Indian Journal of Gynecologic Oncology (2024) 22:82
https://doi.org/10.1007/s40944-024-00840-x

ORIGINAL ARTICLE

BRCA1 and BRCA2 Mutations in Carcinoma Ovary: A Prospective Cohort Study

Priyanka Makkar[1] · Amita Naithani[2] · R. Pratima[3] · Anurag Sharma[4] · Anurag Mehta[5] · Rupinder Sekhon[2]

Received: 28 October 2023 / Revised: 20 February 2024 / Accepted: 7 April 2024 / Published online: 14 May 2024

Abstract

Purpose Epithelial ovarian cancer (EOC) is one of the leading fatal gynaecologic malignancies. The paradigm is shifting towards understanding of the molecular biology and genomics of the disease and exploiting them as a therapeutic potential.

Methods We conducted a prospective observational analysis with newly diagnosed EOC unselected for a family or personal history of breast or ovarian cancer to estimate the period prevalence of germline BRCA1 and BRCA2 mutations in patients with EOC and compare the clinicopathological characteristics and outcome.

Results Between October 2019 and October 2021, 100 consecutive patients of newly diagnosed EOC who agreed to undergo BRCA mutation testing after counselling were enrolled in the study. Our study reported 30% incidence of germline BRCA mutation, whereas variants of undetected significance (VUS) was reported in 4 patients. 73.33% of the patients had (22/30) BRCA 1 gene mutations and 26.67% (8/30) of the patients harboured mutations in BRCA 2 gene. Expectedly frameshift mutations were the commonest among pathogenic alterations. 22.7% patients of BRCA1 associated ovarian cancer had positive family history for ovarian cancer. 25% of BRCA 2 associated ovarian cancer had family history of breast cancer as compared to 4.5% BRCA wild type tumours (p value = <0.001).

Conclusions Our study indicates that there is a high prevalence of germline BRCA1/2 mutations amongst patients of EOC so it is of paramount importance in recent times to improve knowledge and awareness about the gBRCA testing among clinicians and patients.

Keywords BRCA · Ovarian malignancy · Platinum

Introduction

Epithelial ovarian cancer (EOC) is a fatal gynecologic malignancy. 70% of patients present at an advanced stage at diagnosis. Despite the evolution of new treatment strategies for ovarian cancer, overall survival statistics have remained unaffected. The paradigm has shifted towards understanding the molecular biology and genomics of the disease and exploiting them as a therapeutic potential. Upcoming evidence has shown the role of mutations in BRCA genes in the pathogenesis of ovarian cancer. In India, there is a paucity of data on germline BRCA mutations in ovarian cancer patients because of the limited uptake of genetic testing. BRCA1/2 mutations are associated with improved progression-free survival (PFS) and overall survival (OS) as compared to patients who are not harbouring the mutation [1, 2]. This increase in survival in patients harbouring BRCA mutations may be due to increased chemosensitivity or due to the development of new therapeutic agents such as poly (ADP-ribose) polymerase (PARP) inhibitors.

In the last decade, there has been progressive improvement in the understanding of these genetic mutations and there has been an evolution in treatment options that particularly target these mutations.

✉ Amita Naithani
dramita2006@yahoo.co.in

1 Department of Gynae Oncology, TMH, Mullanpur, Punjab, India

2 Department of Gynae Oncology, Artemis Hospital, Gurugram, India

3 Department of Gynae Oncology, Manipal Hospital, Bengaluru, India

4 Department of Statistics, Ram Lal Anand College, University of Delhi, Delhi, India

5 Department of Laboratory, Molecular Diagnostics & Research, Rajiv Gandhi Cancer Institute & Research Centre, Delhi, India

In India, uptake of genetic testing by the affected population is very low; both due to a lack of knowledge at the patient as well as care provider's end and due to financial constraints [3].

We conducted an observational prospective study in women diagnosed with epithelial ovarian cancer to determine the prevalence of BRCA1 and BRCA2 mutations, with a focus on the clinicopathological characteristics and outcomes of the patients harbouring these genetic mutations.

Methods

This was a prospective observational single-centre study. The study was conducted in a tertiary care centre in North India and was part of the DrNB thesis. The study was approved by the scientific research and institutional ethics committee (Enclosure 1).

Hundred consecutive patients of newly diagnosed epithelial ovarian cancer were prospectively enrolled. (There was no sampling technique. Patients who met the inclusion criteria and who were willing to undergo the test were consecutively enrolled in the study).

Patients with non-epithelial ovarian cancers and borderline ovarian cancers were excluded.

Patients were not selected for a family or personal history of breast or ovarian cancer. The primary objective of the study was to estimate the period prevalence of germline BRCA1 and BRCA2 mutations in patients with EOC and compare the clinicopathological characteristics and outcomes between BRCA 1/ 2 mutated and BRCA wild-type tumours. A secondary objective was to calculate the frequencies and compare clinicopathological characteristics like family history of ovarian and breast cancer, personal history of breast cancer, FIGO staging, histology, residual disease, primary treatment strategy among patients with BRCA wild type, BRCA 1 and BRCA 2 mutated epithelial ovarian cancer.

All germline BRCA mutations were analysed using blood samples. Blood samples were either taken at the time of diagnosis of epithelial ovarian cancer or at the time of completion of primary treatment. BRCA mutations were detected by NGS(next-generation sequencing) using an oncomine BRCA panel (Life technologies) that covers all the coding regions with padding of an average of 50 nucleotides to ascertain splice site and branch site mutations along with all other coding sequence mutations. All negative cases for deleterious mutations were further tested for Large genomic rearrangements (Big Insertions and deletions) using a Multiplex Ligation Probe Amplification (MLPA) assay. The Ion Reporter Software which has all the five classes of mutations, and polymorphisms was used for variant calling.

Patients were informed about their BRCA mutation test results and appropriate post-test genetic counselling was provided as per the standard of care.

The diagnosis of ovarian carcinoma was confirmed by the World Health Organization (WHO) classification for histological subtyping [4] and International Federation of Gynecologists and Obstetricians (FIGO) criteria for stage determination [5] were used. The completeness of cytoreduction (CC) was scored as proposed by Sugarbaker [6]. Patients either underwent neoadjuvant chemotherapy (NACT) or primary debulking surgery (PDS) depending on the disease burden, performance status of the patient and the surgeon's preference.

Data for all the patients regarding BRCA status, familial and personal history, primary treatment strategy received, completeness of cytoreduction score, pathological report and follow-up status were recorded in the database.

Patients were followed up for 6–9 months after completion of primary treatment. Patients underwent clinical examination, CA 125 and appropriate radiological imaging every three months. Platinum-sensitive recurrence (PSR) was defined when the treatment-free interval (TFI) was 6 months or longer, whereas platinum-resistant recurrence (PRR) was defined as TFI shorter than 6 months.

Statistical Analysis

All the data were collected prospectively. Statistical package for social sciences (SPSS Inc version 23.0 for Windows) was used for analyses of data and Excel 2007 for data entry was used. To compare the age and BMI among BRCA wild type, BRCA 1 mutation and BRCA 2 mutated epithelial ovarian cancer patients, ANOVA was used after checking the normality of the data using the Shapiro- Wilk test. To compare the categorical variables, viz., age group, Family history of ovarian, breast cancer, Personal history of breast cancer, FIGO stage, histology, primary treatment strategy, residual disease and recurrence among BRCA wild type, BRCA1 and BRCA2 mutated epithelial ovarian cancer patients using Fisher's exact test and chi-Square test. All the reported p values were two-sided and p values < 0.05 were considered to indicate statistical significance.

Results

A total of 30 pathogenic or likely pathogenic germline alterations were observed in our cohort. Variants of undetected significance (VUS) were reported in 4 patients. 73.3% of the patients had (22/30) BRCA 1 gene mutations and 26.6% (8/30) of the patients harboured mutations in BRCA 2

Table 1 Incidence of germline BRCA mutation in ovarian cancer

BRCA mutation	
No	66 (66%)
Yes (pathogenic or likely pathogenic)	30 (30%)
VUS	4 (4%)
Type of BRCA mutation N = 34	
BRCA1	22 (64.7%)
BRCA 2	8 (23.5%)
BRCA 1 VUS	3 (8.8%)
BRCA2-VUS	1 (2.9%)

Table 2 Mutational spectrum of BRCA genes in ovarian cancer

	BRCA1 ($n=22$)	BRCA 2 ($n=8$)	*P* value
Type of mutation			0.639
FRAMESHIFT	9 (40.91)	5 (62.5)	
NONSENSE	4 (18.18)	1 (12.5)	
MISSENSE	2 (9.09)	0 (0)	
DELETION	1 (4.55)	1 (12.5)	
SPLICESITE	6 (27.27)	1 (12.5)	

gene. The majority of the patients were BRCA 1 mutated (Table 1).

Frameshift mutations were the most common among pathogenic alterations. Nine of the 22 cases of BRCA1 had deleterious frameshift mutations. Splice site alterations were the 2nd most common mutations. The splicesite mutations were recurrent with c.4547G > A and c.5137G > A accounting for 3 and 2 cases of pathogenic alterations in the BRCA1 gene (Table 2).

BRCA2 genes harboured a lesser number of pathogenic alterations (8/30). The nature of the oncogenic variant, however, retained the same proportions as observed in BRCA1 with frameshift and splicesite alterations being the most important pathogenic classes.

The mean age at diagnosis for EOC was 51.2 ± 11.2 years. Mean ages for BRCA1, BRCA wild type and BRCA 2 were 47.6 ± 9.9 years; 56.6 ± 10 years and 61.1 ± 8.5 years, respectively. In all, 54.5% of patients with BRCA 1 mutated ovarian cancer patients were below the age of 50 years and all patients with BRCA 2 mutation were above the age of 50 ($p < 0.001$). There was no difference in BMI among the three groups.

5 (22.7%) patients had a family history of ovarian cancer in BRCA 1 mutated patients. Two sisters presented with EOC to us within 6 months of one another. 25% had a history of breast cancer in the family in BRCA 2 mutated patients but none with BRCA 1 mutation ($p < 0.001$). Four among 30 patients (13.3%) with BRCA mutations had a personal history of breast cancer compared to 1.5% in BRCA wild-type tumours ($p = 0.012$).

23 of 30 BRCA-associated EOC presented at stage III c and 5/30 (16.7%) were at stage IV at presentation. BRCA wild type tumours similarly presented in advanced stage: stage 3c: 77.27%, stage IV A: 13.64%, Stage IV B: 6.06%. All 30 patients who had BRCA mutation (100%) had high-grade serous histological subtype.

47% of patients with germline BRCA mutation and 38% of patients in the wild-type cohort underwent primary debulking surgery ($p = 0.211$). In 75% of the patients with wild type, 86.4% of BRCA 1 mutated and in 75% of patients with BRCA 2 mutations CC-0 was achieved ($p = 0.194$). Neoadjuvant chemotherapy (NACT) was offered as the primary treatment strategy in the majority of the patients in all three cohorts.

44/66 patients with wild type recurred. 19 (43%) patients had platinum-sensitive recurrence. 15 (50%) patients both among both BRCA 1/2 mutated cohorts had recurrence during follow-up. Seven of 11 patients had platinum-sensitive recurrences in the BRCA 1 cohort. 3 out of 4 patients had platinum-sensitive recurrences in the BRCA 2 cohort ($p = 0.29$).

Discussion

The uptake of genetic testing for ovarian cancer in a population-based study in Ontario was only 19% [7]. In India, with limited resources associated with a lack of awareness and the social stigma attached to the results, the proportion of people opting for genetic testing is far less. This study aimed at determining the prevalence of germline BRCA mutation in patients with EOC and exploring the clinicopathological characteristics and clinical outcomes of patients harbouring these mutations.

According to the literature prevalence of germline BRCA mutations varies from 3 to 27% in patients who have been diagnosed with epithelial ovarian cancer [8]. The frequency of BRCA mutations varies with race and region. Incidence of germline *BRCA* mutation in Asian countries has been reported between 12 and 29% in some studies [9, 10]. A study from our institute of total of 206 unrelated patients with breast and/or ovarian cancer, who met the National Comprehensive Cancer Network (NCCN) recommendations,[14] were comprehensively tested for germline mutations in a time frame of 3 years (2015–2018).

It was observed that BRCA mutations were about twice more common in the ovarian cancer group (42.9%, 31/74) [11].

However, results from our study indicate that the proportion of patients harbouring these genetic mutations can be

as high as 30%, whereas 73.3% of the mutations were noted in the BRCA 1 gene.

Various previous studies have shown that BRCA 1-associated ovarian cancers occur a decade earlier than sporadic ovarian cancer, whereas the age at diagnosis for BRCA 2 is more or less the same as the age for sporadic tumours [12]. Our results suggested that BRCA 1-associated ovarian cancers occurred at a younger age as compared to BRCA 2-associated and sporadic EOC; which was in corroboration with the previous cited literature.

The strongest risk factor for ovarian cancer is a family history of breast or ovarian cancer, and approximately 25% of all ovarian cancers are caused by a heritable genetic condition, of these, mutations in BRCA1 and BRCA2 account for the majority [13]. Our results suggest that a family history of breast and ovarian cancer cannot be used to triage patients for genetic testing. 22.7% of patients with BRCA1-associated ovarian cancer had a positive family history of ovarian cancer. 25% of BRCA 2-associated ovarian cancer had a family history of breast cancer as compared to 4.5% of BRCA wild-type tumours (p value = < 0.001). None of the BRCA 1 and BRCA 2 carriers had a family history of breast cancer and ovarian cancer, respectively. Our results suggest that patients with a prior diagnosis of breast cancer have a high probability of harbouring a germline BRCA mutation. Surprisingly all 30 patients who had BRCA mutation (100%) had high-grade serous histological subtype. None with endometrioid, clear cell, or mucinous carcinoma had g*BRCA* mutation, implying that histological subtype can be used to screen patients for genetic testing.

Table summaries of previously published data. Data from India and Indian subcontinent are still emerging. A recent multicentric study conducted in India reported that germline mutations in BRCA1 and BRCA2 were detected in 15.5% and 5.9% of patients, respectively. 15.1% had a family history of ovarian or breast cancer, and 66.5% had a serous subtype of epithelial ovarian cancer. They concluded that there was a high incidence of germline BRCA1 and BRCA2 mutations in Indian patients with epithelial ovarian cancer [8].

Complete clearance of the disease in the abdominal cavity is the goal of surgery in ovarian cancer. In the early twentieth century, Bristow in a meta-analysis showed that there was a 5.5% increase in the median survival with a 10% increase in the level of maximal cytoreduction [14]. In the present study, complete cytoreduction was achieved in three-fourths of patients in both BRCA wild-type and BRCA-associated ovarian cancer patients at the time of PDS/IDS (interval debulking surgery).

Tan et al. showed that BRCA-positive patients had higher overall (95.5% *v* 59.1%) and complete response rates (81.8% *v* 43.2%) to first-line treatment, higher responses to second-line, (91.7% *v* 40.9%); and third line platinum-based chemotherapy (100% *v* 14.3%) and longer TFIs as compared to BRCA wild type tumours [15]. Kim et al. observed that the BRCA mutation cohort had a longer progression-free survival (median, 22.9 vs. 16.9 months, $p = 0.001$) than the BRCA wild-type cohort. On multivariate analyses, it was identified that germline BRCA1/2 mutation was an independent favourable prognostic factor [16]. Investigators from The Cancer Genome Atlas (TCGA) project concluded that patients with stage II to IV high-grade serous ovarian cancer with germline or sporadic mutations in BRCA1 or BRCA2 had improved outcomes compared to patients without BRCA deficiency (median overall survival 66.5 vs. 41.9 months, $p = 0.0003$) [17].

It has been suggested that the more favourable response to chemotherapy of BRCA-associated ovarian cancers is related to their increased sensitivity to platinum agents, but this leads to better overall survival is still unclear. Studies have also demonstrated that this increased platinum responsiveness is for a transient time, as in due course of time there is the emergence of platinum resistance due to "revertant mutations" where the BRCA 1 and 2 sequence is restored to normal [18]. It was beyond the scope of the present study to follow the patients for a long period. However, during our follow-up of patients, 50% of BRCA mutated and 66.7% of BRCA wild-type tumours recurred. However, 63.6% and 75% were platinum-sensitive recurrences in BRCA 1 and BRCA 2-associated ovarian cancers. Contrary to available literature our study showed comparable results in terms of platinum-sensitive recurrence among BRCA-associated and BRCA wild-type ovarian cancers.

Many studies have suggested that the short-term 5-year survival advantage does not persist if the patients are followed up for 10 years. Reasons why BRCA1/2 carriers have only a short-term survival advantage are yet not clear [19]. In our study, half of BRCA-associated EOC patients had recurrences during follow-up which was comparable to the BRCA wild-type. Evidence suggests that intragenic reversion of germline alleles restores BRCA1 and BRCA2 function in tumor cell lines and this may be associated with a time-dependent loss of the survival advantage associated with germline mutation [20].

Some centres in India have reported BRCA in patients with both Ca ovary and Ca breast. However, data on prevalence of BRCA in Indian patients with isolated Ca ovary are scarce. Our study contributes to the pool of scientific literature.

Strengths and limitations

The strength of our study is the inclusion of patients of EOC of all age groups, irrespective of histologic subtypes, and relevant family history. Germline testing for BRCA was performed in an institutional molecular laboratory with a

standard testing and inference protocol. Financial constraints are fairly common in our country. We are able to identify factors that could be used to screen patients for BRCA mutation in case.

Our study is an observational single-centre analysis. The sample size is small and does not truly represent the affected population as India is a large country with diverse genetic backgrounds. The follow-up period was too short for the extrapolation of results on a larger population.

Conclusion

Our study indicates that there is a high prevalence of germline BRCA1/2 mutations amongst patients of EOC.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s40944-024-00840-x.

Funding None.

Data availability The data used for this research are available from the corresponding author upon reasonable request.

Declarations

Conflict of interest None.

References

1. Huang YW. Association of BRCA1/2 mutations with ovarian cancer prognosis: an updated meta-analysis. Medicine (Baltimore). 2018;97(2): e9380.
2. Sun C, Li N, Ding D, Weng D, Meng L, Chen G, et al. The role of BRCA status on the prognosis of patients with epithelial ovarian cancer: a systematic review of the literature with a meta-analysis. PLoS ONE. 2014;9(5): e95285.
3. Pramanik R, Vats S, Mitra S, Deo S, Kumar L, Gogia A, et al. Assessment of knowledge and attitude of breast and ovarian cancer patients regarding hereditary breast-ovarian cancer syndrome at a tertiary cancer institute: a cross-sectional observational study. Indian J Med Paediatr Oncol. 2023;45:28.
4. Meinhold-Heerlein I, Fotopoulou C, Harter P, Kurzeder C, Mustea A, Wimberger P, et al. The new WHO classification of ovarian, fallopian tube, and primary peritoneal cancer and its clinical implications. Arch Gynecol Obstet. 2016;293(4):695–700.
5. Prat J. FIGO's staging classification for cancer of the ovary, fallopian tube, and peritoneum: abridged republication. J Gynecol Oncol. 2015;26(2):87–9.
6. Sugarbaker PH Peritoneal Carcinomatosis: Principles of Management. Boston: Kluwer Academic; 1996.
7. Metcalfe KA, Fan I, McLaughlin J, Risch HA, Rosen B, Murphy J, et al. Uptake of clinical genetic testing for ovarian cancer in Ontario: a population-based study. Gynecol Oncol. 2009;112(1):68–72.
8. Gupta S, Rajappa S, Advani S, Agarwal A, Aggarwal S, Goswami C, et al. Prevalence of BRCA1 and BRCA2 mutations among patients with ovarian, primary peritoneal, and fallopian tube cancer in India: a multicenter cross-sectional study. JCO Global Oncol. 2021;7:849–61.
9. Wu X, Wu L, Kong B, Liu J, Yin R, Wen H, et al. The first nationwide multicenter prevalence study of germline BRCA1 and BRCA2 mutations in Chinese ovarian cancer patients. Int J Gynecol Cancer. 2017;27(8):1650.
10. Chao A, Chang T-C, Lapke N, Jung S-M, Chi P, Chen C-H, et al. Prevalence and clinical significance of BRCA1/2 germline and somatic mutations in Taiwanese patients with ovarian cancer. Oncotarget. 2016;7(51):85529.
11. Mehta A, Vasudevan S, Sharma SK, Kumar D, Panigrahi M, Suryavanshi M, et al. Germline BRCA1 and BRCA2 deleterious mutations and variants of unknown clinical significance associated with breast/ovarian cancer: a report from North India. Cancer Manag Res. 2018;10:6505–16.
12. Risch HA, McLaughlin JR, Cole DE, Rosen B, Bradley L, Kwan E, et al. Prevalence and penetrance of germline BRCA1 and BRCA2 mutations in a population series of 649 women with ovarian cancer. Am J Hum Genet. 2001;68(3):700–10.
13. Walsh T, Casadei S, Lee MK, Pennil CC, Nord AS, Thornton AM, et al. Mutations in 12 genes for inherited ovarian, fallopian tube, and peritoneal carcinoma identified by massively parallel sequencing. Proc Natl Acad Sci USA. 2011;108(44):18032–7.
14. Bristow RE, Tomacruz RS, Armstrong DK, Trimble EL, Montz FJ. Survival effect of maximal cytoreductive surgery for advanced ovarian carcinoma during the platinum era: a meta-analysis. J Clin Oncol. 2002;20(5):1248–59.
15. Tan DS, Rothermundt C, Thomas K, Bancroft E, Eeles R, Shanley S, et al. "BRCAness" syndrome in ovarian cancer: a case-control study describing the clinical features and outcome of patients with epithelial ovarian cancer associated with BRCA1 and BRCA2 mutations. J Clin Oncol. 2008;26(34):5530–6.
16. Kim SI, Lee M, Kim HS, Chung HH, Kim J-W, Park NH, et al. Effect of BRCA mutational status on survival outcome in advanced-stage high-grade serous ovarian cancer. J Ovarian Res. 2019;12(1):40.
17. Integrated genomic analyses of ovarian carcinoma. Nature. 2011;474(7353):609–15.
18. Norquist B, Wurz KA, Pennil CC, Garcia R, Gross J, Sakai W, et al. Secondary somatic mutations restoring BRCA1/2 predict chemotherapy resistance in hereditary ovarian carcinomas. J Clin Oncol. 2011;29(22):3008–15.
19. Candido-dos-Reis FJ, Song H, Goode EL, Cunningham JM, Fridley BL, Larson MC, et al. Germline mutation in BRCA1 or BRCA2 and ten-year survival for women diagnosed with epithelial ovarian cancer. Clin Cancer Res. 2015;21(3):652–7.
20. Sakai W, Swisher EM, Karlan BY, Agarwal MK, Higgins J, Friedman C, et al. Secondary mutations as a mechanism of cisplatin resistance in BRCA2-mutated cancers. Nature. 2008;451(7182):1116–20.