ORIGINAL ARTICLE

GENETIC ANALYSIS OF FOUR SELECTED EXONS OF BRCA1 GENE IN DIFFERENT ETHNIC GROUPS IN PAKISTAN

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ABSTRACT

OBJECTIVE: To analyze the BRCA1 gene for mutations in patients with breast and ovarian cancer belonging to different ethnic groups of the Pakistan with the aim to spot out recurrent founder mutations.

STUDY DESIGN: Descriptive study,

PLACE AND DURATION: This study was conducted over a period of two years at the Centre for Research in Applied and Experimental Medicine, National University of Science and Technology (NUST), Rawalpindi.

METHODOLOGY: In this study mutational analysis of 4 major exons of BRCA1 gene was performed in 40 diagnosed cases of female breast and ovarian cancer belonging to various ethnic backgrounds. The genomic DNA was isolated from the blood samples. Primers of BRCA1 gene were designed and used for amplification of the region of interest. Results were recorded on BIORAD Gel Documentation System. Sequencing of the gene for variants was done using Automated DNA Sequencer. Interpretation of chromatograms was done using Bioinformatics software.

RESULTS: The study group comprised of familial cases of breast and ovarian cancer with diverse ethnic composition that included Punjabis n=40 (40%), Kashmiri n=20 (20%) and Pathans n=20 (20%), Balouchi n=10 (10%) and Muhajirs n=10 (10%). PCR amplified DNA from the samples revealed bands having both high quality and quantity of DNA. After extensively embarking on all four exons no functional sequence variant was detected in these chosen exons.

CONCLUSION: Our results suggest the involvement of other coding sequences of this gene apart from those assessed in our study group. This emphasizes the need for assessing the complete BRCA1 gene in all ethnic groups located in Pakistani territory.

KEY WORDS: BRCA1, Ethnicity, Pakistani Population

INTRODUCTION

Most of the hereditary breast and ovarian cancers result due to mutational disruption of different genes located on various human chromosomes ¹. Germline mutations in BRCA1 gene, located on chromosome 17, are the most significant and well characterized genetic risk factors so far identified for this disease ². Identification of recurrent mutations specific to isolated populations results due to the phenomenon of a founder effect and are more common in specific ethnic groups ³-⁴. These founder mutations in the genes may be the cause of existence of differences in genetic predisposition to cancer among different populations ⁵. Occurrence of founder mutations in BRCA1 gene has been described in several Western European populations ⁶. Ashkenazi Jewish, Norwegian, Dutch and Icelandic people have a higher rate of certain genetic alterations in BRCA1 ⁷. Pakistan, with highest frequency of breast and ovarian cancer ⁸ offers an excellent opportunity to study the role of ethnic diversity on the prevalence of mutations in BRCA1 gene. The Pakistani population shows a wide spectrum and variable frequency of mutations in BRCA1 gene as a cause of disease ⁹. Pakistani population is a blend of local and migratory haplogroups who have settled in its four provinces over the past many years. Present study is a small reflection of this diversity and was designed to determine the genetic differences between different Pakistani ethnic groups in reference to BRCA1 mutations with an aim of finding founder mutations. Four most prevalent exons of the BRCA1 gene Exon 2, exon 10, exon 11 and exon 13 of were selected for this study, keeping in view the fact that mutations have previously been reported in exons 2, 10 and 11 ⁸, ¹⁰, while exon 13 was added to increase the probability of finding mutations. This will help health care providers to determine the most appropriate preventive and therapeutic strategy targeted for specific Pakistani ethnic groups, hence saving precious time and money.

METHODOLOGY

This is a descriptive study (case study type) approved by the Institution Ethical Committee (IEC) of NUST, conducted over a period of two years. Forty female patients with breast and
ovarian cancer, who presented at the outpatient department (OPD) or were receiving treatment (radiation or chemotherapy) from the allied hospitals, were included in this study after informed consent. Patients without a biopsy report confirming the presence of the cancers, patients not belonging to the under study ethnic groups and foreign patients were excluded. Patients were divided into two equal groups, group A having breast cancer patients and group B having ovarian cancer patients. Questionnaire containing information about the personal, family and disease history was filled.

The study included women from diverse ethnic backgrounds the ethnic roots of each candidate were established in detail and percentage distribution of each ethnicity was determined. Figure - I shows the ethnic distribution of the study group in which n=40 subjects (40%) were Punjabis, n=20 subjects (20%) Kashmiris, n=20 subjects (20%) Pathans, n=10 subjects (10%) Balouchis and n=10 subjects (10%) Muhajirs. This inclusion was necessary in order to detect mutations, especially founder effects, from a specific ethnic group.

The forward and reverse oligonucleotide primer sequences were designed for the respective exons of BRCA1 gene according to their base pair amplification lengths to carry out Polymerase Chain Reaction (PCR) and DNA sequencing. The annealing temperatures for the respective exons were set (Table - I). Five milliliter (ml) venous blood was collected from the selected patients. For extraction of DNA, phenol extraction method was used. The quantitative and qualitative analysis of both the extracted and diluted DNA was checked by horizontal gel electrophoresis in the wells of 50 and 200 ml horizontal tank on 1 percent Agarose gel. The optical density of the extracted DNA was checked with the help of Spectrophotometer and visualization of DNA was done with UV transilluminator. BIORAD Gel Documentation System Data of Quantity One Software was used to analyze and save the results.

The selected exons and their intron-exon boundaries were amplified by oligonucleotide primer sets using the conventional PCR machine with Palmycycler software. Amplified PCR products were analyzed on 2% Agarose gel and results recorded. After purification of these products they were subjected to cycle sequencing using GenomeLab Dye Terminator Cycle Sequencing (DTCS) Quick Start Kit. The samples were sequenced using capillary array and gel cartilage through Automated Genetic Analyzer.

The BRCA1 gene was then assessed by doing mutational analysis for the selected exons using DNA of all the probands. Genome Browser database was used to obtain full length control gene sequence. This was used as a comparison with the obtained sequencing chromatograms to identify any mismatch sequence variant. Genomic and cDNA sequence were counter checked and biological software was utilized to do alignment and mutational analysis.

RESULTS

In present study, four exons (2, 10, 11 and 13) of the BRCA1 gene of forty unrelated probands were analysed by molecular genetic analysis. Visualization of the extracted DNA and further PCR amplification revealed bands having both high yield and quality of DNA. All coding region and exon-intron boundaries of the four exons were sequenced. Figure - II shows a chromatogram from an affected patient generated by sequencing of Ex 11 of BRCA1 gene by Automated Genetic Analyzer. The arrow indicates the position for insertion of nucleotide A at position 2041 which would result in 2041insA novel mutation of the Pakistani population. No genetic alteration or variation was detected in the representative chromatograms of the effected individuals from various ethnic backgrounds of the Pakistani population. Hence founder effects could not be established.

DISCUSSION

The discovery of BRCA1 gene led to an outflow of knowledge

<table>
<thead>
<tr>
<th>Exon No.</th>
<th>Primers (5'→3')</th>
<th>Amplification Length (bp)</th>
<th>Annealing Temp. (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forward primer</td>
<td>Reverse primer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E-2</td>
<td>GCGTCAGTAGTAACAGATGC</td>
<td>GGTCATTCTGTTCTTTGCG</td>
<td>450</td>
</tr>
<tr>
<td>E-10</td>
<td>GGGAACTAAACCAACCGGACG</td>
<td>AGTGCTAACTTCCAGTAACG</td>
<td>360</td>
</tr>
<tr>
<td>E-11</td>
<td>CACACAGCTAGGAGCAGTCATC</td>
<td>TCCATCAAGGTGCTTACAGTC</td>
<td>1140</td>
</tr>
<tr>
<td>E-13</td>
<td>TGTCGTTGCGATTGCTTGTG</td>
<td>ACCGCACATTTCATGTTG</td>
<td>420</td>
</tr>
</tbody>
</table>
regarding the constructions and major functions of this protein and its implication in breast and ovarian cancer. Along with this, incidence rates of these cancers have also continued to increase worldwide, with an overall annual increase of approximately 0.5% since 1990. Most countries, especially those where the occurrence rates of these cancers were previously low are showing a rapid increase in these cancers with changes in incidence rates being greater in developing countries, attaining annual increases of 3-4%. Pakistan is amongst these countries. Worldwide these high rates represent a change in reproductive patterns. In Pakistan, however, traditional reproductive patterns are the norm. Different clinical and molecular level studies have revealed that genetic factors have significantly increased the incidence rate of these cancers in Pakistan. Keeping these facts in mind it can be concluded that genetic factors must definitely be playing a major role in the etiology of these cancers. Hence, any study in to the etiology of breast and ovarian cancer, regions into Pakistan have further diversified this population. The Pakistani population is a diverse mix of people and Belgian ethnic groups.

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Initially exon 2 was tested for any pathogenic variant as it holds historic importance because the first founder mutation in BRCA1 gene was detected here. Shattuck-Eidens et al. identified thirty-eight distinct mutations in the complete BRCA1 screening of patients with breast and ovarian cancer, out of which the most common alteration was a 2-bp deletion found at base 185 in exon 2 at an AG repeat region of BRCA1 gene. This deletion mutation (185delAG) of exon 2 has been described in different populations that include non-Ashkenazi Jewish groups across the Middle East, as well as for Greek, Turkish, English and some Indo-Pakistani families settled in Britain. This mutation has also been reported from neighboring India and from Pakistan. An insertion mutation of exon 2, 185insA has been reported in the Pakistani population. This mutation has previously been reported in Ashkenazi Jewish, Latin-American, Dutch, Italian and Belgian ethnic groups.

During second phase of this study, we focused on exon 11 for sequence analysis as it is the largest exon of this gene having a product size of 1140 base pairs and is the site of majority of the known mutations detected in the Pakistani population including the 2041insA novel mutation. Amongst these, 2080insA and 2388delG have been reported in Western-European ethnic groups, 4184delTCAA has been reported in British, French and European and Indo-Pakistani families, while 3889delAG has been reported in the Dutch population. Extensive sequence analysis of this exon with its intron exon boundary revealed no pathogenic mutation. In order to enhance probability of finding mutation, we had selected two more exons that include Ex 10 and Ex 13. Previously, no mutation was found in these exons. Sequence analysis of these exons revealed no functional variant. The absence of finding any sequence variants in the above mentioned exons of the BRCA1 gene of the probands suggests either the involvement of any other region of this gene and the diversity of mutations.
gene or any other gene in our selected samples. Therefore founder effect could not be established, despite the fact that ethnicity was a key factor in the study. However, the importance of focusing on population based mutational screening of this gene in our ethnically diverse country has been highlighted through this study. This trend of screening mutational position along the BRCA1 coding region may have significant impact on treatment decisions for carriers of BRCA1 mutations especially in newly diagnosed patients as it is known that among carriers of BRCA1 mutations, 56-80% and 10-30% develop breast or ovarian cancer respectively. In the Pakistani population where effective resource management is the need of the day, physical and financial losses of patients can be decreased and better prognosis can thus be guaranteed.

CONCLUSIONS

Our results suggest the involvement of other coding sequences of this gene in the etiology of these cancers in our population as no sequence variant with founder effect could be established in the exons under study. A more detailed insight into the relationship between breast and ovarian cancer and mutations in BRCA1 gene in relevance to our country's varied ethnic composition appears to be the most effective method to plan future screening policies for these diseases especially for developing countries like Pakistan.

REFERENCES

mutation originated before the dispersion of Jews in the Diaspora and is not limited to Ashkenazim. Hum. Molec Genet 1998; 7: 801-5.