Screening of BRCA 1 - 185delAG mutation in Ovarian Cancer patients in a Tertiary care centre from Telangana

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Abstract

Aim: Ovarian cancer (OC) occurs due to genetic alterations and mutations in BRCA gene. The aim of the study is to assess the frequency of genetic alterations that persist in Ashkenazi founder mutation BRCA1, 185delAG in patients with ovarian cancer from South Indian origin.

Materials and Methods: A total of 100 ovarian cancer patients and an equal number of control subjects were included in the present study. Screening of 185delAG mutation BRCA1 gene was carried out by ARMS PCR followed by agarose gel electrophoresis. Statistical analysis was applied to test for the significance of the results obtained.

Results: The genotype distribution of WW, WM, MM showed a significant difference between the two subjects, 95%, 4% and 1% in controls and 52%, 36% and 12% in cases respectively. An increased frequency of homozygotic mutant genotypes (MM) were found in patients compared to controls. Similarly, a significant difference in the distribution of M allele in cases and control subjects (W v/s M: χ² P<0.0001, OR 18.06, 95% CI 6.31-51.65) was observed.

Conclusion: The demographic details of the patients and controls revealed that females of age greater than 40 years are associated with high risk of ovarian cancer. The postmenopausal women have a very high susceptibility to OC (6.5 times riskier). Therefore, 185delAG mutation BRCA1 has a possible association in the etiology of ovarian cancer.

Keywords: BRCA1; 185delAG mutation; Ovarian Cancer

Introduction

Ovarian cancer (OC) is the most common forms of hereditary cancer in women and is the leading cause of death by gynecological malignancy. It has a hostile phenotype and a relatively poor prognosis. More than two thirds of patients were showing late stage disease [1,2]. It is predominantly a disease of postmenopausal women and has an overall 5-year survival of less than 30% [3]. Earlier studies indicate that hereditary cancers constitute 5%-10% and may be up to 14% [4,5] epidemiologic and molecular genetics analyses indicate that about 10% of all epithelial ovarian carcinomas are associated with autosomal dominant genetic predisposition, conferred primarily by inherited mutations in BRCA1 or BRCA2, they account for >90% of hereditary cancers. In general population the frequency ranges between 2-12% and 2-6% respectively [6]. The cumulative life risk of developing epithelial ovarian carcinoma ranges from 20%-30% [7]. BRCA1 cloned in 1994 and 2 in 1995 both are very large genes most known mutations lead to premature termination of protein, leading to loss of tumor suppression [8]. Mutations in BRCA1 are present in approximately one-half of the early-onset breast cancer families and 80% of the early-onset breast and ovarian cancer families [9] whereas BRCA2 mutations are believed to account for a comparable percentage of inherited breast cancer cases [10]. Both the genes are detrimental and acts as a sensor for DNA damage [11,12] hence resulting in defects in DNA repair, transcription, abnormal centrosome regulation, impaired spindle checkpoint and chromosome damage. In view of the above the present study is aimed to evaluate the role of the founder mutation in the etiology of ovarian cancer.

Materials and Methods

Study population

A total of 200 individuals were included in the present study. 100 clinically and histopathologically confirmed ovarian cancer patients from Yashoda Hospital, Secunderabad during the years 2012-14 and an equal number of age matched healthy control subjects were also incorporated for comparative studies. A structured proforma was used to seek information on dietary habits, smoking, alcohol consumption, family history etc. Written informed consent was obtained from all the subjects. The study was also approved by our Institutional Ethical committee.

DNA isolation

Five ml of venous blood was drawn from each individual in EDTA vacutainers. Genomic DNA was isolated from whole blood using the salting out method [13].

Mutation analysis of 185delAG

The mutation of 185delAG was analyzed based on Amplification Refractory Mutation System-Polymerase Chain Reaction (ARMS-PCR). The BRCA1 185delAG exon 2 primers are as follows: forward primer, 5’- GGTGGCCAGCATATGTGGAA-3’ (P1), Wild-type reverse 5’-GCTGACTTACCGATGGGACTCTC-3’ (P2) and Mutant

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The age of incidence of ovarian cancer seems to be population specific. Our ethnic group includes 100 OC patients of which 18% (≤ 40 years of age) and 82% (>40 years of age). Patients and control subjects have been represented on various variables like Age, Menopause status, Consanguinity, Diet Intake and Marital Status.

**Results**

The study population consist of 100 Ovarian cancer patients, comprising 32 familial and 68 sporadic cases including 100 age-matched controls were screened for the presence of 185delAG of BRCA1 mutation. Data related to age, marital status, consanguinity, menopause status, familial history, taken into consideration and (Table 1) derived from the same geographic location and are representative of South Indian population from Telangana. There was a significant difference in allele distribution between patients and control subjects (W v/s M: χ² P<0.0001, OR 18.06, 95% CI 6.31-51.65).

The demographic features of ovarian cancer patients and controls subjects have been represented on various variables like Age, Menopause Status, Consanguinity, Diet Intake and Marital Status.

The age of incidence of ovarian cancer seems to be population specific. Our ethnic group includes 100 OC patients of which 18% (≤ 40 years of age) and 82% (>40 years of age). Patients and control subjects were derived from the same ethnic group. A significant association of ovarian cancer patients with age (p<0.000), menopausal status (p<0.000), consanguinity (0.043) and marital status (p=0.022). On the other hand, there was no significant association with diet intake. Histopathologically, staging plays a major role, the ovarian tumors falls under major categories (Scully, 1987; Serov, 1973); epithelial, germ cell and sex cord-stromal cell tumors. According to our study, Epithelial (85%); Germ cell (04%); Sex cord-stromal (03%) and Unknown/ unclassified (08%). When taking family history into consideration, the study reflected over 32% are of familial cases and 68% are sporadic cases.

**Mutation frequency (185delag) based on family history**

Figure 1 shows the mutation-detection rate was dependent on different Cancer Case History that shows: Breast & Ovarian Cancer 7/9 (77.7%); Only Ovarian Cancer 3/4 (75%); History of Other Cancer 10/19 (52.6%); Sporadic Cases 31/68 (45.50%).

**Frequency of mutations in cases by age**

Age has been considered as one of the potential factors for ovarian cancer as the risk increases with the advancement of age and the survival rate decreases accordingly. The average age at diagnosis of ovarian cancer was 52.5 years and the average age of menarche is 12.2 years, respectively. This graph is obtained in accordance with the age specific incidences of Ovarian cancer in south-Indian population. The age of the patients have been categorized into 4 groups ≤ 30 yrs (4), 31-40 yrs (14), 41-50 yrs (29) and ≥ 50 age group (53). However, the overall incidences in different groups were inconsistent and showed peak in ≥ 50 age group (Figure 2). Table 2 shows the distribution of 185delAG mutation of BRCA1 gene in control subjects and patients with ovarian cancer.
to cancer by inactivating BRCA1 ubiquitin protein ligase activity [23].

In our present study, an increased frequency of homzygotic mutant genotype (MM) was found and a statistical significant association was noticed in the distribution of 185delAG in patients compared to control subjects. Further studies are required based on mutational analysis for identification of related mutations and polymorphisms. The biological effects of the protein related to polymorphisms can be understood which will help in predicting the etiology of ovarian cancer in different geographical regions. Proper counselling of patients and pre-symptomatic mutation carriers will help them make better decisions about medical and surgical preventive options.

**Conclusion**

From this study, we found that the prevalence of 185delAG of BRCA1 gene have shown some similarities and difference when compared with other populations. This study also emphasizes the importance of demographic details which play major role in mutation screening. Patients who are at a high risk of early-onset disease, with appropriate awareness i.e. genetic counselling, counselling should include a discussion of the basic principles of hereditary cancer susceptibility as well as an evaluation of the woman’s own risk of cancer, and how testing would add to the characterization of those risks, and how medical management would be affected by a positive and a negative test result so that patients and carriers would be in safe zone.

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**References**


