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GENETIC RISK FOR BREAST CANCER DEVELOPMENT

Dr. Kausar Abbas Saldera[1]*, Dr. Taseer Ahmed Khan[2], Dr. Syed Mehboob Alam[3].

[1]*Ph.D. Scholar, Basic medical science institute (BMSI), JPMC, Karachi
[2]Professor, Department of Physiology, University of Karachi, Karachi
[3]Professor, Department of Pharmacology, Jinnah Postgraduate medical center, Karachi

***Corresponding Author:** - Dr. Kausar Abbas Saldera
*Email: - @ k.salderabbas@gmail.com Contact number: 0300 2184661

Abstract
Objective: studies in the relation of moderate risk susceptibility genes in the onset of breast ca
Methods: Systematic random samples are done on selected Breast cancer patients from tertiary care hospitals in Karachi. The sample size was calculated using an online sample size calculator, Open Epi version 3.01 for case-controls, after inserting a 9.6% proportion of late menopause among cases, at a 5% margin of error and 95% confidence interval. The required sample size for our study was 74 with 37 participants in each group, but we took 100 samples in each group, making it a total of 200. This type of moderate-risk gene research and population-based study in correlation with high-risk gene variation is being conducted first time in the Pakistani population, therefore to see more effective results, we increase the sample size. Subjects were divided into two groups: Cases Group, which included 100 Known Naïve cases of Breast Ca (Females), and Control Group, which included 100 healthy individuals.
Results: The results of our study were from 100 breast cancer patients from a homogeneous population (Sindh). We found a correlation between age at diagnosis, family history, and mutation detection rate. Though our study has a limited number of patients, a low frequency of mutation in ATM, CHEK2, and BARD1 genes was reported.
Conclusion:
Around 20% of hereditary breast cancer is account for variants in the high and moderate breast cancer susceptibility genes BRCA1, BRCA2, TP53, CHEK2, and RAD51C, in Pakistan (Muhammad et al, 2018). Convincingly identified five moderate-risk breast cancer susceptibility genes are: CHEK2, ATM, BRIP1, PALB2, and NBS1 (Rashid et al. 2013). Attempts should be made to develop a real-time assay for the diagnosis of mutations in moderate-risk (ATM, BARD1, and CHEK2) genes.

Keywords: Genetic, Breast Cancer ATM, CHEK2, BARD1

INTRODUCTION:

Breast cancer (BRCA) is a highly prevalent and heterogeneous disease. However, it is anticipated that 627,000 women died from breast cancer which is around 15% of all cancer deaths among women in 2018 (WHO report, 2018). Pakistan is the major contributor to commonly diagnosed cancer among women, while the rate of occurrence is more or less similar to those cases reported in the West (Fan et al 2015). Numerous genetic, ecological, and biological factors drastically affect high-risk breast cancer suppressor genes i.e. BRCA1 and BRCA 2. These high-risk genes extensively act as a major

contributor to suppressing tumor development. But mutations in these genes extensively cause BRCA. Approximately 38% of female malignancies are related to BRCA in Pakistan (Iqbal et al., 2015). According to the World Health Organization [WHO], each year 1.2 million people are diagnosed with BRCA. Certain risk factors are involved in BRCA but the biologist could not explicate the exact reason why a woman develops breast cancer. It is suggested that high energy intake, body lethargy, high body mass index (BMI), and obesity accelerate breast growth. Globally, the high prevalence of breast cancer incidence is also characterized by type II diabetes dyslipidemia, high blood pressure, visceral obesity, and imbalance in blood cholesterol ((Chen et al., 2018, Vainio, *et al*., 2002). Besides all these reasons, genetic makeup also plays a major part in women causing BRCA. Extensively, human genes related to breast cancer or metastasis BRCA1 and BRCA2 have been studied. Gene BRCA1 positioned at the long (q) arm of chromosome 17 from base pair 38,429,551 to base pair 38,551,283 built-in many women with BRCA, (Hall, *et al.,* 1990). BRCA1 consists of 84 kilobases and a large-sized gene in a genomic sequence. 7800 nucleotide functions and pre-arranged in 22 exons and 2 introns (Thompson, *et al.,* 1994). These genes encoded 1863 amino acids having a molecular weight of about 200 KDa which encodes the protein responsible for the growth of breast epithelial cells. The BRCA2 gene encloses 91193 nucleotides that are structured in 26 exons and one intron and do not articulate any structural homology with the BRCA1 gene. This gene codes multifunctional proteins involved in DNA repair cell cycle/checkpoint control transcriptional regulation (Rashid *et al.,* 2013). An alteration in any of the respective genes may attribute to an extensive share of breast and ovarian cancer (Ford, *et al.,* 1998). Around 20% of hereditary breast cancer is account for variants in the high and moderate breast cancer susceptibility genes BRCA1, BRCA2, TP53, CHEK2, and RAD51C, in Pakistan (Muhammad et al., 2018). Convincingly identified five moderate-risk breast cancer susceptibility genes are CHEK2, ATM, BARD1, BRIP1, and PALB2. However, a 10% risk of BRCA in the general population is due to moderate-risk genes and it has been progressively increasing BRCA risk two to fourfold. As the similarities and their key correlations reported in previous studies between BARD1 and BRCA1, it is unpredictable yet that the BARD1 mutations compared to BRCA1 mutation are relatively rare in BRCA patients. The role of *BARD1* mutations with BRCA1/2 in cancer predisposition research has not been provided up till now solid supported data (Suszynska et al., 2019). So, our investigation with moderate-risk genes ATM, CHEK2, *and BARD1 and their* correlation with BRCA1/*2* regarding standard clinical genetic testing practice in the Pakistani population will be provided supplementary data. It is also evident that the BRCA1 interacting protein C-terminal helicase 1 (BRIP1) gene is a breast cancer susceptibility gene. Mutational screening and its sequencing of the whole BRIP1 gene were extensively studied in British familial breast cancer. Finally, for moderate-risk breast cancer susceptibility genes, the important aspects are the discovery of BRIP1 and PALB2. These two genes are both associated with the breast cancer protein BRCA1, and BRCA2 respectively. Although, 20%-40% of the lifetime risk of breast cancers are the mutations in moderate-risk genes including homozygous ataxia-telangiectasia (ATM) while tumor suppressor genes like CHEK2, and BRCA1 and BRCA2 modifier genes BRIP1 and PALB2 confer somatic mutations. Numerous moderate low-risk common alleles have been recognized principally through genome-wide association studies and the clinical application in the occurrence of these mutations is up till now to be unexplored (Hollestelle et al., 2010).This study is helpful for the findings of moderate-risk genes involved in the Pakistani population to inspect the contribution of breast cancer-predisposing genes. Furthermore, the allegation of the current studies related to moderate-risk genes and future research potentials will be discussed. But presently data on the contribution of low-or moderate risk variants to the disease are lacking. Though, it is yet to be unpredictable that at what extent the effects of these moderate genes are involved in BRCA.

AIM OF STUDY

This study is helpful for the findings of moderate-risk genes involved in the Pakistani population to inspect the contribution of breast cancer-predisposing genes. But presently data on the contribution

of low-or moderate risk variants to the disease are lacking. Though, it is yet to be unpredictable that at what extent the effects of these moderate genes are involved in Breast cancer.

PATIENTS AND METHODS

The study was a case-control, and Non-probability purposive sampling was used as the patients were selected according to predetermined criteria and obtaining ethical clearance. The study was carried out within one (1) year after the approval of the synopsis and obtaining ethical clearance at the Department of Physiology (BMSI), Department of Radiology, Department of Surgery, Jinnah Post Graduate medical center (JPMC), Karachi, Pakistan in collaboration with the Department of Physiology, University of Karachi, Karachi, Pakistan. The required sample size for our study was 74 with 37 participants in each group, but we took 100 samples in each group, making it a total of 200 as this type of moderate-risk gene research and population-based study in correlation with high-risk gene variation is being conducted first time in the Pakistani population, therefore to see more effective results, we increase the sample size. The study was approved by the Jinnah Post Graduate Medical Centre's ASRB (Advance Studies and Research Board) in Karachi, Pakistan. During the study's execution, the Helsinki Declaration was followed. Written permission was obtained in both English and the local language. All of the participants agreed to participate in the study voluntarily. The consent form was completed, signed, and thumb printed. The information was kept completely confidential. Subjects were divided into two groups: Cases Group, which included 100 Known cases of Breast Ca (Females), and Control Group, included 100 healthy individuals. The recruitment of the patients and controls employed a simple random sampling technique. Naïve known cases of Breast Ca (Females), between 15-60 years of age, Before medical and surgical intervention, Before Chemotherapy & Radiotherapy, and agreed to take part in the study were included. Radiological assessment, Detailed Hx were taken to make a diagnosis. Individuals less than 15 and above 60 years and those failing to give consent were excluded from the study. Also, patients suffering from any other medical or Surgical Comorbidity were excluded due to the different and complex pathophysiology of these conditions which was unrelated to our study point. The recruitment of the patients and controls employed a simple random sampling technique. The participants were questioned /assessed for Anthropometric parameters. Detailed medical history, Symptoms, presenting complaints, ethnicities, Family history of breast cancer, family and residential history, diagnosis, socioeconomic status, BMI, co-morbidities, and a detailed dietary profile was recorded. Primers synthesis for ATM and Chek2 was designed following the online software to detect the Specific SNVs (point mutations) in the subject's DNA. The primers were optimized, and PCR was done. The subjects and controls were optimized with ARM-PCR for BARD1. The primers were optimized before processing samples, similar to ATM and Chek2. The primer pairs were ordered for amplification of ATM, CHEK2, BARD1, and sequencing of targeted Exon from the BARD1 gene was sent for sequencing to see the susceptibility of selected variant (SNP) towards breast cancer. From peripheral blood samples genomic DNA was extracted by using the kit method. PCR of moderate-risk genes (CHEK2 and ATM) was carried out using primers specific to the Pakistani population.

Statistical analysis

The five age groups were compared statistically using the chi-squared test. Multivariate logistical. IBM SPSS Statistics version 26 was used for the data analysis. For numeric values, the means and standard deviations were calculated. When calculating qualitative variables, frequency and percentage were used. When necessary, the chi-square analysis and Fisher exact test were used to stratify the data based on risk factors and determine how these modifiers affected the results. A one-way analysis of variance (ANOVA) and independent sample t-test were used as necessary to analyze mean differences between quantitative variables. The risk factors for the result were examined using logistic regression. $p \leq 0.05$ were considered as significant.

RESULTS:

Data were logged and computed in SPSS and Excel formats. The sample size that reached Hardy Weinberg equilibrium was evaluated to determine genotype and allele frequency. The common chi-squared statistics on one degree of freedom were used to determine the p-value for each variant. Logistic and linear regression analysis found significant and sizeable associations with study characteristics.

This case-control study was accomplished in the Department of Physiology BMSI, JPMC, Department of Radiology, Department of Surgery, Jinnah Post Graduate medical center (JPMC), Karachi, Pakistan in collaboration with the Department of Physiology, University of Karachi, Karachi, Pakistan. 200 individuals were interviewed and collected samples after applying inclusion and exclusion criteria and divided into two groups: i) Group A (cases) diagnosed naive breast cancer patients, Group B (controls) all healthy individuals falling in age limit, without any existing co-morbidity All cases were selected from Surgery and Radiology departments, JPMC, Karachi, while the healthy individual's, age and weight-matched, selected from friends, and family patient attendants. Data were collected on predefined Performa. Further, it was analyzed and processed by using IBM SPSS version 26.

The age range of patients was between 15 to 65 years. 3rd and 4th decade is commonly observed in the diseased group (Table No.1). The mean age was 42.61±10.81 years for the disease group and 35.20± 11.45 years for the control group, the difference between two means was statistically insignificant (p-value = 0.056). In this study, in the disease group, overweight (24.6-29.9) BMI in 44% of cases, followed by normal weight (18.6 to 24.5) in 34% of cases and 19% of cases in obesity. However in the Control Group, overweight BMI in 34% of cases, followed by normal weight 30% of cases and 26% of cases in obesity. Most women were housewife 84% in the disease group, and 69% in the control group. Followed by private job were 11% in the disease group and 12% in the control groups. 89% of women were married in the disease group and 80% in control group. While unmarried was 11% in the disease group and 20% in the control groups. In our study most women were given the history of pre-menopause 53% in disease group and 80% in the control group. While post Menopause status were 45% in the disease group and 20% in control group. History of hysterectomy were in 2% in the disease group. In our study most of the women were observed menarche between 10 to 15 years in 91% cases f the disease group and 96% cases of the control group. While common age of marriage was 2nd decade of life in 55% cases in each group. Followed by between 22 to 31 years in 29% of cases in disease group and 21% of cases in control group. In our study, most of the women have more than one child like 4-6 children 35% in the disease group while in 25% in the control group, while in both groups almost the same 42% and 43% of women have children 1 to 3, respectively. 84% of women were breastfeeding children in the disease group while 67% of women were in the control group. In our study, 52% of women in the disease group had a history of miscarriage/abortion compared to 60% in the control group. Contraceptive history was almost the same in both groups. No, any history of contraceptives was 79% in the disease group and 62% in the control group, pills 4% in the disease group and 7% in the control group, implants were 1% in the disease group and 2% in the control group, IUCD was 3% in both groups. Family history of breast cancer was 25% in the disease group and 28% in the control group.

The most common symptom in disease group of patients is Breast Lump in 93%, followed by pain and burning sensations were 50% cases and 40% of cases respectively. While nipple discharge was in 23% cases, we observed nipple retraction in 11% of cases, redness in 38% cases, tenderness in 35% of cases, and cyst-like feeling in 29% of cases (Table No.2). The most common sampling technique used in our study was Turcotte biopsy in 89% of cases followed by ultrasound-guided biopsy in 6% of cases. In our study observed both sides were commonly involved. 50% lesion was found in the left breast, 49% in the right breast, and 1% in both breasts.

According to bird classification. 31% of cases were observed in Category IV while 17% and 13% were in Category III and Category VI respectively (Table No.3). This tables the analysis of immunohistochemistry finding of studied partcipants. In respect to ER status, 55% were strong positive, while major chunk were negative for Estrogen receptor (Table No.4). Similarly 34% were positive for Progesterone receptor (PR). While major cases were negative for HER 2 neu. This table shows the BI-RADS category of mutated cases with all genes. In our study, ATM gene was expressed in 2 cases, Chek2 was expressed in 3 cases and none showed positive results for BARD gene (Table No.5).

Table No.1 - Age Distributions

Age in year	**Disease Group**		**Control Group**	
	No:of Patients	Percentage	No: of Participants	Percentage
15 – 25	2	2%	26	26%
26 – 35	25	25%	26	26%
36 – 45	33	33%	32	32%
46 – 55	24	24%	12	12%
56 – 65	16	16%	4	4%
Total	100	100%	100	100%
Mean	42.61		35.20	
Std. Deviation	10.81		11.45	

Table No. 2- PRESENTATION/SYMPTOMS OF PATIENTS (DISEASE GROUP)

SYMPTOMS	No: of Patients	Percentage
• Breast Lump	93	93%
• Pain	50	50%
• Nipple Discharge	23	23%
• Redness	38	38%
• Burning sensation	40	40%
• Nipple Retraction	11	11%
• Cyst like feeling	29	29%
• Tenderness	35	35%

TABLE NO.3- BIRAD CLASSIFICATION (DISEASE GROUP)

CATEGORY	**No: of Patients**	**Percentage**
• CATEGORY I	16	16%
• CATEGORY II	23	23%
• CATEGORY III	17	17%
• CATEGORY IV	31	31%
• CATEGORY V	13	13%
Total	100	100%

TABLE NO.4- IHC FINDINGS OF ER, PR, and HER2 (DISEASE GROUP) (n=100)

VARIABLE	NO: OF PATIENTS	PERCENTAGE
Estrogen Receptor (Intensity Score)		
Negative		
• **0**	12	12%
• **1**	30	30%
• **1+**	1	1%
Intermediate		
• **2 (Weak)**	0	0
• **2+ (Weak Positive)**	2	2%
• **3 (Positive)**	0	0
Strong Postive		
• **3+ to 8**	55	55%
Total	100	100%
Progesterone Receptor (Intensity Score)		
Negative		
• **0**	35	35%
• **1**	6	6%
• **1+**	4	4%
Intermediate		
• **2 (Weak)**	7	7%
• **2+ (Weak Positive)**	4	4%
• **3 (Positive)**	0	0%
Strong Postive		
• **3+ to 8**	34	34%
Total	100	100%
HER2 NEU (Intensity Score)		
Negative		
• **0**	51	51%
• **1**	18	18%
• **1+**	12	12%
Intermediate		
• **2 (Weak)**	2	2%
• **2+ (Weak Positive)**	13	13%
• **3 (Positive)**	2	2%
Strong Postive		
• **3+ to 8**	2	2%
Total	100	100%

TABLE NO.5 - CLASSIFICATION OF CASES PRESENTED IN EVERY CATEGORY, EXPRESSED WITH ALL GENE

CATEGORY	GENES		
	ATM (Sequence Method)	CHEK 2 (Sequence Method)	BARD 1 (ARM PCR Method)
	Mutated	Mutated	Mutated
CATEGORY I	0	0	0
CATEGORY II	0	0	0
CATEGORY III	1	2	0
CATEGORY IV	1	1	0
CATEGORY V	0	0	0
TOTAL	2	3	0

DISCUSSION:

Breast cancer is the most common malignancy in women. It is a complex disease that involves the accumulation of genetic, and epigenetic alterations, environmental, personal habits, and lifestyle factors. The purpose of this study was to characterize genetic variations in tumor suppressor genes associated with breast cancer in the study population. In 2022, Stacy Simon narrated in her study in the American Cancer Society, that increased weight and obese women are more prone to develop breast cancer. Our study also supports the above results. In our study, a huge number of cases are found to have increased BMI. Disease progression is seen in all age groups which clearly showed that the disease onset is not age dependent, one can develop the disease at any stage of life. (According to the National Cancer Institute, in the 30s, the risk of breast cancer is 1 in 204 or about 0.4 percent. By age 40, the risk is roughly 1 in 65 or about 1.5 percent. By age 60, the chance increases to 1 in 28 or 3.5 percent) the breast cancer cases presented in our study are of different ages, no particular age is found to have many cases. In our study, the unmarried women of the diseased group, although in small numbers, showed more aggressive breast cancer lesions compared to the married group, which confirms the involvement of hormones in disease progression. Forty-nine international publications were reported in the meta-analysis. Compared with married women, unmarried and lifelong single women had an elevated risk of breast cancer, and the pooled case-control studies were 1.20 (95% CI: 1.07 to 1.35) and 1.24 (95% CI: 1.05 to 1.45), respectively. This claim is further confirmed by their bad obstetrics history, those who have Hx of abortions, and miscarriages, have more suspicious lesions, leading to increase chances of cancer development. In 2019, an international study by the National Cancer Institute reported, miscarriages and treatment of threatened abortion build a platform for breast cancer development in those women, who have positive hx for breast cancer. Our study also showed that bad obstetrics history increases the risk of a suspicious lesion. The analysis of 54 international epidemiologic studies conducted on more than 150,000 women showed that the use of oral contraceptives had a slight (7%) increase in the relative risk of breast cancer compared with women who had never used oral contraceptives. A Study by Lei Liu, et all, 2021 narrates that, having a first-degree relative diagnosed with breast cancer approximately doubles the risk of breast cancer. This risk is higher when more close relatives have breast cancer, or if a relative developed breast cancer under the age of 50. In our study, we have found that 75% of subjects have no family history of breast Ca, while only 25% have a positive family history, which suggests that despite having no background, breast cancer can develop at any stage of life and to anyone. Subjects in the disease group were presented with a breast lump, pain, Nipple Discharge, redness, Burning sensation, Nipple Retraction, Cyst like feeling, and Tenderness, and many had none. Tissue samples were also taken from subjects selected in the study, samples were mostly trust biopsy, Cone excision, wedge biopsy, and samples from mastectomies. Immunohistochemistry of cases for Estrogen receptor was positive in 59 cases, while negative in 41 cases 53 cases were positive for progesterone receptor while negative in 47 cases For Her 2 Neu 40 cases were positive while 60 were negative; Our study results verify international studies, that ratio of ER incidence is high as compared to PR and HER 2NEU. According to Li Wang, Li-Jun D et al, the overall incidence of ER is more in breast cancer patients, followed by progesterone and HER2. The analysis also showed greater the lesion grade, the more ER, PR, and HER2. Mutation of ATM was seen in the genome of 02 cases, while the rest showed normal ATM optimization. Mutation of CHEK2 was seen in the genome of 3 cases, while 97 showed a normal appearance of CHEK2 in their genome. Mutated BARD1 was seen in only the genome of the 01 cases, while the remaining showed a normal presence of BARD1. Hence concluded that the incidence of mutated chek2 is more than ATM followed by BARD1, which is still seen in very few. The hormone status of breast cancers includes Estrogen receptor (ER) positive.

CONCLUSION:

The results concluded from our study of over 100 breast cancer patients from a homogeneous population (Sindh). We found a correlation between age at diagnosis, family history, and mutation detection rate. Though our study has a limited number of patients, a low frequency of mutation in ATM, CHEK2, and BARD1 genes was reported. We were able to monitor 5 cases of genetic

mutations that are predisposing factors for the development of breast cancer, only by taking blood samples, which is a non-invasive technique. However, many cancer markers have previously been used to diagnose cancer such as ovarian, colon, and many others. Analysis on genetic grounds using sequencing is a very authentic and important step towards early diagnosis of breast cancer.

FUTURE PROJECTION:

In the industrialized and developing world, breast cancer lines as the principal cause of death (Muhammad et al, 2018). Around 20% of hereditary breast cancer is account for variants in the high and moderate breast cancer susceptibility genes BRCA1, BRCA2, TP53, CHEK2, and RAD51C, in Pakistan (Muhammad et al, 2018). Convincingly identified five moderate-risk breast cancer susceptibility genes are: CHEK2, ATM, BRIP1, PALB2, and NBS1 (Rashid et al. 2013). Attempts should be made to develop a real-time assay for the diagnosis of mutations in moderate-risk (ATM, BARD1, and CHEK2) genes.

Conflict of Interest for all authors – The authors declared no conflicts of interest concerning the research, authorship, and/or publication of this article.

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Ethical approval: Ethical Approval was taken from the Advanced research and ethical committee of Jinnah postgraduate medical center, Karachi

Informed Consent: Informed consent was collected from all 200 participants, included in the study.

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