FAMILY HISTORY STUDIES

CHAPTER FOUR
4.1 INTRODUCTION

'Pedigree' is a diagrammatic representation of a genealogy, a set of familial relationships. It is a method of recording genetic information in a form that can be readily and unambiguously interpreted. The word Pedigree is derived from the Latin words 'pes' and 'grus' meaning literally "foot of crane", a reference to the lines in the pedigree specifying particular relationships. Pedigrees are usually constructed with reference to a particular phenotypic (potentially genetic) trait [1].

Family history can be represented in the form of a pedigree chart. This pedigree chart can be used as a ready reference to see the role of genetic factors in breast cancer. From the pedigree chart different mutations and their inheritance can be detected. Family history can thus be helpful in diagnosing genetic disorders. It can also provide information about the natural history of the disease and about variation in its expression. It can clarify the pattern of inheritance, indicate which other family members are at risk and also allow the estimation of the recurrence of this in them.

In medical science, role of pedigree chart is self-evident. It provides a lot of valuable information for counselling the family at the basic level, including genetic counselling. Genetic counseling, can be
defined as a process by which patients or relatives at risk of a disorder that may be hereditary, are advised of the consequences of the disorder, the probability of developing and transmitting it and the ways in which this may be prevented or ameliorated.

Sporadic breast cancer: A breast cancer case with no other family history of breast carcinoma through two generations including siblings, offspring, parents, aunts and uncles and both sets of grandparents [2].

Familial breast cancer: A breast cancer case with a family history including one or more first or second degree relatives with breast cancer that does not fit the hereditary breast cancer definition given later. It is important to note that a patient with one or more first-degree relatives with breast cancer in this familial breast cancer category has an approximate three fold excess lifetime risk when compared with patients in the general population who do not have affected first-degree relatives.

Hereditary Breast Cancer (HBC): HBC is characterized by a significantly earlier age of breast cancer onset (average age of onset, 45 years), an excess of bilateral breast cancer, a greater frequency of multiple primary cancer such as cancer of the breast and ovary in the HBOC syndrome and an autosomal dominant inheritance pattern for cancer susceptibility. Surveillance and management strategies for HBC must be in accord with these clinical features and therefore clearly differ from that required for sporadic cases [3].
The terms ‘familial breast cancer’ and ‘hereditary breast cancer’ are frequently interchanged. Familial breast cancer implies that two or more first-degree relatives have had breast cancer and some familial factors are thought to underlie development of the disease. Hereditary breast cancer refers to cancer that occurs when the family history suggests a highly penetrant major gene for breast cancer, characterized by early age of onset, high incidence of bilateral disease and association with other tumours and autosomal dominant inheritance [4].

About one-third of women with breast cancer have a positive family history of one or more first-degree relatives with the disease [5]. Positive family history is an important risk factor for the development of carcinoma of the breast. The autosomal dominant transmission can be inferred from the appearance of breast cancer in multiple generations, with multiple family members being affected. As with all autosomal dominant conditions, children of an affected parent have a 50% risk of inheriting the mutated allele. The term familial breast cancer is used to describe the appearance within a family of multiple cases of breast cancer, but may be with insufficient evidence for autosomal dominant transmission [6].

The study of inherited predisposition to cancer is of clinical relevance, because family members at high risk may be helped by screening or by advice about prevention. It is also of biological interest, because the families offer a means to identify genes that may have important normal
roles in the control of growth and differentiation and which when faulty can predispose to malignancy.

Rare cases may be instructive in themselves and inherited cases may have lessons for cancers in general because mutation of the same genes may be involved in the development of the inherited and the more common non inherited forms of the same cancer.

Patients from Hereditary Breast Cancer-prone (HBC) families provide one of the most powerful and potentially cost effective models for cancer prevention and control. These families illustrate several important clinical/genetic features such as age of cancer onset, bilaterality and/or multiple primary cancer occurrences, incomplete gene penetrance, and the identification of putative obligate gene carriers. Interpretation of HBC pedigrees is dependent upon the understanding of these issues, which may enable the physician to more readily identify those individuals who might benefit from highly targeted breast cancer control measures.

Cancers generally arise from a single cell that has been changed dramatically by a series of genetic alterations. Hence, calculation of a theoretical risk for a condition with a Mendelian mode of inheritance is simple. However, in some cases it is possible to derive further information from pedigree, from consideration of additional factors and/or from supplementary tests that will permit a more accurate calculation of a specific risk.
Hereditary cancer-prone families provide one of the most powerful and potentially cost-effective models for cancer prevention and control. There are more than 200 Mendelian inherited disorders which predispose to cancer. The magnitude of the hereditary cancer problem may also be viewed in the context that about 5 to 10% of the total cancer burden is clearly hereditary, while approximately 15 to 25% is familial. Furthermore, of the remaining so-called sporadic occurrences of cancer, an unknown fraction of these will represent new germline mutations wherein, in the absence of genetic biomarker, their genetic etiology will only be recognized prospectively. Advances in biomarker research might enable highly targeted surveillance and management strategies for cancer control benefit among at-risk patients [7].

On the basis of genetic model of breast cancer and on the specific pattern of affected relatives and their age at diagnosis, the counselor can offer calculated probabilities. This strategy has the disadvantage of ignoring other individual risk factors and assuming a genetic model for every family. Thus it is hoped that having a quantitative estimate of risk in a clinical setting may motivate women at high risk to undergo surveillance [8].
The BRCA1 gene was initially localized to chromosome 17q21 in families with breast-ovarian cancer and early-onset breast cancer and cloned in 1994. BRCA1 is a large gene and is composed of 22 coding exons, distributed over roughly 100 kilo bases of genomic DNA [9]. Genetic linkage studies have shown that almost all multiple-case breast-ovarian cancer families are counted segregating mutations in BRCA1. At age 40 years, women who inherit a mutation in BRCA1 are at roughly 200-fold greater risk of breast cancer than the general population. This risk decreases somewhat with age but remains elevated roughly 15-fold to age 70 years.

The BRCA2 gene was localized to chromosome 13q and cloned in 1995 [10]. BRCA2 gene is composed of 26 coding exons distributed over roughly 70 kilo bases of genomic DNA. Like BRCA1, BRCA2 appears to be a tumor suppressor gene. (11). Like BRCA1, BRCA2 is thought to account for approximately 40% of the families with apparent hereditary predisposition to breast cancer and to confer a high risk of early-onset breast cancer in females. [12]

However, knowledge regarding all possible mutations and the relationship between risk factors and mutations is incomplete [3]. The identification of genetic mutations thought to be directly responsible for the development of breast cancer presents a major advance in our
understanding of this disease. Mutations in BRCA1 and BRCA2 are thought to be responsible for the majority of inherited breast cancer [13].

**Polymerase Chain Reaction**

Polymerase Chain Reaction (PCR) an ingenious and highly sensitive new tool in molecular biology was originally developed by Mullis and Faloona (1986). [14] The technique is based on the in vitro enzymatic synthesis / amplification of new DNA strands on the template using oligonucleotide sequences as primers.

### 4.2 MATERIALS AND METHODS

In a retrospective study of breast cancer from the Department of Radiology at Goa Medical College, Bambolim, Goa, as well as from other nearby hospitals from July 1997 to April 1999, the family histories of 95 patients were collected. Pedigree charts were made as per the guidelines of Water Fuhrmann and Fredrick Vogel [15]. Probands were contacted through self or physician referral, or through their identification in the various hospitals in Goa during the course of their breast cancer evaluation and treatment. Questionnaires were distributed to the
probands, to their first and second degree relatives (both sets of grandparents, aunts, and uncles) if available. Personal interview was made with selected relatives in order to increase the accuracy of our dataset. Information was gathered from every patient employing a questionnaire and pedigree charts were constructed and analysed. Normal, healthy females who were free from any chronic or acute diseases and those who did not have any family history for any type of cancer consisted the control group. The questionnaires included the information on hereditary as well as environmental aspects. Among environmental aspects, dietary habits and other habits such as consumption of alcohol, smoking, consumption of tobacco, pan-masala was studied. Other aspects such as mental tension, type and period of contraceptive used, exposure to radiation, to heat and to electromagnetic waves was included. Exposure to smoke (polluting gases, automobile smoke, etc.), exposure to pesticides was also included.

Blood samples were collected from Goa. Phenol-chloroform extracted DNA from blood was amplified using PCR reaction for exon 2, 5, 11, 13 and 20 of BRCA1 gene and exon 2, 9, 11, 18 and 20 of BRCA2 gene. These exons were selected because it is reported that these are frequently mutated in breast cancer. The PCR amplified products were subjected to SSCP analysis.
4.2.1 Extraction of genomic DNA from peripheral blood:

5 ml peripheral venous blood was collected in heparinised blood collection vial (15ml, Coming, USA). To this was added 3 volumes of blood lysis buffer (155mM NH₄Cl, 10mM KHCO₃, 0.1 mM EDTA pH 8.0), kept on ice for 15 min and centrifuged at 3,000 rpm for 10 min at 4°C. The supernatant was discarded and the lymphocyte cell pellet was suspended in 5ml SE solution (75mM NaCl, 20mM EDTA pH 8.0) to which was added 1ml 10% SDS and Proteinase K (100µg/ml) and incubated overnight at 37°C. After phenol, phenol: CIA and CIA extractions and precipitation in 2.5 volume of ethanol with 1/10 volume of 3M sodium Acetate. Vacuum dried DNA pellet and dissolved in TE (Tris-EDTA). The estimation of genomic DNA concentration was done by electrophoresis of 1 µl or 2 µl of DNA solution on an ethidium bromide stained 1% agarose gel with 1 µg Hind III-digested lamda DNA molecular weight marker and stored at -20°C.

4.2.2 Determination of genomic DNA concentration

The estimation of concentration of genomic DNA solution, was done using a dual beam UV spectrophotometer (Cecil, USA) at wavelengths 260 nm and 280 nm and the concentration of DNA was calculated using the following formula:

\[(\text{Absorbance} \times 260 \times \text{Dilution factor} \times 50) = \text{DNA} \mu\text{g/ml}\]

Alternatively the quality and the approximate quantity of genomic DNA was determined by electrophoresis of 1µl or 2µl of DNA solution on an
ethidium-bromide stained 1% agarose gel in 1xTE buffer with 1μg Hind III-digested lamda DNA molecular weight marker as reference.

Fig. 4.1: Determination of quality and quantity of genomic DNA from breast cancer biopsies, visualized on an ethidium bromide-stained 1% agarose gel.
Lane 1-Hind III-digested λ-DNA molecular weight marker.
Lane 2-12- Sample genomic DNA from breast cancer biopsies.

The primers which were synthesized in an ABI 381A DNA synthesizer (Applied Biosystems, Foster City, California, USA) and purified by a reverse phase HPLC (Shimadzu, Japan), were procured and amplification of different BRCA1 and BRCA2 exons was done (for primers see Table-1). A typical PCR amplification was performed in 25 μl reaction volume containing 10 mM Tris-HCl (pH 8.4), 50 mM KCl, 1.5 mM MgCl2, 200 μM of each dNTP (dATP, dCTP, dGTP, dTTP), 5 pmoles of each oligonucleotide primers, 100-500 μg of genomic DNA and 0.5U Taq DNA polymerase. (Perkin-Elmer Cetus, Norwalk, CT, USA). The temperature profiles used for amplification of exons of BRCA1 and BRCA2 are provided in Table 2.
After amplification, 10µl of PCR products was electrophoresed and visualised on an ethidium bromide strained 3% agarose gel under a short wave UV transilluminator.

Table 4.1: Oligonucleotide primers used for amplification of different exons of BRCA1 and BRCA2 gene:

<table>
<thead>
<tr>
<th>Primer</th>
<th>Nucleotide position in genomic DNA</th>
<th>PCR amplified fragment size (bp)</th>
<th>Primer Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRCA 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exon 2</td>
<td>4557-4580 4793-4814</td>
<td>258 (+)5'-GAA GTT GTC ATT TTA TAA ACC TTT-3' (-)5'-TGT CTT TTC CCT AGT ATG T-3'</td>
<td></td>
</tr>
<tr>
<td>Exon 5</td>
<td>22092-22111 22307-22326</td>
<td>235 (+)5'-CTC TTA AGG GCA GTT GTG AG -3' (-)5'-TTC CTA CTG TGG TTG CTT CC -3'</td>
<td></td>
</tr>
<tr>
<td>Exon 11</td>
<td>37187-37208 37355-37374</td>
<td>188 (+)5'-AAA GCC AGG GAG TTG GTC TGA G-3' (-)5'-GTG CTC CCA AAA GCA TAA A -3'</td>
<td></td>
</tr>
<tr>
<td>Exon 13</td>
<td>46100-46120 46399-46419</td>
<td>320 (+)5'-AAT GGA AAG CTT CTC AAA GTA -3' (-)5'-ATG TTG GAG CTA GGT CCT TAC -3'</td>
<td></td>
</tr>
<tr>
<td>Exon 20</td>
<td>71518-71537 71899-71918</td>
<td>401 (+)5'-ATA TGA CGT GTC TGC TGC TCC AC -3' (-)5'-GGG AAT CCA AAT TAC ACA GC -3'</td>
<td></td>
</tr>
<tr>
<td>BRCA 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exon 2</td>
<td>440-462 677-697</td>
<td>258 (+)5'-CTC AGT CAC ATA AGG AAT GC -3' (-)5'-CAA CAC TGT GAC GTA GGT GGT -3'</td>
<td></td>
</tr>
<tr>
<td>Exon 9</td>
<td>399-418 645-664</td>
<td>266 (+)5'-TAA CTG AAA TCA CCA AAA GT -3' (-)5'-TCA AAA CAA CAA C AA A -3'</td>
<td></td>
</tr>
<tr>
<td>Exon 11</td>
<td>4458-4478 4532-4554</td>
<td>97 (+)5'-GGG AAG CTT CAT AAG TCA GTC -3' (-)5'-TTT GTA ATG AAG CAT CTG ATA CC -3'</td>
<td></td>
</tr>
<tr>
<td>Exon 18</td>
<td>435-457 883-902</td>
<td>468 (+)5'-CTT GTT TAA ACA GTG GAA TTC TA -3' (-)5'-TAA CTG AAT CAA TGA CTG AT -3'</td>
<td></td>
</tr>
<tr>
<td>Exon 20</td>
<td>425-443 696-718</td>
<td>294 (+)5'-ACT GTG CCT GGC CTG ATA C-3' (-)5'-TGT TAA ATT CAA TCG GAG ATT CT -3'</td>
<td></td>
</tr>
</tbody>
</table>
Table 4.2: Temperature profile for amplifications of different exons of BRCA1 and BRCA2 gene.

<table>
<thead>
<tr>
<th>1 Cycle</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial denaturation</td>
<td>95°C</td>
<td>4 min</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>30 Cycles</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Denaturation</td>
<td>95°C</td>
<td>30 Sec</td>
</tr>
<tr>
<td>Primer annealing</td>
<td>58°C</td>
<td>30 Sec</td>
</tr>
<tr>
<td>Synthesis</td>
<td>72°C</td>
<td>30 Sec</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>1 Cycle</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Final extension</td>
<td>72°C</td>
<td>5 min</td>
</tr>
</tbody>
</table>

4.2.3 Single strand confirmation polymorphism (SSCP):

![Image of PCR amplification of exon 2 of BRCA1 tumor suppressor gene showing amplimer of 258 bp.](image)

Fig. 4.2: PCR amplification of exon 2 of BRCA1 tumor suppressor gene showing amplimer of 258 bp.

Lane 1: HaeIII-digested φx174 DNA molecular weight marker.
Lane 2-12: Breast cancer DNA.

SSCP was performed by radiolabelling the PCR products following regular amplification of 25 cycles for an additional 10 cycles where dCTP was replaced with $[^{32}P]dCTP$ (specific activity, 4000 Ci/m mole) (BARC, India) in the PCR mix. Thus for a typical PCR mix of 100 µl meant...
for 10 samples (10 µl each) 1 µl (10µCi) of [α-32P]dCTP was added with other reaction component being the same. For loading each sample, 1 µl radiolabelled PCR product was diluted with 9 volumes (10 times) of denaturing solution (95% formamide, 20 mM EDTA pH 8.0, 0.05% xylene cyanol and 0.05% bromophenol blue) and heat denatured for 5 min at 95°C, chilled on ice for 5 min. Three microliters of this diluted products was applied to non-denaturing electrophoresis in a 6% polyacrylamide gel with 10% glycerol. The gel was run in 0.5xTBE for 12 hr at 200V in Base Ace™ sequencing gel apparatus (Stratagene GmbH, Germany) at 17±1°C, dried and exposed to x-ray film with intensifying screen at -70°C. Alterations in electrophoretic mobility shifts in single strand DNA bands were analysed by their comparison to that of normal controls.

4.2.4 Automatic sequencing

PCR products that showed altered mobility in SSCP were sequenced using an ABI Prism 310 Automated Sequencer. Before sequencing the PCR products were purified using Ammonium Acetate / Ethanol precipitation method. The cycle sequencing of the purified products was performed using BIG Dye Terminator Cycle Sequencing Ready Reaction Mix with AmpliTaq DNA Polymerase FS, o the Gene AMP PCR 9700. The PCR conditions were set as follows: 96°C for 10 sec, 50°C for 5 sec, 60°C for 4 min., for 25 cycles. After cycle sequencing extension products were purified to remove the unincorporated dye-labeled terminators using the Ethanol / Sodium Acetate precipitation method. The air-dried labeled
purified products were resuspended in 20 μl Template Suppressor Reagent, chilled on ice and loaded on the 310 Sequencer. The sequence were analyzed using Sequencing Analysis Software 3.4.1 on a Mac OS 9.1

4.2.5 Ammonium acetate purification of PCR products for DNA sequencing

Briefly, it involves addition of an equal volume of 5M ammonium acetate (pH 5.0) and twice the volume of isopropanol to the PCR products. The mixture was vortex and incubated at room temperature for 10 min. The mixture was centrifuged for 15 min at 14,000g at 4°C, the pellet washed with 500μl 70% ethanol, dried for 15 min in vacuum and dissolved in either distilled water or TE (pH 7.4). The estimation of DNA concentration was done either by gel electrophoresis or spectrophotometrically. For checking the quality of PCR products an aliquot of purified PCR product was analyzed by electrophoresing it along with 1μg Hae-III-digested DNA molecular marker on 3% ethidium-bromide stained agarose gel (FMC Bioproducts, USA) and visualized under a UV transilluminator.

The database was created in MS-Word 2000 and the pedigree charts were prepared in MS-Powerpoint 2000.
4.3 RESULTS

The Frequency of positive family history of cancer in BC patients and controls is represented in table 4.3.

Table 4.3: Frequency of positive family history of cancer in BC patients and controls

<table>
<thead>
<tr>
<th>OBSERVATIONS</th>
<th>NUMBER</th>
<th>PERCENTAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of BC patients</td>
<td>95</td>
<td>-</td>
</tr>
<tr>
<td>Total BC Patients with positive family history</td>
<td>13</td>
<td>13.7%</td>
</tr>
<tr>
<td>of BC including other cancer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total BC Patients with positive family history</td>
<td>7 (Cases I to VII)</td>
<td>7.4%</td>
</tr>
<tr>
<td>of only BC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BC Patients with positive family history</td>
<td>6 (Cases VIII to XIII)</td>
<td>6.3%</td>
</tr>
<tr>
<td>of cancer other than BC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total no. of controls</td>
<td>66</td>
<td>-</td>
</tr>
</tbody>
</table>

Out of 95 Breast Cancer (BC) patients, 7 BC patients had positive family history of breast cancer (7.4%), whereas, 6 BC patients had family history of other types of cancer (6.3%) thus, 13 BC patients (13.7 %) were having family history of cancer and the remaining 82 BC patients (86.32 %) were sporadic.
Pedigree of BC patients with positive family history of BC

Case I:

Proband is diagnosed with BC. All the three generations were affected by breast cancer. The proband’s maternal aunt and the proband’s maternal grandmother’s sister were affected by breast cancer.
Case II:

Proband is diagnosed with BC. One generation is affected with BC. Proband’s sister was also diagnosed with BC.

Case III:

Proband is diagnosed with BC. One generation is affected by cancer. Proband’s eldest sister with BC, followed by her eldest brother who was diagnosed with liver cancer. Further, her 2nd as well as 3rd elder sisters were diagnosed with BC. Proband’s maternal uncle’s son was diagnosed with throat cancer at the age of 25. Thus, 5 out of 9 members, of the same sibling suffer from cancer.
Case IV:

Proband is diagnosed with BC. One generation is affected with breast cancer. Proband's eldest sister and her immediate younger sister had BC. The proband's paternal aunt's daughter had BC. Proband's daughter is normal.
Case V:

One generation is affected by BC. Proband was diagnosed with BC and her younger sister was also diagnosed with BC.
**Case VI:**

Proband is diagnosed with BC. One generation is affected by BC. Proband's maternal aunt's daughter was also affected with breast cancer.

**Case VII:**

Proband is diagnosed with BC. One generation is affected by BC. Proband's elder sister developed BC.
Pedigree of BC patients with positive family of cancer other than BC

Case VIII:

Proband is diagnosed with BC. Two generations were affected by cancer. Proband's maternal aunt was affected by brain tumor.
Case IX:

Proband is diagnosed with BC. Two generations were affected by cancer. Proband's father was affected by intestinal cancer.
Case X:

Proband is diagnosed with BC. Two generations are affected by cancer. Proband's maternal uncle was affected by cancer of the cheek.

Case XI:

Proband is diagnosed with BC. Proband's maternal uncle was affected with cheek cancer at 57 years. Thus, two generations are affected by cancer.
Case XII:

Proband is diagnosed with BC. Two generations are affected by cancer. Proband's maternal and paternal grandmother had cancer.
Case XIII:

Proband is diagnosed with BC. Two generations are affected by cancer. Proband's father was diagnosed with certain type of cancer.

BRCA gene analysis of BC patients: Cases III to VII were analysed for BRCA1 and 2 mutation analysis.

BRCA1 and BRCA2 mutation analysis:

BRCA1 gene mutation was detected in 2 (28.5%) out of 7 breast cancer patients analysed. This mutation occurred only in exon
2 of BRCA1 gene. Sequencing of both these samples revealed a heterozygous A-T transversion/substitution at nucleotide 4706 of BRCA1 gene leading to conversion of codon 23 from GAG to GTG showing change of amino acid from Glutamic acid to Valine. The pedigree of these two cases are shown in Case III. No mutations were detected neither in other exons of BRCA1 gene nor any of the exons of the BRCA2 gene.

<table>
<thead>
<tr>
<th>Codon</th>
<th>Nucleotide change</th>
<th>Amino acid change</th>
<th>Age</th>
<th>Family history</th>
</tr>
</thead>
<tbody>
<tr>
<td>23</td>
<td>GAG ----GTG</td>
<td>Glutamic acid ----</td>
<td>37</td>
<td>+</td>
</tr>
<tr>
<td>23</td>
<td>GAG ----GTG</td>
<td>Glutamic acid ----</td>
<td>39</td>
<td>+</td>
</tr>
</tbody>
</table>

Figure 4.3: A red peak is seen in the mutant (see arrow) indicating T which is not there in normal.
Among environmental aspects, dietary habits and other habits such as consumption of alcohol, smoking, consumption of tobacco, pan-masala was studied. Most of the breast cancer patients were not consuming alcohol, tobacco and pan-masala. One of the patients reported that there may be a co-relation between mental tension in the conscious or subconscious mind and the induction of breast cancer. Most of the patients were also not exposed to radiation, to heat, to electromagnetic waves, to smoke (polluting gases, automobile smoke, etc.) or to pesticides.

4.4 DISCUSSION

Majority of the reports available endorse the view that besides the environmental factors, genetic factors play a very important role in the induction of breast cancer. Our present observation of 7% of positive cases of family history of breast cancer is in agreement with other similar reports where 5% to 9% of breast cancers are reported to be hereditary. Hereditary breast cancer is believed to account for 5-9 per cent of all breast cancers [16] and inherited factors are thought to contribute to 25-35 per cent of cases diagnosed before the age of 30 years [17].
Figure 4.4: PCR-SSCP analysis of exon 2 of BRCA1 gene in breast cancer.

Lane 1: Normal control
Lane 2: Sample showing altered band mobility indicative of presence of a mutation.
Lane 3 to lane 7: Breast cancer samples
In Case I where three generations are affected by breast cancer, the risk of breast cancer has repeatedly been found to be increased in close relatives of breast cancer [18].

Occurrence of BC in 4 out of 9 siblings of a normal couple without BC (case III) may indicate that these siblings might have inherited the BC alleles in a recessive form from both of their parents. Hence, a recessive mode of inheritance of BC may be genetically predisposed to BC. Further, several kinds of environmental impact might have played a role in the induction of cancer in these individuals.

In cases which had two or more generations affected with breast or other type of cancer had a higher risk of breast cancer in Goan cases with family history of breast cancer. Thus, the relative risk in case one where three generations are affected by breast cancer must be very high.

Sattin et al. (1985) [19] found that the relative risk (RR) of a woman with an affected first degree relative was 2.3; for a woman with an affected second degree relative, it was 1.5 and for women with both an affected mother and sister, the RR was 14. Similarly, the relative risk according to Sattin et al can be applied to Goan BC patients.

The familial cases of BC exhibit that there is an inherited component in BC [20]. Although only a few exons were analysed in the present study, the trend shows a lower prevalence of mutation of BRCA gene in these
patients. This is in agreement with many references from various parts of
globe. [21], [22], [23], [24]. Reports on BRCA1 familial breast cancer
cases showed that 21% mutation observed in Southern region of India
[24].

Data based on genetic linkage analysis of breast cancer families
suggest that 45% of all hereditary cases of breast cancer are associated
with BRCA1 mutation [25] and 35% to BRCA2 mutation [26], [27].
However, our result shows that only 29% of the breast cancer families
carry mutation in BRCA1 gene. This prevalence is certainly lower than the
predicted values by the above groups. The higher frequency of BRCA
mutation reported by above authors [25, [26, [27], [28] [29] may be due to
the fact that those studies considered extremely high-risk families that
included multiple affected individuals for several generations. Further, the
smaller size of the family and less number of affected individuals in the
family of most of the patients of our study may perhaps explain the lower
BRCA mutation frequency.

We could observe heterozygous A-T transversion/substitution at
nuclotide 4706 in exon 2 of BRCA1 gene leading to conversion of codon
23 from GAG to GTG showing change of amino acid from Glutamic acid
to Valine. This change in BRCA1 gene of 2 cases, two first degree
relatives of particular case III may indicate the molecular level change
responsible for the induction of BC in these individuals.
The molecular mechanism of BRACA gene in case of BC is yet to be analysed fully. However, both BRCA1 and BRCA2 genes also play a crucial role in DNA repair, maintenance of genomic integrity and transcriptional regulation. Therefore any alteration in BRCA1 or BRCA2 genes may lead to loss of control of DNA repair, transcriptional activation and cell cycle regulatory activities of the cell and thereby promote the initiation of BC.

4.5 CONCLUSION

Seven percent of BC patients exhibit a positive family history of BC. A mutation was found in exon 2 of BRCA1 gene in 2 sisters represented diagrammatically in the text of case III. No mutations were there in BRCA2 gene.

Thus, case III and case IV, there is a high risk of breast cancer as studied from the pedigree chart. If complete family history is known upto many generations, the mode of inheritance also can be studied.

Thus pedigree charting is the starting point for any genetic study as far as cancer is concerned. In Indian circumstances, where the surveillance for breast cancer is quite poor, this study can be a good beginning to understand the importance of surveillance.

Genetic counseling is an important tool in the prevention of breast cancer, as well as, for its early detection. This has a very important role especially at places where the incidence of breast cancer is relatively
high. The counselor can offer calculated probabilities of the risk involved in the concerned individuals.

4.6 REFERENCES


