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Genetic Studies of Breast Cancer Patients in Goa, India

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ABSTRACT Chromosomal Aberrations (CA) may play a key role in tumor initiation, promotion and progression stages of carcinogenesis. Presence of such CA in the normal Peripheral Blood Culture (PBC) could be used to identify individuals in high-risk groups. Therefore, early diagnosis, cure and in some cases, prevention of such tumors can be achieved. Molecular analysis at population level is a tedious and very costly technique. Hence, a primary screening of Breast Cancer (BC) patients with highly sensitive marker(s) using a less expensive methodology namely PBC, was carried out in the present study. Our earlier studies indicated that there is a high frequency of BC compared to other types of cancers in females reported in Goa Medical College (GMC). Hence, present work was undertaken to analyze the chromosomal instability in BC patients using PBC. Altogether, 79 subjects were studied involving BC patients (47 subjects) [comprising two groups, (i) Radiotherapy treated (RT) group (19 subjects) and (ii) Surgery (S) group (28 subjects)] and control (C) group (32 subjects). We found that there is a high frequency of dicentrics (27.65 %) in BC patients compared to that of controls (3.13%) and a high frequency of dicentrics in RT group (36.84%) compared to the S group (21.43%). The observation of dicentrics in S group indicates that there may be inherent chromosomal instability in these patients, which may be inducing tumorogenecity.

INTRODUCTION

Breast Cancer (BC) is a complex disease in which several Chromosomal Aberrations (CA) are involved and thus many CA are observed in breast tumors (Dahiya and Deng 1998). Cytogenetic analysis of peripheral blood lymphocytes (PBL) of patient with ductal breast carcinoma by Hrvoje et al. (2004) indicated that chromosomal aberration analysis in PBL might be a useful technique for a better preoperative and postoperative definition of the biologic characteristics of breast carcinoma. Some of these CA are located on specific genetic loci that directly contribute to one or more forms of transformation, i.e., deregulated proliferation and invasion (which is the hallmark of carcinogenesis), while other changes confer genetic instability that increases the possibility of acquiring subsequent, specific genetic lesions relevant to tumorigenesis. The knowledge of specific genetic changes and their biological consequences is critical to an understanding of the natural history of breast tumors and the development of rational means to prevent and treat them.

Recent studies (El-Zein 2005) indicate that spontaneous chromosome instability could be a risk for prostate cancer. In addition, individuals with inherited predisposition to spontaneous chromosome breakage (Bloom's syndrome, Fanconi's anaemia and ataxia talangiectasia) (German 2000) have an increased risk of various cancers such as of upper aero digestive tract (Spitz et al.1989), hereditary mega duodenum (Doneda et al.1995), cervix uteri (Dhillon et al.1996), ovary (Dhar et al.1996), breast (Dhillon et al.1995) and prostate (Dhillon and Dhillon 1998).

Chromosomal rearrangements play an important role in the activation of proto-oncogenes and inactivation of tumor suppressor genes (Hagmar et al. 1994). Several types of genetic predisposition to cancer may be associated with constitutional chromosome instability. Thus, it is generally accepted that CA are causal events in the development of neoplasia. The conceptual basis for using CA in peripheral blood lymphocytes (PBL) as a biomarker has been the hypothesis that the extent of genetic damage in PBL reflects similar events in the precursor cells for carcinogenic processes in the target tissues (Dahiya and Deng 1998). The somatic mutation theory of cancer, i.e., the concept that neoplasia originates in a single cell by an acquired genetic change, remains the paradigmatic view of cancer pathogenesis, supported by a wealth of experimental evidence (Hagmar et al. 1994).

Genetic predisposition to cancer may be caused by several mechanisms. One of the possibilities is genetic instability, which in some

cases is expressed as chromosome instability. Individuals with genetic instability may generate more cells with mutations or CA than those with more stable genomes. One of these aberrant cells in a target tissue may happen to possess a genetic constitution equivalent to the first step of carcinogenesis (Pathak 1991).

Chromosomal abnormalities, both structural and numerical types, have long been speculated of playing a key role in tumor initiation and progression. In certain neoplasias, it is evident that there are two types of chromosomal changes, viz. a) primary – responsible for neoplastic transformation and b) secondary – responsible for tumor growth, heterogeneity and metastasis. Primary anomalies can be effectively tested by cytogenetic methods. By recognizing a primary anomaly, it could be possible to identify high-risk individuals and then the early diagnosis or establishment of genetic predisposition to neoplasia (Hagmar et al. 1994).

Peripheral lymphocytes of the family members have also revealed similar CA to those present in the breast carcinoma cells (Mitelman 2000). Collectively, these findings suggest that a few anomalies at the chromosomal level may already be present in the individuals much before the initiation of the disease. One may remain asymptomatic or may develop BC in the lifetime depending on the interactions of genetic make-up and other confounding risk factors (Pathak 1991).

CA in circulating lymphocytes may have predictive value for cancer onset (Susi et al. 2000, Shambhu et. al. 2000). Lymphocyte cultures of BC patients have exhibited phenomena like premature chromosome condensation and double minutes which generally are reported in tumor cells. This indicates that the genetic / molecular mechanisms usually expressed in tumor tissue are at times manifested in circulating lymphocytes (Rao 1996).

Chromosomal alterations play a key role in tumor initiation and progression. Presence of such anomalies in the normal Peripheral Blood Culture (PBC) could be used to identify individuals in high-risk groups. Therefore, early diagnosis, cure and in some cases prevention of such tumors can be achieved.

Many reports from all over the globe, including from India, confirm the presence of CA in human BC (Dahiya and Deng 1998, Hagmar et al. 1994, Mitelman 2000). However, no detailed reports are available on the studies of BC in Goa, especially on the cytogenetic aspects of BC. Our preliminary study showed that the state has high frequency of BC (Deshpande et al. 2002). Hence, to know the status of the genetic etiology of cancer in Goa, we carried out cytogenetic studies of cancer, especially BC, for the first time in Goa (Deshpande 2003). Present work is undertaken to analyze the chromosomal instability in BC patients using PBC as the major objective.

Analysis of mutations in BRCA1 (Pathak 1986) and BRCA2 (Teixeira 1996) may help significantly in identifying the lifetime risk of BC (Hopper 1997). Such molecular analysis at population level is costly and hence a primary screening of BC patients with highly sensitive marker(s) using less expensive methodology is necessary. Thus, initial cytogenetic studies on breast cancer patients in Goa were carried out as it is a cost effective approach. Subsequently, our preliminary study on mutations in BRCA1 and BRCA2 genes in familial breast cancer patients from Goa was carried out in collaboration with Indian Council of Medical Research (ICMR). Of eight familial breast cancer patients from Goa, two sisters from a family showed protein truncating frameshift mutation 185delAG leading to formation of TGA at codon 39 which is one of the founder BRCA1 mutation reported in Ashkenazi Jews (Hedau et al 2004).

MATERIALS AND METHODS

Fine chemicals such as Phytohaemagglutinin M (DIFCO), McCoy's 5a Medium (HIMEDIA), and Fetal Calf Serum (CENTRON) were used.

Subjects: Histopathologically confirmed BC patients reported to the major hospitals in Goa [(i) Goa Medical College, Bambolim, (ii) Hospicio Hospital, Margao and Manipal-Goa Cancer and General Hospital, Dona Paula)] during September 2000 to August 2001 were selected for the study. Normal, healthy females who were free from any chronic or acute diseases and those not exposed to X-ray treatment in the recent past (5 years) consisted of the control group (C group).

Altogether, 79 subjects were studied. This consisted of a total of 47 females with ages ranging between 26 years and 81 years, consisting of 28 patients from the Surgery Department (S group) and 19 patients from the Radiotherapy Department (RT group). Peripheral blood was collected from the patients of S group

before they underwent any kind of treatment including chemotherapy. The Control (C) group consisted of 32 females with ages ranging from 20 years to 65 years. Informed consent was taken from the patients and controls prior to the collection of their blood samples. This study was approved by the local ethical committee of GMC.

Culturing of Leucocytes: Peripheral blood of BC patients and controls was cultured following the method of Moorhead et al. (1960).

Collection of Blood Samples: About 2.0 ml of venous blood was drawn separately into a sterile, heparinized, disposable syringe from each of the subjects cited above (patients and controls). 0.5 ml of this blood was inoculated into the culture vial (30 ml) containing 5.0 ml of culture medium, 1.0 ml of FCS, 0.2 ml of PHA, 0.02 ml of Benzyl penicillin and 0.1 ml of Streptomycin sulphate under aseptic conditions. The cultures were incubated for a period of sixty nine and half hours at 37^0C with intermittent mixing up once a day. For each test sample, cultures were set up in duplicate. At the end of incubation period, 0.2 ml of colchicine (0.01%) was added to each culture vial in order to arrest the dividing cells at metaphase stage and each culture was further incubated for a period of 45 minutes.

Chromosome Examination: Metaphase plates were prepared following the Acetic-Flame dry technique and the chromosomes were stained with Giemsa. Well spread metaphase plates were screened under oil immersion objective of Zeiss Trinocular Research microscope. CA were identified and recorded. The frequency of the CA observed in C group, RT group and S group were calculated in percentages. Selected metaphase plates were micro photographed. For each sample, 10 metaphase plates were studied.

Statistical Analysis: The data was statistically analyzed using student T test. The database was created in MS-Word 2000.

RESULTS

Dicentrics were the common chromosomal anomaly observed in BC patients. The frequency of dicentrics found in the BC patients is represented in the table 1 and the distribution of dicentrics in different groups of BC patients and controls is represented in figure 1. Dicentrics were seen in 7 out of 19 (36.84%) RT patients. There were dicentrics in 6 subjects from S group out of 28 (21.43%) S group patients. Dicentrics were more (24) in RT group compared to that in S group (7).

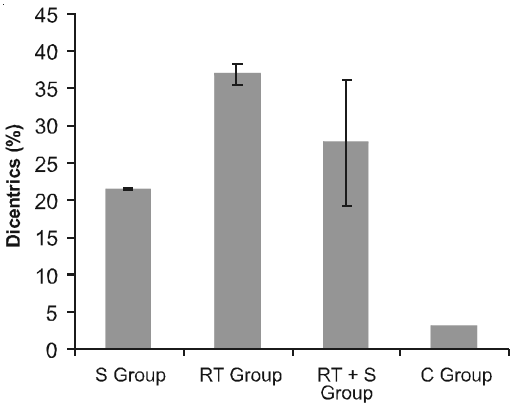

Fig. 1. The distribution of dicentrics in different groups of breast cancer patients and controls.
Note: In C group, dicentric was seen only in 1 sample.

Comparison Between BC Patients and C Group: A statistically significant higher percentage of dicentrics is observed in BC patients (27.65%) compared to the controls (3.13%) (t = 2.804) (P is significant at 0.05 level) P=0.006.

Comparison Between S Group and C Group: A statistically significant higher percentage of

Table 1: Frequency of dicentrics in breast cancer patients and controls

Groups	*Total subjects*	*Subjects with dicentrics(No.)*	*Subjects with dicentrics(%)*	*Total dicentrics*
S group	28	6	21.43*	7
RT group	19	7	36.84	24
RT+S group	47	13	27.65*	31
C group	32	1	3.13	1

Where RT - Radiotherapy group, S - Surgery group, C - Control group
*Significant at 5 % level

dicentrics is observed in S group (21.43%) compared to the controls (3.13%) (t = 2.205) (P is significant at 0.05 level) P=0.0314.

Comparison Between RT Group and S Group: The percentage of dicentrics (14.89%) (7 RT out of total 47 BC) was slightly higher in RT group compared to the percentage of dicentrics (12.76%) (6 S out of total 47 BC) in S group. However, this difference is not statistically significant.

DISCUSSION

Significant increase of CA observed in the present study of S group of BC patients compared to the C group may indicate their role in the induction of BC by chromosomal instability. Similar observations were made recently by Rossner et al.(2005) and reported that CA may be associated with the induction of stomach cancer and that CA may act as a predictive marker for risk of stomach cancer.

Further, the high increase in the frequency of dicentrics observed by us indicates that dicentrics may be a major kind of CA causing the induction of BC.

Although it is reported from earlier studies (Cvetka and Marjan 2001, Sreedevi et al. 2001) that radiation increases the frequency of dicentrics, we could not find a statistically significant increase of dicentrics in the RT group of BC patients in comparison with the S group patients who were not exposed to radiation or any other kind of therapy. This is supplementary to our observation of dicentrics induced BC in Goan BC patients.

Further, the frequency of dicentrics is not significant in the RT+S group compared to that of S group. This again supports that dicentrics may be responsible for the induction of BC.

From the present study, we may conclude that dicentrics, seen in BC patients in Goa may be associated with chromosomal instability and cancer induction in these patients. Further, we may even presume that radiation alone may not be associated with chromosome instability for induction of tumorigenesis in Goan BC patients although a slight increase of dicentrics were observed in RT group compared to the S group.

All these observations indicate the presence of certain level of chromosome / genetic instability in BC patients. Although chromosomal instability is a well-known characteristic of a number of recessive disorders, none of our BC patients showed any of the signs usually associated with them. This indicates that the genetic etiology and genetic predisposition of BC may be due to chromosome instability in these patients. Similarly, Venkatchalam et. al. (1999) observed higher frequency of dicentrics in peripheral blood lymphocytes of 25 cancer patients prior to chemo and radiotherapy compared to 21 controls indicating that induction of cancer is due to chromosomal instability.

Concurrent breaks in two different chromosomes may either give rise to translocations or dicentrics (Gisselsson D. May 2001). Whereas translocation derivatives are stably transmitted through cell division, the dicentric chromosomes may be stretched out between the spindle poles to form bridges at anaphase.These bridges may subsequently break, and the chromosomes are transmitted to the daughter cells with broken ends that may recombine further during the subsequent interphase (McClintock 1940). The broken chromosome ends may fuse into novel dicentrics and rings, which may again break at the next cell division. Thus, chromosomal damage may not only result in static aberrations, such as translocations, inversions, deletions, and duplications; it may also result in mitotically unstable chromosomes, which may trigger a series of breakage-fusion-bridge (BFB) events. Such BFB cycles have been shown to occur in many malignant solid tumours with complex chromosome abnormalities, including head and neck, pancreatic, and ovarian carcinomas, as well as leiomyosarcoma, osteosarcoma, malignant fibrous histiocytoma, and atypical lipomatous tumours (Gisselsson et al. 2000; Saunders et al. 2000). Thus, the formation of dicentrics may be associated with the etiology of breast cancer in Goan BC patients (S group) in this study. However, more studies of this nature are necessary to confirm the findings.

Several earlier workers have reported a close relationship between a high frequency of spontaneous CA and some forms of cancer (Czeizel et al. 1974, Hsu et al. 1981, Wang et al. 1982, Delhanty et al. 1983, Han et al. 1993, Tzancheva and Komitowski 1997). The phenomenon of increased chromosome fragility deserves further study because it might be involved in the pathogenesis of BC. Thus, chromosome instability may be considered as a reaction to environmental agents which through genome alteration, may play a (fundamental) role in mammary cells proliferation and eventually, also in the increased risk of malignancy (Pathak 1991).

The results of this study highlight the importance of practical monitoring of latent chromosome instability and identification of individuals at high risk. To confirm the hypothesis that high frequency of dicentrics may be responsible for inducing tumorigenecity in BC patients (especially from S group) in Goa, a detailed cytogenetic study involving greater number of BC patients from operated and unoperated group, untreated group, treated (with chemotherapy and radiotherapy) group and the follow up of BC patients with many genetic markers is essential for better understanding of the process of induction of tumorigenesis in these groups.

CONCLUSION

Dicentrics are the commonly observed CA in BC patients compared to controls. This indicates that genetic instability prevailing in BC patients. Thus, it is proposed that an increased level of chromosomal breakage may be a relevant biomarker of cancer risk assessment. This is supported by different studies showing an increase in CA among cancer patients compared with controls

Further studies in BC patients from Goa on genetic instability are to be carried out to know which chromosomes are involved and thereby which genes are involved in the induction of BC. Once the type of mutations at chromosomes and at gene level (genetic etiology) in sporadic as well as familial BC are known, appropriate measures concerning the management of the disease and preventive measures such as gene therapy could be designed. Thus, attempts can be made to improve the prognosis of the BC patients and increase their survival.

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