Mutational Analysis of BRCA2 Gene in the Pakistani Breast Cancer Patients

Tatheer Fatima*1,2 & Hajra Sadia2
1,2 Atta-Ur-Rahman School of Applied Biosciences, National University of Science and Technology (NUST), 44000, Islamabad, Pakistan

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ABSTRACT

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Breast carcinoma is one of the most frequent malignant tumors and about one in every nine females has the probability to suffer from it in Pakistan. BRCA1 and BRCA2 genes function in DNA repair mechanism and function as guardian of genomic stability. BRCA1 and BRCA2 genes are tumor suppressor elements and polymorphic changes in these are intrinsic factor of both hereditary breast and ovarian carcinoma. The objective of this collaborative research is to estimate the probability of common mutation (6174delT) in the BRCA2 gene in a Pakistani population by conventional PCR. Genomic DNA samples of cancer patients were tested for the presence of BRCA2 gene polymorphism (6174delT) in Pakistani female population. It was estimated that 26% of the samples were positive for BRCA2 polymorphism. It is analysed that hereditary and consanguinity is a significant risk element for recurrent population specific mutations. The identification of the distinct BRCA2 gene mutation trends in breast cancer patients might help in the improvement of designing therapeutic options for patients.

Keywords:
Breast Cancer, Pakistani Women, BRCA2, Mutational Analysis

1. Introduction

Breast carcinoma is one of the recurrent diagnosed cancers in female population globally with approximately 1.7 million new sufferers diagnosed in 2012 (American Cancer Society; 2015).

According to Centre for Disease Control and Prevention Breast cancer causes 25% of invasive cancers in women, whereas the root cause of 6% deaths worldwide is breast cancer including men and women [1]–[5]. About 11% of all new incidents of breast carcinoma have been observed among younger women (below 45 years) in United States[6]–[8]. According to a study 1–2% of Pakistani women would be given treatment for breast cancer by the time she is 65 years of age [9].

Key factors that affect breast carcinoma are reproductive cycle, exogenous and endogenous hormones in females, immunity status and genetics [10]. Several breast cancer subtypes have been observed depending upon gene expression which includes HER2+/ER, Luminal A and B [11]–[13].

According to [14], [15] women with polymorphic variant of BRCA1 or BRCA2 have approximately 40–80% chance to become infected with invasive breast carcinoma in one’s lifetime. BRCA1/BRCA2 genes function as “guardian of genome” hence, for this purpose they are expressed in all cells and

* Corresponding author.
E-mail address: Tatheerfatima93@gmail.com (Tatheer Fatima)
tissues where DNA repair mechanism is required for chromosomal double strand breaks. BRCA1/BRCA2 genes are intrinsic for integrity and stability of genetic material.

If genes themselves are damaged, then proper repair mechanism of DNA cannot occur. BRCA1 and BRCA2 genes causes suppression of tumors and polymorphism in these genes greatly increase the probability of malignant breast carcinoma/ovarian carcinoma and several other malignancies such as prostate cancer [16].

At UC Berkeley Mary-Claire King's laboratory analysed the very first evidence of BRCA1 in 1990 [17]–[22]. This dominant BRCA1 belongs to the family of RNF (RING-type zinc fingers). On chromosome 17 (17q21), BRCA1 is responsible for double strand repair. BRCA1 protein consists of 1863 amino acids. Human BRCA1 gene has four major domains; the BRCA1 serine domain, Znf C3HC4- RING domain and two BRCT domains. BRCA1 gene combines with multiple other signal transducer molecules to form complex subunit known as “BRCA associated genome surveillance complex”. Other names of BRCA1 gene are IRIS, PPP1R53, PSCP, and RNF5.

Professor of 1994, Dr. Richard Wooster and Dr. Michael Stratton demonstrated the presence of BRCA2 in 1994. This dominant BRCA2 gene belongs to the family FANC (Fanconi anemia, complementation groups). BRCA2 location is chromosome 13 (13q12.3) and codes for a protein of approximately 3420 amino acids. Three major domains of BRCA2 are: OB1 domain, OB3 domain and tower domain. According to study about 1800 mutations have been analyzed in BRCA2. Other names for BRCA2 are BRCA2_HUMAN, BRCC2 [23]. BRCA1/BRCA2 mutations are autosomal dominant. Each child having a BRCA1/BRCA2 polymorphism has a 45%-50% probability of having a polymorph resulting in abnormal protein product or no protein product in some cases [24]–[26]. Two polymorphic alteration in BRCA1 (185delAG and 5382insC) have been estimated in tumorigenic patients having BRCA1 related breast and ovarian cancer. A common frame shift polymorphism of BRCA2 gene is 617delT. BRCA1 gene polymorph (185delAG) produces an altered protein BRAT, causing a deletion of two nucleotide bases in exon 2 of the BRCA1 gene resulting in a frame shift and a stop codon at position 39. 185delAG mutation exhibit enhanced apoptosis, caspase-3 activation, diminished levels of phosphorylated Akt and serve as a cellular inhibitor of apoptosis 1 [27]–[29].

Another important mutation in BRCA1 gene is 5382insC mutation and this polymorphic abnormality has widely affected BRCA1 normal function. 5382insC BRCA1 mutation produces truncated protein producing 1830 amino acids and missing second BRCT repeat [28]–[30].

Deleterious germ line polymorphism in BRCA2 may account for 85% probability of breast carcinoma and 26% probability of ovarian carcinoma in women (Ford et al., 1998). In hereditary breast carcinoma 6174delT is a common frame shift polymorphism of BRCA2. Truncated protein product is produced as a result of 6174delT mutation. This cancer associated mutation removes 224 residues out of the 3,420 at terminal C in BRCA2 gene reporting that these amino acids at the end are intrinsic for BRCA2 functionality [31]–[33].

2. MATERIALS AND METHODS

2.1 Sample Collection

Samples of blood were collected from Benazir Bhutto hospital and NOORI during February- May 2012 from patients who were diagnosed with breast cancer. Tissue sample size was fifty whereas blood samples were 20. The tissue samples were collected in FFPE tubes whereas, blood specimen was
stored in EDTA vacutainer. Blood specimen were kept at -4°C whereas, tissue specimen were kept at -20°C. For each patient the data was reviewed, and the pathological results were recorded prior to the study. Tumor grade, tumor size, existence of multifocal or multicentric disease, number of lymph node metastases, Progesterone receptor Estrogen receptor status was recorded for each of the patient.

Blood samples were collected from applied biosciences department during February-March 2016. These 30 blood samples collected randomly from subjects were used as control samples. These blood samples were stored at -4°C. DNA extraction was carried out by standard phenol- chloroform protocol. All the samples were fresh blood samples from which DNA was extracted later. For each subject certain data was recorded which included parameters such as name, age and gender of the subject and the familial inherited history of individuals.

2.2 DNA Extraction

Formalin-fixed, paraffin-embedded tissue samples are a significant source of nucleic acid for DNA extraction. For this purpose FFPE tissue biopsy technique was used for the DNA extraction. Phenol Chloroform DNA extraction protocol described by (Chan et al., 1999) was used for Blood samples.

2.2.1 DNA Extraction from Blood

Extraction of DNA was carried out through phenol-chloroform protocol [34], [35]. 1mL of blood was mixed with 9 mL Buffer A. Iced Incubation was done for 4 minutes and centrifuged at 1500 rpm for 15 minutes at 4°C. Then, suspension of pellet (pink coloured) of DNA in 320µL Buffer B (cooled). Add 32µL of 10% of SDS and 3.5µL of Proteinase K. Incubate at shaker at 36°C for 24 hours. Equal quantity of buffer (equilibrium equivalent to phenol) was mixed for about 10 minutes. Centrifuge (2500 rpm for 15 minutes) at 4°C. To the supernatant and mix equal quantity of Isoamyl and chloroform and mix properly. Centrifugation was done at 14,000 rpm for 25-30 minutes to carry our precipitation of DNA. Finally, wash with ethanol (70%) and dry it in the air. Mix DNA (pallet) in the buffer (Tris-EDTA) and store it at 20°C. Lastly check DNA quality at 0.7% Agarose gel.

2.2.2 DNA Extraction from Tissue Biopsies

Extraction of DNA from formalin fixed paraffin embedded tissues was preceded. Pieces of 5-10 µm from the tissue were obtained and kept in micro centrifuge tube. Xylene was added in an amount 1 mL to remove Paraffin. This step was led by mixing it for 25-30 minute at 35°C. Centrifugation was done at 14000 rpm for 10 minutes to decant the supernatant and to eliminate mixed paraffin. Paraffin was completely removed by carrying out repeatedly addition of xylene step. Next step was to wash the segments with 100% ethanol and then centrifuge at 14000 for 10 minutes again. It was then dried by air. Then, added 500 µL of buffer (Tissue digestible) and kept at about 45°C for 24 hours. This step was followed by the halting activity of Proteinase K by keeping the vessels at 96 °C for 8 minutes on heat.

In each tube 120 µL of Sodium Chloride (6 Molar) was mixed into pellet down proteins and then centrifugation of tubes was done for 8 minute. Supernatant then shifted to a separate vessel and Sodium Acetate (3 Molar) was added and then mixed manually. A pallet of DNA was observed by mixing 600µL isopropyl alcohol after incubation at -20 °C for 24 hours. After incubation,
centrifugation of tubes at 15,000 rpm for 20 minute with a temperature of 4°C, to separate the nucleic acid. The pallet was then washed using Ethanol (70%). Lastly we diluted DNA with Tris-EDTA buffer (50 µL). Nucleic acid was refrigerated at -20°C.

2.3 Determination of DNA Concentration

Optical density was calculated at 270 nm through a device called “spectrophotometer”. Absorbance estimate was 270-280 nm to evaluate the concentration of extracted nucleic acid. A value defined as “pure” for Genomic DNA is ~1.8; hence the specimen having the absorbance lesser than 1.7 were added in phenol-chloroform to remove contamination.

2.4 Analysis of DNA Through Agarose Gel Electrophoresis

Evaluation of the genomic DNA quantity isolated from blood, 0.7 % gel was produced in TBE and run it in the prepared buffer. 1 litre volume of TBE buffer was prepared by mixing 55 g of boric acid, 109 g of Tris-base, 7.5 g of EDTA and finally 800 mL of distilled water was added to make its volume to 1 litre as mentioned above. Gel was prepared by taking 1.4 g of Agarose and 70 mL of TBE buffer which was microwaved for 2 minutes. Gel was then kept at fairy low temperature to ~50 °C and then, 5 µL of ethidium bromide was mixed for gel staining.

Next step was to run DNA (4 µL) from specimen on a gel by mixing it with loading dye. For about 25-30 minutes sample was run and current given for running it was 80mA. Last step was to visibly see gel under UV light. Gel Doc was used to take photographs.

2.5 Optimization of PCR

2.5.1 β-Globin PCR

Analysis of DNA was carried out by PCR for β-globin gene. PCR volume was 12.5 µL and constituted of 2mM dNTPs, forward and reverse primers as mentioned in Table 1, 1 µL of DNA template sample, MgCl2 (2mM) Taq polymerase buffer (1X) and thermostable Taq polymerase (1 U). To make up its final quantity up to 12.5 µL we added PCR water to the reaction tube. Table 3.1 highlights the nucleotide sequence of BRCA2 primers (reverse and forward).

Standard profile of PCR was designed to process Polymerase Chain Reaction. This is carried out to amplify DNA sample in an efficient manner and to eliminate contamination. This is performed by amplification of β-globin gene which is of 119-bp size. PCR Profile included Initial denaturation at 95°C for 5 minutes, Denaturation at 95°C for 40 seconds, Annealing at 57°C for 40 seconds, Extension at 72°C for 50 seconds and Final Extension at 72°C for 10 minutes. Following is the Primer sequence and Tm of β-globin primers.

<table>
<thead>
<tr>
<th>Serial No.</th>
<th>Primer Code</th>
<th>Primer Sequence(5’---3’</th>
<th>Tm(°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>β-globin–F</td>
<td>ACACACTGTGGTTCACTAGC</td>
<td>58.4</td>
</tr>
<tr>
<td>2.</td>
<td>β-globin-R</td>
<td>CAACCTCATCCACGTCACC</td>
<td>60.4</td>
</tr>
</tbody>
</table>
Forward and reverse primers for β-globin housekeeping gene were designed to screen this gene in DNA. This analysis was important to perform PCR.

2.5.2 PCR Product Analysis by Gel Electrophoresis

Agarose gel (2%) is used for the visualizing final results of PCR. Agarose gel is run in a buffer (TAE). Recipe of gel is to mix 70 mL of 1X buffer (TAE) in 1.4 grams of Agarose and to heat it properly for 2 minutes in a flask. Gel is kept at fairly low temperature of 45°C and then 5μL of ethidium bromide was added. Screening of amplified PCR product was done on a gel.

Constant current of 80 mA was suitable for 23-25 minutes to run gel. Under UV light gel was visualized. At -20°C PCR product was refrigerated.

2.6 BRCA2 AMPLIFICATION BY PCR

DNA that was extracted and amplified through PCR for the analysis of BRCA2 polymorphism of BRCA2 gene for each of the diseased and control samples. For detection of BRCA2 polymorphism following set of primers was used (Table: 2).

Standard PCR profile was used to reduce contaminants and BRCA2 polymorphism was tested by amplifying a 151-bp fragment of the BRCA2 gene. Amplification yielded 151-bp or B1 allele (without duplication) and the mutant 171-bp fragments or B2 allele (with 20-bp mutation). Primers set for BRCA2 polymorphism detection were as designed by (Chan et al., 1999).

PCR reaction tube volume of 12.5 μL consisted of 2mM dNTPs, forward and reverse primers as mentioned in Table 3.2, 1 μL of DNA template sample, MgCl2 (2mM) Taq polymerase buffer (1X) and thermostable Taq polymerase (1 U). To make up its final quantity to 12.5 μL we added PCR water to the reaction tube. Table 3.2 highlights the nucleotide sequence of primers designed.

Table 2
Primer for the amplification of desire nucleotide bases

<table>
<thead>
<tr>
<th>Oligo Name</th>
<th>Sequence (5'–3')</th>
<th>Fragment size</th>
</tr>
</thead>
<tbody>
<tr>
<td>B2P7</td>
<td>AGCTGGTCTGAATGTTCTTACT</td>
<td>23</td>
</tr>
<tr>
<td>B2P8</td>
<td>GTGGGATTTTAGCACAGCAAGT</td>
<td>23</td>
</tr>
<tr>
<td>B2P9</td>
<td>CAGTCTCATCTGCAAATACTTCAGGGATTTTGCACAGCAAGTG</td>
<td>44</td>
</tr>
</tbody>
</table>

2.7 ANALYSIS OF PCR PRODUCT BY GEL ELECTROPHORESIS

Agarose gel (2%) was used for visualizing final results of PCR. Agarose gel was run in a buffer (TAE). Recipe of gel is to mix 70 mL of 1X buffer (TAE) in 1.4 grams of Agarose and heat it properly for 2 minutes in a flask. Gel was kept at fairly low temperature of 45°C and then 5 μL.

3. RESULTS & DISCUSSION

3.1 β-Globin Gene Screening
For the sufficiency of Polymerase Chain Reaction, DNA that was extracted was confirmed by analyzing β-globin gene. DNA was confirmed on the basis of 121-bp sized β-globin gene. The results are shown in Figure 1.

Lane M; 100 bp DNA marker (Fermatas); Lane 1: Negative Reagent, Lane 2-19: 191 bp β-globin gene fragments amplified from extracted DNA from breast cancer tissue and blood samples.

3.2 Amplification Of BRCA2 Gene Fragment By PCR

Extracted DNA from samples was used as a template, 171bp BRCA2 gene mutation was amplified through specific primers by PCR. Size of amplicon of wild type forward was 151bp. The present study included 50 case subjects with invasive breast cancer.

Lane M; 100 bp DNA marker (Fermatas); Lane 1-19: 171bp BRCA2 gene fragment.
3.3 Screening Of Control Samples For BRCA2 Mutation

The present study included control sample size of 30 female individual. The average age of control samples was 22 years (range: 19-25 years). DNA extraction from blood samples was performed by Phenol-Chloroform protocol. Only 4 women had the history of breast cancer, out of total 30 individuals. β-globin gene was analyzed and results suggested all positive DNA extracted from blood samples. Blood samples DNA was evaluated for the wild type BRCA2 gene polymorphism using primers set to carry out amplification of 151-bp gene.

Agarose gel (2%) was used for visualizing final results of PCR. Agarose gel was run in a buffer (TAE). Recipe of gel is to mix 70 mL of 1X buffer (TAE) in 1.4 grams of Agarose and heat it properly for 2 minutes in a flask. Gel was kept at fairly low temperature of 45°C and then 5 μL.

Lane A; 100-bp Ladder; Lane B: Negative reagent, Lane C: 171-bp BRCA2 gene fragment amplified from extracted DNA from breast cancer tissue and blood samples.

**Figure. 3.** Control DNA Samples screened for BRCA2 gene

Lane 1-16: control samples screened for 151-bp BRCA2 gene; Lane 1: Negative reagent and Lane 2: Positive reagent and Lane L: 50-bp ladder. Following is the gel image of control samples analyzed for the presence of wild type BRCA2 gene by using specific set of primers to carry out amplification of 151-bp gene.

**Figure. 4.** BRCA2 gene screened for breast cancer control DNA samples.

Lane L: 50-bp ladder; Lane 17-30: control samples; Lane 17: Negative Reagent; Lane 30: Positive Reagent.
3.4 Breast Cancer Samples Screening For BRCA2 Mutation

The present study included 50 case subjects with invasive breast carcinoma. The average age at diagnosis of breast cancer was 46.2 years (range 21-60). Among 50 women diagnosed with breast cancer, 13 (26%) BRCA2 mutations were identified in patients. Larger number of women who had breast cancer diagnosed was at the age of 45 and also they were carriers. 11 patients were heterozygous mutants whereas 3 patients were homozygous mutants for BRCA2 6174delT mutation. Patients with Breast cancer were evaluated on the basis of DNA that was extracted from either tissue sample or blood sample for the presence of BRCA2 polymorphism by using specifically designed primers (Table 3.2) to carry out amplification of 171-bp BRCA2 gene fragment. Figure 4 shows the amplified BRCA2 gene fragment.

Figure 5. Samples screened for BRCA2 gene polymorphism

Lane 1-19: samples screened for presence of 151-bp BRCA2 gene; Lane 1: Negative reagent; Lane 2: Positive reagent L: 50-bp ladder

3.5 Statistical Analysis

The data obtained in the study is estimated statistically using SPSS Statistical Package for the Social Sciences Software. BRCA2 gene polymorphism is compared with multiple clinical parameters like Age, Gender, Disease Category, Grading of cancer and Lymph node. Following graphs were obtained in the analysis carried out.

Table 3
BRCA2 gene comparison with clinical parameters like Gender, Age, Disease Category, Grading of cancers and Lymph Nodes

<table>
<thead>
<tr>
<th></th>
<th>Gender</th>
<th>Age</th>
<th>Disease Category</th>
<th>Grading of Cancers</th>
<th>Lymph Node</th>
<th>BRCA2</th>
</tr>
</thead>
<tbody>
<tr>
<td>N Valid Missing</td>
<td>63</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>13</td>
<td>13</td>
<td>13</td>
<td>13</td>
<td>0</td>
</tr>
</tbody>
</table>

In a given study, 63 patients were analyzed for BRCA2 polymorphism. Out of 63, 50 patients were found positive for BRCA2 gene. Age, Disease category, Grading of cancer, Lymph node analysis was done for these 50 patients.

In this study sample size of breast cancer patients was 50. Out of 50 patients, 49 were female whereas single patient was male. Following table depicts the frequency and percentages of patients on the basis of gender.
Table 4
Frequency estimate of BRCA2 Polymorphism in comparison to Gender

<table>
<thead>
<tr>
<th>Gender</th>
<th>Frequency</th>
<th>Percent</th>
<th>Valid Percent</th>
<th>Cumulative Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valid Male</td>
<td>13</td>
<td>20.6</td>
<td>20.6</td>
<td>20.6</td>
</tr>
<tr>
<td>Female Total</td>
<td>49</td>
<td>77.8</td>
<td>77.8</td>
<td>100.0</td>
</tr>
<tr>
<td>Total</td>
<td>63</td>
<td>100.0</td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>

Out of 50 patients, 49 were female and 1 was male patients. Frequency, percentage and their cumulative percentage is given for both male and female breast cancer patients.

Table 5
Frequency estimate of BRCA2 Polymorphism in comparison to Age

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Frequency</th>
<th>Percent</th>
<th>Valid Percent</th>
<th>Cumulative Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>30-39 Valid</td>
<td>7</td>
<td>11.1</td>
<td>14.0</td>
<td>14.0</td>
</tr>
<tr>
<td>40-49</td>
<td>18</td>
<td>28.6</td>
<td>36.0</td>
<td>50.0</td>
</tr>
<tr>
<td>50-59</td>
<td>16</td>
<td>25.4</td>
<td>32.0</td>
<td>82.0</td>
</tr>
<tr>
<td>Greater than 59</td>
<td>8</td>
<td>12.7</td>
<td>16.0</td>
<td>98.0</td>
</tr>
<tr>
<td>Total Missing</td>
<td>7</td>
<td>1.6</td>
<td>2.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Total System</td>
<td>13</td>
<td>20.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>63</td>
<td>100.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5 Frequency estimate of BRCA2 Polymorphism in comparison to Age Commutative frequency of BRCA2 polymorphism was found to be higher in patients within Age limit of 40-49. It was also deduced that BRCA2 polymorphism is associated with Age of patient.

Breast cancer patients BRCA2 gene mutation was analyzed with respect to their age. Following bar chart was obtained. Bar chart clearly demonstrated that majority of patients belonged to age limit that is 40-49 years of age.
Bar chart demonstrate majority of patients belonged to age of 40-49 years. Both heterozygous and homozygous mutants belonged to this age limit.

**Table 6**
Frequency estimate of BRCA2 Polymorphism in comparison to Disease Category

<table>
<thead>
<tr>
<th></th>
<th>Frequency</th>
<th>Percent</th>
<th>Valid Percent</th>
<th>Cumulative Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valid</td>
<td>Ductal Carcinoma in situ</td>
<td>35</td>
<td>55.6</td>
<td>70.0</td>
</tr>
<tr>
<td></td>
<td>Invasive Ductal Carcinoma</td>
<td>9</td>
<td>14.3</td>
<td>18.0</td>
</tr>
<tr>
<td></td>
<td>Medullary Carcinoma</td>
<td>3</td>
<td>4.8</td>
<td>6.0</td>
</tr>
<tr>
<td></td>
<td>Papillary Carcinoma</td>
<td>3</td>
<td>4.8</td>
<td>6.0</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>50</td>
<td>79.4</td>
<td>100.0</td>
</tr>
<tr>
<td>Missing</td>
<td>System</td>
<td>13</td>
<td>20.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>63</td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>

Above shown table clearly demonstrate the category of disease with respect to BRCA2 mutation samples. Majority of patients had Ductal Carcinoma in situ.
Statistical results clearly showed that BRCA2 Polymorphism is more prevalent in Ductal Carcinoma in situ category of patients.

Table 7
Frequency estimate of BRCA2 Polymorphism in comparison to Grading of Cancer

<table>
<thead>
<tr>
<th></th>
<th>Frequency</th>
<th>Percent</th>
<th>Valid Percent</th>
<th>Cumulative Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valid</td>
<td>grade 1</td>
<td>2</td>
<td>3.2</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>Grade 2</td>
<td>25</td>
<td>39.7</td>
<td>54.0</td>
</tr>
<tr>
<td></td>
<td>Grade 3</td>
<td>17</td>
<td>27.0</td>
<td>88.0</td>
</tr>
<tr>
<td></td>
<td>Grade 4</td>
<td>6</td>
<td>9.5</td>
<td>100.0</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>50</td>
<td>79.4</td>
<td>100.0</td>
</tr>
<tr>
<td>Missing</td>
<td>System</td>
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<td>20.6</td>
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</tr>
<tr>
<td>Total</td>
<td></td>
<td>63</td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>

In a given statistical analysis, Grade 2 breast cancer patients were most prevalent among the patient sample given which was led by Grade 3 and then Grade 4. It is important to note that Grade 1 patients are least common. Frequency, percentage and cumulative percentages are shown in above mentioned table.

Statistical analysis was performed to estimate grade of breast cancer with respect to breast cancer mutation of BRCA2. Bar chart clearly represented majority of patients had Grade 2 type breast cancer.
Upon analysis of the BRCA2 polymorphism association with the grade of the breast tumor, the following results were obtained (Figure 4.7). The results indicate that the said polymorphism is more prevalent in the higher grades of the breast cancer i.e. Grade 2, Grade 3 and Grade 4, whereas none of the Grade 1 patients presented with the BRCA2 polymorphism.

Table 8
Frequency estimate of BRCA2 Polymorphism in comparison to Lymph Node

<table>
<thead>
<tr>
<th></th>
<th>Frequency</th>
<th>Percent</th>
<th>Valid Percent</th>
<th>Cumulative Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valid Positive</td>
<td>12</td>
<td>19.0</td>
<td>24.0</td>
<td>24.0</td>
</tr>
<tr>
<td>Negative</td>
<td>38</td>
<td>60.3</td>
<td>76.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
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<td>100.0</td>
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</tr>
<tr>
<td>Missing System</td>
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<td>20.6</td>
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<tr>
<td>Total</td>
<td>63</td>
<td>100.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Statistical analysis was done to estimate lymph node status of breast cancer patients positive for BRCA2 mutation. Frequency estimate of BRCA2 Polymorphism in comparison to Lymph Node In a given statistical analysis, Lymph node status frequency was higher is Negative samples as compared to Positive samples.

It is important to analyze lymph node status of breast cancer patients and for that reason, SPSS analysis was performed. Results clearly demonstrated that lymph node status was negative for breast cancer patients.
Lymph node status for breast cancer patients was analyzed to determine its correlation with BRCA2 mutation.

**Table 9**

Wild type, Heterozygous, Mutant and cumulative percent of BRCA2 polymorphism

<table>
<thead>
<tr>
<th></th>
<th>Frequency</th>
<th>Percent</th>
<th>Valid Percent</th>
<th>Cumulative Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valid</td>
<td>13</td>
<td>20.6</td>
<td>20.6</td>
<td>20.6</td>
</tr>
<tr>
<td>Wild Type</td>
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<td>57.1</td>
<td>57.1</td>
<td>77.8</td>
</tr>
<tr>
<td>Heterozygous</td>
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<td>15.9</td>
<td>15.9</td>
<td>93.7</td>
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<tr>
<td>Mutant</td>
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<td>6.3</td>
<td>6.3</td>
<td>100.0</td>
</tr>
<tr>
<td>Total</td>
<td>63</td>
<td>100.0</td>
<td>100.0</td>
<td></td>
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</tbody>
</table>

Frequency and percentage of wild type BRCA2 gene was highest, heterozygous polymorphism was comparatively less frequent and Mutant samples frequency and percentage was least high in given breast cancer samples.
4. Conclusions

The high prevalence of the BRCA2 mutations especially 6174delT in Pakistani women population proves the importance of counselling. It is concluded that family history is a significant risk factor for Pakistani population mutations (6174delT). There are multiple factors such as obesity, polycystic ovaries, failure to breast feed, older age pregnancy and most importantly poor diet like passive or active smoking habits, nulliparity lack of vitamins in body, has a significant role in causing breast carcinoma in population. Stress, exposure to traffic pollution, anxiety is also important factors for breast carcinoma.

References


