RESEARCH ARTICLE

Editorial Process: Submission:03/09/2024 Acceptance:12/13/2024

Mutation Spectrum Analysis of *BRCA1/2* Genes for Hereditary Breast and Ovarian Cancer in the Indian Population

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Abstract

Objective: The objective of this study was to determine the prevalence and spectrum of genetic mutations linked to inherited breast and ovary cancer (HBOC) in the Indian population, and to evaluate the correlation of *BRCA* mutation types, frequency, and incidence with age, gender, and personal and family history. **Methods:** A retrospective cohort of 500 Indian HBOC patients, meeting NCCN criteria who underwent *BRCA1/2* testing from 2017 to 2023 were shortlisted for this study. The anonymized data was retrieved from medical records. Genetic analysis was conducted using Next Generation Sequencing (NGS) on the Thermo Ion GeneStudio™ S5 System, with positive mutations confirmed via Sanger sequencing. Peripheral blood samples were processed for DNA extraction, library preparation, and variant classification following ACMG guidelines. **Results:** Out of the 500 patients, 119 (23.8%) were positive for *BRCA* mutations, and 381 (76.2%) were negative. The prevalence of BRCA pathogenesis, likely pathogenicity, and variants of uncertain significance (VUSs) were 14.8%, 1.6%, and 7.4%, respectively. A total of 128 mutations were detected in the positive *BRCA1/2* patients. A statistically significant correlation was found between *BRCA* mutations with the patient and family history. A total of 38.8% of the patients with mutations had a family history of BC, OC or BC/OC, while 7.6% had other cancers. *BRCA* mutations were predominant (26.2%) in the age group of 46-65 Y. Among the 128 mutations, 59.3% (76/128) and 40.6% (52/12) of the patients had mutations in *BRCA1* and *BRCA2*, respectively. Missense mutations were the most common in both the *BRCA1* (30.26%) and *BRCA2* (55.77%) genes, followed by frameshift (22.3%) and nonsense (17.3%) mutations in *BRCA1* and *BRCA2*, respectively. Conclusion: BRCA positivity was detected in 23.8% of the patients. A statistically significant association was shown between *BRCA* mutations and patient and family history.

Keywords: Next generation sequencing- developing countries- breast cancer- ovarian cancer

Asian Pac J Cancer Prev, **25 (12)**, 4145-4151

Introduction

Breast cancer (BC) is the most common cancer among women worldwide. BC is responsible for approximately 11.6% of all cancer cases and 6.6% of all cancer-related deaths (Bray et al. 2018). Ovarian cancer (OC) is less common than breast cancer and accounts for approximately 1.6% of all cancers and 1.9% of cancer-related deaths [1]. Approximately 90–95% of breast cancers and 80% of ovarian tumors are sporadic, while 5–10% and 20% are familial or hereditary, respectively. Among BC/OC cases, 20–25% are caused by the highly penetrant cancer susceptibility genes *BRCA1* and *BRCA2* [2]. *BRCA1/2* mutations are found in different ethnic groups and geographical locations, with frequencies in white European and Australian populations (17.6–29.8%) and lower frequencies in Asian countries (9.4–21.7%) [3]. The frequency of breast cancer in Asian countries, specifically India, is lower than that in developed countries because of the large population size. However, the number of deaths from new cases reported in India is much greater (48.3%) than that in the United States (18.9%) and European Union (25.4%) [4]. Delayed implementation of effective breast cancer screening programs, lifestyle changes, and limited access to treatment are reasons cited for higher mortality rates in India [5]. It is critical to identify high-risk populations and provide them with timely genetic consultation to determine risk, mutation carrier status, genetic variation analysis, preventive care, and prophylactic surgery for reducing mortality.

Next-generation sequencing (NGS) technology offers a strong approach for identifying novel genes linked to BRCA cancer susceptibility for early diagnosis and the opportunity to counsel patients with a family history for screening and surveillance and as a risk-reduction strategy [6]. NGS-based gene sequencing is widely used as a

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high-throughput and cost-effective screening method for sequencing specific targets of interest; this method allows for massive parallel multigene analysis within significant time, cost savings, and a small amount of nucleic acid [7]. Moreover, sequencing in combination with Sanger sequencing was used to evaluate important hotspot mutations in breast cancer, and 98.4% concordance was found between the two sequencing methods [8]. Sequencing technologies are powerful tools for identifying novel factors involved in familial BC/OC. The discovery of new genetic factors would have significant translational value by assisting pathologists in identifying potential risks and in developing prevention strategies [9].

Increased demand for hereditary breast and ovarian cancer (HBOC) testing is putting pressure on diagnostic laboratories to expand their mutation screening capacity and classify BRCA sequence variants for clinical significance, such as by interpreting pathogenic mutations and variants of unknown significance and determining large genomic rearrangements accurately. In this study, 500 patients of Indian nationality were found to have BRCA mutations. The association of BRCA mutation type, frequency, and incidence with age, gender, patient, and family history was evaluated.

Materials and Methods

Study population

A retrospective study was conducted in an Indian HBOC cohort comprising 500 patients who met the National Comprehensive Cancer Network (NCCN) criteria. *BRCA1/2* genetic testing was performed for these patients, whose samples were submitted to Metropolis Healthcare Limited (Vidyavihar, Mumbai, India) between April 2017 and March 2023. The HBOC cohort is divided into four age groups <=25 Y, 26-45 Y, 46-65 Y and >65 Y. Patient and family history and clinical information were retrieved from the Metropolis medical records, and their information was masked.

BRCA mutation spectrum analysis using gene sequencing

BRCA1 and *BRCA2* genetic analysis was performed via NGS using the Ion GeneStudio™ S5 System (Thermo Fisher Scientific, USA), and confirmation of the positive mutations was performed via Sanger sequencing (3500Dx Genetic Analyzer, Applied Biosystems, Thermo Fisher Scientific, Inc., USA). A 3 mL peripheral blood sample in ethylenediaminetetraacetic acid (EDTA) was collected from the patient, and genomic DNA was extracted using a standard QIAamp DNA Mini Kit (QIAamp Kit, Qiagen, CA, USA) according to the manufacturer's protocol. For library preparations, capture probes targeting the *BRCA1* and *BRCA2* gene coding domains with 10 bp flanking intronic sequences were used. These were selectively used to enrich the regions of interest in the genomic DNA that had been enzymatically fragmented. The libraries were created using adaptors that were compatible with Ion GeneStudio S5, and each amplicon had a minimum coverage of 20 bases [10].

Sanger sequencing was used to confirm the mutations detected by the NGS platform [11]. The extracted DNA was quantified using a NanoDrop OneTM UV/Vis Spectrophotometer (Thermo Fisher Scientific, Inc., USA) and amplified using polymerase chain reaction (PCR), and the fragments were verified using agarose gel electrophoresis. Sequencing was carried out on a Big Dye Terminator v3.1 Cycle Sequencing Kit on the 3500Dx Genetic Analyzer. The reference sequences *BRCA1* – NM_007294.3 and BRCA2 ¬– NM_000059.3 were used to identify the variants via BioEdit Sequence Alignment software (BioEdit v7.1.11; Ibis Biosciences, Carlsbad, CA). The classification of the variants was performed as per the guidelines of the American College of Medical Genetics (ACMG) [12].

Data analysis

Continuous data were recorded and are reported as the mean ± standard deviation (SD), median (interquartile range or IQR) and range. Discrete variables such as age group, gender, history of cancer, and family history were summarized in terms of frequencies and percentages. Variants were classified into five classes, namely, benign (class 1), likely benign (class 2), variant of uncertain significance (class 3), pathogenic (class 4) and likely pathogenic (class 5) [13]. The chi-square test was used to determine the association of BRCA (detected/not detected) with age group, gender, and history of cancer in the patient and family. All the statistical analyses were performed using "R Studio version 1.4.1103". A two-tailed p value of <0.05 was considered to indicate statistical significance.

Results

Among the 500 patients, 119 (23.8%) had *BRCA1/2* genetic mutations according to the NGS method, while 381 (76.2%) had negative results. The prevalence of BRCA pathogenesis, likely pathogenicity and VUS were 14.8%, 1.6% and 7.4%, respectively (Figure 1). Among the 119 patients with a positive *BRCA1/2* genetic mutation, 110 had single mutations, while 9 had double mutations, resulting in a total of 128 mutations. 53.125% (68/128) mutations were detected in *BRCA1* gene and 32.8125% (42/128) mutations in *BRCA2* genes. The prevalence of the *BRCA1* gene was 4.6875% (6/128) in three patients, the prevalence of the *BRCA2* gene was 6.25% (8/128) in four patients, and the prevalence of both the *BRCA1* and *BRCA2* genes was 3.125% (4/128) in two patients (Figure 2).

Most of the individuals in HBOC cohort belonged to the age range of 46-65 (48.80%), the median age of the individuals was found to be 50 Y. Distribution pattern of cancer based on patient history and family in the study cohort (n=500) (Figure 3a and 3b). The association between *BRCA* gene mutations and a history of cancer (both family and patient history) was statistically significant (p value 0.0004), where individuals with a history of breast and ovarian cancer (BC/OC) had the highest detection rate (66.67%). A significant association (p value 0.0218) was also detected between mutations in *BRCA* genes and a family history of cancer. A total of 38.8 of the patients who were reported to be positive for *BRCA* mutations had a family history of BC and/or

Table 1a. BRCA Gene Patient History

Cancer	*BRCA* Detected n (%)	*BRCA* Not Detected n (%)	p Value *BRCA*	*BRCA1* Detected n (%)	*BRCA 1* Not Detected n (%)	p Value BRCA1	*BRCA2* Detected n (%)	*BRCA2* Not Detected n (%)	p Value *BRCA2*
Breast	45 (22)	155 (77)	0.0004	28 (14)	172 (86)	< 0.0001	19 (9)	181 (90)	0.5337
Ovarian	35 (33)	70 (67)		27 (26)	78 (74)		8 (8)	97 (92)	
Breast and Ovarian	6 (67)	3 (33)		5 (55)	4 (44)		1 (11)	8 (89)	
Pancreatic	0	15 (100)		0	15 (100)		0	15 (100)	
Prostate	0	7 (100)		0	7 (100)		0	7 (100)	
No history	33 (20)	131 (80)		13 (8)	151 (92)		20 (12)	144 (88)	

Statistical analysis of correlation between patient history with overall *BRCA* positivity & *BRCA 1* was found to statiscally significant (p value <0.05), while *BRCA 2* was found to statiscally insignificant (p value >0.05).

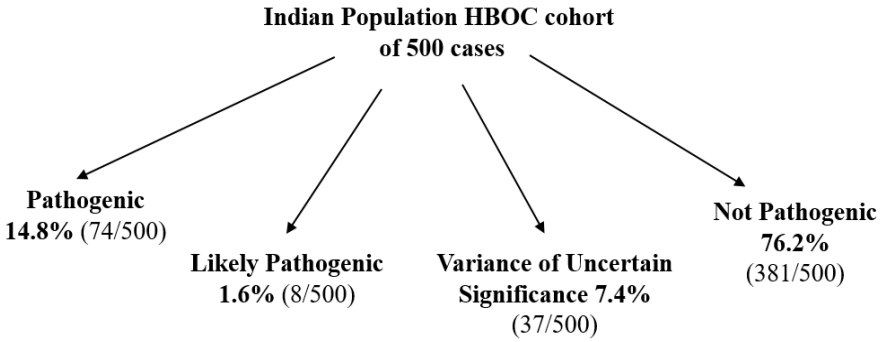

Figure 1. Distribution of Indian Population HBOC Cohort in Four Category i.e. pathogenic, likely pathogenic, variance of uncertain significance and not pathogenic.

OC, while 7.6% had other cancers. In the HBOC cohort, 28.4%, 6.4% and 4% of the individuals with mutations had a family history of BC, OC or BC/OC, respectively (Figure 3b). BRCA detection was highest in the 46-65 Y

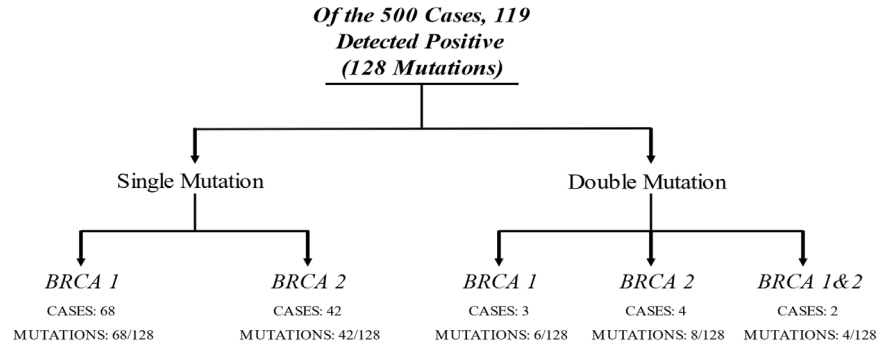

Figure 2. Distribution pattern of *BRCA1* and *BRCA2* gene mutations in studied HBOC cohort.

Table 1b. BRCA Gene Family History

	BRCA Detected n (%)	*BRCA* Not Detected n (%)	p Value *BRCA*	*BRCA1* Detected n (%)	*BRCA 1* Not Detected n (%)	p Value *BRCA1*	*BRCA2* Detected n (%)	*BRCA2* Not Detected n (%)	p Value *BRCA2*
Breast	39 (27)	103 (72)	0.0218	21 (15)	121 (85)	0.2512	19 (13)	123 (87)	0.0051
Ovarian	12 (37)	20 (62)		7 (22)	25 (78)		5 (16)	27 (84)	
Breast and Ovarian	8 (40)	12 (60)		4 (20)	16 (80)		5 (25)	15 (75)	
Pancreatic	0	2 (100)		0	2 (100)		0	2 (100)	
Prostate	2 (33)	4 (67)		0	6 (100)		2 (33)	4 (67)	
No history	48 (18)	220 (82)		33 (12)	235 (88)		15 (6)	253 (94)	
Other Cancer	10 (33)	20 (67)		8 (27)	22 (73)		2 (7)	28 (93)	

Statistical analysis of correlation between family history with overall *BRCA* positivity & *BRCA 1* was found to statiscally insignificant (p value >0.05), while *BRCA 2* was found to statiscally significant (p value <0.05).

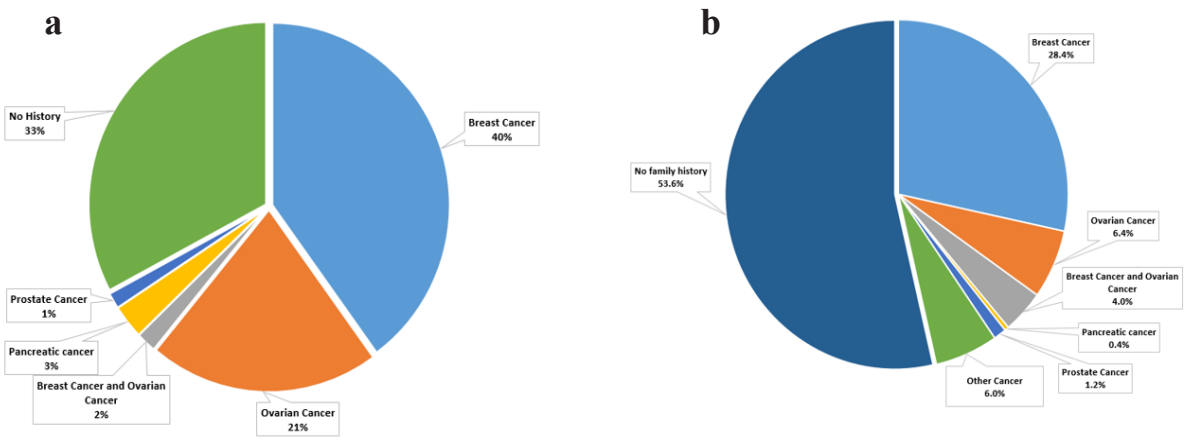

Figure 3. Patient History (a) and Family History (b) Analysis in the Positive Cases.

age group at 26% (67/255), followed by 25% (41/165) in the 26-45 Y age group, 14% (9/65) in those over 65 Y, and 13% (2/15) in those aged 25 Y or younger. In HBOC cohort, BRCA mutation was detected in 24% (114/473) of females & 18.5% (5/27) of males.

A significant relationship (p value <0.0001) was detected among patients with a history of cancer and *BRCA1* gene mutations. Among individuals with a malignancy, the BC/OC patients had significant rates of *BRCA1* mutations (55.5%) (Table 1a). Gender, age

Table 2. Correlation between Various Combinations of Patient & Family History with Overall *BRCA1, BRCA2 , BRCA1/2* Detection Rate

Cancer History Combinations		*BRCA1*	*BRCA2*	*BRCA1/2*	Not Detected
Patient	Family	n (%)	n (%)	n (%)	n (%)
Breast	Breast	7 (17)	7 (17)	1 (2)	26 (63)
Breast	Breast and Ovarian	0	1 (17)	1 (17)	4 (67)
Breast	Ovarian	0	0	0	3 (100)
Breast	Other Cancer	4 (27)	1 (7)	0	10 (67)
Breast	Prostate	0	1 (50)	0	1 (50)
Breast and Ovarian	Breast	4 (80)	0	0	1 (20)
Ovarian	Breast	5 (50)	0	0	5 (50)
Ovarian	Ovarian	5 (45)	2 (18)	0	4 (36)
Ovarian	Other Cancer	4 (29)	1 (7)	0	9 (64)

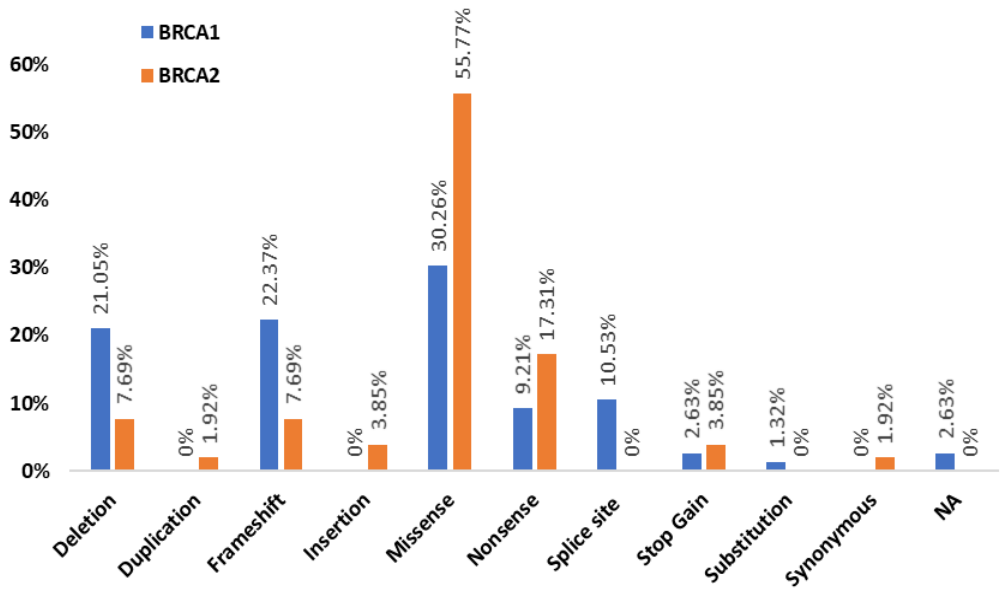

Figure 4. Types of Mutations among *BRCA1* and *BRCA2* Genes.

group and family history of cancer were not associated with mutations in the *BRCA1* gene. Among all the parameters, a significant association was observed among the individuals with a family history and a *BRCA2* gene mutation (p value 0.0051). Patients with a family history of cancer were more likely to be diagnosed with *BRCA* mutations (Table 1b). Among patients with a family history of cancer, 40% had *BRCA* mutations in BC/OC patients, whereas 37.5% had a family history of only OC, 27.4% had BC, and 33.3% had prostate cancer (Table 1b). In the population in which the patients were affected but had no family history, the maximum prevalence of *BRCA* mutations was 50% in the BC/OC cohort, whereas 26.09% of patients had only OC and 16.54% had only BC. Among the 42 patients without a personal or family history of cancer, 13.64% were positive.

Of the various combinations, the highest number (41) of patients had a personal and family history of BC, as shown in Table 2. Seven (17.07%) had equal numbers of mutations in the *BRCA1* and *BRCA2* genes, with 1 individual showing mutations in both. Eleven patients had a family history of ovarian cancer. Of the seven patients with mutations, 5 (45.45%) had *BRCA1* mutations and 2 (18.18%) had *BRCA2* mutations. Notably, 50% (5 out of 10) of *BRCA1* mutations were found in patient with a history of ovarian cancer and family history of breast cancer (Table 2).

The mutation spectra of 128 different variants were segregated into 11 types. Missense mutations were predominant in both the *BRCA1* (30.26%) and *BRCA2* (55.77%) genes. In the *BRCA1* gene, frameshift, deletion, splice site and nonsense variants accounted for 22.37%, 21.05%, 10.53% and 9.21%, respectively. Similarly, in *BRCA2*, the other common variant types, nonsense, frameshift and deletion, accounted for 17.31%, 7.69% and 7.69%, respectively (Figure 4). A total of 52 patients had the *BRCA1* pathogenic variant. The rate of pathogenic mutations in the *BRCA1* gene was the highest (68.42%), followed by 23.68% for VUS variants and 7.89% for likely pathogenic variants. Among the *BRCA2* gene mutations, VUS was the most prevalent (53.85%), followed by 42.31% pathogenic mutations and 3.85% likely pathogenic mutations.

The frequency of single nucleotide variants (SNVs), insertions and deletions (INDELs) and large genomic rearrangements (LGRs). Among all the mutations, SNVs were predominant in both the *BRCA1* and *BRCA2* genes, with 53.95% and 75%, respectively. In total, 44.74% of the INDELs were in the *BRCA1* gene, and 25% were in the *BRCA2* gene. There was only one (1.31%) *LGR* mutation in the *BRCA1* gene. The supplementary tables summarize 76/128 and 52/128 mutations detected in the *BRCA1* and *BRCA2* genes, respectively, with clinical indications for the patients (Supplementary Tables 1 and 2). The most common mutation was found to be c.68_69 del (popularly known as 185delAG) in the *BRCA1* gene, while c.7505 G>A in the *BRCA2* gene.

Discussion

NGS along with Sanger sequencing was used to detect the HBOC-related pathogenic mutations in the familial *BRCA1, BRCA2* and *BRCA1/2* genes. It was reported that with an NGS-based multigene panel, it was possible to detect *BRCA1/2* mutations in 25.7% (19/74) of patients, which were found to be negative by the conventional PCR-dHPLC method [14]. In the studied Indian HBOC cohort, *BRCA* mutations were most frequent (26%) in patient aged 46-65 Y, followed by those aged 26-45 Y (25%) age group. Another study performed in an Indian population reported that patients in the 40–50 Y of age group had 53.4% *BRCA* mutations and was found to be statically significant (p value 0.03) [5]. Patients in the 44–60 Y age group are at high risk of *BRCA* mutations [15]. The mean age at which 50 Y were observed was similar to that in a previous study [11]. Eerola et al. [16] reported that *BRCA1* and *BRCA2* patients exhibit typical features at a younger age (<50 Y) and older age (>50 Y), respectively No consistent correlation between *BRCA* mutation positivity and age was found [17], and was found to be not a reliable predictor of *BRCA1/2* mutation status [18, 19]. Other factors like family history and genetic profiles are more significant in identifying *BRCA* mutation carriers [20]. B.P. Koirala Memorial Cancer Hospital, found low (10%) regular screening (mammograms) rates among 150 first-degree female relatives of breast cancer patients [21]. Family history significantly increases breast cancer risk for first-degree relatives, highlighting the need for targeted interventions to enhance screening and awareness in high-risk group.

Almost half (46.4%) of the patients whose *BRCA* mutation was detected had a family history of cancer, while 38.8% had specific BC, OC or BC/OC cancer. Another study conducted in an Indian population cohort of 239 individuals reported that 15.1% of patients had a family history of BC or OC [22]. A total of 42.86% of patients had a family history of breast cancer and ovarian cancer according to the *BRCA1* and *2* genes, while 50% had a history of breast and ovarian cancer. These findings correlate with the study by Mehta et al. [23]. *BRCA1/2* mutations significantly increase breast and ovarian cancer risk, though other rare gene variants like Li-Fraumeni and Cowden also contribute to hereditary cancer [24].

The overall percentage of patients with mutations in the *BRCA* genes was 23.80% in the current Indian population cohort, while the percentages of patients with mutations in the *BRCA1* and *BRCA2* genes were 14.6 (73/500) and 9.6% (48/500), respectively. In our earlier study, a greater number of *BRCA1* gene mutations than *BRCA2* gene mutations were detected [11]. Gupta et al. [22] reported the prevalence of *BRCA2* and *BRCA1* mutations in the Indian population to be 5.9% and 15.5%, respectively. A study conducted in southern Indian women reported that 24.6% and 3.28% of patients had *BRCA1* and *BRCA2* mutations, respectively, and an overall 28% of patients were BRCA positive with a family history of BC or OC [25]. Similarly, individuals with a family history of both breast and ovarian cancer have maximum chances of exhibiting mutations in *BRCA* genes.

The mutation spectrum of our studied cohort revealed missense mutations as the most common in the *BRCA1* and *BRCA2* genes, while another study of 141 Indian patients

revealed the most dominant mutations as insertions and deletions [5]. A total of 14.8% of the pathogenic variants were detected in the Indian HBOC cohort, 68.4% of which were identified in the *BRCA1* gene and 42.3% in the *BRCA2* gene. In our previous study, it was found that there are more pathogenic variants in the *BRCA1* gene than in the *BRCA2* gene [11]. In the studied Indian cohort, out of 128 mutations, 7.4% were VUSs. Laboratories in Europe reported 15% VUS, while individuals of African-American ancestry reported up to 21% VUS in *BRCA1* or *BRCA2* [26]. The overall prevalence of novel (pathogenic and VUS) and recurrent mutations in the *BRCA1/BRCA2* genes was 18% among high-risk breast cancer patients in Jordan [27], while in this study, the overall prevalence (pathogenic, likely pathogenic and VUS) was 23.8%.

Among the 128 mutations identified, c.68_69 del (popularly known as 185delAG) in the *BRCA1* gene was found in 13 patients, the most common mutation prevalent in Ashkenzi Jews [28]. The most characterized mutations in the *BRCA1* gene are found to be c. 68_69delAG (185delAG) and c. 5266dupC (5382insC), while in the BRCA2 c. 5946delT was prevalent [29]. In our study, the c.7505 G>A mutation in the *BRCA2* gene was more prevalent compared to findings from a 2019 study [30].

In conclusion, of the 500 patients, 119 (23.8%) tested positive for *BRCA* mutations. Pathogenic variants were found in 14.8% of cases, likely pathogenic in 1.6%, and variants of uncertain significance (VUS) in 7.4%. A total of 128 *BRCA1/2* mutations were identified, with 59.3% affecting *BRCA1* and 40.6% affecting *BRCA2*. Missense mutations were the most frequent in both *BRCA1* (30.26%) and *BRCA2* (55.77%), followed by frameshift and nonsense mutations. A significant link was observed between *BRCA* mutations and cancer history, 38.8% had a family history of breast or ovarian cancer, and 7.6% had other cancers. The highest mutation rate (26.2%) occurred in patients aged 46-65 Y. Nearly half (46.4%) of mutation carriers had a family history of cancer. *BRCA* mutations are associated with early onset, poorer prognosis, and higher recurrence rates. Targeted genetic testing of family members is crucial for early detection, enabling timely therapeutic decisions. Genetic counseling, surveillance, and possible prophylactic measures can aid in early cancer detection and treatment.

Author Contribution Statement

Kunjal Lila designed the study and analyzed the results. Harshita Bhanushali, Milind Chanekar and Monisha Banerjee conducted the experiments. Raj Jatale conducted statistical analysis. Rakhi Bajpai Dixit, Aparna Rajadhyaksha and Kirti Chadha interpreted the results, prepared figures, tables, and manuscript draft. All authors reviewed and approved the final manuscript.

Acknowledgements

The authors are thankful to Mr. Yogeshwar Vijay Gawali and Dr. Shaikhali M Barodawala for helping in the processing of NGS samples and for providing suggestions for editing the manuscript.

Ethical Clearance

Ethical approval was granted by Conscience Independent Ethics Committee (24AAKFC1503G1Z0) – Gujarat.

Conflict of interest

The authors declare that they have no conflicts of interest.

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