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DNA Extraction From Formalin-Fixed, Paraffin-Embedded Tissue Sections of Breast Carcinoma Patients

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ABSTRACT

Globally, breast cancer is the most common cause of cancer-related death in women, with around 327,000 deaths each year. Around 1.35 million cases of breast cancer have been found each year and 4.4 million women are believed to be live with breast cancer worldwide. DNA isolated from formalin-fixed paraffin-embedded tissue is often fragmented and cross-linked and is therefore difficult to analyse. Isolation of DNA from Formalin-fixed, Paraffin-embedded Tissue Sections in adequate quantities is an integral part of Biological research and analysis. The present study was performed to determine the quality of DNA extracted from paraffin embedded tissue sections of breast cancer patients. Based on our findings, we can safely conclude that, we developed a rapid, cost-effective, and noninvasive method of sample collection and simple DNA extraction from breast cancer tissues using the phenol-chloroform- Isoamyl alcohol method which is very much useful for researcher in near future for breast cancer diagnosis.

KEYWORDS : Breast carcinoma, formalin-fixed paraffin-embedded tissue, Histopathology.

INTRODUCTION- 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) to distant areas of the body. Several environmental risk factors that may contribute to or hasten the development of breast cancer have been identified, including mainly lifestyle and reproductive factors. The factor with the strongest breast cancer risk association is a family history of breast and/or ovarian cancer, the associated risk being even higher for family history of early-onset disease (< age 40)[1]. Genetic susceptibility to breast cancer is triggered in several ways; the best understood causal mechanism being due to germline mutations in tumor suppressor genes. Together, mutations in BRCA1 and BRCA2 genes account for the great majority of families with hereditary susceptibility to breast and ovarian cancer[2]. BRCAI gene located on chromosome no 17.a DNA repair gene inherited case BRCAI deletion. Due to mutated BRCAI Prostate cancer occur. The normal BRCAI genes encode 1863 amino acids[3]. BRCAII gene located on chromosome no 13 another DNA repair gene. The normal BRCAII genes encode 3418 amino acids. The contribution of mutations in these two genes to breast cancer patients in the Indian population remains relatively unexplored apart from a few small studies[4]. Generally, breast cancer can occur at any age but younger women are less susceptible to ward's breast cancer[5]. The probable reason for the early onset of this dreaded disease in the younger women may be due to personal history with a breast cancer/ovary cancer[6], family history of breast cancer, particularly in a mother, sister and daughter[7]. In this research work we can do pathological as well as molecular analysis of DNA samples.

MATERIALS AND METHODS-

Collection of samples:- 10 number of breast carcinoma cases were collected from the clinical suspected patients during the year 2013 (Figure-1). The age range of the patient was between 23 to 65 yrs (*Mean= 39 years*).

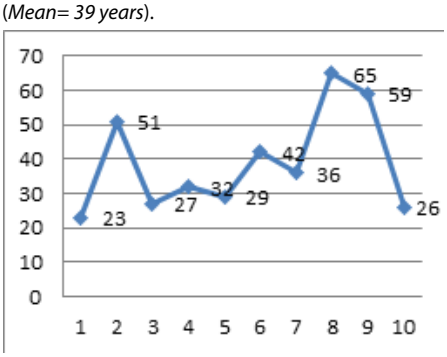

Figure 1- Age (in years) of breast carcinoma patients

Histopathology of Breast carcinoma tissue:- Histopathological study was done for confirmation of the breast carcinoma**.** At first, cutting the suspected tissue samples by microtome machine (3-5 µm) and taking the tissue samples on poly L Lysine coating slides. After that Dewaxing or Deparaffinization by Xylene and follow the rehydration step by 100% - 70% - 50% , each for 5 minute. Hematoxyline stain step was done for 1-2 minute (Figure-2) following the 1% acid alcohol step for differentiation and used 2-4 drops Eosin as a counter stain for 5 mints (Figure-3).

DNA isolation from Formalin-Fixed, Paraffin-Embedded Tissue- Cut paraffin block at 10 µm and collected in an autoclaved plastic microtube (1.5 ml). Add 1 ml xylene to the microtube for 30 min, for two changes and add 100% and 75% ethanol for 30 min with two changes. Wash with PBS for 15 min with two changes. Add 500 µl of lysis buffer containing proteinase K and incubated at 52°C overnight until all tissue fragments were dissolved completely. Add 500 µl phenol: chloroform: isoamyl alcohol at 25:24:1 to the dewaxed tissue Mix by vortex and centrifugation at 4^{0}C, 12,000 rpm for 10 minute. Transfer the supernatant to the another microtube and add similar volume of chloroform to the supernatant, mixed by vortexing, centrifuged at 12,000 rpm for 5 min and carefully remove the upper aqueous supernatant to another fresh microtube. Adding 0.1 volume of 3 M sodium acetate to the new tube and add 1 volume of isopropanol, and incubate at -70^{0}C for 3 hour. Discard the supernatant fluid after centrifuged and wash once with 75% ethanol. Dissolve the final yield of DNA in nuclease and protease free molecular grade water and store at 4^{0}C.

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- 1.83 (260nm/ 280nm) from the extracted DNA samples of 10 breast carcinoma patients by Phenol: Chloroform: Isoamylalcohol method.

RESULT AND DISCUSSION- A total of 10 number suspected breast carcinoma tissue samples were subjected to hematoxyline and eosin stain for confirmation and found to be positive under microscope. The hematoxyline is a basic dye and stained the nucleus thats why it was appear blue (Figure-1). On the other side, eosin is a acidic dye and stained the cytoplasm thats why it was appear pink (Figure-2).

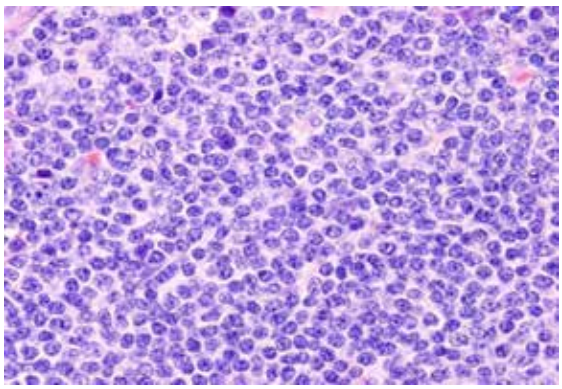

Figure 2 - Hematoxyline staining of breast cancer sl ide.

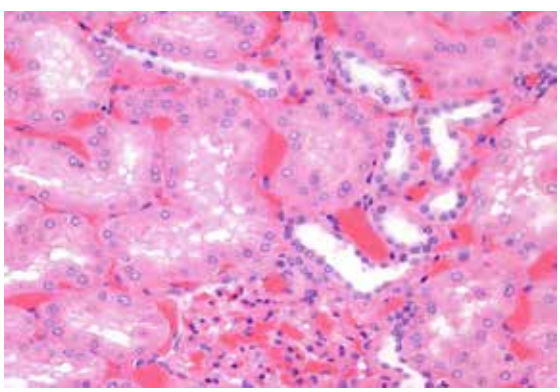

Figure 3- Eosin staining of breast cancer slide

The DNA samples were isolated from the tissue samples of breast carcinoma patients. The Purity of DNA samples by spectrophotometer were suggested the quality of DNA. The isolated DNA samples were electrophoresed on 0.8% agarose gel (Figure-4).

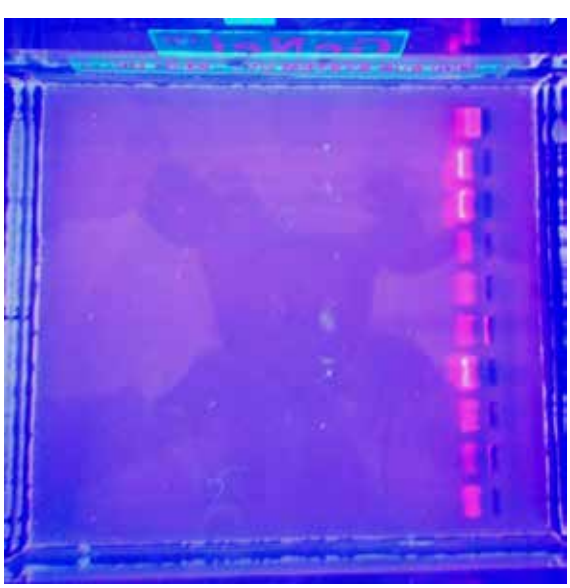

Figure 4- DNA samples on 0.8% agarose gel.

CONCLUSION- This article describes a very simple and efficient method for DNA extraction from Formalin-Fixed, Paraffin-Embedded tissue blocks in order to carry out Molecular analysis. Most clinical tissue samples are routinely fixed in formalin and embedded in paraffin wax. This process is essential for archiving purposes and to maintain excellent cell morphology. Regarding the tissue fixation by formalin, it is known that denaturation and modification of macromolecule by formalin (e.g., alkylating and cross-linking of functional groups), leads to an insolubilization of the macromolecules, thereby minimizing the loss of nucleic acids from fixed tissues. On the other hand, the solubilization of DNA from formalin-fixed specimens is negatively correlated with the duration of formalin treatment and the yield of DNA extractions may be seriously reduced when compared to an unfixed specimen. We choose the Breast cancer in our work, because the Breast cancer remains the most common cause of death from cancer worldwide. One goal of cancer researchers has been to extract DNA from archival tissue blocks. Formalin-Fixed, Paraffin-Embedded samples are routinely and widely used for Molecular Biology research and Pathology examinations.

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