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BRCA and Breast Cancer Risk: A Bio-Environmental Susceptibility Study, India

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Abstract

Breast cancer is considered to be the most frequent malignancy among women all over the world. The present study attempts to understand the molecular heterogeneity of BRCA1 and BRCA2 genes, as well as to understand the association of various lifestyle and reproductive variables for the risk of the disease. The study was conducted in a total of 110 patients and 128 controls that revealed DNA sequencing of ten Single Nucleotide Polymorphisms (SNPs) (6 novel). Significant ($p<0.005$) molecular heterogeneity is revealed in terms of SNPs in BRCA1 (4 exonic & 1 intronic variants) and BRCA2 (2exonic and 3 intronic variants) genes. The augmentation study revealed significant ($p<0.005$) association with positive family history, early age at menarche, irregular menstrual periods, menopause, prolong contraceptive use, nulliparity, history of abortions, consumption of alcohol and smoking towards disease risk. This study being the first of its kind, envisaged that the identification of the SNPs and modification of the lifestyle factors might aid to minimize the risk among the Bengalee Hindu females.

Keywords: BRCA1; BRCA2; Breast Cancer Risk; Bengalee Hindu Females; Lifestyle and Reproductive Variables; Snps

Introduction

Breast cancer, a complex multifactorial disease, is one of the most common malignancies among women worldwide. Breast cancer is the most common form of cancer and the second most common cause of death from a neoplastic disease affecting women. Breast cancer is a disease in which breast cells become abnormal and multiply to form a malignant tumor. One in eight women will develop breast cancer in her lifetime [1,2]. Breast cancer is now the most common cancer in both developed and developing countries. The disease ranks as the fifth cause of death from cancer, but it is still the most frequent cause of cancer death in women in both developing countries and developed regions [3]. Most malignancies are not due to the heredity. In general, about 10% of breast cancer and 5% of ovarian cancer cases are contracted by carriers of defective genes [4].

There are several ways that trigger the genetic susceptibility to cancer; the best understood cause is the inactivating germline mutations in tumor suppressor and DNA repair genes, which leads to an accumulation of mutations in oncogenes and cell-cycle checkpoints that

are required for uncontrolled cell division [5]. Germline mutations of the BRCA1 and BRCA2 genes represent the most significant and thus so far the best characterized genetic risk factors for breast cancer development [6]. Most of the reported mutations in tumor suppressor genes are characterized by deletions, insertions, nonsense mutations and splice variants that result in a truncated protein [7]. Nevertheless, human breast cancer results from a number of genetic and environmental interaction, and genes, which interacts with environmental carcinogens and lifestyle factors [8]. Therefore, breast cancer eventually demonstrated uneven spatial distribution in occurrence reflecting the influence of local environmental conditions, lifestyle, hormonal, reproductive pattern and genetic predisposition in the development of the condition [9,10]. A number of studies have attempted to understand the possible association between certain lifestyle variables and risk for breast cancer. Women, who underwent more menstrual cycles having early age at menarche or underwent late menopause were found to have elevated risk of breast cancer [11]. Similarly, nulliparous women were also found to have increased risk of the disease, might be due to nulliparous women have undifferentiated cells without differentiation, that retains high concentration of epithelial cells that are targets for carcinogens and therefore undergoes neoplastic transformation [12-15]. However, studies also reported that breast feeding reduces breast cancer risk regulated by hormonal mechanisms [12,16,17].

Effect of Mutations in the genes leading to breast cancer from India remains relatively unexplored apart from a few small studies [18-23]. However, mutations in susceptible BRCA1 and BRCA2 genes for breast cancer among Bengalee population are yet to be carried out.

Objectives

The present study aims to study the polymorphisms in BRCA1 and BRCA2 genes and their association with certain lifestyle variables that modify the risk of Breast cancer and susceptibility.

Materials and Methods

Collection of samples: The study includes 110 patients with histopathologically confirmed Breast Carcinoma visiting the Cancer Centre Welfare Home and Research Institute, Kolkata, India and National Medical College and Hospital, Kolkata. Ethical approval of the research project using human subjects was obtained from the Institutional Ethical Committee. The socio-demographic data (age, caste, origin, occupation, family history, educational status, etc) , reproductive (age at menarche, regularity of menstrual periods, age at menopause, parity, number of issues) and lifestyle (post-menopausal hormone therapy, abortions, use of oral contraceptives, alcohol consumption and smoking practices) data were collected using specially prepared pre tested schedule. Age at menarche was defined as the chronological age when the women first had her menses; age at menopause was defined as the chronological age when the natural period ceases without any effect of medical or pathological interference. Apart from the patients age, sex and ethnic matched 128 controls were collected without any family history of breast cancer for the present study.

DNA Isolation: Genomic DNA was prepared from fresh whole blood (5ml) by using the conventional phenol–chloroform method [24].

PCR Amplification: Polymerase chain reaction (PCR) was carried out to amplify exons and flanking regions in a Thermocycler (GeneAmp-9700; PE Applied Biosystems, Foster City, CA). PCR amplified DNA fragments were analyzed on 2% agarose gel and then visualized by ethidium bromide staining.

Mutation and Polymorphism Detection: The amplified products were directly sequenced in forward and reverse direction in DNA Analyzer 3730 (Applied Biosystems, USA). Nucleotide changes were detected by comparing sequence obtained in chromatogram with the normal gene sequences using pair-wise BLAST and SeqScape software v2.5

Descriptive and inferential statistics has been applied in appropriate places for analyzing the data using the statistical software SPSS 17.0 version. Cut off was set as p=0.005.

Results

A total of 238 participants (110 histopathologically confirmed breast cancer patients and another 128 age, sex and ethnic group matched controls, without any personal and family history of breast cancer) were included in the present study and presented in table 1.

	Mean ± SD	Range (Years)	Total
Patients	54.037 ± 0.383	30 – 78	110
Controls	54.609 ± 8.005	38 – 72	128

Table 1: Present study of participants.

The molecular sequencing of BRCA2 gene identifies 3 intronic variants and two exonic variants (Table 2). 19.4% of the patients and 1.57% of the controls is analyzed with exon 2 mutation (rs1799943) with significant association ($p<0.005$), which changes the amino acid from Serine to Asparagine. Whereas the variation in the coding region of exon 9 changes the amino acid from Proline to Leucine among 7.05% of the patients only. The intronic variants doesn't exhibit any amino acid changes as they are present in the non-coding region of the DNA, but all of them have significant associations ($p<0.005$) for Breast cancer in the studied cohort. Intron 9 (rs2126042) mutations is present among 22.2% of the patients and 6.29% of the controls. Two novel variants are identified in Intron 9, one (IVS9+139 T>C) is present among 8.33% of the patients and 0.787% of the controls, another (IVS9+145 T>C) is present among only 11.1% of the patients.

The function of the non-coding regions of the DNA is less understood than that of the coding DNA, researchers are left to speculate the functional effect of non-synonymous polymorphisms. Since polymorphism and variations are the themes in anthropology, therefore, the present study certainly possess anthropological interest with regard to the significance of both synonymous and non-synonymous variants for breast cancer risk among the studied cohort.

Exon/ Intron	Nucleotide Change	Amino Acid Change	Patient (%) N=108	Control (%) N=127	SNP Status	P Value
Exon 2	AGC>AAC	Ser> Asn	21 (19.4)	2 (1.57)	Reported (rs1799943)	0.0001
Intron 9	CCT>CTT	NA	24 (22.2)	8 (6.29)	Reported (rs2126042)	0.0002
Exon 9	CCT>CTT	Pro>Leu	8 (7.407)	0	Reported (rs80359633)	0.0001
Intron 9	IVS9+139 T>C	NA	9 (8.33)	1 (6.787)	NOVEL (rs04)	0.0001
Intron 9	IVS9+145 T>C	NA	12(11.11)	0	NOVEL (rs05)	0.0001

Table 2: Identifies of three Intronic variants and two Exonic Variants.

Socio demographic characteristics (Table 3) revealed significant differences in occupation ($p<0.005$), family history ($p<0.005$) and marital status ($p<0.005$) among the breast cancer patients in comparison to controls. Interestingly, 66.7% of the patients had positive family history of breast cancer, whereas no controls had any family history of cancer.

Characters	Status (Patients)
Education	NS
(Graduate)	
Occupation	0.30**
(Service)	(0.129 – 0.682)
Marital Status	NS
(Unmarried)	
Family History	0.028***
(No)	(0.0009 – 0.092)
Age at Menarche	NS
(Below 12 years)	
Regularity of Menstrual Periods	9.40***
(Irregular)	(2.092 – 42.22)
Use of Oral Contraceptive Pills	2.578***
(Yes)	(1.00 – 6.65)
Abortions	17.02***
(Yes)	(2.03 – 143.15)
Parity	NS
(Nulliparity)	

Number of Issues	NS
(Only One)	
Breast Feeding Duration	0.261**
(>3months)	(0.09 – 0.690)
Which Breast Fed	NS
(Only one)	
Menopausal Status	NS
(Post-menopause)	
Age at Menopause	NS
(Below 50 years)	
Post Menopausal Therapy	0.191*
(No)	(0.034 – 1.07)
Hysterectomy	6.03**
(Yes)	(1.426 – 25.48)
Smoking/Alcohol	0.31*
(No)	(0.141 – 0.669)

Table 3: Socio demographic characteristics among the breast cancer patients.

Stepwise logistic regression (backward conditional) analysis revealed that there are some lifestyle and reproductive variables, which can significantly predict a person's risk of developing the disease. A person in service as means of occupation is 0.3 times less likely to have breast cancer (OR-0.30, 95% CI=0.129 – 0.682, p<0.001), likewise having no family history of breast cancer reduces the risk of developing the disease by 0.028 times (OR-0.028, 95% CI=0.0009 – 0.092, p<0.0001). Irregular menstrual periods are seen to increase the risk of breast cancer by 9.40 times (OR-9.40, 95% CI= 2.092 – 42.22, p<0.0001). Prolonged used of oral contraceptives is likely to elevate the breast cancer risk by 2.59 times (OR-2.578, 95% CI= 1.0 – 6.65, p<0.0001). A women's risk of having breast cancer gets 17.02 times increased if she has history of abortions (OR-17.02, 95% CI= 2.3 – 143.15, p<0.0001). The potential risk of breast cancer gets increased to 0.26 times if a woman feed her breast for less than 3 months (OR- 0.26, 95% CI= 0.09 – 0.69, p<0.001). Likewise, the risk is modified to 0.19 times (OR- 0.19, 95% CI=0.034 – 1.07, p<0.01) if a woman undergoes post menopausal hormone therapy and 6.03 times if she has been operated with hysterectomy (OR-6.03, 95% CI=1.43 – 25.48, p<0.001). Consumption of alcohol and smoking increases the risk of developing breast cancer by 0.31 times (OR-0.31, 95% CI=0.141 – 0.669, p<0.01).

Discussion

The present study revealed substantial variation in breast cancer risk among the mutation carriers, particularly in terms of age variation and cancer type which basically envisaged that the concomitant effect of genetic variability and environmental factors which eventually modify the expression of the status.

The human genome has millions of SNPs, but relatively few have been shown to have functional significance. In cancer genomics, numerous SNPs have been reported, with synonymous and non-synonymous changes. A striking feature of the newly associated variants is that the top signals often occur at DNA sites (Splice, UTR, Introns, IVS) that do not encode amino acids [24].

Nevertheless, the effect of mutations in BRCA1 and BRCA2 genes to the incidence and prevalence of breast cancer (BC) has been well established worldwide. In other word, BRCA1 and BRCA2 genes are well established Breast Cancer susceptibility gene, which when mutated are inherited and strongly predisposes to breast cancer. Some of them directly influence breast cancer risk, whereas others are involved in the general process of cancer growth and metastasis. However, the role of these genes in pre-disposing Bengalee Hindus to breast cancer has not been explored and there is no reported study till date.

In an attempt to screen the BRCA1 and BRCA2 genes in Bengalee population, 10 Single Nucleotide Polymorphisms has been identified; out of which 5 were novel variants, and has already been submitted to GenBank (NCBI). The present study also revealed that the mutations in BRCA1 and BRCA2 genes were apparently low among the studied population which is contrary to the earlier studies reported from Southern and Northern

India. This might be due to ethnic differences vis-à-vis the genetic structure [19-21,25]. The present study demonstrated a significant association ($p<0.005$) of breast cancer among the BRCA1 mutation carriers, similarly BRCA2 and breast cancer revealed also significant association ($p<0.005$) for the disease.

Nevertheless, the implication of natural hormones specially the sex hormones on developing cancers such as endometrial cancer, breast and prostate cancer (among sex organ related neoplasm) or colon cancer, gall bladder cancer, kidney cancer etc. (non sex organ related neoplasm) have been reported globally [26-30]. Furthermore, breast cancer risk is enhanced by increasing the duration of exposure to endogenous hormones (Endogenous Hormones and Breast Cancer Collaborative Group, 2011). It has also been reported that age at menarche, parity and age at first full-term pregnancy are risk factors for breast cancer [31,32]. In addition to that cancer risk breast is associated with several reproductive factors. It is well established that breast cancer risk increases with early age at menarche [33]. This association is consistent with the hypothesis that breast cancer risk is related to the extent of breast mitotic activity. This activity is driven by estrogen and progesterone exposure during the luteal phase of the menstrual cycle, which determines the probability of tumorigenic somatic events [34,35]. Therefore, an early age at menarche increases the period during which the breast is mitotically active and subsequently increases breast cancer. Therefore, early menarche or late menopause increases the risk of breast cancer. In this context, the present study also observed that an early age at menarche (Table 4) is significantly associated ($p<0.005$) with an elevated risk of breast cancer in Bengalee population. Irregularity of menstrual periods was also seen to be significantly ($p<0.0002$) associated with breast cancer risk. Similarly many studies also reported the increased risk associated with irregular menstrual cycles [36-39]. However, a number of studies have reported little association with irregularity and increased breast cancer risk [40-43].

Menopausal status and characteristics are known to be induced by hormonal factors, which are the key factors for breast cancer and may synergistically interact with genetic factors in triggering the development and progression of breast cancer through estrogen synthesis, metabolism and signal transduction [44]. The present finding demonstrated no significant association of menopausal status and characters with breast cancer risk, which is contrary to the result from Pakistan [44].

Induced and spontaneous abortion increases the risk of developing breast cancer. In early pregnancy, levels of estrogen increase, leading to breast growth in preparation for lactation. The hypothesis proposes that if this process is interrupted by an abortion before full maturity in the third trimester then more relatively vulnerable immature cells could be left than there were prior to the pregnancy, resulting in a greater potential risk of breast cancer over time. Though many studies have reported association between abortion and breast cancer risk, the exact influence is still unclear. Abortions increases the risk of having breast cancer (Table 4), which is significantly ($p<0.005$) demonstrated in the present study. There are a very few studies on abortions and breast cancer risk from India, but the few available reports also showed similar finding [45].

It has been known for decades that nulliparity is associated with an increased risk for certain reproductive malignancies, including breast, ovarian and uterine cancers. A recent commentary in The Lancet summarized the available evidence based on data in nulliparous women and concluded that the risk of nulliparity was related to the increased number of ovulatory cycles, and so might be preventable by utilization of oral contraceptives [46]. Furthermore, long-term users of Oral Contraceptives (OCs) were at a higher risk of breast cancer than never users. Current/recent use of OCs is associated with an increased breast cancer risk. [47-51]. In contrary studies also reported no or weak association of OCs use among BRCA1 mutation carrier in Breast Cancer [52-55]. In this context, the present study revealed significant association ($p<0.005$) of prolong use (more than six months) of OCs and breast cancer in comparison to the controls [56-61].

Because hormones are considered to play a role in the etiology of breast cancer, it seems likely that BRCA1 may be important regulators of growth and differentiation in hormonally responsive epithelial cells [62-67]. Breast and ovary being the main estrogen receptor sites, the increased levels of the estrogen due to prolonged consumption of oral contraceptives gets accumulated in these sites [68-72].

The present study being the first report from the Bengalee Hindu Caste Females of West Bengal revealed that the spectrum and prevalence of the BRCA1 and BRCA2 genes in the Bengalee Hindu Caste Females were found to be variable compared to other populations. It is evident from the above findings that having a mutation in tumor suppressor genes cannot solely trigger a person's risk of developing the disease during the lifetime. Certain

other environmental factors modify the risk for the same [72-75].

This study also emphasizes the importance of a positive family history and other lifestyle factors for the breast cancer predisposition [76]. Therefore, the present study envisaged that appropriate genetic counseling and modification of lifestyle factors, symptomatic mutation carriers would be able to minimize the risk for disease susceptibility among the Bengalee Hindu Caste Females of West Bengal, India [77-79].

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