Genotyping of Exon 2 BRCA1for Breast Cancer in Iraqi Women by Sanger Sequencing

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Abstract

Background: Sporadic, familial and hereditary kinds account for approximately (70-75%), (10-15%) and (five-10%) of all breast cancers respectively. Although numerous chance factors predispose to hereditary breast cancer, the maximum mighty is mutations within the breast cancer susceptibility genes, BRCA1.An important example for tumor suppressor genes are BRCA1 .

Material and methods: We examined the frequency of the BRCA1 Exon 2 in 80Iraqi breast cancer (BC) patients, and 60 controls and then the previously genotyped variants in ages between 25 to 45 and also 45 to 70,who attended the Oncology Hospital in the City of Medicine during the period from June 2018 to April 2019, The products of PCR from amplification of BRCA gene analyzed by PCR-sequencing method.

Result: Our study refer to the genotyping of BRCA1 that distributed to (GG and TT genotyping while TG equal zero.). A significant change was observed in the comparison between the newly diagnosis group and the control group. T allele was associated with 3.0 fold higher risk for cancer than the G allele (OR=2.38; p=0.041). In BRCA-1 Polymorphism, the odd ratio for the mutant GG genotype was 2.12 with p=0.0141 indicating that Patients with an allele GG genotype a higher risk factor of newly diagnosis group compared to patients who carry allele TT genotype and control group. Also BRCA have a positive correlation with age and BMI in newly and treatment group also with some biomarkers.

Conclusion:SNP variants and different genotypes of BRCA1 have important roles in the development of cancer. Their application on such patients would discriminate those with high susceptibility to DNA damage from those with low susceptibility. This is specially applied for the GG and TT variants of BRAC1 Exon 2.

Key words: *breast cancer , BRCA1 gene , sanger sequencing, polymorphism.*

Introduction

Breast cancer is a multi-factorial ailment with contributions from both genetic and environmental elements. Worldwide, it's far the most typical malignancy in girls accounting for 23% of all cancers [1][2]. In Sri Lanka, breast cancer in ladies diagnosed at a mean age of 50 years, approximately 27% of all woman cancers [3]. Sporadic, familial and hereditary kinds account for approximately (70-75%), (10-15%) and (five-10%) of all breast cancers respectively. Although numerous chance factors predispose to hereditary breast cancer, the maximum mighty is mutations within the breast cancer susceptibility genes, BRCA1[4].

Molecular profiling of different subtypes of breast cancer has discovered that TNBC subtype is substantially related with basal-like functions with the aid of expressing basal cytokeratin markers like CK-5, [5,6]. Furthermore, subsequent-technology sequencing studies have shown that notably high mutational fee in both BRCA1 and BRCA2 genes and a defective DNA repair mechanisms are hallmarks of a TNBC subtype of breast cancer [7,8,9,10].

An important example for tumor suppressor genes are BRCA1 and BRCA2 genes . though BRCA gene mutations are inherited in an autosomal dominant manner, their expression relies upon on acquiring a 2nd

mutation within the wild kind allele in somatic cells. According to Knudson's ("Two Hit") speculation, each alleles of a tumor suppresser gene need to be mutated so as for a tumor to expand, therefore a patient who manifests a tumor, inherits one mutation from a parent, and develops the second mutation inside the same gene in the affected organ as a somatic mutation, at which factor the tumor starts off evolved to appear. Even though children of mutation companies are at 50% risk of inheriting the mutation, the age of onset in their cancer is hard to are expecting[11,12].

Material and Methods

This study designs as prospective study, One hundred and forty (140) Iraqi women subject were participated in this study in ages between 25 to 45 and also 45 to 70, who attended the Oncology Hospital in the City of Medicine, present studyweredivided into two groups patients with breast cancer (80) blood samples and sixty (60) blood samples are collected from healthy women as a control group.

BRCA-1 gene polymorphisms were genotyped with a PCR method by confronting primers briefly, genotyping was also performed by PCR-sequencing . Genomic DNA was amplified by up strand, 5'- GAA GTT GTC ATT TTA TAA ACC TTT- 3' and down strand, 5'- TGT CTT TTC TTC CCT AGT ATG T- 3' .PCR contained 2µl genomic DNA, 5µl Go Pre Master Mix,1µl of Primer Forward , 1µl of Primer Reverse and 16µl distilled water. Thermal cycling condition for the BRCA1 were: initial denaturation 1 step for 3 minutes at95°C, followed by 1 cycles and {denaturation 2 step for 45 seconds at 95°C , Annealing step for 45 seconds at 56°C and extension 1 step for 45 seconds at 72 °C} followed by 35 cycle. The final extension 2 step was performed at 72°C for 7 minutes. The PCR products (amplicon) were sequencing successfully via Macrogen Corporation/Korea (Sanger sequencing method). Homology search was conducted by using BLAST option, which is available online in NCBI By using BioEditprogramand NCBI, the SNPs were determined. The data were tested for normality, homogeneity and normal distribution. Also, it was expressed as median ± SD, the probability was tested using IBM SPSS version 25.0. While, the genotyping and alleles frequencies were tested through Hardy-Weinberg equilibrium calculator. Such, the Odd ratio and Fisher exacts' probability were tested by using WinPepi version 11.65. The probability was significant when it $p< 0.05$.

Results

The result of this research represented that two grouped (newly diagnosis of breast cancer and control group) showed the compatibility 99% with gene band under number MH043259.1, score 389 and expected 1e-105, Sequencing study of our groups showed a Transversion substitution in location 203 and Nucleotide T>G especially in newly diagnosis group. While control grouped showed no change in sequencing of BRCA1 Exon 2 its remain TT.

Group newly diagnosis breast cancer Figure (1): Matching of the primers sequences on the bioinformatics programs/ NCBI blast sequence were matched by the bioinformatics programs NCBI,

Sequence ID: MH043259.1

Score	Expect	Identities	Gaps	Strand
389 bits(431)	1e-105	217/218(99%)	0/218(0%)	Plus/Plus

Query 190 GAACAGAATTGACCTTACATAC**G**AGGGAAGAAAAGACA 227

||||||||||||||||||||||||||||||||||||||

Sbjct 181 GAACAGAATTGACCTTACATAC**T**AGGGAAGAAAAGACA 218

Group newly diagnosis breast cancer and control group Figure (2): **Homo sapiens clone I103_P-10_EXON2-F_D04 breast cancer 1 (BRCA1) gene, partial cds**

Sequence ID: MH043259.1

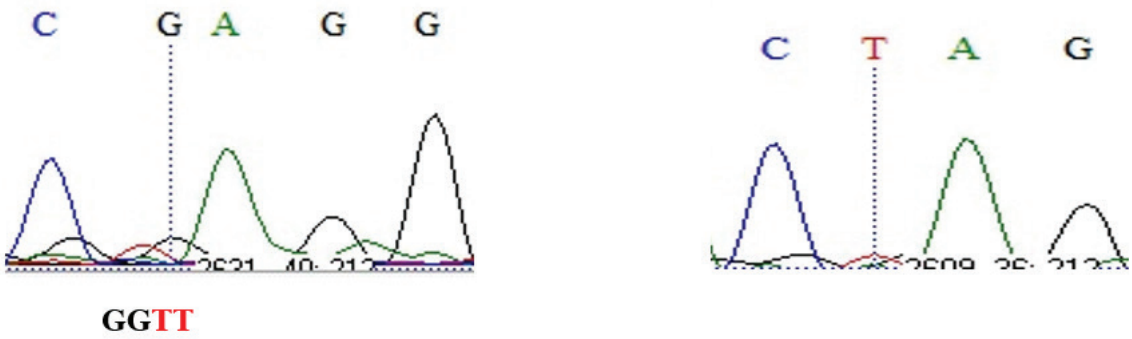
Score	Expect	Identities	Gaps	Strand
374 bits(414)	2e-101	207/207(100%)	0/207(0%)	Plus/Plus

Query 187 GAACAGAATTGACCTTACA**T**ACTAGGG 213

||||||||||||||||||||||||||

Sbjct 181 GAACAGAATTGACCTTACA**T**ACTAGGG

The genotyping by graphic of sequencing Microgen of BRCA1 illustrate below figure and illustrated the GG and TT genotyping while TG equal zero.



The distribution of genotype and allele frequencies among newly diagnosed and control groups is shown in table (1) . The genotypic frequencies of cancer patients were 72% (n=58) normal TT and 0.0 % (n=0) heterozygous TG. Mutant homozygous was found in GG 27% (n=22).In controls, the results demonstrate 77.97% (n=46) wild type TT, 0.0 %(n=0) heterozygous TG and mutant homozygous GG 22.0% (n=13).In BRCA-1 Polymorphism, the odd ratio for the mutant GG genotype was 2.34 with p=0.055 indicating that Patients with an allele GG (homozygous) genotype a higher risk factor of a newly diagnosis group compared to patients who carry allele TT (homozygous) genotype, and control group. Total statistical manual allele frequency. The ratio was allele T for the patients 72%, while allele G 28%, whilst control allele T 78 %, allele G 22 %.A significant change was observed in the comparison between the newly diagnosis group and the control group. T allele was associated with 3.0 fold higher risk for cancer than the T allele (OR=2.38; p=0.041).

Table (1): genotyping and allele frequency of BRCA1 gene between newly diagnosed and control groups.

Genotypes	Hardy-Weinberg frequency								OR	P
	Newly diagnosed				Control					
	Observed		Expected		Observed		Observed			
	No.	%	No.	%	No.	%	No.	%		
TT	58.0	72.50	42.05	52.56	46.0	77.97	35.86	60.79	0.75	0.554
TG	0.0	0.00	31.90	39.88	0	0.00	20.27	34.36	-	-
GG	22.0	27.50	6.05	7.56	13.0	22.03	2.86	4.85	2.34	0.0554
Total	80.0	100.00	80.00	100.00	59.0	100.00	59.00	100.00		
P-HWE	4 x 10-19				2 x 10-14					
Allele frequency										
T	0.72				0.78				2.38	0.041
G	0.28				0.22				0.73	4.414

Our study assessed whether BRCA-1 gene and associated with an increased risk of breast cancer, especially Iraqi women patient with various types of breast cancer (newly malignant diagnosis, and control). The genotyping of BRCA-1genes were classified by PCR- and the results showed a no significant change when comparing control group with newly diagnosis according to risk factor especially in age table (2).

Table 2-: The age median distribution according to the genotyping for BRCA-1 of the studied groups.

	Newly diagnosed 2			P value	Control 3			P value
	TT	TG	GG		TT	TG	GG	
AGE	54.71 ± 10.70a		50.07 ± 15.07a	NS	48.30 ± 10.75b		49.92 ± 8.43a	NS
BMI	33.29 ± 3.95b	-	25.68 ± 6.0a	0.019	27.57 ± 3.67a		23.77 ± 2.83a	0.007⁎⁎

While a positive correlation was found between TT and GG in control group (p value = 0.007) and newly diagnosis (p value = 0.019) while no significant in another group according to BMI.

Discussion

Complete sequencing of the BRCA1 is a favored procedure in hereditary directing of a breast cancer patient's sufferers in various Western European nations, what's more in the US, Australia, and Japan. In any case, these investigations are cost and work expending; thus, it is exceptional that clinicians routinely utilize screening for known harmful germline changes. Under this study, it is critical to distinguish transformations of the best an incentive informative value for diagnostics, just as to build up the methods for their proficient detection our examination refers to a change in BRCA1 and agreement with numerous investigations that the effect of genotype on relative malignancy hazard has been evaluated in a few investigations up to now [13].

This was seen in the frequency of TT genotype and T allele in disease understanding remembered for the present examination in correlation with the control gathering. It is obvious that the polymorphic allele and genotype prevailed in malignant growth patients gathering. It appears that the search for such a polymorphism is a delicate test for scanning for DNA damage by radiation particularly in the heterozygote group GG in the two both patients and radiation groups. In any case, it is specific for malignancy patients whereby both GG and TT genotype were related with significant differences.[[14, 15].

Also contrasting the frequencies of BRCA1-TT genotype in this examination with different investigations, result discovered TT homozygosity by and large much the same as with Nigeria (YRI) (1.8%), Kenya (LWK) (1.1%), Japanese (JPT) (7%), Tuscan (TSI) (11%), (Kinyawa, Kenya) MKK (2.8%) and, (Mexican heritage in Los Angeles) MEX (8.2) (Brewster BL et al.,2012 [15]. This information proposes that the BRCA1- variation G>T presents an expanded danger of creating malignancy in our gathering of patients. One could think from these detection that the BRCA1 -variation works likewise to that of standard BRCA1 open-perusing outline variations, which are all the more usually connected with advancement of the disease rather than the different subtypes. [16].

BRCA-1 gene and related with an expanded danger of disease, particularly Iraqi ladies quiet with different sorts of malignant growth (newly malignant diagnosis and control) The genotyping of BRCA-1genes were characterized by PCR-and the outcomes demonstrated a no noteworthy change when contrasting control group and newly diagnosis as indicated by the risk factor particularly in age, this investigation agreement with many examination [17]. They no found a critical or we found no distinction in mean age among ladies with a BRCA1 transformation in general just as for cases analyzed under age 45. Furthermore, disagreement with [18], as of late studied shown a positive relation of an age on disease in BRCA change transporters analyzed under age 50. In their examination which included 2424 BRCA mutation carrier , Britain with 990 episode malignancies, the auther detailed breast cancer rates as indicated by age bunch at disease analysis [overall (20–49), 20–29, 30–39, 40–49] by age from the outset full-term birth class (nulliparous, < 21, 21–29, 31–39, >39) additionally our investigation disagreement with women who carry the BRCA1 transformation have a more serious danger of creating malignancy before they arrive at 40 years old when contrasted with women having the BRCA2 transformation. In ladies beyond 55 years old years the ladies carry the BRCA2 transformation who stay at higher danger of creating malignant growth while ladies that have the BRCA1 change have a lesser hazard [19].

This examination pointed that a positive connection be tween's a BRCA1 genotyping with BMI especially in newly diagnosis and control group and this outcome agreement with numerous investigations in different populace Ricks-Santi, L. J. 2013 [20]. Because the rate of transformation increase with increment BMI that influenced were related with DNA repair limit in ladies with additionally refer to build danger of BRCA1 transformation with increasing 5Kg in corresponding. This examination demonstrated that the danger of a gene change in BRCA1 expanded by 25% when the weight was expanded to 5 Kg from the ordinary weight limit BMI>25 [20] .

Conclusion :SNP variants and different genotypes of BRCA1 have important roles in the development of cancer. Their application on such patients would discriminate those with high susceptibility to DNA damage from those with low susceptibility. This is specially applied for the GG and TT variants of BRAC1 Exon 2.

Conflict of Interest: There is no conflict of interest among the authors.

Funding: Self

Ethical Clearance: This study is ethically approved by the Institutional ethical Committee.

References

1) Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. International journal of cancer. 2010 Dec 15;127(12):2893-917.
2) Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA: a cancer journal for clinicians. 2005 Mar;55(2):74-108.
3) De Silva W, Karunanayake EH, Tennekoon KH, Allen M, Amarasinghe I, Angunawala P, Ziard MH. Novel sequence variants and a high frequency of recurrent polymorphisms in BRCA1gene in Sri Lankan breast cancer patients and at risk individuals. BMC cancer. 2008 Dec 1;8(1):214.
4) Craig DW, O'Shaughnessy JA, Kiefer JA, Aldrich J, Sinari S, Moses TM, Wong S, Dinh J, Christoforides A, Blum JL, Aitelli CL. Genome and transcriptome sequencing in prospective metastatic triple-negative breast cancer uncovers therapeutic vulnerabilities. Molecular cancer therapeutics. 2013 Jan 1;12(1):104-16.
5) Jonasson JG, Stefansson OA, Johannsson OT, Sigurdsson H, Agnarsson BA, Olafsdottir GH, Alexiusdottir KK, Stefansdottir H, Mitev RM, Olafsdottir K, Olafsdottir K. Oestrogen receptor status, treatment and breast cancer prognosis in Icelandic BRCA2 mutation carriers. British journal of cancer. 2016 Sep;115(7):776-83.
6) Vincent-Salomon A, Macgrogan G, Charaffe-Jauffret E, Jacquemier J, Arnould L. Identification of basal-like carcinomas in clinical practice:" triple zero/BRCA1-like" carcinomas. Bulletin du cancer. 2010 Mar;97(3):357-63.
7) Aksoy A, Odabas H, Kaya S, Bozkurt O, Degirmenci M, Topcu TO, Aytekin A, Arpaci E, Avci N, Pilanci KN, Cinkir HY. Hormone receptor status and survival of medullary breast cancer patients: A Turkish cohort. Saudi medical journal. 2017 Feb;38(2):156.
8) Muendlein A, Rohde BH, Gasser K, Haid A, Rauch S, Kinz E, Drexel H, Hofmann W, Schindler V, Kapoor R, Decker T. Evaluation of BRCA1/2 mutational status among German and Austrian women with triple-negative breast cancer. Journal of cancer research and clinical oncology. 2015 Nov 1;141(11):2005-12.
9) Satagopan JM, Boyd J, Kauff ND, Robson M, Scheuer L, Narod S, Offit K. Ovarian cancer risk in Ashkenazi Jewish carriers of BRCA1 and BRCA2 mutations. Clinical Cancer Research. 2002 Dec 1;8(12):3776-81.
10) Liede A, Narod SA. Hereditary breast and ovarian cancer in Asia: genetic epidemiology of BRCA1 and BRCA2. Human mutation. 2002 Dec;20(6):413-24.
11) Petrucelli N, Daly MB, Pal T. BRCA1-and BRCA2-associated hereditary breast and ovarian cancer. InGeneReviews®[Internet] 2016 Dec 15. University of Washington, Seattle.
12) Tabchy A, Hennessy BT, Gonzalez-Angulo AM, Bernstam FM, Lu Y, Mills GB. Quantitative proteomic analysis in breast cancer. Drugs of today (Barcelona, Spain: 1998). 2011 Feb;47(2):169-82.
13) Nakamura S, Kwong A, Kim SW, Iau P, Patmasiriwat P, Dofitas R, Aryandono T, Hu Z, Huang CS, Ginsburg O, Rashid MU. Current status of the management of hereditary breast and ovarian cancer in Asia: first report by the Asian BRCA consortium. Public health genomics. 2016;19(1):53-60.
14) -Alemar B, Herzog J, Netto CB, Artigalás O, Schwartz IV, Bittar CM, Ashton-Prolla P, Weitzel JN. Prevalence of Hispanic BRCA1 and BRCA2 mutations among hereditary breast and ovarian cancer patients from Brazil reveals differences among Latin American populations. Cancer genetics. 2016 Sep 1;209(9):417-22.
15) - Brewster BL, Rossiello F, French JD, Edwards SL, Wong M, Wronski A, Whiley P, Waddell N, Chen X, Bove B, Hopper JL. Identification of fifteen novel germline variants in the BRCA1 3′ UTR reveals a variant in a breast cancer case that introduces a functional miR□103 target site. Human mutation. 2012 Dec;33(12):1665-75.
16) -Holm K, Melum E, Franke A, Karlsen TH. SNPexp-A web tool for calculating and visualizing correlation between HapMap genotypes and gene expression levels. BMC bioinformatics. 2010 Dec 1;11(1):600.

17) Kotsopoulos J, Gronwald J, Lynch HT, Eisen A, Neuhausen SL, Tung N, Ainsworth P, Weitzel JN, Pal T, Foulkes WD, Eng C. Age at first full-term birth and breast cancer risk in BRCA1 and BRCA2 mutation carriers. Breast cancer research and treatment. 2018 Sep 1;171(2):421-6.

18) -Evans DG, Harkness EF, Howel S, Woodward ER, Howell A, Lalloo F. Young age at first pregnancy does protect against early onset breast cancer in BRCA1 and BRCA2 mutation carriers. Breast cancer research and treatment. 2018 Feb 1;167(3):779-85.

19) Al-Mulla F, Bland JM, Serratt D, Miller J, Chu C, Taylor GT. Age-dependent penetrance of different germline mutations in the BRCA1 gene. Journal of clinical pathology. 2009 Apr 1;62(4):350-6.

20) Ricks-Santi LJ, Nie J, Marian C, Ochs-Balcom HM, Trevisan M, Edge SB, Freudenheim JL, Shields PG. BRCA1 polymorphisms and breast cancer epidemiology in the Western New York exposures and breast cancer (WEB) study. Genetic epidemiology. 2013 Jul;37(5):504-11.