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Association Between *BRCA1/2* Polymorphism and HPV in Breast Cancer Patients in Thi-Qar/South Iraq

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Abstract

Breast cancer is presently the greatest common malignant disease detected in women worldwide. The breast cancer occurs as a result of transformations in *BRCA1* and *BRCA2* when the cells injured, by chemicals in tobacco, exposure to radiation and infected with oncogenic viruses such as a Human papilloma virus, *BRCA1* and *BRCA2* are human genes which make tumor silencer proteins. This study was carried out to investigate the presence of mutation (5382insC and 6174delT) in *BRCA1* and *BRCA2* genes, respectively (to determent the effect of HPV of human genes) by gene polymorphism amplification-refractory mutation system-PCR (ARMS-PCR) technique. The ARMS-PCR assay was performed for detection BRCA1 (185delAG) and BRCA2 (6174delT) gene mutation in breast cancer blood patients infected with HPV. The ARMS technique which revealed that out of 32 malignant breast tumors 5 (15.6%) cases carry a 5382insC mutation in BRCA1 gene, and no 6174delT mutation was recorded in BRCA2 gene. The 5382insC polymorphisms recorded in BRCA1 gene, and the HPV may induce it, while no evidence of the BRCA2 6174delT polymorphism, This may indicate that there is no correlation between this mutation and HPV.

Keywords: HPV, Breast Cancer, *BRCA1/2*, Polymorphism, ARMS-PCR.

Introduction

Breast cancer is the communal neoplasm detected in women around the world at present (Khodabandehlou *et al.,* 2019). The breast cancer occurs as a result of transformations in *BRCA1* and *BRCA2* when the cells injured, by chemicals in tobacco, exposure to radiation and infected with oncogenic viruses such as a human papilloma virus, *BRCA1* and *BRCA2*

are human genes which make tumor silencer proteins. When DNA damage, these proteins contribute to reform it, and in this manner, these protein help in maintaining the stability of the genetic material. When these genes exposures to mutation or changed, the tumor suppreser proteins do not make or do not work accurately. Subsequently, cells will probably develop additional genetic transformation that can increase the risk of cancer. (Morris *et al*., 2010; Kuchenbaecker *et al*., 2017). More than 2000 distinct changes detailed in BRCA1and BRCA2 genes, including insertions, deletions, and substitutions in structural or non-structural sequences. The most widely recognized types of transformation assign to small insertion/deletion mutation, and the disarray of the splice region promoting whole non-functional BRCA proteins (Rassi *et al*., 2014). The BRCA1 5382insC mutation has an Ashkenazi originator influence. Also the second record frequent mutation in Eastern European people, having been previously recognized in numerous Romanian HBOC patients (Negură *et al.*, 2015). The mutation in BRCA2 (breast cancer), well-known in 1994. Harmful germline transformations in BRCA2 may converse up to 84% threat of breast cancer and 27% risk of ovarian cancer in women, and are commonly related with Ashkenazi Jewish (Petrucelli *et al.*, 2010). The carrier ratio of the BRCA2 6174delT frameshift mutation in persons of Ashkenazi Jewish descent is assessed to be 0.9%, and is designed to have ascended about 90 generations ago (Greenwood *et al.*, 2010). Individuals who have acquired mutations in BRCA1 and BRCA2 have a tendency to generate breast cancer at more youthful ages than individuals who don't have these transformations (Petrucelli *et al*., 2016). The HPV was classified as oncogenic viruses, and known by its tropisms to epithelial cell and causes about 99.7% of malignant cervical lesions (DüzgüneŞ *et al*., 2016). All these explanations make us believe that the virus has a role in emerging breast cancer and should be studied and knowledge of its impact on BRCA1/2 human genes.

This study was carried out to investigate the presence of mutation (5382insC and 6174delT) in BRCA1 and BRCA2 genes, respectively, to determine the effect of HPV in human genes by gene polymorphism ARMS-PCR technique.

Subjects and Methods

Patients: A total of 37 out of 100 breast formalin-fixed paraffin-embedded (FFPE) tissue of the female patients (diagnostic by histological methods) were included in this study, 32 FFPE with malignant breast tumors and 5 FFPE of benign breast tumor were considered as a control group in this study, were diagnosed by conventional PCR assay and give positive results to HPV, then took 1 ml blood sample from each patients infected with HPV. The ages of patients range from 15 to 75 years with a mean of (45.5 ± 21.0).

ARMS PCR Primers for Human BARCA1 and BARCA2

This primers were used for detection *BRCA1*-5382insC and BRCA2-6174delT wild type and mutant types alleles. This primers were designed by (El-Debaky *et al.,* 2011) and provided by Macrogen/ Korea as showed in table (1):

(Table 1): Human BARCA1 and BARCA2 Primers and Probes.

Primer	Sequence		Amplicon size
BRCA1-5382insC	**Common forward (P4)**	GACGGGAATCCAAATTACACAG	-
	Wild-type reverse (P5)	AAAGCGAGCAAGAGAATCGCA	271bp
	Mutant reverse (P6)	AATCGAAGAAACCACCAAAGTC CTTAGCGAGAAGAGAATCACC	295bp
BRCA2-6174delT	**Common forward (P7)**	AGCTGGTCTGAATGTTCGTTACT	-
	Wild-type reverse (P8)	ATGGGATTTTTAGCACAGCTAGT	151bp

	Mutant reverse (P9)	CAGTCTCATCTGCAAATACTTCA GGGATTTTAGCACAGCATGG	171bp

The Amplification-Refractory Mutation System (ARMS)

The amplification-refractory mutation system- PCR assay was performed for detection BRCA1 (185delAG) and BRCA2 (6174delT) gene mutation in blood of breast cancer patients infected with HPV. This method was carried out according to described by (Chan *et al.*, 1999).

Genomic DNA Extraction

Genomic DNA was extracted from blood samples (Frozen Blood) by using Genomic DNA extraction kit (Geneaid. USA), and done according to company instructions.

Genomic DNA estimation

The extracted blood genomic DNA was checked by using Nanodrop spectrophotometer (THERMO. USA).

Preparation of ARMS-PCR master mix

The ARMS-PCR master mix was prepared by using (Maxime PCR PreMix Kit) and this master mix done two reactions for each sample according to company instructions.

The Conditions of ARMS-PCR Thermocycler

The conditions of PCR thermocycler were done for each gene independent as following tables:

PCR step	Temp.	Time	Repeat
Initial denaturation	95°C	5min.	1
Denaturation	95°C	30 sec.	35cycle
Annealing	50°C[1] 52°C[2]	30 sec.	
Extension	72°C	30 sec.	
Final extension	72°C	5min	1

Hold	4°C	Forever	-

1- BRCA1 (185delAG), 2- BRCA2(-6174delT)

The product analysis of ARMS-PCR

The products of ARMS-PCR were analyzed by 1% agarose gel electrophoresis.

Finding

The detection of 5382insC and 6174delT mutation in BRCA1 and BRCA2 genes, respectively in breast cancer patients infected with HPV done by ARMS technique, this technique which revealed that out of 32 malignant breast tumors 5 (15.6%) cases carry a 5382insC mutation in BRCA1 gene as shown in fig. (1 A and B), and no 6174delT mutation was recorded in BRCA2 gene as shown in fig. (2 A and B).

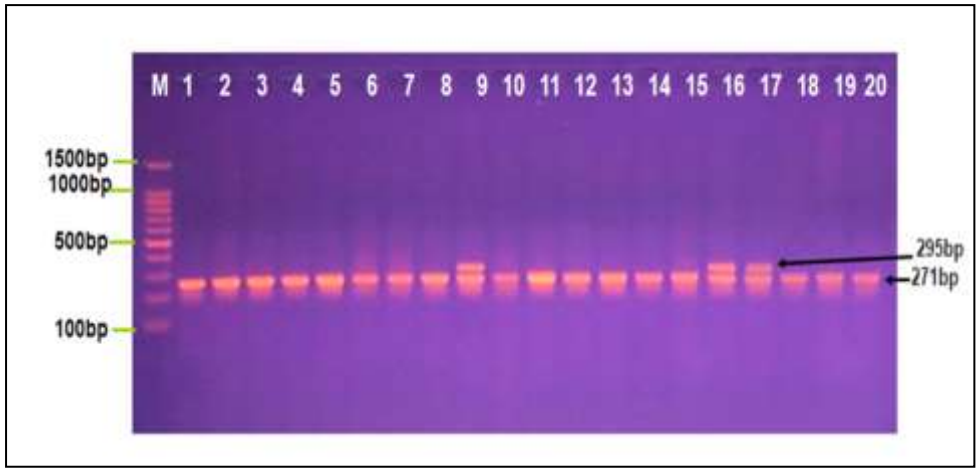


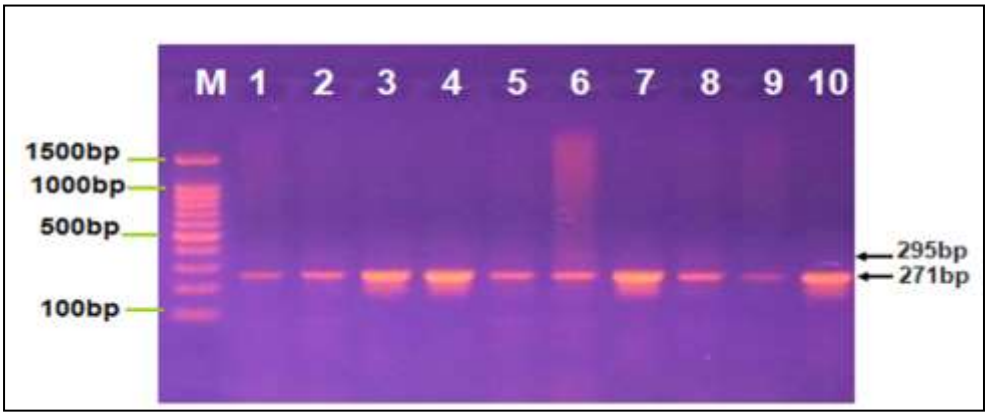
Figure (1A): Agrose gel electrophoresis for BRCA1-5382insC gene in patient samples.

*** Lanes 9,16 and17 show the positive results of BRCA1-5382insC mutation.**

*** Lanes of other wells show negative results.**

Figure (1B): Agrose gel electrophoresis for BRCA1-5382insC gene in control samples.

*** All lanes show no evidence of BRCA1-5382insC mutation.**



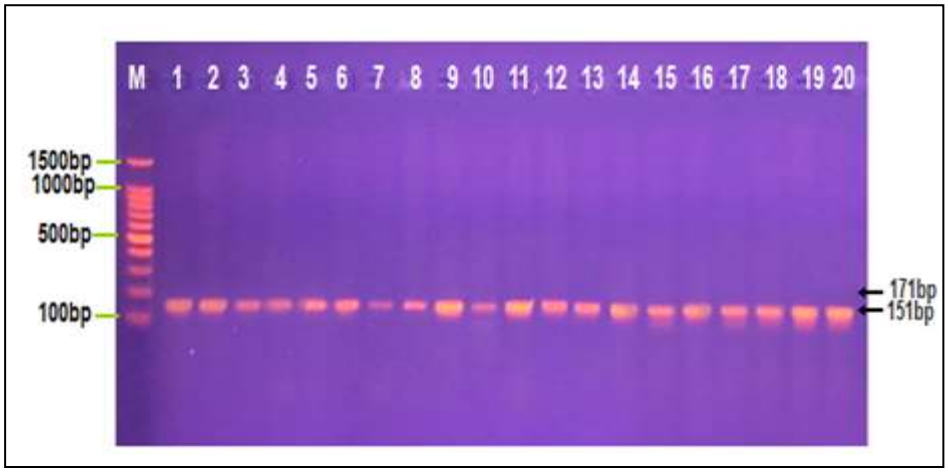


Figure (2A): Agrose gel electrophoresis for BRCA2-6174delT gene in patient samples.

*** All lanes show negative results of the BRCA2-6174delT mutation.**

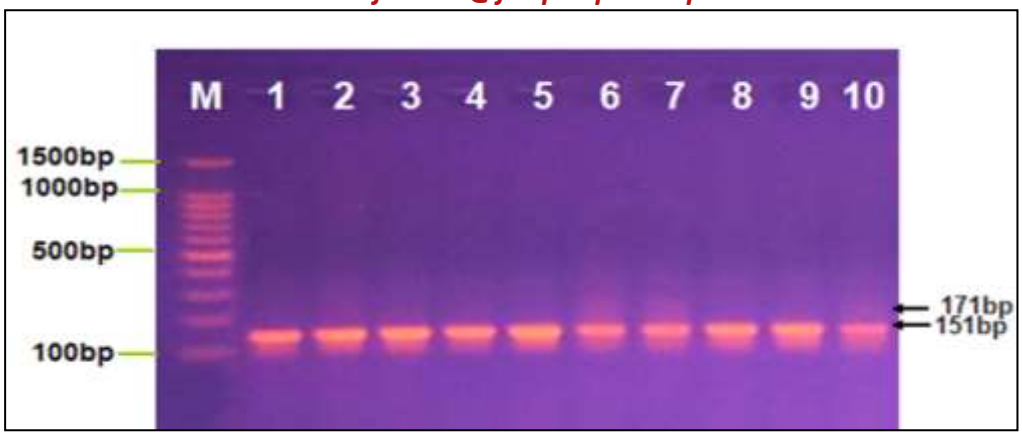


Figure (2B): Agrose gel electrophoresis for BRCA2-6174delT gene in control samples.
*** All lanes show negative results of the BRCA2-6174delT mutation.**

Discussion

The BRCA1 and BRCA2 are human genes that classified a type of genes called as tumor silencers. In normal cells, BRCA1 and BRCA2 contribute to maintaining the stability of cellular DNA and preventing the growth of cancer cells. (Mehdipour *et al.,* 2006). This study was prepared to distinguish the frequency of two single nucleotide polymorphisms (SNPs) variants in BRCA1 gene and BRCA2, The 5382insC mutation in BRCA1 and 6174delT in the BRCA2 gene, which is the most serious and predominant mutation in European countries, Asian and American BC individuals rarely demonstrate it (Hamel *et al.*, 2011; Bansal *et al*., 2014).

In the current study, we observed five 5382insC polymorphisms out of 32 malignant breast cancer, this result agrees with previous studies in Iran by Mohtasebi *et al.*, 2016, they recorded also five 5382insC polymorphisms out of 38 breast cancer cases.

The BRCA2 is known to be implicated in the BC, as BRCA2 6174delT polymorphism, a mutation frequency in a 20-year-old BC patient (Brooks *et al.*, 2006). Also, this mutation recorded in breast cancer patients at Thi-Qar province by Alghaliby *et al*., (2015), but in our study by using the ARMS technique did not reveal its presence in the malignant

breast cases. This may indicate that there is no correlation between this mutation and HPV.

And there are no previous studies on the relationship of the HPV with BRCA2 6174delT polymorphism.

Conclusion

The 5382insC polymorphisms verified in BRCA1 gene, and the HPV might induce it, though no evidence of the BRCA2 6174delT polymorphism, This may point out that there is no association between this mutation and HPV.

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