A study of The Frequency and Type of Mutation in Exon 11 in BRCA1 and BRCA2 Genes In Breast Cancer Women With Positive Family History

Noor Jassim M(Msc)[1], **Ammar Ahmed Sultan(**PhD)[2] **and Fhadil Ahmed Dalaf**(F.I.BMS)[3]

Abstract

Background: Both BRCA1 and BRCA2 are tumor suppressor gene and are inherited as an autosomal dominant. The cumulative lifetime risk of developing invasive breast cancer for those with BRCA1 or BRCA2 mutations ranges from 53% to 89%. Familial breast cancer represent less than 10% of all cancers of the breast, and cancers related to BRCA1 and BRCA2 familial disease account only for three-fourths to two-thirds of these cases. In women younger than 35 year old with CA breast, 10% to 15% have a BRCA1 mutation. Females with mutations involving BRCA 1/2 who are already affected by breast cancer have an increased risk of breast cancer involving the other breast of 52% and 66%.

Objective: To identify the frequency and the type of mutation in exon 11 in the genes BRCA1 and BRCA2 in breast cancer women with positive family history.

Patients and Methods: This is a prospective study of fourteen females having breast cancer with positive family history of breast cancer. The study done in Baquba Teaching Hospital over a period of eight months (October 2016-July 2017). The age range of the patients was 40-70 years. Genomic DNA was extracted from lymphocytes yielded from the peripheral blood using the salting out procedure. Primers were used to amplify exon 11 region of the genes BRCA1 and BRCA2 by using polymerase chain reaction (PCR) cycling.

Results: A total of eight variants in the BRCA1 gene and four variants in the BRCA2 gene were seen. Only one deleterious germline mutation in BRCA1 was detected in 1/14 (7.14%). The patient with deleterious mutation was 31 year-old and was having strong family history of the disease (two relatives, first and second degree). The sequence variant of the mutation was c.795_789delTT with an effect as p.Val256-Ser261ValLys. The remaining 11 identified variants belonging to BRCA1 and BRCA2) are classified as polymorphisms or unknown variants.

Conclusion:BRCA1 and BRCA2 mutations in females with breast cancer with positive family history of the disease is never low and cannot be neglected. Therefore screening for these mutations is important for strict follow up of those with positive results.

Key words: Breast cancer, BRCA 1, BRCA2.

Corresponding Author: noor.gassim88@gmail.com.

Received: 24th September 2017
Accepted: 14th November 2017
https://doi.org/10.26505/DJM.

[1,2] Biology Department-College of Education for Pure Science University of Diyala- Diyala- Iraq.
[3]Baquba Teaching Hospital- Diyala- Iraq.

Introduction

Carcinoma of the breast is the commonest cancer affecting women [1]. It represents phenotypically and heterogeneous so complex disease, with many biological sub-types that is presenting specific responses to therapy [2]. Despite the pathophysiology of breast carcinogenesis is not yet fully understood, many risk factors have been mentioned, including those inducing direct DNA damage [3]. Two known genes shown to be involved in the development of breast cancer when they undergo mutations; these are BRCA1 and BRCA2 which are tumour suppressor gene. Mutation occurring in these two genes increase the susceptibility of developing breast CA in up to 85%. Also, these genes have been shown as being causative factor in breast cancer with family history [4, 5].

It is known that BRCA genes have an important role in DNA repair, regulation of transcription in response to damaged DNA and control of cell cycle. A recent studies suggest that BRCA proteins are needed for protecting the genome against damage [6].

A mutations which is deleterious in either of these two genes, causing protein dysfunction, was established to increase women's risk for having breast cancer over their life (average 70 years). Studies revealed a cumulative risk for having breast cancer for mutation carriers of about 57 for BRCA1 and 47% for BRCA2 mutation carriers [7-9].

The numbers and spectrum of mutations occurring in BRCA1 or BRCA2 genes differ widely among different populations. In some ethnic groups or some geographically selected groups, founder mutations can explain large number of inherited breast and ovarian cancer cases [10-13].

Most of breast cancers are sporadic, meaning occurring in females with out family history of breast malignancy. About 16-21% of CA breast are associated with family history of breast CA, but there is no evidence of autosomal transmission. Studies reported only 5-10% attribution to germline mutation in BRCA1 and BRCA2. Cancers resulted from mutation of the above mentioned genes are shown to be transmitted as an autosomal dominant fashion [14].

BRCA1 or BRCA2 genes have been shown to be the cause of about 45% breast cancer susceptibility syndromes (breast cancer and/or ovarian cancer) that are transmitted as a dominant autosomal trait, which the later account for approximately 40% of the cases in families with both early onset breast cancer and ovarian cancer [15, 16].

A hundreds of different types of mutations in BRCA1 and BRCA2 genes identified, some of these mutations are said to be harmful, and others have no proven impact. Harmful mutations may result in a hereditary breast-ovarian cancer syndrome in persons who are affected[17].Females with harmful mutations in BRCA1 or BRCA2 have an increased risk of developing breast cancer

that is about four to five times the normal risk, and an increased risk of cancer of the ovaries reaching up to eleven to thirty times normal [18].

Therefore, not all mutations are high-risk mutations; some seen to be harmless types. The risk of having cancer that is associated with any given mutation differ significantly and is depending on the exact location and type of the mutation in addition to possibly other individual factors. There are different variations that is occurring in BRCA genes; not all changes have the same risks. Some of these variants are harmless and other variants are known to be very harmful. In other cases screened, whether the variant is harmful, it is unknown. Variants are classified as follows: [19].

Deleterious mutation: Here the changes are proved to cause significant risks.

Suspected to be deleterious: Here, nothing proved. The changes is believed to be harmful.

Variant of uncertain significance: The changes have uncertain effect and this is a common result of the test. These are re-classified into:

Variant which favor polymorphism: Nothing is proved, the changes are currently believed to be harmless.Benign polymorphism: Here the changes are classified as harmless and may be said as having no mutation.

We should know that deleterious mutations have high, but not complete, genetic penetrance. This means that females with the mutation have a high risk of developing breast cancer and others will not develop cancer in spite of carrying the harmful mutation.

Patients and Methods

This is a cross sectional study of fourteen females having breast cancer with positive family history of breast cancer ranging from first degree to third degree relatives. The study done in Baquba Teaching Hospital over a period of eight months (October 2016-July 2017). The age range of the patients was 30-70 years.

The methodology was as follow: [20].

Blood samples (5 ml each) were collected from the patients (11 women) in EDTA tubes.

Isolation of DNA

Genomic DNA extracted from lymphocytes in the peripheral blood using the standard salting out procedure [20].

DNA extraction: This is achieved by using extraction tools (genomic DNA mini kit blood) by taking 200µl blood in tubes capacity 1.5 Eppendorf and then 20µl proteinase K added. The tubes then placed in water bath for five minutes at 60C°. After that, a 200µl GSB (GeneFix Blood-Prep) material added and the tubes taken back to the water bath for another five minutes with continuous shaking every minute. Then 200µL absolute alcohol has been added and the resulting contents of the tubes was replaced in Genomic DNA column tubes which then put in the centrifuge to be centrifuged at 1400 rpm for one minute. After that, washing done of the tube contents using two solution which are pre-wash/protein-binding buffers (W1) and

Wash buffer W2 (ethanol added), then the tubes centrifuged again at 1400 rpm for one minute. Finally, 50µl Elution solution added and again centrifuged at 1400 rpm for one minute. Then the sedimented DNA that look transparent at the bottom of the tube extracted.

Electrophoresis of extracted DNA: 450ml of distal water put in 500ml scaled glass beaker followed by adding 50ml of TBE (Tris/Borate/EDTA) Buffer solution. After that, 100ml of the resulted mixture aspirated and added to one gram of agarose and mixed well using Hutplate at 100°C until it completely dissolved. The resulted gel left to cool. Then ethidium bromide added. After that, the gel put in a suitable basin. Then holes made in the gel for the DNA specimens extracted and left until completely solid. 5µl is taken from DNA extracted and mixed with 6µl loading dye and put in the gel holes which are immersed in the space found in the electrophoresis device. Then, the electrodes attached to the gel holes containing the extracted DNA, then we can see the band of electrophoresed DNA on the device screen. Figures (1, 2).

Genotyping

Primers which are universal used to amplify all regions of the genes BRCA2 and BRCA1 prepared. The genomic DNA was amplified by PCR (polymerase chain reaction) with initial denaturation at 96°C for 10 minutes. This is followed by 30 rounds of 95°C for 20 seconds. Exons 11 of the BRCA1 and BRCA2 gene was analyzed. Nine fragments of the BRCA1 exon 11 were separated, and for the BRCA2 exon 11, thirteen fragments were separated. Reaction products then purified with Sephadex G50 which then electrophoresed on an agarose gels to 2%. After that, the purified DNA used in the sequencing reaction (PRISM Ready Kit Reaction, Applied Biosystems) who has the product deposited on polyacrylamide gel which is denaturing to 6%. The migration to be revealed then performed in an automatic sequencer. The sequence analysis was performed using SEQMAN (DNAstar, Madison, WI) and SEQSCAPE V2.5 (Applied Biosystems) software.

Results

Fourteen patients with family history of breast cancer with an age range of onset of the disease from 30 to 70 (median age 50 years) were analyzed for the presence of hereditary breast cancer related to mutation in exon 11 in the genes BRCA1 and BRCA2. Thirteen cases were diagnosed with one sided breast cancer and one case with bilateral breast cancer (1).

Table (1): Distribution of breast cancer patients with regard to their age at onset of the disease and their relatives with breast cancer.

Patient	Age	First degree relatives n(age)	Second or Third degree relatives n(age)	Comments/other cancers
1	45	1(43)	0	-
2	47	0	1(55)	-
3	41	1(40)	1(43)	One bilateral breast cancer
4	47	1(34)	1(43)	-
5	52	0	1(45)	-
6	44	0	1(55)	-
7	44	0	1(65)	-
8	49	1(60)	0	-
9	54	0	1(70)	-
10	66	1(56)	0	-
11	69	0	1(55)	-

Analysis of the mutation of the exon 11 in BRCA1 and 2 was performed in those fourteen cases. A total of eight variants in the BRCA1 gene and four variants in the BRCA2 gene were seen.

Only onedeleterious germline mutation in BRCA1was detected (7.14%) familial cases.The patient with deleterious mutation was 31 year-old and was having strong family history of the disease (two relatives, first and second degree). The sequence variant of the mutation was c.795_789delTT with an effect as p.Val256-Ser261ValLys. The remaining eleven identified variants belonging to BRCA1 and BRCA2) are classified as polymorphisms or unknown variants.

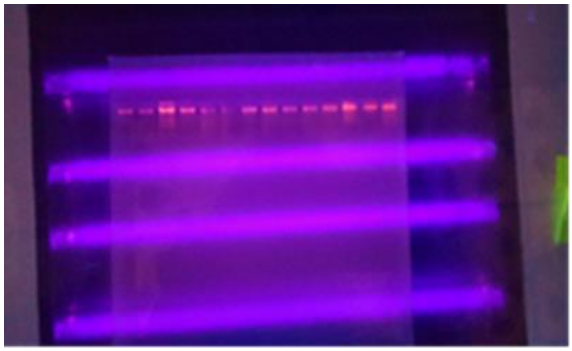

Figure (1): DNA phase bundles on the agarose gel.

Figure (2): Connecting DNA band with Primer designed.

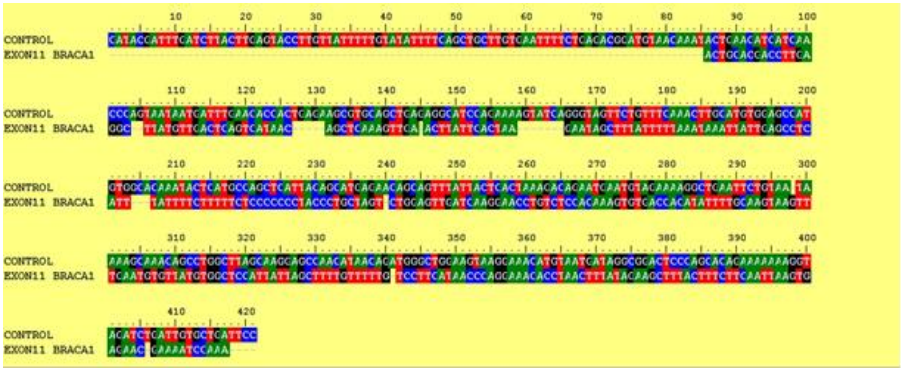

Figure (3): Shows the deleterious mutation in exon 11 in BRCA1

Discussion

The BRCA1 gene is a large gene and is consisting of 22 coding exons responsible for generating a protein composed of 1863 amino acids. Exon 11 encode about 61% of the entire BRCA1 gene [21].

The study revealed one BRCA1 deleterious germline mutations in patient with a strong positive family history of breast cancer (7.14%). The germ line mutation was affecting exon 11. This is in agreement with a study done by Mahfoudh et al. who reported a significant mutation affecting more than 78% of exon 11 in 6.32% of Tunisian females affected by breast cancer with family history of the disease. Sequence variant of exon 11 mutated was c.798_799delTT [22]. In this study the sequence variant mutated was c.795_789delTT. The Tunisian mutation variant was also observed in two Algerian families but the rate of mutation affecting Algerian families was 18% [23].

Another study in Belgium by G Goelen et al. where 42 families with both breast and ovarian cancer were screened for mutations in both BRCA1 and BRCA2. The result of the study was that 14 patients out of 41 families (34.1%) were having mutation; 10 mutations were found in the BRCA1 gene [2 (20%) affecting exon 11] and four in the BRCA2 gene which indicate raised possibility of finding a BRCA gene

mutation in patients with ovarian cancer in addition to the breast cancer. Exon 11 of the gene BRCA1 was screened by protein truncation test. The mutation affecting it was 3780GtoT [24].

In the current study, no patient was having both breast and ovarian cancer. This is explained by the short period of the study which result in small sample size (only fourteen cases with positive family history).

The above findings in the different studies indicate that the prevalence of BRCA1 mutation and the mutation type might vary among different populations which may result from different environmental factors and genetic backgrounds. This means that the origin of the patient might significantly account for the different mutation frequency and type in the genes tested.

Conclusions

BRCA1 and BRCA2 mutations in females with breast cancer with positive family history of the disease is never low and cannot be neglected. Therefore Screening for these mutations is important for strict follow up of those with positive results. Females with mutated BRCA1 or BRCA2 is liable for developing another cancer in the body or cancer involving the other breast.

The absence of deleterious mutations in the BRCA2 in the current study can be explained by the small sample size.

Recommendations

The sample size need to be larger, and also should include a cases with both breast and ovarian cancer.Negative results for the presence of deleterious mutation in exon 11 in the remaining eleven cases with breast cancer for both BRCA1 and BRCA2 shouldn't exclude the potential risk for having mutation in other exons other than exon 11 and the tested patients should be informed for that. Therefore another exons need to be tested for the presence of mutation.

References

[1]Marcus JN, Watson P, Page DL, Narod SA, Lenoir GM, et al. (1996) Hereditary breast cancer: pathobiology, prognosis, and BRCA1and BRCA2 gene linkage. Cancer 77(4): 697-709.

[2]Banin-Hirata BK, Losi-Guembarovski R, Maeda Oda JM, Coral de Oliveira CE, Campos CZ, et al. (2015) CCR2-V64I genetic polymorphism: a possible involvement in HER2+ breast cancer. Clin Exp Med.

[3]Shadrina AS, Ermolenko NA, Boyarskikh UA, Sinkina TV, Lazarev AF, et al. (2014) Polymorphisms in DNA repair genes and breast cancer risk in Russian population: a case–control study. Clin Exp Med.

[4]Wooster R, Weber BL (2003) Breast and ovarian cancer. New Eng J Med 348(23): 2339-2347.

[5]Chen J, Silver P, Walpita D, Cantor B, Gazdar F, et al. (1998) Stable interaction between the products of the BRCA1 and BRCA2 tumor suppressor genes in mitotic and meiotic cells. Mol Cell 2(3): 317- 328.

[6]Yoshida K, Miki Y (2004) Role of BRCA1 and BRCA2 as regulators of DNA repair, transcription, and cell cycle in

response to DNA damage. Cancer Sci 95(11): 866-871.

[7]Chen S, Parmigiani G (2007) Meta-analysis of BRCA1 and BRCA2 penetrance. J Clin Oncol 25(11): 1329-1333.

[8]Troudi W, Uhrhammer N, Sibille C, Dahan C, Mahfoudh W, et al. (2007) Contribution of the BRCA1 and BRCA2 mutations to breast cancer in Tunisia. J Hum Genet 52(11): 915-920.

[9]Mahfoudh W, Bouaouina N, Ben Ahmed S, Gabbouj S, Shan J, et al. (2012) Hereditary breast cancer in Middle Eastern and North African (MENA) populations: identification of novel, recurrent and founder BRCA1 mutations in the Tunisian population. Mol Biol Rep 39(2): 1037-1046.

[10]McClain MR, Nathanson KL, Palomaki GE, Haddow JE (2005) An evaluation of BRCA1 and BRCA2 founder mutations penetrance estimates for breast cancer among Ashkenazi Jewish women. Genet Med 7(1): 34-39.

[11]Kadouri L, Hubert A, Rotenberg Y, Hamburger T, Sagi M, et al. (2007) Cancer risks in carriers of the BRCA1/2 Ashkenazi founder mutations. J Med Genet 44(7): 467-471.

[12]Sokolenko AP, Mitiushkina NV, Buslov KG, Bit-Sava EM, Iyevleva AG, et al. (2006) High frequency of BRCA1 5382insC mutation in Russian breast cancer patients. Eur J Cancer 42(10): 1380-1384.

[13] Antoniou AC, Pharoah PD, Narod S, Risch HA, Eyfjord JE, et al. (2005) Breast and ovarian cancer risks to carriers of the BRCA1 5382insC and 185delAG and BRCA2 6174delT mutations: a combined analysis of 22 population based studies. J Med Genet 42(7): 602-603.

[14]Claus EB, Schildkraut JM, Thompson WD, Risch NJ (1996) The genetic attributable risk of breast and ovarian cancer. Cancer 77, 2318-2324.

[15] Wooster R, Weber BL (2003) Breast and ovarian cancer. N Engl J Med 348, 2339-2347.

[16]Hall JM, Lee MK, Newman B, Morrow JE, Anderson LA, Huey B, King MC (1990) Linkage of early breast cancer to chromosome 17q21. Science 250, 1684-1689.

[17]Holly Yan (2013-05-14). "What's the gene that led to Angelina Jolie's double mastectomy?" Health. CNN.

[18]Jeffrey N. Weitzel, Veronica I. Lagos, Carey A. Cullinane, Patricia J. Gambol, Julie O. Culver, Kathleen R. Blazer, Melanie R. Palomares, Katrina J. Lowstuter, Deborah J. MacDonald (2007). "Limited Family Structure and BRCA Gene Mutation Status in Single Cases of Breast Cancer". *Journal of the American Medical Association*. 297 (23): 2587–2595. PMID 17579227. doi:10.1001/jama.297.23.2587.

[19] Morris, Joi L.; Gordon, Ora K. (2010). Positive Results: Making the Best Decisions When You're at High Risk for Breast or Ovarian Cancer. Amherst, N.Y.: Prometheus Books. ISBN 978-1-59102-776-8.

[20]Parzer, S. and C. Mannhalter. 1991. A rapid method for the isolation of genomic

DNA from citrated whole blood. Biochem. J. 273(Pt1):229-231.

[21]Breast Cancer information Core (BIC) database. 5 October 2002. U S.A.

[22]Mahfoudh W, Bouaouina N, Ben Ahmed S,Gabbouj S, Shan J, et al. (2012) Hereditary breast cancer in Middle Eastern and North African (MENA) populations: identification of novel, recurrent and founder BRCA1 mutations in the Tunisian population. Mol Biol Rep 39(2): 1037-1046. [23]Uhrhammer N, Abdelouaheb A, Lafarge L, Feillel V, Ben Dib A, *et al.* (2008) BRCA1 mutations in Algerian breast cancer patients: high frequency in young, sporadic cases. Int J Med Sci 5(4): 197-202.

[24]G Goelen, E Teugels, M Bonduelle, B Neyns, J De Grève High frequency of BRCA1/2 germline mutations in 42 Belgian families with a small number of symptomatic subjects J Med Genet 1999;36:304–308.