Prevalence of Somatic BRCA1 and BRCA2 Mutations in Ovarian Cancer among Filipinos Using Next Generation Sequencing*

Charles Joseph Bernardo, Claire Anne Therese Hemedez, Jose Jasper Andal, Rubi Li, Yancel Mascardo, Alizza Mariel Espiritu, Josephine Matudan Babida, Daphne Ang

Institute of Pathology, St. Luke's Medical Center - Quezon City, Philippines

ABSTRACT

Introduction. Ovarian cancer is one of the leading causes of mortality in women. In 2020, 5,395 (6.2%) of diagnosed malignancies in females were ovarian in origin. It also ranked second among gynecologic malignancies after cervical cancer. The prevalence in Asian /Pacific women is 9.2 per 100,000 population. Increased mortality and poor prognosis in ovarian cancer are caused by asymptomatic growth and delayed or absent symptoms for which about 70% of women have an advanced stage (III/IV) by the time of diagnosis. The most associated gene mutations are Breast Cancer gene 1 (BRCA1) which is identified in chromosome 17q21 and Breast Cancer gene 2 (BRCA2) identified in chromosome 13. Both proteins function in the double-strand DNA break repair pathway especially in the large framework repair molecules. Olaparib is a first-line drug used in the management of ovarian cancer. It targets affected cells by inhibition of poly (ADP-ribose) polymerase (PARP) activity which induces synthetic lethality in mutated BRCA1/2 cancers by selectively targeting tumor cells that fail to repair DNA double-strand breaks (DSBs).

Objectives. The study aims to determine the prevalence of pathogenic somatic mutations in BRCA1 and BRCA2 among patients diagnosed of having ovarian cancer, to characterize the identified variants into benign/ no pathogenic variant identified, variant of uncertain significance (VUS), and pathogenic, and to determine the relationship of specific mutations detected with histomorphologic findings and clinical attributes.

Methodology. Ovarian cancer tissues available at the St. Luke's Medical Center Human Cancer Biobank and formalin-fixed paraffin-embedded (FFPE) tissue blocks diagnosed as ovarian cancer from the year 2016 to 2020 were included. Determination of the prevalence of somatic BRCA1 and BRCA2 mutations using Next Generation Sequencing (NGS).

Results. A total of 60 samples were processed, and three samples were excluded from the analysis due to an inadequate number of cells. In the remaining 57 samples diagnosed ovarian tumors, pathogenic BRCA1/2 variants were identified in 10 (17.5%) samples. Among the BRCA1/2 positive samples, 3 (5.3%) BRCA1 and 7 (12.3%) BRCA2 somatic mutations were identified.

Conclusion. Identification of specific BRCA1/2 mutations in FFPE samples with NGS plays a big role in the management of ovarian cancer, particularly with the use of targeted therapies such as Olaparib. The use of this drug could provide a longer disease-free survival for these patients. Furthermore, we recommend that women diagnosed with ovarian cancer should be subjected to genetic testing regardless of the histologic subtypes or clinical features. Lastly, genetic testing should be done along with proper genetic counseling, especially for patients who are susceptible to these mutations.

Key words: ovarian cancer, BRCA somatic mutations, BRCA1, BRCA 2, NGS

ISSN 2507-8364 (Online)
Printed in the Philippines.

Received: 15 May 2023. Accepted: 15 May 2023.
Published online first: 25 May 2023.
https://doi.org/10.21141/PJP.2023.05

Corresponding author: Charles Joseph L. Bernardo, MD
E-mail: charles.joseph.bernardo@gmail.com
ORCiD: https://orcid.org/0009-0005-3363-5867

**This paper won third place during the Research Competition of the 72nd Annual Convention of the Philippine Society of Pathologists, Inc.*

INTRODUCTION

Ovarian cancer is one of the leading causes of mortality in women. In 2020, 5,395 (6.2%) of diagnosed malignancies in females were ovarian in origin. It also ranked second among gynecologic malignancies after cervical cancer.[1] The prevalence in Asian /Pacific women is 9.2 per 100,00 population. Increased mortality and poor prognosis in ovarian cancer are caused by asymptomatic growth and delayed or absent symptoms[2] for which about 70% of women have an advanced stage (III/IV) by the time of diagnosis. There are several histologic types of ovarian cancer, each with distinct characteristics. Among the different types, surface epithelial tumors, particularly high-grade serous carcinoma, are the most aggressive subtype. It is also the most diagnosed surface epithelial tumor.[3] One of the most

significant risk factors for the occurrence of this tumor is family history.

High-grade serous carcinoma is a genetically unstable malignancy that carries different mutations. The most associated gene mutations are Breast Cancer gene 1 (BRCA1) which is identified in chromosome 17q21 and Breast Cancer gene 2 (BRCA2) identified in chromosome 13. Both proteins function in the double-strand DNA break repair pathway, especially in the large framework repair molecules.[4] A mutated BRCA1/2 gene that is inherited from either parent is defined as germline mutation. While a mutated gene that occurs in a single body cell after birth and cannot be inherited is defined as somatic mutation. Age discrepancy also plays a role in the onset of disease between BRCA1/2, with BRCA1 patients having an increased risk after age 40 and BRCA2 patients after age 50. Somatic mutations were reported in 5-9% and 3-4% of BRCA1 and BRCA2 genes, respectively.[5] Recent advances in the clinical trials for targeted therapy included Olaparib (a poly (ADP-Ribose) polymerase 1 (PARP1) inhibitor). Olaparib is a first-line drug used in the management of ovarian cancer. It targets affected cells by inhibition of poly (ADP-ribose) polymerase (PARP) activity which induces synthetic lethality in mutated BRCA1/2 cancers by selectively targeting tumor cells that fail to repair DNA double-strand breaks (DSBs).[6] This drug provides therapeutic benefits for germline as well as somatic BRCA mutations.

This study aims to determine the prevalence of pathogenic somatic mutations in BRCA1 and BRCA2 among patients with ovarian cancer. We also aim to characterize the identified variants into benign/no pathogenic variants.

METHODOLOGY

A retrospective study was conducted. Sixty (60) cases of ovarian cancer tissues available in St. Luke's Medical Center Human Cancer Biobank and formalin fixed paraffin embedded (FFPE) tissue blocks diagnosed as ovarian cancer from 2016 to 2020 were included in the study. Samples included were primary ovarian cancer with tumor tissue and ovarian masses with metastatic gynecologic origin (fallopian, endometrial, and cervical). All pertinent clinical information from the databank were retrieved and collated. Determination of the prevalence of somatic BRCA1 and BRCA2 mutations using Next Generation Sequencing (NGS) was done. Sample size was calculated based on the estimation of the population proportion. Assuming that the prevalence of ovarian BRCA mutation is 28%[6] with a maximum allowable error of 7.5% and a reliability of 90%, the sample size required was 60.

Genomic DNA was extracted from formalin fixed paraffin embedded (FFPE) ovarian cancer tumor blocks. Four (4) sections, "10 μm thickness" were cut from the blocks. These sections were deparaffinized and DNA was extracted using the QiaAMP DNA MiniKit®. Briefly, 200 μl of the buffy coat was lysed using the lysis solution (Buffer AL) and proteinase K was added to degrade proteins. Cells were incubated at 560C for 10 mins or until complete lysis. To precipitate the isolated DNA, ethanol (EtOH) was added to each sample. Wash Buffers (AW1 and AW2) were added separately to the spin columns to facilitate the removal of contaminants. To elute purified genomic DNA (gDNA) Buffer AE was added to the spin columns. Using Nanodrop® v1000 spectrophotometer the DNA quality and quantity of the extracted eluent were assessed. A final working concentration of 50 ng/μl of gDNA was used for each sample.

Table 1. Pathogenic somatic mutations in BRCA1 and BRCA2 genes

		N	%
Somatic Mutation	Yes	10	17.5
	No	47	82.5
	Total	57	100.0
Mutation	BRCA1 somatic	3	5.3
	BRCA2 somatic	7	12.3
	No pathogenic mutation/ possible germline	47	82.5
	Total	57	100.0

Table 2. Clinicopathologic features and somatic mutations

Characteristics	Somatic mutation		*p*
	Yes, n=10 n (%)	No, n=47 n (%)	
Age group			.786[a]
Less than 50	4 (40.0)	21 (44.7)	
50 and above	6 (60.0)	26 (55.3)	
Diagnosis			.530[a,b]
Serous carcinoma (High-grade)	5 (50.0)	17 (36.2)	
Endometrioid carcinoma	1 (10.0)	14 (29.8)	
Clear cell carcinoma	3 (30.0)	7 (14.9)	
Mucinous carcinoma	0 (0.0)	5 (10.6)	
Mixed carcinoma	1 (10.0)	3 (6.4)	
Undifferentiated malignancy	0 (0.0)	1 (2.1)	

Results are based on nonempty rows and columns in each innermost subtable.
[a]More than 20% of cells in this subtable have expected cell counts less than 5. Chi-square results may be invalid.
[b]The minimum expected cell count in this subtable is less than one. Chi-square results may be invalid.

RESULTS

A total of 60 samples were processed, and three samples were excluded from the analysis due to the inadequate number of cells. In the remaining 57 samples diagnosed with ovarian cancer, pathogenic BRCA1/2 variants were identified in 10 (17.5%) samples. Among the BRCA1/2 positive samples, 3 (5.3%) BRCA1 and 7 (12.3%) BRCA2 somatic mutations were identified while 47 samples (82.5%) had no pathogenic or possibly germline mutations (Table 1).

Of the 10 samples that showed somatic mutation, 60% samples were noted in age 50 and above with most of the cases presenting with high-grade serous carcinoma (50%) (Table 2).

Somatic mutations in the BRCA2 gene were more frequently found in patients diagnosed at age 50 and above compared to younger individuals – 71.4% (5/7) versus 28.6% (2/7), respectively. While somatic mutations in the BRCA1 gene were more frequently found in younger patients compared to older individuals – 66.7% (2/3) versus 33.3% (1/3). High-grade serous carcinoma was the most common epithelial ovarian neoplasm presenting with somatic mutations, and these were identified in 3 samples for the BRCA2 gene and 2 samples for the BRCA1 gene (Tables 3 and 4).

Table 3. BRCA1 and BRCA2 somatic mutations in different age groups

		Mutation						p
		BRCA1 somatic		BRCA2 somatic		No pathogenic mutation/ possible germline		
		n	%	n	%	n	%	
Age group	Less than 50	2	66.7	2	28.6	21	44.7	
	50 and above	1	33.3	5	71.4	26	55.3	
	Total	**3**	**100.0**	**7**	**100.0**	**47**	**100.0**	.519[a]

Results are based on nonempty rows and columns in each innermost subtable.
[a]More than 20% of cells in this subtable have expected cell counts less than 5. Chi-square results may be invalid.

Table 4. BRCA1 and BRCA2 somatic mutations in different histologic subtypes

		Mutation						p
		BRCA1 somatic		BRCA2 somatic		No pathogenic mutation/ possible germline		
		n	%	n	%	n	%	
Diagnosis	Serous carcinoma (High-grade)	2	66.7	3	42.9	17	36.2	
	Endometrioid carcinoma	0	0.0	1	14.3	14	29.8	
	Clear cell carcinoma	0	0.0	3	42.9	7	14.9	
	Mucinous carcinoma	0	0.0	0	0.0	5	10.6	
	Mixed carcinoma	1	33.3	0	0.0	3	6.4	
	Undifferentiated malignancy	0	0.0	0	0.0	1	2.1	
	Total	**3**	**100.0**	**7**	**100.0**	**47**	**100.0**	.429[a,b]

Results are based on nonempty rows and columns in each innermost subtable.
[a]More than 20% of cells in this subtable have expected cell counts less than 5. Chi-square results may be invalid.
[b]The minimum expected cell count in this subtable is less than one. Chi-square results may be invalid.

DISCUSSION

In this study, we detected the frequency of somatic BRCA1/2 mutations in ovarian cancer patients. The use of tumoral tissues can detect the presence of both germline and somatic mutations but germline variants are primarily detected through blood samples or buccal swabs. Molecular analysis of the BRCA1/2 genes revealed that out of the 57 samples, 10 (17.5%) of which demonstrated the presence of somatic mutations. In one study, our percentage is higher – 17.5% versus 4.1%.[6] While in another study, as high as 39% of somatic BRCA1/2 mutations were detected.[7] These results can be attributed to varying numbers of samples. However, the prevalence of somatic BRCA1 and BRCA2 mutations in relation to the age of diagnosis was comparable to previous studies. BRCA1 mutations were frequently detected in younger individuals and BRCA2 mutations were more associated with older individuals. This study also identifies that serous carcinoma (high-grade) was the most common epithelial tumor associated with BRCA1/2 mutations comprising 66.7% and 42.9%, respectively. Somatic mutations in the BRCA2 gene were also noted in clear cell carcinoma (3 samples, 42.9%) and endometrioid carcinoma (1 sample, 14.3%). Goodheart et al., demonstrated that clear cell carcinoma showing BRCA2 mutations has shown to have a better prognosis compared to clear cell carcinoma with wild-type mutations.[5]

The guidelines from the American Society of Clinical Oncology and the European Molecular Genetics Quality Network (EMQN) recommend genetic testing for BRCA1 and BRCA2 mutations in every patient diagnosed with ovarian cancer. With the application of NGS as a standard diagnostic tool, we can detect the presence of mutations in each patient.[6] Olaparib, a PARP inhibitor is used as a drug for cases with BRCA1/2 germline as well as somatic mutations. SOLO-1 trial data from the 5-year follow-up demonstrated that Olaparib reduced the risk of disease progression or death by 67 percent. At 5 years, 48.3 percent of patients on Olaparib remained free from disease progression versus 20.5 percent of those who received a placebo (Society of Gynecologic Oncology 2021 Annual Meeting).[7-11]

CONCLUSION AND RECOMMENDATIONS

Identification of specific BRCA1/2 mutations in FFPE samples with the use of NGS plays a big role in the management of ovarian cancer, particularly with the use of targeted therapies such as Olaparib. The use of this drug could provide a longer disease-free survival for these patients. Furthermore, we recommend that women diagnosed with ovarian cancer should be subjected to genetic testing regardless of the histologic subtypes or clinical features. Lastly, genetic testing should be done along with proper genetic counseling, especially for patients who are susceptible to these mutations.

STATEMENT OF AUTHORSHIP

The authors certified fulfilment of ICMJE authorship criteria.

AUTHOR DISCLOSURE

The authors declared no conflict of interest.

FUNDING SOURCE

None.

REFERENCES

1. International Agency for Research on Cancer. World Health Organization. The Global Cancer Observatory; 2020. https://gco.iarc.fr/today/data/factsheets/populations/608-philippines-fact-sheets.pdf.
2. Momenimovahed Z, Tiznobaik A, Taheri S, Salehiniya H. Ovarian cancer in the world: epidemiology and risk factors. Int J Womens Health. 2019;11:287-99. PMID: 31118829. PMCID: PMC6500433. https://doi.org/10.2147/IJWH.S197604.
3. Natanzon Y, Goode EL, Cunningham JM. Epigenetics in ovarian cancer. Seminars Cancer Biol. 2018;51:160-9. PMID: 28782606. PMCID: PMC5976557. https://doi.org/10.1016/j.semcancer.2017.08.003.
4. Ramus SJ, Gayther SA. The contribution of BRCA1 and BRCA2 to ovarian cancer. Mol Oncol. 2009;3(2):138-50. PMID: 19383375. PMCID: PMC5527889. https://doi.org/10.1016/j.molonc.2009.02.001.
5. Goodheart MJ, Rose SL, Hattermann-Zogg M, Smith BJ, De Young BR, Buller RE. BRCA2 alteration is important in clear cell carcinoma of the ovary. Clin Genet. 2009;76(2):161-7. PMID: 19656163. https://doi.org/10.1111/j.1399-0004.2009.01207.x.
6. Koczkowska M, Zuk M, Gorczynski A, et al. Detection of somatic BRCA1/2 mutations in ovarian cancer - next-generation sequencing analysis of 100 cases. Cancer Med. 2016;5(7):1640–6. PMID: 27167707. PMCID: PMC4867663. https://doi.org/10.1002/cam4.748.
7. Hennessy BT, Timms KM, Carey MS, et al. Somatic mutations in BRCA1 and BRCA2 could expand the number of patients that benefit from poly (ADP ribose) polymerase inhibitors in ovarian cancer. J Clin Oncol. 2010; 28(22):3570–6. PMID: 20606085. PMCID: PMC2917312. https://doi.org/10.1200/JCO.2009.27.2997.
8. Faraoni I, Graziani G. Role of BRCA mutations in cancer treatment with poly (ADP-ribose) polymerase (PARP) inhibitors. Cancers (Basel). 2018;10(12):487. PMID: 30518089. PMCID: PMC6316750. https://doi.org/10.3390/cancers10120487.
9. George A, Banerjee S, Kaye S. Olaparib and somatic BRCA mutations. Oncotarget. 2017;8(27): 43598–9. PMID: 28611314. PMCID: PMC5546426. https://doi.org/10.18632/oncotarget.18419.
10. Nalley C. Benefit of olaparib for brca-mutated advanced ovarian cancer, Oncology Times. 2020;42(22):23,30. https://doi.org/10.1097/01.COT.0000723552.86174.ff
11. Montemorano L, Lightfoot MD, Bixel K. Role of olaparib as maintenance treatment for ovarian cancer: the evidence to date. Onco Targets Ther. 2019;12:11497-506. PMID: 31920338. PMCID: PMC6938196. https://doi.org/10.2147/OTT.S195552.