Original Research Paper

Volume - 15 | Issue - 01 | January - 2025 | PRINT ISSN No. 2249 - 555X | DOI : 10.36106/ijar

Oncology

DETECTION OF INHERITED PATHOGENIC VARIANTS BRAC1/2 IN BREAST AND OVARIAN CANCER

Kalidas Ponnusamy	Professor and Head, Department of Social and Preventive Medicine, Coimbatore Medical College, Coimbatore-14, Tamil Nadu, India.
Sureshkumar Muthusamy	Research Scientist, Multi-Disciplinary Research Unit, Coimbatore Medical College, Coimbatore-14, Tamil Nadu, India.
Manonmani Ramasamy	Professor and Head of Obstetrics and Gynaecology, Coimbatore Medical College and Hospital, Coimbatore-18, Tamil Nadu, India.
Nirmala Appadurai*	Dean, Coimbatore Medical College and Hospital, Coimbatore-18, Tamil Nadu, India. *Corresponding Author

ABSTRACT The occurrence of breast and ovarian cancer among the Indian population is gradually increasing. It is important to determine the major predisposing factors involved in hereditary breast and ovarian cancer (HBOC). In-depth genomic and functional analyses are necessary to identify the pathophysiology of inherited BRCA1 and BRCA2 mutations, which can shed light on the processes underlying cancer development. Hence, this study intends to screen the variants of BRCA1/2: 185delAG, Exon 11 (1307delT), c.5946del and Exon 21 (c.8680C>T) among breast and ovarian cancer patients using the PCR-RFLP method. Patients with early onset of cancer between the ages of 25 to 50 were selected to screen for the inherited predisposing traits as these genes are believed to be inherited. The prevalence of BRCA1 (185 DelAG) mutation was found to be 4 among breast cancer and 3 among ovarian cancer patients. The incidence of BRCA1 Exon 11 (1307delT) was 12%. The results show that the deletion mutation variant (c.5946delT) of BRCA 2gene was 12%. The occurrence of T for C substitution (mutant c.8680C>T of BRCA2) was detected in 14% of amplified samples. The statistical significance was obtained due to the smaller sample size. In conclusion, this study shows that the selected variants have certain involvement in cancer development. We have screened other reported inherited variants to conclude their role in the development of breast/ovarian cancer. This will help us in categorizing high-risk populations and postulating treatment and preventive modalities.

KEYWORDS :

INTRODUCTION

Breast and ovarian cancers are the most frequent and serious tumors that put women at higher risk globally with an average of over 2 million new cases reported each year [1,2,3]. About, 238,700 new cases were diagnosed with deadly ovarian cancer which accounts for about 151,900 deaths worldwide [4,5,6]. Among the Indian population, both breast and ovarian cancers were the predatory risk of women as per the National Cancer Registry Programme [7]. Despite intensive efforts in early detection and treatment, these cancers remain one of the leading causes of death [8,9,10]. In recent years, the increasing incidence of ovarian cancer and mortality has been reported due to a lack of early diagnosis [11]. It accounts for about 42,700 deaths in Europe in 2012, 7.3/100.000 mortality in the Romanian population and 37,829 prevalent cases in Italy [8,9,12,13].

Many studies have reported the role of hereditary components in the development of cancer. It is estimated that about 5 to 10% of breast cancer and 10% of ovarian cancer occur due to these hereditary components [14,15]. The most common hereditary components involved in the development of cancer were BRCA1 (17q chromosome) and BRCA2 (13q chromosome) [16,17]. In particular, about 1800 to 2000 BRCA mutations were distinctly involved in promoting early-onset carcinogenesis [1,18,19,20]. The majority of these risk factors were described in a decade [21]. These germline deleterious mutations of BRCA account for an elevated risk of breast/ovarian cancer syndrome with the probability of ~80% and ~40%, respectively [22,23]. Regardless of family history or age at diagnosis, the incidence of BRCA mutations in breast and ovarian cancer is less than 7% for BRCA1 and 3% for BRCA2 [24]. Compared to patients with sporadic disease, those with germline BRCA mutations have a shorter median time (in age under 50) of diagnosis for breast cancer [25].

The predicted lifetime risk of ovarian cancer (OC) is 54% for BRCA1 carriers and 23% for BRCA2 carriers, while the lifetime risk of breast cancer (BC) for carriers of these inactivating mutations is 82% [26,27,28]. In general populations, the carrier frequency ranges from 1/40 to 1/800, and the incidence and spectrum of mutations differ between populations [29]. Examining family history is the most popular method for diagnosing hereditary cancer. However, because of the small size of families and the unreliability of family history records, it is frequently challenging to identify hereditary breast/ovarian cancer (HBOC) via family history [30].

Determining mutations will assist us in developing a panel that may help screen individuals who are at high risk of developing cancer and in performing oncological surveys among the vulnerable population [31]. Hence, the present study is designed to screen the variants of BRCA1/2: 185delAG, Exon 11 (1307delT), c.5946del and Exon 21 (c.8680C>T) among breast and ovarian cancer patients for determining the incidence of mutation among the existing patients.

MATERIAL AND METHODS

Study Design

The study was conducted at Coimbatore Medical College and Hospital, Coimbatore, Tamil Nadu, Southern part of India. It's a cross-sectional study, conducted on breast and ovarian cancer patients attending Tertiary care hospital from October 2022 to November 2023. The study was approved by the Institutional Human Ethics Committee. After getting informed consent from the patients the blood sample was collected by the trained technician. Individuals between the age group of 25 to 50 who were diagnosed with breast and ovarian cancer were included in the study. This study was conducted at the Multi-Disciplinary Research Unit (Under DHR) available at our college.

Blood Sampling

Three ml of peripheral blood sample was collected in EDTA-coated tubes based on a purposive, non-randomized sampling method for this study. A total of 50 samples from patients diagnosed with breast cancer and 50 from ovarian cancer patients were collected based on the socio-demographic data of the patients for analyzing the incidence of inherited pathogenic variants BRAC1/2 among the patients. All samples were transported and analyzed as per the standard testing protocol.

Extraction of genomic DNA

The genomic DNA from each blood sample was extracted using Qiagen DNeasy® Blood and Tissue Kit. The quantification and quality of the extracted DNAs were measured using Multiscan™ GO Spectrophotometer, Thermoscientific Inc., Wilmington, DE, USA. The extracted DNA samples were stored at -20 °C for further analysis.

Genotyping

Genotyping was performed using Polymerase chain Reaction Restriction Fragment Length Polymorphism (PCR-RFLP). The variants of BRCA1/2: 185delAG, Exon 11 (1307delT), c.5946del and Exon 21 (c.8680C>T) were screened using published primers [32,33,34,35]. Primers used for determining inherited pathogenic variants BRAC1/2 were tabulated in Table 1. PCR reaction was performed with the reaction volume of 25 µL containing 2x Taq DNA master mix Red (Ampliqon), 20mM $MgCl_2$, 50 µM dNTPs, 100 µM primers and 100 ng template DNA. The annealing used for screening all the variants is 52 ºC, 55 ºC, 42 ºC and 54 ºC respectively. The PCR products were analyzed with 1.5 % agarose gel using an Electrophoretic apparatus (BioRad). The PCR products were subjected to RFLP to determine the presence of pathogenic inherited variants using a specific restriction enzyme. RFLP was performed using Taq1, HpyF3I, HindIII and TaaI respectively. All the restriction reactions were performed as per the manufacturer's instructions (Thermo Fisher Scientific, USA). After restriction, the products were electrophoresed in 2% agarose, visualized and photographed using Vilber Lourmat, France.

Table 1 Primers Used For Determining The Mutations

Gene	Primers	Product	Mutant
BRCA1 (185delAG)	F: 5′ TTAATGCTATGCAGAAAATCTTCG 3′ R: 5′ GTGGATGGAGAACAAGGAATC 3′	223 bp	200 and 23 bp
BRCA1 Exon 11 (1307delT)	F: 5′ GCAGCATTCAGAAAGTTAATGAG 3′ R: 5′ TCTGAAGAACCAGAATATTCATCT ACC 3′	151 bp	55 and 96 bp
BRCA2 (c.5946del)	F: 5′ CGAAAATTATGGCAGGTTGTTAC G 3′ R: 5′ GCTTTCCACTTGCTGTACTAAATC CA 3′	534 bp	534, 231 and 303 bp
BRCA2 exon 21 (c.8680C>T)	F: 5′ TTGGTTCTTTAGTTTTAGTTGCTT TTG 3′ R: 5′ TATTCCTTCCTGTGATGGCC 3′	281 bp	166, 80 and 35 bp

Data Analysis

SPSS version 25 was used for statistical analysis. A chi-square test was employed to determine the Hardy-Weinberg Equilibrium (HWE) and the difference in the existence of genotype and allele frequencies among breast and ovarian cancer patients. The odds ratio (OR) with 95% CI was computed to ascertain the relationship between BRCA1/2 hereditary mutations and the risk of breast and ovarian cancer. A genetic model analysis was performed to determine the association of the BRCA1/2 variants with breast and ovarian cancer risk. A p-value of less than 0.05 was deemed statistically significant [7].

RESULTS

PCR-RFLP was performed to detect the frequency of inherited pathogenic variants BRAC1/2 that existed among breast and ovarian cancer patients. About 100 patients, 50 patients with breast cancer and 50 patients with ovarian cancer were selected based on clinical and histopathological confirmation. Patients with early onset of cancer between the ages of 25 to 50 were selected to screen for the inherited predisposing traits. Most of the patients selected in the study were diagnosed with Stage II and Stage III. The majority of the patients were categorized with sporadic breast cancer (91%) and serous ovarian cancer (89%). The variants of BRCA1: 185delAG and Exon 11 (1307delT) and BRAC 2: c.5946del and Exon 21 (c.8680C>T) were screened among breast and ovarian cancer patients.

The amplified PCR products were subjected to restriction digestion to detect the presence of gene mutation. Taq1 enzyme used for exon 2 of BRCA1 (185 DelAG). The wild type will fragmented into two fragments with the size 200 bp and 23 bp whereas, the mutant 185delAG allele will be intact without any fragmentation which yields 223 bp. The results show about 93 % of samples exhibit two fragments upon restriction digestion and 7 % of samples were found to be mutant. The prevalence of BRCA1 (185 DelAG) mutation was found to be 4 among breast cancer and 3 among ovarian cancer patients. The PCR product of BRCA1 Exon 11 (1307delT) was digested with a DdeI restriction enzyme. The restriction digestion will yield 151 bp uncut fragment for the wild type and 55 and 96 bp fragments for the mutant type. About 82% of samples yield uncut fragment with a size of 151 bp. Among 18 mutants, 10 were breast cancer patients and 8 were ovarian cancer patients.

The deletion mutation variant (c.5946delT) in BRCA 2 was tested for all the study participants by restriction digestion using the HindIII restriction enzyme. The gel electrophoresis of the digested product exhibits an undigested PCR product of the mutant allele at 534 bp and the wild-type allele as two fragments (231 and 303 bp). The results show the presence of mutant c.5946delT variant of the BRCA2 gene was 12%, among which 8 were breast cancer patients and 4 were ovarian cancer patients. In the case of BRCA2 exon 21, digestion of the wild-type amplicon generated fragments using TaaI restriction enzyme generated 246 and 35 bp, while the c.8680C>T mutation generated 166, 80, and 35 bp fragments. The presence of T for C substitution (mutant c.8680C>T) was detected in 14% of amplified samples, among which 9 were from ovarian and 5 from breast cancer patients. The values were not statistically significant to determine the genotypic frequency among the breast and ovarian cancer patients and also for comparing with other socio-demographic data.

DISCUSSION

Understanding the pathogenesis of inherited BRCA1/2 mutation and the mechanism of cancer development provides us insight into the management of disease. Various methods like Sanger sequencing, Next Generation Sequencing and PCR-RFLP-based detection of SNPs, were incorporated for assessing the genetic mutations. As sequencing of the gene is expensive and time-consuming, in this study we performed PCR-RFLP for detection of BRCA1/2 mutation. As 10-15% of Breast and ovarian cancer were reported to occur due to hereditary, this study was designed to detect the mutation among existing cancer patients. This helps us in postulating a diagnostic practice for screening the carriers among the family members susceptible to developing cancer.

Earlier, many studies have reported that the median time of germline mutation detection was lower than 50 [24,25]. Similarly, in the present study patients with early-onset of cancer between the ages of 25 to 50 were selected to screen the inherited predisposing traits. Various studies have been conducted to determine the role of the BRCA1/2 gene and its variants in the development of cancer. In a meta-analysis, the author has identified a total of 11 BRCA1 and BRCA2 distinct common mutations, reported more frequently [36]. In another study, the authors investigated the involvement of BRCA1 founder mutations c.4034delA and c.5266dupC in conventional breast and ovarian cancer patients. The study showed that 57.5% of carriers with mutation did not correlate with the clinical criteria [1, 2].

Abdel-Mohsen et al. [37] determined BRCA1/2 gene mutations to provide insight into their roles as risk factors for breast and ovarian cancer and reported the incidence of BRCA1 mutation was 5 (16.7%) among controls and 44 (99.8%) cancer patients. They also reported that the most common variant was 5382insC followed by C61G and 185 delAG. In the present study, 7 % of the samples were found to be mutants while screening for the prevalence of BRCA1 (185 DelAG) mutation.

In a study, Gajalakshmi et al. [33] reported a BRCA1 gene exon 11 (1307delT) mutation in a 43-year-old woman with breast cancer. They screened the family members of the patients for the presence of this mutation. The study revealed that close family members also possess this mutation. Similarly, in the detection of BRCA1 Exon 11 (1307delT) facilitated among 18% of patients. Doraczynska-Kowalik et al. [38] tested five BRCA1 founder variants among 3,400 patients of the Polish population using NGS. They conclude that BRCA1/2-c.5266dup, c.181T>G, and c.4035del were found to be significant and reported screening complete HBOC genes will be better insight for therapeutic and prophylactic practices.

In the present study, the presence of deletion mutant c.5946delT variant of the BRCA2 gene was 12%. In a similar study, 2% incidence of c.5946delT pathogenic variant in the BRCA2 gene was reported in screening breast cancer patients [34]. Similarly, various studies have reported the presence of c.5946delT pathogenic variant among various

study groups for incidence, among the Jewish population the presence of this mutation was reported among 4.1% of participants [39]. On the contrary, a few reports are also stating that their study population has no reported c.5946delT pathogenic variant [40,41].

In screening of BRCA2 exon 21, the presence of T for C substitution (mutant c.8680C>T) was detected in 14% of amplified samples, among which 9 were from ovarian and 5 from breast cancer patients in the present study. Berzina et al. [2] conducted a study to distinguish HBOC patients in the sporadic breast and ovarian cancer group. In the study, the authors enrolled 50 HBOC patients in the Latvian Cancer Registry with negative BRCA1 founder mutation for targeted BRCA1 and BRCA2 genes resequencing and reported the BRCA2 mutations in two uninterrupted breast cancer patients.

Górski et al. [42] detected BRCA1/2 in families of HBOC patients using the Real-time PCR method. The authors reported 111 families possess 3 founder mutations in common and also reported this test can be considered as a rapid test and facilitates the large-scale community screening for determining potential inherited traits. Alvarez et al. [43] also designed 9 pairs of probes and primers targeting BRCA1/BRCA2 gene mutation detection by real-time PCR. Their study gives a better understanding of allelic discrimination by identifying the nine founder mutations. This study was also validated among patient and control groups which correctly categorized carriers and non-carriers.

CONCLUSION

The results of the present study indicate the role of BRCA1/2 mutations in the development of breast and ovarian cancer. The study should be performed with a large group for better understanding. Other reported BRCA variants are also incorporated for determining the inherited traits involved in inherited cancer development. Further study should also be performed in the view of screening role of other genes involved in inherited cancer development. This will results in developing a rapid and inexpensive mutation detection method that can be instigated in Hospitals for early detection and implementation of therapeutic and prophylactic modalities.

ACKNOWLEDGEMENT

The study was performed in Multi-Disciplinary Research Unit supported by Department of Health Research. We acknowledge DHR was for providing funds and infrastructure for doing the study. Authors would like to thank all the members who have participated in the research.

REFERENCES

1. *Plakhins G, Irmejs A, Gardovskis A,* Subatniece S, Rozite S, Bitina M, Keire G, Purkalne G, Teibe U, Trofimovics G, Miklasevics E and Gardovskis J. *Genotype-phenotype correlations among BRCA1 4153delA and 5382insC mutation carriers from Latvia. BMC Med Genet., 2011;12:147.*
2. Berzina D, Nakazawa-Miklasevica M, Zestkova J, Aksenoka K, Irmejs A, Gardovskis A, Kalniete D, Gardovskis J, Miklasevics E. BRCA1/2 mutation screening in high-risk breast/ovarian cancer families and sporadic cancer patient surveilling for hidden high-risk families. BMC Med Genet., 2013; Jun 14; 14:61.
3. Cardoso F, Kyriakides S, Ohno S, Penault-Llorca F, Poortmans P, Rubio IT, Zackrisson S, Senkus E. Early breast cancer: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. Ann. Oncol. 2019; 30, 1194-1220.
4. Kurman RJ, Carcangiu ML, Herrington CS and Young RH. (2014). WHO Classification of Tumours of Female Reproductive Organs. (Lyon: International Agency for Research on Cancer (IARC), 2014; 978-92-832-2435-8.
5. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J and Jemal A. Global cancer statistics, 2012. CA Cancer J Clin. 2015; 65:87-108.
6. Tewari KS and Monk BJ. (2015). The 21st century handbook of clinical ovarian cancer. Springer International Publishing, 2015; 10.1007/978-3-319-08066-6.
7. Kour A, Sambyal V, Guleria K, Singh NR, Uppal MS, Manjari M and Sudan M. Screening of BRCA1 variants c.190T>C, 1307delT, g.5331G>A and c.2612C>T in breast cancer patients from North India. Genetics and Molecular Biology, 2020; 43, 2, e20190014.
8. Ferlay J, Steliarova-Foucher E, Lortet-Tieulent J, Rosso S, Coebergh JW, Comber H, Forman D and Bray F. Cancer incidence and mortality patterns in Europe: estimates for 40 countries in 2012. Eur J Cancer. 2013; 49:1374-1403.
9. AIOM-AIRTUM. The numbers of cancer in Italy. 2015. (http://www. registri-tumori.it /cms/it/node/3993).
10. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A and Bray F. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 71(3):209–249.
11. Chicos A, Negura L, Braescu R, Morariu A, Negura A, Chicos A and Lupascu C. High frequency of BRCA recurrent mutations in a consecutive series of unselected ovarian cancer patients. Revista Română de Medicină de Laborator. 2020;28(3).
12. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA and Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018; 68(6):394-424.
13. Ferlay J, Colombet M, Soerjomataram I, Mathers C, Parkin DM, Pineros M, Znaor A, Bray F. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. International journal of cancer. 2019; 144(8):1941-53.
14. *Claus EB, Schildkraut JM, Thompson WD and Risch NJ. The genetic attributable risk of breast and ovarian cancer. Cancer. 1996; 77:2318-2324.*
15. Oosterwijk JC, de Vries J, Mourits MJ and de Bock GH. Genetic testing and familial implications in breast-ovarian cancer families. Maturitas. 2014; 78:252-7.
16. Miki Y, Swensen J, Shattuck-Eidens D, Futreal PA, Harshman K, Tavtigian S, Liu Q, Cochran C, Bennett LM, Ding W. A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. Science. 1994; 266(5182):66-71.
17. Wooster R, Bignell G, Lancaster J, Swift S, Seal S, Mangion J, Collins N, Gregory S, Gumbs C and Micklem G. Identification of the breast cancer susceptibility gene BRCA2. Nature. 1995; 378(6559):789-792.
18. Szabo C, Masiello A, Ryan JF and Brody LC. The breast cancer information core: database design, structure, and scope. Hum Mutat. 2000; 16:123-31.
19. Fackenthal JD and Olopade OI. Breast cancer risk associated with BRCA1 and BRCA2 in diverse populations. Nat Rev Cancer. 2007; 7(12):937-948.
20. Ferla R, Calo V, Cascio S, Rinaldi G, Badalamenti G, Carreca I, Surmacz E, Colucci G, Bazan V and Russo A. Founder mutations in BRCA1 and BRCA2 genes. Ann Oncol. 2007; 18(6):93-98.
21. Doherty JA, Jensen A, Kelemen LE, Pearce CL, Poole E, Schildkraut JM, Terry KL, Tworoger SS, Webb PM and Wentzensen N. Current Gaps in Ovarian Cancer Epidemiology: The Need for New Population-Based Research. J Natl Cancer Inst. 2017; 1:109(10).
22. Baretta Z, Mocellin, S, Goldin, E, Olopade OI and Huo D. Effect of BRCA germline mutations on breast cancer prognosis: A systematic review and meta-analysis. Medicine (Baltimore) 2016; 95, 4975.
23. Szczerba E, Kamińska K, Mierzwa T, Misiek M, Kowalewski J and Lewandowska MA. BRCA1/2 Mutation Detection in the Tumor Tissue from Selected Polish Patients with Breast Cancer Using Next Generation Sequencing. *Genes*, 2021; *12*(4), 519.
24. Balmaña J, Díez O, Rubio LT and Cardoso F. ESMO Guidelines Working Group: BRCA in breast cancer: ESMO clinical practice guidelines. Ann Oncol. 2011; 22 (6):31-34.
25. Huszno J, Kołosza Z and Grzybowska E.BRCA1 mutation in breast cancer patients: Analysis of prognostic factors and survival". Oncology Letters. 2019; 1986-1995.
26. Antoniou AC, Gayther SA, Stratton JF, Ponder BA and Easton DF. Risk models for familial ovarian and breast cancer. Genet Epidemiol. 2000;18(2):173-90.
27. King MC, Marks JH and Mandell JB. New York Breast Cancer Study Group Breast and ovarian cancer risks due to inherited mutations in BRCA1 and BRCA2. Science. 2003; 302:643-646.
28. Muinao T, Pal M and Deka Boruah HP. Origins based clinical and molecular complexities of epithelial ovarian cancer. Int J Biol Macromol. 2018; 118:1326-45.
29. Brozek I, Ratajska M, Piatkowska M, Kluska A, Balabas A, Dabrowska M, Nowakowska D, Niwinska A, Rachtan J, Steffen J and Limon J. Limited significance of family history for presence of BRCA1 gene mutation in Polish breast and ovarian cancer cases. Fam Cancer. 2012; 11:351-4.
30. Vanags A, Strumfa I, Gardovskis A, Borošenko V, Aboliņš A, Teibe U, Trofimovičs G, Miklaševičs E and Gardovskis J. *Population screening for hereditary and familial cancer syndromes in Valka district of Latvia. Hered Cancer Clin Pract. 2010; 8:8.*
31. *Ponnusamy K, Muthusamy S, Narayanaswamy D and Appadurai N. Evaluation of Vitamin D Polymorphism in Assessing the Susceptibility of Breast Cancer Patients. International Journal of Life Sciences, Biotechnology and Pharma Research, 2014;13: 4.*
32. Mary FA, Rajana A, Selvia R and Paula SFD. A pilot study on screening for 185 delAG mutation in breast cancer patients in a Tertiary Care Center from South India. Sri Ramachandra Journal of Medicine, 2010; 3(2).
33. Gajalakshmi P, Natarajan TG, Rani DS and Thangaraj K. A novel BRCA1 mutation in an Indian family with hereditary breast/ovarian cancer. Breast Cancer Res Treat, 2007; 101:3-6.
34. Berhane N, Chekol Z and Seid A. Detecting the Frequency of c.5946delT Pathogenic Variant in the BRCA2 Gene and Associated Risk Factors Among Breast Cancer Patients Visiting Felege Hiwot Referral Hospital and University of Gondar Comprehensive Specialized Hospital. Breast Cancer: Targets and Therapy, 2023; 421-427.
35. Negură L, Azoicăi D, Matei M, Popoiu G and Negură A. Screening of a novel BRCA2 mutation by rapid in-house PCR-RFLP. Revista Română de Medicină de Laborator, 2011; 19: 4/4.
36. Forat-Yazdi M, Neamatzadeh H, Sheikhha MH, Zare-Shehneh M and Fattahi M. BRCA1 and BRCA2 common mutations in iranian breast cancer patients: a meta analysis. Asian Pac J Cancer Prev. 2015; 16(3):1219-24.
37. Abdel-Mohsen1 MA, Ahmed OA and ElKerm YM. BRCA1 Gene Mutations and Influence of Chemotherapy on Autophagy and Apoptotic Mechanisms in Egyptian Breast Cancer Patients. Asian Pac J Cancer Prev, 2016; 17 (3), 1285-1292.
38. Doraczynska-Kowalik A, Michalowska D, Matkowski R, Czykalko E, Blomka D, Semeniuk M, Abrahamowska M, Janus-Szymanska G, Mlynarczykowska P, Szynglarewicz B, Pawlak I, Maciejczyk A and Laczmanska I. Detection of BRCA1/2 pathogenic variants in patients with breast and/or ovarian cancer and their families. Analysis of 3,458 cases from Lower Silesia (Poland) according to the diagnostic algorithm of the National Cancer Control Programme. Front Genet. 2022; 13:941375.
39. Tonin P, Weber B, Offit K, Couch F, Rebbeck TR, Neuhausen S, Godwin AK, Daly M, Wagner-Costalos J, Berman D, Grana G, Fox E, Kane MF, Kolodner RD, Krainer M, Haber DA, Struewing JP, Warner E, Rosen B, Lerman C, Peshkin B, Norton L, Serova O, Foulkes WD and Garber JE. Frequency of recurrent BRCA1 and BRCA2 mutations in Ashkenazi Jewish breast cancer families. Nat Med. 1996; 2(11):1179–1183.
40. Elfandi L, Said G, Saleh SS, Marwan M and Enattah N. Analysis of 6174delT mutation in BRCA2 gene by mutagenically separated PCR among Libyan patients with breast cancer. Arch Breast Cancer. 2016; 2016:8-13.
41. Sitompul Y, Syukur S, Syafrizayanti S, Purwati E. Absence of BRCA1 185delAG, BRCA1 5382InsC and BRCA2 6174delT among hereditary breast cancer patients in North Sumatera, Indonesia. Am Acad Sci Res J Eng Technol Sci. 2017; 38(2):152-160.
42. Górski B, Jakubowska A, Huzarski T, Byrski T, Gronwald J, Grzybowska E, Mackiewicz A, Stawicka M, Bebenek M, Sorokin D, Fiszer-Maliszewska Ł, Haus O, Janiszewska H, Niepsuj S, Góźdź S, Zaremba L, Posmyk M, Płuzańska M, Kilar E, Czudowska D, Waśko B, Miturski R, Kowalczyk JR, Urbański K, Szwiec M, Koc J, Debniak B, Rozmiarek A, Debniak T, Cybulski C, Kowalska E, Tołoczko-Grabarek A, Zajaczek S, Menkiszak J, Medrek K, Masojć B, Mierzejewski M, Narod SA, Lubiński J. A high proportion of founder BRCA1 mutations in Polish breast cancer families. Int J Cancer. 2004; 110(5):683-6.
43. Alvarez C, Ortega-Hernández V, Cortez A and Carvallo P. BRCA1 and BRCA2 screening of nine Chilean founder mutations through allelic-discrimination and real-time PCR in breast/ovarian cancer patients. Mol Biol Rep 2022; 49, 7531-7539.