

A PILOT STUDY ON SCREENING FOR 185 DeLAG MUTATION IN BREAST CANCER PATIENTS IN A TERTIARY CARE CENTER FROM SOUTH INDIA

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ABSTRACT

Background and Objective: The incidence of breast cancer in India is on the rise and is rapidly becoming the number one cancer in females pushing the cervical cancer to the second spot. In India an average of 80,000 women are diagnosed with carcinoma of the breast, and 40,000 women die of this disease every year. Most of the predisposition to hereditary breast and ovarian cancer has been attributed to inherited defects in two tumor suppressor genes BRCA1 and BRCA2. The 185delAG mutation in the BRCA1 gene is a founder Jewish Ashkenazi mutation that is carried by 1% of this population and has been reported in Indian studies also. The present study was initiated to assess the presence of the common Ashkenazi founder BRCA1 mutation, 185delAG in Indian breast cancer patients.

Methodology: Clinical information and peripheral blood leucocytes were obtained from 15 breast cancer patients

with a positive family history, and 10 patients with sporadic breast cancer and age group matched control subjects. Mutational analysis of BRCA1 exon 2 for 185DEL_{AG} was carried out by polymerase chain reaction using specific primers followed by restriction digestion with Taq1.

Results: PCR products of 200 bp were obtained from the control sample as expected. Similar results were obtained with the DNA of breast cancer subjects. Molecular analysis revealed that the 185delAG mutation was not found in any of the samples analyzed. Statistical evaluation was not possible due to small sample size and absence of mutation.

Conclusion: The sample size and the less number of affected individuals in the family of the patients, which may perhaps explain the absence of the mutation in the samples.

Key words: Mutation, BRCA1 gene, Breast cancer, 185DelAG mutation.

SRJM 2010;3:9-11

INTRODUCTION

Breast cancer is a cancer of the glandular breast tissue. It has been reported as the most prevalent malignancy and primary cause of cancer death in women worldwide accounts for 14.1% of female cancer deaths and is the second most common cancer overall when both sexes are considered together. Most alarmingly, incidence rates have continued to increase worldwide, with an overall annual increase of approximately 0.5% since 1990. However, changes in incidence rates are high in developing countries, attaining annual increase of 3-4%.^[1] In India an average of 80,000 women are diagnosed with carcinoma of the breast, and 40,000 women die of this disease every year and it is the second most common cancer among Indian women (19%) after cervical cancer (30%).^[2]

Most inherited cases of breast cancer have been shown to be associated with two genes: BRCA1 and BRCA2, breast cancer gene one and breast cancer gene.^[2] The mutation 185delAG has been coined as the "Ashkenazi Mutation" because it was predominantly detected among Ashkenazi Jews.^[3] It is estimated to be present in 1% of the Ashkenazi Jewish Population. Though, association of 185delAG in the BRCA1 gene with breast cancer has also been found in Jews it has been reported in people with non-Jewish origins.

Haplotype analysis has shown that there is likely a single origin for this mutation since all tested Ashkenazi mutation carriers displayed the same allelic pattern.^[4] A study conducted showed that BRCA1 gene is a strong candidate for breast and ovarian cancer.^[5] Certain frequently mutated exons were analysed in sporadic and familial breast cancer patients from India, six patients (25%) with BRCA1 mutation were identified.^[6] Investigations on the genetic polymorphisms of BRCA1 and BRCA2 gene in a cohort of 204 Indian breast cancer patients, deleterious frame shift mutation 185DelAG mutation was detected.^[7] A study conducted in breast and ovarian cancer families and sporadic breast cancer patients of Indian origin revealed 185DelAG mutation in patients with family history of breast cancer and no mutation was found in sporadic cases.^[8] Analysis of PCR amplicons in members of a family with multiple cases of breast cancer in the BRCA1 gene revealed deletion of AG nucleotide at the 185th position, haplotype analysis suggested an independent origin for this mutation.^[2] Thus the presence of 185DelAG mutation in Indian population has been proved by other studies. Hence in the present study the breast cancer patients were screened for 185DelAG mutation so that the frequency of 185DelAG mutation may be estimated in breast cancer patients of south India.

MATERIALS AND METHODS:

A formalized informed consent form was obtained from the subjects to proceed with the sampling. About 3-4ml of peripheral blood was collected from 15 breast cancer patients with a positive family history, and 10 patients with sporadic breast cancer and 6 age group matched control subjects by veinpuncture in K₂-EDTA vacutainers. The genomic DNA isolated from the peripheral blood was subjected to *in vitro* amplification using PCR reaction. Standardization of the PCR program was done, a gradient PCR was performed between

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the temperature 50°C-68°C and a positive band was observed at temperature 52°C. All the samples were then subjected to PCR amplification using specific primers.^[9]

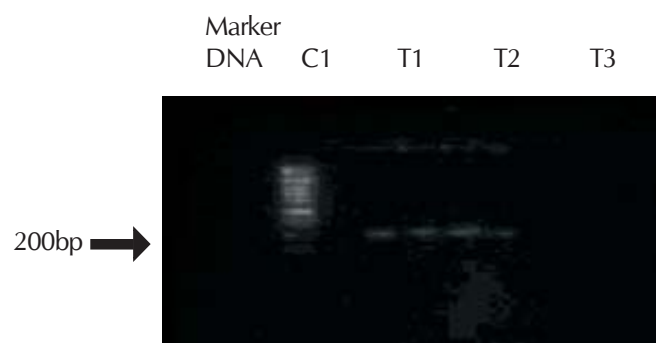
Forward primer F1 – 5' TT AA TG CT AT GC AG AA AA TC TT CG 3'

Reverse Primer R1- 5' GT GG AT GG AG AA CA AG GA AT C 3'

PCR with primer set F1, R1 was performed with 35 cycles each cycle consisting of denaturation at 95°C for 1 minute, annealing at 52°C for 30 seconds and polymerization at 72°C for one minute. Prior to cycles initial denaturation at 95°C for 5 minutes and after completing the cycles final extension at 72°C for 7 minute was carried out. PCR products were obtained and the quality of the PCR amplicons was analyzed using 2% agarose gel electrophoresis. To detect the presence of mutation in exon 2 of BRCA1 gene all the samples were subjected to restriction digestion using *Taq1* enzyme.

RESULTS

The mutation analysis using PCR-RFLP was performed to detect the presence of 185delAG BRCA1 mutation 15 blood samples were obtained from familial breast cancer patients, 10 were obtained from sporadic breast cancer patients and 6 were obtained from age group matched controls. The breast cancer cases ages ranged from 30 to 50 years and they were from South Indian origin. High molecular weight genomic DNA was successfully isolated by salting out method from the blood samples. The quality of the DNA was checked by 0.8 % agarose gel electrophoresis. The quantity of the DNA was measured spectrophotometrically. The genomic DNA isolated from the peripheral blood was subjected to invitro amplification using PCR reaction. The quality of the PCR products were analysed using 2% agarose gel electrophoresis. To detect the presence of mutation in exon 2 of BRCA1 gene all the samples were subjected to restriction digestion using *Taq1* enzyme. In case of wild type *Taq1* cuts the 223 bp amplicon giving rise to two fragments of size 200 bp and 23 bp. The 185delAG allele is intact when treated with the restriction enzyme giving rise to fragment of size 223bp. All the samples on restriction digestion with *Taq1* gave rise to two fragments of size 200bp and 23bp. The bands



C1- Control Sample, T1,T2,T3- Breast cancer patient sample.

Figure 1: Agarose gel electrophoresis of PCR products subjected to restriction digestion by *Taq 1* enzyme of Control subjects and breast cancer patients

generated of the patient DNA were compared to that of the normal controls. The results obtained revealed that no mutation was present in any of the samples analyzed (Figure 1). The mutation was not detected in either group. Statistical evaluation was not possible as it is a pilot study. The results of our study demonstrated that the small sample size and the less number of affected individuals in the family of the patients, which may perhaps explain the absence of the mutation in the samples.

DISCUSSION

In this study, 25 Indian breast cancer patients comprising 15 familial cases and 10 sporadic cases including 6 age-matched controls were screened for the presence of 185delAG BRCA1 mutation using PCR-RFLP technique. The mutation was not detected in either group. The contribution of mutations in these two genes to breast cancer patients in the Indian population remains relatively unexplored apart from a few studies.^[10] BRCA1 as being responsible for approximately 50% of inherited breast cancer, 80 to 90% of hereditary breast-ovarian cancer and the majority of hereditary ovarian cancer.^[11] The 185delAG mutation is one of the common, ancient mutations; it is located at the 5' end of the gene and predicted to cause truncation at the beginning of the zinc-binding region of the RING of the putative polypeptide. It was proposed that mutations within the BRCA1 RING domain predispose to cancer by inactivating BRCA1 ubiquitin protein ligase activity.^[12] The mutation 185delAG has been coined as the "Ashkenazi Mutation" because it was predominantly detected among Ashkenazi Jews having attained a 1% carrier frequency within the population since origin of the ancestral mutation.^[4] In India, 185delAG has been reported in all populations studied. This deleterious frame shift mutation was first reported in a family residing in a part of Trivandrum not far from the small towns with settlement of Jewish people. It was later reported in two south Indian families from Kerala province, as well as two sisters from Goa, where a multi-ethnic population exists.^[14] Surprisingly, the mutation was also found in north Indian Hindu patient residing in New Delhi who claimed to have no Jewish ancestry.^[2] In addition to the clearly established founder effect for 185delAG, this mutation has been shown to have arisen independently at least twice, thus it would be interesting to evaluate the origin and population genetics of these disease susceptibility alleles in the Indian population through haplotype analysis.^[6] Considering the Indian history and taking into account the multiethnic, multi religion status and population structure of this country; and due to waves of immigrants and colonization in the past, it is highly conceivable that this ancient mutation would have migrated to India.

The presence and higher frequency of mutation in the previous studies may perhaps be due to the fact that those studies considered extremely high-risk families that included multiple affected individuals in the family. But in this study, the sample size and the number of affected individuals in the family of the patients were much smaller, which may perhaps explain the absence of mutation in the samples. The lack of 185delAG mutation in BRCA 1 among the breast

cancer patients may be due to mutation in the non-coding region and other coding regions of BRCA1 and the existence of other breast cancer susceptible genes namely BRCA2, p53 and PTEN. Rare mutations in a number of other genes, such as CHEK2, ATM, BRIP1, and PALB1 predispose to breast cancer^[15-18] as do more common variants in CASP8 and TGF β 1.^[19] The results also suggest that for statistically significant contribution of this mutation to the breast cancer risk there is a need for studies with larger sample size. In the long run, identification of BRCA1 mutations and other cancer susceptibility genes should permit the development of new and more effective therapies, so that physicians can not only predict future risks, but can reduce those risks reliably and safely before disease occurs.

ACKNOWLEDGEMENTS:

We would like to thank Dr. P. Venkatachalam, Professor, SRU, Department of Human Genetics, for sparing his valuable time. I would like to thank Dr. V. Vetri Selvi and Dr. K. Vijayalakshmi Department of Human Genetics, SRU for helping me with molecular techniques. I also thank Dr. LVKS.Bhaskar Assistant Professor Department of Biomedical science for his continuous support, inputs and suggestions, his timely help will never be forgotten.

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