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### RESEARCH ARTICLE

#### DNA EXTRACTION AND PCR DIAGNOSIS OF BREAST CANCER PATIENTS AMONG THE POPULATION OF TRIPURA.

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#### Abstract:

Breast cancer is a malignant tumor that starts in the cells of breast. A malignant tumor is a group of cancer cells that can grow into (invade) surrounding tissues or spread to distant areas (metastasize) of the body. Several environmental risk factors that may contribute to or hasten the development of breast cancer have been identified including mainly lifestyle, reproductive factors and family history. Mutations in the *BRCA1* and *BRCA2* genes profoundly increase the risk of developing breast cancer among women. *BRCA1* gene is located on chromosome No. 17. The normal *BRCA1* genes encode 1863 amino acids. *BRCA2* gene located on chromosome No. 13 is another DNA repair gene. The normal *BRCA2* genes encode 3418 amino acids. To explore the contribution of *BRCA1* and *BRCA2* mutations in the development of breast cancer among Indian women, we carried out DNA extraction & PCR analysis of the *BRCA1* and *BRCA2* genes in thirty eight (38) number of breast cancer female patients from Tripura, India.

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#### Introduction:-

Genetic predisposition for familial early-onset breast cancer accounts for approximately 5–10% of all breast cancers and 7–10% of all ovarian cancers [1]. Mutations in two autosomal dominant genes *BRCA1* and *BRCA2* have been linked to familial breast or breast and ovarian cancer [2-4]. Women who carry *BRCA1* or *BRCA2* genes mutations have an estimated lifetime risk of between 60% and 85% for developing breast cancer and a lifetime risk of between 26% and 54% for developing ovarian cancer for *BRCA1*, and between 10% and 23% for *BRCA2* [5-8]. Every year 75,000 new cases of breast cancer are diagnosed in Indian women [9]. In India, breast cancer accounts for about 19-34% of all cancer cases among women. As per national and regional cancer registries, it is the commonest cancer amongst women in Delhi, Mumbai, Ahmedabad, Kolkata and Trivandrum [10-12]. A recent study on breast cancer risk in India, revealed that one in twenty eight women develop breast cancer during her life time. This is higher in urban areas. In Tripura state too breast cancer is the second leading site among females, the first and third being carcinoma cervix and lungs [13]. Because of the continuing rise in breast cancer incidence, an urgent need to develop strategies for prevention is very much required. Carcinoma of breast has a complex etiology some of which are hormonal, genetic and environmental factors operating over a long period [14].

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## Materials & Methods:-

This study was carried out in the DBT Health Care Laboratory, Department of Biochemistry, Agartala Government Medical College in collaboration with Pathology Department, Agartala Government Medical College & Regional Cancer Center, Agartala during the year March 2016 to September 2017.

### Collection of Samples:

A total fifty eight (58) number of cases were collected from Department of Pathology, Agartala Government Medical College & Regional Cancer Center, Agartala. Among fifty eight (58) no. of total cases thirty eight (38) cases were positive of breast cancer and twenty (20) no. of cases were using as a control. The age range of the patient was between 22 to 45 years. Five ml of peripheral blood sample were collected for the proposed test using EDTA vials and stored at 4°C till further use if not done immediately.

### Histopathology:

Histopathological study was done for confirmation of the breast cancer. At first, tissue samples were excised by microtome machine (3-5µm). Then the tissue samples were placed on poly L Lysine coating slides. Thereafter Dewaxing or Deparaffinization by Xylene was done which was further rehydrated by 100% - 70% - 50% alcohol, for 5 minutes each. Hematoxyline stain step was done for 1-2 minutes following the 1% acid alcohol step for differentiation and used 2-4 drops Eosin as a counter stain for 5 minutes.

### DNA Extraction from Blood Samples:

5 ml of EDTA blood is centrifuged at 1500 rpm for 30 minutes. Serum is partially decanted. To the tube Lysis buffer is added and mixed. Tubes are kept in ice for 30 minutes. Then tubes are centrifuged at 2500 rpm for 20 minutes and supernatant is decanted. 10 ml of Lysis buffer is added then centrifuged at 2500 rpm for 20 minutes and supernatant is decanted. 200 µl of 10% SDS and 500 µl of Proteinase K are added to the tubes. Overnight incubated at 37°C or 3-4 hours shaking at 56°C. One ml 6M NaCl is added and mixed. Tubes are centrifuged at 4000 rpm for 20 minutes. Supernatants are collected in separate tubes. Equal volume of absolute ethanol is added to the supernatant and mixed. 1 ml of 70% ethanol is added and mixed by inverting the tubes. Tubes are spinned at 6000 rpm and supernatant are discarded carefully. DNA is collected in 1.5 ml microfuge tube.

### DNA Purity Test By Spectrophotometer:

The extracted DNA samples by Phenol: Chloroform: Isoamylalcohol method was subjected to spectrophotometer as per the standard laboratory procedure. The average DNA purity was obtained- 1.87 (260nm/ 280nm) from the extracted DNA samples of 38 breast carcinoma patients by Phenol: Chloroform: Isoamylalcohol method.

### PCR Analysis:

*BRCA1* & *BRCA2* genes of the extracted genomic DNA will be amplified in PCR by using two sets of primers (BioChain Cat.12210861, BioChain Cat. 12210862), taq polymerase kit supplied by ( QIAGEN Cat nos. 201443 & 201445) following given steps :-

- i) Initial denaturation: at 95°C for 2 minutes.
- ii) Denaturation: At 95°C for 30 seconds.
- iii) Annealing: At 60° C for 30 seconds.
- iv) Extension: At 72° C for 1 minute.
- v) Final extension: At 72°C for 5 minutes

Number of cycles: - 30

## Results:-

A total thirty eight (38) no. of histopathological positive samples belonging to the age range between 22 to 45 years were amplified by PCR. Earlier studies reveal that among the different risk factors of causing breast cancer, age of the individual plays an important role. The age distribution among breast cancer patients are shown in the Table-1.

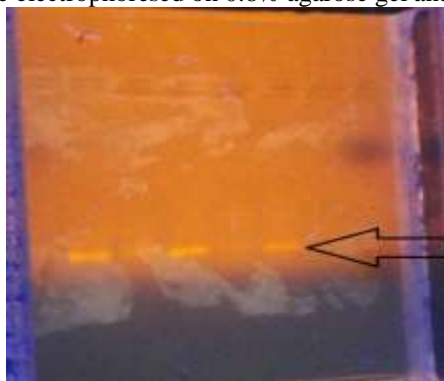
Age less than 35 years	Age more than 35 years
25 nos.	13 nos.

**Table 1:-** Age distribution of breast cancer patients.

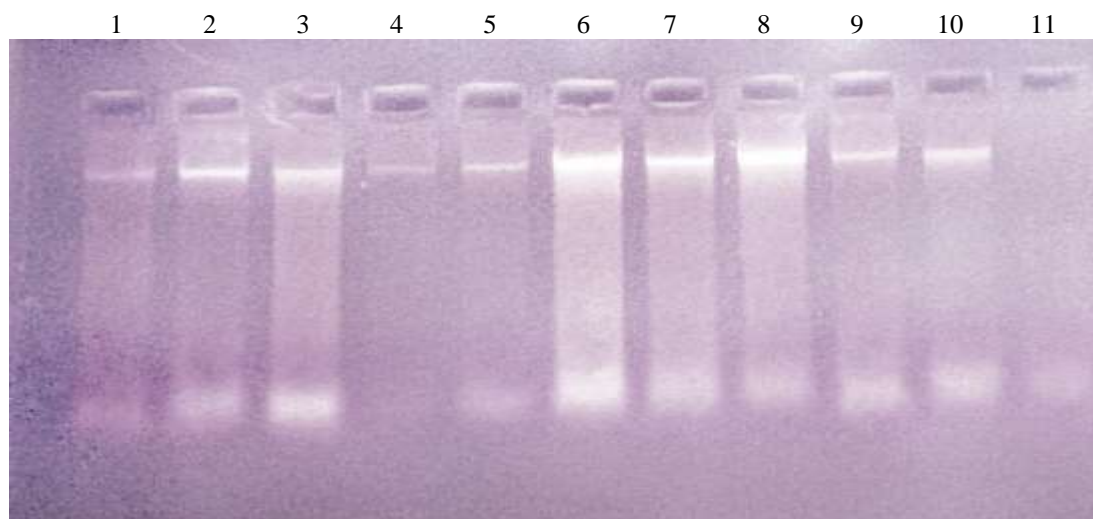
In our study, it is revealed that among the 38 positive samples, 25 no. of samples were below 35 years and 13 no. of samples were above 35 years. This suggests that the mutation of *BRCA1* and *BRCA2* genes are predominant among

the younger females in compare to older group of female population of Tripura. This is a major concern among the female population of Tripura.

Then, the isolated DNA samples were electrophoresed on 0.8% agarose gel and shown in Fig-1.



**Fig1:-** DNA samples on agarose gel (0.8%). The arrow indicates the DNA samples. Samples were analysed by spectrophotometer and was followed by PCR amplification.



**Fig-2:-** PCR products on 1.8% agarose gel.

Then, Electrophoresis was done for the PCR products with 1.8% agarose gel using Tarsons electrophoresis unit. The amplification of *BRCA1* and *BRCA2* gene confirmed the diagnosis of breast cancer. DNA amplification by PCR technique using specific primers of breast cancer (*BRCA1* & *BRCA2*) is a widely used technique for diagnosis of breast cancer and one of the reliable techniques in the modern day medicinal field. The amplification of DNA using PCR technique are shown in Fig – 2, including Sl. No.1 to 10 as positive among cases and Sl. No. 11 as negative (control) among cases. The different type of concentrations of DNA represents different type of bands in agarose gel. The results of this study would help to develop to diagnose breast cancer patient and subsequently will provide a milestone in treating the patient with breast cancer among the population.

### Discussions:-

Not many progresses have been made in the past few years in understanding the mutations of *BRCA1* & *BRCA2* gene in breast cancer patients of Tripura population. But it is considered that mutation in the *BRCA1* and *BRCA2* genes profoundly increase the risk of developing breast and/or ovarian cancer among women. Although their mechanism of action is not yet fully elucidated it is assumed that these genes play a key role in important cellular pathways including response to DNA damage, transcription, and interaction with other proteins involved in DNA repair and apoptosis [15-16]. This study revealed that PCR amplification that was carried out by using specific

primers (*BRCA1* and *BRCA2*) are shown positive in case of breast cancer and shown negative in control sample (Fig-2). Hence, this positive PCR amplification clearly confirms the diagnosis of breast cancer patients.

Genetic testing helps in identifying high-risk individuals in families with inherited breast cancer, and there are various management options available for mutation carriers. Significant variations in the relative contribution of mutations in *BRCA1* and *BRCA2* to the development of inherited familial breast and/or ovarian cancer have been reported [17]. Breast cancer is the most frequently diagnosed cancer accounting for 23% of total cancer cases and 14% of cancer deaths. The incidence in India is also alarming. Every year about 1.5 lakh new cases of breast cancer have been reported by World Health Organization in India [18].

### Conclusion:-

Thirty Eight (38) number of histopathologically confirmed cases of breast cancer planned for cancer treatment were taken up for study from the Department of Pathology, Agartala Government Medical College & Regional Cancer Center, Agartala. A total of 20 numbers of female individuals without having any clinical signs & symptoms of breast cancer were taken as control. We choose the Breast cancer patients in our research work, because the Breast cancer remains the most common cause of death from cancer worldwide including Tripura state. Our goal of being cancer researcher was to extract DNA from blood in order to find out the cause of breast cancer. Here we discussed about the DNA extraction and PCR diagnosis of breast cancer patients by *BRCA1* and *BRCA2* genes, which plays a major concern among female worldwide.

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