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Relation between Mutation in BRCA1&2 Carriers and Histopathlogical Characteristics of Breast Cancer Patients in Erbil City

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Abstract

Mutation of breast cancer susceptibility gene1(BRCA1) and breast cancer susceptibility gene2 (BRCA2) are responsible for an increased risk of developing breast cancer. This study was planned to correlate probable occurrence of sequence variant in three exons (exon 2 and exon 20) of BRCA1 and (exon 11) of BRCA2 with other clinicopathological variables like family history, grade, stage of breast cancer. Fifty breast cancer women patients which randomly selected. The age, size of tumer, grade, stage and presence of family history were measured. Pathological analysis using H and E staining method, while conventional PCR and Direct Nucleotide Sequencing Techiques were applied for detection of BRCA1 and \ or BRCA2 mutations. Molecular analysis revealed that among 50 breast cancer patient 8 of 50 harbored deleterious mutation and 9 of 50 harbored non sense mutations. The result of these experiments also revealed that there is strong relationship between mutation and each of grade and family history, but in the same time no relation was found between mutation and stage of breast cancer.

Introduction

Breast cancer, which is considered to be most prevalent female malignancy, is a main cause of death in middle-aged women and its incidence is elevated [1]. The malignant breast tumors are usually caused by their abnormal growth and uncontrolled proliferation of cells within the terminal duct and lobular ports of the breast. The cancerous cells can attack and destroy surrounding normal tissue, and spread throughout all parts of the body via blood stream or lymph fluid to metastasize at new sites. Breast cancer mainly occurs in females, although less commonly, males can also develop this type of cancer. Worldwide, breast cancer is the second most common cancer in the world and represents 9% of global cancer burden [2]. It has been proposed that breast cancer is a multifactorial disease and **its** etiology is the interaction of genetic and environmental factors [3]. Almost 90% of breast cancers occur sporadically, but without known predisposing genetic alterations, the remaining 10% are linked to genetic causes which include mutations in tumor suppressor genes, mostly *BRCA1* and *BRCA2* [4]. The incidence of breast cancer depends on the regions and countries, likely due to differences in racial and ethnic make-up, economy and social situations health resources, and lifestyle patterns [5, 2].

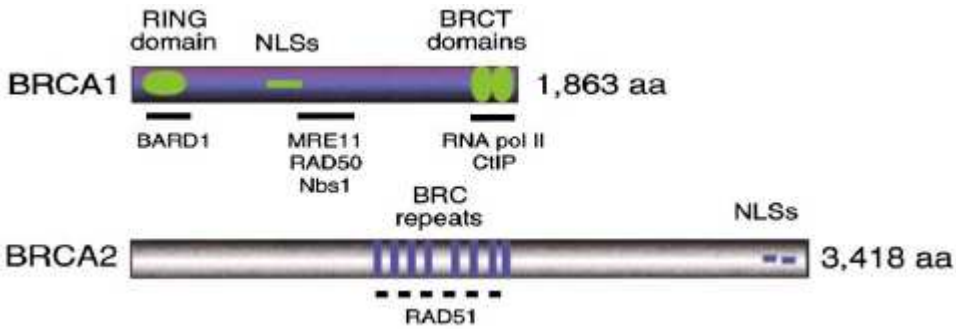
BRCA1 gene at 17q21 was cloned in 1994 [6]. The *BRCA1* gene includs 81 kilobases (kb) of genomic DNA on chromosome 17q21 with 24 exons, 22 of which are encoding [6, 7]. The other 2 non-coding genes

are exon 1 and the Alu repitive motif, exon 4. *BRCA1* coding region starts in exon 2. The central exon 11 corresponds to over 50% of the *BRCA1* coding region, while most of the other exons are small [6].

BRCA 1 contains several functional domains including: N-terminal RING a Zn-finger special type domain, BRCT domain and nuclear localization sequences (NLS) [8, 9].

BRCA2 gene, which is located at 13q12 was cloned by [10] and completely sequenced in 1996 [11]. The BRCA2 is a 70 kb gene, it contain 27 Exons and 26 are encoding [10, 11]. Like BRCA1, BRCA2 is very AT-rich, possesses a large exon 11 and a translational start site in exon 2 [11].

BRCA2 has 2 distinct domains, BRC repeats and a binding site at the C-terminal region of BRCA2, that bind with RAD51. RAD51 is a key component in which DNA is repaired by homologous recombination [12, 10].



It is believed that double strand breaks (DSB) in the structure of DNA are the major cause of genomic instability and chromosomal aberrations that lead to cell transformation. In eukaryotes, two primary mechanisms of DNA DSB repair are described: homologous recombination (HR) and non-homologous end joining (NHEJ). HR is the cellular repair mechanism during the S and G2 phases of the cell cycle as the template of intact sister chromatid is available. NHEJ is a process of ligation of DSB ends together without a homologous template; therefore it is considered an error-prone mechanism. The protection of the DNA from HR includes damage recognition, signal mediation and initiation of repair [13]. *BRCA1* has a key role in signal mediation while *BRCA2* and *RAD51* play roles in initiation of repair, which suggests that it is the *BRCA1*-*BRCA2*-HR pathway that suppresses the process of tumor genesis [14, 15].

BRCA1 suggested to has majority role in transcription regulation of genes related to response against DNA damage or genes regulate the cell cycle checkpoints.

Activation or inhibition of these genes depends on the interaction between *BRCA1* and the transcriptional repressor CtIP [16, 17]. *BRCA1* up-regulates tumor suppressor genes like *p53* and *p53*- regulated genes (*p21*) [16], while it represses cell proliferation genes [18].

Invasive ductal carcinoma (IDC) is the most common histological type among sporadic breast cancer, comprising 70-80% of hereditary breast cancer and it seems to be significantly more frequent in *BRCA1* and *BRCA2* mutation carriers than in non-carriers [19].

BRCA1 tumors are mostly grade3 tumors because they show less tubule formation, higher nuclear pleomorphism and a higher count of mitosis. Therefore, the recorded incidence of high-grade tumours in BRCA1 mutation carriers ranged from 66% to 84% according to different epidemiological studies [20].

BRCA2 tumours are showed to be of higher grade than sporadic controls, although this association is weaker than for BRCA1 cases. Mostly, the BRCA2 tumours are between grade 2/3, and show less tubule formation but similar cellular pleomorphism and mitotic count than sporadic control cases [20].

Interestingly, frequent allelic loss of *BRCA1* in *BRCA2*-associated tumors and vice versa has also been noticed [21]. Unlike to tumors in *BRCA1*-carriers, *BRCA2*-associated breast tumors show to be more heterogeneous with lack of the pathological and clinical feature compared to sporadic tumors [22]. *BRCA1*-

and *BRCA2*-associated cancers are both found at younger age and with appearance higher incidence of bilateral tumors than sporadic cases [23].Women with *BRCA1*-associated tumours have worse clinical pathological characteristics than women with sporadic case control [24, 25]. The progression of *BRCA1*-associated breast tumours can be diffentiated from sporadic cases in that there is a no significant correlation between the size of the primary tumour and the number of axillary lymph nodes [26]. The other difference between *BRCA1* associted tumour and sporadic tumours or tumours with *BRCA2* mutations, is that *BRCA1* tumors much less likely to be node-positive than otherwise expected. Women carrying *BRCA1* mutations who are negative node are associated with a poor prognosis [27].

Previous studies reported that breast cancer genetic inheritance (such as *BRCA1* and *BRCA2* genes) account for approximately 5% of breast cancer cases, although some authors reported a higher rateup to 10% [28]. The majority of studies on BRCA gene mutations have focused on the western population
Only a relatively small number of investigations on the role of the BRCA genes have been undertaken in Asian sporadic breast cancer populations [29].

In this study, our aim is to evaluate the role of BRCA1 and BRCA2 germline mutations in kurdish patients with sporadic breast cancer.

Subject and Methods

The study included 50 breast cancer patients which randomly selected from cases of Nanakally and Rizgari hospitals/Erbil-Iraq in period between October 2014 to march 2015. The age of breast cancer patient ranged between 25-68 years. For pathological investigation we divided patients into two groups of age ≤ 40 and age > 40 years.

Pathological Investigation

Slides prepared from 50 breast cancer tumor blocks in Rizgari hospital by using special protochol. Hematoxylin and Eosin stained breast tissue sections (4-5μm) were examined to determine tumor type, grade and stage. Tumor size and number of lymph nodes involvement, while family history also obtained from patients directly or from hospitals documents records.

Molecular Investigation

Total DNA was extracted from peripheral blood from 50 cases using EZ High™ DNA extraction kit (Texas Biogene, USA) according to manufacturer's instructions. The quantification and purity of extracted DNA was examined using Nanophotometer instrument. To detect mutations in BRCA 1&2 we use two molecular methods the first one (conventional PCR for detecting three common point mutation and the second was direct nucleotide sequencing for three exons (exon 2, 20 for BRCA1 and exon11 for BRCA2).

Results and Discussion

Grade and Age

Grading was done on all malignant cases of this study and the most predominant grade for breast cancer in general was grade II (16% of 30% and 58% of 70%) for both ages (≤ 40 and > 40), respectively followed by grade III (Figure 1) .This result agreed with study performed by [30] who found that breast cancer was characterized by an early age of onset (younger than the ages estimated in the developed countries), This result was also in agreement with study of [31] on Caucasian women who concluded that the proportion of Grade III tumors was significantly less than the proportion of Grade II tumors.

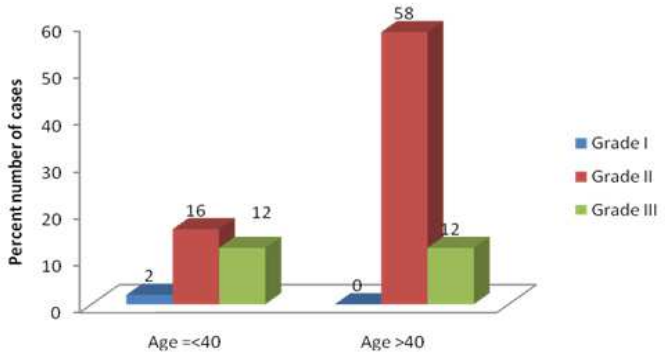


Figure -1: Age distribution of studied breast cancer female patients in relation to grade of their tumor.

Stage and age

The result of this experiment showed that most patients of (age > 40) were presented stage II (20 / 35 cases, 40%) followed by stage III (16%), then stage I and stage IV (8% and 6%, respectively). For age ≤ 40 most of the cases were in stage III (7 of 15 cases, 14%) followed by stage II(12%). In general patients presented with stage II breast cancer 52% followed by stage III 30% (Figure 2). Such findings have also been documented in some Arabic countries, while in developed countries, most patients present with an early stage [30]. This is mainly due to the lack of health education, delay medical consultation, absence of screening programs, rejection of management strategy and defects in follow-up. This result was also in agreement with study of [32] in Iran who concluded that stage II is the most prevalent followed by stage III.

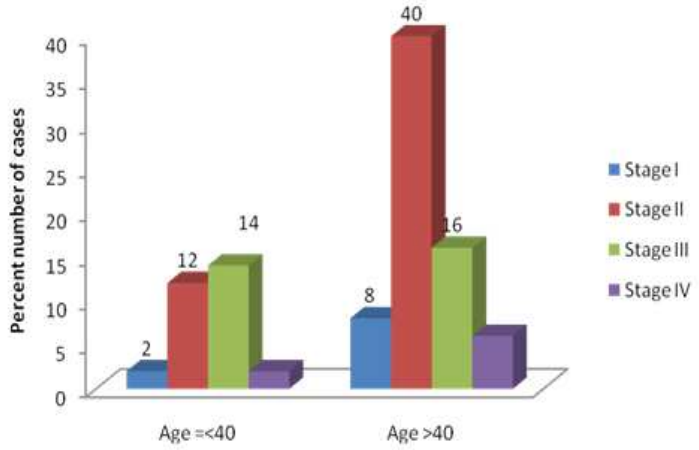


Figure -2: Age distribution of studied breast cancer female patients in relation to the stage of tumors**.**

Location and Age

50 female patients have been included in this study. Left side of the breast was involved in 30 patients (60%) versus 20 patients (40%) in which right side of the breast was involved Figure (4). This finding was also noted by [33, 34] among Malaysian, Egyptians and USA population [35] in Pakistan also found that right sided breast cancer has lower incidence than left sided but had an aggressive behavior.

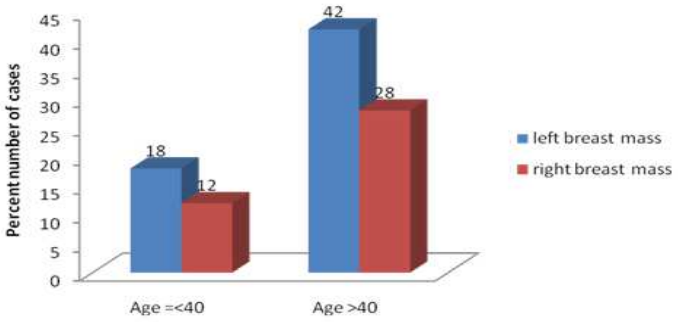


Figure -3: Age distribution of studied breast cancer female patients in relation to the location of breast tumors**.**

Age and size

The result of this experiment show that there was not any significant differences in tumor size between the two groups of age. The size of 0-4cm was the most predominant in both age groups (Figure 4). This result was found to be similar to previous study in Saudi Arabia [36] who observed that tumor size did not differ significantly between the patients above and less than 40 years old.

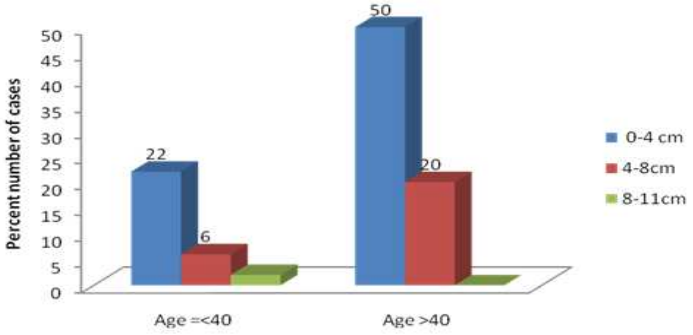


Figure -4: Age distribution of studied breast cancer female patients in relation to breast tumor size.

Family History and Age

For age group ≤ 40 the number of patients with positive family history were (8 / 15 cases, 16%) followed by negative family history 7 / 15 cases, 14%), while for (age > 40), the family history negative was predominant (25 / 35 cases, 50%) followed by family history positive (10 / 35 cases, 20%) (Figure 5). The result of this experiment agreed with a research reported by [37] in Italia who observed that patient under 40 yeares old more frequently had familly history of breast cancer than did other patients.

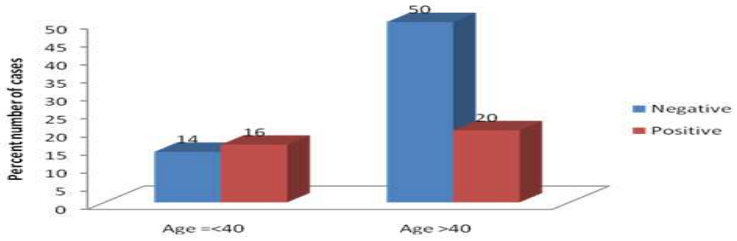


Figure -5: Age distribution of studied breast cancer female patients in relation to family history**.**

Age and tumor

The result of our experiment reveald that for all ages the most common histological type of breast cancer was ductal carcinoma (Figure 7, 8, 9) which constitutes about (45 / 50 cases, 90%) followed by infiltrative lobular carcinoma 2 / 50 cases, 4%) (Figure 6). This result agreed with [38] who observed that the most tumor developed in the carrier of BRCA mutation was an invasive ductal carcinoma, which is the most common histological type in all forms of hereditary breast cancer and seems to be significantly more frequent in BRCA1- and BRCA2-mutation carriers than in non-carriers. Also this result was in agreement with study of [39], who concluded that histopathological analysis of hereditary breast cancers shows that the majority are ductal carcinomas was associated to BRCA mutations; however, medullary carcinomas are overrepresented in patients with germline mutations in BRCA1.

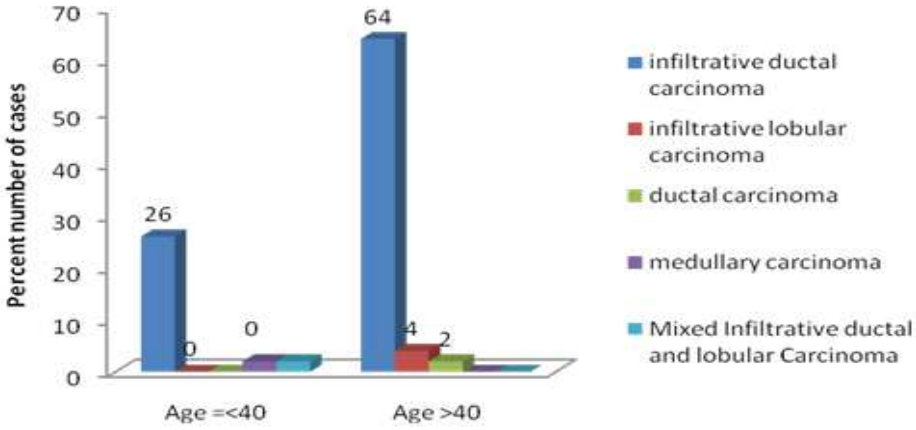


Figure -6: Age distribution of dtudied breast cancer female patients in relation to tumor type.

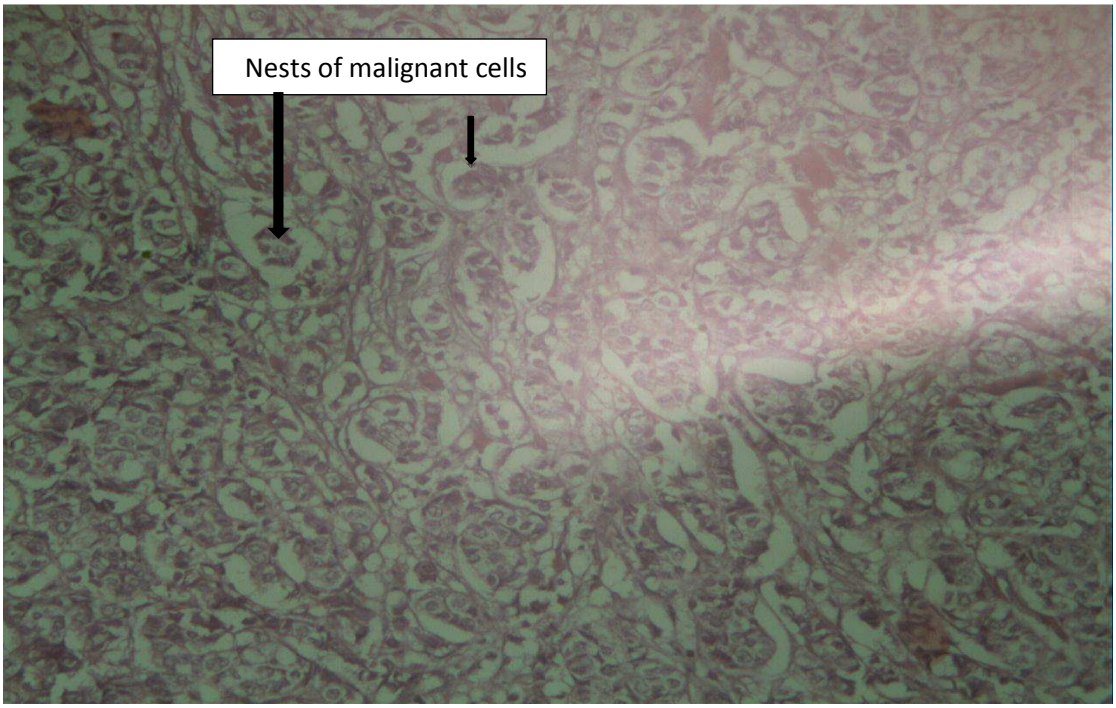


Figure -7: Infilterative ductal carcinoma (H&E) X40, showing nests of malignant cells infilterating the stroma.

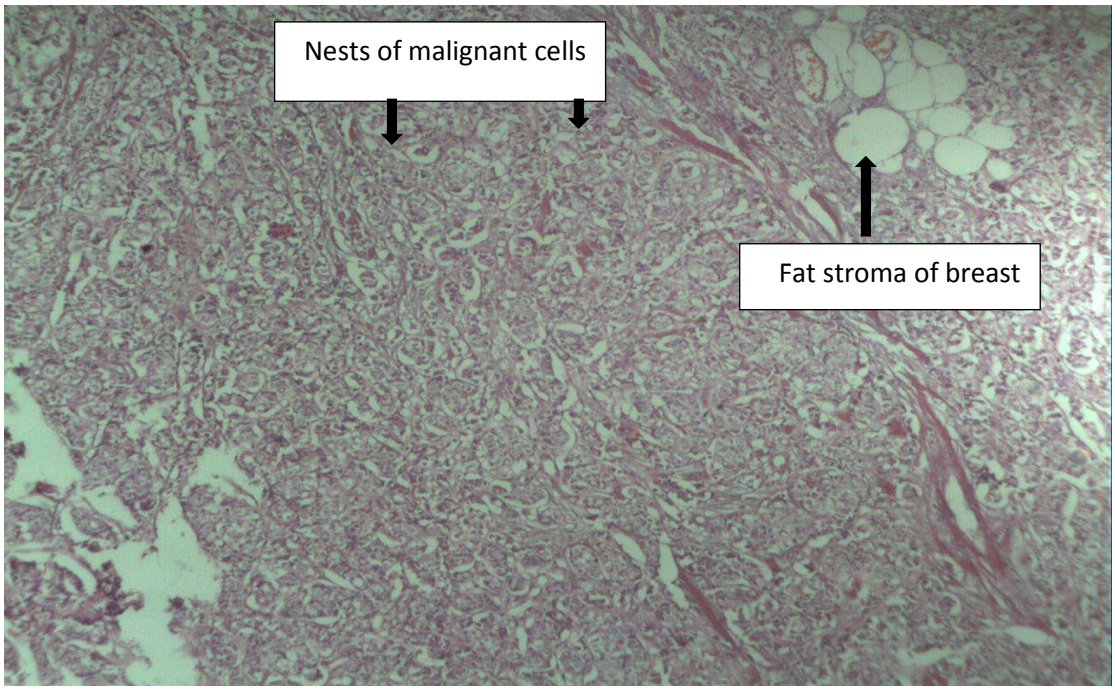


Figure -8: Infilterative ductal carcinoma (H&E)X100 ,nests of malignant cells infilterating fat stroma & connective tissue of the breast.

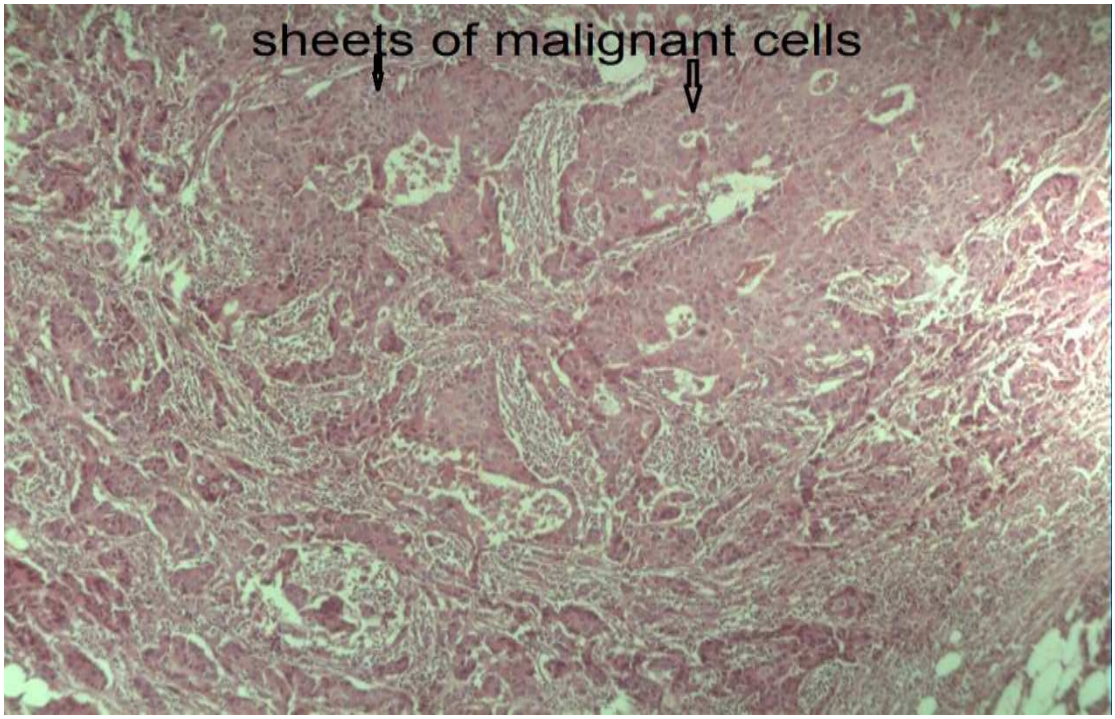


Figure -9: Infilterative ductal carcinoma(H&E) X100 showing sheetes of malignant cells.

In the present study, the prevalence of BRCA1/2 gene mutation carriage rate was investigated among Kurdish women using conventional PCR technique and direct nucleated sequencing. The results of our investigation showed that from 50 breast cancer patient, 8 / 50 deleterious mutation has negative effect breast cancer 9 / 50 non sense mutation.

Table -1: Mutation found in exon 2 & 20 for BRCA1 and exon 11 for BRCA2 in ethnic Kurdish population.

Disease	Gene	Exon	Nucleotide change	Amino Acid change	Mutation type	Interpretation
Breast Cancer	***BRCA1***	***2***	**185del AG**	**Glu23Val**	**Truncated protein**	**Pathogenic**
Breast Cancer	***BRCA1***	***20***	**5382ins C**	**1829 stop**	**frameshift**	**Pathogenic**
Breast Cancer	***BRCA2***	***11/1***	**T→C**	**His743HIS**	**Silent substitution**	**Non pathogenic**
Breast Cancer	***BRCA2***	***11/2***	**A→G**	**Lys1132Lys**	**Silent substitution**	**Non pathogenic**
Breast Cancer	***BRCA2***	***11/2***	**A→ G**	**Asn991 Asp**	**Missense**	**Pathogenic**
Breast Cancer	***BRCA2***	***11/3***	**T→C**	**Val1269Val**	**Silent substitution**	**Non pathogenic**
Breast Cancer	***BRCA2***	***11/5***	**C→T**	**Thr1915Met**	**Missense**	**Pathogenic**

Recent studies have detected the 185delAG mutation in non-Jewish individuals in populations of other countries. Figure (10) shows the lanes 1WT, 2WT, 4WT, 6WT, 335bp ASPCR product were detected due to the presence of wild type alleles in these samples. In the lane 5MT the 354bp amplified PCR products were detected (presence of 185delAG mutation this sample). In Lane 5WT 335bp fragment not detected may be due to hemizygous (one allele is missing). In lanes 3WT and 3MT neither 335bp nor 354bp ASPCR product was detected. Hence DNA was not amplified in these two lanes because all germline mutations in the BRCA1 and BRCA2 genes are expected to be occured in heterozygotes since mutant homozygotes are lethal [40].

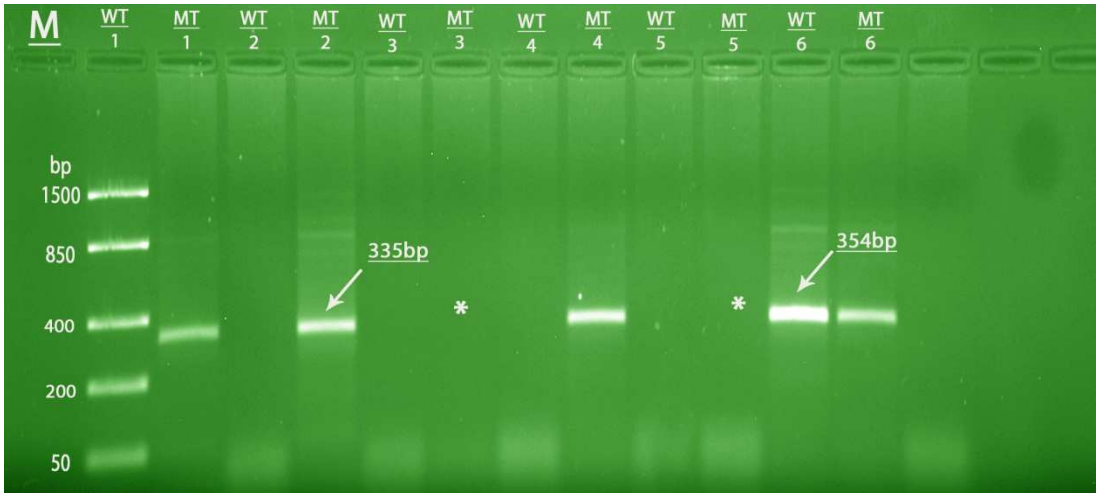


Figure -10: Amplification Products of BRCA1 Exon 2; including wild-type alleles with 335bp by specific primers (p_1, p_2) with DNA samples and mutant type alleles 354bp by (p_1, p_3) with DNA sample 5 in Group C. Electrophoresis was performed on 2% agarose gel and run with 5-8V/cm, for 2 hours. Lane M contained DNA molecular marker (1500-50 bp). Asterisks represent that DNA was not amplifiable [41].

Figure (11) shows that two samples (1 and 2) are heterozygous for this type of mutation, the four first lanes represent the 2 sample; lanes 1WT and 2WT (wild type specific amplification, 271bp fragment and 1MT and 2MT (mutant-specific amplification 295bp fragment), other two samples 3 and 4 lanes 271bp fragment detected in lanes 3WT and 4WT, but in lanes 3MT and 4MT 295bp fragments not detected due to absence of mutation in these samples.

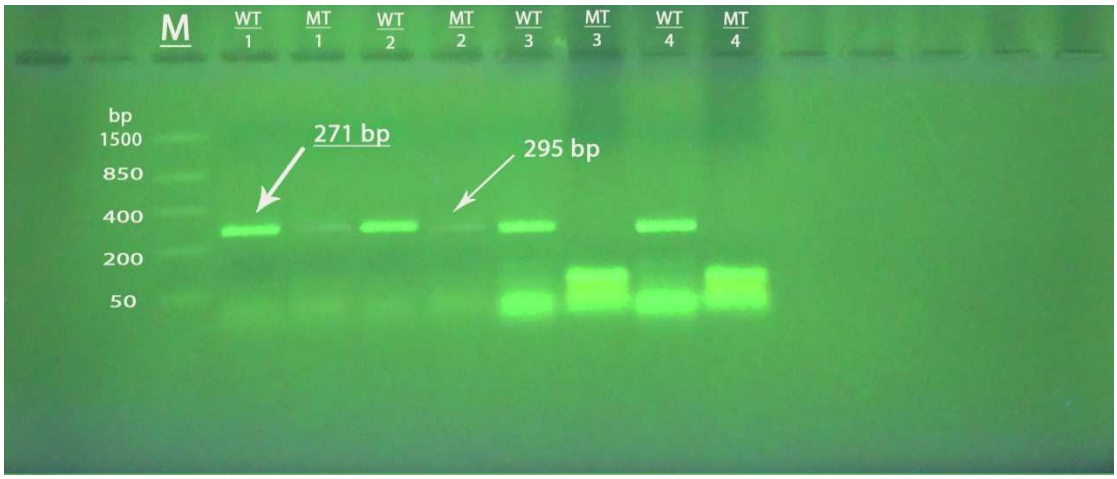


Figure -11: Amplification Products of BRCA1 Exon 20; including wild-type alleles with 271bp by wild-type specific primers (p_4, p_5) with DNA samples and mutant type alleles 295bp by mutant-type specific primers (p_4, p_6) with DNA samples in Group C. Electrophoresis was performed on 2% agarose gel and run with 5-8V/cm, for 2 hours. Lane M contained DNA molecular marker (1500-50bp) [41].

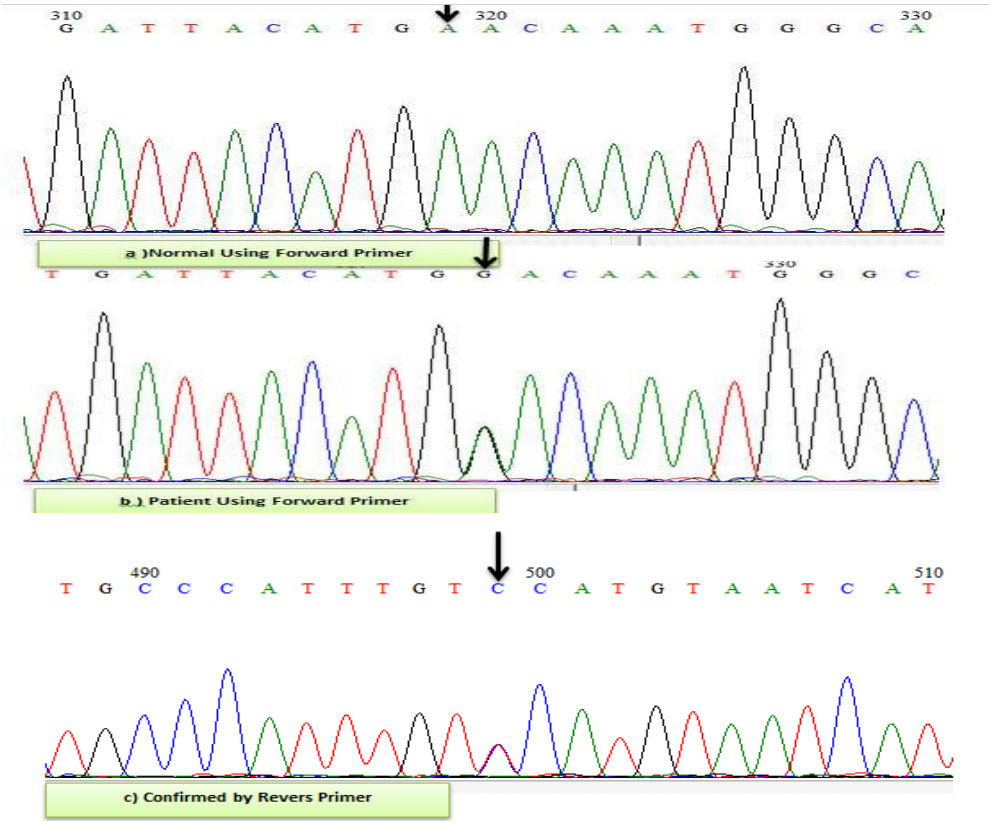


Figure -12: Partial chromatograms representing the sequence in BRCA2 Exon 11 (part 2) profile, showing base substitution A→ G in codon AAC at position 991 (shown by arrow) a) Normal sequence using Forward primer b) Heterozygote patient sequence using Forward Primer show the position of base substitution A to G c) confirmed by Revers primer.

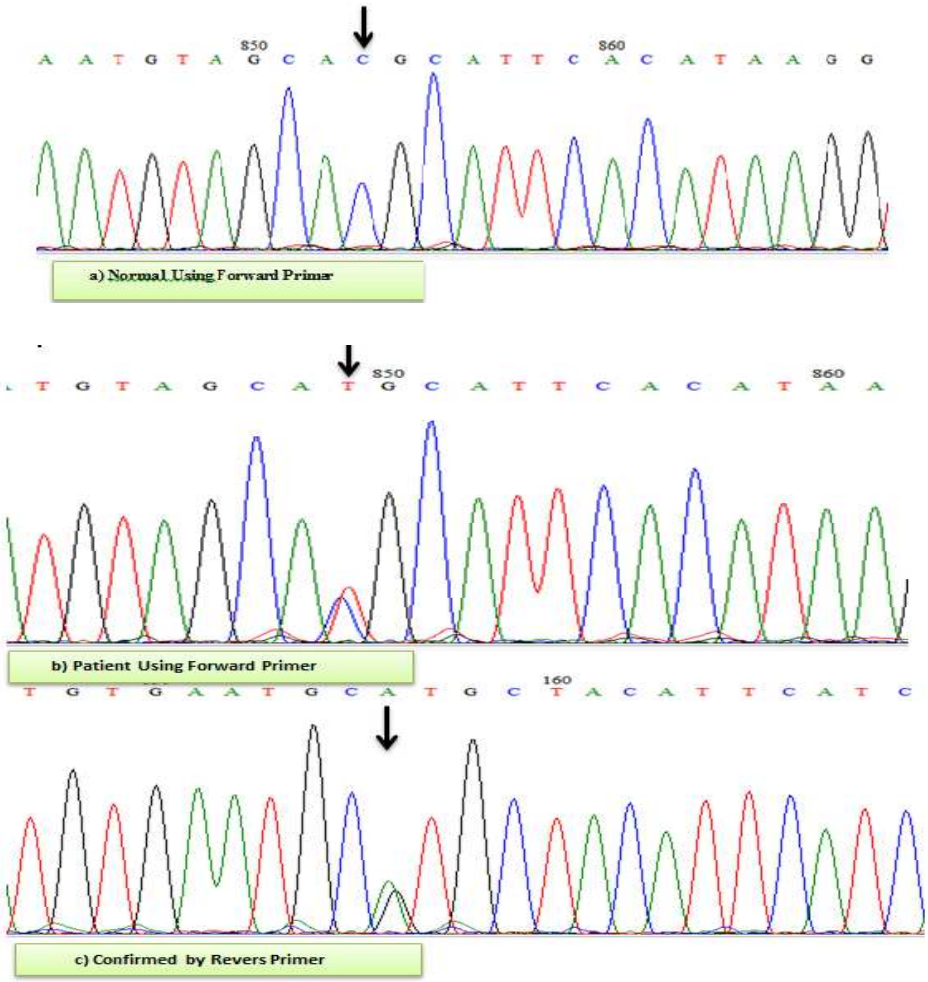


Figure -13: Partial chromatograms representing the sequence in BRCA2 Exon 11 (part 5) profile, showing base substitution C→ T in codon ACG at position 1915 (shown by arrow) a) Normal sequence using Forward primer b) Heterozygote patient sequence using Forward Primer show the position of base substitution C to T. c) Confirmed by Reverse primer.

[42] concluded that one in 500 women carries both a CHEK2 mutation and the T1915M missense variant in BRCA2. According to the results from [43] study these women may face a risk of breast cancer that is almost seven-fold greater than average.

The BRCA2 T1915M variant is located in exon 11, coding for the domain that interacts with RAD51 and this interaction is necessary for the acti-vation of the BRCA2-mediated homology-directed repair [44]. A wide variation in the BRCA1 and BRCA2 mutation spectrum and frequency has been reported for different population [45].

Mutation and grade

The result of this experiment that showed there is positive relationship between mutation and grade of breast cancer disease as shown in Table (2) from 8 deleterious mutation 15.3% (5, 9.6%) were in grade III followed by grade II (3, 5.7%) (Figure 15).In this study, the most important markers that distinguished BRCA1 carrier tumors from familial non-BRCA1/2 tumors were the earlier age of diagnosis and higher grade. This results was agreed with studies reported in Iraq and in China by [46], these studies also reported that young age, family history and a prevalence of more aggressive tumors were common in BRCA carrier.

Table -2: The relation between BRCA mutations and grade of tumors in studied breast cancer patients.

Crosstab

			Grade			Total
			I	II	III	
G. Age	<= 40	Count	1	8	6	15
		% of Total	2.0 %	16.0%	12.0%	30.0%
	> 40	Count	0	29	6	35
		% of Total	0.0%	58.0%	12.0%	70.0%
Total		Count	1	37	12	50
		% of Total	2.0%	74.0%	24.0%	100.0%

Recent molecular studies of breast cancer have demonstrated the importance of the genetic makeup of tumors and showed associations between molecular portrait of breast cancer and tumor biologic and clinical behavior [47]. Furthermore, they have demonstrated that histologic grade, when compared to LN stage and tumor size, represents the predominant contributor to the molecular makeup of breast cancer [48], they showed an association between genetic grade signature of breast cancer and patients' outcome independent of LN status or tumor size [49].

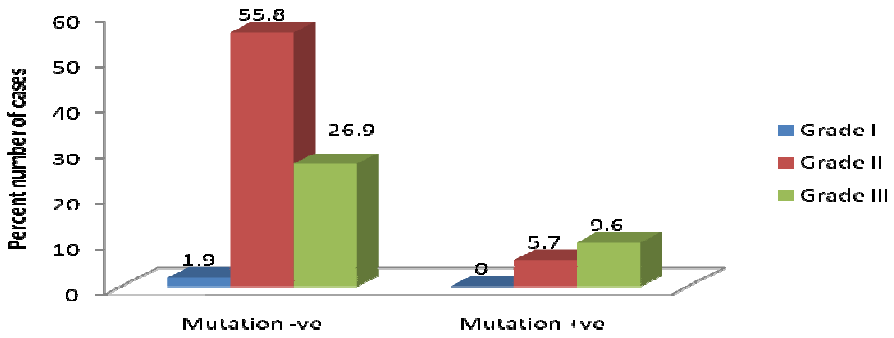


Figure-14: Relation between BRCA mutation and gade of tumors in studied breast cancer patients.

Mutation and stage

The result of our experiment showed (Table 3) that there is no relation between mutations and stage of breast cancer (Figure 15). This result was in agreement with [50] who concluded that patients with BRCA carrier did not have significant higher prevalence of metastasis than those without mutation [51], also noted that there is no significant differences between BRCA carrier and non-carrier according to the stage and disease.

Table -3: Relation between Mutation and Stage.

Crosstab

			Stage				Total
			I	II	III	IV	
Mutations	<= 40	Count	1	6	7	1	15
		% of Total	7.7%	12.0%	14.0%	2.0%	30.0%
	> 40	Count	4	20	8	3	35
		% of Total	8.0%	40.0%	16.0%	6.0%	70.0%
Total		Count	5	26	15	4	50
		% of Total	10.0%	52.0%	30.0%	6.0%	100.0%

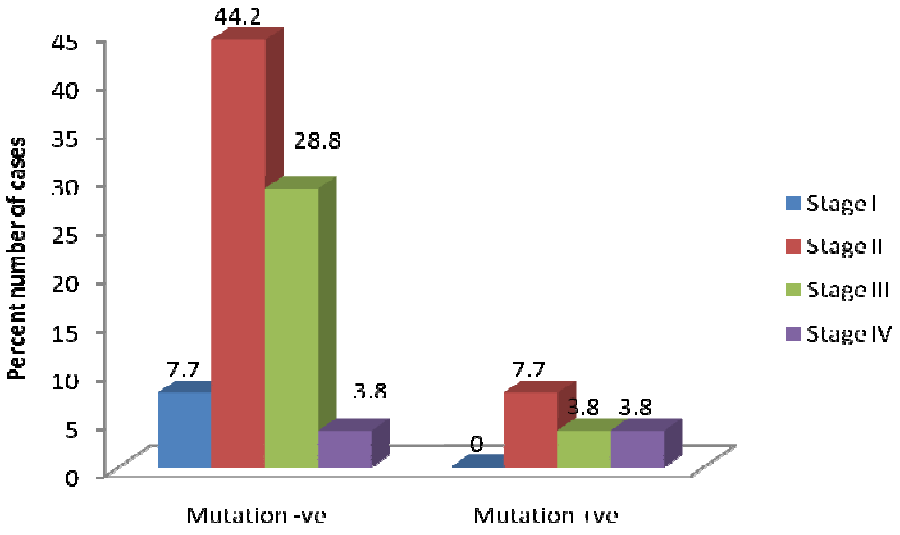


Figure -15: Relation between BRCA mutation and the stage of tumors in studied breast cancer patients.

Mutation and family history

Strong relation between mutations and family history was observed as illustrated in Table (4), among eight out of fifty patients 15% with deleterious mutation (6, 11.5%) was positive for family history (Figure 16).

Table -4: Relation between BRCA mutations and family history of studied breast cancer patients.

Crosstab

			familly history		
			-ve	+ve	Total
Mutations	<= 40	Count	7	8	15
		% of Total	14.0%	16.0%	30.0%
	> 40	Count	25	10	35
		% of Total	50.0%	20.0%	70.0%
Total		Count	32	18	50
		% of Total	64.0%	36.0%	100.0%

This result was in agreement with [52] who concluded that higher percentage of breast cancer incidence was among familial breast cancer females compared to non-familial breast cancer [53, 54] also found that germline BRCA2 mutation frequency has been observed to be higher in patients with cancer family history than in patients without cancer family history.

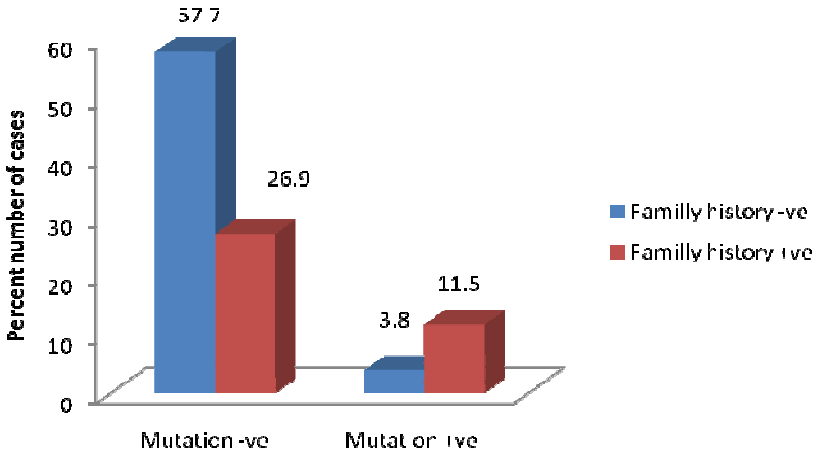


Figure -16: Relation between BRCA mutation and family history of studied breast cancer patients.

Conclusion

We can conclude that it would be advantageous to combine the result of clinical histopathological characteristic with result of molecular techniques (PCR & Direct nucleotide sequencing) in detecting hereditary breast cancer to avoid false results. In this study hereditary breast account for 9% of breast cancer cases, grade and family history have strong relation with mutation in BRCA carrier. Also we concluded that the peak age frequency of breast cancer occurred between ages (35-45years).

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