Original Article

BRCA1 AND BRCA2 MUTATIONS IN OVARIAN CANCER PATIENTS

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Background: To find the frequency of germ line BRCA gene mutations among our patients with epithelial ovarian cancer, unselected for age and family history of cancer.

Methods: A total of 75 women with histologically proven epithelial ovarian cancer were accrued and 20 cc of peripheral blood sample was collected from each subject and sent to the molecular laboratory of Sunnybrook and women's college Health Science centre in Toronto, Canada for detection of BRCA genes.

Results: TBRCA 1 & 2 genes mutations were found in 9 (12%) patients. BRCA 1 mutation was more common found in eight (88.8%) patients as compared to BRCA2 in 1(11.2%) patient. Out of 9, six mutations were unique to our subjects and remaining three have also been reported in Dutch and Belgian families. All but one BRCA 1 mutation were found in exon 11. BRCA gene mutation was detected in 35.7% patients of with positive family history of breast and ovarian cancer. All four patients who had ovarian cancer as a second malignancy after breast cancer were positive for BRCA mutation.

Conclusions: The frequency of ovarian cancer in our patients is comparable to what has been reported in Western literature. The correlation between family history and probability of finding gene mutation in these patients can be used to identify the families to be tested for the gene mutations. Genetic testing can identify women and their families at increased risk of ovarian cancer.

Keywords: Breast and ovarian cancer, BRCA 1 and BRCA 2 and gene multation.

Introduction

Carcinoma of the ovary is the second most common malignancy among Pakistani women, being the most common cancer of gynecological origin.[1]The ranking varied from second to fifth among women in various Pakistani studies. Age Standardized Ratio (ASR) of ovarian cancer in Karachi in 10.2 per 10,000per year, which is comparable to that of Ontario, Canada (10.7 per 10,000per year) but higher as compared to Bombay, India (7.2 per 10,000per year).[2] The reasons for high incidence of ovarian cancer is not known. Risk factors such as early menarche, late menopause and low parity are uncommon among Pakistani women.[3] Consanguinity is known to increase the risk of breast cancer and it is quite common in Pakistan.[4,5] So it is possible that genetic factors may also contribute to significant proportion of ovarian cancer in our patients.[6]

Sporadic ovarian cancer accounts for 90-95% of all ovarian cancer. While hereditary ovarian cancer constitutes 5-10% of all cases.[7,8]Germ line mutations in BRCA 1 and BRCA2 are responsible for 90% of hereditary ovarian cancer. BRCA1 and BRCA2 has been mapped chromosome 17q21 and 13q12, respectively.[9] These genes are associated with transcription regulation as well as recognition and repair of certain forms of DNA damage. Mutations in BRCA genes can lead to uncontrolled cell growth and ultimately carcinogenesis.

Women carrying deleterious mutation face lifetime risk of breast cancer ranging from 36-90% and of ovarian cancer between 16-63%.[10, 11] According to various studies, BRCA gene mutations have been found in 14-22% of patients with epithelial ovarian cancer, while those who have history of ovarian cancer in one or more first degree relatives, the frequency of BRCA gene mutation is 50-70%. The frequency and spectrum of these mutations is different in different regions and ethnic groups across the world. Majority of data comes from studies done in western countries. Despite high incidence of ovarian cancer in Pakistan, very little is known about the frequencies of mutations in BRCA genes among ovarian cancer patients in Pakistan. So far, few studies have been done to determine the prevalence of these mutations in Pakistan and only two studies[3,12] mentioned the frequencies of these mutations in ovarian cancer patients 15.8 and 9% respectively, but In later study patients were selected on the basis of age and family history of breast/ovarian cancer. Therefore, we conducted this study to find out the frequency of BRCA genes in our patients which are unselected for age and family history of cancer.

Objectives:

To find out the frequency of germ line BRCA gene mutations among our patients with epithelial ovarian cancer

Material and Methods

Seventy-Five women with histologically proven epithelial ovarian cancer were accrued from August 1999 to August 2001 at Department of oncology, Jinnah hospital, Lahore. After getting informed consent, subjects were interviewed for family history of cancer, consanguinity, prior history of cancer, menstrual and reproductive history. These subjects were unselected for age and family history of cancer. Twenty milliliter of peripheral blood sample was collected from each subject and sent to the molecular laboratory of Sunnybrook and women's college Health Science centre in Toronto, Canada for detection of BRCA genes.

Exon11 of BRCA1 and exon 10 & 11 of BRCA 2 were screened by protein truncation testing (PTT) in all subjects.13 All mutated bands detected by PTT were confirmed by direct sequencing. Subjects with strong family history of breast and ovarian cancer in whom no mutation was found were selected for complete sequencing of all coding regions at Myriads Genetics .

Analysis of BRCA1 exon 2, 12, 15, 20 and BRCA 2 exon 22 was performed by denaturing gradient cell electrophoresis. Confident interval was calculated with assumption of Poisson distribution.

Results

The characteristics of our patients are given in table 1. Median age of our subjects was 47 years. Family history of cancer was found in 14 (18.7%) patients. BRCA 1 & 2 genes mutations were found in 9 patients, which constituted 12% (95% CI, 6.24-22.06) of all patients. Median age of the patients who were positive for BRCA mutation was 46 years (36.8-74.2). BRCA 1 mutation was more common found in eight (88.8%) patients as compared to BRCA2 mutation which was positive in only 1out of 9 (11.2%) patients. Out of 9, six mutations were unique to our subjects and other three (3889del A→G exon 11, 185insA exon 11, 2722C→G exon11) have also been reported in Dutch and Belgian families.14 Recently the 185insA exon 11 mutation has also been reported in three Punjabi subjects with ovarian cancer by Muhammad U. Rasheed et al.12 All but one BRCA 1 mutation were found in exon 11. BRCA 2 mutation was also found on exon 11. BRCA gene mutation was detected in five out of 14 (35.7%) patients of ovarian cancer with positive family history of breast and ovarian cancer. Four patients in our study had ovarian cancer as a second malignancy after breast cancer and all of them were found positive for BRCA mutation.

Discussion

Ovarian cancer is the second most common malignancy in Pakistani women. Traditional risk

Table-1 Characteristics of Patients.

All patients charactertistic (n=75)	No.	Percentage	BRCA Cariers (n=9)	Percentage
Age (median 47 years)			Age (Median 46 years	
22-75 (years)			(36.80-74.20)	
< 50 Years	47 Years	62.60	06	66.60
? 50 Years	28 Years	37.40	03	33.30
Menopausal status Premenopausal				
	67	83.33	07	77.70
Postmenopausal	08	100.0	02	33.30
Nulliparity	11	100.0	-	
Consanguinity	30	40	04	44.40
Family history of cancer	14	18.70	05	55.50
Stage				
I/II	09	83.33	01	11.20
II/IV Grade	66	37.40	08	88.80

Well differentiated	19	25.30	04	44.40
Moderately Diff.	27	36.00	02	33.30
Poorly Differentiates	29	38.60	02	22.20
Histopathology				
Serous	40	53.30	08	88.80
Macniou	22	29.30	0	00.00
Endomertniod	13	17.30	01	12.20

Table-2: Detected BRCA mutations.

Subject No. (N=9)	Age	Mutations	F.H. OF Breast Cancer	F.H of Ovarian Cancer	Other Cancers
5	46.00	195delA;exon 1140	1	0	0
9	36.80	204insA;exon 1140	1	2	1
11	40.40	185insA;exon 2	0	0	0
17	72.20	3889delAG;exon 11	0	0	0
22	40.709	4627C? A[S1503X];exon 15	1	0	0
24	45.90	1127del;exon11	1	0	0
33	52.20	2266delG;exon11	0	0	0
38	52.80	2722C? G;exon11	1	0	0
47	50.00	6679ins A;expm11	0	0	0

factors like early menarche, late menopause, and low parity are not common in our population; so the genetic factors may be responsible for majority of hereditary breast and ovarian cancers in Pakistan.[3] BRCA1 and BRCA2 genes accounts for majority of breast and ovarian cancers, therefore, we conducted this study to determine the frequency of BRCA genes mutation in ovarian cancer patients presenting to Jinnah hospital, Lahore.

BRCA genes mutation was found in 12% of our ovarian cancer patients and this frequency is comparable to the frequencies reported in foreign and local studies.[2,3,12] The screening method used in this study for the detection of these mutations has sensitivity of about 80%, so there is possibility that some mutations might have missed in about 20% of the cases, therefore actual frequency may be 10-20% higher than reported in our current study.[13]

Patients in whom BRCA gene was detected were younger (median age 46 years) than those without gene mutation (median age 47 years). This is in agreement to the notion that hereditary cancers tend to present at earlier age as compared to the sporadic cancers. However this is in contrast to the two local studies which reported median age at presentation of ovarian cancer is higher in BRCA carriers.[3, 12] This controversy could be due to the difference in study population and selection criteria.

Out of nine mutations reported in our subjects, 6 were unique to our patients as well as non recurrent. (1956delA; exon 11, 2041insA; exon 11, 4627C→A; exon 15, 1127delA; exon11, 2266delG; exon11, 6679 insAA; exon11) While other three mutations (3889 del AG exon 11, 185insA exon 11, 2722C→G exon11) were reported earlier in local as well as foreign literature.[3, 12, 14] In contrast to our study, Muhammad U. Rashid et al reported 4 recurrent BRCA mutations in their patients.[12] This can be explained on the basis of larger sample size and inclusion of the patients with strong family history of breast and ovarian cancer.

Studies have shown correlation between presence of BRCA mutation and the strength of family history of breast and ovarian cancer. BRCA gene mutations are seen in 50-70% of patients with strong family history of breast and ovarian cancer.[15,16] In one recent study, prevalence of BRCA 1 and BRCA2 mutations was estimated in our patients with breast and ovarian

cancer, selected on the basis of strong family history and age of diagnosis. This study has shown a prevalence of BRCA 1 and BRCA2 to be 50% in patients from breast cancer families and 9% for single cases of early onset ovarian cancer (<45 years age).[12]

In our study patients with family history of cancer in their first and second degree relatives BRCA gene was found 35.7% of the patients and this prevalence rose to 55% in patients having history of breast and ovarian cancers in their first degree relatives. Four of our patients had prior history of breast cancer, all of them found to have BRCA mutation. Therefore, chances of detecting BRCA gene mutation increases either with strong family history of cancer or having breast and ovarian cancer in the same patient. [17]

The average risk of developing breast cancer is 12.5% and that of ovarian cancer is 1.5%. However, in the presence of germ line BRCA 1 mutation and strong family history cancer, these risk rise to about 90% and 40% for breast and ovarian cancers, respectively.[10, 11] It is important to recognize that these risk estimates are from families with multiple cases of breast and/or ovarian cancer. The risk for women with BRCA mutations from families with less impressive family history is probably lower, ranging from15-20% for ovarian cancer. On the basis of family history, we can identify families which should be tested for these mutations.

The ability to identify individuals at higher risk of cancer holds the promise of prevention and early detection of cancer. Family members who are found to be "non carriers" after genetic testing can be spared the anxiety and the need for increased surveillance. Those who are found to have the deleterious gene mutation can be offered strict surveillance for breast and ovarian cancer according to ASCO guidelines.

Conclusion

The data from this study suggests that the frequency of ovarian cancer in our patients is comparable to what has been reported in Western literature. The correlation between family history and probability of finding gene mutation in these patients can be used to identify the families to be tested for the gene mutations. Genetic testing can definitely identify women with germ line mutations that place them and their families at increased risk of ovarian cancer. It can enhance medical management when used appropriately and should be accompanied by patient education and counseling.

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