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Study of BRCA1 and BRCA2 Gene Mutations and Clinicopathological Criteria of Breast Cancer in Thi-Qar

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Abstract

Breast cancer is the commonest type of malignancy in Iraq. For this reason we study risk factors that associated with this disease in Thi Qar province patients. Such as mutations of breast cancer susceptibility genes 1 and 2 (BRCA1 and BRCA2), clinicopathological parameter (age, family history and tumor site) .

Eighty five blood samples were taken from patients who attended Al-Hussain teaching hospital and oncology unit in Al-Habboby hospital during the period (from August /2014 to April /2017) , fifty blood samples were collected from healthy women as a control. Blood samples were using in genetic study. For histopathological analysis fifty tissue samples were collected from fifty patients with breast cancer who were undergoing surgical resection (mastectomy) to prepare paraffin embedded blocks which have been used for histopathological diagnosis.

The present study results revealed that a highest incidence of breast cancer occur in the age group between 40-49 years of age (41%),there is a significant difference among patients age groups ($P \leq 0.01$).

Family history of breast cancer indicates a strong association with risk of developing breast cancer. The present study showed that family history positive in 31 cases (36.47%), and 54 cases (63.52%) had negative family history of breast cancer. Also the results of present study showed that the 34 cases (41.5%) had first birth at age (20-29years), 18 cases (23.37%) had their first birth in age groups (15-19 years) and (30-39 years), and 7 cases (9.09%) had first birth at age (≥40 years). Most of patients (50.5%) breast cancers were located in right breast and (38.8%) of cases breast cancers were located in left side.

Histopathologically, carcinoma was divided into 44 cases (88%) were ductal carcinoma {from which 42 cases(84%) were invasive ductal carcinoma, and 2 cases (4%) were comedocarcinoma}, and 6 (12%) cases were invasive lobular carcinoma. Results revealed that 2 cases (4%) were stage I, 13 cases (26%) were stage II, 24 cases (48%) were stage III and 11 cases (22%) were stage IV, also our results showed that 2 cases (4%) were grade I, 23 cases (46%) were grade II, and 25 cases (50%) were grade III.

The DNA was extracted from blood samples by using Accupower® genomic DNA extraction kit. The concentration and purity of all DNA samples have been measured by Nanodrop. Samples with a purity ranged from 1.7 to 1.9 have been enrolled in this study

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for the molecular detection of *BRCA1/2* gene mutation in patients and control groups. In 85 cases of breast cancer, detection of *BRCA1/2* gene mutation by multiplex PCR done for the serum revealed that; (185 del AG) mutation in *BRCA1 gene* was detected in 6 patients (7.05%), and (5382 ins C) mutation in the same gene was detected in 2

patients (2.35%). Regarding (6174 del T) mutation in *BRCA2* gene was detected in 3 patients (3.52%). In the control patients gene mutations of any types didn't detected.

Key words:breast cancer,BRCA1, BRCA2, gene analysis, PCR.

Medical College/Thi-Qar University

Introduction

Breast cancer is the most common cancer among women, comprising 18% of all female cancers, and worldwide, breast cancer is the fifth most common cause of cancer mortality.(1) Iraqi National Cancer Research Center (INCRC) considered breast cancer is common type of Iraqi female cancer, account for approximately one third of the registered female cancers in Iraq.(2)

Breast cancer risk factors have been reported by epidemiological studies; for instance age, family history, genetic, age of menarche, duration of lactation, parity, age of menopause , diet and hormonal levels are known risk factors for the development of breast cancer.(3) Hereditary breast cancer contributes to about 5-10% of all cases with an earlier age of onset. Mutations of BRCA1 and BRCA2 genes (Breast cancer susceptibility genes 1 and 2) are the most well recognized mutations responsible for an increased risk of breast cancer, this mutations include 185del AG and 5382ins C in (BRCA1) gene and 6174del T in (BRCA2) gene.(4) The aims of the current study is to analyze the clinico-pathological features of breast cancer and to do molecular study for the presence of 185del AG and 5382ins C in (BRCA1) gene and 6174del T in (BRCA2) gene.

Materialsand methods

This study was designed as prospective study, all samples were taken from patients who attended the Al-Hussain teaching hospital and oncology unit inAl-Habooby hospital during the period from August 2014 to April 2017, including eighty-five blood samples from patients with breast cancer, fifty blood samples were collected from healthy women as a control. For histopathological analysis fifty tissue samples were collected from patients with breast cancer who were undergoing surgical resection (mastectomy). Blood samples were obtained by venipuncture, using a 5 ml disposable syringe, 2 ml was dispensed in a sterilized tube with EDTA to prevent coagulation and kept in the freezer (-

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20C) until use in genetic studies. The tissue samples were collected from the 50 patients with breast cancer were kept in 10 % of buffered natural formalin to prepare paraffin embedded blocks which have been used for histopathological diagnosis.

Molecular study

Genomic DNA was extracted by using Accupower®Genomic DNA extraction kit (Bioneer. Korea).

Determination of DNA concentration and purity

The extracted DNA was checked by using Nanodrop spectrophotometer (THERMO.USA),which measured DNA concentration (ng/µL) and check the DNA purity by reading the absorbance at (260 /280 nm) .

Detection of *BRCA 1/2* gene mutations:

Multiplx Polymerase Chain Reaction technique was performed for detection of BRCA1 and BRCA2 mutations in blood samples of breast cancer patients and normal healthy control samples. The method was carried out according to method described by (Fattahi *et al*., 2009) as following steps:[(5)]

PCR master mix preparation

Multiplex PCR master mixwas prepared by using (AccuPower® Gold Multiplex PCR PreMix Kit) that contain:Top DNA polymerase, dNTPs (dATP, dCTP, dGTP, dTTP), Tris-HCl pH 9.0, KCl,$MgCl_2$, and Stabilizer and Tracking dye. This master mix done according to company instructions as following table (1).

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Table 1: PCR reaction mix and their volume.

PCR Master mix	Volume
DNA template	**5µL**
P1 primer (10pmol)	**1µL**
P2 primer (10pmol)	**1µL**
P3 primer (10pmol)	**1µL**
P4 primer (10pmol)	**1µL**
P5 primer (10pmol)	**1µL**
P6 primer (10pmol)	**1µL**
P7 primer (10pmol)	**1µL**
P8 primer (10pmol)	**1µL**
P9 primer (10pmol)	**1µL**
PCR water	**6µL**
Total volume	**20µL**

After that, these PCR master mix component that mentioned above placed in standard AccuPower PCR PreMix Kit, then, all the PCR tubes transferred into Exispin vortex centrifuge at 3000rpm for 3 minutes. Then placed in PCR Thermocycler (Mygene. Bioneer. Korea).

- **PCR Thermocycler Conditions**

PCR thermocycler conditions were done by using convential PCR thermocycler system as following table (2).

Table 2 : Condition of PCR .

PCR step	Temperature	Time	Repeat
Initial Denaturation	94 C	5 min	**1**
Denaturation	94 C	30 sec	**35 cycles**
Annealing	57 C	30 sec	
Extension	72 C	30 sec	
Final extension	72 C	5 min	**1**
Hold	4 C	Forever	**-**

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▪ **Primers**

The primers were used in PCR technique for detection of *BRCA1* and *BRCA2* mutation in blood samples of breast cancer patients and normal healthy control samples. These primers were designed by (Chan *et al*., 1999),[6] and provided from Bioneer company, Korea as following table (3).

Table 3: Primers and their sequence and size.

Genes	Primer Sequence		Products size
BRCA 1-185 del AG	Common Forword P1	GGTTGGCAGCAATATGTGAA	**-**
	Wild type Reverse P2	GCTGACTTACCAGATGGGACTCTC	**335 bp**
	Mutant Reverse P3	CCCAAATTAATACACTCTTGTCGTGACTTACCAGATGGGACAGTA	**354 bp**
BRCA 1 – 5382 ins C	Common forward P4	GACGGGAATCCAAATTACACAG	**-**
	Wild type reverse P5	AAAGCGAGCAAGAGAATCGCA	**271 bp**
	Mutant reverse P6	AATCGAAGAAACCACCAAAGTCCTTAGCGAGCAAGAGAATCACC	**295 bp**
BRCA 2 - 6174 del IT	Commn forward P7	AGCTGGTCTGAATGTTCGTTACT	**-**
	Wild type reverse P8	GTGGGATTTTTAGCACAGCTAGT	**151 bp**
	Mutant reverse P9	CAGTCTCATCTGCAAATACTTCAGGGATTTTTAGCACAGCATGG	**171 bp**

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Gel electrophoresis:

The mPCR products were analyzed by agarose gel electrophoresis following steps:

i. A 3% Agarose gel was prepared in using 1X TBE and dissolving in water bath at 100 °C for 15 minutes, after that, left to cool 50°C.

ii. Then 3µL of ethidium bromide stain were added into agarose gel solution.

iii. Agarose gel solution was poured in tray after fixed the comb in proper position after that, left to solidified for 15 minutes at room temperature, then the comb was removed gently from the tray and 10µL of PCR product were added into each comb well and 10µL of (100bp Ladder) in one well.

iv. The gel tray was fixed in electrophoresis chamber and fill by 1X TBE buffer. Then electric current was performed at 100 volts and 80 AM for 1hour.

v. The mPCR products were visualized by using UV Transilluminator.

Results

Age distribution

In this study the distributing of patients into age groups revealed that most patients 35 cases (41.17%) were at the age group (40-49) years old, 22 cases (25.88%) were at the age group (50-59)years old, 17 (20%) cases were at the age group (30- 39) years old, 6 cases (7.05%) were at the age group (60-69)years old, and 5 cases (5.88%) were in the age group (≥ 70) years old, table 4.

Table 4: Distribution of breast carcinoma patients according to age groups.

Age groups (years)	Number of patients with breast cancer	%
30 - 39	١٧	20
40 - 49	٣٥	41.17
50 - 59	٢٢	٢٥,٨٨
60 - 69	٦	7.05
≥ 70	٥	5.88
Total	٨٥	١٠٠

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Family history

In this study we found that 31 cases (36.47%) had positive family history of breast cancer, while 54 cases (63.52%) had negative family history of breast cancer. table (5).

Table 5: Distribution of patients according to family history of breast cancer.

Family history	Number of patients	%
Positive history of breast cancer	٣١	36.47
Negative history of breast cancer	٥٤	63.52
Total	٨٥	١٠٠

We found in this study that 34 cases (41.5%) had their first birth at age (20-29years), 18 cases (23.37%) had their first birth in age group (15-19 years) and (30-39 years), and 7 cases (9.09%) had their first birth at age (≥40 years), table (6).

Table 6: Distribution of patients according age groups of patients at first birth.

Age at first birth of patients	Number of patients	%
15 – 19	١٨	٢٣,٣٧
20 – 29	٣٤	٤١,١٥
30 – 39	١٨	٢٣,٣٧
≥ 40	٧	٩,٠٩
Total	٧٧	١٠٠

Most patients 43 cases (50.5%) had malignant mass in right breast, 33 cases (38.8%) were left breast, and 9 cases (10%) were bilateral., table (7).

Table 7: Distribution of patients according to tumor site.

Location	Number of patients	%
Right	٤٣	٥٠,٥
Left	٣٣	٣٨,٨
Bilateral	٩	١٠,٥
Total	٨٥	١٠٠

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Genomic DNA concentration

The current study was detected genomic DNA concentration for 135 samples (85 patients and 50 control) shows a significant differences within the groups ($P \leq 0.05$) .our results show that most cases (43 case) around percentage (31.85%) had DNA concentration (10-19 ng/µl) , whereas 30 cases were show percentage (22.22%) in concentration between (20-29 ng/µl), followed by 17 cases have (12.59%) were between (1-9 ng/µl) , while 16 cases (11.85%) were between (30-39 ng/µl) concentration , and then 15 cases were have percentage (11.11%) were between (40-49 ng/µl) concentration and in the last 14 cases (10.37%) were have (≥50 ng/µl)concentration,there was a significant difference in DNA concentrations among patientsp value ≤ 0.01,table (8).

Table 8: The concentration of extracted DNA.

DNA concentration ng/µl	Samples	
	Number of patients	%
1 – 9	١٧	12.59
10 – 19	٤٣	31.85
20 – 29	٣٠	٢٢,٢٢
30 – 39	١٦	11.85
40 – 49	١٥	11.11
≥ 50	١٤	10.37
Total	١٣٥	١٠٠

($X^2 = 30.111$, df = 5, P value ≤ 0.01).

Genomic DNA purity

The purity of genomic DNA for 135 samples (85 patients and 50 control) revealedthat the DNA purity was 1.80 in (42.22%) of patients, followed by (31.85%) of patients the purity of DNA was (> 1.80), while DNA purity (< 1.80) in (25.92 %), there was a significant difference among the groups (P value ≤ 0.05) table (9).

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Table (9): The DNA purity percentage.

DNA purity	Samples	
	Number of patients	%
< 1.80	٣٥	25.92
1.80	٥٧	42.22
>1.80	٤٣	31.85
Total	١٣٥	

(X^2 = 5.511 , df = 2 , P value ≤ 0.05)

Multiplex PCR results

The study results showed that (185delAG) mutation in *BRCA1* gene was detected in 6 cases (7.05%) out of 85 cases of the breast carcinoma, (5382insC) mutation was detected in 2cases (2.35%) in *BRCA1* gene. While 3 cases (3.52%) had mutation (6174delT) in *BRCA2* gene. In these analyses, control group casesdidn't show mutation in the (185delAG, 5382insC and 6174delT genes) of BRCA*1/2* genes. There was significant difference among the studied groups and control (P value ≤ 0.05), table (10).

Table (10): BRCA1 and BRCA2 gene mutations distribution between patients and controls.

Gene	Mutation	Patient			Control		
		Total	No.	%	Total	No.	%
BRCA1	٥٣٨ ins C	85	2	2.35	50	0	0
	185 del AG	85	6	7.05	50	0	0
BRCA2	6174 del T	85	3	3.52	50	0	0

(X^2 =2.470 , df = 2 , P ≤ 0.0٥)

Multiplex allele-specific PCR

In the patient's samples figure (4) agrose gel electrophoresis image of Multiplex allele-specific PCR products analysis shows *BRCA1* and *BRCA2* gene mutation in breast cancer patient samples. M: DNA Marker (1500-100bp), and Lane (1-3) are not mutant alleles and only found wild allele at 335bp in (185delAG), 271bp in (5382insC), and 151bp in (6174delT); lane (4-7): found mutant allele in (185delAG) at 354bp, lane (8): found

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mutant allele in (5382insC) at 295bp, and lane (9-10): found mutant allele in (6174delT) at 171bp.While control samples figure (5) agrose gel electrophoresis image of Multiplex allele-specific PCR products analysis that show *BRCA1* and *BRCA2* gene mutations in healthy control samples. Where, M: Marker (1500-100bp), lane (1-10) not mutant alleles and only found wild allele at 335bp in (185delAG), 271bp in (5382insC), and 151bp in (6174delT).

Histopathological studies

Histological type of breast cancer

In fifty studied cases carcinoma was divided into 44 cases (88%) were ductal carcinoma (from which 42 cases (84%) were invasive ductal carcinoma, figure (1) and 2 cases (4%) were comedocarcinoma, figure (2), and 6 (12%) cases were invasive lobular carcinoma, figure (3),table (11).

Table 11: Distribution of patients according to histological type.

Histological type	Number of patients	%
Ductal carcinoma	٤٤	٨٨
Invasive Lobular Carcinoma	٦	١٢
Total	٥٠	١٠٠

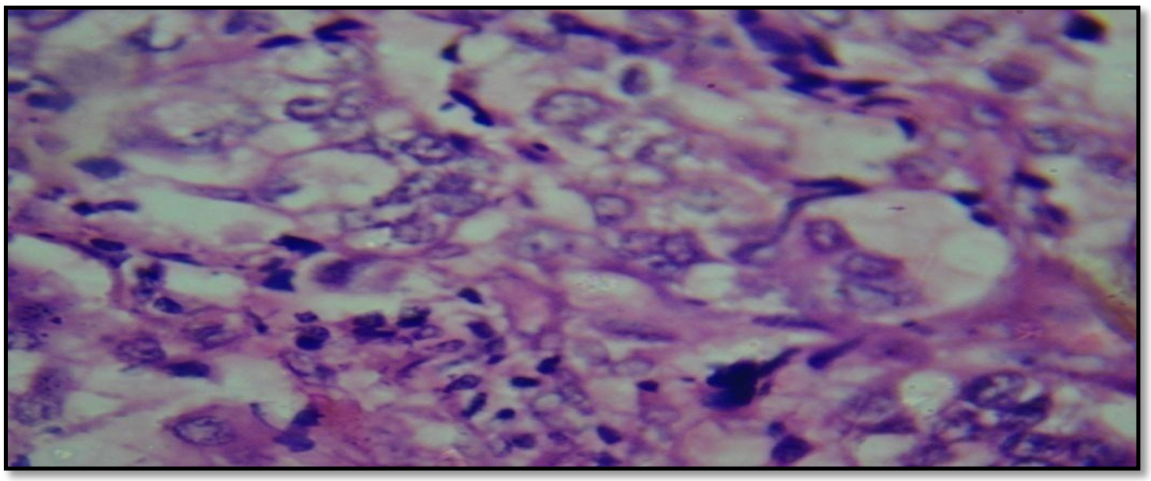

Figure 1: Invasive Ductal Carcinoma (H&E 40x).

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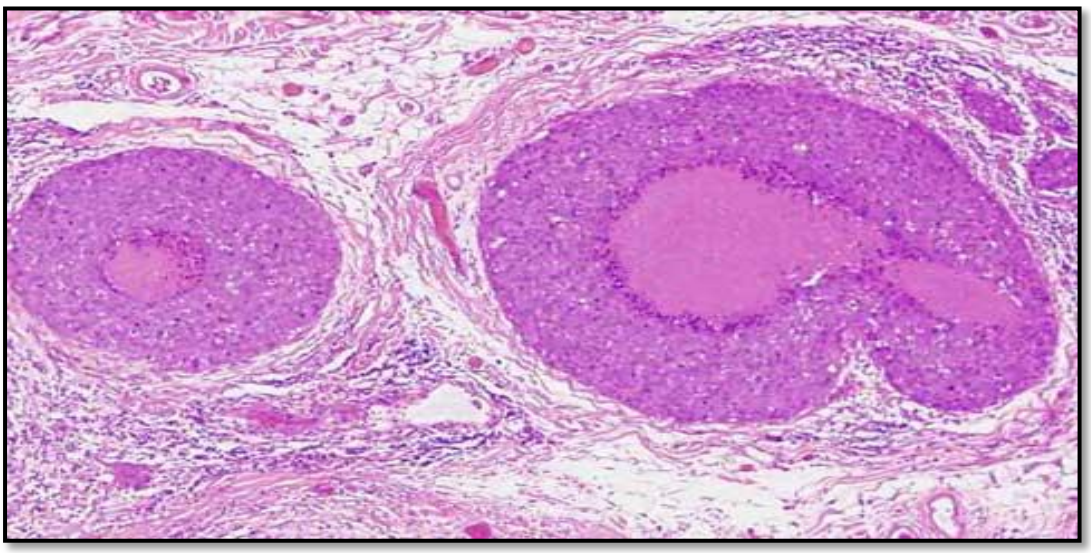

Figure 2: Comedocarcinoma H&E 40x.

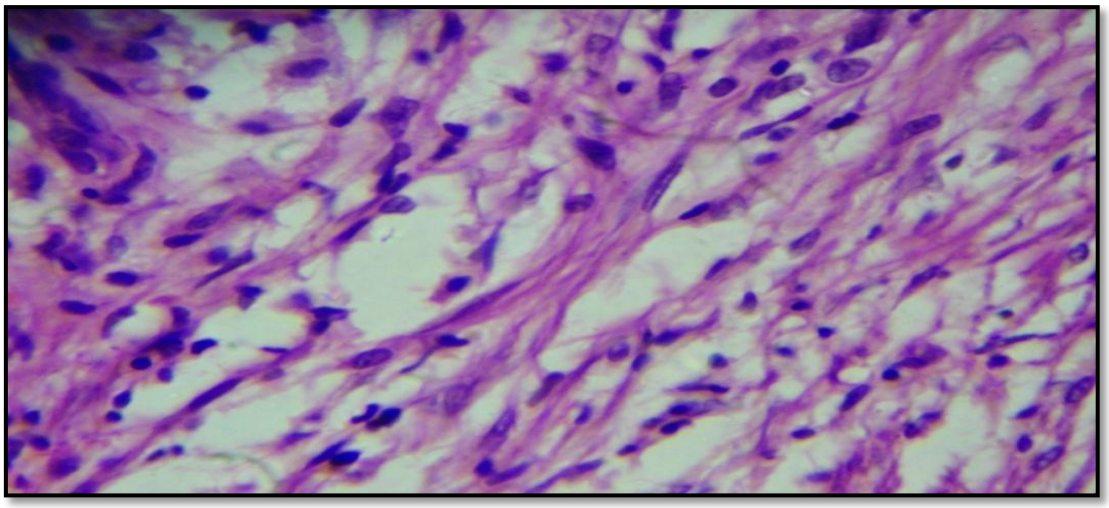

Figure 3: Invasive Lobular Carcinoma H&E 40x.

Staging of breast cancer

Out of 85 cases, staging system can be applied only to 50 cases, and we found that 2 cases (4%) were stag I, 13 cases (26%) were stage II, 24 cases (48%) ere stage III and 11 cases (22%) were stage IV. The differencewas a significant (P value ≤0.05), (table 12).

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Table 12: Distribution of patients according to stage.

Stage	Number of patients	%
Stage I	٢	٤
Stage II	١٣	٢٦
Stage III	٢٤	٤٨
Stage IV	11	22
Total	٥٠	١٠٠

(X^2 = 5.627 , df = 12 , P value ≤0.05)

Grading of breast cancer

Out of 85 cases, grading system can be applied only to 50 cases, and we found that 2 cases (4%) were grade I, 23 cases (46%) were grade II, 25 cases (50%) were grade III. There was significant difference among patients (Pvalue≤ 0.05), table (13).

Table 13: distribution of patients according to grade.

Grade	Number of patients	%
Grade I	٢	٤
Grade II	٢٣	٤٦
Grade III	٢٥	٥٠
Total	٥٠	١٠٠

(X^2 = 5.511 , df = 8 , P value ≤ 0.05)

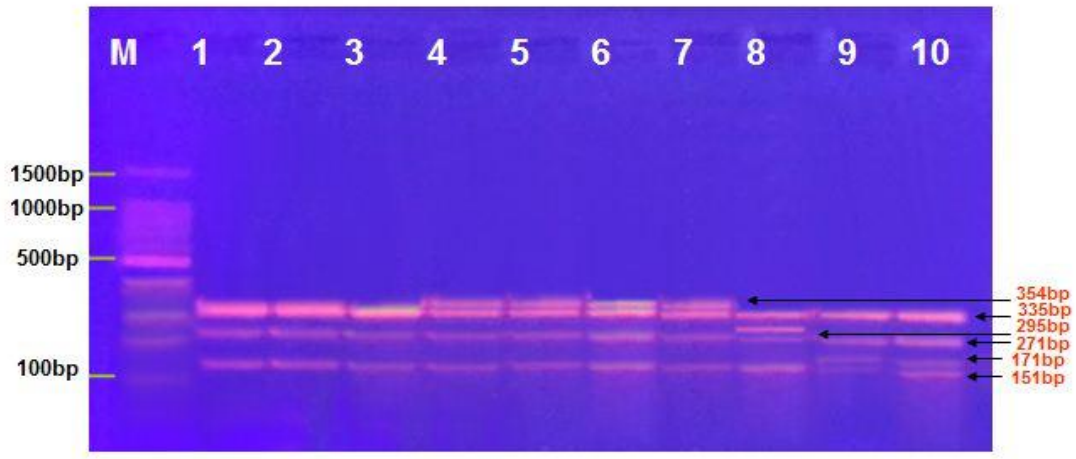

Figure 4: Agarose gel electrophoresis image of Multiplex allele-specific PCR products analysis.

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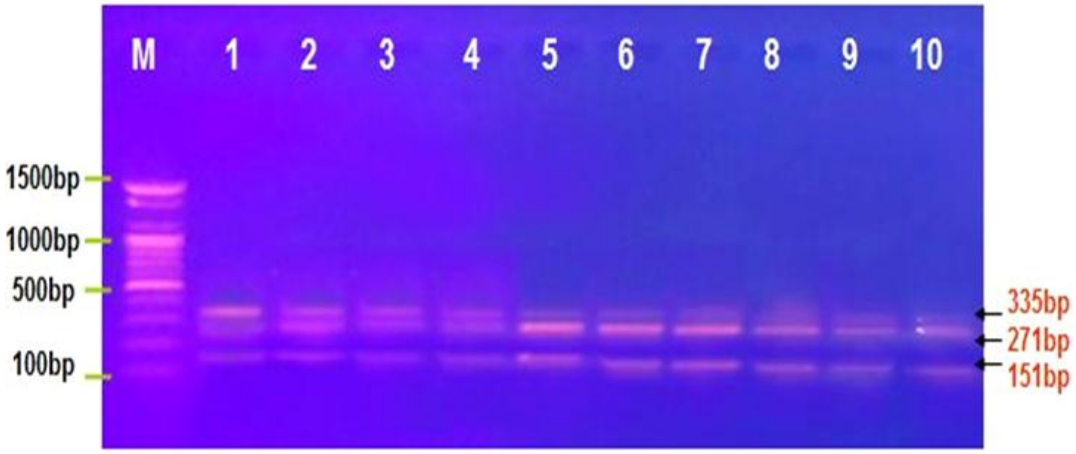

Figure 5: Agarose gel electrophoresis image of Multiplex alleles PCR products analysis.

Discussion

increase in younger women who had first birth than the older 34 cases (41.5%) had first child at age between (20–29years) in comparison with 7 cases (9.09 %) had first child at age (≥ 40 years) as low percentage. This result agree with two other studies.[10,11]

Breast cancer are more common in the left side of breast than the right side breast. In this study, breast cancer was more frequent in the right side of breast (50.5%) than the left side (38.8%) with only (10 %) was bilateral. This finding agree with a study done byFayaza *et al*., (2013) who observed that the (53%) of breast cancer were in the right side while (46%) were in the left side of breast. Our result agree with [8] and disagree with[12] other studies. Several studies have documented the peculiar fact the breast carcinoma is slightly more frequent in the left breast than in the right. In one recent series, the excess for the left side was 13% .[13]

The result of the present study recorded that the ages of the breast cancer patients ranged from 30 to ≥ 70years, the peak age frequency of (40-49 years) and (50-59 years). Our results agree with results of other studies.[2,7]

The higher incidence of breast cancer in age group between (40-49years) is related to many factors which have collectively a cumulative effects including reproductive factors, genetic factors, hormonal factors, exposure to radiation and life style.The present study had shown that family history was positive in 31 cases (36.47%), and negative in 54 cases (63.52%). This finding agreed with other studies.[8,9]

Table (6) in the present study, shows that the incidence of breast cancer

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280 nm can provide validation of the purity of nucleic acid samples.[16]

In this study, the quantities of DNA obtained from blood samples of normal women and breast cancer patients were in normal range (> 4 < 4000 ng / µL), as shown in table (8). AlKhinji, (2011), indicates that the nanodrop has broad linear range, and accuracy drops off (error > 10%) for concentration < 4 ng /µL and > 4000 ng /µL.Low *A*260/*A*280 ratios are typically due to the presence of protein, phenol or surfactant. In addition, nucleic acids that are not fully resuspended can scatter light, resulting in low*A*260/*A*280 ratios. Any precipitates, whether they are silica particles, salt crystals (i.e., guanidine and thiocyanate) or surfactant micelles can result in abnormal *A*260/*A*280 ratios.[16]

Also our results demonstrated that DNA purity of most cases was (1.8) in percentage about (42.2%), as shown in table (9). We deduce that purity can be considered in normal range. If the ratio is appreciably lower, it may indicate the presence of protein, phenol or other contaminants that absorb strongly at or near 280 nm.

The 185 del AG mutation in *BRCA1* Gene results, the results in this study showed that (185 del AG) was present in 6 patients (7.05%) out of 85 patients, this study agree with previous studies in Iraq and India.[17,18]and Concerning the microscopical types of breast cancer, invasive ductal carcinoma was the predominant type (84%). This result agree with other study done on Iraqi women.[14]

In the current study, most of patients 24 (48%) out of 50 cases were stage III, followed by stage II in 13 cases (26%). SO most of women came late when the cancer in late stage this correlated with other study[15],and disagree with other.[8]

In this study, grade III tumor constituted the highest number of patients (25) cases (50%), followed by grade II that present in 23 cases (46%) of cases. This results agree with other study.[8]

We concluded most cases in present study were advance stage and grade. These observations obviously reflect the poor health education of the general population and their ignorance regarding the significance of clinical breast examination, breast self-examination and early medical consultation.

Both DNA and RNA absorb maximally at 260 nm, while most proteins absorb strongest at 280 nm. However, nucleic acids also absorb significantly at 280 nm and most proteins can absorb strongly at 260 nm (the absorbance varies, depending on the protein). Thus, it can be difficult to accurately measure the concentrations of DNA, RNA and protein in complex mixtures. However, measuring absorbance at 260 nm and at

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5382insC mutation in 2 cases (2.35%) with breast cancer. All control cases were negative for (5382insC) mutation expression. These results are compatible to pervious study done by Armaou *et al.*,[19]and disagree with Mehdipour *et al.* study.[21]

The 6174delT mutation *BRCA2* gene result, in current study, 6174delT mutation expressed in 3 cases (3.52%). These findings agree with one study and disagree with another.[22,23]

disagree with another study who showed that all samples were negative for 185 del AG mutation by multiplex PCR in breast cancer patients.[19]
The most frequent *BRCA1* mutation is 185AGdel in exon 2 and was described in all the ethnics including Asia, America, Africa, and European populations. Since, it was replicated in various populations with Arabic ethnics including Syria, Iraq, and Yemen.[20]

The 5382 ins C mutation in*BRCA1* gene result, the present study has shown that the expression of

Conclusions

2.The (185 del AG and 5382 ins C mutation in BRCA1 gene, and 6174 del T mutation in BRCA2 gene expression were an important risk factor for the development of breast cancer compared with control.

3.The incidence of *BRCA1* mutations (185AG and 5382C mutations) was more than *BRCA2* mutation (6174T mutation).

According to the results of the present study, the following conclusions could be elucidated:

1. The age between (40-49) years and over represents a risk factor for breast cancer, in addition to other important risk factors such as family history of breast cancer, age at first birth, and tumor site.

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دراسة الطفرة الوراثية لجيني BRCA2, BRCA1 والصفات المرضية والسريرية لسرطان الثدي في محافظة ذي قار

أ. م. د. مها شاكر حسن

الخلاصة

سرطان الثدي هو النوع الأكثر شيوعاً من الأورام الخبيثة في العراق. طفرات الجينات قابلية سرطان الثدي (BRCA1 و BRCA2) هي طفرات الجينات الأكثرشيوعا المسؤولة عن زيادة خطر الإصابة بسرطان الثدي. وقد أجريت الدراسة الحالية لتقييم الدور المحتمل للجينات BRCA1 و BRCA2 في عينات سرطان الثدي للمرضى في محافظة ذي قار.

تم أخذ عينات الدم خمسة وثمانين من المرضى الذين حضروا مستشفى الحسين ووحدة الاورام السرطانية في مستشفى الحبوبي خلال الفترة (من آب/أغسطس في/٢٠١٤ إلى/٢٠١٧ نيسان/أبريل)، مع ٥٠ عينة دم من اشخاص اصحاء كمجموعة سيطرة لهذه الدراسة. لغرض التحليل النسيجي جمعت ٥٠ عينة نسيجية من مرضى سرطان الثدي الذين يخضعون للاستئصال الجراحي (استئصال الثدي). تم الحصول على عينات الدم بواسطة السحب الوريدي لأستخدامها فيما بعد في الدراسة الوراثية ودراسة بعض معايير الدم. العينات النسيجية جمعت من ٥٠ مريضه مصابه بسرطان الثدي وحفظت في ١٠% فورمالين لتحضير كتل البارافين التي تم استخدامها للتشخيص النسيجي والمناعي.

وكشفت نتائج الدراسة الحالية عن وجود تكرار للمرض يحدث للأعمار مابين ٤٠-٤٩ عاماً (٤١%). وكان تاريخ عائلي إيجابي في ٣١ حالة (٣٦,٤٧ %)، و ٥٤ حالة (٦٣,٥٢ ٪) سالبه للتاريخ العائلي لسرطان الثدي. كما أظهرت نتائج هذه الدراسة أن ٣٤ حالة (٤١,٥%) كانت الولادة الأولى في سن (٢٠-٢٩ سنة)، ١٨ حالة (٢٣,٣٧%) كانت ولادتهم الأولى في سن (١٥-١٩ سنة) و (٣٠-٣٩ عاماً)، و ٧ حالات (٩,٠٩%) كانت الولادة الأولى في السن (سنة ≤٤٠).

اعتماداً على تشخيص النسيجي الحالات السرطانية قسمت الى ٤٤ حالة (٨٨%) كانت السرطان الأنبوبي (منها ٤٢ حالة (٨٤%) كانوا السرطان الأنبوبي المتسرب، وحالتين (٤%) كانوا كوميدوكارسينوما، وكانت ٦ حالات (١٢%) السرطان المفصص المتسرب.

أظهرت النتائج إلى أن (٤%) من المريضات كانوا في المرحلة الأولى من المرض ، (٢٦%) كانوا في المرحلة الثانية، (٤٨%) كانوا في المرحلة الثالثة وكانوا (٢٢%)في المرحلة الرابعة،

وأيضا النتائج التي توصلنا إليها أظهرت أن ٢ حالة (٤%) كانوا grade الأول، ٢٣ حالة (٤٦%) كانوا grade الثاني، ٢٥ حالة (٥٠%) كانوا grade الثالث.

تم استخراج الحمض النووي من عينات الدم باستخدام أدوات استخراج الحمض النووي الجينوم ® Accupower®. وتم قياس كل من التركيز والنقاء لجميع عينات من الحمض النووي بواسطة جهاز المطياف الضوئي النانو دروب. تم تسجيل العينات بدرجة نقاء تتراوح من ١,٧ إلى ١,٩ في هذه الدراسة للكشف عن الطفرات في الجينات BRCA1/2 في المرضى ومجموعات السيطرة.

لقد تم كشف الطفرات في الجينات BRCA1/2 باستخدام تفاعل البلمره المتسلسل المتعدد البرايمر حيث تم الكشف عن الطفرة (185 del AG) في الجين الاول في ٦ مرضى (٧,٠٥%) من أصل ٨٥ حالة المصابات بسرطان الثدي، و تم الكشف عن طفرة(5382 ins C) في الجين نفسه في ٢ من المرضى (٢,٣٥%) .اما بخصوص الطفرة (6174 del T) تم الكشف عنها في الجين BRCA2 في ٣ من المرضى (٣,٥٢%). اما بالنسبة لمجموعه للسيطره ، لم تكن موجودة هذه الطفرات في الجينات على حد سواء.