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ORIGINAL ARTICLE

FREQUENCY OF BRCA MUTATIONS IN BREAST CANCER PATIENTS: EXPERIENCE FROM SHIFA INTERNATIONAL HOSPITAL, PAKISTAN: A DESCRIPTIVE CROSS-SECTIONAL STUDY

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ABSTRACT

Background: Breast cancer in Pakistan includes 5–10% hereditary cases linked to BRCA1/2 mutations. This study aimed to identify BRCA1/2 mutations and their variants in breast cancer patients and assess their association with clinical and histopathological features for improved personalized care.

Materials & Methods: This retrospective study at Shifa International Hospital analyzed 123 breast carcinoma cases tested for BRCA1/2 mutations over 5.5 years. Data were evaluated using SPSS 26, applying descriptive statistics, Chi-square, and Fisher's exact tests ([P < .05 significant]).

Results: *BRCA* positivity was detected in 13% ([n=16]), with *BRCA1* in 8.9% ([n=11]) and *BRCA2* in 4.1% ([n=5]). All cases had invasive breast carcinoma of no special type. Family history was reported in eight *BRCA1* and two *BRCA2* cases. Consanguinity was found in seven *BRCA1* and two BRCA2 cases, showing a significant association for *BRCA1* ([P=.037]). The most common molecular subtype was triple-negative in *BRCA1* and HER2-neu overexpressed in *BRCA2*. Nonsense mutations were predominant in both genes. Class 1 pathogenic variants were found in nine *BRCA1* and four *BRCA2* cases, while class 2 variants were seen in two *BRCA1* and one *BRCA2* case. Two *BRCA1*-positive patients shared the c.5566C>T mutation. Mutation type ([P=.001]) and gene variant ([P=.001]) were significant for both genes.

Conclusions: *BRCA* mutations were detected in 13% of cases, with *BRCA1* more prevalent than *BRCA2*. Nonsense mutations were most common, and consanguinity was significantly associated with *BRCA1* positivity.

KEY WORDS: Breast; Cancer; Consanguinity; Features; Gene; Mutation.

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INTRODUCTION

Breast cancer is the most frequently occurring malignancy among women worldwide, accounting for 24.4% of all cancer cases.[1] In 2020, approximately 2.3 million new cases were reported[2] ,representing 11.6% of all new cancer cases.[3] By 2040, the incidence is expected to rise to 33.8%.[4] Globally, breast cancer is the fifth leading cause of cancer-related deaths in women[5], accounting for 16% of all cancer-related deaths[2] Pakistan faces an alarming situation, with the highest incidence of breast cancer in Asia. Breast cancer constitutes 47.3% of all cancer cases in the country, making it the third most prevalent cancer overall.[4] The age-standardized incidence rate ([ASIR]) for breast cancer in Pakistan is 34.4 per 100,000, which is significantly lower compared to the United States' rate of 90.3 per 100,000.[6] Pakistan reports an annual incidence of 90,000 cases and 16,000 deaths, with the mortality rate predicted to increase by 62% by 2030.[4]

While breast cancer is primarily sporadic, 5–10% of cases are hereditary and linked to specific gene mutations. Among these, BRCA1 and BRCA2 are the most studied and clinically significant genes, discov-

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ered in 1994 and 1995, respectively.[7] Mutations in these tumor suppressor genes significantly increase the risk of developing breast and ovarian cancers, making them critical markers for genetic risk.[8] Although rare in the general population ([affecting 1 in 300–800 individuals]),[9] these mutations confer a 55–72% risk for BRCA1 carriers and a 45–69% risk for BRCA2 carriers of developing breast cancer by the age of 70–80 years.[10] Studies have shown that BRCA mutation carriers have poorer overall survival rates and a higher risk of distant recurrence compared to non-carriers.[11] In Pakistan, the prevalence of BRCA1 mutations in breast cancer is higher than BRCA2, differing from other Asian countries like China, Indonesia, Malaysia, and the Philippines.[12] Next-generation sequencing (NGS) is a powerful tool for identifying mutations and theranostic markers, widely used since the early 2000's. It detects various genetic alterations and supports complex diagnoses. [13] However, its application in Pakistan is limited, with scarce population-specific data despite global integration into routine diagnostics.

Population-specific studies are essential to understand genetic mutation patterns. Identifying variants helps assess cancer risk and guides personalized treatment, improving outcomes and risk management for breast and other cancers, ultimately benefiting both patients and their families. This study aimed to evaluate the prevalence of BRCA1 and BRCA2 mutations and their variants in Pakistani breast cancer patients. It also seeks to analyze their association with clinicopathological factors and provide insights into genetic counseling and personalized medicine, enhancing our understanding of the genetic landscape of breast cancer in Pakistan.

MATERIALS AND METHODS

This cross-sectional retrospective study was conducted in Pathology department, Shifa International hospital from Jan 2019 to June 2024. Purposive sampling was done and total 123 patients were selected that were all diagnosed cases of breast cancer. BRCA1/BRCA2 genetic testing done on non-breast cancer cases were excluded. The study was IRB-approved at Shifa International Hospital. Breast cancer cases over 5.5 years were retrieved via Electronic Medical Record, and data were analyzed using SPSS version 26. Descriptive statistics summarized clinical, histopathological and molecular characteristics of BRCA1/2 carriers. Chi-square and Fisher's exact tests assessed associations with clinicopathological parameters, considering p-values <0.05 as statistically significant.

RESULTS

A total of 123 cases were included, with a mean patient age of 48 years; from which 98.4% were female. Modified radical mastectomy([MRM]) was performed in 52%, and lumpectomy in 48% cases. Most tumors were of histological grade 2 ([62.6%]) and Invasive breast carcinoma, no special type ([NST]), was the most common subtype. Luminal A was the predominant molecular subtype ([39.8%]). Ductal carcinoma in situ ([DCIS]) and lobular carcinoma in situ ([LCIS]) was observed in 26% and 7.3% of cases, respectively. Estrogen receptor ([ER]), progesterone receptor ([PR]), and HER2-neu positivity were found in 54.5%, 55.3%, and 34.2% of cases, respectively (see Table 1).

BRCA mutations were found in 16 of 123 cases ([13%]), with 11 ([8.9%]) BRCA1 and 5 ([4.1%]) BRCA2 positive cases. All patients were female, with a mean age of 44.8 years. Most tumors were in the right breast (62.5%), averaging 4.9 cm. MRM was performed in 62.5% of cases. Histological grade 2 and 3 were seen in 50% and 43.75%, respectively. All cases were invasive carcinoma NST. ER and PR positivity were found in 37.5%, HER2-neu in 43.75%. Triple-negative and HER2-neu overexpressed subtypes were most common (31.25% each), followed by Luminal A (25%) and Luminal B (12.5%). Nottingham scores were 6–7 in 50% and 8–9 in 43.75%, with high Ki-67 in all. A family history was noted in 10 cases, and consanguinity in 9 cases. Nonsense mutations predominated. BRCA1 cases had mostly class 1 variants; BRCA2 cases included both class 1 and 2. Both shared and unique BRCA1/2 gene variants were identified as shown in Figure 1.

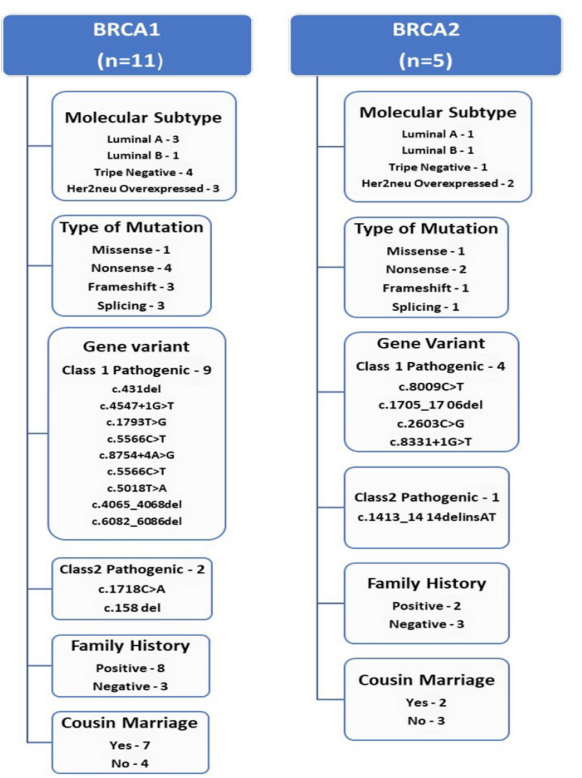

Figure 1: Characteristics of BRCA 1 and BRCA 2 positive cases

Chi-square and Fisher's exact tests showed significant associations of BRCA1 with consanguinity, mutation type, and gene variant. For BRCA2, mutation type and variant were significant (shown in table 2).

Table1: Clinical, surgical and histopathological characteristics of all enrolled (n=123) and BRCA positive (n=16) patients

Variables	All patients n = 123 (%)	BRCA positive n = 16(%)
Age		
Mean age, years (min-max)	48.18 (18-84)	44.8 (27-70)
Mean size of Tumor, cm (min - max)	3.74 (0.23-14.30)	4.90 (0.5 -12.1)
Gender		
Male	2 (1.6)	0 (0.0)
Female	121 (98.4)	16 (100)
Specimen Laterality		
Left Breast	61 (49.5)	6 (37.5)
Right Breast	62 (50.5)	10 (62.5)
Surgical margins		
Involved	1 (0.8)	0 (0.0)
Uninvolved	122 (99.2)	16 (100)
Type of surgery		
Lumpectomy	59 (48)	6 (37.5)
MRM	64 (52)	10 (62.5)
Histological Grade		
1	10(8.1)	1(6.25)
2	77(62.6)	8 (50.0)
3	36 (29.3)	7(43.75)
DCIS		
Not seen	91 (74)	14 (87.5)
Seen	32 (26)	2 (12.5)
LVI		
Absent	85(69.1)	12 (75)
Present	38 (30.9)	4 (25)
LCIS		
Absent	114 (92.7)	16(100)
Present	9(7.3)	0 (0.0)
ER		
Positive	67 (54.5)	6 (37.5)
Negative	56 (45.5)	10 (62.5)
PR		
Positive	68 (55.3)	6 (37.5)
Negative	55 (44.7)	10 (62.5)
Her2neu		
Positive	42 (34.2)	7 (43.75)
Negative	81 (65.8)	9 (56.25)
Molecular Subtype		
Luminal A	49 (39.8)	4 (25.0)
Luminal B Her2 (+)	18 (14.6)	2 (12.5%)
Her2neu (+)	24 (19.5)	5 (31.25)
Triple Negative	32 (26.1)	5 (31.25)
Histologic Subtype		
Invasive Breast Carcinoma NST	111 (90.4)	16(100)
Invasive Apocrine Carcinoma	2 (1.6)	0(0.0)
Invasive Cribriform Carcinoma	1 (0.8)	0(0.0)
Invasive Lobular Carcinoma	6 (4.8)	0(0.0)
Invasive Micropapillary Carcinoma	1 (0.8)	0(0.0)
Pleomorphic Breast Carcinoma	1 (0.8)	0(0.0)
Mixed (mucinous + NST)	1 (0.8)	0(0.0)
Lymph Nodes		
Positive	32 (26)	2 (12.5)
Negative	91 (74)	14 (87.5)
Nottingham Score		
Less than 5	19 (15.4)	1 (6.25)
Between 6 to 7	67 (54.4)	8 (50.0)
Between 8 to 9	37 (30.2)	7 (43.75)
Ki67%		
Less than 14	16 (13.1)	0 (0.0)
Greater than 14	107 (86.9)	16 (100.0)
BRCA		
Positive – (BRCA1, BRCA2)	16 (13.9)	11,5 (68.7,31.2)
Negative	107 (86.1)	0 (0.0)

Table: 2 Correlations between BRCA1/2 mutations and clinicopathological characteristics

		BRCA1			BRCA2		
Variables	**n = 123**	**Yes (%)**	**No (%)**	**p**	**Yes (%)**	**No (%)**	**p**
Age							
<35 years	24	2 (8.3)	22 (91.7)	0.626	2 (8.3)	22 (91.7)	0.729
35 - 45 years	29	4(13.7)	25 (86.3)		1 (3.4)	28 (96.6)	
45 - 55 years	29	3(10.3)	26 (89.7)		0(0.00)	29 (100)	
>55 years	41	2 (4.8)	39 (95.2)		2 (4.8)	39 (95.2)	
Pathological Subtype							
Invasive Breast Carcinoma NST	111	11(9.9)	100(90.1)	0.998	5 (4.5)	106 (95.5)	1.000
Invasive Apocrine Carcinoma	2	0 (0.0)	2 (100)		0 (0.0)	2 (100)	
Invasive Cribriform Carcinoma	1	0 (0.0)	1 (100)		0 (0.0)	1 (100)	
Invasive Lobular Carcinoma	6	0 (0.0)	6 (100)		0 (0.0)	6 (100)	
Invasive Micropapillary Carcinoma	1	0 (0.0)	1 (100)		0 (0.0)	1 (100)	
Pleomorphic Breast Carcinoma	1	0 (0.0)	1 (100)		0 (0.0)	1 (100)	
Mixed (mucinous + NST)	1	0 (0.0)	1 (100)		0 (0.0)	1 (100)	
Histological Grade							
1	10	1(10)	9(90)	0.137	0 (0.0)	10(100)	0.793
2	77	4(5.1)	73(94.9)		4(5.1)	73(94.9)	
3	36	6(16.6)	30(83.4)		1(2.7)	35(97.3)	
Molecular Subtype							
Luminal A	50	3(6)	47(94)	0.772	1 (2.0)	49 (98.0)	0.412
Luminal B Her2 (+)	18	1(5.5)	17 (94.5)		1(5.5)	17 (94.5)	
Her2neu (+)	24	3(12.5)	21(87.5)		2(8.3)	22(91.7)	
Triple Negative	31	4(12.9)	27(87.1)		1(3.2)	30(96.8)	
Nottingham Score							
Less than 5	19	1 (5.2)	18 (94.8)	0.485	0 (0.0)	19 (100)	
Between 6 to 7	67	5 (7.4)	62 (92.6)		3 (4.4)	64(95.6)	
Between 8 to 9	37	5(13.5)	32 (86.5)		2 (5.4)	35 (94.6)	
Ki67%							
Greater than 14	107	9 (8.4)	98 (91.6)	0.434 #	5 (4.6)	102 (95.4)	0.568 #
Less than 14	16	2(12.5)	14 (87.5)		0 (0.0)	16 (100)	
Cousin Marriage							
Yes	42	7(16.6)	35 (83.4)	0.037 #	2 (4.7)	40 (95.3)	0.578 #
No	81	4 (4.9)	77 (95.1)		3 (3.7)	78 (96.3)	
Type of Mutation							
Missense	2	1 (50)	1 (50)	0.000	1 (50)	1 (50)	0.000
Nonsense	6	4(66.6)	2(33.4)		2 (33.4)	4 (66.6)	
Frameshift	4	3 (75)	1 (25)		1 (25)	3 (75)	
Splicing	4	3 (75)	1 (25)		1 (25)	3 (75)	
BRCA Negative Cases	107	0 (0.0)	107 (100)		0 (0.0)	107 (100)	
Family History							
Positive	71	8(11.2)	63(88.8)	0.234 #	2 (2.8)	69 (97.2)	0.203 #
Negative	52	3 (5.7)	49 (94.3)		3 (5.7)	49 (94.3)	
Gene Variant							
Class1 Pathogenic	13	9(69.2)	4 (30.8)	0.000	4 (30.8)	9 (69.2)	0.000
Class2 Pathogenic	3	2(66.6)	1 (33.4)		1 (33.4)	2 (66.6)	
BRCA Negative Cases	107	0 (0.0)	107 (100)		0 (0.0)	107 (100)	

#Fisher's exact test.

DISCUSSION

Hereditary breast and ovarian cancer syndrome, caused by BRCA1/2 mutations, impairs DNA repair and increases cancer risk. [14] BRCA testing guides prevention, prognosis, and personalized treatment. Guidelines like NCCN and ASCO recommend testing for high-risk individuals, including those with early-onset or triple-negative breast cancer or a family history.[15] In Pakistan, many eligible patients remain untested due to financial barriers and limited healthcare coverage, hindering early detection and effective management.

In our study, 13% of cases were BRCA-positive, with 8.9% BRCA1 and 4.1% BRCA2, indicating a higher prevalence of BRCA1 mutations in the Pakistani population. This aligns with findings by Priyanka et al., [11] Liede et al.,[16] and multiple studies by Rashid et al,.[17,18,19] all showing BRCA1 predominance. In contrast, BRCA2 mutations are more frequently reported in other Asian populations, such as Bangladesh, China, Hong Kong, Korea, and the Philippines, highlighting regional genetic differences. [7]

Among BRCA1-positive cases, one recurrent mutation (c.5566C>T) was found in two patients, while other variants were unique ([e.g., c.431del, c.4065_4068del, c.1793T>G]). BRCA2-positive cases showed both class 1 and 2 pathogenic variants, all distinct ([e.g., c.8009C>T, c.2603C>G]). Notably, mutations like c.4065_4068del and c.1793T>G (BRCA1), and c.2603C>G (BRCA2) were also reported by Liede et al.[16] and Rashid et al.[19] in Pakistani families. These shared findings highlight some regional mutation patterns, yet the overall data on BRCA variants in the Pakistani population remains limited, emphasizing the need for further research and expanded genetic screening.

In our study, BRCA1 cases were mostly triple-negative, while BRCA2 cases showed HER2-neu overexpression. This aligns with study done by Bao S et al.[20] reporting triple-negative subtype in BRCA1 cases. However, BRCA2 was more often Luminal B in other studies, including one by Azim et al.[21] Our study found a significant association between consanguinity ([P = .037]) and BRCA1 positivity, with no significant links to age, tumor features, or family history. This contrasts with a Chinese study [20] but aligns with Turkish research [22] showing no correlation between BRCA mutations and various tumor or pathological parameters.

This study adds to limited Pakistani data on BRCA1/2 mutations, despite the country's high breast cancer incidence. Understanding local mutations aids in identifying hereditary risk, guiding preventive strategies, and personalizing treatment. Additionally, an Asian BRCA1/2 registry is essential for advancing region-specific research and improving cancer management.

CONCLUSION

BRCA mutations were found in 13% of cases ([8.9% BRCA1, 4.1% BRCA2]). Triple-negative molecular subtype was common in BRCA1, while it was HER2-neu in BRCA2. Nonsense mutations predominated. Consanguinity was significantly associated with BRCA1, while mutation type and gene variant were significant in both BRCA1 and BRCA2 cases.

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CONFLICT OF INTEREST
Authors declare no conflict of interest.
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AUTHORS' CONTRIBUTION

The following authors have made substantial contributions to the manuscript as under:

Conception or Design:	ZA, MN
Acquisition, Analysis or Interpretation of Data:	ZA, MN, AM, NM, AR
Manuscript Writing & Approval:	ZA, MN, AM, NM, AR

All the authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.