ABSTRACT

World wide, Breast Cancer is the most common cancer in women and comprises 18% of all female malignancies with a life time risk of 10% in general population. Breast cancer occurs in a more common sporadic form and a less common hereditary form. Inheritance of germline mutation(s) in two cancer susceptibility gene(s) designated as BRCA1 and BRCA2 accounts for a significant proportion of all familial breast and ovarian cancers. BRCA2 is the second major breast cancer susceptibility gene and individuals who carry germline mutations in BRCA2 gene have increased risk of breast cancer in both women and men. Despite the large number of BRCA2 gene mutations reported worldwide, the contribution of this gene to the development of breast/ovarian cancer as well as the clinical and pathological characteristics and prognostic outcome of patients with BRCA2 mutations remains completely unexplored among Kerala population. Hence the present study was undertaken at the Regional cancer Centre, Thiruvananthapuram, Kerala, during 2001-2006 period, to identify the genetic heterogeneity and prevalence of germline BRCA2 gene mutations in 102 breast/ovarian cancer patients from Hereditary Breast Ovarian Cancer (HBOC) families, 72 early age onset sporadic breast cancer patients and 90 age matched controls and to correlate the mutation status with clinical characteristics and overall survival. Peripheral blood from the study subjects were collected in ACD solution, after getting written informed consent and DNA was extracted. All the 26 coding exons of BRCA2 gene were PCR amplified and analyzed for mutations employing Conformation Sensitive Gel Electrophoresis and characterized by DNA sequencing. The associations between the BRCA2 mutation status and clinicopathological characteristics of breast/ovarian cancer patients were examined by unconditional logistic regression analyses to calculate the Odds Ratios (ORs) and 95% Confidence Intervals (CIs). Survival curves were generated according to the method of Kaplan-Meier using Log Rank test and Cox proportional hazard regression method.

Mutation analysis of BRCA2 gene in 102 HBOC patients showed sequence variations in 31.3% of patients. A total of 19 distinct germline BRCA2 sequence variants were detected in these patients, including two frame shifts (c.4642delAA and c.4926insGACC), nine missense, one nonsense, four silent mutations and three intronic variants. Twelve distinct pathogenic mutations (two frame shifts, one
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nonsense and nine missense) directly leading to an altered BRCA2 protein were identified in 18.62% (19/102) patients from 18 HBOC families. 52.63% (10/19) of sequence variants identified in the present study have not been previously reported elsewhere, and hence could be considered as novel. Interestingly, 68.4% of the variants identified were detected in exon 11 of the BRCA2 gene. In sporadic breast cancer patients, BRCA2 sequence variants were detected only in 6.94% of patients. Apart from these alterations, two common polymorphisms in exon 10 of the BRCA2 gene (N289H and S455S) were also identified in the present study with either one of the polymorphisms present in 11.8%, 27.8% and 13.33% of HBOC, sporadic and control subjects respectively. A significant association of BRCA2 gene polymorphisms with sporadic breast cancer susceptibility risk (OR=2.5; 95% CI=1.1257-5.547, P= 0.02) was observed.

No statistically significant correlation was observed for clinicopathologic characteristics and mutation status of the gene. In survival analysis, BRCA2 gene mutation status (P=0.02) and various clinicopathologic parameters such as tumour size (P = 0.01), metastasis (P=0.01), disease stage (P=0.03) and laterality (P=0.02) were significantly associated with poor prognosis of HBOC cases. In the case of sporadic breast cancer patients, only tumour size (P = 0.03), metastasis (P=0.02) and disease stage (P=0.01) were found to be significantly associated with poor prognosis. No significant difference in overall survival was noted between the HBOC and sporadic breast cancer cases.

In conclusion, BRCA2 germline mutation analysis of Hereditary Breast Ovarian Cancer (HBOC) patients showed mutation frequency rate of 18.62%, with a unique pool of novel BRCA2 mutations in Kerala population. Hereditary Breast Ovarian Cancer patients who were inferred carriers of BRCA2 mutations seemed to have a worse prognostic outcome than the non mutated group. Genetic screening for mutations of BRCA2 gene in patients from HBOC families may help to advance our understanding of the mutation spectra, tumour biology of these cancers and to develop adequate screening programmes for high risk young women in such HBOC families. Ultimately, comprehensive genetic testing for breast cancer risk, offered as part of routine clinical practice, will translate into breast cancer prevention at the population level.