PRINT: ISSN 0972-3757 ONLINE: 2456-6360
Int J Hum Genet, 13(1): 1-6 (2013)
DOI: 10.31901/24566330.2013/13.01.01

Chromosomal Aberrations in Breast Cancer Patients in West Bengal, India

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KEYWORDS Breast Cancer; BRCA; Chromosomal Aberrations; Karyotyping. West Bengal

ABSTRACT Breast cancer is the most frequent malignancy among women. Since genetic factors such as BRCA1 and BRCA2 as well as reproductive history constitute 30% of the cause, environmental exposure may play a significant role in the development of breast cancer. Likewise, the relevant enzymes involved in the biotransformation of xenobiotics (from tobacco smoke, diet or other environmental sources) might play a role in breast carcinogenesis. We have carried out cytogenetic studies, using the G-banding technique in peripheral blood lymphocytes of 10 patients affected by breast carcinoma. A variety of chromosomal aberrations including aneuploidy, polyploidy, cluster of cells, acrocenteric associations, chromosomal breaks and gaps were seen in the peripheral blood leucocytes of the patients. The frequency of aberrant metaphases varied from 5% to 69% in cultured leucocytes. The frequency of aneuploidy in sporadic breast cancer was 43% to 69% and in hereditary breast cancer was 65%to 68 %(P= 0.25). The frequency of other aberrations like polyploidy, cluster of cells formation and acrocentric association was significantly found in hereditary breast cancer compared to sporadic breast cancer. Random chromosomal breaks and gaps was found significantly in hereditary breast cancer ($p<0.0001$).Chromosomal aberrations were not seen in controls. Mitotic index in breast cancer was higher than in controls ($P = 0.0038$). The present study lays the emphasis on initiating karyotyping as screening recommendations for detecting early age of onset in Hereditary Breast Cancer (HBC) because molecular analysis is a tedious and very costly technique.

INTRODUCTION

Cancer research in the past 20 years has generated a rich and complex body of knowledge, revealing cancer to be a disease involving dynamic changes in the genome. The genomes of tumour cells are invariably altered at multiple sites, having suffered disruption through lesions as subtle as point mutations and as obvious as changes in chromosome. The early notion that cancer was caused by mutations in genes critical for the control of cell growth implied that genome stability is important for preventing oncogenesis (Charles.Burnicardi 2004). Genomic instability in the peripheral blood lymphocytes has been correlated with tumour progression. Various reports indicate a significant increase in the chromosomal aberrations in peripheral blood lymphocytes of cancer patients with solid tumours. PBLs of patient with breast cancer and other solid tumours show simple chromosomal lesions that may be a stable marker in cancer cells. Hence, it is proposed that lymphocyte may be used as a surrogate tissue model for studying genomic instability in case of solid tumours and the frequency of chromosomal aberrations in PBLs can be used as a predictor of cancer risk (Harsimaran et al. 2009). Breast Carcinoma is among the most common and lethal malignancies in women. Currently, India reports roughly 1,00,000 cases annually (Bagchi 2008). As against as estimated 48,710 women who died of breast cancer in 2007, the number breached the 50,000 mark in 2010. The figure for the year 2011 was put at 50,821 (Sinha 2011). In the present study, our aims were to 1) To detect chromosomal aberrations (CAs) in Breast Cancer patients and compare it with controls (healthy individuals). 2) To compare chromosomal aberrations found in Sporadic Breast Cancer (SBC) and Hereditary Breast Cancer (HBC) and its key role in early breast cancer detection, prevention and treatment.

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MATERIAL AND METHODS

The present study was carried out as a single centre study and single blinded study over a period of 1 year from July 2010 to June 2011. The study was carried out involving the Department of General Surgery, Department of Genetics and Pathology Department of Ramakrishna Mission Seva Pratishthan, V.I.M.S, Kolkata. The

total number of patients that formed the study group was 10 and the control group of 5 healthy individuals i.e. without breast cancer. All the 10 patients were thoroughly assessed both in the Surgical OPD and Genetics OPD. A pedigree chart was made and followed (Pedigree chart of a family.Wikipedia.org.) A sample Questionnaire was designed according to which the questions were asked to the patients and patient's relative accompanying her. Questionnaire was followed by a detailed clinical examination for new patients and for operated patients local examination for recurrence and condition of the operated site was taken into consideration. All the patients who accepted to undergo the karyotyping procedure were insisted to give consent. A consent form was signed by the patient and patient relative. Collection of blood 3ml was done at the same time in the OPD. The control groups were also sent to the same OPD for collection of blood. This was followed by karyotyping procedure which was performed at the Genetics laboratory V.I.M.S. For each subjects 100 clear metaphases were assessed for chromosomal aberrations. The mitotic index (MI) was calculated as the number of mitosis per 1000 nuclei (Buerger and Ellen 2000).

RESULTS AND DISCUSSION

All the 10 patients that were involved in the study have been classified according to their age, family history and religion, operative procedure and pathology report background in Table 1. The results obtained by analysis of the CAs and MI found in SBC and HBC patients and controls, Karyotype and Giemsa trypsin banding have been presented in Tables 2 and 3. A variety of CAs including aneuploidy, polyploidy, terminal deletions, acrocentric associations, chromosomal breaks and gaps, cluster of cells formation were seen in peripheral blood lymphocytes of the patient.The frequency of aberrant metaphases varied from 5% to 69% in cultured leucocytes of patients with breast cancer but in controls no CAs were found. The frequency of aneuploidy in SBC was 43% to 69% and in HBC was 65%to 68%. The mean value of percent aneuploidy in SBC was 59.4% and HBC was 66.5% (P =0.2573). According to (Roy et al. 2001) numerical abnormalities i.e. aneuploidy was observed in 33.33% of HBC as compared to 13.14% in healthy blood relatives. The frequency of polyploidy, cluster of cells was higher in HBC. The difference was statistically significant ($p<0.0001$). One of the interesting thing we noted in our study was chromosomal breaks. Chromosomal breaks were not found in SBC. However, it was significantly found in HBC ($p<0.0001$). We found chromosomal break at 1q in one patient and random chromosomal breaks in the second patient. The identification of chromosomal breaks and their association with HBC was an unusual finding. We know that BRCA1 is localized to chromosome 17 and the gene was cloned in 1994. Mutations in BRCA1 gene are associated with 50 percent to 85 percent lifetime risk of developing breast cancer as seen in various studies. Carriers of BRCA1 gene mutation often develop breast cancer at a younger age compared to the general population. BRCA2 gene is located on chromosome13. The risks of development of breast cancer in women carrying BRCA2 mutations are similar to the risk of BRCA1 carriers. Previous studies showed that, a phenotypic hallmark in cells mutated for genes involved in DNA double-strand break repair is spontaneous chromosome instability. Dramatic CAs were noted in BRCA1 and BRCA2-deficient embryonic tissue (Tutt et al. 2002; Xu et al. 2001b) and in cells deficient in BRCA1 and BRCA2 (Shen et al. 1998; Turner et al. 2002; Tutt et al. 1999; Yu et al. 2000). All the tumors displayed numerical and structural CAs, including chromatid and chromosome breaks, insertions, deletions, and re-arrangements (Moynahan 2002; Christopoulou and Spiliotis 2006; Palma M et al. 2006)

The cost of the BRCA gene test ranges from $300 to $3000 (Lawrence 2001). In our study, MI in SBC and HBC was found and compared with each other. The results were statistically significant (P equals 0.0423) with mean values of (4.04) and (2.10) respectively. It was also compared with healthy individuals (controls). The result was highly significant (P equals 0.0038) (Buerger 2000)

CONCLUSION

The pathogenesis of BRCA1 and BRCA2 gene and their association with repair of DNA and CAs in breast cancer patients formed the main basis of our study. Finding of chromosomal breaks and other chromosomal aberration in the breast cancer can help us segregate only those

Table 1: Background of the Breast cancer patients

No. of patients	*Age*	*Family history*	*Religion*	*Operation/Procedure underwent*	*Histopathological report*
1	40yrs	N A	Muslim	MRM + chemotherapy + Radiotherapy	Invasive Ductal carcinoma
2	42yrs	N A	Hindu	MRM + Chemotherapy	Poorly differentiated Invasive Ductal carcinoma
3	50yrs	N A	Hindu	MRM	Poorly differentiated Invasive Ductal carcinoma
4	55yrs	N A	Hindu	Simple Mastectomy	Ductal Carcinoma in situ
5	43yrs	N A	Hindu	Simple Mastectomy	Ductal Carcinoma in situ
6	45yrs	N A	Hindu	Excision Biopsy	Atypical Ductal Hyperplasia
7	60yrs	N A	Hindu	MRM	Invasive Ductal Carcinoma
8	54yrs	Cousin sister was affected with breast cancer	Hindu	MRM + chemotherapy + Radiotherapy	Adenocarinoma Breast
9	45yrs	Elder sister died of breast cancer at 35yrs	Hindu	Pre- Operative Chemotherapy	Invasive Ductal carcinoma
10	35yrs		Muslim	Pre- Operative Chemotherapy	Invasive Ductal carcinoma

Table 2: Chromosomal analysis results

No. of Patients	*Slide No.*	*Mitotic Index*	*Aneuploidy*	*Polyploidy*	*Breaks in chromosomes/ chromatid*	*Cluster of cells*
1	2608	3.67	54%	-	-	-
2	2697	6.17	43%	5%	-	5%
3	2621	2.88	62%	-	-	-
4	2624	3.66	60%	8.6%	-	-
5	2626	2.52	63%	-	-	-
6	2732	4.88	67.7%	-	-	10.5%
7	2731	2.15	65%	17%	20%	36.5%
8	2743	2.06	68%	22%	45%	80%
9	2734	4.04	69%	-	-	7.1%
10	2767	4.55	57%	-	-	6%

Controls (healthy individual) n=5	*Mitotic index*
1	1.56
2	1.20
3	2.54
4	2.11
5	1.8

patients whose family members and relatives are highly susceptible to develop breast cancer from the huge population of breast cancer. This could implement a cost effective management of detecting early breast cancer in family member and take necessary preventive measure and treatment as the case may be. From the above study, the emphasis is on initiating karyotyping as a screening recommendation for detecting early age of onset in hereditary breast cancer because

Table 3: Mitotic index, chromosomal aberrations and p value between controls and sporadic and hereditary breast cancer patients.

		Sporadic breast cancer n= 8	*Hereditary breast cancer n=2*	*Controls*	*P value*
Mitotic Index		4.04	2.10	*1.84	*0.00380.0423
Chromosomal Analysis	Aneuploidy	59.4%	66.5%	Nil	0.2573
	Polyploidy	1.7%	19.5%		<0.0001
	Breaks in chromosomes/chromatids	Nil	32.25%		<0.0001
	Cluster of cells	3.57%	58.25%		<0.0001

*P-value of mitotic index between controls and patient.

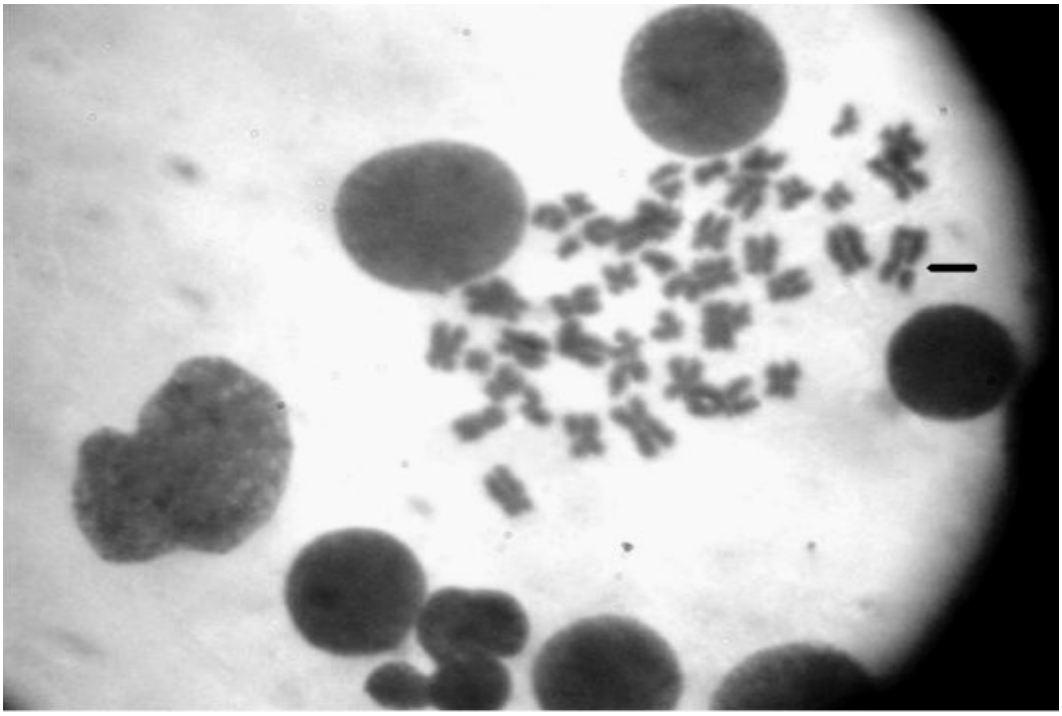

Fig. 1. Chromosomal break and cluster of cells

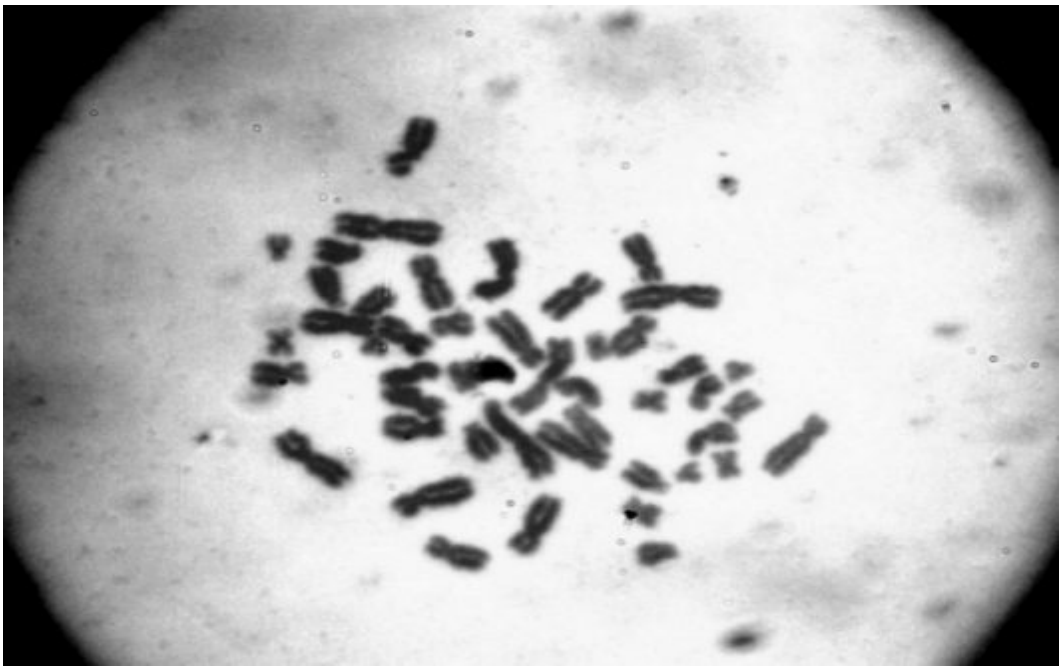

Fig. 2. Aneuploidy

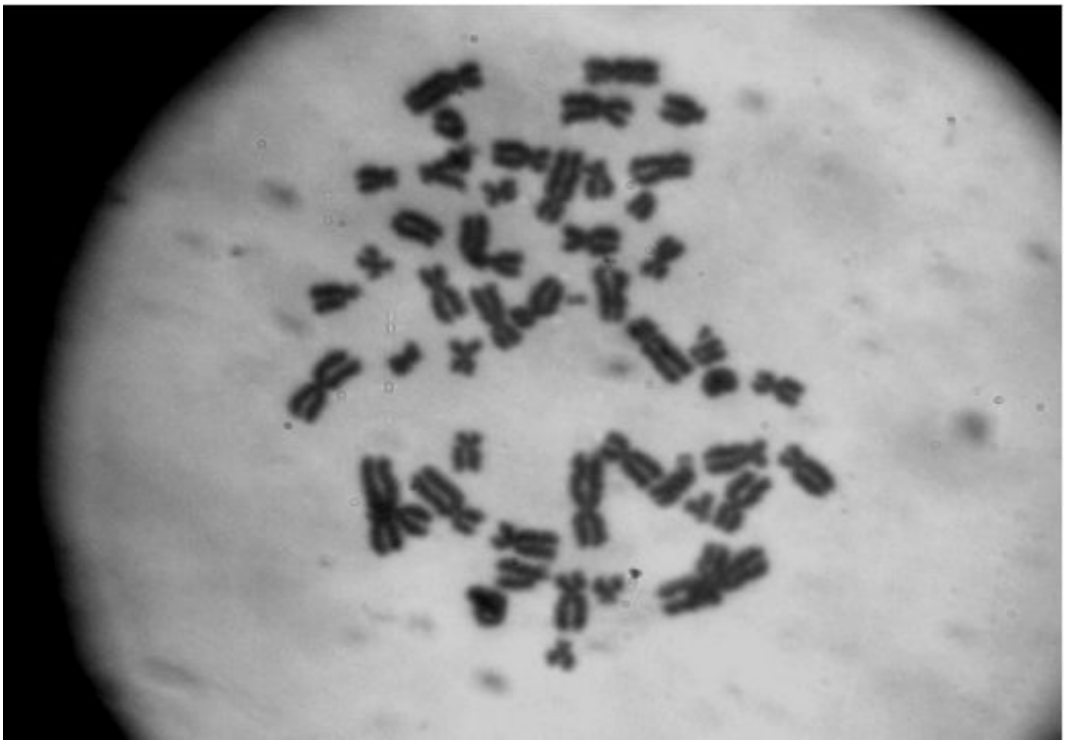

Fig. 3. Polyploidy

Molecular analysis is a tedious and very costly technique.

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