PREVALENCE OF BRCA1 (185delAG AND 5382insC) AND BRCA2 (6174delT) MUTATIONS IN OVARIAN CANCER OF SOUTH INDIAN COHORT

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HIGHLIGHTS

Ovarian Cancer (OC) an asymptomatic gynaecologic malignancy, has over the decade is the prime form of cancer to affect the postmenopausal women, wherein the highest incidence rate, with an alarming survival rate of only 20 - 30% which is threatening their existence. Our study analysed the possible causes and identifies the demographic profiles in understanding the potential growth and assessing the frequency of BRCA1 (185delAG and 5382insC) & BRCA2 (6174del T) from South Indian cohort

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ABSTRACT

Background: Ovarian cancer (OC) is the most common cancer among women worldwide. Mutations in BRCA1 &BRCA2strongly increase the risk of developing breast and/or ovarian cancer. Several factors contribute to the increased frequency, and the proportion varies widely among various populations. The aim of the study is to assess the frequency of founder mutations of BRCA1 (185delAG and 5382insC) and BRCA2 (6174delT) in OC from South Indian cohort. **Materials and Methods:** A total of 100 clinically and histopathologically confirmed ovarian cancer patients and an equal number of control subjects were included in the present study. The screening was carried out by ARMS-PCR using appropriate primers followed by 2% agarose gel electrophoresis. Statistical analysis was carried out to test for the significance of the results obtained. **Results:** Age >40ys and postmenopausal women are at an increased risk for OC. Genotype distribution showed an increased frequency of 185del AG of BRCA1 with an odd's ratio of eighteen-fold and 5382ins C of BRCA1 with sixteen-fold increased risk whereas 6174del T of BRCA2 revealed seven-fold increased risk to ovarian cancer. **Conclusion:** The present study highlights the role of BRCA1 and BRCA2 founder mutations in the etiology of ovarian cancer patients of South Indian cohort.

KEY WORDS *BRCA1; BRCA2; founder mutations; ovarian cancer; South Indian cohort*

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INTRODUCTION

Ovarian cancer (OC), a malignant neoplasm is the number one killer amongst gynaecological malignancies with more than two third of patients present with late stage symptoms [1]. It is the fourth most common cause of cancer-related death among women globally, (Boyd et al, 2000) with a lifetime risk of 1.71% [2]. In India, the age-adjusted rate (AAR) of OC is 5.3/100,000 women (ICMR 2002) and often result as a consequence of a multistep genetic process due to an accumulation of mutations. The aetiology of Ovarian cancer is influenced by various epidemiological and genetic susceptibility factors which play a vital role in the causation of the disease. Hereditary cancers often show an autosomal dominant genetic predisposition due to mutations in BRCA1 and BRCA2 genes with high penetrance [3]. It is well known that mutations in these genes account for a large number of familial ovarian cancers [4].The BRCA1&2 mutations are commonly found in Ashkenazi Jewish individuals, as founder mutations of 185delAG and 5382insC (BRCA1) and 6174delT (BRCA2). To date, several studies have reported lifetime risk to OC due to inherited BRCA1 (28–66%) and BRCA2 (16–27%) mutations based on familial status and population series of ovarian cancer probands [5-11]. In view of the above, the present study is aimed to evaluate the role of founder mutations185delAG and 5382insC of BRCA1 and 6174delT of BRCA2 genes in the aetiology of ovarian cancer of south Indian cohort.

MATERIALS AND METHODS

Study population

A total of 200 individuals comprising of 100 clinically and histopathologically confirmed ovarian cancer patients and 100 female healthy controls of the same ethnicity were enrolled for the present study. All the subjects were collected from the oncology unit of Yashoda Hospitals, Secunderabad during the years 2012-16. Information regarding the demographic details such as age, menopause status, consanguinity, gravida, dietary habits and family history etc., were obtained with the help of a structured proforma. Informed written consent was obtained from all the subjects and the study was approved by an ethical committee of Institute of Genetics, Osmania University, and Yashoda Hospitals, Secunderabad. Clinical variables such as age at onset, tumor stage (T1–4), duration of disease (DOD), i.e. duration of time from the initial clinical symptoms to sample collection were also obtained from all the patients. Patients who had a history of other malignancies, chronic diseases, cysts, and fibroids were excluded from the study. Healthy women with no family history of cancer were considered as controls for the present study. Pedigree information was obtained from all the probands and/or their first-degree relatives through multiple interviews. Informed written consent was obtained from all the subjects.

Sample collection

Five ml of venous blood was drawn from each individual in EDTA vacutainers and genomic DNA was isolated from whole blood using the phenol-chloroform method [12] and stored at -20 0C for further use. The concentration of the DNA was measured spectrophotometrically.

Molecular analysis

Detection of 185delAG, 5382insC, and 6174delT founder mutations was carried out using the oligonucleotide primers listed in Table 1. In each PCR reaction, 25ng of genomic DNA was added to 20µl of reaction mixture consisting of 10 × PCR reaction buffer (10mMTris-HCl, pH 8.3, 50mMKCl, 10 µg/ml gelatin), 3.25mM MgCl2, 0.2mMdNTPs, and 3 U Taq DNA polymerase (Roche, Penzberg, Germany). The concentrations of primers used were 2.0 µM for P1 and P3, 0.4 µM for P2, 0.12 µM for P4, P5, and P6, 0.31 µM for P7 and P9, and 0.24 µM for P8. Each PCR reaction consisted of an initial 4 min at 94°C, followed by 35 cycles of 94ºC for 30 sec, 57ºC for 30 sec, and 72ºC for 45 sec, and a final extension step of 10 min at 72°C.The amplified products were electrophoresed on 2% agarose gel stained with ethidium bromide. The genotypes were determined based on the band pattern.

Statistical analysis

The detailed statistical analysis was carried out to compute percentages, mean ± SD, Chi- square test of significance to compare the allele and genotype frequencies between patients and control subjects based on different variables. The risk associated with genotypes was calculated using SSCP18 version, online odds ratio calculator and logistic regression analysis with 95 % confidence interval (CI). Statistical significance was defined as a two-tailed p-value <0.05.

Gene	Exon	Primer name	Sequence	Product size
185delAG (BRCA1)	2	1B185 CF(P1) 1B185 WR(P2) 1B185MR(P3)	5'ggttggcagcaatatgtgaa3' 5'gctgacttaccagatgggactctc3' 5'cccaaattaatacactcttgtcgtgacttaccagatgggacag ta3'	335 bp
5382insC *(BRCA1)*	11	1B5382 CR(P4) 1B5382 WF(P5) 1B5382 MF(P6)	5'-gacgggaatccaaattacacag-3' 5'-aaagcgagcaagagaatcgca-3' 5'aatcgaagaaaccaccaaagtccttagcgag caagagaatcacc-3'	271bp 295bp
6174del T (BRCA2)	11	2B6174 WF(P7) 2B6174 MF(P8) 2B6174 CR (P9)	5' ctcttgagacctttgcttttacgttt 3' 5' ctcttgagacctttgcttttacgttt 3' 5' gtcaaaatccatagaaccatacaaagg 3'	393bp

Table 1. Primers used for detection of founder mutations. 1B-BRCA1,2B-BRCA2,CF- Common Forward; WR- Wild Reverse; MR- Mutant Reverse; CR- Common Reverse; WF- Wild Forward; MF- Mutant Forward, P1-P9 are primer code

RESULTS

Demographic information of patients and controls was presented in Table 2, wherein a statistically significant association of demographic variables was observed with respect to age (p=0.048), menopausal status (p=0.035), and on the other hand, no such significant association was observed with consanguinity, diet intake. The subjects were classified into two groups based on age i.e. ≤40yrs and. >40yrs. A total of 31% of the patients were below or equal to 40yrs of age and the remaining 69% belong to more than 40yr of age indicating the late onset of the disease.

Variables	Controls/Patients N=100/ N=100	Odd's Ratio	95% CI	p- Value
Age (yrs.)				
≤ 40	31/18	2.047	(1.054-3.973)	**0.048***
> 40	69/82			
Menopause Status				
Pre	41/26	1.978	(1.087-3.599)	**0.035***
Post	59/74			
Consanguinity				
+	17/23	0.862	(0.424-1.751)	0.817
-	83/77			
Diet Intake				
Veg	19/16	1.231	(0.592-2.561)	0.709
Non-Veg	81/84			
*p ≤ 0.05, **p ≤ 0.01				

Table 2. Demographic details of control and ovarian cancer groups

Cases with >40yrs of age revealed around two-fold increased the risk for developing OC when compared to≤40yrs. Of the total patients, 26 % were premenopausal women, and 74 % were postmenopausal with a mean age of 24±7 and 56±8yrs, respectively. The fluctuation in the levels of progesterone in postmenopausal women may lead to increased risk for the disease. In the present study, high parental consanguinity was observed in the patients (23%) compared to controls (17%) suggesting the role of common and/or rare recessive genes leading to homozygosity of mutant alleles.

Table 3 gives clinical and histological variables within patient group. An interesting observation is that the women with early menarche (< age of 12yrs) are at high risk for the disease which strengthens the role of estrogen in women at early menarche. OC patients were sub-categorized based on the cell type and found 85% of epithelial cell type, 4% germline, 3% sex cord stromal and 8% unclassified. Positive family history was observed in 32% of cases whereas 68% belong to sporadic type, indicating a strong genetic basis for the occurrence of OC. Patients were categorized based on the parity and found 4% of them with nulliparous and the remaining 96% parous women.

Table 4 represents the genotype and allele frequencies of 185delAG, 5382insC of BRCA1 and 6174delT of BRCA2 genes in control and OC cases, wherein 185delAG showed 95% of WW, 4% of WM and 1% MM in controls while 52 % were WW, 36% of WM and 12% MM in OC cases respectively. A statistically significant increased frequency of homozygotic mutant (MM) and heterozygotic (WM) mutant genotypes were found in patients compared to controls. There was a statistically significant difference in the distribution of allele frequencies in cases and controls (W v/s M: $\chi 2$ p<0.0001, OR 18.06, 95 % CI 6.31-51.65), with an eighteen-fold increased the risk for ovarian cancer.

Variables	Patients (n=100)	%
Mean Age (Years ± SD)	44.0 ± 12.05	
Mean Age at Menarche (Years ± SD)	12.2 ± 1.61	
FIGO Stage		
I	8	08
II	14	14
III	60	60
IV	18	18
Histological Cell Type		
Epithelial	85	85
Germ Cell	04	04
Sex Cord Stromal	03	03
Unknown/ Unclassified	08	08
Family History of Ovarian Cancer		
+	32	32
-	68	68
No. of Full-term Births		
Nulliparous	04	04
1-3	34	34
≥ 4	60	60
Unknown	02	02

Table 3. Clinical and histological variables within OC patient group

Genotype	Controls N=100 (%)	OC n=100 (%)	Odds Ratio	95% CI	p-Value
185delG					
WW	95 (95)	52(52)			
WM	04 (4)	36(36)	16.4	5.54-48.7	< 0.001**
MM	01 (1)	12(12)	21.9	2.77-173.3	<0.0001**
Alleles					
W	194(0.98)	140(0.7)	18.05	6.31-51.6	<0.0001**
M	06 (0.03)	60 (0.3)			
5382insC					
WW	95 (95)	61 (61)			
WM	03 (3)	32 (32)	16.61	4.58-71.3	< 0.001**
MM	02 (2)	07 (7)	5.451	0.99-39.3	0.034*
Alleles					
W	193(0.96)	154(0.77)	8.236	3.41-15.6	< 0.001**
M	07 (0.04)	46 (0.23)			
6174delT					
WW	92 (92)	54 (54)			
WM	06 (3)	37 (37)	10.506	3.91-29.7	< 0.001**
MM	02 (2)	09 (9)	7.667	1.46-53.5	0.007**
Alleles					
W	190(0.95)	145(0.72)	7.207	3.41-15.6	< 0.001**
M	10 (0.05)	55 (0.28)			

Table 4. Genotype and allele frequencies of BRCA1 (185delAG and 5382insC) and BRCA2 (6174delT) in controls and ovarian cancer group. **p <0.01is significant WW-homozygous wild, WM-heterozygous, MM-homozygous mutant genotypes

The distribution of 5382ins C of BRCA1 gene in control subjects and OC groups revealed 95% WW, 3%WM and 2% MM in controls and 61 % of WW, 32% of WM and 7%MM in OC cases. An increased frequency of homozygotes (MM) and heterozygotic (WM) mutant genotypes was found in patients compared to controls with a statistically significant difference in the distribution of allele and genotype frequencies (W v/s M: $\chi 2$ P<0.0001,OR 8.23, 95 % CI 3.45-20.58) respectively. The frequency of 5382ins C of BRCA1 showed eight fold increased risk with OC.

Parameter	WW (%)	WM (%)	MM (%)	χ2 (p-Value)	W(%)	M(%)	χ2 (p-Value)
AGE		185delAG					
≤40 (n=18)	10 (55.5)	06 (33.3)	02 (11.2)		0.72	0.28	0.09(0.75)
>40 (n=82)	42 (51.2)	30 (36.6)	10 (12.2)	0.11 (0.94)	(0.69)	(0.31)	
OR (95% CI),	Ref (1)	1.19(0.39-3.63)	1.19(0.22-6.30)				
		5382 insC					
≤40 (n=18)	12 (66.7)	05 (27.7)	01 (05.6)	0.30 (0.85)	0.80	0.20	0.26(0.60)
>40 (n=82)	49 (59.7)	27 (33)	06 (07.3)		(0.76)	(0.24)	
OR (95% CI)	Ref (1)	1.32(0.42-4.15)	1.46(0.16-13.3)				
		6174del T					
≤40 (n=18)	07 (38.9)	09 (50)	02 (11.1)	2.04 (0.36)	0.63	0.37	2.31(0.12)
>40 (n=82)	47 (57.3)	28 (34.2)	07 (8)		(0.74)	(0.26)	
OR (95% CI)	**Ref (1)**	**0.46(0.15-1.38)**	**0.52(0.08-3.03)**				
STAGE							
		185delAG					
T1+T2(n=22)	10 (45.5)	09 (40.9)	03 (13.6)	0.56 (0.75)	0.66	0.34	1.12(0.29)
T3+T4(n=78)	41 (52.6)	30 (38.5)	07 (8.9)		(0.72)	(0.28)	
OR (95% CI),	Ref (1)	0.81(0.29-2.24)	0.56(0.12-2.59)				
		5382 ins C					
T1+T2(n=22)	11 (50)	08 (36.4)	03 (13.6)	5.05 (0.07)	0.5	0.5	17.1(<0.001)**
T3+T4(n=78)	50 (64.1)	24 (30.8)	04 (5.1)		(0.79)	(0.21)	
OR (95% CI),	Ref (1)	0.66(0.23-1.85)	0.29(0.05-1.50)				
		6174del T					
T1+T2(n=22)	10 (45.5)	07 (31.8)	05 (22.7)	6.49 (0.03)	0.61	0.39	3.88(0.048)*
T3+T4(n=78)	44 (56.4)	30 (38.5)	04 (05.1)		(0.75)	(0.25)	
OR (95% CI),	Ref (1)	0.97(0.33-2.84)	0.18(0.04-0.80)				
DOD(Duration of Disease)							
		185delAG					
<6MONTHS(n=26)	17 (65.4)	07 (26.9)	02 (07.7)	2.55 (0.27)	0.79	0.21	3.61(0.05) *
>6MONTHS(n=74)	35 (47.3)	29 (39.2)	10 (13.5)		(0.66)	(0.34)	
OR (95% CI),	Ref (1)	2.01(0.73-5.51)	2.42(0.47-12.3)				
		5382 ins C					
<6MONTHS(n=26)	16 (61.5)	06 (23.1)	04 (15.4)	4.70 (0.09)	0.73	0.27	1.48(0.22)
>6MONTHS(n=74)	45 (60.8)	26 (08.1)	03 (04.1)		(0.64)	(0.36)	
OR (95% CI),	Ref (1)	1.54(0.53-4.42)	0.26(0.05-1.32)				
		6174del T					
<6MONTHS(n=26)	17 (65.3)	05 (19.3)	04 (15.4)	2.95 (0.22)	0.73	0.27	0 (>0.99)
>6MONTHS(n=74)	41 (55.5)	28 (37.9)	05 (06.6)		(0.74)	(0.26)	
OR (95% CI),	Ref (1)	2.32(0.83-6.75)	0.51(0.12—2.16)				
Menopause status							
		185delAG					
PRE (n=26)	17 (65.4)	06 (23.1)	03 (11.5)	2.93 (0.23)	0.76	0.24	0.63 (0.42)
POST (n=74)	37 (50)	31 (41.9)	06 (08.1)		(0.7)	(0.3)	
OR (95% CI),	Ref (1)	2.37(0.83-6.75)	0.91(0.20-4.11)				

		5382 ins C					
PRE (26)	20 (76.9)	04 (15.4)	02 (07.7)	4.51 (0.10)	0.84	0.16	2.44(0.11)
POST (74)	41 (55.4)	28 (37.8)	05 (06.8)		(0.74)	(0.26)	
OR (95% CI),	Ref (1)	3.41(1.05-11.0)	1.22(0.21-6.84)				
		6174del T					
PRE (26)	13 (50)	11 (42.3)	02 (07.7)	0.81 (0.66)	0.71	0.29	0.02(0.87)
POST (74)	38 (51.4)	26 (35.1)	10 (13.5)		(0.69)	(0.31)	
OR (95% CI),	Ref (1)	0.80(0.31-2.08)	1.71(0.33-8.84)				

Table 5. Genotype and allele distribution with respect to demographic details within OC group. *p<0.05, **p<0.01is significant WW-homozygous wild, WM-heterozygous, MM-homozygous mutant genotypes

The distribution of 6174del T mutation of BRCA2 gene in control and ovarian cancer patients showed 92% of WW, 6% of WM and 2% of MM in controls and 54 % of WW, 37% of WM and 9% of MM in cases. There was a statistically significant difference in the distribution of genotype and allele frequencies in cases compared to controls (W v/s M: $\chi 2$ P<0.0001,OR 7.20, 95 % CI 3.41-15.63), indicating an increased frequency of homozygote (MM) and heterozygote (WM) mutant genotypes in patients group compared to controls, with a seven-fold increased risk with OC.

Further, genotype and allele frequencies are compared with respect to demographic variables within the OC group and presented in Table5. Age wise comparison revealed no significant difference with respect to BRCA1 and BRCA2 founder mutations in patients less than or equal to 40yrs of age compared to patients more than 40yrs. A statistically significant increased frequency of 5382insC of BRCA1 and 6174del T of BRCA2 was observed in T1+T2 cases compared to T3 +T4 OC cases. Patients were further categorized based on the duration of the disease [Table 5] and found increased frequency of 185del AG in cases with more than 6 months duration compared to less than or equal to 6 months and no significant difference was observed with respect to 5382insC of BRCA1 and 6174del T of BRCA2.

DISCUSSION

Several studies based on the frequency estimates of BRCA1/2 mutations revealed a correlation between susceptibility and/or development of cancer. The relative contribution of each of the mutations may be the result of their relative frequency in the general population or differences in their mode of expression and penetrance. Mutations near the 5'end of the coding region are thought to confer a higher risk than mutations near the 3' end. The 185delAG mutation is located near the 5' end, and the 5382insC mutation is located near the 3' end of the BRCA1 coding region. The 6174delT of BRCA2 mutation is located in the ovarian cancer cluster region (OCCR) in BRCA2 gene with truncating mutations which play a crucial role in the causation of breast and ovarian cancer. Gayther et al (1997) reported a correlation between the location of a truncating mutation in the BRCA1 coding region and the chances of developing breast and/or ovarian cancer using data from 60 families and reported that mutations in exon 11 of BRCA2 were associated with a higher risk of OC than mutations outside the OCCR region [13].

The present study showed homozygous mutations of 12%of 185delAG (12/100) and 7% of 5382ins C (7/100) of BRCA1 whereas 9% of 6174del T of BRCA2. Other ethnic groups also revealed high frequencies of founder mutations in breast and ovarian cancer patients. Moslehi et al (2000) reported 86 founder mutations in Jewish women including 57 in BRCA1 (43 in 185delAG and 14 in 5382insC) and 29 in BRCA2(6174delT) [14]. In Finland, the frequency of BRCA1 (4.7%) and BRCA2 (0.9%) are more similar to those reported in the UK and US [15,16]. In 233 Finnish ovarian carcinoma patients 13 (5.6%) germline mutations of BRCA1/2 were detected [17]. In Netherlands large genomic deletions were identified in BRCA1 [18]. In Canadian ovarian carcinoma patients, 26 distinct BRCA1 and 19 BRCA2 mutations were detected with the frequency of 7.6% (39/515) and 4.1% (21/515), respectively [6]. In Icelandic, one BRCA2 founder mutation was present in 7.9% (3/38) of ovarian carcinomas,[19] and in Hungary, three BRCA1 founder mutations accounted for 11.1% (10/90)[20] In UK, 3.5% (13/374) of ovarian cancer patients were found to be mutation-positive in the screening for BRCA1 mutations[21]. Dvorah et al.(1997) reported 61.9% of ovarian cancer patients, with either 185delAG or 6174delT mutations and no mutations with 5382insC [22].

In Indian scenario, contradictory results were observed in various studies, which could be due to ethnic variation. A study was carried out by Saxena et.al on the entire gene and found lowered prevalence of these gene mutations compared to other Asian countries [23], but is similar to that reported by Suter et.al (2004) from Shanghai [24]. Previous studies have shown a higher frequency of BRCA1 and BRCA2 mutations in South Indian population compared to north and eastern states. A study conducted by Vaidyanathan et al [25] found 185delAG at a very high frequency of 16.4% (10/61), it occurs at a high frequency of 18% among breast/ovarian cancer families of Ashkenazi Jews [26,27].In BRCA2 gene, two mutations were identified, and the frequency was 3.28% (2/61). In families with the history of breast and ovarian cancer, about 40% families with ovarian cancer and 23.7% of breast cancer families shown these mutations, while 30.8% of families with both breast and ovarian cancers, suggesting the association of these mutations with OC [24]. However, An Eastern Indian study comprising 130 familial and 101 sporadic breast cancer cases revealed no association with 185delAG of BRCA1and 6174delT of BRCA2 mutations.

Thus, the present study contributes to understand the importance of BRCA1 and BRCA2 founder mutations in ovarian cancer from south Indian cohort. The study revealed a statistically significant association between founder mutations and ovarian cancer, which may be considered as the biomarkers in the prognostic evaluation of ovarian cancer. However, studies on a larger sample size are required to confirm the results obtained.

CONCLUSIONS

The present study revealed a significant association of BRCA1 and BRCA2 founder mutations in the etiology of ovarian cancer in south Indian cohort. The identification of BRCA1 and BRCA2 mutation carriers are important in the clinical management of ovarian cancer in families with breast and/or ovarian cancer. Currently, only women with a family history of ovarian and/or breast cancer are offered genetic testing. Since the mutations are also prevalent in sporadic cases, it is mandatory for genetic diagnosis for better management and therapeutic intervention and personalized therapies.

CONFLICT OF INTEREST

The author declares no competing interests.

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