Detection of Breast Cancer Related Gene Polymorphisms By a New Modified PCR-RAPD-PCR Technique

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Research Article

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Abstract

Breast cancer is one of the most aggressive cancers with high estimated mortality worldwide; many studies around the world have involved polymorphisms in drug resistance genes, tumor suppressor genes, estrogen receptor genes, and regulatory genes related to the development and prognosis of breast cancers. It is worth noting that this study was the first to include these genes as PCR templates to determine the relationship between their polymorphisms and breast cancer incidence using RAPD of amplified genes. The study was designed first to evaluate the association of *ABCG2*, *ABCB1* and *BRCA1* gene polymorphisms in addition to *miRNA-152* and *ER-a* using the RAPD technique with breast cancer incidence in women in Maysan, Province and then to employ those genes in the future as biomarkers in breast cancer prediction and diagnosis. This study included 100 patients with breast cancer and 30 healthy control women, and then all samples were amplified by conventional PCR with specific F and R primers for *ABCG2*, *ABCB1*, *BRCA1*, *ER-α*, and *miRNA-152* genes. Then, the best PCR product

(20) was chosen as the template for the RAPD technique. The results revealed that all RAPD primers showed polymorphisms with higher values in patient samples, and revealed several mutations in patient samples. Our study proved the relationship between genetic polymorphisms of breast cancer related genes and a higher incidence of cancer. The results of the statistical analysis by using Chi-square showed that there were no significant differences in the types of bands of the *ABCG2*, *ABCB1*, *BRCA1* between the patient sample and the control sample, at the probability level of P >0.05, and significant differences in the types of bands of the ER-*α*, and mi-RNA 152 between the patient sample and the control sample, at the probability level of P <0.05. The current study recommends employing these results for future prediction and diagnosis of breast cancers.

1. Introduction

Cancer is a group of diseases involved in abnormal growth of cell with potential spread to other parts of the body (Chandira et al., 2019). Breast cancer (BC) is the most common cancer in women around the world, accounts for 15% of cancer deaths among women, and is the leading cause of cancer death in females worldwide (Jin .,2020). In Iraq, breast cancer is the most common pattern of female malignancy, accounting for approximately one-third of the registered female cancers according to the Iraqi cancer registry in 2004 (Mohammed., 2011; Ismaeel., 2013). Some forms of cancer are associated with a family history of the disease, but in most instances, there is no definitive pattern of inheritance is observed (Sas-Korczyriska et al., 2017; Mampunye., 2020).

Many genes are found related to breast cancer (Sun et al., 2017). The susceptible genes are critical risk factors for both hereditary and sporadic breast cancer (Xie., 2020). ABCB1 gene is located on chromosome 7q21.12 (Sheng X et al., 2012; MUTLU İÇDUYGU et al., 2020). *ABCB1* encoding a transmembrane glycoprotein called permeability glycoprotein (P-gp), the physiologic role of P-gp an ATP-driven transporter mediates to transport many metabolites and harmful substances from inside to outside through plasma membrane (Abuhaliema et al., 2016; MUTLU İÇDUYGU et al., 2020). *ABCG2* gene is located on human chromosome at the locus 4q 22.1. The genetic variants of the ATP-binding cassette, superfamily G, member 2 (*ABCG2*) is known to be involved in developing cancer risk and interindividual differences in chemotherapeutic response

(Wu et al., 2015). *BRCA1* is situated on chromosome 17q21 (Rebbeck et al., 2016 ; Ndiaye et al., 2020) ; is the major cause of familial breast cancer(ALHannan et al., 2019); BRCA1 gene is produce tumor suppressor proteins, so that its called as (TSGs gene), any changes or mutations in *BRCA1* gene can lead to an increased risk of developing breast, ovarian, and prostate cancer (Ayub et al., 2014; Mehrgou and Akouchekian., 2016). ER-α gene is located on chromosome 6q25.1, Estrogen receptor alpha (ER-α) is plays important role in development and progression of cancer, it also stimulates breast epithelial tissue proliferation and differentiation through combining with estrogen (Abd Ellatif et al., 2016; AL-Amri et al., 2020). *miRNA-152* gene is situated on chromosome 17q21.32 (Zhou et al., 2012 ; Liu et al.,2016), *miRNAs* can regulate gene expression at the posttranscriptional level by repressing translation of protein coding gene, *miRNA-152* is abnormally expressed in a variety of diseases, including various cancers, more evidence suggests that miRNA-152 is a tumor suppressor related to cell proliferation, migration, and invasion of human cancer (Kindrat et al., 2016).

RAPD can be defined as a DNA fingerprinting technique based on polymerase chain reaction amplification of random fragments of genomic DNA with single short primers of arbitrary nucleotide sequences (Kumar and Guru Subramanian, 2011; Sharma and Singh, 2017). RAPD analysis is used in studies in the field of genetic instability in breast cancer (Novikov et al., 2016). In this study, we performed a new procedure with some modifications to the traditional RAPD technique when we used the amplified gene as a template in RAPD. This procedure resulted in more accuracy and yielded more specific data. We named it PCR-RAPD-PCR (PRP). The aim of this study was to evaluate the genetic polymorphism of the BC gene in women with breast cancer in Maysan Province, southern Iraq.

2. Material And Methods

Study sites

All methods were carried out in accordance with relevant guidelines and regulations. The experimental protocols were approved by ethics committee/IRB [scientific committee of Biology Department/college of science/Misan university]. The study was undertaken in the genetic engineering laboratory/ Department of Biology / College of science/Misan University. A total of (100) blood samples were collected from the breast cancer women patients and (30) healthy in Maysan Health Directorate/AL-Sader Teaching Hospital /AL-Shifa Tumor Treatment Center in/ AL-Amarah city (sub-districts of Maysan province/south of Iraq).

Samples collection and identification

100 blood samples (2-3 mL) were collected from each breast cancer female patient (in case patient woman in good healthy) or blood samples (1mL) were collected from each breast cancer patient woman (in case the patient woman in critical condition). Blood samples were collected from each woman patient

through the median cubital vein or carpel veins (according to a health condition) into EDTA containing tubes and were stored at -20 ◦C till DNA extraction.

Genomic DNA isolation

All samples were collected from the breast cancer women patients at a different time for three months (September to November). All samples were kept under the same condition. DNA was extracted from whole blood by using the gSYNCTM DNA Extraction Kit Functional Test Data (Geneaid, Taiwan).

Column purification

Genomic DNA was isolated from blood sample. The amount of isolated DNA was varied from 52.392 to 88.712 ng/µl and an absorbance ratio of A260/280 was obtained in the range of 1.71–1.98.

Polymerase chain reaction for isolated PCR product (specific BC genes)

Isolated DNA from all 100 women BC Patient and 30 normal ; genomic DNA samples were prepared by pooling the same amount of genomic DNA from each individual. The DNA fragments of the PCR product of BC genes were amplified through the polymerase chain reaction technique. The following components: 2.5 µl primer F, 2.5µl primer R, 2.5µl Nuclease free

water, 12.5µl GO Taq ®G2 Green master mix and 5µl DNA template were added for each 25 reaction mixture. The primer sequences are shown in the Table 1.

Table (1) Sequence of ABCG2, ABCB1, BRCA1, miRNA-152, and ER-α primers, GC% and length

primer	Sequence	GC%	Length (Base)		References
ABCG2	5-AAAT GTTCATAG CCAGTTTCTTGGA-3 3-ACAGTAATGTCGAAGTTTTTA TCGCA-5	35.29%	F:25	R:26	Wu et al.,2015
ABCB1	5-TTGATGGCAAAG AAATAAAGC-3 3-CTTACATTAGGCAGTGAC TCG-5	40.47%	F:21	R:21	Tazzite et al., 2016
BRCA1	5-CAC CTC CAA GGT GTA TGA AG-3 3-CTC TAG GAT TCT CTG AGC ATG G-5	50%	F:20	R:22	Gholipoorfeshkechen and Arjunan.,2014
miRNA-152	5-TCTGTCATGCACTGACTGCTC-3 3GGGCATGCTTCTGGAGTCTA-5	53.65%	F:21	R:20	Nguyen-Dien et al .,2014
ER-α	5-ATG CGC TGC GTC GCC TCTAA-3 3-CTG CAG GAA AGG CGA CAG CT-5	60%	F:20	R:20	Sato et al .,2008

The PCR reaction consisted of 35 cycles following three essential steps: initial denaturation (5 min at 94◦C), DNA denaturation (1 min at 94 ◦C), primer annealing ABCG2 (45 sec at gradient 53.5 -58.5 ◦C), primer annealing ABCB1 (45 sec at 53 ◦C), primer annealing BRCA1 (45 sec at 55 ◦C), primer annealing ER-α (45 sec at gradient 52-58 ◦C), primer annealing miRNA -152 (15 sec at 65 ◦C), and primer extension (1 min at 72 ◦C). There was a Final extension cycle for 7 min at 72 ◦C. The size of the amplified PCR product was subjected to electrophoresis in 1.2% agarose gel, 1X TBE buffer with ethidium bromide 2%, at 80 V for 60 min. The bands were visualized under ultraviolet trans-illumination and photographed in Gel- Doc equipment. The PCR product that having a clear band was later used as a template in the PCR-RAPD-PCR Technique.

Purify PCR Product by gel extraction

PCR products were obtained after a conventional PCR run; PCR products were extracted from an agarose gel after gel electrophoresis for breast cancer-related genes by using the E.Z.N.A.® Gel Extraction kit protocol (OMEGA BIO-TEK ,USA).

Column Purification

PCR product (specific gene) was isolated from the DNA gel extraction ,the amount of isolated DNA PCR product (specific gene) was varied from 52.392-88.712 ng/µl and absorbance ratio of A260/280 was obtained in the range of 1.71-1.98.

PCR-RAPD-PCR Technique

The isolated PCR product from all women BC Patient and normal DNA; the same best 40 PCR product (20 patient and 20 control) of *ABCG2, ABCB1, BRCA1, ER-α, and miRNA-152* genes were chosen for PCR-RAPD-PCR. The RAPD primer sequencing in the PCR-RAPD-PCR as shown in the Table 2.

Table 2
Sequence of RAPD primers, GC% and length

RAPD primers	Primers Sequences	GC%	Length (Base)
OPAA11	5-ACCCGACCTG-3	80%	10 Base
OPU15	5-ACGGGCCAGT -3	70%	10 Base
OPAA17	5-GAGCCCGACT -3	% 70	10 Base
OPD18	5-GAGAGCCAAC- 3	% 60	10 Base

The reaction mixture (20 µL) consisted of template PCR product 2.5 µl, 2.5 µL (OPAA11, OPU15, OPAA17, OPD18) RAPD primer, 7.5µl Nuclease free water, and 7.5 µl GO Taq ®G2 Green master mix. The mixture was incubated in the TECHNE prime thermal cycler (with heating lid) programmed for (35-40) cycles, each one consisting of as following: a denaturation step (1 min at 94 ◦C), one annealing step (30 sec at gradient 36-39 ◦C) and an extension step (1 min at 72 ◦C). After the cycling, a final extension for 1.30 min at 72 ◦C was followed by slow cooling to 10 ◦C. Four RAPD primers: (OPAA11, OPU15,

OPAA17, and OPD18) were used in the amplifications .The primers were obtained from the company of AUGCT DNA SYN Biotechnology /china.

Electectrophoretic analysis

The reaction products were separated by electrophoresis on an agarose gel (2%) containing ethidium bromide (2%) were prepared in 1 X TBE buffer. The DNA ladder size marker used in this study contained many discrete bands (in base pair) 1500, 1000, 900, 800, 700, 600, 500, 400, 300, 200,100,75,50 and 25 bp from nearest distance to the well to the far one from the well respectively. This ladder was used as a molecular size indicator in the experiments of this study. The DNA fragments obtained were visualized under ultraviolet light and the molecular sizes of DNA bands were estimated according to standard curve represented the relationship between band molecular size of the ladder measured by base pairs and distance of migration bands (Sambrook and Russell., 2006).

Statistical analysis

The relationship between the polymorphic, monomorphic, and unique band and RAPD primer of the breast cancer patient and control was assessed by using the X^2 test, $P \leq 0.05$ was considered statistically significant.

3. Results

The results of the DNA gel extraction of PCR product are showed that the amount DNA of PCR product (specific gene) ranged from 52.3.92-88.712 ng /µl and absorbance ratio of A260/280 was obtained in the range of 1.71-1.98 nm which is within the normal range which between 1.7-2.0 nm which was detected by the NanoDrope device. The results of the DNA gel extraction are showed the success of the specific gene amplification process. Therefore, PCR products can be used instead of DNA as a template in the RAPD technique. Therefore, this technique is called PCR-RAPD-PCR (PRP).

The results of the electrophoresis of the BC gene (PCR product) by using RAPD primers showed a clear difference in the number of amplified gene bands and a clear difference in their molecular weights, depending on the primer used. The four primers OPAA11, OPU15, OPAA17, and OPD18 were used to study the possibility of determining the genetic polymorphisms of the *ABCG2, ABCB1, BRCA1*, *ER-α, and mi-RNA 152* genes between the patient and the control group. The results of the study of genetic polymorphisms in the two groups depended on the presence or absence of bands resulting from the amplification of certain segments of the genes of samples and the molecular weights of those bands. The results of gene amplification showed that all primers (*OPAA11, OPU15, OPAA17, and OPD18)* amplified the *ABCG2, ABCB, BRCA1, ER-α, and mi-RNA-152* gene of the patient and the control group and type of band was determined depending on the size and site of the bands in the field of the gel, as shown in the figure 1.

In our current study, our results showed that the total number of total bands of *ABCG2* in the patient group was 214 band, the primer OPAA 11 was given the highest number of bands 58 bands out of the total bands, in the patient group the OPU15 has the highest efficiency 0.028. It is noted that this primer gave the highest percentage of polymorphisms amounted to 10.714 with a primer discriminatory power of 40%, which is the highest discriminatory power, while with the control group the primer OPAA11 gave the highest number of polymorphic band 6 bands and it has the highest efficiency 0.037. It is noted that this primer gave the highest percentage of polymorphisms amounted to 13.333 with a primer discriminatory power 42.85%, which is the highest discriminatory power. Our results showed that the total number of total bands of *ABCB1* gene in the patient sample was 215 band, the primer OPAA11 was given the highest number of bands 57 bands out of the total bands. in the patient group, the primer OPD18 has the highest primer efficiency 0.037. It is noted that this primer gave the highest percentage of polymorphisms amounted to 15.0943 with a primer discriminatory power of 32%, which is the highest discriminatory power, Compared with control sample OPAA11, OPU15, and OPD 18 have the highest efficiency 0.035, it is noted that the primer OPU15 was given the highest percentage of polymorphisms amounted to 18.1818 with a primer discriminatory power of 30.76%, which is the highest discriminatory power. our results showed that the total number of total bands of *BRCA1* gene in patient sample was 206 band, the primer OPU 15 was gave the highest number of bands (68) bands out of the total bands, in the patient group, the OPD18 primer has the highest efficiency 0.029. It is noted that this primer gave the highest percentage of polymorphisms amounted to 14.285% with a primer discriminatory power of 31.57%, which is the highest discriminatory power, compared with the control group the primer OPAA11 and OPAA17 have the highest primer efficiency 0.023. It is noted that the OPAA17 primer gave the highest percentage of polymorphisms amounted to 10.3448 with a primer discriminatory power of 37.5%, which is the highest discriminatory power, as shown in table 3, 4 and figure (2).

Table (3) The numbers and percentages of the total band ,polymorphic ,unique ,monomorphic band, and primer efficiency and primer discriminatory power that produced from amplified four primer RAPD in *ABCG2, ABCB1, and BRCA1* gene of patient sample.

Gene	RAPD Primer	Total No. of bands	Total No. of polymorphic bands	Polymorph % ism	Primer efficiency	Primer discriminato ry power %	Total No. of unique bands	Uniqueness, %	Total No. of Monomorp hic bands
ABCG2	OPAA11	58	4	6.8965	0.018	26.66	1	1.724	3
Patient	OPU 15	56	6	10.714	0.028	40	0	0	3
	OPAA17	46	3	6.5217	0.014	20	5	10.86	3
	OPD 18	54	2	3.7037	0.009	13.33	4	7.407	3
Total		214	15	7.1770			10	4.784	12
ABCB1	OPAA11	57	6	10.5263	0.027	24	2	3.5087	1
Patient	OPU 15	51	5	9.80392	0.023	20	1	1.9607	1
	OPAA17	54	6	11.111	0.027	24	2	3.7037	1
	OPD 18	53	8	15.0943	0.037	32	3	5.6603	1
Total		**215**	**25**	11.6279			**8**	3.7209	**4**
BRCA1	OPAA11	51	3	5.8823	0.014	15.78	3	5.88235	3
Patient	OPU 15	68	5	7.3529	0.024	26.31	3	4.41176	3
	OPAA17	45	5	11.11	0.024	26.31	2	4.4444	3
	OPD 18	42	6	14.285	0.029	31.57	3	7.14285	3
Total		206	19	9.2233			11	5.3398	12

Table (4) The numbers and percentages of the total band ,polymorphic ,unique ,monomorphic band, and primer efficiency and primer discriminatory power that produced from amplified four primer RAPD in *ABCG2*, *ABCB1*, and *BRCA1* gene of control (healthy) sample.

Gene	RAPD Primer	Total No. of bands	Total No. of polymorphic bands	Polymorphism %	Primer efficiency	Primer discriminatory power %	Total No. of unique bands	Uniqueness, %	Total No. of Monomorp hic bands
ABCG2	OPAA11	45	6	13.333	0.037	42.85	2	4.4444	4
Control	OPU15	39	4	10.256	0.024	28.57	2	5.1282	4
	OPAA17	40	2	5	0.012	14.28	0	0	4
	OPD18	38	2	5.2631	0.012	14.28	2	5.2631	4
Total		162	14	8.6419			6	3.7037	16
ABCB1	OPAA11	32	4	12.5	0.035	30.76	0	0	1
Control	OPU15	22	4	18.1818	0.035	30.76	1	4.5454	1
	OPAA17	26	1	3.84615	0.008	7.69	2	7.6923	1
	OPD18	34	4	11.7647	0.035	30.76	0	0	1
Total		114	13	11.4035			3	2.6315	4
BRCA1	OPAA11	42	3	7.14285	0.023	37.5	1	2.38095	3
Control	OPU 15	30	2	6.66666	0.015	25	2	6.66666	3
	OPAA17	29	3	10.3448	0.023	37.5	1	3.44827	3
	OPD 18	25	0	0	0	0	4	16	3
Total		126	8	6.34920			8	6.34920	12

In the patient group, the smallest size fragments of *ABCG2* were recorded at OPD18 (20-30) bp while the highest size fragments were recorded at OPAA11and OPU15 (990-1000) bp in size, compared with control group the smallest size fragments were recorded at (60 -70) bp at all primers and the highest size fragments were recorded at OPU15 (1090-1100) bp. Our results showed that the primer OPD 18 showed the molecular weight (20-30) bp of the *ABCG2* bands in the patient group and its absence from the control group, as shown in figure (3).

In the patient sample, the smallest size fragments of *ABCB1* were recorded at OPAA17 (20-30) bp, while the highest size fragments were recorded at OPAA11 (990-1000) bp. The control sample revealed smallest size fragments at OPAA11, OPU15, and OPD18 (20-30) bp and the highest size fragments were recorded at

OPAA11, OPD18 (290 - 300) bp. Our results showed that the primer OPAA17 showed that the molecular weight (20-30)bp in the patient group and its absence from the control group, as shown in figure (4).

In patient group, the smallest size fragments of *BRCA1* were recorded at OPAA17 20-30 bp while the highest size fragments were recorded at OPU15 990-100 bp in size, compared with the control group the smallest size fragments were recorded at all primer 50-60 bp and the highest size fragments were recorded at OPAA17 (1090 -1100) bp in size. Our results showed that the primer OPAA17 showed the molecular weight 20-30 bp in the patient group and its absence from the control group, as shown in figure (5).

Our results showed that the highest total number of polymorphic band was 38 in *ABCB1* gene, and the least one was 27 in *BRCA1* gene. While the total number of unique band 19 in *BRCA1* gene, and the least one was 11 in *ABCB1* gene. The highest total number of monomorphic band 29 in *ABCG2* gene and the least one was 8 in *ABCB1* gene. The results of the statistical analysis by using Chi-square showed that there were no significant differences in the types of bands of the *ABCG2, ABCB1, BRCA1* between the patient sample and the control sample, at the probability level of P >0.05, as shown in table (5), figure (6).

Table (5) statistical analysis for polymorphic, unique and monomorphic bands of *ABCG2, ABCB1, BRCA1* gene between patient and control samples.

Gene	Type of band	Overall total band	Total No.of band (patient)	Total No.of band (control)	X2	P-value
ABCG2	Polymorphic band	29	15	14	1.593	0.451
	Unique band	16	10	6		
	Monomorphic band	28	12	16		
	Overall total	73	37	36		
ABCB1	Polymorphic band	38	25	13	1.089	0.580
	Unique band	11	8	3		
	Monomorphic band	8	4	4		
	Overall total	57	37	20		
BRCA1	Polymorphic band	27	19	8	2.245	0.325
	Unique band	19	11	8		
	Monomorphic band	24	12	12		
	Overall total	70	42	28		
Significant *P<0.05,**P<0.01,***P<0.005, NS=No significance P>0.05						

Our results showed that the total number of total bands of *ER-α* in the patient group was 298 band, the primer OPD18 gave the highest number of bands (86) band. In the patient group, the primer OPU15 was the least efficient in generating polymorphic bands 0.010, as it gave only 3 polymorphic bands, this primer gave the lowest percentage of polymorphisms 3.7974% with a primer discriminatory power of 16.66%, and it has the least primer discriminatory power, compared with the control group, the primer OPAA17 was the least efficient in generating polymorphic bands 0.054, as it gave 7 polymorphic bands. Despite this, it is noted that the OPAD18 primer gave the lowest percentage of polymorphisms amounted to 20.4545% with a primer discriminatory power of 26.47%. The lowest discriminatory power was 20.58% for OPAA17. The results showed that the total number of total bands of miRNA-152 gene was 149 band, the primer OPAA 11 was given the highest number of bands(43) bands out of the total bands, compared with control sample the total number of total bands was 93 band, the primer OPAA11 and OPAA17 were given the highest number of bands 24 bands out of the total bands, In the patient sample, the total number of the polymorphic band was 8 band, polymorphisms percentage was 5.369127%, the four primer OPAA11, OPU15, OPAA17, and OPD18 were given the same number of polymorphic band (2) band, compared with the control sample, the total number of the polymorphic band was 0 band, polymorphisms percentage was 0% In the patient sample, the four primers have the same primer efficiency (0.013). It is noted that the primer OPD18 was given the highest percentage of polymorphisms amounted to (6.25%) with a primer discriminatory power of (25%), whereas the four primers have the same discriminatory power (25%), compared with the control sample the four primers have the same primer efficiency (0), the primer discriminatory power for all primers was 0%, as shown in table (6), figure (7).

Table (6) The numbers and percentages of the total band ,polymorphic ,unique ,monomorphic band, and primer efficiency and primer discriminatory power that produced from amplified four primer RAPD in *ER-α* gene of control (healthy) sample.

Gene	RAPD Primer	Total No. of bands	Percentage of primer band %	Total No. of polymorphic bands	Primer efficiency	Polymorphisms %	Primer discriminatory power %	Total No. of unique bands	Uniqueness, %	Total No. of monomorphic bands	Mor ms%
ER-α	OPAA11	81	27.18	5	0.016	6.1728	27.77	2	2.46913	4	4.93
patient	OPU15	79	26.51	3	0.010	3.7974	16.66	0	0	4	5.06
	OPAA17	52	17.44	4	0.013	7.6923	22.22	1	3.84615	4	7.69
	OPD18	86	28.85	6	0.020	6.9767	33.33	2	2.32558	4	4.65
Total		298		18		6.0402		5	1.67785	16	5.36
ER-α	OPAA11	18	14.06	8	44.4444	0.062	23.52	1	5.5555	0	0
Control	OPU15	41	32.03	10	24.3902	0.078	29.41	1	2.43902	0	0
	OPAA17	25	19.53	7	28	0.054	20.58	2	8	0	0
	OPD18	44	34.38	9	20.4545	0.070	26.47	3	6.8181	0	0
Total		**128**		34	26.5625			7	5.46875	0	0
miRNA	OPAA11	43	28.85	2	4.651162	0.013	25	1	2.32558	2	4.65
patient	OPU15	38	25.50	2	5.263157	0.013	25	1	2.631578	2	5.26
	OPAA17	36	24.16	2	5.555555	0.013	25	0	0	2	5.56
	OPD18	32	21.48	2	6.25	0.013	25	0	0	2	6.25
Total		149		8	5.369127			2	1.342281	8	5.36
miRNA	OPAA11	24	25.81	0	0	0	0	0	0	3	12.5
Control	OPU15	23	24.73	0	0	0	0	0	0	3	13.0
	OPAA17	24	25.81	0	0	0	0	0	0	3	12.5
	OPD18	22	23.66	0	0	0	0	0	0	3	13.6
Total		93		0	0	0	0	0	0	12	12.9

In the patient group, the smallest size fragments of ER-α were recorded at OPAA11, OPD18 (40-50) bp while the highest size fragments were recorded at all primer (890-900) bp in size, compared with control sample the smallest size fragments were recorded at OPU15, OPAA17, and OPD18, the highest size fragments were recorded at OPAA11, OPU15, OPD18 (890-900) bp in size. The results showed that the primer OPAA11 showed the molecular weight (40-50) bp of the *ER-α* bands in the patient group and its absence from the control group, as shown in figure (8).

In the patient sample, the smallest size fragments miRNA-152 were recorded at OPAA 17, OPD 18 (20-40) bp, while the highest size fragments were recorded at OPAA 11, OPU 15(290-300) bp in size, as shown in figure (4- 28A), compared with control sample the smallest size fragments were recorded at all primer (50-60) bp and the highest size fragments were recorded at all primer (170-180) bp. Our results showed that the primer OPAA 17, OPD 18 showed the molecular weight (20-40) bp of the mi-RNA 152 bands in the patient group and its absence from the control group, as shown in figure (9).

Our results showed that the highest total number of polymorphic band was 52 in *ER-α* gene, and the least one was 8 in *mi-RNA 152* gene. While the total number of unique band 12 in *ER-α* gene, and the least one was 2 in *mi-RNA 152* gene. The highest total number of monomorphic band 20 in *mi-RNA 152* gene and the least one 16 in *ER-α* gene. The results of the statistical analysis by using Chi-square showed that there were significant differences in the types of bands of the ER- α, and miRNA-152 between the patient sample and the control sample, at the probability level of $P < 0.05$, as shown in table (7), figure (10).

Table (7) statistical analysis for polymorphic, unique and monomorphic bands of *ER-α* gene between patient and control samples

Gene	Type of band	Overall total band	Total No.of band (patient)	Total No.of band (control)	X2	P-value
ER-α	Polymorphic band	52	18	34	21.220	0.000***
	Unique band	12	5	7		
	Monomorphic band	16	16	0		
	Overall total	80	39	41		
mi-RNA 152	Polymorphic band	8	8	0	10.000	0.007**
	Unique band	2	2	0		
	Monomorphic band	20	8	12		
	Overall total	30	18	12		
Significant *P<0.05,**P<0.01,***P<0.005, NS=No significant P>0.05						

4. Discussion

Our PRP technique is a simple polymerase chain reaction product based gene polymorphism assay system that is used for study and analyzes genomic instability or genomic alteration. Novikov et al., (2016) mentioned that the RAPD is used in the field of studying genetic alteration of breast cancer. Boysen et al., (2019); Sabra et al., (2020) found that the genetic progress towards transformation from normal to malignant cells can be achieved by comparing the RAPD profiles obtained from healthy and malignant. RAPD technique is considered random with reduced reproducibility, we chose it as a rapid and low price technique (Bidet et al., 2000). However, we performed a new procedure with some modifications in the traditional RAPD technique when we used the amplified gene as a template in RAPD. This procedure resulted in a higher number of bands yielded with more accuracy and specific data. In the current study, we found that the number of bands per primer for each gene was high. This improved RAPD successfully increased the number of RAPD bands produced from a given PCR product. Therefore, we applied the RAPD technique with 5 specific genes (*ABCB1, ABCG2, BRCA1, miRNA152,and ER-α*) to generate more bands for detecting genomic alterations in human breast cancer.

The results showed that the total bands of the BC genes in the patient group were higher than the control group. Rocco et al., (2014) mentioned that the differences in the number of bands and the variations in their intensity, in the RAPD-PCR profile, are associated with alterations of genetic material. Liu et al., (2005) suggested that the modifications of band intensity and lost band are likely to be due to one or a combination of the following events: changes in oligonucleotide priming sites due mainly to genomic rearrangement, point mutations and DNA damage in the primer binding sites is only 10 base long, and interactions of DNA polymerase with damaged DNA. Our results showed there were significant differences (P <0.05) between the patient and control group among polymorphic, unique, and monomorphic bands in the ER-α and mi-RNA 152 gene may be due to genetic alteration between the two groups. Singh and Roy (2001) stated the disappearance (deletion) or appearance (insertion) of an amplified DNA fragment might be associated with genetic rearrangements, or unequal mitotic recombination. Perrone et al., (2016) reported that the inherently predisposed reaction of DNA damage due to exogenous and endogenous factors that can be the development of hereditary diseases and sporadic cancer.

The results showed marked differences in the primer discriminatory power between patients and control group of the (*ABCG2, ABCB1, BRCA1, ER-α, and mi-RNA 152*) genes, this differences may be due to the dependence of the primer discriminatory power on the number of polymorphic bands generated from each primer and the sum of the polymorphic bands generated from all primers. Ismaeel (2013) reported that the primer capacity to show polymorphisms in comparison to polymorphisms shown by all primers is called primer discriminatory power.The results showed marked differences in the primer efficiency between patients and the control group of the (*ABCG2, ABCB1, BRCA1, ER-α, and mi-RNA 152)* genes, these differences may be due to the dependence of the primer efficiency on the number of polymorphic bands generated from each primer and the sum of the bands generated from all primers, so the primer efficiency was variable between the patient and control group. Newton and Graham., (1997); Ismaeel (2013) stated that the primer efficiency values range from (0-1) and are defined as the measure of the primer's ability to produce polymorphisms. Ibrahim and his team (2010) reported that the efficiency of the primer can be demonstrated by its ability to give the highest percentage of polymorphic bands compared to the total number of amplified bands, the important characteristic of the efficient primer is the ability to illustrate the polymorphism between normal individuals and patient individuals such as leukemia.

The results showed marked differences in the molecular size of fragments produced from primers between the patient and control group. The primer OPD18 showed the molecular weight (20-30) bp of the ABCG2 bands in the patient group and its absence from the control group. The primer OPAA 17 showed the molecular weight (20- 30) bp of the (*ABCB1, BRCA1*) bands in the patient group and its absence from the control group. The primer OPAA 11 showed the molecular weight (40-50) bp of the ER-α bands in the patient group and its absence from the control group. The primer OPAA 17, OPD 18 showed the molecular weight (20-40) bp of the (mi-RNA-152) bands in the patient group and its absence from the control group. Thus, the bands with their primers may rely upon in distinguishing between the patient and the control group genetically after studying them more in the future. Papadopoulos et al., (2002); Xian et al., (2005); Ibrahim and his team (2010) found that the polymorphism also includes the differences in molecular weights of amplified bands which resulted from multiple types of mutations and translocations that occurred, thus causing mobility shift of bands and might cause the addition of new band (s).

Conclusions

Our results proved the accuracy of our modification in the traditional RAPD technique, which yielded more specific bands related to genes, we conclude the success of use PCR-RAPD-PCR methods for first time in the studying genetic polymorphisms of the specific breast cancer related genes. Additionally, this test gave us an indication of high polymorphism in patients when compared to controls, which may be related to mutations or modifications. There were significant differences between patients and healthy women in regard to the expression of breast cancer related genes. High levels of estrogens may be the direct effector in the induction and promotion of breast cancer, and this hormone can be inserted into the body by different routes, such as contraceptive pills, cosmetics and xenoestrogens present in the environment. we suggested from our data that most breast cancers in Iraqi women are not family related but rather drug resistance and estrogen response. We conclude the presence molecular weights of some bands in the patients group and its complete absence from the control group can be considered as an indicator to breast cancer after studying it more at the molecular level. Therefore, We can predict and diagnose breast cancer early upon analysis of our chosen genes, and our study allows us to employ these genes in the early prediction of breast cancer.

Declarations

Ethical approval:

Informed consent: all patients and healthy women participated in the study were signed an informed consents prior the study (samples attached at the end of this file).

Consent of publication:

Authors declare their consent of publication

Availability of data:

Data available with correspondence author Maytham A Dragh, maithamdragh@uomisan.edu.iq

Competing interests:

Authors show no competing interests

Funding:

No funding was available

Authors contributions:

MAD designed the study and suggest the ideae of research. ZZG carried out the molecular genetic work and performed the statistical analysis . ZZG write the manuscript. All authors read and approved the final manuscript.

Ethics of research:

The study was performed under the rules of ministry of higher education and scientific experiments all the patients in which samples were obtained were awaring of the goals of the study and were agree for giving samples.

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Figures

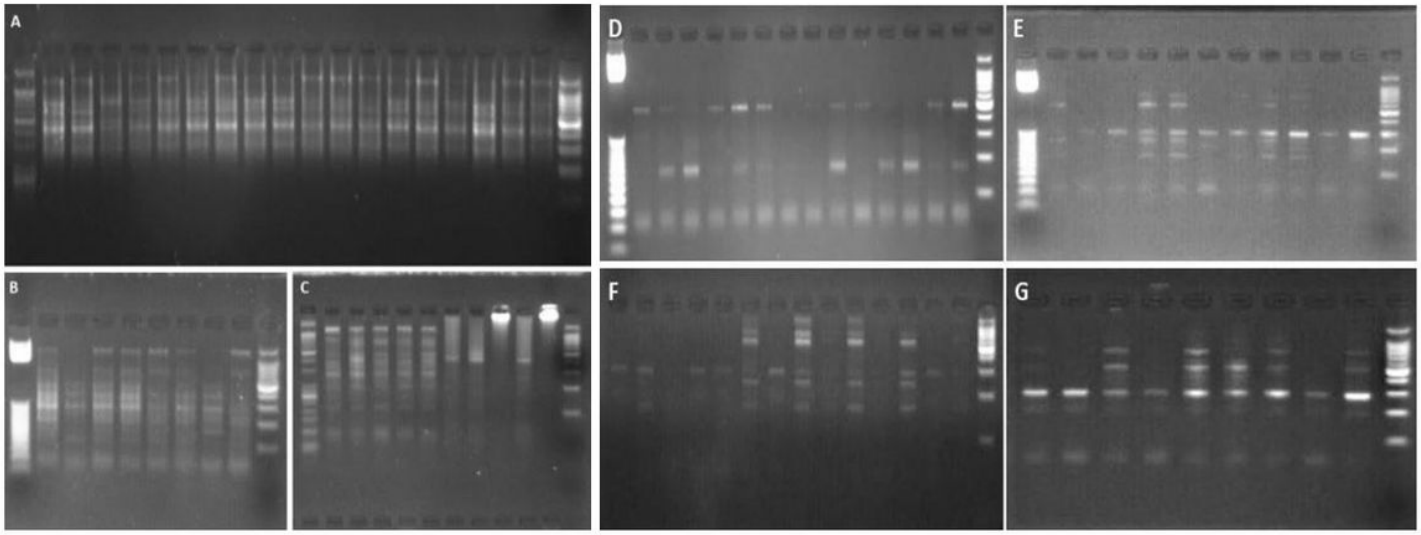

Figure 1

PCR assay for BC related genes with RAPD primer. Agarose gel of 2% loaded with DNA ladder of (25-1100 bp) and four RAPD primers OPAA11, OPU 15, OPAA17 and OPD18 were used.

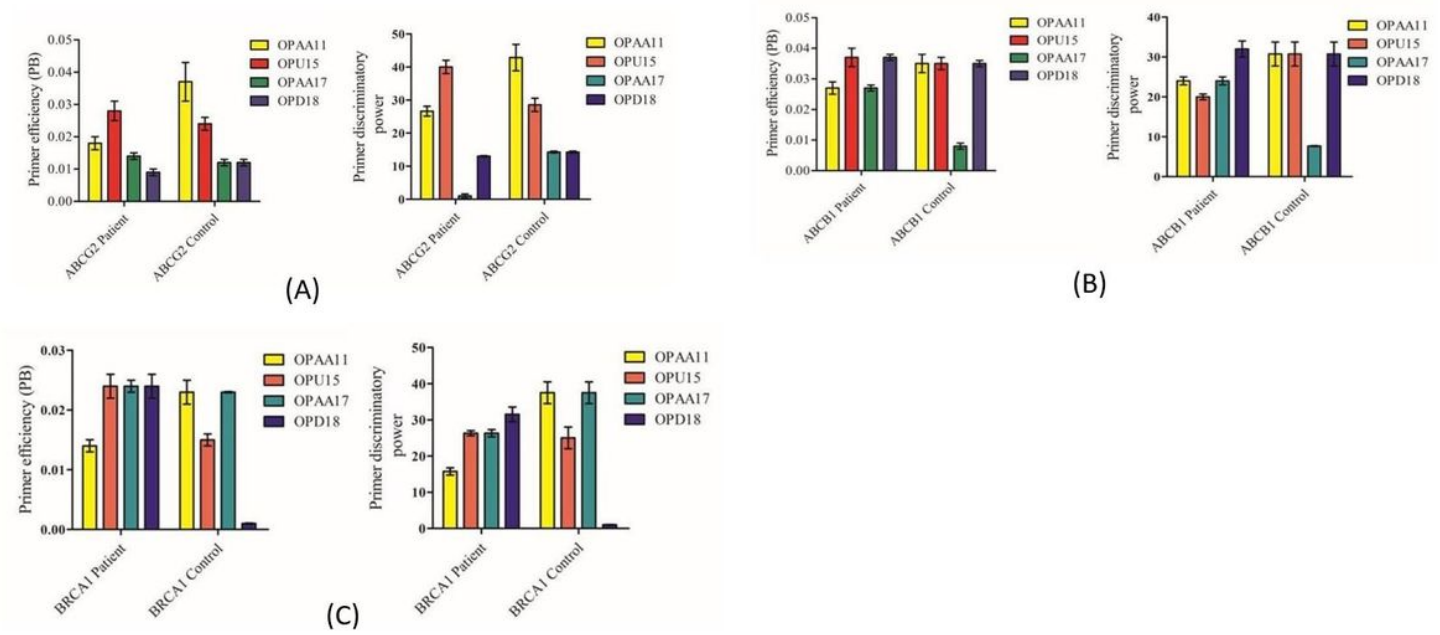

Figure 2

A primer efficiency and discriminatory in A*BCG2* gene of BC patient and control. **B** primer efficiency and discriminatory in *ABCB1* gene of BC patient and control. **C** primer efficiency and discriminatory in *BRCA1* gene of BC patient and control.

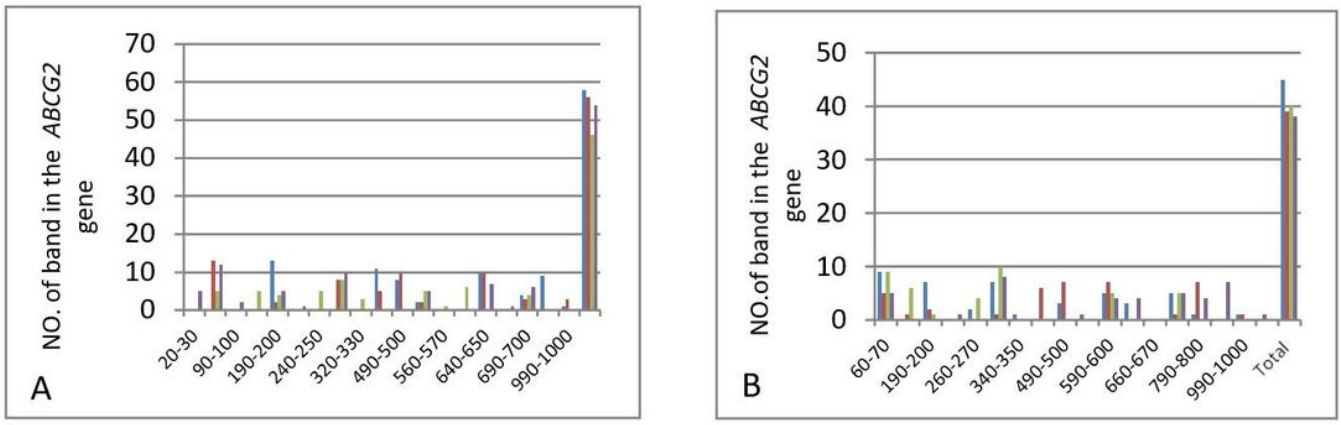

Figure 3

A size range of the fragments in *ABCG2* gene of BC patient. **B** size range of the fragments in *ABCG2* gene of control

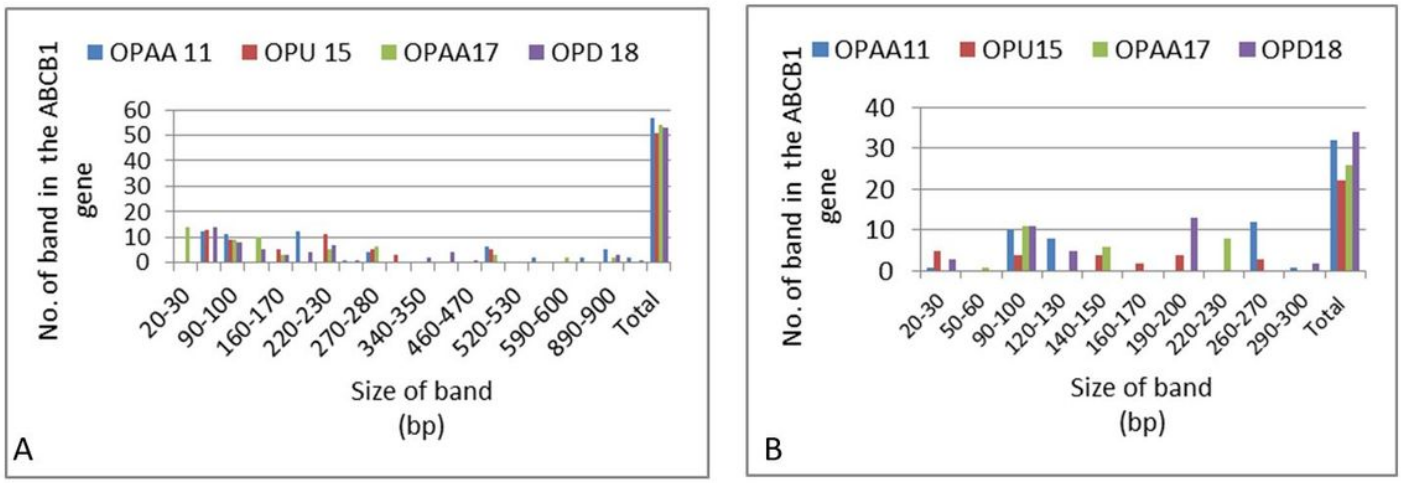

Figure 4

A size range of the fragments in *ABCB1* gene of BC patient. **B** size range of the fragments in *ABCB1* gene of control

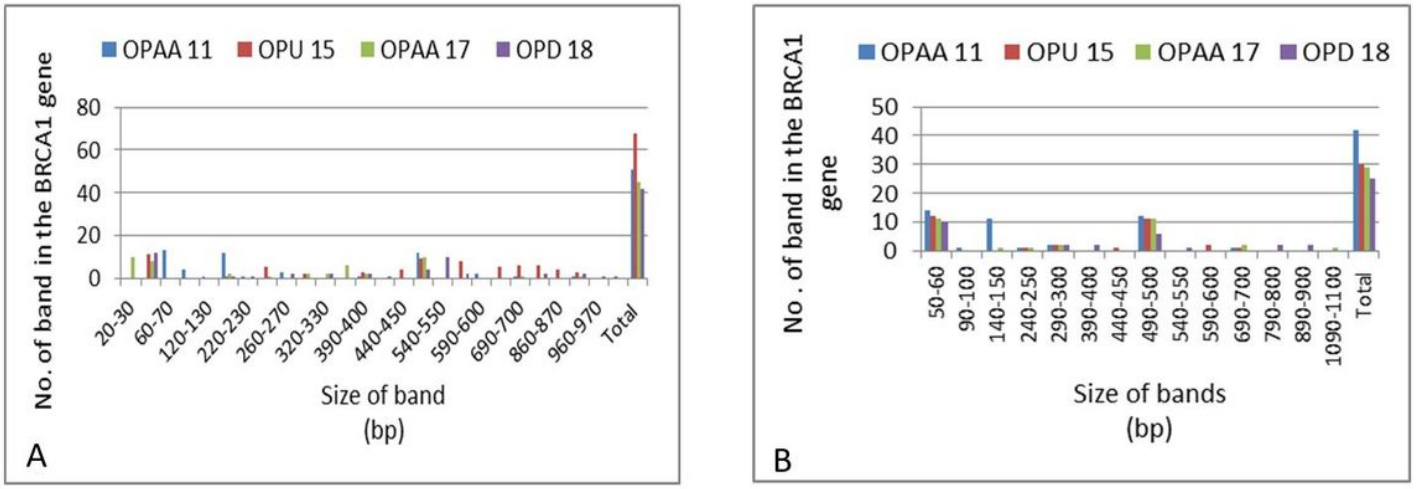

Figure 5

A Size range of the fragments in *BRCA1* gene of BC patient. **B** Size range of the fragments in *BRCA1* gene of control

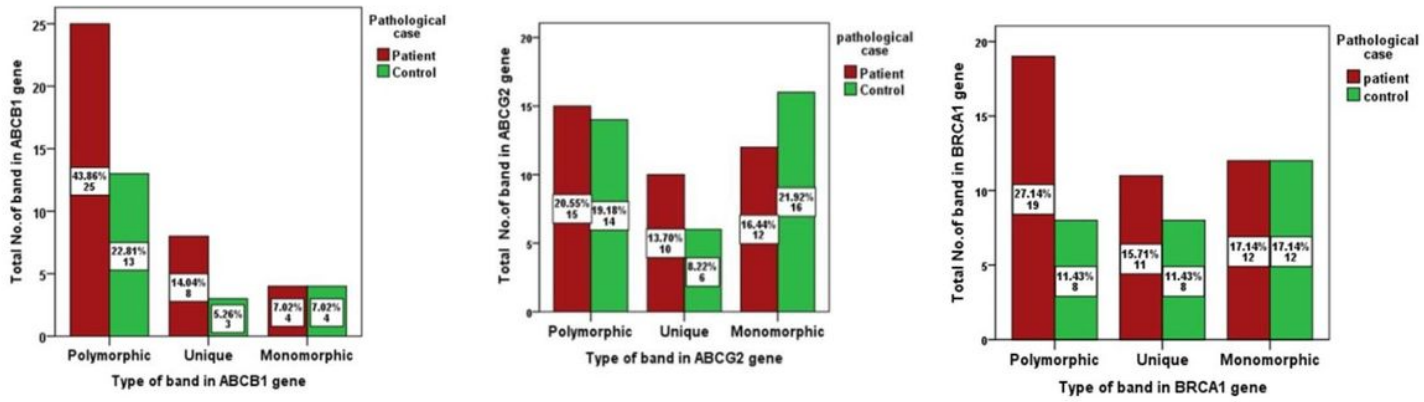

Figure 6

Statistical numbers and percentages of the total polymorphic, unique and monomorphic bands of *ABCG2, ABCB1, BRCA1* gene between BC patient and control.

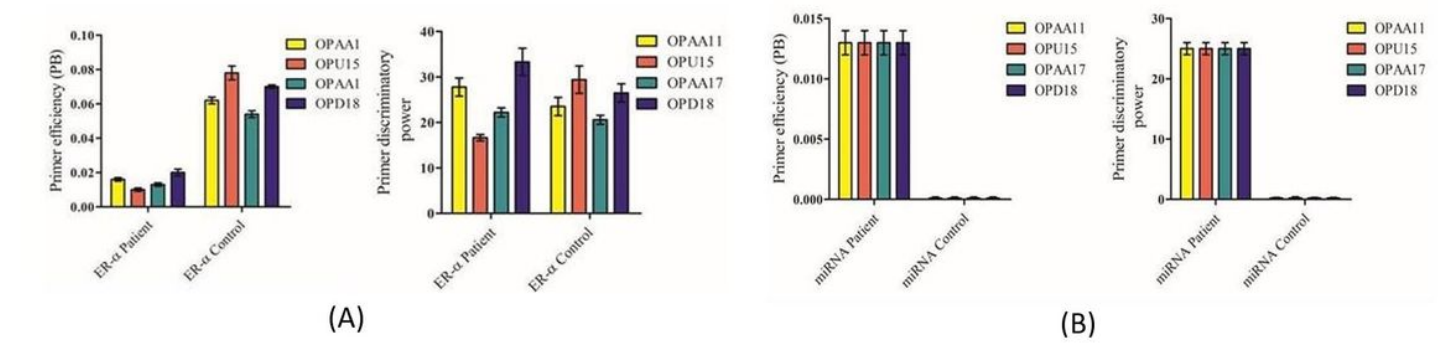

Figure 7

A. primer efficiency and discriminatory in *ER-α* of BC patient and control .(B) primer efficiency and discriminatory in miRNA-152 genes of BC patient and control .

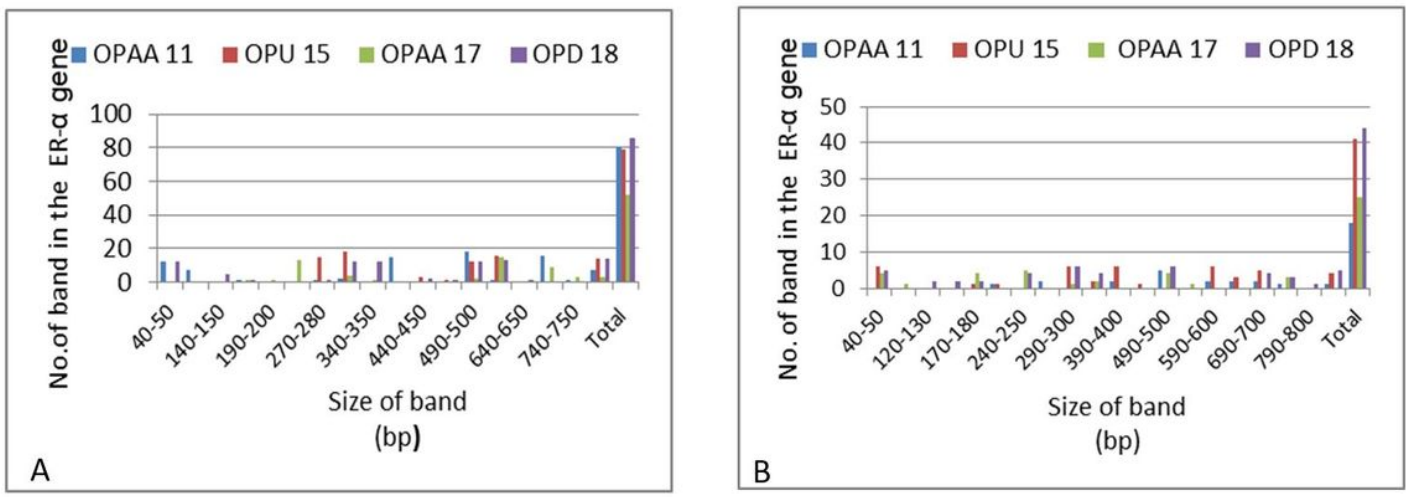

Figure 8

A size range of the fragments in *ER-α* gene of BC patient. **B** size range of the fragments in *ER-α* gene of control

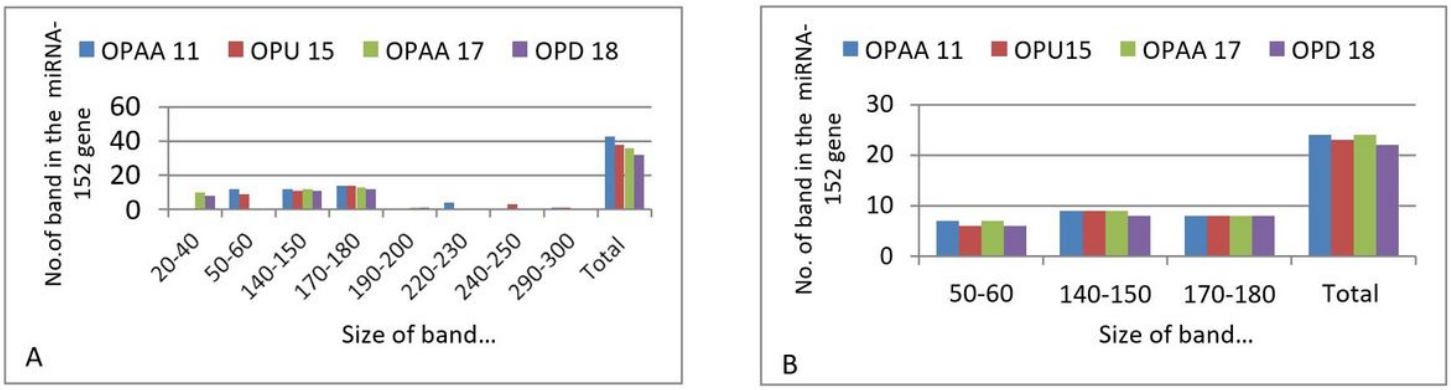

Figure 9

A size range of the fragments in *miRNA 152* gene of BC patient. **B** size range of the fragments in *miRNA 152* gene of control.

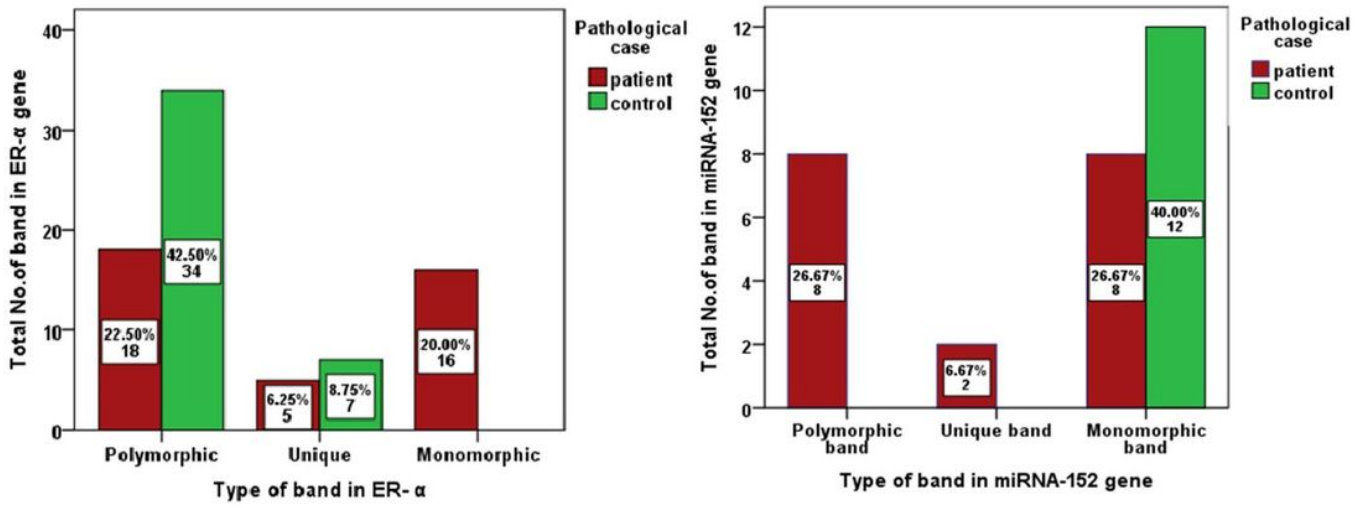

Figure 10

Statistical numbers and percentages of the total polymorphic,unique and monomorphic bands of *ER-α*, and *miRNA-152* gene between BC patient and control.