CHAPTER THREE

CHROMOSOMAL STUDIES
3.1 INTRODUCTION

Breast cancer is a complex disease in which numerous genetic aberrations occur and accordingly a number of genetic changes have been identified in breast tumors [1]. Some of these involve specific genetic loci that directly contribute to one or more attributes of transformation, i.e., dysregulated proliferation and invasion, 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.

Chromosomal rearrangements play an important role in the activation of protooncogenes and inactivation of tumor suppressor genes [2]. Several types of genetic predisposition to cancer may be associated with constitutional chromosome instability. Thus, it is generally accepted that chromosomal alterations are causal events in the development of neoplasia. The conceptual basis for using chromosomal aberrations 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 [1]. 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 [2].

Many reports from all over the globe, including from India, confirm the presence of chromosomal aberrations in human breast cancer [1], [2], [3], [4]. However, no detailed reports are available on the studies of breast cancer in Goa, especially the genetic aspects. Our preliminary study shows that the state has high frequency of breast cancer. Spontaneous chromosomal instability has been correlated with cancer predisposition [4]. 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 chromosomal aberrations 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 [5].

Spontaneous chromosomal instability is being evaluated by employing various cytogenetic techniques, wherein all the chromosomal aberrations are analysed. 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.

Peripheral lymphocytes of the family members have also revealed similar chromosomal abnormalities to those present in the breast carcinoma cells [3]. Monakhov et al. (1996) [4] have reported heritability of marker chromosomes. 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 breast cancer in the life time depending on the interactions of genetic make-up and other confounding risk factors [5].

Chromosomal aberrations in circulating lymphocytes may have predictive value for cancer onset [6], [7]. Lymphocyte cultures of breast cancer 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 [8]. Analysis of mutations in BRCA1 [9] and BRCA2 [10] may help significantly in identifying the lifetime risk of breast cancer [11]. Such molecular analysis at population level is a costly affair and hence a primary screening of breast cancer patients with highly sensitive marker(s) using inexpensive methodology is necessary.

Cytogenetic 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.

Present work is undertaken to analyse the chromosomal instability in breast cancer patients using peripheral blood culture as the major objective.

**3.2 MATERIALS AND METHODS**

Chemicals and salts used were of a good grade. Fine chemicals such as Phytohaemagglutinin M (DIFCO), McCoy's 5a Medium (HIMEDIA), and Fetal Calf Serum (CENTRON), were used.
Subjects:

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

Altogether, 79 subjects were studied. This consisted 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 Surgery Department 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.

Culturing of leucocytes:

Peripheral blood of BC patients and controls was cultured following the modified method of Moorhead et. al. (1960) (12).
A) Materials:

1. McCoy's 5a Medium:
   McCoy's 5a medium powder (Himedia) 11.9 g was dissolved in one litre double distilled water and the pH was adjusted to 7.4 with the a pinch of Sodium bi carbonate. The solution was filtered using 0.22 micron filter in a Tarson's sterile filter assembly. Antibiotics were added to the medium and this medium was stored at -20°C.

2. Antibiotics:
   a) Benzyl penicillin: Stock solution of this was prepared by dissolving the contents of the vial in 5 ml of made in sterile distilled water. This solution was added to culture medium to achieve a final concentration of 0.02 mg/ml.
   
   b) Streptomycin sulphate: Stock solution of this was prepared by dissolving the contents of the vial in 5 ml of made in sterile distilled water. This solution was added to culture medium to achieve a final concentration of 0.1 mg/ml.

3. Fetal Calf Serum:
   Sterile Fetal Calf Serum (Centron) was procured and stored at -20°C.

4. PHA-M: Stock solution of this was prepared by dissolving 5 mg in
5 ml of made in sterile distilled water. This solution was added to culture medium to achieve a final concentration of 0.1 mg/ml.

5. Heparin: Preservative free heparin (commercially available 5ml vial) 0.1 ml was drawn into a sterile disposable syringe aseptically and blood was collected in this heparinized syringe.

6. Colchicine: The stock solution (0.1 mg/ml) was prepared in sterile distilled water and was stored under aseptic conditions at 4°C.

B) Methodology:

Collection of Blood Samples:

About 2.0 ml of venous blood was drawn separately into a sterile, heparinized, disposable syringe; from each of the patients, as well as, from those of control individuals, the subjects cited above. 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 mine and half hours at 37°C with intermittent mixing up and releasing of CO₂, 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.

C) Harvesting:

Materials:

1. Hypotonic Solution (0.075 M):
   - Potassium chloride (KCl) ........ 5.59 gm
   - Glass Distilled Water ........ 1000 ml

2. Carnoy's fixative: Methanol : Glacial acetic acid (3:1, V:V).
   - This was prepared fresh everytime and chilled before use.

Methodology:

1. The contents of the culture vial were centrifuged at 1000 rpm for 5 minutes.

2. The supernatant was discarded and the pellet was resuspended in 8 ml of hypotonic KCl solution prewarmed to 37°C, incubated for 12 min. at 37°C and then centrifuged at 1000 rpm for 10 min.

3. After removal of supernatant, the cells were fixed in freshly prepared fixative and kept at 4°C overnight.

4. Next day, the cells were centrifuged at 1000 rpm for 10 min.
5. Two or three changes were given with the fresh fixative each exposure to fixative lasting for 20-30 minutes until a colourless cell pellet was obtained.

D) Preparation of metaphase plates:

After the last centrifugation the pellet was dissolved in small amount of fresh fixative to form a thick homogenous suspension. A test slide was prepared by placing a drop of the cell suspension on a pre-cleaned, grease-free, chilled, slide and dried immediately over a slide warmer kept at 40°C for 2 minutes.

The slide was examined under a microscope to see whether the concentration of cells and the spreading of chromosomes enabled detailed examination of metaphases.

The rest of the slides were prepared after making suitable concentrations of solutions with fresh fixative.

E) Staining:

Materials:

Giemsa Stain: a) Stock solution:

<table>
<thead>
<tr>
<th>Material</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Giemsa powder</td>
<td>1.0 gm</td>
</tr>
<tr>
<td>Glycerol</td>
<td>54 ml</td>
</tr>
<tr>
<td>Methanol</td>
<td>84 ml</td>
</tr>
</tbody>
</table>

1.0 g of Giemsa powder was dissolved in 54 ml of glycerol which was mixed thoroughly over a magnetic stirrer kept at 56°C for two hours. The solution was allowed to cool till it reached room
temperature and then 84 ml of methanol was added and mixed well on the stirrer overnight. It was filtered using Whatman No.1 filter paper and the filtrate formed the stock solution.

b) Working Solution: Working solution of stain was prepared by dissolving 2 ml of stock solution of Giemsa and 2 ml of 10% disodium hydrogen phosphate buffer and in 46.0 ml of distilled water (pH 6.8).

The metaphase plates were stained in this solution for 10 min. in distilled water and air dried.

F) Chromosome examination:

Well spread metaphase plates were screened under oil immersion objective of Zeiss Trinocular Research microscope.

G) Chromosomal analyses:

Different types of Chromosomal aberrations (CA), were identified, analysed and recorded as per the descriptions outlined in the criteria recommended by WHO [13]. The percentages of the various types of CA in observed in C group, RT group and S group were calculated. Selected metaphase plates were microphotographed.

H) Statistical Analysis:

The data was statistically analysed using student T test. The database was created in MS-Word 2000.
3.3 RESULTS

The frequency and variety of chromosomal abnormalities (CA) found in the BC patients are represented in the table 3.1.

Apart from dicentrics (27.65%), other CA such as Telomeric Associations (TA) (34.04%), chromatid breaks (25.53%), Premature Centromeric Separation (PCS) (12.76%), ring chromosomes (8.15%) and gaps (4.25%) were found in BC patients and in controls, except a dicentric these CA were not observed.

Dicentrics, were seen in 7 subjects from RT group out of 47 (RT+S) BC patients (14.89%) and in 7 out of 19 (36.84%) RT patients. There were dicentrics in 6 subjects from S group out of 47 BC patients (RT+S) (12.76%) and in 6 out of 28 (21.42%) S group patients.

Table 3.2 showing the frequency of dicentrics in Breast Cancer patients and Controls

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Total no. of subjects</th>
<th>Subjects with dicentrics</th>
<th>Total no. of metaphase plates</th>
<th>Total dicentrics</th>
<th>Dicentrics / metaphase plate</th>
</tr>
</thead>
<tbody>
<tr>
<td>RT</td>
<td>19</td>
<td>7</td>
<td>50</td>
<td>24</td>
<td>0.025</td>
</tr>
<tr>
<td>S</td>
<td>28</td>
<td>6</td>
<td>50</td>
<td>7</td>
<td>0.005</td>
</tr>
<tr>
<td>RT+S</td>
<td>47</td>
<td>13</td>
<td>50</td>
<td>31</td>
<td>0.013</td>
</tr>
<tr>
<td>C</td>
<td>32</td>
<td>1</td>
<td>50</td>
<td>1</td>
<td>0.0006</td>
</tr>
</tbody>
</table>

Note: RT – Radiotherapy group
S - Surgery group
C - Control group
<table>
<thead>
<tr>
<th>Subjects</th>
<th>Total subjects studied</th>
<th>Subjects with Dicentrics</th>
<th>Subjects with TA</th>
<th>Subjects with chromatid break</th>
<th>Subjects with PCS</th>
<th>Subjects with ring chromosome</th>
<th>Subjects with gap</th>
<th>Total CA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>RT group</td>
<td></td>
<td>19</td>
<td>7</td>
<td>36.84</td>
<td>8</td>
<td>42.10</td>
<td>6</td>
<td>31.57</td>
</tr>
<tr>
<td>RT + S group</td>
<td></td>
<td>47</td>
<td>13</td>
<td>27.65 *</td>
<td>16</td>
<td>34.04 *</td>
<td>12</td>
<td>25.53 *</td>
</tr>
<tr>
<td>C group</td>
<td></td>
<td>32</td>
<td>1</td>
<td>3.13</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Note: Total number of metaphases observed in each subject under such group is 50. Frequency of CA is measured per cell.
The no. of dicentrics were more (24) in RT group compared to that in (7) S group. See tables 3.4 to 3.9. See plates for CA.

**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 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.

Telomeric Associations (TA) were seen in 8 subjects from RT group out of 47 (RT+S) BC patients (17.02 %) and in 8 out of 19 (42.11%) RT patients. There were TA in 8 subjects from S group out of 47 BC patients (RT+S) (17.02 %) and in 8 out of 28 (28.57%) S group patients.
Table 3.3 showing the frequency of TA in Breast Cancer patients and Controls

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Total no. of subjects</th>
<th>Subjects with TA</th>
<th>Total no. of metaphase plates</th>
<th>Total TA</th>
<th>TA / metaphase plate</th>
</tr>
</thead>
<tbody>
<tr>
<td>RT</td>
<td>19</td>
<td>8</td>
<td>50</td>
<td>12</td>
<td>0.012</td>
</tr>
<tr>
<td>S</td>
<td>28</td>
<td>8</td>
<td>50</td>
<td>18</td>
<td>0.012</td>
</tr>
<tr>
<td>RT+ S</td>
<td>47</td>
<td>16</td>
<td>50</td>
<td>30</td>
<td>0.012</td>
</tr>
<tr>
<td>C</td>
<td>32</td>
<td>0</td>
<td>50</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Note: RT — Radiotherapy group
      S — Surgery group
      C — Control group

The no. of TA were more (18) in S group compared to that in (12) RT group.

Comparison between BC patients and C group:

The TA was not found in control individuals and was found in 34.04% of BC patients compared to total (47) BC patients. This difference is statistically significant. (t = 3.7) (P is significant at 0.05 level) P=0.0004

Comparison between S group and C group: A statistically significant higher percentage of TA is observed in S group (28.57%) compared to the controls (0%) (t = 3.25) (P is significant at 0.05 level) P=0.001.
Table 3.4 showing the frequency of chromatid break in Breast Cancer patients and Controls

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Total no. of subjects</th>
<th>Subjects with chromatid break</th>
<th>Total no. of metaphase plates</th>
<th>Total chromatid break</th>
<th>Chromatid break / metaphase plate</th>
<th>Chromatid break / 50 metaphase plates</th>
</tr>
</thead>
<tbody>
<tr>
<td>RT</td>
<td>19</td>
<td>6</td>
<td>50</td>
<td>8</td>
<td>0.16</td>
<td>0.008</td>
</tr>
<tr>
<td>S</td>
<td>28</td>
<td>6</td>
<td>50</td>
<td>8</td>
<td>0.16</td>
<td>0.005</td>
</tr>
<tr>
<td>RT+ S</td>
<td>47</td>
<td>12</td>
<td>50</td>
<td>16</td>
<td>0.32</td>
<td>0.006</td>
</tr>
<tr>
<td>C</td>
<td>32</td>
<td>0</td>
<td>50</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Note: RT – Radiotherapy group
S - Surgery group
C - Control group

Table 3.5 showing the frequency of PCS in Breast Cancer patients and Controls

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Total no. of subjects</th>
<th>Subjects with PCS</th>
<th>Total no. of metaphase plates</th>
<th>Total PCS</th>
<th>PCS / metaphase plate</th>
<th>PCS / 50 metaphase plates</th>
</tr>
</thead>
<tbody>
<tr>
<td>RT</td>
<td>19</td>
<td>2</td>
<td>50</td>
<td>5</td>
<td>0.1</td>
<td>0.005</td>
</tr>
<tr>
<td>S</td>
<td>28</td>
<td>4</td>
<td>50</td>
<td>5</td>
<td>0.1</td>
<td>0.003</td>
</tr>
<tr>
<td>RT+ S</td>
<td>47</td>
<td>6</td>
<td>50</td>
<td>10</td>
<td>0.2</td>
<td>0.004</td>
</tr>
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<td>C</td>
<td>32</td>
<td>0</td>
<td>50</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Note: RT – Radiotherapy group
S - Surgery group
C - Control group
Table 3.5 Age and CA in controls

<table>
<thead>
<tr>
<th>Age group</th>
<th>No. of subjects</th>
<th>No. of Dicentrics</th>
<th>No. of subjects</th>
<th>No. of TA</th>
<th>No. of subjects</th>
<th>No. of chromatid break</th>
<th>No. of subjects</th>
<th>No. of PCS</th>
<th>No. of subjects</th>
<th>No. of Ring chromosome</th>
<th>No. of gap</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-30</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>-</td>
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<tr>
<td>31-40</td>
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<td>-</td>
<td>-</td>
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<td>-</td>
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<td>61-70</td>
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<td>-</td>
<td>-</td>
<td>-</td>
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</tr>
</tbody>
</table>

Table 3.7 Age and CA in BC patients

<table>
<thead>
<tr>
<th>Age group</th>
<th>No. of subjects</th>
<th>No. of Dicentrics</th>
<th>No. of subjects</th>
<th>No. of TA</th>
<th>No. of subjects</th>
<th>No. of chromatid break</th>
<th>No. of subjects</th>
<th>No. of PCS</th>
<th>No. of subjects</th>
<th>No. of Ring chromosome</th>
<th>No. of gap</th>
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<tr>
<td>25-35</td>
<td>1</td>
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<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>36-45</td>
<td>5</td>
<td>6</td>
<td>5</td>
<td>7</td>
<td>4</td>
<td>6</td>
<td>1</td>
<td>2</td>
<td>1</td>
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<td>2</td>
</tr>
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<td>46-55</td>
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<td>6</td>
<td>8</td>
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<td>2</td>
<td>1</td>
<td>4</td>
<td>1</td>
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<td>1</td>
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<td>56-65</td>
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<td>8</td>
<td>3</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
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<td>66-75</td>
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<td>-</td>
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<td>3</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
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Table 3.8 showing the frequency of Ring chromosome in Breast Cancer patients and Controls

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Total no. of subjects</th>
<th>Subjects with Ring chromosome</th>
<th>Total no. of metaphase plates</th>
<th>Total Ring chromosome</th>
<th>Ring chromosome / metaphase plate</th>
<th>Ring chromosome / 50 metaphase plates</th>
</tr>
</thead>
<tbody>
<tr>
<td>RT</td>
<td>19</td>
<td>3</td>
<td>50</td>
<td>3</td>
<td>0.06</td>
<td>0.003</td>
</tr>
<tr>
<td>S</td>
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<td>50</td>
<td>1</td>
<td>0.02</td>
<td>0.0007</td>
</tr>
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<td>RT + S</td>
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<td>50</td>
<td>4</td>
<td>0.08</td>
<td>0.001</td>
</tr>
<tr>
<td>C</td>
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<td>50</td>
<td>0</td>
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</tr>
</tbody>
</table>

Note: RT – Radiotherapy group
S - Surgery group
C - Control group
Table 3.9 showing the frequency of gap in Breast Cancer patients and Controls

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Total no. of subjects</th>
<th>Subjects with gap</th>
<th>Total no. of metaphase plates</th>
<th>Total gap</th>
<th>Gap / metaphase plate</th>
<th>Gap / 50 metaphase plates</th>
</tr>
</thead>
<tbody>
<tr>
<td>RT</td>
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<td>50</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>S</td>
<td>28</td>
<td>2</td>
<td>50</td>
<td>3</td>
<td>0.06</td>
<td>0.002</td>
</tr>
<tr>
<td>RT+ S</td>
<td>47</td>
<td>2</td>
<td>50</td>
<td>3</td>
<td>0.06</td>
<td>0.001</td>
</tr>
<tr>
<td>C</td>
<td>32</td>
<td>0</td>
<td>50</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Note: RT – Radiotherapy group  
S - Surgery group  
C - Control group
Comparison between RT group and S group:

The percentage of TA (17.021%) (8 RT and 8 S out of total 47 BC) was similar in both the RT and S groups. This difference is not statistically significant.

Chromatid break were seen in 6 subjects from RT group out of 47 (RT+S) BC patients (12.76%) and in 6 out of 19 (31.57 %) RT patients. There were chromatid break in 6 subjects from S group out of 47 BC patients (RT+S) (12.76%) and in 6 out of 28 (21.42%) S group patients.

Comparison between BC patients and C group:

The chromatid break was not found in control individuals and was found in 25.53% of BC patients compared to total (47) BC patients. This difference is statistically significant (t = 3.1) (P is significant at 0.05 level) P=0.002.

Comparison between S group and C group: A statistically significant higher percentage of chromatid break is observed in S group (28.57%) compared to the controls (0% ) (t = 2.2) (P is significant at 0.05 level) P=0.03.

Comparison between RT group and S group:

The percentage of chromatid break (12.76%) (6 RT and 6 S out of
total 47 BC) was similar in both the RT and S groups. This difference is not statistically significant.

PCS were seen in 2 subjects from RT group out of 47 (RT+S) BC patients (4.25 %) and in 2 out of 19 (10.52 %) RT patients. There were PCS in 4 subjects from S group out of 47 BC patients (RT+S) (8.51%) and in 4 out of 28 (14.28%) S group patients.

Comparison between BC patients and C group:
The PCS was not found in control individuals and was found in 12.76% of BC patients compared to total (47) BC patients. This difference is statistically significant. (t = 2.1) (P is significant at 0.05 level) P=0.03.

Comparison between S group and C group: A statistically significant higher percentage of PCS is observed in S group (14.28%) compared to the controls (0%) (t = 2.2) (P is significant at 0.05 level) P=0.03.

Comparison between RT group and S group:
The percentage of Premature Centromere Separation (PCS) (8.510%), (4 S out of total 47 BC) in S group is higher than in RT group PCS (4.25%). (2 RT out of total 47 BC) This difference did not reach statistical significance. This difference is not statistically significant.

Ring chromosome were seen in 3 subjects from RT group out of 47 (RT+S) BC patients (6.38 %) and in 3 out of 19 (15.78 %) RT patients.
There were Ring chromosome in 1 subject from S group out of 47 BC patients (RT+S) (2.12%) and in 1 out of 28 (3.57%) S group patients.

**Comparison between BC patients and C group:**

The ring chromosome was not found in control individuals and was found in 8.52% of BC patients compared to total (47) BC patients. This difference is not statistically significant.

**Comparison between S group and C group:** A higher percentage of ring chromosome is observed in S group (3.57%) compared to the controls (0%). However, this difference is not statistically significant.

**Comparison between RT group and S group:** The percentage of ring chromosome (6.38%) (3 RT out of total 47 BC) was higher in RT group compared to the percentage of ring chromosome (2.126%) (1 S out of total 47 BC) in S group. This difference is not statistically significant.

Gaps were not seen in subjects from RT group but they were seen in 2 out of 28 (7.14%) S group patients. There were gaps in 2 subjects from S group out of 47 BC patients (RT+S) (4.25%).

**Comparison between BC patients and C group:**

The chromatid gap was not found in control individuals and was found in 4.25% of BC patients compared to total (47) BC patients. This difference is not statistically significant.
Metaphase plates of Breast Cancer patients

Figure 3.1: A normal metaphase plate of breast cancer patient.

Figure 3.2: A metaphase plate of breast cancer patient with dicentrics.

Figure 3.3: The enlarged view of fig. 3.2 highlighting dicentrics.
PLATE 2

Breast Cancer patients

Figure 3.4 : A metaphase plate of breast cancer patient with TA.

Figure 3.5 : The enlarged view of TA.
Breast Cancer patients

Figure 3.6: A metaphase plate of breast cancer patient with chromatid break.

Figure 3.7: The enlarged view of chromatid break.
Breast Cancer patients

Figure 3.8: A metaphase plate of breast cancer patient with PCS.

Figure 3.9 and 3.10: The enlarged view of PCS.
Breast Cancer patients

Figure 3.11: A metaphase plate of breast cancer patient with ring chromosome.

Figure 3.12: The enlarged view of ring chromosome.
Comparison between S group and C group: A higher percentage of gap is observed in S group (7.14%) compared to the controls (0%). However, this difference is not statistically significant.

Comparison between RT group and S group:

The percentage of gap in S group is (4.25%) (2 S out of total 47 BC) and in RT group gap were not observed. This difference is not statistically significant.

3.4 DISCUSSION

Significant increase of CA's observed in the present study of S group of BC patients compared to the Control group clearly indicates their role in the induction of BC by chromosomal instability.

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

Although it is reported from earlier studies [14],[15] that radiation increases the frequency of dicentrics, we could not find a statistically significant increase of dicentrics in the radiation treatment 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 Goa.
Further, the frequency of dicentrics is not significant in the RT+S group compared to that of S group. This again supports the active role of dicentrics in the induction of BC.

High incidence of chromatid breaks was observed in the S group of BC patients in the present study in comparison to the C group. This indicates that breaks represent a major kind of chromosomal instability responsible for the induction of BC. Further, the statistically insignificant increase of chromatid breaks in the RT group of patients compared to the S group, indicates that radiation has not induced high number of chromatid breaks in these BC patients. It has been suggested that chromosome breaks and gaps is the primary event of chromosome instability [16].

Chromosomal breaks can also be observed as the result of the clastogenic effect of physical or chemical environmental agents. However, none of our patients was known to have been exposed to such agents.

The association between exposure to viral agents and chromosomal break has been suspected for a long time [17], [18] and had recently been confirmed [19]. However, no preclinical viral infection was observed in our BC patients.
TA is found to be another important kind of chromosomal aberration seen both with S group and RT group of patients [20], [21]. They are in slightly higher frequencies in both groups compared to frequencies of breaks in them. This increase may either be due to the breaks induced at the telomeric regions or due to the sticky ends of chromosomes. Telomeric Associations (TAs) induced chromosome instability in patients with esophageal squamous cell carcinoma [22]. TAs observed in BC patients compared to controls is in agreement with Xiao et al, Paz-y-Mino et al 2001[23], Sharma HW et al 1996 [24] and is also indicative of chromosome instability in BC patients.

TAs are reported to form dicentric, multicentric and ring chromosomes in pediatric solid tumors [25]. Hence, the ring chromosomes observed in BC patients, although not statistically significant, may be due to TAs.

However, the frequency of other CAs viz gaps, ring chromosomes and PCS are very low in the BC patients of both S and RT groups. This may indicate that they are less important in the induction of BC. Further, it may also indicate that radiation may not induce such aberrations in BC patients in significant quantities.

From the present study, we may conclude that dicentrics, chromatid breaks and TA are the major kinds of chromosomal aberrations seen in
BC patients in Goa and are responsible for chromosomal instability and cancer induction in these patients. Further, we may even presume that radiation may not induce significant levels of CA in the BC patients although a slight increase of all the varieties of CAs were observed in RT groups compared to the S group.

All these observations indicate and confirm 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 clearly indicates the genetic etiology and genetic predisposition of BC in these patients.

The relationship between a high frequency of spontaneous chromosomal aberrations and some forms of cancer is reported [26], [27], [28], [29], [30], [31]. The phenomenon of increased chromosome fragility deserves further study because it might be involved in the pathogenesis of breast cancer. 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 [5].

The results of this study raise, however, important questions concerning practical monitoring of latent chromosome instability and
recognition of individuals at high risk. In this respect investigation of a greater number of patients appears to be important.

3.5 CONCLUSION

TA and dicentrics are the commonly observed chromosomal aberrations in BC patients compared to controls. This indicates that genetic instability is there 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. In addition, individuals with inherited predisposition to spontaneous chromosome breakage (Bloom's syndrome, Fanconi's anaemia and ataxia telangiectasia) have an increased risk of leukaemias and other cancers.

3.6 REFERENCES


[23] Mino C. P., Perez J. C., Davalos V., Sanchez M. E. and Leone P. E.


