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ORIGINAL ARTICLE

Detection of BRCA-1 gene mutations in epithelial ovarian tumors at tertiary care hospital Karachi

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ABSTRACT

Background: Ovarian cancer is the most common and lethal gynecological malignancy. Ovarian malignancy is the 3rd most common cancer in Pakistan. More than 90% ovarian cancers are of epithelial cell origin, about 24-40% such cancers have hereditary or sporadic mutation in the BRCA-1 or BRCA-2 genes. The present study was designed to detect BRCA-1 gene mutations in different histological sub-types of epithelial origin of benign, borderline and malignant ovarian tumors.

Methods: This cross-sectional study was based on the analysis of BRCA-1 gene mutations in the epithelial origin of benign, borderline and malignant ovarian tumors received at the department of Pathology, Basic Medical Sciences Institute, Jinnah Post graduate Medical Centre, from 01-01-2011 to 31-12-2015. A total of 80 diagnosed cases were selected and analyzed for PCR.

Results: BRCA-1 gene mutations were detected in 22 (27.5%) cases out of the 80 randomly selected epithelial ovarian tumors. Serous cyst adenocarcinoma was the commonest including 63% cases. BRCA-1 gene mutations were also positive in other epithelial ovarian tumors, including Mucinous cyst adenocarcinoma (13.6%), Endometroid adenocarcinoma and Mixed Mullerian tumor (4.5% each). In addition mutations were also detected in borderline tumors including Mucinous borderline tumor (9.09%) and Seromucinous borderline tumors (4.5%).The observations and results of the study were elaborated with the assistance of tables, figures and photomicrographs.

Conclusion: BRCA-1 gene mutations manifestations were identified in a large number of serous malignant ovarian tumor cases. Small percentages of borderline and malignant mucinous tumors, endometroid adenocarcinoma and mixed mullerian tumor were also positive for BRCA-1 gene mutations.

Key Words: Benign, BRCA-1, Cyst Adenocarcinoma Malignant, Mucious, Polymerase Chain Reaction (PCR), Tumor.

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Introduction

Benign ovarian tumor encounter in younger age, borderline in middle age while malignant is the lesion of older age groups (1). Majority of ovarian malignancies are

categorized as epithelial, sex cord-stromal and germ cell tumors (2). Numerically most significant one is originanted from the outer lining epithelium of the ovary (3). Although majority of malignant ovarían tumors at the times of diagnosis have distant metástasis (1). Ovarian neoplasm is the commonest and fatal gynecological disease (4). The incidence of this malignancy is higher in postmenopausal females, majority of women affected belong to 50 to 75 years age group (5).

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Ovarian malignancy is the 3rd most widespread tumor in Pakistan. Frequency of ovarian cancer is uppermost in Pakistan amongst the entire South Asian countries, including India, Srilanka and Bangladesh (6).

More than 90% ovarian cancers are of epithelial cell origin, about 24-40% such cancers have hereditary or sporadic mutation in the BRCA-1 or BRCA-2 genes (7). When sporadic epithelial ovarian cancers display BRCA-1/2 gene dysfunction it is known as BRCAness (8). BRCA associated hereditary and sporadic ovarian cancers are pathologically similar but hereditary cancers are associated with better survival and develop 10 to 15 years earlier as compared to non-hereditary cases (9).

The Breast Cancer Susceptibility Gene-1 gene was first identified in 1995. It is a tumor suppressor gene, positioned on chromosome 17q21, containing 24 exons and encodes for 1863 amino acids (10). BRCA-1 mutations can exist in a diversity of mechanism, counting germline, somatic mutations and promoter hyper methylation (11).

Mucinous ovarian tumors arise from tubal-mesothelial junction by a process of metaplasia (12). BRCA gene mutations are rarely considered in borderline ovarian tumors, which mean that BRCA-1 mutations do not take part in the evolution of such type of tumors (13).

This study was planned to detect BRCA-1 gene mutation in various histological sub-types of epithelial origin benign, borderline and malignant ovarian tumors and its association with tumor grading.

Methods

This cross-sectional study was based on the analysis of BRCA-1 gene mutations in the epithelial origin of benign, borderline and malignant ovarian tumors received at the department of Pathology, Basic-Medical-Science-Institute (BMSI), Jinnah-Postgraduate-Medical-Center (JPMC) Karachi. This study was conducted from 1stJanuary 2011 to 31st December 2015).It was approved by the institutional review board of Basic Medical Sciences Institute, JPMC Karachi via letter number F.1-2/2018/BMSI-E.COMT/062/JPMC dated 29-03-2018.

A total of 324 histo-pathologically proven cases of ovarian tumors were acknowledged in five years. Ovarian surface epithelial tumors were 267 (82%) out of 324 cases. However due to financial constraints total of 80 diagnosed cases were analyzed for PCR.

Epicenter Kit (mcd 85201) was used to isolate DNA from formalin fixed paraffin embedded tissue (FFPE).We followed the protocol by taking 50 mg of tissue from paraffin block in 1.5 ml eppendorf cup. T & C lysed solution 600 µl was added along with diluted 01 µl of 50µg Protease K to all the sample tubes. Mixed and incubated overnight at 37°C. The tubes were placed on ice for 03-05 minutes. Tubes were then vortexed and 200µl of MPC (Protein Precipitation Reagent) was added

and vortexed again vigorously. Centrifugation was done at 13000 RPM for 10 minutes and supernatant was obtained. It was transferred into another tube containing 500 µl of Isopropanol. Tubes were then inverted gently 30-40 times so as to recover the DNA from supernatant. Centrifugation was done at 13000 rpm for 10 minutes. Discard the supernatant without disturbing the pellet. Add 500 µl of 100% ethanol and centrifuged the pellets at 13000 rpm for 07 minutes so that all the Isopropanol is removed. Discard the supernatant and placed the tubes in inverted position slowly. Air dried the pellet and re-suspended it in 35 µl of TBE buffer. Samples were then stored at -80°C.

Primers used for amplification of the BRCA-1 gene

Exon 02 and parts of Exon 11 of BRCA-1 gene

Forward: 5′-AAACCTTCCAAATCTTCAAA-3′ Exon 02 443 bp
Reverse: 5′-GTCTTTTCTTCCCTAGTATGT-3′
Forward: 5'-AACACCACTGAGAAGCGTGCAG-3'
Exon 11 A 309 bp
Forward: 5'-CAACATAACAGATGGGCTGGAAG-3'
Exon 11 B 300 bp
Reverse: 5'-ACGTCCAATACATCAGCTACTTTGG-3'

PCR was performed on all samples. Total of 20 µl reaction volume was used for PCR reaction. It was composed of 0.5µl DNA template, , 20 pmol/L of each primers (onward and reverse), 2.5units Taq DNA polymerase, and 0.5µl of each DNTP in a solution of 10 Mm Tris- HCl, 50 mM KCl and 1.2 mM MgCl2. The thermal cycler (Master Gradient PCR system, Eppendorf AG, Germany) was used for this purpose. It was programmed as to first incubate samples at 95°C for 05 minutes, followed by 45 cycles composed of 94°C for 45 seconds, 55°C for 50 seconds and 72°C for 01 minute, 30 seconds with a final extension at 72°C for 08 minutes. The PCR amplified products were identified by gel electrophoresis.

Gel was prepared by dissolving 02 g of agarose in TBE buffer and boiled to 100°C for one minute. On cooling to 60°C,it is poured into gel casting block after adding 10µl of Ethidium bromide. Sample comb was placed to make the sample space in the gel. Add 03 µl of gel loading buffer in the entire amplified product and mixed. On solidification load the samples and run the electrophoresis for 50 minutes at 100 volts in 1x TBE buffer. The gel was under trans illuminator gel doc and bands of amplified product were evaluated. The amplified product was compared with 100-bp DNA ladder (Gibco BRL, Life Technologies) and mutation was detected by comparing with BRCA-1 positive human breast cancer taken as a positive control and water as negative control.

Results

The table 01 showed the morphological distribution of 80 cases taken for detection of BRCA-1 gene mutations, total benign cases were 06 including 03 serous cystadenonoma, 02 cases of mucinous cyst adenoma and a single case of benign brenner tumor. Total 14 borderline tumors included 04 serous, 09 mucinous and one seromucinous borderline tumor. Out of 60 malignant ovarian tumors 31 cases were of serous cyst adenocarcinoma, 16 cases of mucinous cyst adenocarcinoma, 03 cases of endometroid adenocarcinoma and 02 cases of clear cell carcinoma. Poorly differentiated tumor cases were 05 and one cases of each mixed-mullerian tumor, signet ring carcinoma and undifferentiated

carcinoma. This was observed that the 22 (27.5%) cases were BRCA-1 gene mutations positive, the most common ovarian tumor was serous cyst adenocarcinoma (63.3%) followed by mucinous cyst adenocarcinoma (13.6%), mucinous borderline tumor (9.09%) endometroid adenocarcinoma (4.5%), mixed-mullerian-tumor (4.5%) and seromucinous borderline tumors(4.5%) .

Table 1: Morphological distribution of selected (80) cases for BRCA-1 Gene mutations

Morphological Types	Benign	Borderline	Malignant	No of cases
Serous	**03**	**04**	**31**	**38**
Mucinous	**02**	**09**	**16**	**27**
Seromucinous	**00**	**01**	**00**	**01**
Endometroid Adenocarcinoma	**00**	**00**	**03**	**03**
Clear cell Carcinoma	**00**	**00**	**02**	**02**
Signet Ring Carcinoma	**00**	**00**	**01**	**01**
Brenner Tumor	**01**	**00**	**00**	**01**
Mixed-Mullerian Tumor	**00**	**00**	**01**	**01**
Poorly Differentiated Tumor	**00**	**00**	**05**	**05**
Undifferentiated Tumor	**00**	**00**	**01**	**01**
Total	**06**	**14**	**60**	**80**

Table 2: Distribution of BRCA-1 Gene mutations positive cases among all selected cases (n=80)

Histology	Benign (n=06)		Borderline (n=14)		Malignant (n=60)	
	Positive	Negative	Positive	Negative	Positive	Negative
Serous	00	03	00	04	14	17
Mucinous	00	02	02	07	03	13
Seromucinous	00	00	01	00	00	00
Endometroid	00	00	00	00	01	02
Clear cell	00	00	00	00	00	02
Signet ring	00	00	00	00	00	01
Brenner tumors	00	01	00	00	00	00
Mixed Mullerian Tumor	00	00	00	00	01	00
Poorly differentiated	00	00	00	00	00	05
Undifferentiated	00	00	00	00	00	01
Total	**00**	**06**	**03**	**11**	**19**	**41**

Table 3: Distribution of BRCA-1 Gene mutations positive cases according to histological types (n=22)

Histology	Benign	Borderline	Malignant	Total no of BRCA-1 Mutation Positive cases
Serous	00	00	14 (63.3%)	22 (27.5%)
Mucinous	00	02 (9.09%)	03 (13.6%)	
Seromucinous	00	01 (4.5%)	00	
Endometroid Adenocarcinoma	00	00	01 (4.5%)	
Mixed-Mullerian-Tumor	00	00	01 (4.5%)	

Table 4: Distribution of BRCA-1 Gene mutation positive cases according to histological grade (n=22)

Morphological Types	Low Grade Tumors		High Grade Tumors
	Benign	Borderline	
Serous	00	00	14
Mucinous	00	02	03
Seromucinous	00	01	00
Endometroid	00	00	01
Mixed-Mullerian Tumors	00	00	01
Total	00	03 (13.6%)	19 (86.3%)

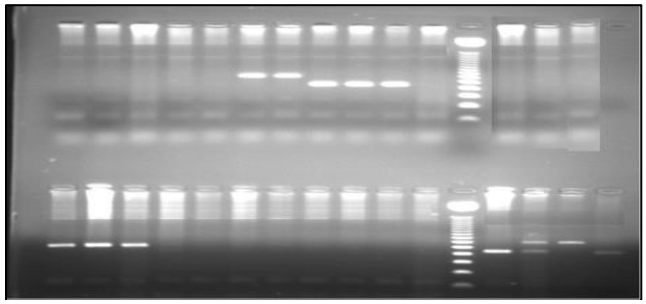

Figure 1: The gel analysis of BRCA-1 (exon 02, 11A and 11 B) DNA sample. Lane 6, 7, 15 to17, 31 and 32 were exon 02 mutation at 443 bp. Lane 8 to 10, 30, 31 and 33 were detected for 11A mutation at 309 bp lane 31 shows 02 mutations i.e. exon 02 and 11 A. Whereas lane 01 to 05, 11 to 13, 18 to 29 were not detected. Lane M is the DNA ladder of 100 bp and lane N was the negative control.

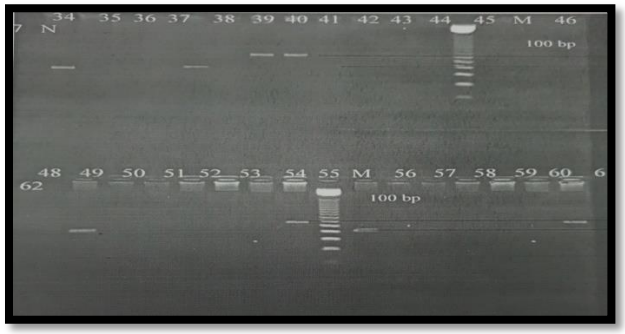

Figure 2: Lane 40, 41, 55 and 62 show exon 02 mutations at 443 bp. Lane 34, 38, 49 and 56 are detected for 11Bexon mutation at 300bp whereas lane 35 to 37, 42 to 48, 50 to 54 and 57 to 61 are not detected. Lane M is the DNA ladder of 100bp (Positive control). Lane N is the negative control.

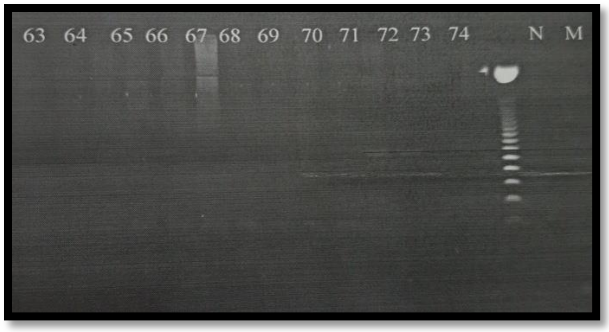

Figure 3: The gel analysis of BRCA-1 (exon 02, 11A and 11B) DNA sample. Lane 73 and 74 are exon 02 mutation at 443 bp whereas lane 63 to 72 is not detected. Lane M is the DNA ladder of 100 bp and lane N is the negative control.

Discussion

BRCA-1 gene mutations were observed in total 22 (27.5%) cases out of 80 randomly selected ovarian tumor samples analyzed by standard PCR. This figure was comparable with Vaidyanathan et al (2009) (14) on a South Indian population based study reported 24.6% and Geisler et al (2002) (15) observed 23.1% BRCA-1 gene mutations in FFPE ovarian tumor samples.

Ellison et al (2015) (16) and Mafficini et al (2016) (17) reported that the germline as well as somatic BRCA gene mutations may lead to breast or ovarian cancers in higher percentages and give good therapeutic response to platinum based chemotherapy and recently approved PARPi (Poly ADP Ribose Polymerase inhibitor). DNA is extracted from the blood samples, saliva sample or buccal smear for germline BRCA gene mutations in most of the medical genetics laboratories, but these investigations are not useful to detect somatic mutations in BRCA linked ovarian tumors. In contrast DNA extract from tumor cells of FFPE ovarian tumor samples are useful to detect genetic as well as the somatic BRCA-1gene mutations, so that patients with somatic mutations may also benefit from the

platinum based chemo therapeutic agents and PARPi (16,17).

The current study showed an expected result that the high grade serous malignant tumors were the commonest ovarian tumors associated with BRCA-1 gene mutations. We observed 63.3% of high grade serous cyst adenocarcinoma showing BRCA-1 gene mutations. Similarly, Bjorge et al (2004) (18)and Candido-dos-reis et al (2014) (19) reported 74% and 73% cases of high grade serous cyst adenocarcinoma showing BRCA-1 mutations, respectively.

In additions we also observed that endometroid adenocarcinoma and mixed mullerian tumors were 4.5% of total BRCA-1 positive cases. Pal et al (2005) (20) reported 05% and 20% of these tumors showing BRCA-1 positivity. The difference in the frequency was due to the larger sample size in his study. In this study, Contrary to popular dogma observed by different researchers, include Pal et-al (2005) (20) and Bjorge et-al (2005) (18) that BRCA gene mutations were not associated with mucinous malignant or borderline tumors, we also found such tumors showing BRCA-1 gene mutations including 13.6% mucinous cyst adenocarcinoma, 9.09% borderline mucinous tumor and 4.5% of seromucinous borderline.

The reasons behind the presence of BRCA-1 gene mutations in mucinous tumors may be due to the high risk geographical regions and ethnicity. Since the present study was done on paraffin embedded formalin fixed ovarian tumor samples therefore it could be due to the somatic BRCA-1 gene mutations.

Conclusion

Current study concludes that BRCA-1 gene mutation associated ovarian tumors are more prevalent in our population. BRCA-1 gene mutation was recognized in large number of high grade malignant serous ovarian neoplasms. Small numbers of borderline and malignant mucinous tumors, endometroid adenocarcinoma and the mixed-mullerian-tumor were also positive for BRCA-1 gene-mutations.

Recommendations

We suggest that more molecular based studies using a large sample size provided with complete clinical and family history are recommended to further evaluate the presence of somatic and germ line mutations in BRCA associated ovarian tumors.

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C. Interpretation/ Analysis and Discussion