Review of literature:

It has been recognized for many years that family history is an important risk factor for the development of carcinoma of the breast. However, all women are not at equal risk for breast cancer. The highest known risk for breast cancer occurs to a woman whose first degree relative has had breast cancer and who is in direct lineage of hereditary breast cancer-prone (HBC) family. The significance of this risk factor is frequently missed, since the family history of cancer is often given short shrift in the clinical practice setting (Lynche/l et al, 1990). This is unfortunate since HBC's natural history typically differs from ordinary breast cancer's family history. These differences make it possible to differentiate families where multiple breast cancers were likely to have occurred by chance from those where HBC is more likely. Individuals from HBC families provide one of the most powerful and potentially cost effective models for early detection of cancer.

Breast cancers (ICD.9: 174) are responsible for 20% of all female cancer deaths worldwide. It is exceptional before the age of 20 years and is rare before 30 years but then, the incidence rises very steadily up to the age of 50 years after which the rate of increase slows down, although the incidence rate continues to rise. It is the leading cause of mortality in women aged 35-55 years, being second only to the cardiovascular diseases (Logan, 1975).

Incidence and mortality rates of breast cancer are universally increasing at a mean rate of 1-2 percent annually and soon nearly 1 million women will develop this disease every year throughout the world (Ranstam et al, 1990). Even in countries where the incidence was reported to be significantly lower.
than Western countries, it is now being reported to be on a rise. In India, this conventionally "a cancer of Western women", has revealed increased incidence over the last 20 years (Jayant, 1986). It has been recorded as the most frequent cancer among Indian women at Bombay Cancer Registry (ICMR, 1990). At the Gujarat Cancer and Research Institute (GCRI), Ahmedabad, breast cancer ranks first among all female cancers and the data of the hospital based registry reveals an age adjusted rate of 21.4 per 100,000 population (Annual report, 1994). At the national level, however, it is the second leading site among females, constituting 18% of female specific cancers. Although, these figures are generally lower than the corresponding figures from Western countries, they represent a significant proportion of the disease burden.

In spite of significant progress made regarding early detection and better management of breast cancers, there is no significant improvement in overall survival after detection (U.S. Department of Health and Human Services, National Cancer Institute, 1989). Hence, like any other cancer, prevention would be a better alternative to control the morbidity and mortality. Control of any disease depends on the sound knowledge of the causative factors. As most cancers occur as a result of interactions between environmental and genetic factors, cancer prevention programs can be targeted on any one of them.

Breast cancer can be classified in one of three categories:
1. Sporadic Breast Cancer (SBC)- Breast cancer patients with no family history of breast cancer through two generations, including maternal and paternal lineages.
2. Familial Breast Cancer (FBC)- Breast cancer patients with one or more first- or second-degree relatives who have breast cancer. The average age
of disease onset is not certain. It may also be present with diverse anatomic sites of cancer.

3. Hereditary Breast Cancer (HBC)- Breast cancer patients with one or more first- or second-degree relatives who have breast cancer, with the following characteristics:

- younger age at onset than that for familial breast cancer (average age at onset, 45 years);
- an excess of bilateral breast cancers;
- greater frequencies of breast-ovarian cancers;

It has been estimated that causation of up to 80% of cancers is related to external environmental factors (Doll and Peto, 1981). However, breast cancers are unique in this regard since the mammary epithelium is never directly exposed to the external environment. In this disease, the traditional external environmental factors have very little role to play. Most of the environmental carcinogens are acted upon by various detoxifying mechanisms before they reach the breast tissue. Hence, it can be safely presumed that the development of breast cancer is largely decided by genetic factors, interacting with internal environment of the mammary epithelium in form of various hormonal levels. Epidemiological studies have shown certain common denominators which may be associated with the risk of breast cancer. Some of them have protective effect while others increase the risk. Table 1 provides brief account of different risk factors associated with breast cancer. Reproduction associated risk factors
Table 1: Brief account of different risk factors associated with breast cancer

<table>
<thead>
<tr>
<th>Factors</th>
<th>Risk</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family history</td>
<td>Increase</td>
<td>Warmuth et al 1997</td>
</tr>
<tr>
<td>Early menarche</td>
<td>Increase</td>
<td>Petridou et al 1996</td>
</tr>
<tr>
<td>First full term pregnancy (&lt;25 years)</td>
<td>Protective</td>
<td>Canty L. 1997</td>
</tr>
<tr>
<td>Last full term pregnancy (&gt;35 years)</td>
<td>Increase</td>
<td>Hsieh et al 1996</td>
</tr>
<tr>
<td>Number of pregnancies</td>
<td>NA</td>
<td>Dietz et al 1995</td>
</tr>
<tr>
<td>Lactation</td>
<td>Protective</td>
<td>Brinton et al 1995b</td>
</tr>
<tr>
<td>Abortion (Spontaneous &amp; induced)</td>
<td>NA</td>
<td>Wingo et al 1997</td>
</tr>
<tr>
<td>Oral contraceptive</td>
<td>Increase</td>
<td>Brinton et al 1995a</td>
</tr>
<tr>
<td>Menopause (&gt;55 years)</td>
<td>Increase</td>
<td>Petridou et al 1996</td>
</tr>
<tr>
<td>Alcohol consumption</td>
<td>Increase</td>
<td>Levi et al 1996</td>
</tr>
<tr>
<td>Smoking</td>
<td>NA</td>
<td>Braga et al 1996</td>
</tr>
<tr>
<td>Height (short)</td>
<td>Protective</td>
<td>Palmer et al 1995</td>
</tr>
<tr>
<td>Physical activity</td>
<td>Protective</td>
<td>Coogan et al 1997</td>
</tr>
<tr>
<td>Body mass</td>
<td>Protective</td>
<td>De-Stefani et al 1997</td>
</tr>
<tr>
<td>Dietary fat</td>
<td>Controversial</td>
<td>Kohlmeier and Mendez 1997</td>
</tr>
</tbody>
</table>

NA = Not associated
e.g. age at menarche, age at child bearing, parity, abortions, age at menopause etc., chiefly governed by endogenous sex hormones. These factors play a more decisive role in the breast carcinogenesis than other risk factors. The duration of action and intensity of estrogens and prolactin also affect the physiology of breast epithelium.

It has been hypothesized that the level of endogenous hormones essentially determine the risk of breast cancer (Henderson et al., 1982). It is noteworthy, however, that hormone level alone cannot explain the occurrence of breast cancer. The complexity of the disease becomes more evident by different clinical forms associated with different biological activities. The postmenopausal disease is associated with late menopause, obesity etc., whereas, premenopausal disease is closely related to early menarche, spinsterhood, late childbearing and genetic factors.

There is a huge discordance in the population so far as the different risk factors are associated with the breast carcinogenesis, which may be due to nature of individual susceptibility (Miller, 1980). Therefore, the population may be divided into four different categories with respect to development of breast cancer:

1. An irreducible background level of breast cancer, "a least possible cancer incidence", is inevitable because of the inherent instability of the genetic material (Lindahl, 1993) and the unavoidable exposure to a certain low level of the mutagens (e.g. cosmic radiation) in the environment.

2. Breast cancers that result from mutagenic exposure in excess of baseline level. The person's constitutional capacity to handle mutagens may be
sufficient to deal with the background load but is unable to cope with the additional requirements.

3. Breast cancer that results from the relative genetic insufficiency to tolerate the carcinogenic exposure. This shows individual variation.

4. Cancer for which environmental influence seems to be insignificant. This is the case, in particular, for autosomal dominant neoplasms, where the initiating mutation is passed on from the germline. Inactivation of tumor suppressor gene belongs to this category.

Inherited genomic factors thus, are the major contributors intimately involved in the causation of hereditary breast cancer. The earliest evidence of the involvement of genetic factors dates back to Roman medical literature around 100 A.D., where the familial clustering of the disease has been recorded. Broca (1866) observed a family with high incidence of breast cancer, and proposed that familial aggregation of malignancy was due to some inherited factors in the affected tissue. The concept is further supported by epidemiological surveys showing increased risk of breast cancer among close relatives of HBC patients (Lynch and Lynch, 1986; Parker et al, 1996).

About one third of women with breast cancer have a positive family history of one or more first-degree relatives with the disease (Lynch and Lynch, 1986). Hereditary breast cancer is believed to account for 5-9% of all breast cancers and inherited factors are thought to contribute to 25-35% of cases diagnosed before the age of 30 years (Lynch and Lynch, 1986; Newman et al, 1988; Lynch et al, 1994; FitzGerald et al, 1996). Recently it has been reported that 10% of the patients with newly diagnosed breast cancer will have the hereditary type of this carcinoma (Parker et al, 1996). The relative risk of developing breast
cancer in a first-degree relative of a woman with the disease increases from 3.1 in those who have early-onset disease, to 5.4 in those with bilateral disease, to 8.8 in those with bilateral early-onset breast cancer (Anderson, 1992). It, therefore, appears unlikely that selection of patients for high-risk clinics based simply on a positive family history would identify susceptible individuals with great specificity or sensitivity. The sites of mutations responsible for familial breast cancer need to be determined and then woman who carry them need to be identified.

The major breast cancer susceptibility gene, BRCA1, is located on chromosome 17q12-21. Genetic linkage analysis, using polymorphic markers in this region to type more than 200 families, has determined that the odds in favor of a breast cancer susceptibility gene existing in close proximity to these markers are many millions to one (Easton et al, 1993). This consortium analysis has calculated that BRCA1 gene mutation is likely to be implicated in the aetiology of malignancy in approximately 50% of families with breast cancer.

There is a great pausity of cytogenetic data regarding the HBC. There are some reports but they are mainly concerned with sporadic type of breast cancer. The reports include:

**C-band heteromorphism, Sister chromatid exchange and Chromosome aberration:**

Whether an individual develops cancer depends to a varying degree on constitutional, perhaps hereditary factors. These are of great importance in the congenital aneuploidies such as Down's syndrome, and, chromosome breakage
syndromes such as Bloom's syndrome. These conditions have been well-studied, but they are uncommon, little is known concerning possible variation in susceptibility among the population at large. Recent reports, however, have suggested that there may be a relationship between the common heterochromatin polymorphisms and cancer susceptibility (Atkin and Brito-Babapulle, 1981). Individual's particular heterochromatin variants may have a bearing on the risk of breast cancer, and these variants might facilitate the cytogenetic changes that accompany neoplastic transformation.

Heterochromatin represents a polymorphic system in which individual chromosomes commonly show an apparently continuous variation with respect to the size of the heterochromatin region, which may also vary to some extent in location. All human chromosomes have heterochromatic region adjacent to the centromere that stain with the C-band technique. Size variation is particularly apparent among those chromosomes that normally have the largest amounts: #1, #9, and #16. The heterochromatic regions may also show "inversions", thus, some of the C-band material may be situated on the short as well as the long arm.

It is clear that individuals with heterochromatin variants show no detectable phenotypic effect. However, two aspects of heterochromatin variability, easily recognizable by current techniques, might be considered in relation to possible deleterious effects: one is where individual chromosomes constitute extreme variants, e.g., possess especially large C-band regions; and the other is where there are differences between homologs, neither of which, however, necessarily shows extreme variation from the norm.
C-band regions are generally stable and show the same appearances in different tissues (Hoehn et al., 1977). Variants usually show Mendelian inheritance, although there is some evidence for the preferential segregation of chromosomes 9qh+ (Driscoll et al., 1979). Nevertheless, new variants occasionally appear both in germ and somatic cells (Verma and Dosik, 1980).

The variants particularly involve chromosome #1, #9 and #16 but other chromosomes may also be implicated. The evidence seems to favor heteromorphism as the critical condition tending to promote neoplastic change. In view of the possible practical importance of this association in delineating high and low risk groups, and because of the light it may throw on the chromosomal events associated with neoplastic transformation, it seems desirable that a detailed investigation be made of heterochromatin polymorphism in all its forms in patients with hereditary breast cancer and their relatives.

Sensitivity to genotoxic agents is a complex phenomenon dependent on many factors emerging at the level of cell, tissue and organism. Individual differences in sensitivity result in a polymorphic response to a given genotoxicant with some individuals exhibiting increased sensitivity compared to general population (Denekamp and Rojas, 1989; Powell and McMillan, 1990). Inter-individual variability in DNA repair activity has been reported (Takano et al., 1991; Preuss et al., 1995). Some human cancer-prone syndromes such as xeroderma pigmentosum, Fanconi's anemia and ataxia telangiectasia have been shown to have both, an increased susceptibility towards DNA damaging agents and a decreased ability of cells to repair DNA damage induced by genotoxicants (Hanawalt and Surasin, 1986). The sensitivity of cells to
ionizing radiation is of practical importance (Schwartz and Vaughan, 1989). Radiosensitivity can be indicative of cancer-proneness in individuals. In addition, radiosensitive cancer patients may require an alternative treatment rather than the routine radiotherapy protocols.

Bleomycin (BLM) seems to be a perfect agent when studying DNA damage and repair, both because of its radiomimetic character and its widespread use in the treatment of many types of cancer (Ostling and Johanson, 1987; Lefterov and Koldamova, 1992). We focused our studies on hereditary breast cancer subjects and their healthy female relatives because of report indicating an increased sensitivity towards gamma radiation (Taylor et al., 1989). In addition, women heterozygous for ataxia-telangiectasia, a radiosensitive syndrome, may constitute a sizable group among breast cancer patients (Swift et al., 1987; Borresen et al., 1990). The main goal of this study was to evaluate cellular sensitivity to bleomycin and efficiency of repair of bleomycin induced DNA damage in HBC patients and their FBR.

Review of literature shows a strong association between C-band heteromorphism and occurrence of malignancies (Atkin, 1977; Petkovic, 1983; Shabtai et al., 1985). Positive reports are also available for sporadic breast cancer patients (Berger et al., 1985; Kivi and Mikelsaar, 1987). Instability of chromosome in terms of sister chromatid exchange, and, spontaneous and mutagen induced chromosome aberration is widely used as cancer risk assessment (Hsu et al., 1991, Hagmar et al., 1994, Bonassi et al., 1995). Unfortunately less attention have been given to the HBC patients. Only a few reports are available and those are for the sporadic breast cancer patients (Adhvaryu et al., 1988; Husain et al., 1992). Higher sensitivity of lymphocytes
Review of literature

to bleomycin (BLM) in sporadic breast cancer patients has been reported by Jaloszynski et al (1997). Such a study has been not carried out in case of hereditary breast cancer patients and their families.

Karyotypic abnormalities:

It is now generally accepted that tumor tissues are heterogeneous not only across the parenchyma-stroma borderline, but also among subpopulations of truly neoplastic parenchymatous cells (Heppner, 1984; Wolman and Heppner, 1992). Analyses of tumor behavior and biology must take into account the causes and consequences of this phenotypic heterogeneity, if a full understanding of the mechanisms of tumorigenesis is to be achieved.

Breast carcinomas are among the most heterogeneous human neoplasms (Patchefsky et al 1989). Several lines of research, taking the central tenet of the somatic mutation theory of cancer as their starting point, have provided evidence that genetic mechanisms are crucially involved in the generation of cell-to-cell and clone-to-clone variations in breast tumors (Meyer and Witliff, 1991; Bonsing et al, 1993; Lonn et al, 1994). In contrast to most chemistry-based investigative methods which yield pictures of an idealized average tumor cell, cytogenetic techniques reveal the karyotypic constitution of individual cells and thus are uniquely well suited to shed light on the question of intratumor genetic heterogeneity (Heim, 1992). Multiple karyotypically related as well as unrelated clones have indeed been detected in a high proportion of breast carcinomas (Pandis et al, 1995). This is all the more remarkable because these analyses probably severely underestimate the actual karyotypic variability present. The single, often small, sample routinely
examined is unlikely to be representative of all tumor areas (Pandis et al., 1994). The spatial distribution of cytogenetically disparate tumor cell subpopulations and their possible relation to zonal phenotypic heterogeneity are issues that have received practically no attention in the past.

Karyotypic abnormalities in breast cancer may be of three kinds:
- primary abnormalities, which are essential steps in establishing the tumor,
- secondary abnormalities, which occur in addition to the primary abnormality and may be important in tumor progression, and,
- cytogenetic noise, which is the background level of non-consequential aberrations distributed randomly throughout the genome (Heim and Mitelman, 1989).

About 400 carcinomas of breast with clonal karyotypic abnormalities characterized by banding technique have been reported (Mitelman, 1994). However, a large proportion of the data concerns the highly advanced tumor cells of pleural effusions, and many karyotypes have been incompletely described. The largest series was reported by Wolman and Dawson (1991) and more recently by Dutrillaux et al. (1993). Many of the differences seen among the results reached by various groups, can be put down to differences in investigative techniques. Indeed, the proportion of highly abnormal tumor karyotypes seems to be higher in direct harvesting methods (Dutrillaux et al., 1993) than when short-term cultures are relied upon (Trent et al., 1993; Pandis et al., 1995).

The most common cytogenetic aberrations in breast cancer are rearrangements of chromosome #1. In particular, whole arm translocations with chromosome
#16 and others (Kokalj-Vokac et al, 1993; Pandis et al, 1994b). Besides, deletions with breakpoints in the heterochromatic region proximal in the long arm, del(1)(q11-12), are sometimes found in breast carcinomas, either alone or part of complex karyotypes (Pandis et al, 1995). Small interstitial deletions of the short arm of chromosome #3, del(3)(p12p14) and del(3)(p13p14), were established as the defining cytogenetic feature of a subset of breast cancers by Pandis et al (1993b), larger terminal del(3p) are also relatively common and typically occur in complex karyotypes (Mitelman, 1994). Interstitial or terminal deletions of the long arm of chromosome #6, often with the proximal breakpoint in 6q21, have been described both as sole anomalies and together with other aberrations in breast carcinomas (Pandis et al, 1995). Dullrillaux et al (1990) found a remarkably high frequency of homogeneously staining regions (hsr) in 8p. Very recently, Muleris et al (1994) used another approach, a modified comparative genomic hybridization technique, to examine the primary breast carcinomas and found recurrent amplifications of the whole chromosome arms 8q and 1q as well as of smaller stretches of DNA in the chromosomal bands- 11q13, 9p13, 17q21, 1q21, 16p11, 8q22, 8q24, 10q22, 15q26, 17q23 and 20q13.

Trisomies have been described both as the only chromosome-level aberrations and as a part of complex karyotypes. Bullerdiek et al (1993) have reported trisomy 8 as the only or first aberration in primary breast carcinomas whereas, trisomies 7, 18 and 20 have been described by Pandis et al (1995) as the sole change. At least for the time being, these trisomies have to be accepted as potential primary chromosome anomalies in breast carcinogenesis.
The cytogenetic abnormalities mentioned above mainly represent the tumor tissue and effusion material of the sporadic breast cancer patients. Petersson et al (1996) have described 3p14 et al (1991) first rearrangement in the breast tissue of normal women belonging to hereditary breast cancer families. Pathak described the abnormality of chromosome #1 in the peripheral blood lymphocytes of breast cancer patients and their first degree relatives. Monakhov et al (1996) have reported constitutional abnormality of chromosomes (1)(q11-q12) and (14)(p12-pter) in the cultured lymphocytes of familial breast cancer patients and their healthy relatives. Very recently, we have reported the abnormality of chromosomes #5, #12, #16 and #17 in the cultured lymphocytes of sporadic breast cancer patients and their first degree relatives (Trivedi et al, 1998). Thus, cytogenetic findings from the cultured lymphocytes of breast cancer patients are very limited.

Positive family history is an established risk factor but, all female relatives in HBC families are not at equal risk for developing breast cancer. It is of prime importance for the preventive measure to identify the high risk relatives belonging to HBC families. Two major breast cancer susceptibility genes BRCA1 and BRCA2 have been recently mapped to a region on chromosome #17 and #13 respectively, through linkage studies of HBC and hereditary breast and ovarian cancer syndrome. It is yet not possible to study BRCA1 and BRCA2 in a routine cytogenetic laboratory because of high cost of the test. Analysis of these genes need highly sophisticated techniques and the procedure is very tedious. Besides, as more than 200 mutations are known in two genes, the analysis is very difficult.
How can we differentiate relatives at high-risk and low-risk for the development of breast cancer? Do we have simpler, sensitive and specific cytogenetic techniques for the day-to-day management of such individuals? In this context, it is interesting to study how many FBR of patients have genetic instability. Do some individuals have hidden genetic instability? Does this form a gradient in the family with the chromosome instability syndrome at one end of the spectrum? If so, what is the distribution pattern? Some of the common cytogenetic abnormalities have been described and discussed in detail in this thesis.

Considering the clinical utility of the above mentioned literature review the thesis has been divided into three sections:

Section I describes and discusses the role of size and location variation in the C-bands of HBC patients and FBR. C-bands are generally localized on the q arm of chromosomes in juxtacentromeric position. In fact, centromere of all chromosomes contain heterochromatin (C-band) but chromosomes #1, #9 and #16 are easily identifiable and large enough for precise measurement. We have attempted to quantify the C-band patterns in these chromosomes and their possible association with HBC as well as their inheritance pattern in the FBR.

In section II of the study, baseline frequencies of chromosomal aberration (CA), sister chromatid exchange (SCE) and mutagen (BLM) induced frequencies of chromosomal aberration have been analyzed to evaluate the genetic instability of the subjects. This approach may be helpful to identify the individual more susceptible to environmental mutagen which may predispose to breast cancer.
The III section deals with the karyotypic changes in patients and their relatives, to find out the common chromosomal abnormalities which may be associated with breast cancer predisposition in HBC families. Simultaneous analysis of chromosome anomalies from tumor tissue and blood lymphocytes may provide site specific (breast cells) as well as constitutional chromosomal abnormalities (lymphocytes) in the HBC patients.

Subjects were classified into three groups as follows:

1. Hereditary breast cancer (HBC) patients: Breast cancer patients who have family history of similar cancer for at least three generations.
2. Female blood relatives (FBR): Mother, maternal grandmother, maternal aunt, sister, daughter, and granddaughter were included.
3. Controls: Age matched healthy women without history of any cancer.

**Selection of cells:**

In the present study, peripheral blood lymphocytes have been selected as a test sample. It can be easily obtained from relatives. Breast tumor cells were selected for karyotypic analysis which may represent the tumor specific abnormalities in the patients. Peripheral blood lymphocytes were selected to find out the constitutional abnormalities. It was not feasible to obtain the breast cells from the healthy relatives, therefore, lymphocytes were considered as the representative somatic cells. Moreover, it has been hypothesised that the extent of genetic damage in PBL reflects similar events in the precursor cells of
carcinogenic process in the target tissues. Other advantages over selection of PBL are:

- easy to procure sample
- repeat sample is possible
- large number of scorable metaphases can be obtained

Detail questionnaire was duly filled-up by all subjects. Pedigree chart was drawn to see the penetrance of breast cancer in each family. Blood samples from patients were taken before any therapy. Similarly, samples from healthy relatives and controls were taken only when they had no medication history and major health problem for the past three months.

Well-established cytogenetic parameters have been used in the study. We have described here, nine families and well-documented HBC kindred in our resource so that, we could illustrate common cytogenetic features which appear to be pervasive in HBC kindred. Attention was then focused on how this knowledge might enable high risk relatives to be more readily identified, thereby enabling their opportunity for participation in highly organ-targeted surveillance and management strategies.