INTRODUCTION
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Cancer is the second leading cause of death among populations after cardiovascular disease and one of the most important health problems of the current era (Siegel et al. 2012). Breast cancer starts in tissues of the breast, usually the ducts (tubes that carry milk to the nipple) and lobules (glands that make milk). Generally it occurs in women but rarely in men (American Cancer Society, 2013). Breast cancer is the most common form of cancer in women and the second most common cause of cancer death for women worldwide. Breast cancer is expected to account for 29% (226,870) of all new cancer cases among women. Majority of new breast cancers are diagnosed as a result of an abnormality seen on a mammogram as a lump or change in uniformity of the breast tissue felt with the fingertips can also be a warning symptom of the disease (Jorgensen and Gotzsche 2006). In spite of the higher degree of awareness of breast cancer, it is the most prevalent cause of fatality in women between the ages of 45 and 55 (Khan and Bui 2010).

As far as the prevalence of breast cancer is concerned, an age standardized incidence rate is 19.3 per 100,000 women in Eastern Africa to 89.7 per 100,000 women in Western Europe and are more than 80 per 100,000 in developed regions of the world (except Japan) and less than 40 per 100,000) in most of the developing regions. Breast cancer accounts for 23% of all newly occurring cancers in women worldwide and represents 12.7% of all cancer
deaths. Although incidence rates are higher in the West, the disability adjusted life years (DALY’s) show the highest burden for breast cancer in middle income countries, where there are increasing incidence rates and a higher proportion with later stages of disease at diagnosis. Male breast cancer is rare compared to female breast cancer (Ferlay et al. 2010).

Although breast cancer is the second most common cancer in all Indian women (Cervix cancer is the most common one), but it is the most common cancer in metropolitan cities (Delhi, Chennai, Mumbai, Kolkata, and Bangalore etc.). It is predicted to be the most common type of cancer in the coming decade (SANCD 2009). An age standardized incidence rate is 22.9 cases per 100,000 women. It represents 12.1% of overall cancer incidences. Breast cancer death rate is 11.1 cases per 100,000 women. There were 53592 deaths in 2008 due to breast cancer which represents 8.5% of all cancer deaths (Ferlay et al. 2010).

Breast cancer begins from the terminal duct lobular unit of breast tissue. Breast cancer that has not invaded the basement membrane and thus confined within the terminal duct lobular unit and is coined as carcinoma in situ. Mainly there are two different types of in situ cancers; lobular carcinoma in situ and ductal carcinoma in situ. On the other hand, breast cancers that penetrate the basement membrane are called invasive cancers. The two main types are synonymously called invasive lobular and ductal carcinoma. The main difference between invasive and in situ cancers is the ability of the invasive forms to spread through the lymphatic and vascular vessels located under
basement membrane leading to regional lymphatic and distant organ metastases. Four major molecular subtypes of breast cancer are Luminal A, Luminal B, Triple negative/basal like and Her-2 type. This classification is based on the presence or absence of estrogen, progesterone or human epithelial growth factor receptors on the tumor cells (Pusztai et al. 2006).

As in the case of most of the cancers, staging of breast cancer considers the size of the tumor (T), the number and location of metastatic lymph nodes (N), and distant organ metastasis (M) (Greene et al. 2002). According to the TNM staging system, breast cancer patients are divided into stages from I to IV. Stages I and II are identified as early stages while stage III designates locally advanced breast cancer and stage IV is regarded as late stage.

Genetically it is categorized as familial and sporadic breast cancer. Familial breast cancer is those who arise as consequences of family history of the disease. For this cancer mostly BRCA1 and BRCA2 gene mutations are regarded as the causative factor. In addition to this BRCA1 and BRCA2 gene mutations in the PTEN, TP53, and ATM etc. are known or suspected to be associated with an increased risk of breast cancer (Liaw et al. 1997; Birch et al. 1994). Sporadic breast cancers arise spontaneously or sporadically, rather through inheritance of certain genes. This may occurs due to the epigenetic effects (DNA methylation) or the defects of DNA repair mechanisms that allow environmentally triggered mutations to accumulate.
After a sincere genetic linkage examination of 23 early onset of breast cancer, BRCA1 was first located to chromosome 17 (Hall et al. 1990). Subsequent researches localized it to BRCA1 is a 100 kb long gene and found at 17q21 position. BRCA1 is comprised of 24 exons, including 2 nontranslating exons. It encodes a protein of 1863 amino acids which constitute two characteristic domains, a zinc binding RING finger domain at the amino terminus and BRCA1 carboxyl terminal (BRCT) domain at the carboxyl terminus. BRCA1 is a tumor suppressor gene and plays a pivotal role in maintenance of cell cycle and repair of DNA damage. After ionizing radiation BRCA1 protein is phosphorylated by the checkpoint kinase ataxia telangiectasia mutated (ATM) protein (Cortez et al. 1999). Mediator of DNA damage checkpoint protein 1 (MDC1) can regulate BRCA1 to the sites of DNA lesions and phosphorylate it through ATM dependent pathways (Lou et al. 2003). After activation, BRCA1 can bind to p53, RAD50-MRE11-NBS1 (R-M-N) complex and RAD51 then conducting homologous recombination or nonhomologous end joining (NHEJ) which is of great importance in repair of DNA damage. The zinc binding RING finger domain of BRCA1 can interact with BRCA1 associated RING domain 1 (BARD1) (Alshatwi et al. 2012a) forming a heterodimeric complex that has ubiquitin ligase activity and the complex itself may be involved in DNA damage repair.

Due to their key role in maintaining genomic integrity and supervising cell cycle, mutations in BRCA1 is found strongly associated with hereditary
breast cancers. But the homozygous mutations may cause the sporadic counterpart. \textit{BRCA1} mutation frequency is 0.4% and 7% in Finnish and Swedish population, respectively (Teng \textit{et al.} 2008). Most of the genetic alterations in \textit{BRCA1} yield a truncated protein (Struwing \textit{et al.} 1995). The cells therefore have a decreased ability to repair damaged DNA.

Single nucleotide polymorphisms frequently called SNPs are the most common type of genetic variation. Each SNP represents a difference in a single nucleotide. They occur once in every 300 nucleotides on average which means there are roughly 10 million SNPs in the human genome. Presently dbSNP (NCBI-dbSNP build 138, 2013) database records more than 5.3 million active human SNPs. All common SNPs have only two alleles. The genomic distribution of SNPs is not homogenous. SNPs usually occur in noncoding regions more frequently than in coding (Barreiro \textit{et al.} 2008). SNPs occur within a gene or in a regulatory region near a gene may act as biological markers and may help to locate genes that are associated with disease. SNPs also help to predict an individual’s response to certain drugs, susceptibility to environmental factors such as toxins and risk of developing particular diseases. SNPs can also be used to track the inheritance of disease genes within families.

SNPs occurring in coding region of a gene are synonymous and non-synonymous. Synonymous SNPs do not change the amino acid in proteins while non-synonymous cause changes in amino acid. dbSNP database have records of 108 SNPs in the coding region of \textit{BRCA1}. Among them 65 are nonsynonymous
SNPs (nsSNPs) which may affect the structure and function of protein. In addition non-coding regions such as 3’ and 5’ untranslated regions (UTRs), intronic region (e.g. splicing sites of RNA transcript) may also affect the function of genes. For example activity of a gene promoter can be affected by the SNPs in promoter sequence of a gene. The change in nucleotide may affect the affinity between transcription factors involved in regulation of gene expression and promoter (Lu et al. 2015; Yu et al. 2004). Although BRCA1 SNPs such as c.-2613G>C (rs799907) and c.-2004G>A (rs799906) do not make any substantial effect on breast cancer occurrence (Freedman et al. 2005). But c.-2265C>T (rs11655505) has been found to enhance the promoter activity (Chan et al. 2009).

In normal cells actively transcribing chromatin is hypomethylated whereas non-transcribing chromatin is methylated in such a way that it makes compact structures that hinder RNA Pol II activities (Lorincz et al. 2004). The methylation of DNA is found to be involved in the stabilization of chromatin structure in an inactive conformation and inhibits gene transcription (Keshet et al. 1986). Therefore it is believed that the mechanism of gene regulation through DNA methylation which involves essential genetic events including differentiation, genomic imprinting and X-chromosome inactivation (Bernardino-Sgherri et al. 2002; Richardson and Yung 2002).

Changes in DNA methylation patterns may contribute to the development of cancer. Aberrant global methylation of DNA is frequently found in tumor
cells. Global hypomethylation can result in chromosome instability and hypermethylation has been associated with the inaction of tumor suppressor genes. The main target for DNA methylation is cytosine. The majority of 5’-methylcytosine in mammalian DNA is in cytosine–guanine (CpG) island which are present in promoter regions of many genes. Among all human promoters, 72% contain high CpG content and 28% of promoters are those with CpG content characteristic to the overall genome, i.e. low CpG content (Saxonov et al. 2006). As far as the whole human genome is concerned, CpGs are present approximately once per 80 dinucleotides in 98% of the genome; however the CpG islands comprise 1–2% of the human genome and the frequency is five times higher (Bird 1986).

Hypermethylation of the BRCA1 promoter has been reported for inactivation of BRCA1. That occurs in 7–31% of breast and ovarian cancer patients with no familial history. BRCA1 methylated sporadic tumors display pathologic features similar to those of BRCA1 mutated hereditary breast cancers. Sporadic tumors with aberrant methylation of the BRCA1 promoter can be clustered with tumors derived from women with inherited BRCA1 germ line mutations because of similarities in their global gene expression profiles (Hasan et al. 2013).

Since SNPs in BRCA1 promoter region may affect the promoter activity and the change in methylation pattern which may cause the gene silencing. Hence the present study was undertaken to find out the effect of c.-2265C>T
(rs11655505) on the occurrence of breast cancer and; effect of \textit{BRCA1} promoter methylation pattern and c.-2265C>T (rs11655505) SNP on \textit{BRCA1} expression and occurrence of sporadic breast cancer was studied.

The outcomes of this study are expected to provide us an insight in to the association of c.-2265C>T (rs11655505) with occurrence of breast cancer and association of \textit{BRCA1} promoter methylation with rs11655505 (c.-2265 C/T) variation and gene expression in sporadic breast cancer.