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Role of BRCA1 and BRCA2 Gene Mutation in Clinical and Histological Aspect of Breast Carcinoma- An Observational Study.

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Abstract

Background: Breast is one of the commonest sites of malignancy in women. Together BRCA1 and BRCA2 account for nearly all high risk families with breast and ovarian cancer. The present study was undertaken with the aim of screening of breast carcinoma patients for BRCA1 and BRCA2 gene mutation and to study nuclear grades in aspirated cytology smears and histological sections in carcinoma breast. **Subjects and Methods:** This study was carried out in the Department of pathology and General Surgery in a tertiary care centre , over a period of one year. Proper examination was done and the diagnosis of carcinoma breast was then confirmed by fine needle aspiration cytology (FNAC) needle biopsy or incisional/excisional biopsy. 20 cc of whole blood was taken in EDTA vial from the patients and preserved in liquid nitrogen at – 190 degree celsius. The blood samples obtained from patients were used for mutation detection. **Results & Conclusion:** Most of the cases in BRCA mutant group had high histological grade. No male case was found in BRCA1 mutant group whereas in BRCA2 mutant group. Out of 4 cases, one was male patient. In BRCA2 mutant group only mis-sense type of mutation was found whereas in BRCA1 group frame shift mutation, non-sense mutation and mis-sense mutation found.

Keywords: BRCA1, BRCA2, Mutation, Breast carcinoma.

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Introduction

Breast is one of the commonest sites of malignancy in women. Its incidence shows a wide variation throughout the world with a low Asian incidence. Breast cancer is uncommon in women under age of 30 years and thereafter goes up the age increases.[1]

Incidence rates are highest among Parsi women reaching 2.1, 1.7 and 1.4 times the rates reported among Hindu, Muslim and Christian women respectively. The last decade has seen tremendous advances in our knowledge of special genes which confer increased susceptibility to breast cancer.[2]

Two major genes conferring hereditary predisposition to breast and ovarian cancer have recently been identified. The BRCAI gene is located on chromosome 17 (Hall et al, 1999) and encodes a large protein of 1863 amino-acids which is thought to play an as yet unidentified role in the maintenance of genomic integrity. Cloning of BRCAI gene in 1994 has led to identification of nearly 300 distinct genetic alterations that confer a high risk of disease (BIC Database).[3]

Together BRCA1 and BRCA2 account for nearly all high risk families with breast and ovarian cancer (i.e. three or more cases among first degree relatives and approximately 60% of families with site specific female breast cancer. Among individuals who are genetically predisposed to breast cancer generally represent 5-10% of all breast cancers, in certain population with strong founder effects, or which have undergone population bottlenecks, the frequency of specific mutations have been found to be significantly higher.[4]

This study would focus on the identification of BRCA1 and BRCA2 mutations found specifically in Indian women with breast cancer. It is anticipated that both mutations unique to specific ethnic groups and those which occur in diverse population worldwide will be identified.

The various prognostic factors in breast cancer are tumor size, tumor type, tumor grade, cell kinetics, aneuploidy (DNA index and S fraction), lymph node status, lymphatic or vascular invasion, tumor staging and hormone receptor status (ER/PR). Of these the nuclear grading is a cytopathological parameter that can be easily performed and is reproducible. Combined with histologic tumor type it is a powerful predictor of tumor aggressiveness.[5-7]

With these considerations, the present study was undertaken with the following aims:

1. Screening of breast carcinoma patients for BRCA1 and BRCA2 gene mutation.
2. To study nuclear grades in aspirated cytology smears and histological sactions in carcinoma breast.

Subjects and Methods

This study was carried out in the Department of pathology and General Surgery in a tertiary care Centre, over a period of one year. The study group included 46 patients of breast cancer and 30 subjects of comparable age with no malignant disease were included as controls.

Criteria for Selection of Patients

1. Any patient with early onset breast cancer diagnosed under the age of 45 years or late onset cancer diagnosed after the age of 45 years.
2. Any patient with breast cancer (any age) having previous or subsequent overian cancer.
3. Any patient with breast cancer (any age) having family history previous of breast or overian cancer in the first degree relatives (mother-daughter, sister-sister).
4. Any patient with breast cancer (any age) having a male member in the family with breast cancer.
5. Any male patient with cancer having family history of breast or overian cancer in first and/or second degree relatives.

The patients were then subjected to a thorough clinical examination with special relevance to stage of tumor by finding out:

1. Tumor size, skin involvement, fixity to skin or underlying structures and inflammatory signs.
2. Involvement of nipple.
3. Palpability and fixity of axillary lymph nodes
4. Status of supraclavicular lymph nodes
5. Examination of abdomen, respiratory and skeletal system.

The diagnosis of carcinoma breast was then confirmed by fine needle aspiration cytology (FNAC) needle biopsy or incisional/excisional biopsy.
The tumor specimen and axillary lymph nodes removed after surgery was subjected to histopathological examination for:

1. Confirmation of diagnosis, tumor size
2. Confirmation of nodal status
3. Histological grading according to classification of bloom and Richardson.

20h cc of whole blood was taken in EDTA vial from the patients and preserved in liquid nitrogen at – 190 degree celcius. The blood samples obtained from patients were used for mutation detection by following methods:

1. Isolation of genomic DNA from peripheral blood lymphocytes using established phenol chloroform extraction procedure.
2. PCR amplification of genomic DNA to screen the entire coding regions of the BRCA1 and BRCA2 genes using exon specific primers.
3. Heteroduplex analysis (HAD) of coding sequences and splice junctions of both are BRCA1 and BRCA2 genes. Electrophoretic mobility changes introduced by sequence alterations in PCR fragments amplified from genomic DNA enabled detection of mutations by this method.
4. DNA sequencing: The exact sequence alterations, detected by the screening methods, were determine using conventional DNA sequencing. Thus it was possible to determine whether a specific genetic variant was a disease associated mutation, a sequence variant of unknown functional significance or a neutral polymorphism.

Results

Table 1: Sex distribution of cases

Distribution of cases	No.	Female	Male
Total cases	46	41 (89.13%)	5 (10.86%)
Cases with +ve family history	8	8 (100.0%)	0 (0%)
Early onset (age < 45 years)	27	27 (100.0%)	0 (0%)
Late onset (age > 45 years)	11	6 (54.54%)	5 (45.45%)

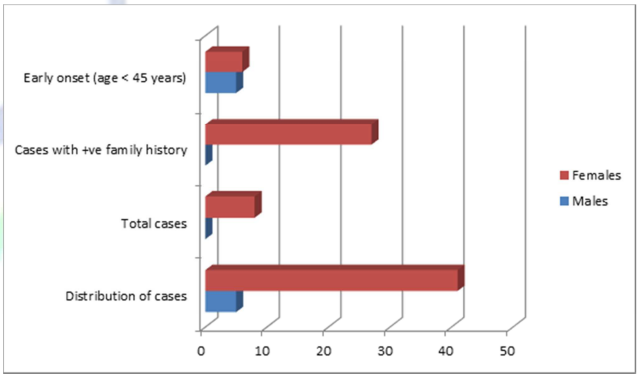

Figure 1: Sex distribution of cases.

Among the familial cases and early onset sporadic breast cancer cases no male patient was present, whereas cases with late onset sporadic breast cancer had five male patients (45.45%). Overall male to female ratio was 1:8.
Table 2 shows that out of 46 cases, majority (82.60%) were nonfamilial and 127.39%) were familial. In both the categories, most common variety was infiltrating duct carcinoma followed by carcinoma breast not otherwise specified. Among the familial group most of the cases were of grade II and grade III whereas in familial group most of the cases were of grade I [Table 1, Figure 1].

Table 2: Morphological Patterns and Grade among Familial and Nonfamilial Cases

	Total	Morphological Pattern	Number of Cases	Grade		
				I	II	III
Familial cases	8 (17.39%)	Infiltrating duct carcinoma	3 (37.5%)	-	2	1
		CA Breast not otherwise specified	2 (25.0%)	2	-	-
		Medullary carcinoma	1 (12.5%)	-	-	-
		Adenocarcinoma	1 (12.5%)	-	-	-

		Malignant fibrous histiocytoma	1 (12.5%)	-	-	1
Nonfamilial	38	Infiltrating duct carcinoma	25(65.78%)	8	7	8
cases	(82.60%)	Ca Breast – not otherwise specified	7 (18.42%)	4	3	-
		Medullary carcinoma	2 (5.26%)	1	-	1
		Malignant phylloid tumour	1 (2.63%)	-	1	-
		Mucinous carcinoma	1 (2.63%)	-	1	1
		Mixed ductal and lobular carcinoma	1 (2.63%)	1	-	-
		Bronchogenic carcinoma (Locally invasive)	1 (2.63%)	-	-	1

Table 3: BRCA 1 Gene Mutation in the Study Group

EXON	No. of Patients			Type of Mutation	Codon	Detail
	ID No.	Age Yrs	Sex			
11	L-4	32	F	Frame shift mutation	655	208- ins →After 672
11	L-21	55	F	Non-sense	826	Cys 826 Glyceine Stop Codon 29
16	L-14	32	F	Mis-sense	1637	Prolone 1637 Leu
13	L-27	37	F	Splice	4476+2T>C	Aberrant splicing
1	L-35	45	F	Splice		Aberrant Splicing
7				Polymorphism frequent		

In this study after complete analysis of BRCA1 genes by DNA sequencing, 5 cases (L-4, L-21, L-14, L-21 and L-35) were found to have mutation in BRCA1 gene (10.86% of total breast cancer cases). All the cases were female. 3 cases belonged to early onset age group. In one case there was frame shift mutation, involving the codon 655. In one case there was non-sense mutation, involving the codon 826 and in one case there was mis-sense mutation involving the codon 1637.

The rest of the two cases mutations were of splice junction type, resulting in aberrant splicing of BRCA1 transcript [Table2,3].

Table 4: BRCA 2 gene mutation in the study group

ID No.	Age Yrs	Sex	Gene	Exon	Sequence of alteration in DNA	Effect on protein	Type of Mutation
L-26	70	F	BRCA2	18	8345 A>G	D2707S	Mis-sense
L-42	26	F	BRCA2	18	8345 A>G	D2707S	Mis-sense
L-43	48	M	BRCA2	11	5007 A>C	E1593D	Mis-sense
L-8	35	F	BRCA2	11	5007 A>C	E1593D	Mis-sense

After complete analysis of BRCA2 genes by DNA sequencing in all the 46 cases, four cases (L-26, L-42, L-43, L-8) were found to have mutation in BRCA2 gene (8.6% of total breast cancer cases).

Among these cases three were female and one was male. One case (L-26) had positive family history. 2 cases belonged to early onset age group and one male patient belonged >45 years age group. Two cases (L-26, L-42) had A>G 8345 alteration in the exon 18, causing mis-sense alteration and other two cases (L-43, L-8) had A>C 5007 alteration in the exon 11 also causing mis-sense mutation [Table 4].

Comparison of various features among mutant carriers and non-mutant breast cancer cases.

1. BRCA mutant – Total nine cases were found (L-4, L-8, L-14, L-21, L-26, L-27, L-35, L-42 and L-23). Among these, 8 cases were females and one case (L-43) was male, five cases had BRCA1 mutation (L-4.L-14, L-21, L-27 and L-35) and four cases had BRAC2 mutation (L-26, L-42, L-43 and L-48). Out of the nine cases with BRCA mutations 4 cases (L-14, L-21, L-26 and L-35) were from familial group (44.44%) and 4 cases (L-42, L-8, L-4 and L-27) were from early onset group (44.44%) and only one case (L-43) belonged to late onset group (11.11%).
2. Non- mutants – This group included rest 37 cases in which no BRCA was found

As the number of cases among the BRCA mutant group was very less (only 9 cases) no statistical analysis could be done. Only a simple comparison of various features between mutant and non-mutant cases has been done.

Table 5: Comparison Of Histological Tumour Grade

Grade	BRCA mutant (n=9)	BRCA non-mutant (n=37)
I	2 (22.22%)	15 (40.54%)
II	4 (44.44%)	11 (29.72%)
III	3 (33.33%)	11 (29.72%)

Most of the cases among mutant group were grade II and grade III whereas among nonmutant group majority of cases were grade I [Table 5].

Discussion

Breast cancer is the leading cause of death among women worldwide and is the second most common malignancy in Indian women after carcinoma cervix. Genetic predisposition is responsible for 5-10% of all breast cancer and the breast cancer susceptibility genes, BRCA1 and BRCA2, are thought to account for the majority of breast/overian cancer families. And as much as 62% of inherited breast cancer.[8]

BRCA1 is a tumour suppressor gene localized to chromosome 17q21. It has been demonstrated to be involved in 80-90% of the hereditary breast/ overian cancer families. The role of BRCA1 as a negative growth regulator has been suggested by its ability to induce apoptosis. Decreased levels of expression of a functional BRCA1 gene

product in breast cancer may be responsible for the increased resistance of these cells to apoptosis.[9]

About 85% of all alterations are frameshift or non-sense mutations and lead to truncated gene product. These are spread throughout all the coding sequence leading to heterogeneity in the size of the truncated mutant product. The remaining 15% are either mis-sense alterations which affect the cysteine residue within the RING domain or inferred regulatory mutations that lead to the absence of a stable transcript from the mutant allele. BRCA1 is also associated with increased risk of overian cancer, prostate cancer (6%) and colon cancer (8%).[10]

BRCA2 gene is located to chromosome 13q12. It is composed of 27 exons and is present in normal breast epithelial cells and placenta. Like BRCA1, BRCA2 also functions as a transcriptional activator and is also involved in DNA repair. Mutations in BRCA2 most commonly induce protein truncation and cause loss of protein function. Like BRCA1, BRCA2 appears to confer a high risk of early onset breast cancer in females. In contrast to BRCA1, male carriers have a 6% lifetime risk of developing breast cancer and it has been reported that 14% male breast cancers are attributed to BRCA2 mutations. BRCA2 families appear to have a moderate increased incidence of overian cancer.[11]

The study was carried out in 46 patients of breast cancer attending surgery OPD and those admitted to surgical wards of SVBP Hospital, LLRM Medical College, Meerut. In the present study patients were divided into three groups, I.e. familial cases (17.39%) early onset breast cancer cases (58.69%) and late onset breast cancer cases (23.91%).

Among the BRCA mutant cases majority (44.44%) had grade II tumour followed by grade III (33.33%) tumour. This finding is in accordance with the fact that tumours in BRCA mutants have high histological grade.

Recently identified gene BRCA1 and BRCA2 are associated with increased susceptibility to develop breast cancer. In our study we found BRCA1 mutation in five cases (10.86%) among the total breast cancer cases. All the cases were females. Four out of five cases belonged to early onset age group and only one case to late onset age group. Out of five, 3 mutant cases (60.0%) had a positive family history. So this study is in accordance with ford et al (1998) who reported that mutations in BRCA1 gene account for 56-87% (by age 70) of the families with breast cancer incidence. But in1997, couch and found that only 16% of women with breast cancer and a family history of breast cancer, had detectable BRCA1 mutations.[8]

Four cases were found to have mutation in BRCA2 gene. Three were females and belonged to early onset age group (<45 years) and one was a male, belonging to late onset age group.

Two cases involved the exon 18 (A>G at 8345) and two cases involved the exon 11 (A>C at 5007). So all the four cases had mis-sense type of mutation.

Conclusion

The Present study was conducted on patients with breast cancer attending surgery OPD and the patients admitted to surgical wards of SVBP Hospital, LLRM Medical college, Meerut, in collaboration with the division of tumor biology, Institute of Pathology, Indian Council of Medical Research, Safdarjang Hospital, New Delhi, over a period of one year.

Total 9 (19.56%) BRCA mutant cases were found. Of these 8 (88.88%) were females and one was male. 4 cases (44.44%) had a positive family history, 4 cases belonged to early onset age group and one case belonged to late onset age group. In one case there was frame shift mutation, involving the codon 655. In one case there was noon-sense mutation involving the codon 1637. In rest of the 2 cases mutations were of splice junction type, resulting in aberrant splicing of BRCA1 transcript.

Both the mutations in BRCA2 gene (each present in 2 cases) were mis-sense mutation.

The early onset cases had mainly grade I tumour, whereas most of the late onset group and BRCA mutant cases had high histological grade (Grade II and Grade III).

Based on the results, following conclusions were drawn from the study:

1. Most of the cases in BRCA mutant group had high histological grade.
2. No male case was found in BRCA1 mutant group whereas in BRCA2 mutant group. Out of 4 cases, one was male patient.
3. In BRCA2 mutant group only mis-sense type of mutation was found whereas in BRCA1 group frame fhift mutation, non-sense mutation and mis-sense mutation found.

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