Vol 3, No 2, Jun, 2017, P 67-73
DOI, 10.15562/tcp.45

CANCER PRESS
Vol. 3, No. 2, jun, 2017

Original Article

Correlation of Heterozygote risk, Pathological risk and lifetime risk with Clinicopathological Features in Iranian breast cancer patients

Sahar Ardalan[1,2,5], Mahtab Dastpak [3,5], Fatemeh Homayee Shandiz [4], Narjes Forouzanfar [3], Ariane Sadr-Nabavi1[1,2,3*]

ABSTRACT

Breast cancer is a prevalent malignancy among women worldwide and a principle reason of death in Iranian women. In current study, 64 Iranian women diagnosed with breast cancer and classified into four age groups (<35 years, 35–49 years, 50–64 years and >65 years) were analyzed for correlation between heterozygote risk and lifetime risk with clinicopathological features. Nine patients were also investigated for BRCA1 germline mutations. Our results indicated that people with hetrozygosity risk over 30% more likely to infect invasive ductal carcinoma and utilization of Cyrillic software for Iranian family would open new sights towards the prediction, prognosis and mutation detection.

Keywords: Heterozygote Risk, lifetime Risk, BRCA1, Iran

Author Information

1. Medical Genetic Research Center (MGRC), School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
2. Department of Medical Genetics, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
3. Academic Center for Education, Culture, and Research (ACECR)-Khorasan Razavi, Mashhad, Iran
4. Department of Radiation Oncology, Cancer Research Center, Mashhad University of Medical Sciences, Iran
5. These authors contributed equally to this work.

Submitted: 23-04-2017
Accepted: 23-05-2017
Published : 29-06-2017

Breast cancer is a common malignancy comprising 24.4% of all cancers and is the leading cause of death among Iranian women (1, 2). Approximately 5–10% of all breast cancers are attributable to the strong hereditary susceptibility, highly penetrant genes, such as BRCA1/2 (3). Women carrying germ line mutations in these genes have an extremely high lifetime risk of developing breast and/or ovarian cancer (4). Among Carriers of BRCA1/2 mutations, the Lifetime risk of breast cancer is 56% to 84% by age 70 (4-7). Estimation the mutation probability is important for various

References

1. Mousavi SM, Montazeri A, Mohagheghi MA, Jarrahi AM, Harirchi I, Najafi M, et al. Breast cancer in Iran: an epidemiological review. The breast journal. 2007;13(4):383-91.
2. Harirchi I, Kolahdoozan S, Karbakhsh M, Chegini N, Mohseni S, Montazeri A, et al. Twenty years of breast cancer in Iran: downstaging without a formal screening program. Annals of oncology. 2010;22(1):93-7.
3. Claus EB, Schildkraut JM, Thompson WD, Risch NJ. The genetic attributable risk of breast and ovarian cancer. Cancer. 1996;77(11):2318-24.
4. Antoniou A, Pharoah P, Narod S, Risch HA, Eyfjord JE, Hopper J, et al. Average risks of breast and ovarian cancer associated with BRCA1 or BRCA2 mutations detected in case series unselected for family history: a combined analysis of 22 studies. The American Journal of Human Genetics. 2003;72(5):1117-30.

reasons including the cost of genetic testing, the low probability of mutations that concerned patients and the psychosocial reasons(8). Breast cancer risk determination plays an important role to select an appropriate strategy of disease management (9).

To date, correlation of in vitro heterozygote risk and lifetime risk of breast cancer patients with clinicopathologic features have not been described in Iran population. In this research we estimated the probability of carrying of mutations in BRCA1 by risk evaluation program and correlation between heterozygote risk and lifetime risk of breast cancer patients with age, weight, histological type, menopausal status, lymph node status, histological grade, tumor stage, Diabetes status and expression of five immunohistochemical markers (ER, PR, HER-2, P53 and Ki67) investigated.

Materials and methods

Patients: A study was conducted with 64 Iranian women diagnosed with breast cancer who were referred to the Ghaem Hospital of Mashhad University of Medical Sciences between 2010 and 2013. After genetic counseling of index cases with breast cancer and obtaining a written informed consent, demographic information, family-history and hormone receptor status of breast cancer patients were archived to a database. The individual's risk of heterozygosity and the lifetime risk for breast cancer were assessed by Cyrillic 3.1 (an established pedigree drawing program designed for clinical geneticist).

Data Collection: The patients were classified into four age groups: <35 years, 35–49 years, 50–64 years and >65 years. Age of 35 as cut-off point to define young age breast cancer was regarded. A clinical and pathology information such as patient age, weight, histological type, tumor stage, menopausal status, lymph node status, histological grade, ER, PR, HER-2, P53 and Ki67 status were extracted from medical and pathology records.

DNA Extraction and Polymerase Chain Reaction: Genomic DNA was extracted from peripheral whole blood samples (9 breast or ovarian cancer patients were selected for BRCA1 germline mutations analysis) using standard procedure (salting out). All of 24 exons of BRCA1 gene were amplified by PCR using 34 pairs of exon-specific primers.

References

1. Mousavi SM, Montazeri A, Mohagheghi MA, Jarrahi AM, Harirchi I, Najafi M, et al. Breast cancer in Iran: an epidemiological review. The breast journal. 2007;13(4):383-91.
2. Harirchi I, Kolahdoozan S, Karbakhsh M, Chegini N, Mohseni S, Montazeri A, et al. Twenty years of breast cancer in Iran: downstaging without a formal screening program. Annals of oncology. 2010;22(1):93-7.
3. Claus EB, Schildkraut JM, Thompson WD, Risch NJ. The genetic attributable risk of breast and ovarian cancer. Cancer. 1996;77(11):2318-24.
4. Antoniou A, Pharoah P, Narod S, Risch HA, Eyfjord JE, Hopper J, et al. Average risks of breast and ovarian cancer associated with BRCA1 or BRCA2 mutations detected in case series unselected for family history: a combined analysis of 22 studies. The American Journal of Human Genetics. 2003;72(5):1117-30.
5. Ford D, Easton D, Stratton M, Narod S, Goldgar D, Devilee P, et al. Genetic heterogeneity and penetrance analysis of the BRCA1 and BRCA2 genes in breast cancer families. The American Journal of Human Genetics. 1998;62(3):676-89.
6. Struewing JP, Hartge P, Wacholder S, Baker SM, Berlin M, McAdams M, et al. The risk of cancer associated with specific mutations of BRCA1 and BRCA2 among Ashkenazi Jews. New England Journal of Medicine. 1997;336 (20):1401-8.
7. King M-C, Marks JH, Mandell JB. Breast and ovarian cancer risks due to inherited mutations in BRCA1 and BRCA2. Science. 2003;302(5645):643-6.
8. Gomy I, Diz MDPE. Hereditary cancer risk assessment: essential tools for a better approach. Hereditary cancer in clinical practice. 2013;11(1):16.
9. Amir E, Freedman OC, Seruga B, Evans DG. Assessing Women at High Risk of Breast Cancer: A Review of Risk Assessment Models. JNCI: Journal of the National Cancer Institute. 2010;102(10):680-91.

PCR was performed using GenetBio kit with the following program: 2 min at 94°C, 30 s at 94°C, 30 s at 54-64°C, and 1 min at 72°C for 35 cycles in a 25 µl reaction volume.

BRCA1 Sequence Analysis

For mutational analysis, PCR products were sequenced using forward or reverse primers by a commercial sequencing company (Macrogen, Korea) and the results was analyzed using SeqScape® Software Version 2.7. Finally, all detected variants were checked by BIC and HGMD database.

Statistical analysis

Statistical analysis was performed using the SPSS 11.0 statistical package (SPSS, Chicago, IL). All data were expressed as mean ± s.d. and analyzed by t test or ANOVA. P-value $\leq$ 0.05 was considered statistically significant.

Results

Demographic and Clinicopathological Characteristics

In the overall study group, the mean ages ± SD at diagnosis of studied patients was 44.3±10.0 years. 64 patients were diagnosed with unilateral breast cancer that Invasive Ductal Carcinoma (IDC) was the most common form between studied patients and others were comedo carcinoma, infiltrating ductal carcinoma, medullary carcinoma, invasive papillary carcinoma and mucinous carcinoma. Histological grade of the patients were grade 1 (8 cases), grade 2 (27 cases) and grade 3 (18 cases). Tumor stage classification were stage 1 (8 cases), stage II (32 cases), stage III (9 cases) and stage IV (6 cases). Four patients had diabetes at diagnosis and the remaining patients (60 cases) were categorized as non-diabetic.

Table1: Demographic characteristics of 64 patients by age category

	<35yr / n=7	35-49yr / n=36	50-64yr / =18	65+yr / n=3
Weight(mean)	63.78	69.88	74.60	58.00
Unilateral	7(100%)	36(100%)	18(100%)	2(66.7%)
Histology				
Invasive ductal carcinoma	5(71.4%)	32(88.9%)	13(72.2%)	3(100%)
Comedo carcinoma	1(14.3%)	0	2(11.1%)	0
Infiltrating ductal carcinoma	1(14.3%)	3(8.35%)	0	0
Medullary carcinoma	0	0	1(5.6%)	0
Invasive papillary carcinoma	0	0	1(5.6%)	0
Mucinous carcinoma	0	1(2.8%)	1(5.6%)	0
Grade				
I	0	3(8.3%)	4(22.2%)	1(33.3%)
II	3(42.9%)	20(55.6%)	4(22.2%)	0
III	3(42.9%)	9(25%)	4(22.2%)	2(66.7%)
Unknown/not done	1(14.3%)	4(11.1%)	6(33.3%)	0
TNM-stage				
I	1(14.3%)	2(5.6%)	5(27.8%)	0
II	2(28.6%)	21(58.3%)	8(44.4%)	1(33.3%)
III	3(42.9%)	4(11.1%)	1(5.6%)	1(33.3%)
IV	1(14.3%)	4(11.1%)	1(5.6%)	0
Unknown/not done	0	5(13.9%)	3(16.7%)	1(33.3%)
menopausal status				
Premenopausal	7(100%)	33(91.7%)	4(22.2%)	0
Postmenopausal	0	3(8.3%)	14(77.8%)	3(100%)
Diabetes status				
Positive	0	2(5.6%)	2(11.1%)	0
Negative	7(100%)	34(94.4%)	16(88.9%)	3(100%)

We classified the patients based on menopausal status at diagnosis, 44 pa-tients (68.8%) were premenopausal, while 20 patients (31.2%) postmenopausal (table 1).

Risk Assessment

Heterozygote and lifetime risk of breast cancer patients before the age of 85 years were assessed by Cyrillic 3.1. The heterozygote risk was estimated to be 39.81% (<35 yr), 34.21% (35–49 yr), 9.93% (35–49 yr) and 6.00% (>65 yr). The majority of lifetime risk in 10, 20 and 30 years were assigned in age of <35 (table 2). Furthermore, the risk to age 85 years of patients was 43.11% (<35 yr), 33.43% (35–49 yr), 14.74% (35–49 yr) and 13.53% (>65 yr), respectively (table2).

Table2: Risk assessment of 64 patients by age category

	<35yr n=7 N(%)	35-49yr n=36 N(%)	50-64yr n=18 N(%)	65+yr n=3 N(%)
Heterozygote Risk	39.81	34.21	9.93	6.00
10 years risk	7.51	7.90	3.41	2.70
20 years risk	16.97	15.30	6.83	4.30
30 years risk	25.07	21.26	11.71	7.90
Risk to age 85	43.11	33.43	14.74	13.53

Immunohistochemical Characteristics

Expression of five immunohistochemical markers included ER, PR, HER-2, Ki67 and P53 status in tumors were positive in 73.4%, 64.1%, 14.1%, 23.4% and 20.3% and negative in 23.4%, 33%, 78.1%, 23.4% and 25% cases, respectively (table 3).

Table3: Immunohistochemical of 64 patients by age category

	<35yr n=7	35-49yr n=36	50-64yr n=18	65+yr n=3
ER*				
Positive	5(71.4%)	28(77.8%)	12(66.7%)	2(66.7%)
Negative	2(28.6%)	8(22.2%)	5(27.8%)	0
Unknown/not done	0	0	1(5.6%)	1(33.3%)
PR†				
Positive	3(42.9%)	26(72.2%)	11(61.1%)	1(33.3%)
Negative	4(57.1%)	10(27.8%)	6(33.3%)	1(33.3%)
Unknown/not done	0	0	1(5.6%)	1(33.3%)
HER-2				
Positive	2(28.6%)	1(2.8%)	4(22.2%)	2(66.6%)
Negative	5(71.4%)	32(88.9%)	13(72.2%)	0
Unknown/not done	0	3(8.3%)	1(5.6%)	1(33.3%)
Ki67				
Positive	3(42.9%)	8(22.2%)	3(16.7%)	1(33.3%)
Negative	1(14.3%)	9(25.0%)	5(27.8%)	0
Unknown/not done	3(42.9%)	19(52.8%)	10(55.6%)	2(66.7%)
P53				
Positive	2(28.6%)	7(19.4%)	4(22.2%)	0
Negative	2(28.6%)	10(27.8%)	3(16.7%)	1(33.3%)
Unknown/not done	3(42.9%)	19(52.8%)	11(61.1%)	2(66.7%)

* Estrogen receptor, † Progesterone receptor

CANCER PRESS
Vol. 3, No. 2, jun, 2017

Association between the heterozygote and lifetime risk of breast cancer patients with clinicopathological characteristics

We compared heterozygote and lifetime risk between different groups of population and tumor characteristic features. Our results showed a significant difference between heterozygote risk and lifetime risk of different groups of age (P<0.01). The risk at age 85 was also statistically significant between the two groups of premenopausal and postmenopausal breast cancer patients (P<0.001) (table 4). However, there was no statistically significant difference across the age groups between the heterozygote risk and lifetime risk in breast cancer patients and Weight, histological type, histological grade, tumor stage and Diabetes status (P>0.05) (table 4).

Table 4: Analyzing heterozygote risk and lifetime risk between different variables

	Heterozygote risk P-value	10 years risk P-value	20 years risk P-value	30 years risk P-value	Risk to age 85 P-value
Age (<35,35-49,50-65,+65)	0.004*				0.000*
weight	r=-0.182, p=0.156 ⇑	r=-0.150, p=0.244 ⇑	r=-0.195, p=0.132 ⇑	r=-0.227, p=0.095 ⇑	r=-0.233, p=0.068 ⇑
Side	0.552§	0.338§	0.381§	0.384§	0.742§
Histology	0.602*				0.619*
Grade	0.403*	0.587*	0.605*	0.731*	0.252*
Stage	0.426*	0.436*	0.531*	0.600*	0.533*
ER status	0.367§	0.625§	0.453§	0.404§	0.327§
PR status	0.215§	0.402§	0.358§	0.358§	0.191§
HER-2 status	0.060§	0.281§	0.042§	0.161§	0.265§
Ki67	0.800§	0.453§	0.708§	0.687§	0.802§
P53	0.761§	0.779§	0.821§	0.940§	0.753§
pre/postmenopausal	0.250§	0.045§	0.027§	0.315§	0.000§
Diabetes status	0.201§	0.140§	0.216§	0.145§	0.538§

* One-Way ANOVA

§ Independent-Sample T test

⇑correlation test

CANCER PRESS
Vol. 3, No. 2, jun, 2017

BRCA1 Mutation Analysis

According to inclusion criteria and genetic counseling, nine breast or ovarian cancer patients were analyzed for BRCA1 germline mutations analysis. In patients' pedigree, the information about the proband and the relatives with breast and/or ovarian cancer has been demonstrated (Supplementary 1). Different mutations in BRCA1 gene were identified in the patients (table 5).

Table 5: list of all mutations and variants identified in all nine patients

Patient ID	Exon	DNA Mutation*	Amino Acid Change	Type
1	2	IVS1-115T>C	-	PM[1]
	7	7+18delCTT	-7:c.-19-115T>C	PM
	8	IVS7-34T>C	-	PM
		c.3548 A>G	p. Lys1183Arg	UV[2]
		c.2430 T>C	p. Leu1177Ile	PM
	13	c.4427T>C	p. Ser1305Ser	UV
	18	IVS19+66G>A	-	IVS
2	8	IVS8-33T>C	-	PM
	10	IVS10-34T>G	-	PM
	11	c.1067 A>G	p.Gln356Arg	UV
3	7	7+32delCT	-	PM
4	2	IVS1-115 T>C	-	IVS
	9	IVS9- 49 del T	-	IVS
	11	c.2077 G>A	p. Asp693Asn	UV
5	7	7+32del CT		PM
	11	c.3508 A>T	p.Ile1170Asn	UV
6	7	7+18del CTT	-	PM
	16	c.3113 A>G	p.Glu1038Gly	UV
		c.3548 A>G	p.lys1183Arg	UV
		c.4837 A>G	p. Ser1613Gly	UV
		c.4956 G>A	p.Met1652Ile	UV
7	7	7+32delCT	-	PM
		7+18delCTT	-	PM
	8	IVS8-35T>C	-	PM
	11	c.1186 A>G	p.Gln356Arg	UV
		c.1009 T>A	p.Met297Lys	UV
8	7	7+32delPolyT	-	PM
	14	c.4463-4464 (insA)	p.Asn1488	PM
9	7	7+32delCT	-	PM

Discussion

Breast cancer (BC) is one of the common cancer between women in worldwide and the top malignancy in Iranian women over the past few decades (10). It has been reported that the mutation of BRCA1/2 have been found in 2–6% of breast cancer patients (7, 11, 12). BC at a younger age is also related to the disease advanced stage, higher grade, ER negativity and BRCA1 ectopic expression (13). In this study we compared the heterozygote risk and lifetime risk between different groups of our population and tumor characteristic features. 45–80% lifetime risk of breast cancer has been estimated in carriers of BRCA1 and BRCA2 mutations (4, 7). In our results heterozygote risk and lifetime risk had a significant difference between diverse groups of age ($P<0.01$). The risk to age 85 was statistically significant between the two groups of premenopausal and postmenopausal breast cancer patients ($P<0.001$). Meanwhile, there was no statistically significant difference across the age groups between the heterozygote risk and lifetime risk in breast cancer patients and clinicopathological traits ($P>0.05$).

Association between 20 years risk of breast cancer patients and other clinicopathological characteristics was merely statistically significant for expression of HER-2 and other factor (ER, PR, Ki67 and P53) were not significant.

Different studies in Iran demonstrated various alterations of BRCA1 and BRCA2 genes. In our previous study we had introduced a novel mutation in Khorasan papulation (accession number BankIt1473921 JN686490) (14). However, due to the sequencing of nine patients in present study, not only we found out submitted mutation, but also, no novel mutations did not determine in BRCA1 gene exons.

Conclusion

Our data recommended which utilization of Cyrillic software for Iranian family would open new sights towards the prediction, prognosis and mutation detection. People with heterozygote risk over 30% are more likely to be infect invasive ductal carcinoma and are a good candidate for the BRCA1 and BRCA2 gene mutations. So far, for Iranian Breast Cancer affected Families, the Cyrillic is appropriate Software for the prediction of genetic diagnostic for BRCA1 and BRCA2. However, sample size and limitations of medical records of patients are two major limitations should be considered in future studies.

References

10. Rafiemanesh H, Rajaei-Behbahani N, Khani Y, Hosseini S. Incidence trend and epidemiology of common cancers in the center of Iran. Global journal of health science. 2016;8(3):146.
11. Malone KE, Daling JR, Doody DR, Hsu L, Bernstein L, Coates RJ, et al. Prevalence and predictors of BRCA1 and BRCA2 mutations in a population-based study of breast cancer in white and black American women ages 35 to 64 years. Cancer research. 2006;66(16):8297-308.
12. McClain MR, Palomaki GE, Nathanson KL, Haddow JE. Adjusting the estimated proportion of breast cancer cases associated with BRCA1 and BRCA2 mutations: public health implications. Genetics in Medicine. 2005;7(1):28-33.
13. Zhang Q, Zhang Q, Cong H, Zhang X. The ectopic expression of BRCA1 is associated with genesis, progression, and prognosis of breast cancer in young patients. Diagnostic pathology. 2012;7(1):181.
14. Sadr□Nabavi A, Dastpak M, Homaei□Shandiz F, Bahrami AR, Bidkhori HR, Raeesolmohaddeseen M. Analysis of novel mutations in BRCA1 in Iranian families with breast cancer. hereditas. 2014;151(2-3):38-42.