	70	0.005	Significant (P<0.01)
Breast cancer	32	46.8	12.1	27	70		
Total	**40**						



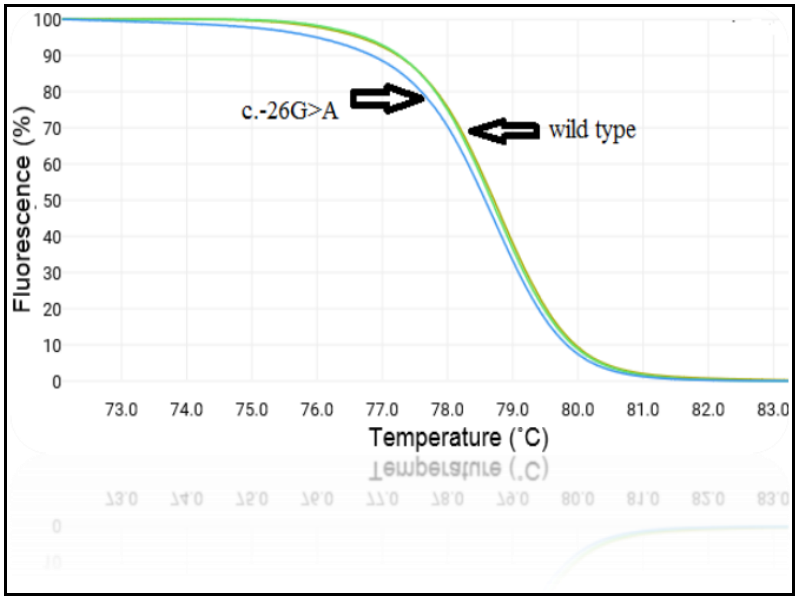


Figure (1):Resolution melting cures of heterozygous of c.211 dupA in BRCA 1 and c.-26G>A in BRCA 2 gene compared with wild-type genotype.

The Sanger DNA sequencing performed for all samples that showed the validation of PCR-HRM assay of BRCA1c.211dupA and BRCA2 c.IVS1-26G>A variations, as shown in figure (2).

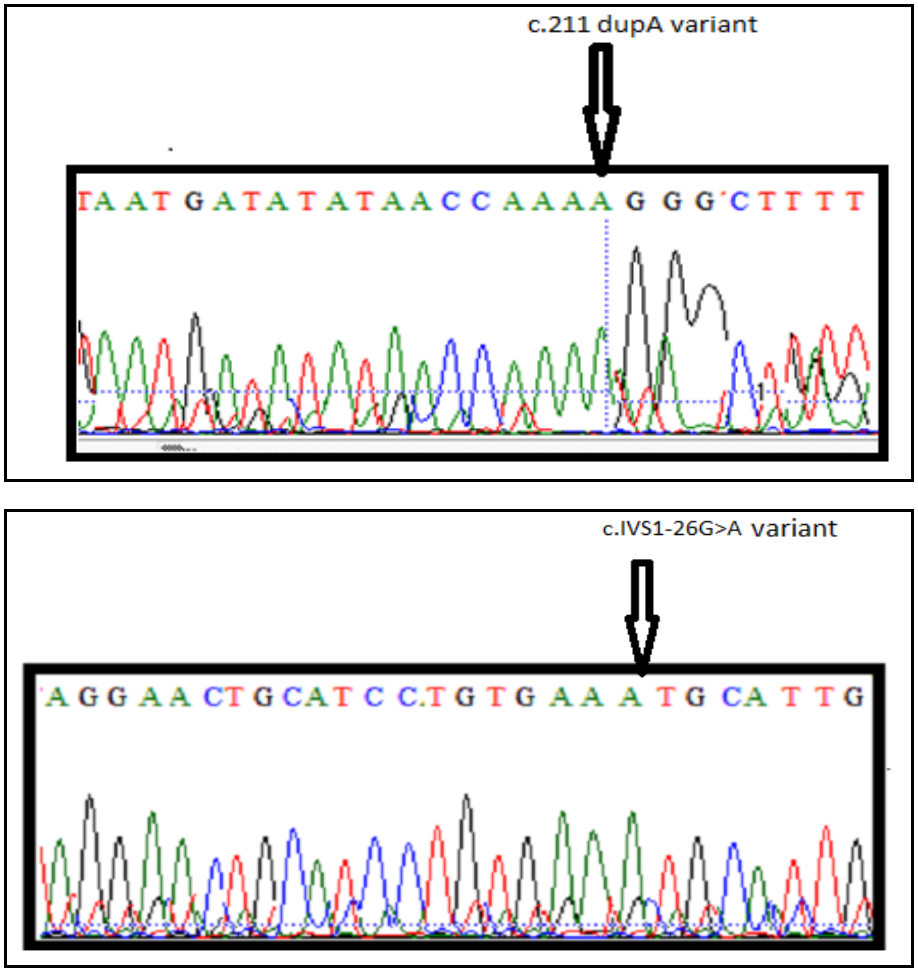


Figure (2): The variants c.211dupA and c.-26G>A of BRCA1 and BRCA2 described by DNA sequencing electropherogram.

The first study is described in Arab Iraqi breast cancer patients of c.211dupA and c.-26G>A mutations in BRCA 2 and BRCA 1 genes, respectively. The genetic test of BRCA1 and BRCA2 mutations have important for positive test results indicate the harmful mutation may be pass though family generation in risk of developing some cancers (15). While in negative cases of BRCA1 or BRCA2 mutations are more complex in breast cancer so that it required the completed scanning of gene mutations of patients and his family (16). Sometimes, a mutation was first recovery and regarded as no significance in cancer developing in certain race of the world, because the belief that BRCA1 or BRCA2 mutations which has not been previously associated with ethic cancer patients (17). Several studies reported that 10% of BRCA 1 and BRCA 2 patients have positive for ambiguous mutations result (18).

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