SECTION II
CHAPTER 1

INTRODUCTION
CHAPTER ONE

INTRODUCTION

Breast cancer is the most common malignancy affecting women worldwide (Parkin, 2004), accounting for 25% of all new cases of cancer. One in eight to one in twelve women is likely to suffer from Breast cancer during her life-time in the developed countries and one in twenty two is likely to have the disease in developing countries. Breast cancer incidence rate varies at least tenfold worldwide (Parkin et al., 2001) largely because of range of socio-economic, reproductive, hormonal, nutritional and genetic factors (Mcpherson et al., 2000). Areas of high-incidence include North America and Europe (Parkin et al., 2001). South-east Asia contributes over 1/5th of the worldwide prevalence of the disease with Pakistan having highest incidence among Asian population after Israel (Bhurgri et al., 2000). In India, breast cancer is the second common cancer in women after cervical cancer; however breast cancer has replaced cervical cancer as the leading site of cancer among women in Indian cities (ICMR, 2001). Kashmir, which lies at an altitude of 1800-2400 km from the sea-level and borders the low-incidence country, India and the high-incidence area of Pakistan, is distinct from other areas in term of its unique geographical locale, intra-community marriages, tradition, culture, food habits and ethnicity. Over the past few years, the valley has witnessed an increase in the incidence of breast cancer and threatens to overtake oesophageal cancer as the most common cancer among Kashmiri women.

Breast Cancer refers to cancers originating from breast tissue, most commonly from the epithelial cells that line the milk ducts or the lobules that supply the ducts with milk (Jensen, 1976). Depending on wherein the glandular or ductal unit of the breast the cancer arises, it develops certain characteristics that are used to sub-classify breast cancer into types. Cancers originating from
ducts are known as ductal carcinomas and those originating from lobules are known as lobular carcinomas. The primary tumor begins in the breast itself but once it becomes invasive, it may progress beyond the breast to the regional lymph nodes or travel (metastasize) to other organ systems in the body and become systemic in nature. Cancer cells that remain confined to the boundaries of the lobular unit or the draining duct are classified as in situ or non-invasive. An invasive breast cancer is one in which there is dissemination of cancer cells outside the basement membrane of the ducts and lobules into the surrounding adjacent normal tissue. There are many different types of breast cancer, with different stages (spread), aggressiveness, and genetic makeup and the survival of the patient varies greatly depending on those factors (6).

Although many risk factors of the disease like ageing (Moolgavkar et al., 1979), early menopause (Magnusson et al., 1998), alcohol consumption (Hamajima et al., 2002), radiation exposure (Grevias-Faqnou et al., 1999), tobacco use (Hamajima et al., 2002), obesity or over weight (Calle et al., 2003), physical activity (Bray et al., 2004), urbanization (McMichael et al., 1998), sedentary life style, dietary fat (Schulz et al., 2008), changes in reproductive patterns (Lord et al., 2008), such as delayed childbearing and having fewer children, nulliparity (Huo et al., 2008; Winer et al., 2000), no breast-feeding (Huo et al., 2008; Lord et al., 2008; Zeng et al., 2010), multiple abortions (Zeng et al., 2010) and post-menopausal hormone replacement therapy (Beral, 2004), stand identified but a higher proportion of breast cancer is associated with a strong family history, with first-degree relatives of patients having an approximately two fold elevated risk (Colditz et al., 1993; Madigan et al., 1993).

Genetic susceptibility as a result of highly penetrant germ-line inactivation in cancer predisposing genes characterizes approximately 5-10% of breast cancer and 25% of the early onset of breast cancer (Palma et al, 2006; Wooster et al., 1994). Several genes have been identified that are associated with inherited susceptibility to breast cancer and have provided means to begin identifying individuals and families with an increased risk of cancer. One of the
most exciting and highly anticipated breakthrough in cancer genetics was the cloning of BRCA1 and BRCA2 in early nineties (Wooster et al., 1994; Miki et al., 1994). BRCA1 and BRCA2 genes appear to account for the majority of hereditary breast cancer cases via autosomal dominant inheritance mechanism. Tumorigenesis in women with BRCA1 or BRCA2 mutations requires the loss or inactivation of the remaining wild-type allele, resulting in expression of a nonfunctional protein and a loss of cell cycle control and DNA repair mechanisms (Welch & King, 2001). BRCA1 and BRCA2 perform multiple discrete functions like regulates DNA repair, gene transcription and maintain genome integrity and tumor is initiated when genetic instabilities lead to increased mutations in these genes (Hall et al., 1990). In addition, many other genes like p53, ATM, CHEK2, MDM2, PTEN, the GADD repair group, the HER2/neu oncogene (Campeau et al., 2008; Lavin, 1998; Nechushtan et al., 2009; Walsh et al., 2006) have also been implicated in the genesis of the disease. However, their involvement account for only a minor fraction of breast cancers. Some of these genes directly influence BC risk, whereas others are involved in the general processes of cancer growth and metastasis.

In families with breast cancer consistent with hereditary breast cancer, it has been reported that BRCA1 mutations account for approximately 45% of families with significantly high breast cancer incidence and at least 80% of families with increased risk of both early-onset breast and ovarian cancer (Ford et al., 1994; Thompson & Easton, 2002). Whereas germ-line mutations of BRCA2 are predicted to account for approximately 35% of families with multiple cases, early onset female breast cancer, and they are also associated with an increased risk of male breast cancer and ovarian cancer (Gayther et al., 1997; Thorlacius et al., 1996). In women the overall risk of breast cancer associated with mutations in the BRCA1 or BRCA2 gene is from 40% - 85% over a lifetime, whereas the lifetime risk in the general population is approximately 12.5%, which differ in populations (Japan (2%) and USA (14%)) (Antoniou et al., 2002; Begg, 2002). BRCA1 or BRCA2 mutation carriers (women) with a history of breast cancer
exhibit an elevated risk of contralateral breast cancer, at 40% to 60% (Begg, 2002). BRCA1 and BRCA2 mutation carriers also have an elevated risk of ovarian cancer, ranging from 15% to 40%, compared with an approximate risk of 2% in the general population (Ford et al., 1998). It is generally accepted, that carriers of mutations in BRCA1 or BRCA2 have an excessive risk for both breast and ovarian cancer, thus screening for and detection of BRCA1/BRCA2 mutations may be helpful in determining the overall risk for the development of breast carcinoma, especially in families with hereditary cases. Individuals who are mutation carriers can undertake different surveillance strategies, chemoprevention interventions, or surgical prophylaxis for carcinomas of the breast and ovary.

BRCA2 was the second breast and/or ovarian cancer susceptibility gene to be discovered, mutated form of which when inherited strongly predispose to breast or breast and ovarian cancers (Sowter & Ashworth 2005; Wooster et al., 1995). BRCA2 plays an important role in the error-free repair of DNA double strand breaks as well as transcriptional regulation (Davies et al., 2001; Moynahan et al., 2001; Pellegrini et al., 2002). In normal cells, BRCA2 ensures the stability of the cell’s genetic material (DNA) and helps to prevent uncontrolled cell growth. The spectrum of BRCA2 mutations has been characterized in different populations worldwide, with significant variation of the relative contribution of these genes to hereditary cancer between populations. Various population-based studies have shown population specific BRCA2 founder mutations and also variable number of novel mutations in different populations, and thus have defined high and low risk subsets for developing breast cancer based on ethnic origin (Oddoux et al., 1996; Thorlacius et al., 1997). Since Kashmiri women represent a cohort of genetically pure population, the mutational pattern of BRCA2 can serve as genetic marker for the identification of women who are at high risk of breast cancer.

Among the other genes likely to be involved in breast cancer is the gap-junction gene Connexin43, which codes for a 43-kd gap-junction protein.
Connexin forms membrane spanning hexameric hemicannels called as connexons that, when in register in apposed cell membranes, couple to form a continuous hydrophilic channel through which ions, metabolites, and molecules involved in signal transduction can move from cell to cell. An array of many such channels constitutes a gap junction. Gap junctions initially identified to serve as passageways for the cell-to-cell exchange of low molecular weight growth regulatory molecules (Flagg-Newton et al., 1997; Loewenstein, 1981; Loewenstein & Kanno, 1966), have now been proposed to play an important regulatory role in cell growth, cell differentiation, cell apoptosis and tissue development (El Sabban et al., 2003; Gramsch et al., 2001; Ma & Dahl, 2006; Vinken et al., 2006; Wiszniewski et al., 2000). Down regulation of intercellular communication via gap junctions, either by down regulation or mistargeting of connexins (due to post-translational processing, germline or somatic mutations), appears to play a role in many types of pathologies, including cancer (Laird et al., 2006; Mesnil et al., 2005; Severs et al., 2004). A large number of studies have indicated that connexins like Cx43, Cx32, Cx26 have tumor suppressing effects due to maintenance of cellular homeostasis via gap junctional intercellular communication and thus are players in the arena of growth regulation and in many types of cancer. (King et al., 2005; Naus et al., 1992). Studies on neoplastic cells using ultrastructural, biochemical and immunological means and by introduction of fluorescent or radio-active tracers have shown that the majority of neoplastic cells have fewer and smaller gap junctions, express less connexins and have reduced GJIC compared to their non-neoplastic counter-parts and up-regulation of Cxs has been shown to restore normal phenotypes and retard tumor cell growth (Qin et al., 2002).

Connexin 43 is the predominantly expressed gap junction protein in normal breast tissue and plays an important role in normal mammogenesis, lactogenesis and involution (Cai et al., 1998; Monaghan et al., 1996). Studies have shown down-regulation of connexin 43 gap-junction protein in human breast cancer tissues or a relocalization of the connexin to intracellular
compartments, resulting in a predicted loss of GJIC compared to matched normal or benign breast tissue (Kanczuga-Koda et al., 2003, 2005; Laird et al., 1999; Lee et al., 1991; Wilgenbus et al., 1992). Cx43 gap junction down-regulation has been observed in breast tissue at various stages of tumorigenesis, including Ductal carcinoma in situ (DCIS), invasive Infiltrating ductal carcinoma (IDC), and infiltrating lobular carcinoma (ILC) and restoration of gap-junction intercellular communication by up-regulation of connexins has been shown to restore normal phenotypes in vitro and reduce tumor growth in vivo (Hirschi et al., 1996; Mehta et al., 1991). Studies have also shown that down-regulation of endogenous Connexin 43 expression by small interfering RNA promoted a more aggressive phenotype in human breast cancer cell lines (Shao et al., 2005). Further more, data obtained with rat mammary carcinoma induced by DMBA also demonstrates that the loss of Connexin 43 gap junction is a common feature of mammary neoplastic transformed. Studies have also shown that Cx43 expression in cancer cells leads to decrease expression of proteins involved in increased motility, invasion and metastases clearly indicating that it contributes to decreased metastasis in vivo. However the molecular mechanisms behind the down-regulation of Connexin 43 and contribution to development of primary tumor and its metastasis in breast remain elusive. A better understanding of the key events that lead to the down-regulation of connexins in breast cancer is necessary to gain information relevant to the designing of anti-cancer treatment models against breast cancer. Multiple mechanisms appear to be responsible for the down regulation of Connexin43 in breast neoplastic tissue, and one of the potent mechanisms can be mutations in gap-junctions genes.

We propose to study the sequence variations in breast cancer patients of the Kashmir region, and workout association of such variations (if any) with the disease phenotype. Despite the existence of a large population of breast cancer patients, there is a paucity of such kind of data from the state of the Jammu and Kashmir. The present study is therefore aimed to investigate the mutation status
Introduction

of BRCA2 and Connexin 43 in Kashmiri breast cancer patients to verify its possible role in breast cancer incidence and development in Kashmiri population.