REVIEW ARTICLE

Role of Single Nucleotide Polymorphisms in BRCA1 and BRCA2 Genes Relative to Previous Studies in Pakistan in the Prognosis of Breast Cancer

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Abstract

Breast cancer is a complex disease characterized by a myriad of genetic alterations. Single nucleotide polymorphisms are particularly relevant due to small allelic variations. BRCA1 and BRCA2 genes are notably associated with inherited breast and ovarian cancers. Alterations in other genes were also identified. Mutations in BRCA1 and BRCA2 tumor suppressor genes play a significant role in development. Studies in the Pakistani population revealed significant differences in gene mutations. In 31 families, 8 had male and 23 had female breast cancer, substantial findings were observed. The analysis encompassed full coding sites of these genetic variations. Among eight families with male BC, changes were detected in 2 BRCA1 and 4 BRCA2 genes. Additionally, eight alterations were noted in 23 females from site-specific families, with 4 in BRCA1 and 4 in BRCA2. These findings underscore the importance of genetic variations, especially in the BRCA1 and BRCA2 genes, in the context of breast cancer susceptibility within diverse populations.

Keywords: *Breast cancer, single nucleotide polymorphisms, mutations, BRCA1, BRCA2, DNA.*

INTRODUCTION

Breast cancer is a complex disease marked by genetic, epigenetic, and phenotypic alterations. Variations in genes associated with various biological pathways have been identified as potential factors contributing to the risk of breast cancer [1]. Individuals carrying these genetic variations exhibit varying levels of disease risk and severity [2]. In the management of breast cancer patients, precise molecular analyses and the identification of predictive biological markers are crucial for effective intervention and treatment planning.

The development of breast cancer is believed to involve both inherited and non-inherited factors. Multiple subdivisions of breast cancer exhibit a range of genetic influences, and recent research suggests that different subtypes of breast cancer may have distinct etiological pathways [3, 4]. Key predictive markers for breast cancer include tumor size, stage, and lymph node metastatic status. However, the diagnosis currently places minimal emphasis on the interpretation of genetic data. Single nucleotide polymorphisms (SNPs) are particularly relevant in this context due to their small allelic variations. The high prevalence of SNPs, coupled with variations among populations and races, makes them valuable for researching various infections or disease characteristics [5].

BRCA1, identified in October 1994 [6], and BRCA2, discovered in December 1995, are pivotal genes associated with inherited breast and ovarian cancers [7, 8]. Both genes exhibit extensive coding regions and intricate genomic structures: BRCA1 comprises 5,592 nucleotides and 22 coding regions, while BRCA2 consists of 10,443 nucleotides and 26 coding regions. The proteins encoded by BRCA1 and BRCA2 play crucial roles in DNA repair, homologous recombination, and various cellular processes [9]. Cells with a disrupted BRCA1 or BRCA2 gene, resulting in non-functional BRCA1 or BRCA2 protein, experience a diminished capacity to repair damaged DNA. In animal models, this deficiency has been observed to lead to gene mutations [10]. In humans, breast tumors in individuals carrying altered BRCA1 or BRCA2 genes are characterized by extensive chromosomal aberrations, with variations depending on the mode of inheritance [11].

Approximately 10–20% of breast cancer patients have at least one affected first-degree relative. Up to 20% of them carry germline variations in the tumor suppressor genes BRCA1 or BRCA2. Many of these variations are frameshift mutations, leading to premature termination codons and reduced synthesis of BRCA proteins [12]. BRCA1 and BRCA2 are crucial tumor suppressor genes that are particularly valuable for prevention since they play a key role in repairing DNA damage through homology-directed repair (HDR). Apart from BRCA, alterations in other cancer-related genes account for less than 1% of all inherited breast cancer cases [13-16]. This study aimed to analyze the role of SNPs in the BRCA1 and BRCA2 genes in the Pakistani population and to compare work to date for their frequency in mutations in different areas of Pakistan and other Asian countries.

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LITERATURE STUDY

BRCA Associated Mutations Among Pakistani Population

Single nucleotide polymorphisms (SNPs) are characterized as mutations that exhibit a specific prevalence within particular ethnic populations, thereby influencing the incidence of diseases, particularly those governed by tumor suppressor genes responsible for preserving genomic stability. Although certain SNPs in BRCA1, BRCA2, and TP53 are recognized globally for their correlation with an increased risk of breast cancer, there is a noticeable gap in research regarding the risk of breast cancer development within the Khyber Pakhtunkhwa population in Pakistan. This study focused on investigating established associative SNPs—specifically, BRCA1 (rs1799950), BRCA2 (rs144848), and TP53 (rs1042522)—to assess the prevalence of these genetic variants within the Pakhtunkhwa population. The primary objective was to further stratify the risk of breast cancer development associated with these polymorphisms in the same population. The findings demonstrated a statistically significant association between the risk alleles of all three selected SNPs and the presence of breast cancer ($p<0.05$ for BRCA1, C- $p=0.001$; BRCA2, C- $p=0.000$; TP53, C- $p=0.000$). Similarly, all genotypes carrying the risk allele were also significantly linked to an elevated risk of breast cancer ($p<0.05$ for BRCA1, TC- $p=0.037$, CC- $p=0.005$; BRCA2, AC- $p=0.000$, CC- $p=0.000$; TP53, GC- $p=0.000$, CC- $p=0.000$). In conclusion, the study revealed a statistically significant association between the risk alleles and genotypes containing these alleles with the risk of breast cancer in the region. Further investigations will be necessary to validate these results using larger datasets and whole-genome sequencing [17].

Single Nucleotide Polymorphisms (SNPs) in BRCA1, BRCA2, and TP53 have been extensively linked to breast cancer risk across various ethnicities, yielding inconsistent findings. To date, no investigation has addressed this association in the Pashtun population of Khyber Pakhtunkhwa, Pakistan. Thus, this study aimed to examine the BRCA1 (rs1799950), BRCA2 (rs144848), and TP53 (rs1042522) polymorphisms in breast cancer risk among the population. A total of 140 breast cancer patients and 80 gender- and age-matched healthy controls were included in this study. Participants provided clinicopathological data and blood samples were collected for DNA extraction. The T-ARMS-PCR protocol was employed to confirm BRCA1, BRCA2, and TP53 polymorphisms. The data revealed a significant association ($P< 0.05$) between the risk alleles of BRCA1, BRCA2, and TP53 selected SNPs, as well as genotypes containing these risk alleles, with breast cancer risk in the population. All three selected SNPs in BRCA1, BRCA2, and TP53 demonstrated a noteworthy association with breast cancer risk in this population. Nevertheless, further investigations on larger datasets are imperative to validate these selected SNPs and explore others within the chosen genes, as well as related genes, about the risk of breast cancer [18].

Pakistan exhibits the highest rates of breast and ovarian cancer among Asian countries. To understand the role of BRCA1 and BRCA2 germline mutations in these elevated rates, the researchers conducted a pioneering study involving 176 Pakistani patients with breast and ovarian cancer, selected based on family history and age of diagnosis. A comprehensive screening for BRCA mutations utilized various techniques, including denaturing high-pressure liquid chromatography, single-strand conformational polymorphism analysis, and the protein truncation test, followed by DNA sequencing. The study identified 30 deleterious germ-line mutations in 17.0% of the 176 families, with 23 in BRCA1 and 7 in BRCA2. Among these, four mutations (185delAG, 185insA, S1503X, and R1835X) recurred, collectively accounting for 52% of all identified BRCA1 mutations. Haplotype analyses suggested founder effects for three of these recurrent mutations. The prevalence of BRCA1 or BRCA2 mutations was notably high, reaching 42.8% in families with multiple cases of breast cancer and 50.0% in families with both breast and ovarian cancer cases. For single cases of early-onset breast cancer (onset at age 30 years), the mutation prevalence was 11.9%, while for early-onset ovarian cancer cases (onset at age 45 years), it was 9.0%. These findings underscore the significant contribution of BRCA mutations to hereditary breast and ovarian cancer cases, particularly in families with multiple instances of these cancers and in cases of early-onset breast and ovarian cancer in Pakistan [19].

MiRNAs (microRNAs) play a pivotal role in the development of breast cancer (BC), where variations in these molecules can impact their maturation, expression, and subsequently, the regulation of target genes. In this study, the researchers genotyped the single nucleotide polymorphism rs11614913 in BC patients ($n = 300$) and 230 controls using tetra primer amplification refractory mutation system PCR and Sanger sequencing. The analysis revealed a noteworthy difference in genotypes, as evidenced by co-dominant ($\chi2 = 42.03$; $p < 0.0001$), additive (odds ratio [OR] = 0.6441 [0.4887–0.8490, 95% confidence interval]; $p < 0.0019$), dominant (OR = 0.3996 [0.2809–0.5686], $p < 0.0001$), and recessive (OR = 0.2993 [0.1220–0.7347], $p < 0.009$) statistical

models. These models collectively indicated a reduced risk association of the C allele with BC. Moreover, the study suggested that females with the CT genotype face a higher risk of BC compared to those with the CC genotype. This emphasizes the potential significance of the rs11614913 polymorphism in miRNAs as a contributing factor to BC susceptibility [20].

The study aimed to assess the BRCA1 status in breast carcinoma patients of Pakistani origin treated at the Oncology Clinics of the Aga Khan University Hospital, Karachi, from May 2005 to December 2009. A total of 53 breast cancer patients were recruited based on clinical and laboratory diagnoses, with inclusion criteria specifying moderate family history as having a close relative (mother, daughter, sister) diagnosed with breast cancer under 45 years. Peripheral blood samples were collected from each patient in a 5 ml tube containing EDTA as an anticoagulant. Following DNA extraction, mutational analysis of BRCA1 exons 2, 5, 6, 16, 20, and 22 were performed using the single-strand conformation polymorphism (SSCP) assay, while mutations in exon 11 were examined using the protein truncation test (PTT). All identified BRCA1 sequence variants were subsequently confirmed by DNA sequencing. Of the 53 patients, 23 were diagnosed with early-onset breast cancer, and 30 had a moderate family history. At the time of diagnosis, the median age of the enrolled patients was 39 years, with a range of 24-65 years. Results from the SSCP assay indicated mobility shift in exon 6, 16, and 20 of three patients, and one patient tested positive for mutation in exon 11 by PTT assays. All BRCA1 mutations were further validated by DNA sequencing analysis. In exon 16, c.4837A > G was confirmed, and a common polymorphism was reported in various populations, including Asians. Additionally, mutations in exon 6 (c.271T > G), exon 20 (c.5231 delG), and exon 11 (c.1123 T > G) were reported for the first time in the Pakistani population. The study observed several BRCA1 mutations in Pakistani breast cancer patients with a moderate family history. Therefore, the identification of mutations through genetic counseling, especially for patients with a moderate family history or apparent early-onset disease in first or second-degree relatives, can aid in the management of these cases [21].

Variations in DNA repair genes have been widely documented as contributors to genetic instability, escalating the susceptibility to breast cancer. The collaborative action of NBS1, MRE11, and RAD50 forms the MRN (MRE11–RAD50–NBS1) complex, crucial for the repair of DNA damage. However, specific genetic modifications in MRE11 and RAD50 can lead to the production of aberrant proteins, disrupting the repair process and potentially leading to malignancy. The study aimed to explore the association between polymorphisms in the DNA repair genes MRE11 and RAD50 and the risk of breast cancer in the female population of Punjab, Pakistan. Blood samples were collected from 100 breast cancer patients and 100 tumor-free females serving as controls. The extracted DNA underwent genotyping using tetra ARMS-PCR, followed by gel electrophoresis. Statistical analyses were conducted using SPSS and SNPstats to assess the association between different clinical factors, single nucleotide polymorphisms (SNPs), and breast cancer risk. Results indicated a significant association between elevated risk of breast cancer and the MRE11 variant rs684507 (odds ratio-OR 3.71, 95% confidence interval-CI 1.68–8.18, p-value < 0.001) and the RAD50 variant rs17166050 (OR 2.85, 95% CI 1.45–5.62, p-value < 0.001). These findings suggest that specific genetic alterations in MRE11 and RAD50 may contribute to an increased risk of breast cancer in the female population of Punjab, Pakistan [22].

Families with BRCA1 and BRCA2 Mutations

Linkage studies suggest that BRCA1 is responsible for 80 percent of families with breast and ovarian cancer, with nearly half of these families exhibiting cases of breast cancer [23, 24]. Variations in BRCA2 genes are associated with a reduced risk of ovarian cancer but an elevated risk of male breast cancer compared to genetic abnormalities [25, 26]. The duplication of BRCA2 has facilitated the exploration of the percentage of breast cancer families affected by alterations in both BRCA1 and BRCA2. It also allows for the assessment of the number of families affected by other genetic variants through the susceptible gene (BRCAX). In a study involving 31 families, 8 of which had cases of male breast cancer and 23 with cases of female breast cancer, the researchers examined the full coding areas of BRCA1 and BRCA2 for variations. An association study was conducted when applicable to gain further insights into the genetic landscape of these families [27].

The risk of breast cancer associated with BRCA1/2 variations varies based on inheritance and genetic factors. About 50 variations have been identified to alter breast cancer risk for carriers of these mutations, with overall research reporting three cases. While new carriers susceptible to variations are also identified, ongoing studies are examining additional variations. A unique case-only genome-wide association study (GWAS) was conducted, analyzing genotypic frequencies in 60,212 breast cancer patients from the general population and

13,007 patients with variations in BRCA1 or BRCA2 genes. This study revealed new associations with breast cancer, including two for BRCA1 and three for BRCA2 alteration carriers, with a significance level of $P < 10-8$, at five locations that pose no threat to the overall population. Genes such as MADD, SP11, and EIF1, previously linked to breast cancer, are anticipated to be potential targets at rs60882887 on chromosome 11p11.2. This contributes to the development of a polygenic risk for breast cancer in BRCA1/2 alteration carriers [28].

Breast cancer development is significantly influenced by mutations in genes such as BRCA1 and BRCA2, which contribute to over 80 percent of inherited breast and ovarian cancers. The p53 suppressor gene, responsible for inhibiting cell growth, also exhibits variations in about 50% of individuals. In India, where breast cancer incidence is rapidly increasing, especially among urban females, alterations in BRCA1/2 and p53 genes play a crucial role. A study was conducted on 124 non-treated initial breast cancer (BC) individuals, including 100 non-sporadic and 24 inherited patients, with a control group of 56 age-matched individuals. The study utilized PCR-SSCP and nucleotide sequencing to analyze alterations in BRCA1/2 and p53 genes. Specific exon regions were examined, including exons 2, 5, 11, 13, and 20 of BRCA1, exons 2, 9, 11 (for 6174delT), 18, and 20 of BRCA2, and exons 4 to 9 of p53 in non-inherited BC individuals. For inherited BC individuals, all 22 coding regions of BRCA1, along with neighbouring intronic areas and specific regions of BRCA2 (exons 2, 9, 11) and p53 (exons 4, 9), were assessed. Among the cases, 6 (25%) showed effectiveness with BRCA1 mutations, including 3 novel mutations: one in exon 16 (4956insG) and two in exon 7 (Lys110Thr and Ser114Pro) from 24 inherited BC cases. These findings contribute valuable insights into the genetic landscape of breast cancer in the Indian population [29].

In a study involving eight relatives from Goa, India, it was found that two sisters from a single family, constituting 12.5% of the group, carried the BRC1 mutated gene (185delAG) along with other variations (IVS7 561)34T>C and (IVS18 5271 + 66G > A), which were traced back to a foreign origin of the founder Ashkenazi Jewish. In New Delhi, among 16 relatives with breast cancer, four cases exhibited BRCA1 alterations, including frame-shift protein-cutting mutation (4956insG), a nonsense mutation (Gln1395Stop), and two amino acid substitutions (Lys110Thr and Ser114Pro). Additionally, one patient was affected by a p53 gene mutation (Val97Ile) in exon 4, along with a BRCA1 mutation (4956insG). No patient was found to have a BRCA2 gene mutation without G203A at 5¢ UTR of exons 2. In two individuals from Goa, silent nucleotide changes in the BRCA1 gene at codon 1436 were observed. Among 100 non-inherited breast cancer patients, no protein-cutting or deleted BRCA1 or BRCA2 gene variations were observed. Only 3% of p53 gene mutations were noted in non-inherited breast cancer patients. In summary, the study indicates a low occurrence of BRCA gene variations in Indian females with a familial history of breast cancer, but it identifies three novel BRCA1 alterations with a founder Ashkenazi Jewish BRCA1 alteration in Indian females with inherited breast cancer [30].

The identification of breast cancer (BC) susceptibility genes, such as BRCA1 and BRCA2, has significantly enhanced the ability of clinicians to identify high-risk individuals, enabling more vigilant investigation and prevention strategies, and leading to the advancement of treatments. However, BRCA1 and BRCA2 variations only account for 25% of inherited breast cancer cases. To comprehensively classify pathogenic variations in inherited BC patients without BRCA1 and BRCA2 alterations, a study was conducted on 312 genes. Using next-generation sequencing (NGS), DNA enhancement was performed on 104 individuals with 'BRCAx' (no BRCA1/2 mutations) and 101 geologically matched controls in Ireland. This approach revealed variations in several well-known high-susceptibility and moderate-susceptibility genes, including ATM, RAD50, CHEK2, TP53, PALB2, and MRE11A. The study identified new pathogenic variations in thirty additional genes, collectively explaining the missing heritability in up to 35% of BRCAx individuals. Notably, these findings involved potential pathogenic alterations in MAP3K1, CASP8, RAD51B, ZNF217, CDKN2B-AS1, and ERBB2, including a splice locus alteration predicted to affect HER2 proteins. Overall, this research contributes to a better understanding of inherited BC gene mutations and provides insights into the development of treatment strategies for breast cancer [31].

To assess the prevalence of BRCA1 or BRCA2 mutations in breast cancer families, a comprehensive analysis was conducted on 31 families, consisting of 8 with male breast cancer (BC) and 23 with site-specific female BC. Various protein deletion tests, single-strand conformation polymorphism (SSCP), and nucleoprotein studies were employed for variation detection. Additionally, the evaluation of BRCA1 and BRCA2 allele expression levels was conducted when deemed applicable. Frame-shift variations were identified in two BRCA1 and four BRCA2 genes within six of the eight families with male

BC. Interestingly, although alterations in BRCA1 or BRCA2 were assumed in all families with female BC, only eight alterations were observed in the collection of 23 families with site-specific female BC (34%), with four in BRCA1 and four in BRCA2. The study estimated that 8-10 site-specific BC families in their research were not attributed to both BRCA1 and BRCA2 genes, suggesting the presence of at least one more susceptible gene for breast cancer in their findings [27].

In a study reporting 1037 potentially influential Single Nucleotide Polymorphisms (SNPs) in 437 females with initial breast cancer (BC) and 2463 control cases, significant associations were identified for various genes linked with BC risk. Notably, BRCA1, BRCA2, ATM, TP53, and CHEK2 genes were found to be associated with BC, with a total of twenty-five SNPs implicated. The SNPs associated with BC risk included those in the BRCA1 gene (rs1799950, rs4986850, rs22279945, rs16942, and rs1799966), BRCA2 gene (rs766173, rs144848, rs4987117, rs1799954, rs11571746, rs11571747, rs4987047, rs11571833, and rs1801426), ATM gene (rs3218707, rs4987945, rs4986761, rs3218695, rs1800056, rs1800057, rs3092856, rs1800058, and rs1801673), CHEK2 gene (rs1787991), and TP53 gene (rs1042522) [32]. In a study examining nine single nucleotide polymorphisms (SNPs) with previous indications of their association with breast cancer (BC), datasets from 9 to 15 studies were utilized. The datasets comprised 11,391 to 18,290 case individuals and 14,753 to 22,670 control individuals. Specifically, the study focused on the relationship between BC and two SNPs, namely CASP8 D302H (rs1045485) and L10P TGFB1 (rs1982073). For CASP8 D302H, the odds ratio was found to be 0.89 for heterozygotes and 0.74 for unique homozygotes, suggesting a potential protective effect against BC. On the other hand, for L10P TGFB1, the odds ratio was 1.07 for heterozygotes and 1.16 for unique homozygotes, indicating a weaker association with BC [33]. In a study involving 11,563 control cases, researchers conducted genotyping of 300,000 Single Nucleotide Polymorphisms (SNPs) in 1,600 individuals from Iceland with breast cancer (BC). The study identified two specific SNPs associated with breast cancer: rs13387042 on chromosome 2q35 and rs3803662 on chromosome 16q12 [4]. In a study, Single Nucleotide Polymorphisms (SNPs) in the 5' UTR of the RAD51 gene, specifically the 135 GgC variation, were investigated as potential modifiers of breast cancer (BC) risk in carriers of BRCA1/2 mutations. Based on earlier indications, these SNPs were explored for their potential influence on BC risk, particularly in individuals with BRCA1/2 variations. The study observed an enhanced BC risk in individuals with CC homozygotes for the 135 GgC variation. Specifically, when analyzing BRCA1/2 alteration carriers individually and studying the cancer probabilities for BRCA2 alteration carriers, the study found that the 135 GgC variation in the RAD51 gene might modify the risk of BC in BRCA2 alteration carriers [34].

Incidence Rate in Pakistan

Breast cancer (BC) in Pakistan presents a significant health concern, with one in nine females developing BC, indicating a high incidence rate in Asia. The death rate associated with breast cancer was notably high in Pakistan in 2012. A recent study conducted at the Shaukat Khanum Memorial Cancer Hospital and Research Center (SKMCH & RC) in Lahore reported that 45.9% of variations occurred in females with breast cancer in December 2009. It's important to note that exact breast cancer statistics may be lacking due to a lack of national-level registration, underscoring the need for comprehensive data collection to better understand and address the prevalence and impact of breast cancer in the region [35-37].

Observation and early identification of breast cancer (BC) face challenges in Pakistan, with over 30% of BC patients being diagnosed at stages 3 and 4. The primary identification of BC, such as through mammography, is not widely available, primarily due to the high ratio of patients in Pakistan and the limited resources to address this issue in the targeted population. A lack of awareness about the disease further complicates the situation, with many females in Pakistan becoming aware of BC only when it has reached an advanced stage. Common methods for detecting BC include Breast Self-Examination (BSE), Clinical Breast Examination (CBE), and mammography. These methods play a crucial role in early detection, but the challenges in access and awareness emphasize the need for comprehensive strategies to improve breast cancer screening and education in the population [36, 38]. Breast Self-Examination (BSE) is a method used at home by females to sense variations in their breasts, offering a simple and cost-effective approach to detecting breast cancer (BC). The initial noticeable sign of BC often involves a mass expansion in the breast. Additionally, changes in breast size, thickening of breast tissues, rashes around the nipples, nipple discharge, regular discomfort, and inflammation in the breast are among the signs that may be observed. The simplicity and low cost of BSE make it an accessible tool for females to monitor changes in their breasts regularly. Consistent Breast Self-Examination can potentially contribute to the early identification of BC, leading to

Table 1: Mutations in BRCA 1 and BRCA 2 genes in Pakistan population.

Sr. no	Title of paper	Gene name	No. of patients studied	Mutations observed/ Significance	Technique used	Publication year	References
1	Contribution of BRCA1 and BRCA2 Mutations to Breast and Ovarian Cancer in Pakistan	BRCA 1	341	15(4.4%)	PTT, DS	2002	[45]
2	Contribution of BRCA1 and BRCA2 Mutations to Breast and Ovarian Cancer in Pakistan	BRCA2	341	8(2.3%)	DS, PTT	2002	[45]
3	Prevalence of BRCA1 and BRCA2 mutations in Pakistani breast and ovarian cancer patients	BRCA 1	176	23(13%)	DHPLC, SSCP, PTT	2006	[43]
4	Prevalence of BRCA1 and BRCA2 mutations in Pakistani breast and ovarian cancer patients	BRCA2	176	7(3.9%)	DHPLC, SSCP, PTT	2006	[43]
5	Contribution of BRCA1 germline mutation in patients with sporadic breast cancer	BRCA 1	150	1(0.67%)	SSCP	2008	[44]
6	BRCA1 status in Pakistani breast cancer patients with moderate family history	BRCA 1	53	3(5.66%)	PTT, SSCP	2011	[46]
7	Identification of Mutations in Gene BRCA1/2 in Breast Cancer Cases from Baluchistan, Pakistan	BRCA1	9	0	Histopathology	2019	[47]
8	Identification of Mutations in Gene BRCA1/2 in Breast Cancer Cases from Baluchistan, Pakistan	BRCA2	4	0	Histopathology	2019	[47]
9	Predictive role of single nucleotide polymorphism (rs11614913) in the development of breast cancer in the Pakistani population	rs11614913	300	Significant	Sanger sequencing	2020	[20]
10	Significant association of BRCA1 (rs1799950), BRCA2 (rs144848) and TP53 (rs1042522) polymorphism with breast cancer risk in the Pashtun population of Khyber Pakhtunkhwa, Pakistan	BRCA1, BRCA2, TP53	140	Significant	T-ARMS-PCR protocol	2023	[18]

higher survival rates among individuals. Encouraging women to perform regular BSE is crucial in promoting early detection and timely intervention [39].

Diagnostic methods for breast cancer (BC) include various approaches such as physical examinations, mammography, breast ultrasounds, magnetic resonance imaging (MRI), biopsies, and ductograms. These methods aid in the accurate diagnosis and staging of BC. Surgeries are often employed as a treatment for BC, and additional therapeutic interventions may include hormonal therapies, chemotherapies, and radiation therapies. The choice of treatment depends on the specific characteristics of the cancer and its stage at diagnosis. The prognosis and survival rates for individuals with breast cancer are significantly influenced by the stage at which the cancer is diagnosed. Early detection through enhanced screening programs has been shown to improve the chances of a cure for BC and reduce mortality rates by about 20%. Regular screenings and awareness initiatives play a vital role in promoting early diagnosis and effective management of breast cancer [40, 41]. The primary emphasis in combating breast cancer (BC) lies in early screening. Early detection significantly improves the survival rate and facilitates more effective treatment for this disease. Detecting BC at an early stage allows for the removal of affected tissues before the cancer has the chance to spread to other parts of the body. Patients must undergo regular screenings and detect BC as early as possible, preventing any delays in identification and treatment. Timely detection is paramount, as delays in diagnosis can lead to the progression of the disease to a more serious condition and may result in fatal outcomes. Encouraging awareness, regular screenings, and proactive healthcare measures are essential components of the strategy to combat breast cancer effectively [42].

In a study discussed in Table **1** of breast cancer patients, the presence of mutations in BRCA1 and BRCA2 genes was investigated. According to the findings reported by [43] among the 176 breast cancer patients analyzed, 13% exhibited mutations in the BRCA1 gene, while 3.9% showed mutations in the BRCA2 gene [44] observed 150 patients showing 0.67% of mutations in the BRCA1 gene [45]. Observed 341 patients showing 4.4% of mutations in the BRCA1 gene and 2.3% in the BRCA2 gene [46]. Observed 53 patients showing 5.66%of mutations in the BRCA1 gene [47]. Observed 9 patients showing mutations in the BRCA1 gene and 4 patients showing mutations in the BRCA2 gene.

SNPs that are analytically notable, show a connection with BC threat in common populations for example modifiers of threat in BRAC1 and BRAC2 alterations carriers, concentrated on SNPs. These modifiers were studied by SNPs in candidate genes and GWAS comprises D302H of CASP8 [48], rs2981522 of FGFR2, rs3803662 of TOX3/TNRC9, rs889312 of MAP3K1 [48], rs3817198 of LSP1, rs13387042 of 2q35 and rs13281615 of 8q24 [3]. In the common population, many loci that had been observed were interconnected with BC. **Fig. (1)** shows mutations in BRCA1 and BRCA2 genes in the graph.

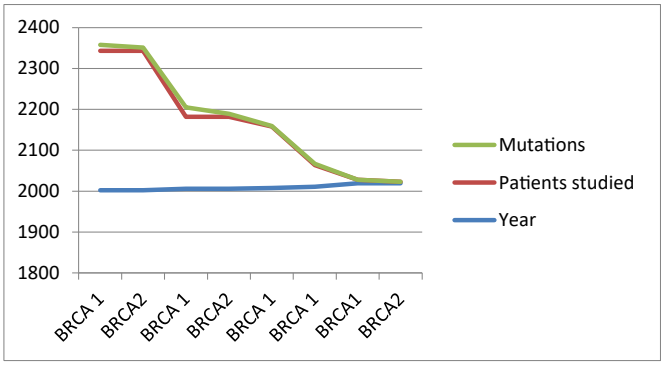

Fig. (1): Mutations in BRCA1 and BRCA2 genes in Pakistani Breast cancer patients.

CONCLUSION

It is concluded that BRCA1 and BRCA2 genes collectively contribute to 25% of inherited breast cancer cases. While these genes normally function as tumor suppressors, their involvement in the development of breast cancer is noteworthy. The study, particularly focusing on families from Goa, India, highlighted the presence of BRCA1 mutated genes in two sisters from one family out of eight families. Among the 31 observed families, eight had male breast cancer (BC) cases, and 23 had female BC cases. Comprehensive analysis revealed variations in two BRCA1 and four BRCA2 genes in six families with male BC and eight alterations in 23 families with site-specific BC (34%), including 4 in BRCA1 and 4 in BRCA2. Single Nucleotide Polymorphisms (SNPs) were also observed in different regions of BRCA1/2 genes, emphasizing the potential threat these variations pose to individuals' lives.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHORS' CONTRIBUTION

All the authors contributed equally to the publication of this article.

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