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Assessment of Loss of Heterozygosity (LOH) in Breast Cancer Patients of Central Indian Region

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Abstract

Breast cancer is the most common malignancy affecting women worldwide. In India breast cancer is the second most common malignancy found in women. Genetic predisposition for familial early onset of breast cancer accounts for approximately 5-10% o all breast cancer. Germline mutation in two major breast cancer susceptibility genes BRCA1 and BRCA2 contribute to the majority of inherited breast cancers. Beside germline mutation, tumour progression also depends on the loss of a wild type allele. The allelic loss is termed as loss of heterozygosity (LOH). LOH is detected in both sporadic and familial breast cancer patients. Eighty six breast cancer patients were analyzed for loss of heterozygosity (LOH) in BRCA1 at two polymorphic markers region D17S1322 and D17S1323. Our study reports an overall loss of 42.85% in the BRCA1 region in the series of 46 sporadic tumours. BRCA1 loss is seen to an extent of 57.14% in the familial cases. Our studies show that sporadic cases represent a low risk group compared to the familial cases. Thus allele loss is an important molecular event in breast tumerogenesis among Indian population.

Keywords: Breast cancer, BRCA1 ,BRCA2, sporadic, familial.

1. Introduction

Breast cancer is the second most common cancer among women worldwide and leading cause of death (Parkin etal.2005).The first major breast and ovarian cancer susceptibility gene BRCA1 was identified in 1994(Miki etal.1994).BRCA1 (MIM#113705) is a tumour suppressor gene and located on chromosome 17q21.About 5% of breast and 20% of ovarian cancer arises in woman carrying germline mutation in BRCA1and BRCA2 gene. Both these genes are involved in double strand break repair. Mutation in these gene leads to genome instability and lead to breast and ovarian cancer.

Tumours from the affected mutation carriers display a loss of wild type allele of the respective BRCA1/2 gene (Cleson Jansen etal.1995; Osorio etl; 2002)

The allelic loss is termed as loss of heterozygosity (LOH) and is visualized as a complete or partial loss of signal intensity of one of the two corresponding allele in tumour DNA from patients heterozygous for a polymorphic marker. Thus in inherited breast tumours the first allele of gene is inactivated by germline mutation,whereas the second is lost during tumour development.

The aim of our study is to examine the role of BRCA1 gene in both sporadic and familial breast cancer cases.

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2. Materials and Methods

2.1 Patients and Samples

The process of sample collection was done in accordance with the ethical standards of Institutional Ethical Committee; following ICMR guidelines. For the present study about 2-5 ml of blood sample was collected from healthy as well as patients of the studying disease related individuals with their informed written consent. Selection of patients was considered with the criteria as, age of diagnosis, family history and any other personal history. Eighty six patients diagnosed with primary breast cancer who belonged to one of the two following categories were included in this study.

2.1a *Sporadic breast cancer* (*n=46*): Forty six breast cancer patients were selected who does not have any family history of breast cancer.

2.1b *Familial breast cancer(n=40)*:Forty breast cancer patients were selected where one first degree relative or second degree relatives were affected with breast cancer

2.2 DNA isolation and PCR amplification at polymorphic regions

Genomic DNA was isolated from all the blood samples by FTA classic card technique (An alternate and advanced method of DNA isolation) following manufacturer's instructions. Universal primers (table-1) were used to amplify two locus of the BRCA1 gene. The polymerase chain reaction was carried out using 100 ng of DNA, 0.2 mM dNTPs (*Sigma-Aldrich Co.*),1xPCR buffer (*Sigma-Aldrich Co.*), 0.5U of Taq DNA polymerase (*Sigma-Aldrich Co.*) and 10 pmol of each primer (*Sigma-Aldrich Co.*) at final volume of 25µl.The PCR cycling parameters were 94°C for 5min followed by 35 cycles of denaturation at 94°C for 1 min, at annealing temperature of 55° C for 1min and extension at 72° C for 30 sec followed by one cycle of 72° C for 10 min.

Table 1. Microsatellite markers and their characteristics.

Locus	Primer	Sequence	Type	Annealing temperature (°C)	Average product size	No. of alleles	Heterozygosity (%)	Reference
D17S1322	s754	5´-CTA GCC TGG GCAACA AAC GA-3´ 5´-GCA GGA AGC AGGAAT GGA AC-3´	(TTG)15	55	130 bp	7	67	Neuhausen et al 1994
D17S1323	s975	5-´TAG GAG ATG GATTAT TGG TG-3´ 5´-AAG CAA CTT TGCAAT GAG TG-3´	(TG)19	55	155 bp	6	44	Neuhausen et al 1994

2.3 Loss of heterozygosity (LOH) analysis

Fifteen ml of each PCR sample was loaded on a 15% polyacrylamide gel with 3 ml of DNA gel loading dye. The gel was run at a constant voltage of 150 V till the dye front reached the bottom of the plates.

2.4 Silver staining

A rapid silver staining method (Neilan *et al* 1994) was used to detect the bands.The gels were washed in deionized water and fixed in 10% ethanol. They were then shaken in 1% nitric acid and thoroughly rinsed in deionized water. Staining was continued in partial darkness, using a solution of 3% sodium carbonate and 0·05% formaldehyde until the desired band intensity was achieved. Development was stopped in 3% acetic acid and the gel was preserved in a solution of 10% ethanol and 5% glycerol.

3. Results and Discussion

Samples that were heterozygous (informative) for any given locus were analyzed for allele losses. LOH was scored as a significant alteration in the relative allele intensities between the two alleles from the tumour samples compared to those from the normal as shown in the figure.1. The figure represents a typical LOH, showing loss of one constitutive allele in the tumour sample. To calculate the total loss at BRCA1 in each patient category, the numbers of samples informative at one or more BRCA1 locus were noted. The percentage LOH at BRCA1 was then calculated as the per cent of informative tumors giving allele loss at any one of these loci. The results are as follows:

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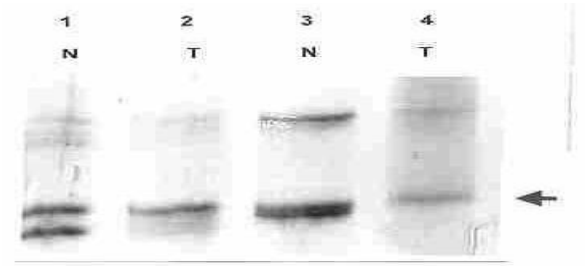

Figure 1. LOH at D17S1322 loci. Normal (N) DNA and tumor (T) DNA from the same individual. Loss of one constitutional allele in tumour DNA is shown by the arrowhead

(i)Sporadic cases: Collectively out of the 46 sporadic cancer patients, 35 were informative at one or more loci and 15 of these (42.85%) presented with LOH at at least one BRCA1 locus.

(ii) *Familial cases*: Collectively out of 40 cancer patients studied, 35 were informative at one or more loci and 20 of these (57.14%) are presented with LOH at BRCA1 locus.

(iii) *Total analysis of all cases*: Out of all 86 cancer patients studied, 70 were informative at one or more loci. Overall, 35 out of these (57.14%) presented with LOH at any one BRCA1 locus. Individual analysis of LOH at each of the three loci in the different patient categories are represented in tables 2-5.

Table-2 : LOH in sporadic cases

Locus	No. of blood sample studied	Number of Non informative cases	Number of informative cases(x)	Cases with LOH(y)	% of samples with LOH(y/x)100
D17S1322	46	10	36	12	33.33%
D17S1323	30	5	25	6	24%

Table-3 LOH in familial cases

Locus	No. of blood sample studied	Number of Non informative cases	Number of informative cases(x)	Cases with LOH(y)	% of samples with LOH(y/x)100
D17S1322	46	20	26	5	19.23%
D17S1323	40	15	25	3	12%

Table 4 Total analysis of LOH in all patients

Locus	No. of blood sample studied	Number of Non informative cases	Number of informative cases(x)	Cases with LOH(y)	% of samples with LOH(y/x)100
D17S1322	92	30	62	17	27.41%
D17S1323	70	20	50	9	18%

Table 5- Summary of LOH analysis

Patient Category	No. of blood sample studied	Number of Non informative cases	Number of informative cases(x)	Cases with LOH(y)	% of samples with LOH(y/x)100
Sporadic	46	11	35	15	42.85%
Familial	40	5	35	20	57.14%
Total	86	16	70	35	50%

BRCA1 is one of the well established breast cancer susceptibility gene, mutated form of which when inherited strongly predisposes to breast cancer. In an attempt to screen the BRCA1 gene in Indian population to establish its role in predisposing population to breast cancer, we have studied total 86 samples of breast cancer patients for microsatellite analysis. Our studies show that sporadic cases represent a low risk group compared to the familial cases. This subdivision of patient samples was done to determine whether the extent of allelic loss at BRCA1 was different in different risk populations. However there a higher rate of BRCA1 loss in the familial cases compared to that seen in the sporadic cases. This suggests that the pathogenesis of breast cancer related to BRCA1 is the same irrespective of risk of developing the disease. Allele losses previously reported in studies on sporadic tumours

have ranged from 21–59% (Futreal et al.,1994) with a high value of 79% LOH reported at THRA1 (Futreal et al., 1992). Another study reported an LOH of 50%, using two intragenic and one extragenic sequence tagged repeats (STRs) (Futreal et al., 1994). A total LOH of 32% had been previously observed in a series of sporadic tumours at the intragenic BRCA1 locus, D17S855 (Dillon et al., 1997). Other study over a span of four years have revealed that 16–45% of breast-ovarian cancer families have BRCA1 mutations (Easton et al., 1993; Couch et al., 1997). Our study report an overall loss of 42.85% in the BRCA1 region in the series of 46 sporadic tumours.BRCA1 loss is seen to an extent of 57.14% in the familial cases. Our estimates may be considered as the "minimum estimates" on BRCA1 LOH analysis, since our study has included only two BRCA1 markers.

After analysis of LOH in microsatellite marker study the samples were shown to have allele loss at BRCA1, confirming its role in molecular pathogenesis of breast cancer. Thus allele loss is an important molecular event in breast tumerogenesis among Indian population

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