PROLOGUE
During recent years, cancer has emerged as one of the major health problems among noncommunicable diseases in India. Based on the data available from National Cancer Registry Programme, it has been estimated that approximately 6,00,000 new cancer cases occur annually (ICMR, 1982-1987). Among males, commonly encountered cancers are those related to tobacco usages, i.e. malignancies of oral cavity and upper aerodigestive tract. Being habit associated, they can be termed as self-induced and are largely preventable. Among females, predominant cancers are those of uterine cervix and breast. Cervical cancer is a disease mainly related to poor personal hygiene, Human Papilloma Virus (HPV) infection and practices' like multiple sexual partners etc. The second common female malignancy, the breast cancer is considered to have multifactorial etiology. Of several factors, hormonal and genetic factors appear to play crucial role in deciding a woman's chances of afflicting breast cancer during her lifetime.

Breast cancers (ICD.9: 174) are responsible for 20.00 % of all female cancer deaths worldwide. 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 steadily increasing universally. 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 last 20 years (Jayant, 1986). It has been recorded as the most frequent cancer among Indian women at Bombay Cancer Registry (ICMR, 1990) and now even at Ahmedabad Cancer Registry (Biennial report of Ahmedabad Cancer Registry, 1991). At the national level, however, it is the second leading site among females, constituting 18.00% 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 programmes can be targeted on any one of them. It can be made more effective either through improvements in external environment, or through identification of individuals at higher risk and providing them with closer surveillance.
It has been estimated that causation of upto 80.00 % 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 the form of various hormone levels.

Epidemiological data has shown that certain common denominators inflicting higher risk of breast cancer are endocrine associated as well as life style related factors. These factors include: age at first childbirth, parity, dietary preferences etc. Of endocrine system associated factors, early menarche (Kelsey and Hilderth, 1983; Byers et al., 1985), late first full term pregnancy (Meirik et al., 1986; Mellemgaard et al., 1990), first trimester abortions (Pike et al., 1981; Ewertz and Duffy, 1988), late menopause (Kvale and Heuch, 1988) and nulliparity (Kampert et al., 1988) have been extensively considered to increase risk of mammary carcinoma. Whereas, full term pregnancy at early age (Paffenbarger et al., 1980; Layde et al., 1989; Pathak et al., 1986), breast feeding (Mc Tiernan and Thomas, 1986; Layde et
al. (1989) and natural or induced early menopause (Negri et al. (1988; Kelsey and Hilderth, 1983; Kampert et al., 1988) have been reported to confer protection against the disease.

Other recognized risk factors, viz. body mass index (De Waard et al., 1964; Micozzi and Schatzkin, 1985) and to a certain extent younger age at menarche (De Waard and Trichopoulos, 1988), are nutrition related. It has been postulated that like reproductive factors, nutrition also alters breast cancer risk via hormonal effect (Hill and Wynder, 1976). However, during last decade the correlation between diet and breast cancer has become controversial. Several case control studies have failed to show relationship between dietary fat consumption and predisposition to breast cancer (Willett et al., 1987; Katsouyani et al., 1987; Rohan et al., 1988).

Reproduction associated risk factors like; age at childbearing, parity, abortions, menarche, menopause etc., suggest that endogenous sex hormones appear to play a decisive role. It has been hypothesized that the levels of endogenous hormones, like estrogens and prolactin essentially determine the risk of breast cancer because of the intensity and duration of their action on the breast epithelium (Henderson et al., 1982). However, hormone levels 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.

The presence of benign breast disease also increase the subsequent risk of breast cancer. These diseases embrace a wide variety of lesions in mammary gland. Though these benign conditions do not represent premalignant condition, they are considered to increase the patient's risk for developing breast cancer (Dupont and Page, 1985; Kelsey and Hilderth, 1983). No clear or consistent abnormality has been reported in women with benign breast diseases (Wang and Fentiman, 1985), however, epidemiological data have shown that females having family history of breast cancer have a higher risk of benign diseases too (Morgan et al., 1974). This possibly indicate involvement of genetic factors common for both, benign as well as malignant breast diseases.

Inherited genomic factors thus, are the other major contributors intimately involved in the causation of 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 patients, and proposed that familial
aggregation of malignancy was due to some inherited factors in the affected tissue. Since then a number of similar reports have accumulated (Anderson and Badzioch, 1985; Knudson, 1989). The concept is further supported by epidemiological surveys showing increased risk of breast cancer among close relatives of breast cancer patients (Adami et al., 1981; Tulinius et al., 1982; Lynch et al., 1984; Schneider et al., 1986). The contribution of genetic factors can be further substantiated by the fact that, unlike most adult cancers such as, carcinoma of lung, ovary, prostate or oesophagus, breast cancer can occur earlier, in third or fourth decade of life.

In spite of lengthy debate, identification of genetic factors and their precise role in the causation of breast cancer still remains enigmatic. Genetic markers for predisposition are yet not precisely known. By using specific probes particular genomic sites and their variations such as amplification, deletion or mutations can be studied at molecular level. Like all other cancers, molecular analysis of breast tumours also repeatedly point to rearrangements at DNA level. However, these highly sophisticated and expensive techniques are not feasible for large scale studies such as, population monitoring for risk assessment.

Since otherwise intricate DNA of a cell organizes into 46 distinct chromosomes only during mitosis, nature of genetic material can be studied precisely for each chromosome with
the help of various cytogenetic techniques. Advances in the field of cytogenetic methods during last two decades have tremendously improved our ability to look into the organization of the genetic material and its interactions with environmental agents. Such methods include: several specific banding procedures, techniques like Premature Chromosome Condensation (PCC) and Fluorescence In Situ Hybridization (FISH), frequencies of Chromosome Aberrations (CA), Sister Chromatid Exchanges (SCE) etc.

By the virtue of being precise, various banding techniques can detect minute interindividual variations in subchromosomal regions of the chromosome complement - known as chromosomal heteromorphism. Many of these variations are unequivocally correlated with human health impairments. Some of the cytogenetic markers are helpful in identifying malignant genotype such as presence of Philadelphia chromosome in Chronic Myeloid Leukemia (C.M.L.) or t(8,14) in Burkitt's lymphoma. However, for the premorbid risk assessment, cytogenetics has not been extensively used. In last two decades some of the chromosomal heteromorphisms, e.g. C-band variants and Ag-NOR, have been studied to see if they can be used in this regard. For the carriers of C-band heteromorphism, it has been hypothesized that they are predisposed to cancer (Atkin, 1977). Other cytogenetic parameters like structural chromosome aberrations and sister chromatid exchange frequencies are helpful in detecting DNA
damage, caused either spontaneously or inflicted by some external agent. The damage can even be quantified according to the end-point used in terms of number of CA or SCE per cell(s). Thus because of its simplicity and precision, cytogenetic approach is appropriate one and we have chosen the same for the present study.

OBJECTIVES AND STUDY DESIGN:

Cytogenetic studies were carried out utilizing short term cultures of somatic cells. In pursuit of studying predisposing genomic alterations, in PART-I it was decided to study prevalence of chromosomal heteromorphism among the individuals studied. C-band heteromorphism, representing heterochromatin variants, was considered as the parameter.

Results of PART-I revealed significantly higher prevalence of C-band heteromorphics among breast cancer patients. Since it has been hypothesized that C-band heteromorphism predisposes the carrier to malignancy (Atkin, 1977), it was important to know how this genetic heteromorphism can be related to neoplastic transformation. We have attempted to quantify genomic damage induced by in vitro treatment with the known mutagen Mitomycin-C (MMC) in PART-II. For quantifying the effects, frequencies of CA were evaluated in lymphocytes of controls as well as of breast cancer patients. Data were analyzed keeping C-band heteromorphism as the variable.
Cytogenetic end-points like SCE and CA are commonly used for genotoxicity testings, however, some papers published in recent past have reported elevation in the frequencies of these two end-points among patients suffering from different malignancies (Adhvaryu et al., 1988a; 1988b; Delhanty et al., 1983; Brown et al., 1985; Heim et al., 1985b). Even application of lymphocytic SCE as a preclinical marker of malignancy has been advocated by some authors (Mitra et al., 1982; Murty et al., 1986). We have studied frequencies of lymphocytic SCE and CA in PART-III to evaluate the validity of such applications.

SELECTION OF CELLS

Since the purpose was to study prevalence of genetic heteromorphisms, which are constitutional factors, any of the somatic tissues can be utilized. However, Peripheral Blood Lymphocytes (PBL) were selected for the present study because of several advantages. Considerations included: ease of sampling, non-invasive nature of collection procedure, possibility of repeat sampling and ease of culturing. Large number of cells in a small sample of venous blood can be obtained and can be readily stimulated to divide in vitro with various mitogens like Phytohaemagglutinin (PHA), Pokeweed mitogen etc. Adequate number of fairly good quality metaphases can be obtained following colchicine/colocond treatment. C-band heteromorphism studied in the present study is a constitutional factor, which is inherited identically in
all the tissue of an individual (Hoehn et al., 1977). Hence, short term cultures of PBL were ideal for present study. Secondly, since they have a very low rate of spontaneous chromosome aberrations and a relatively homogenous sensitivity to external agents, PBL cultures also offer easy access for studying genomic damage, either spontaneous or induced by a great variety of external agents. Thus, in view of its validity and convenience, short term cultures of PBL were used for the work envisaged.

SELECTION OF SUBJECTS

The study was addressed to assess application of cytogenetic markers in identifying individuals at elevated risk of having breast cancer, which is largely a female specific cancer. Hence, only females were included in the study. Considering the clinical conditions of the females included in the study, they were classified into three groups as follows:

(1) **Primary breast cancer patients** (ICD.9: 174) with the histological confirmation of malignancy. Blood samples were collected after the diagnosis but before the start of any anticancer therapy.

(2) **Patients having benign breast diseases**: For the present study, patients suffering from benign conditions like; cystic diseases, epithelial hyperplasia and fibroadenoma were selected.
Controls: For comparison of observations made for patients with breast cancer or with benign diseases, age matched asymptomatic healthy females were also included in this study as controls. They were selected mainly from blood bank donors and staff members of different Institutions in The New Civil Hospital Campus.

PART - I

A review of literature shows a strong association between occurrence of malignancy and presence of C-band heteromorphism in cancer patients (Atkin, 1977; Shabtai et al., 1985; Petkovic, 1983). Earlier reports from our laboratory have also shown higher prevalence of C-band heteromorphism among leukemia patients (Adhvaryu et al., 1987; 1989) and oral cancer patients (Dave et al., 1991). Hence, C-band heteromorphism was selected as the parameter for studying prevalence of chromosomal heteromorphism.

C-banding was performed with the help of C-bands by Barium Hydroxide treatment followed by Giemsa staining (CBG) technique. Although centromeres of all the chromosomes are CBG positive, the C-bands of chromosome #1, #9 and #16 are easily identifiable and are large enough for precise measurement. In addition, difference in the size of C-bands of homologous chromosomes and identification of localization variation is also very convenient, hence, C-bands only of chromosome #1, #9 and #16 were considered. C-banding was
performed for all the three groups of females included in the study. C-bands are generally localized on q arm of the chromosome in juxtaposition to the centromeres. Any deviation involving the localization of the C-band, or sufficient size difference in the C-bands of the homologues of same chromosome, were considered to constitute heteromorphism.

The data obtained were compared with the same obtained for the control group. Our findings were interpreted for following aspects:

i) Incidence of C-band heteromorphism among controls compared with benign breast disease patients and breast cancer patients, would provide information regarding difference in prevalence of C-band heteromorphism, if any, among different groups.

ii) It has been proposed that different factors are operating in causation of premenopausal and postmenopausal breast cancers. Further analysis of data would help in finding whether prevalence of genetic factors such as C-band heteromorphism, differ in premenopausal and postmenopausal patients.

iii) Incidence of C-band heteromorphism events, individually for chromosome #1, #9 and #16, in all the three groups of subjects was also analyzed to assess if any of these chromosomes have specific involvement.
PART II

Even a smallest band in human chromosome contains a large number of genes. In a good metaphase around 400 bands per haploid set can be seen with good quality banding. If we presume that human genome contains approximately 30,000 genes, then each chromosome band, would have an average of 37 genes. Compared to this, on metaphase chromosomes prominent C-bands correspond to a sizable stretch of DNA, which would account for a number of genes. Presence of heteromorphism, in the form of size variant of sufficient order or an event of inversion, in this region may constitute highly potent mutation.

If we accept the presence of heteromorphism as an event of mutation, it would be very important to see whether the carriers of one mutation (C-band heteromorphism) have differential sensitivity to DNA damaging agents, i.e. mutagens and/or clastogens. For the present study, sensitivity towards the external agents was evaluated in terms of induction of DNA damage by a known mutagen, among breast cancer patients as well as among controls. An in vitro treatment of MMC at a fixed concentration (15 ng/ml) was given to PBL cultures for 72 hours. DNA damage was assayed in terms of frequency of chromosome aberrations (CA) per cell.

Premenopausal breast cancer is believed to be more closely related to genetic factors. It was important to see
if they have differential susceptibility to external agents. If so, they would respond differentially to external agents. Hence, an attempt was made to analyze the DNA damaging effects of MMC treatments considering, C-band heteromorphism as well as menopausal status of a woman as variables.

PART III

Cytogenetic end-points like Sister Chromatid Exchange (SCE) and Chromosome Aberrations (CA) have been used for studying genomic damage at cellular level. During recent years, several authors have reported elevated frequencies of SCE and CA in somatic cells of patients having different malignancies (Adhvaryu et al., 1988a; 1988b; 1991; Barrios et al., 1988; Hsu et al., 1981; Murthy et al., 1985; Brown et al., 1985). On the basis of lymphocytic SCE frequencies being higher among cancer patients, some authors have suggested that baseline SCEs in lymphocytes can be used as a preclinical marker of malignancy (Murty et al., 1986; Mitra et al., 1982). In the present study, we have attempted to see if this hypothesis holds true for breast cancer patients as well. Proponents of this theory have observed increase of SCE among patients with cancer of uterine cervix and Human Papilloma Virus (HPV) infection plays a significant role in the etiology of this disease. Since viruses are reported to elevate SCE frequencies, increased lymphocytic SCEs in a patients with cancer of uterine cervix may not be solely
attributed to malignancy. In breast cancer patients however, no such specific external agent is known to affect the causation. Hence, it was considered worthwhile to test the proposed hypothesis in breast cancer patients. Frequencies of SCE and CA were scored from the PBL cultures of the patients with breast cancer as well as benign breast diseases. The values obtained were compared with the same obtained for controls and statistical significance of the difference, if any, was calculated using Student's t' test.

The frequencies of lymphocytic SCE have been reported to be influenced by presence of malignant tumour. In tumour bearing animals it has been suggested that rates of SCE have been elevated due to the metabolic stress imposed by the presence of malignancy in the host (Benerjee et al., 1982; Park and Grimm, 1982). To find out the exact mechanisms, blood samples from some of the breast cancer patients were collected a second time after the surgical removal of the tumour, for studying post-operative lymphocytic SCE and CA frequencies.

It has been reported that certain tumours produce clastogenic substances, which, on release in the circulatory system, inflict chromosomal damage in somatic tissues (Nordenson et al., 1984). To evaluate validity of this assumption, we have carried out short term in vitro experiments using Chinese Hamster Ovary (CHO) cells. For
these experiments, sera from breast cancer patients as well as controls were separated from their blood samples. CHO cells were grown in medium containing sera obtained from breast cancer patients and from the controls. SCE frequencies were examined as the endpoint.