

Molecular study of *BRCA-1,2* and *P53* gene polymorphisms among post-operative breast cancer patients

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Key Words: *BRCA* genes, *P53* and Breast cancer.

Abstract

Breast cancer is a malignant tumor that starts from cells of the breast. A malignant tumor is a group of cancer cells that may grow into (invade) surrounding tissues or spread (metastasize) to distant areas of the body. The burden of breast cancer is increasing in both developed and developing countries, and in many regions of the world, it is the most frequently occurring malignant disease in women; comprising 18% of all female cancers, and worldwide, breast cancer is the fifth most common cause of cancer mortality. This case-control study was arranged to investigate the possible role of selected genetic parameters in a random samples of patients with breast cancer in the Al-Diwaniya province. 5 ml blood samples obtained from fifty females with breast cancer in post-operative stage attending the outpatient department of oncology in Al Diwaniya teaching hospital have been recruited in the study and compared to 50 health control females without any cancer types, ages of patients and control were ranged between 18-80 years. Among the studied three candidate susceptibility genes, *BRCA-1* genotypes had significant predictive power. In *BRCA-1* GG genotype has obviously suggested an risk factor for tumor, as had an (OR 5.3191) and risk factor (EF 0.065), In contrast, the AG & AA genotypes had rather preventive role as it had no risk factor (PF) of 0.0476 & 0.1667 respectively and low OR (0.7619 & 0.7917 respectively), and patient have 16% and 84% of patients have G and A alleles respectively. In *BRCA-2* AG genotype has obviously suggested an etiology for tumor, as had an (OR 13.4146) and risk factor (EF

0.1851), In contrast, the AA genotype had rather no risk factor role as it had Protective Fraction (PF) of 0.9103 and low OR (0.0731) . patients have 10% of G and 90% of A alleles compared with control they have 100% of A only .In P53 CC genotype has obviously suggested an etiology for tumor, as had an (OR 1.2941) and risk factor (EF 0.091) , In contrast, the GC genotype had rather had no risk factor as it had (PF) of 0.087 and low OR (0.4565) and patients have 56% of G allele and 44% of C allele compared with control they have 52% of G and 48% of C.

الخلاصة: سرطان الثدي هو ورم خبيث يحدث في خلايا الثدي , والورم الخبيث هو مجموعة من الخلايا السرطانية تنمو داخل أو حول النسيج أو تنتشر إلى مناطق مختلفة من الجسم. إن ثقل مرض سرطان الثدي يبدأ في جميع دول العالم حيث يعتبر هو أكثر مرض تتعرض إليه الإناث من بين بقية أنواع السرطانات حيث يشكل حوالي 18% من بين السرطانات التي تصيب الإناث ويشكل حوالي خمس حالات الوفيات الناجمة عن أمراض السرطان حول العالم . ترتبت الدراسة الحالية على التحقق من دور بعض الجينات في عينات عشوائية للمرضى الوافدين إلى مستشفى الديوانية التعليمي في محافظة الديوانية . تم جمع 5 ملي لتر دم من 50 مريضة مصابة بسرطان الثدي في مرحلة ما بعد العملية الجراحية كمجموعة مرضى و 50 أنثى لا تعاني من أي نوع من أنواع السرطانات كمجموعة سيرة في الدراسة الحالية , تراوحت أعمار كلا المجموعتين بين 18 إلى 80 سنة. أظهرت نتائج الدراسة الحالية عدم وجود علاقة إحصائية بين الفئات العمرية والإصابة بسرطان الثدي ولكن تكررت الإصابات في الفئة العمرية 40- 50 سنة (50%). في مجال العلاقة بين مرض سرطان الثدي وبعض المعلمات السرطانية أظهرت نتائج دراستنا وجود علاقة إحصائية ($p < 0.05$) بين الإصابة بسرطان الثدي وارتفاع تركيز CA-15.3 و CEA في مصل المرضى إلى مستويات عالية بالمقارنة مع مجموعة السيطرة $P < 0.0001$. كذلك أظهرت نتائج دراستنا عدم وجود علاقة إحصائية بين CA-15.3 و CEA ($p = 0.185$) لكن هنالك علاقة موجبة طفيفة ($r = 0.2432$) . من ناحية أخرى بينت نتائج دراستنا الحالية عدم وجود علاقة إحصائية بين ارتفاع تركيز المعلمات السرطانية والعمر حيث إن تركيز CA-15.3 و CEA لا تتأثر بالعمر ($CA-15.3 \ r = 0.20$ and $CEA \ r = 0.114$) . خلال دراسة التغيرات الجينية لجينات *BRCA-1*, *BRCA-2* و *P53* ضمن حقل الدراسة الجزيئية لدينا , أظهرت نتائج الدراسة وجود علاقة إحصائية بين وجود الطفرات في هذه الجينات والإصابة بسرطان الثدي. في جين *BRCA-1* أظهرت نتائج الدراسة الحالية إن الطراز الوراثي GG له دور كعامل مسبب للورم حيث كان OR (5.3191) و (EF 0.065) , في المقابل أظهرت الطرز الوراثية AG و AA بدلا من ذلك دور وقائي ضد الورم حيث PF (0.7917 و 0.0476 على التوالي) كذلك أظهرت OR نسب منخفضة معنويا حيث كانت (0.7619 و 0.7917 على التوالي) , كذلك أظهرت المريضات المصابات بسرطان الثدي نسب متفاوتة بالنسبة لامتلاكهن الأليلات G و A حيث كانت 16% و 84% على التوالي. في جين *BRCA-2* أظهرت نتائج الدراسة الحالية إن الطراز الوراثي AG له دور كعامل مسبب للورم حيث كان OR (13.4146) و (EF 0.1851) , في المقابل أظهر الطراز الوراثي AA بدلا من ذلك دور وقائي ضد الورم حيث PF (0.9103) كذلك أظهرت OR نسب منخفضة معنويا حيث

كانت (0.0731) , كذلك أظهرت المريضات المصابات بسرطان الثدي نسب متفاوتة بالنسبة لامتلاكهن الأليلات G و A حيث كانت 10% و 90% على التوالي . في جين P53 أظهرت نتائج الدراسة الحالية إن الطراز الوراثي CC له دور كعامل مسبب للورم حيث كان OR (1.2941) و (EF 0.091) , في المقابل أظهرت الطراز الوراثي GC دور وقائي ضد الورم حيث PF (0.087) كذلك أظهرت OR نسب منخفضة معنويا حيث كانت (0.4565) , كذلك أظهرت المريضات المصابات بسرطان الثدي نسب متفاوتة بالنسبة لامتلاكهن الأليلات G و C حيث كانت 56% و 44% على التوالي.

Introduction

Breast cancer is a malignant tumor that starts from cells of the breast. A malignant tumor is a group of cancer cells that may grow into (invade) surrounding tissues or spread (metastasize) to distant areas of the body. The disease occurs almost entirely in women, but men can get it, too. The burden of breast cancer is increasing in both developed and developing countries, and in many regions of the world, it is the most frequently occurring malignant disease in women; comprising 18% of all female cancers, and worldwide, breast cancer is the fifth most common cause of cancer mortality ¹. In 2008, approximately 1.4 million women were diagnosed with breast cancer worldwide with a corresponding of 460,000 deaths ². However, these risk factors have been shown to have different relations to breast cancer in different ethnic populations of the world ⁶. Accordingly, breast cancer is clinically regarded as a heterogeneous and complex disease, encompassing a wide variety of pathological entities and a range of clinical behavior⁷. The scope of genetic anomalies in breast cancer has been impacted through different genetic approaches and one of them is genomic instability evaluation. Genomic instability in cancer can be viewed as chromosomal instability (CIN), in which a majority of the tumors exhibit abnormal karyotypes involving either chromosomal rearrangement and/or aneuploidy and are classified as CIN tumors. In this regard, various reports indicated a significant increase in chromosomal aberrations (CAs) in cultured peripheral blood lymphocytes (PBLs) of cancer patients with solid tumors ^{8&9}. As presented earlier, breast cancer is often initiated by genetic and epigenetic changes in genes that regulate the function of the mammary epithelial cells, and to prevent the development of breast cancer, diverse

intrinsic tumorsuppressor mechanisms induce senescence or apoptosis of neoplastic cells¹¹.

Aim of Study: Study of some predisposing genes and tumor markers to reach to more frequent and dangerous factor among breast cancer patients through the following objective :

Study of genetic variation in *BRCA-1* & -2 , and *P53* as a predisposing genes and response to tumor by using RFLP-PCR.

Materials and Methods

1- Subject : The present study was conducted on 100 females (50 patients group and 50 controls group) The patients were females who had a breast cancer (post-operative stage) . Both groups include females with 18-80 years old. The patients were referred to Al-Diwanya Teaching hospital , department of oncology, during the period March-November 2016. The diagnosis was made by the expert pathologist, all patient in after surgery stage (post-operative). Demographical and risk factor data were collected using a short structured questionnaire, that included information on age, weight, height, marital status, number of pregnancies and children, age at first child birth, average lactation term, family history of breast cancer or other cancers (first degree relatives), age at menarche and age at marriage. Another group include healthy females without any family history of breast cancer also included in this study as a control group.

2-Genomic DNA Extraction: Genomic DNA from blood samples were extracted by using Genomic DNA mini kit extraction kit (Frozen Blood) Geneaid. USA, and done according to company instructions.

3-Genotyping: RFLP-PCR for *BRCA1-185delAG* mix was prepared by using *DdeI* restriction enzyme (New England Biolabs. UK) and this master mix done independent according to company instructions, After that, this master mix placed in Exispin vortex centrifuge at 3000rpm for 3 minutes, then incubation at 37°C for overnight. After that, RFLP-PCR product was analysis by 3% agarose gel electrophoresis methods. The genotyping of BRCA1 gene including AA (homozygous) by two bands at (150, 26bp), GG (homozygous) as non-digested band at 176bp, A/G (heterozygous) of three bands at bp, 150bp, and 26bp.

RFLP-PCR mix for (*BRCA2-A/G*) RFLP-PCR mix was prepared by using *BspHI* restriction enzyme (New England Biolabs. UK) and this master mix done independent according to company instructions, After that, this master mix placed in Exispin vortex centrifuge at 3000rpm for 3 minutes, then incubation at 37°C for overnight. After that, RFLP-PCR product was analysis by 3% agarose gel electrophoresis methods. The genotyping of BRCA2 gene including AA (homozygous) by two bands at 296bp and 50bp, GG (homozygous) three band at 235bp, 61bp, and 50bp, A/G (heterozygous) of four bands at 296bp, 235bp, 61bp, and 50bp.

RFLP-PCR mix for (*p53 intron 6G13964C*) RFLP-PCR mix was prepared by using *HhaI* restriction enzyme (New England Biolabs. UK) and this master mix done independent according to company instructions, After that, this master mix placed in Exispin vortex centrifuge at 3000rpm for 3 minutes, then incubation at 37°C for overnight. After that, RFLP-PCR product was analysis by 3% agarose gel electrophoresis methods. The genotyping of p53 gene including GG (homozygous) by two bands at 33bp and 98bp, CC (homozygous) as non-digested band at 131bp, G/C (heterozygous) of four bands at 33bp, 98bp, and 131bp.

Statistical analysis: Statistical analysis was performed by Social Science Statistics and the Statistical Package For Social Sciences version 19 for Windows Software and Microsoft Excel 2010. Continuous random variables of age and serum concentration of immunological makers that normally distributed are described by mean, SD (standard deviation), SE (standard error), and the parametric statistical tests of significant. ANOVA test are used to analysis the statistical significance of difference in mean between more than 2 groups and when ANOVA model shows statistically significant differences, additional exploration of the statistical significance of difference in mean between each 2 groups was assessed by Bonferonni t-test The statistical significance, direction and strength of linear correlation between 2 quantitative variables was measured by Spearman's rank and Pearson linear correlations coefficient as in state of serum markers. Moreover measure the strength of association between 2 categorical variables, such as the presence of certain genotype and disease status the odds ratio (OR) and Chi-square (χ^2) test were used. P value calculate from different tests depend on

variables and that less than the 0.05 level of significance was considered statistically significant

level of significance was considered statistically

12.

Result

1-Demographic Features Of The Study: The present case- control study were based on the analysis of a random sample of 50 females with precise diagnosis of breast cancer, their ages ranged from 19 to 80 years with a mean of 46.38 (SD 14.31) and 50 (cancer free health) controls females their ages ranged 19 to 80 years with a mean of 45.6 (SD14.34) as in Table 1 , that also show not significant ($p > 0.05$) association between mean age of cases and controls. **Table (1): The case-control difference in mean age**

Demographic features	Case (breast cancer)	Healthy controls
Age Groups (years)	N (%)	N (%)
19-29	5(10)	6 (12)
30-39	10 (20)	9 (18)
40-50	20 (40)	23(46)
51-60	6 (12)	4(8)
61-80	9 (18)	8(16)
Total Number	50	50
Range	19-80	19-80
Mean	46.38	45.6
SD	14.31	14.34
SE	2.023	2.028
P – value	0.9369 (NS)	

❖ NS= Not Significant ($p > 0.05$), SD= Standard Deviation, SE= Standard Error, N= Number

2-Detection of *BRCA-1* Polymorphism

The distribution of *BRCA-1* polymorphism was detected by PCR-RFLP technique, at this locus there're three genotype; homozygote lane (AA) homozygous as non-digested band , lane (GG) homozygous at 150 and 26bp , and lane (G/A) heterozygous at bp, 150bp, and 26bp shown in Figure (1).

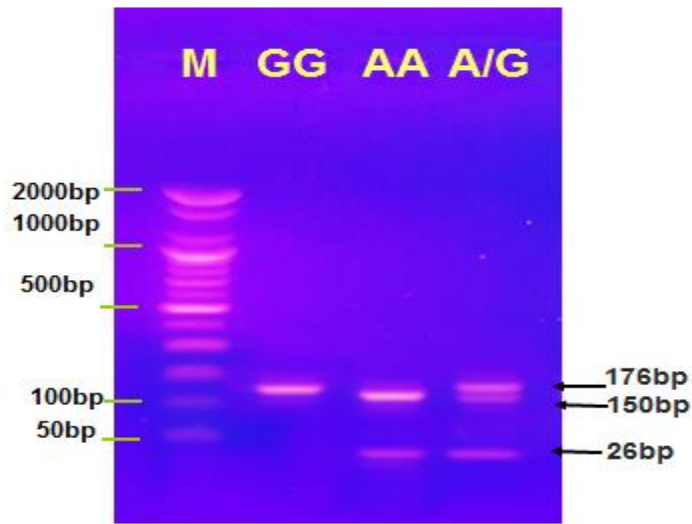


Figure1: Agarose gel electrophoresis image that show the RFLP-PCR product analysis of BRCA1 185delAG gene polymorphism by using *DdeI* restriction enzyme. Where M: marker (2000-50bp), lane (GG) homozygous at 150 and 26bp, lane (AA) homozygous as non-digested band 176bp, and lane (G/A) heterozygous at bp, 150bp, and 26bp.

In *BRCA-1* GG genotype has obviously suggested an etiology for tumor, as had an (OR 5.3191) and Etiologic Fraction (EF 0.065), In contrast, the AG & AA genotypes had rather preventive role as it had Protective Fraction (PF) of 0.0476 & 0.1667 respectively and low OR (0.7619 & 0.7917 respectively). Figure (2) show patient have 76% of AA, 8% of GG and 16% of AG compared with control show 20% of AG, 80% of AA and 0% of GG. Figure (3) show patient have 16% and 84% of patient have G and A respectively compared with control they have 10% and 90% of G and A respectively.

Table (2): distribution of genotypes and alleles of *BRCA1* gene in case & control

<i>BRCA1</i> gene	Patient	Control	OR	95% CI OR	X^2	P (X^2)	EF	PF
	N (%)	N (%)						
BRCA1 genotypes								
AA	38 (76)	40 (80)	0.7917	0.306 -2.046	0.233	0.629	***	0.1667
GG	4 (8)	0 (0)	5.3191	0.599 -47.229	5.233	0.022	0.065	***

AG	8 (16)	10 (20)	0.7619	0.273 -2.125	0.271	0.603	***	0.0476
Total number	50	50						
BRCA1 Alleles								
A	84 (84)	90 (90)	0.5833	0.251 -1.357	1.591	0.208	***	0.3750
G	16 (16)	10 (10)	1.7143	0.737 -3.988	1.59	0.207	0.0667	***
Total number	100	100						

❖ OR=Odd ratio, EF= Etiology fraction, PF=Preventive fraction, X^2 = chi square

3- Detection of *BRCA-2* Polymorphism:

The distribution of *BRCA-2* polymorphism was detected by PCR-RFLP technique, at this locus there're three genotype; lane (GG) homozygous at 296bp and 50bp, lane (AA) homozygous at 235bp, 61bp, and 50bp, and lane (G/A) heterozygous at 296bp, 235bp, 61bp, and 50bp. Figure (2).

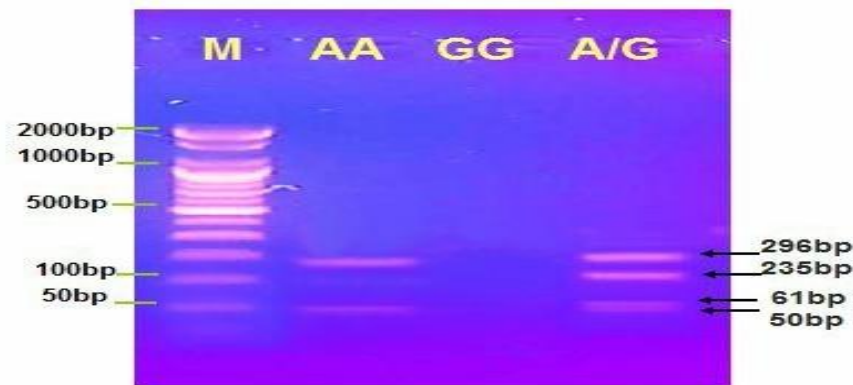


Figure2: Agarose gel electrophoresis image that show the RFLP-PCR product analysis of BRCA2185delAG gene polymorphism by using *BspHI* restriction enzyme. Where M: marker (2000-50bp), lane (GG) homozygous at 296bp and 50bp, lane (AA) homozygous at 235bp, 61bp, and 50bp, and lane (G/A) heterozygous at 296bp, 235bp, 61bp, and 50bp.

In *BRCA-2* AG genotype has obviously suggested an etiology for tumor, as had an (OR 13.4146) and Etiologic Fraction (EF 0.1851), In contrast, the AA genotype had rather preventive role as it had Protective Fraction (PF) of 0.9103 and low OR (0.0731). Figure (9) show patient have 80% of AA and 20% of AG compared with control show 100% of AA and 0% of AG Figure (10) show patient have 10% of G and 90% of A

compared with control they have 100% of A only.

Table (3): distribution of genotypes and alleles of *BRCA2* gene in case & control

<i>BRCA2</i> gene	Patient N (%)	Control N (%)	OR	95% CI OR	X^2	P (x^2)	EF	PF
BRCA2 genotype								
AA	40 (80)	50 (100)	0.0731	0.009 - 0.5897	11.11	0.001	***	0.9103
GG	0 (0)	0 (0)	***	***	***	***	***	***
AG	10 (20)	0 (0)	13.4146	1.662- 108.282	11.10	0.0009	0.1851	***
Total number	50	50						
BRCA2 Alleles								
A	90 (90)	100(100)	0.0819	0.010 - 0.647	10.50	0.0012	***	0.9098
G	10 (10)	0 (0)	12.2088	1.546 - 96.430	10.53	0.0010	0.0918	***
Total number	100	100						

❖ OR=Odd ratio, EF= Etiology fraction, PF=Preventive fraction, X^2 = chi square

4-Detection of *p53* intron 6G13964C Polymorphism:

The distribution of *P53* polymorphism was detected by PCR-RFLP technique, at this locus there're three genotype; lane (GG) homozygous at 33bp and 98bp, lane (CC) homozygous as non-digested band at 131bp, and lane (G/C) heterozygous at 33bp, 98bp, and 131bp. Figure (3).

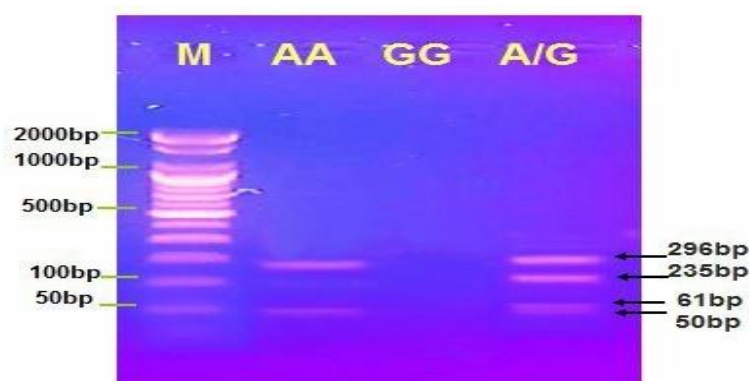


Figure3: Agarose gel electrophoresis image that show the RFLP-PCR product analysis of *p53* intron 6G13964C gene polymorphism by using *HhaI* restriction enzyme. Where M:

marker (2000-50bp), lane (GG) homozygous at 33bp and 98bp, lane (CC) homozygous as non-digested band at 131bp, and lane (G/C) heterozygous at 33bp, 98bp, and 131bp.

In *P53* CC genotype has obviously suggested an etiology for tumor, as had an (OR 1.2941) and Etiologic Fraction (EF 0.091), In contrast, the GC genotype had rather preventive role as it had Protective Fraction (PF) of 0.087 and low OR (0.4565). Figure (3) show patient have 52% of GG, 40% of CC and 8% of GC compared with control show 50% of GG, 34% of CC and 16% of GC. Present study show patient have 56% of G and 44% of C compared with control they have 52% of G and 48% of C.

Discussion

1-Demographic characteristics

The age characteristic of patients who have breast cancer in the present study, revealed that the highest frequency of breast cancer patients among (40-50) years old (40%), followed by the age group of (30-39) years old (20%), and the less frequency in the age (19-29) years (10%), which has no significant differences as compared with control group ($p > 0.05$) mean 46.38 years (SD14.34), so breast cancer is a disease of all ages, considering the entire lifespan¹². The results of our present study are agreed with (Dodova *et al.*, 2015) since the results of their study which included 200 Bulgarian females with breast cancer (post operative and the age ranged from 25 to 74 years) selected by the established genetic testing criteria, the mean age of the patients at diagnosis was 49.5 years, and no significant association between patients group and controls group ($p > 0.05$). So our findings are comparable with a study conducted an average 12% of women worldwide related breast cancer, their ages ranged between <40 - >70 years and showed 48.5 years mean of patients ages¹⁴. Other studies documented an age mean 50.3 years¹⁵. So this results that is consistence with (Barthelemy *et al.*, 2011) who found the mean age of breast cancer patients 45.1 years, and no significant differences with control group ($P = 0.903$), another study performed by¹⁷, stated in their study a mean age 44.7 years of patients with breast cancer which was not different from control group ($p=0.19$), and a similar findings was reported by¹⁸ who found 42.95 years as a mean age of breast cancer patients. The *BRCA-1*, *BRCA-2* and

P53 genotypes were assessed for their roles in predicting the risk of having breast cancer, each compared of control group, (general population without history family for breast cancer in any degree). The results of present study showed the *BRCA-1* genotypes, had significant predictive power. The G allele had the strongest association and significantly increases the risk of having breast cancer 16% (OR= 1.7143, 95% CI OR=0.737 -3.988 , EF=0.0667) compared to general population control. In a lesser degree the A allele had a statistically significant protective effect 84% (OR= 0.5833, 95% CI OR= (0.25 -1.357), PF= 0.3750). the homozygous GG genotype increase the risk of the disease 8% (OR=5.3191, 95% CI OR= 0.599 -47.229, EF=0.065. While the wild AA genotype showed a statistically significant protective effect 76%(OR= 0.7917, 95% CI OR= (0.306 -2.046), PF=0.1667) . So the heterozygous AG genotype showed a statistically significant protective effect 16%(OR= 0.7619, 95% CI OR= (0.273 -2.125), PF=0.047), compared with control group they have (0% GG, 80% AA and 20% AG) . this result have similarity with results of ¹⁹ , she tested (310) patients with breast cancers were recruited from different public and private hospitals of Bangladesh and as controls (250) Bangladeshi women , and found GG genotype increase the risk of malignant tumor in breast (OR=4.9, 95% CI=0.59 to 41.09, p=0.14). So our result that is consistence with study ²⁰ , who study on 106 consecutive breast cancer patients who were admitted to Istanbul Training and Research Hospital, Department of General Surgery and, they found GG responsible for risk to breast cancer (OR=8.54 ,95% CI; 1.07- 68.27). So our present study have similarity with the findings from most other previous studies in breast cancer patients with mutations in *BRCA1* and *BRCA2* such as studies of ^{21&22} they referred to G allele had the strongest association and significantly increases the risk of having breast cancer in GG genotype (OR= 1.812, 95% CI OR=0.691 -3.312) and (OR= 1.911, 95% CI OR=0.599 -3.018). The results in this study showed the *BRCA-2* genotypes, so had significant predictive power. The G allele had the strongest association and significantly increases the risk of having breast cancer (OR= 12.2088, 95% CI OR=1.546 - 96.430, EF=0.0918) compared with control group . In a lesser degree the A allele had significant protective role (OR= 0.0819, 95% CI OR= (0.010 - 0.647), PF= 0.9098) . The heterozygous AG genotype increase the risk of the disease by (OR=13.4146, 95% CI OR= 1.662- 108.282, EF=0.1851. While the wild AA genotype

showed a statistically significant protective effect (OR= 0.0731, 95% CI OR= (0.009 - 0.5897), PF=0.9103) . This results agreed with most studies such as ²⁰, who study on 106 Turkish patients with breast cancer and they reached to AG genotype increase the risk for breast malignancies (OR=12.6 ,95% CI, 43.91-3.67, EF=0.203) , ²³ they , their result showed to (OR= 11.412, 95% CI, 1.20-24.65, EF= 0.154) , So ²⁴ , they found AG increase risk of malignant tumor of breast(OR= 14.211, 95% CI, 2.03-28.55, EF= 0.106). The results in this study showed the *p53* genotypes, so had significant predictive power. The C allele had the strongest association and significantly increases the risk of having breast cancer 44%(OR= 1.0850, 95% CI OR=0.6198 - 1.8996, EF=0.0345) compared with control group . In a lesser degree the G allele had significant protective role56% (OR= 0.9216, 95% CI OR= (0.526 - 1.614), PF= 0.0455) . The homozygous CC genotype increase the risk of the disease by40 % (OR=1.2941, 95% CI OR= 0.573 - 2.921, EF=0.091) , and the heterozygous GC genotype showed a statistically significant protective effect 8% (OR= 0.4565, 95% CI OR= (0.128 - 1.627), PF=0.087. while wild type GG genotype don't have any role in increasing risk or protective effect 52% (OR= 1, 95%CIOR=0.495 - 2.374). this present study agreed with ²⁷ and their result referred CC genotype increase risk for breast cancer (OR = 0.87, 95% CI: 0.78–0.97) while GC have protective effect (OR = 0.91, 95% CI: 0.83–1.00), So there are similarity between our results and ²⁸ who study on Tunisian women , and who found increasing risk of disease by CC and presence of protection belong to GC genotype (OR=0.81 and OR=0.79 respectively). *P53*, which is tumor suppressor gene , creating a protein that repairs DNA and prevents carcinogenesis. Every cell in mutation carriers has been demonstrated to lack one functional allele (i.e. the tumor-suppressor function of that gene is lost); a situation that favors cancer development , so *P53* is a tumor suppressor gene that is mutated or changed in more than 50 percent of tumors²⁹.

Conclusion: Patients how have a history family considered a risk for breast cancer disease because presence of mutations in *BRCA-1* and *BRCA-2* genes, breast cancer considered a disease for all ages.

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