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The Study of Mutations of CDK4 and TP53 Genes in Selected Breast Lesions

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Abstract

Lesions are abnormal changes in tissue. The theories behind tumorigenesis can be genetic, epigenetic, immune surveillance and monoclonal hypothesis, which led to genetic changes. The genetic change can be the inactivation of tumor suppressor genes or the activation of oncogenes in the cell cycle. This study investigated the presence of gene mutation in CDK4 and TP53 genes in breast lesions. Ten (10) formalin fixed paraffin embedded tissue block, which includes 5 cases of fibroadenoma and 5 cases of invasive ductal carcinoma from the pathological archives were used. The nuclei amplification technique was carried out on the formalin fixed paraffin embedded tissue blocks. DNA extraction involves lysing the cells and solubilizing DNA, which is followed by chemical or enzymatic methods to remove macromolecules, lipids, RNA or proteins. Thereafter, the DNA is amplified using reagents and heated (denaturing) and cooling (annealing) steps. PCR requires free nucleotide (dNTPs), template DNA to amplify and unique single-stranded DNA primers that bind upstream (5') and downstream (3') of the DNA region of the tissue blocks. The integrity of the DNA is assessed by loading approximately 100 ng per sample on a 1.5% agarose gel and the sizes of PCR products were estimated by comparison with the mobility of a 100bp molecular weight ladder that was run alongside the samples in the gel. However, the amplified fragment was purified to remove PCR reagents and thereafter it was sequenced. The sequence of single nucleotide polymorphism of each gene was analyzed, and the results were reported. In Fibroadenoma, TP53 gene mutation recorded 0% of SNPs mutation and 0% of functional mutation. This is because fibroadenoma is a benign condition, and if there is an SNPs mutation it does not lead to a harmful functional mutation. However, in Invasive ductal carcinoma gene mutation was detected, revealing 50% of transitions and 50% of transversion of SNPs mutation and 100% missense of function mutation. This is because carcinogens have the potential to substitute one ring easier than 2 rings. In the TP53 gene, the gene mutation of both breast conditions in comparison revealed more mutation in IDC and no mutation in FA. In the CDK4 gene, single nucleotide polymorphism mutation revealed 100% indel mutation and 100% Silent functional mutation. Indel is not compatible with life thus, it proceeds to apoptosis. However, in invasive ductal carcinoma, gene mutation was detected ranging from 100% transition of SNPs mutation and 100% missense of function mutation. However, in comparison to both genes, the Invasive ductal carcinoma gene mutation prevalence is 75% and in Fibroadenoma is 25% prevalence. In cancer signatures, the transition has a rate of 33.3% and transversion has 66.6%. Transition can be point or missense, but it has 33.3% of leading to a harmful function mutation. Transversion has a 66.6% chance of leading to harmful function because the substitution of one ring is easier than 2 rings. The investigation also revealed TP53 gene is more susceptible to missense mutation, which is more susceptible to cancer and in a correlation of both genes a complication of one can lead to another. From this study, the analysis demonstrated missense

mutation was more in both genes compared to the sequence of FA and IDC. From this study, both genes showed segments of mutation which were either SNPs or functional mutation. It showed that both genes were modified from a proto-oncogene to an oncogene and the protein it produces is an oncoprotein and as such it favours the alteration of both genes led to cell proliferation. The data generated from this research has highlighted the importance of carrying out further studies in assessing both genes to understand their involvement in the etiology of carcinogenesis which will help in clinical information about breast cancer. In line with the findings, there is an urgent need to understand carcinogenesis which may serve in early diagnosis and understanding of breast conditions and further help prevent or delay the onset of chronic complications and reduce morbidity and mortality rates to improve and assure the health of individuals.

Keywords: Mutation, TP53, CDK4, Breast Lesion, Fibroadenoma, Invasive Ductal Carcinoma.

Introduction

Background of Study

The word 'lesion' comes from the Latin word 'Laesio' which means 'attack or injury'. Lesions occur due to any disease or injury. They are abnormal changes in a tissue or organ. Breast lesions can be classified into Benign or Malignant lesions, Benign or malignant lesions in the breast are the two most important terms to know for understanding breast injuries (Mohiyuddin *et al*., 2022). Benign breast lesions are non-cancerous, it is characterized by the formation of lumps but do not lead to cancer, they occur in a vast majority of the breast but are often neglected because they are not as dangerous as malignant lesions (Stachs *et al.,* 2019). These types of lesions do not spread but should be removed according to their size and location. They are also removed due to their abnormal appearance. Malignant breast lesions, on the other hand, are cancerous and are of serious threat to health after a biopsy, this is because they can be aggressive (Punitha *et al*., 2018). They are characterized by progressive and uncontrolled growth that spread throughout the body through the circulatory system (Mohiyuddin *et al.*, 2022) and are the leading diagnosed cancer worldwide (Cao *et al*., 2021) and the most common cause of cancer death for women worldwide (Azamjah *et al*., 2019). A Lesion describes any area of damaged tissue. All tumors are lesions, but not all lesions are tumors. A tumor is a mass or group of abnormal cells that form in the body. A tumor isn't necessarily cancer. Many tumors are benign (not cancerous). Tumors can form throughout the body. They can affect bone, skin, tissues, glands and organs. Neoplasm is another word for tumor. Breast cancer is a type of cancer that starts in the breast. It can start in one or both breasts. When cells begin to grow out of control in the breast, Breast cancer develops (Patil & Biradar, 2021). Breast cancer occurs almost entirely in women (Wang *et al*., 2019), on rare occasions, with a low prevalence of about 1% of all diagnosed cases, men can also get breast cancer (Konduri *et al.*, 2020). It's important to understand that most breast lumps are benign (Stachs *et al*., 2019) and not cancer (malignant) (Namazi *et al*., 2017). Non-cancer breast tumors are abnormal growths, but they do not spread outside of the breast. They are not life-threatening, but some types of benign breast lumps can increase a woman's risk of getting breast cancer. Any breast lump or change needs to be checked by a health care professional to find out if it is benign or malignant (cancer), to decrease the death rate, it is important that breast lumps need to be detected in an early stage (Patil & Biradar, 2021). Breast cancers can start from different parts of one or both breasts (Waks & Winer, 2019). The breast is an organ that sits on top of the upper ribs and chest muscles. There is a left and right breast and each one has mainly glands, ducts, and fatty tissue. In women, the breast makes and delivers milk to feed newborns and infants. The amount of fatty tissue in the breast determines the size of each breast. The breast has different parts, the Lobules are the glands that make breast milk and cancers that start here are called lobular cancers. The Ducts are small canals that come out from the lobules and carry the milk to the nipple (Samineni *et al*., 2022), and this is the most common place for breast cancer to start, cancers that start here are called ductal cancers. Lobular cancer and ductal cancers are more commonly diagnosed types of breast injuries (Samineni *et al*., 2022). The nipple is the opening in the skin of the breast

where the ducts come together and turn into larger ducts so the milk can leave the breast. The nipple is surrounded by slightly darker thicker skin called the areola. A less common type of breast cancer called Paget disease of the breast can start in the nipple. The fat and connective tissue (stroma) surround the ducts and lobules and help keep them in place. A less common type of breast cancer called phyllodes tumor can start in the stroma (Samineni *et al*., 2022). Blood vessels and lymph vessels are also found in each breast. Angiosarcoma is a less common type of breast cancer that can start in the lining of these vessels. (Namazi *et al*., 2017). A small number of cancers start in other tissues in the breast. These cancers are called sarcomas and lymphomas and are not thought of as breast cancers.

There are many different types of breast cancer. The type is determined by the specific kind of cells in the breast that are affected. Most breast cancers are carcinomas (Thamilselvam, 2019). The most common breast cancers such as ductal carcinoma in situ (DCIS) and invasive carcinoma are adenocarcinomas since the cancers start in the gland cells in the milk ducts or the lobules (milk-producing glands) (Samineni *et al*., 2022). Other kinds of cancers can grow in the breast, like angiosarcoma or sarcoma, but are not considered breast cancer since they start in different cells of the breast. After a biopsy is done, breast cancer cells are tested for proteins called estrogen receptors and progesterone receptors, and the HER2 gene or protein. The tumor cells are also closely looked at in the laboratory to find out what grade it is. The specific proteins found and the tumor grade can help decide the stage of cancer and treatment options (Vieira & Schmitt, 2018). A mutation is an alteration in the nucleic acid sequence of the genome of an organism, virus, or extrachromosomal DNA (Sfeir *et al*., 2015). Viral genomes contain either DNA or RNA. Mutations result from errors during DNA or viral replication, mitosis, meiosis or other types of damage to DNA (such as pyrimidine dimers caused by exposure to ultraviolet radiation), which then may undergo error-prone repair (especially microhomology-mediated end joining), however, attempted repair may be erroneous, resulting in the DNA repair system placing a wrong nucleotide in place of the lesion or cause an error during other forms of repair, or cause an error during replication such as trans lesion synthesis (Seplyarskiy & Sunyaev, 2021). Mutations may also result from the insertion or deletion of segments of DNA due to mobile genetic elements. (Rodgers *et al*., 2016). Mutation can result in many different types of changes in sequences. Mutations in genes can have no effect, alter the product of a gene, or prevent the gene from functioning properly or completely. Mutations can also occur in non-genic regions. The tumor suppressor genes in a healthy cell work together with another class of genes, called proto-oncogenes, to control cell reproduction. Tumor suppressor genes code for proteins that restrain cell growth and proto-oncogenes specify proteins that stimulate cell growth. Tumor suppressor genes and proto-oncogenes regulate cell division (Nagaraja & Nagarajan, 2021). Cancer occurs as a result of mutations in these genes that are responsible for DNA repair, cellular proliferation, and cell cycle checkpoints, which result from the unbalanced equilibrium of oncogenes and tumour suppressor genes that cause uncontrolled growth and invasive migration of the cells (Coskun *et al.*, 2022). A mutation in one gene alone does not cause a malignant tumor to develop; several genetic insults occurring in a few different genes over time are necessary for a cell to transform into a malignant state, for example, a proto-oncogene becomes a cancer-causing oncogene when mutated in a manner that increases the cell's propensity to divide excessively. For a cell to give rise to cancer, other mutations, such as damage to a tumor suppressor gene, must arise (Wang *et al*., 2018). In breast cancer, alterations in several cell cycle regulatory proteins have been described, including various cyclins, CDKs, and the RB gene product (pRb). Evidence indicates that dysregulation of the cyclin D1:CDK4 axis has a role in breast cancer, with some tumors over-expressing cyclin D1 (Aftab, *et al*., 2018). Additionally, while not necessary for normal mammary gland development, CDK4 and cyclin D1 are required for the induction of breast malignancies in mouse models, suggesting that CDK4 inhibition may inhibit breast cancer cells while sparing healthy tissues (Kashyap *et al*., 2021). Subsequent research

revealed that mutations in this gene also play a role in cancers of the bone, lung, breast, cervix, prostate, and bladder. Several other tumor suppressor genes (such as TP53, which encodes a protein known as p53) and other protooncogenes (CDK4/6) have been identified (Aftab, et al., 2018). The mutated form of TP53 has been implicated in more than 50 per cent of all cancers. Mutations in two other tumor suppressor genes, BRCA1 and BRCA2, are associated with increased susceptibility to breast cancer; they are found in 5 to 10 per cent of all cases and in about 85 per cent of all cases of inherited breast cancer (De-Talhouet *et al*., 2020).

Research Methodology

A total number of ten cases (Five cases of Fibroadenoma and Five cases of Invasive ductal carcinoma) was used for this controlled retrospective study.

DNA Extraction

This protocol used for DNA extraction from human tissue was developed by Dellaporta *et al*. In 1983 and some modifications were added to it according to Odeyemi *et al.,* 2018).

The tissue was ground into powder in a sterile mortar and pestle, 500ul of preheated extraction buffer was added to the powder, the mixture was then transferred into a sterile Eppendorf tube and 33 µl of 20% Sodium Dodecyl Sulphate (SDS) was added to the sample. The sample was subsequent shaken vigorously and allowed to stand on the ice. Thereafter it was incubated in a water bath at 65°C for 30 minutes (with inversion each 5 minutes). The sample was left at room temperature and 10ul of 5 M potassium acetate (pH 8) while vortexing the sample was incubated on ice for 30 min. The sample was left at Room temperature and centrifuged at 12200 × g (13000 rpm) for 30 min at 10°C. The supernatant was transferred to a new sterile 2 ml tube and 5 µl of RNAase (10 mg/ml) was added to it, it was then incubated at 37°C for 30 min. An equal volume of cold isopropanol was added to the tube it was mixed gently and incubated at -20°C for 1 h. It was later Centrifuged at 12200 × g (13000 rpm) for 20 min at 4°C. The supernatant was discarded, and the pellet was washed 2 times with ethanol 70%, (500 µl, 9500 × g (10000 rpm), 5 min). The pellet was allowed to completely dry and dissolved in 600 µl of TE (incubation at 45°C can be used for acceleration). The sample was centrifuged at 12200 × g (13000 rpm) for 10 min at 10°C and the supernatant was transferred to a new 1.5 ml tube, an equal volume of cold isopropanol with 1/10 volume sodium acetate (3 M, pH5.2) was added and the tube inverted gently. Thereafter, incubated at -20°C for 1 hour and centrifuged at 12200 × g (13000 rpm) for 20 min at 4°C. The supernatant was discarded, and the pellet was washed 2 times with ethanol 70%, (500 µl, 9500 × g (10000 rpm), 5 min). The pellet was allowed to completely dry and dissolved in 50 µl of ddH2O (Odeyemi *et al.,* 2018).

Polymerase Chain Reaction (PCR)

PCR sequencing preparation cocktail for all PCR consisted of 10 µl of 5x GoTaq colourless reaction, 3 µl of 25mM MgCl2, 1 µl of 10 mM of the dNTPs mix, 1 µl of 10 pmol each primer (table 1) and 0.3units of Taq DNA polymerase (Promega, USA) made up to 35 µl with sterile distilled water 15µl DNA template. PCR was carried out in a GeneAmp 9700 PCR System Thermocycler (Applied Biosystem Inc., USA) with a PCR profile for each primer (Batista *et al.*, 2020).

Integrity of the Amplified Gene

The procedure for the integrity of the amplified gene fragment was performed according to Odeyemi *et al*. (2018) and it was checked on a 1.5% Agarose gel. The buffer (1XTAE buffer) was prepared and subsequently used to prepare 1.5% agarose gel. The suspension was boiled in a microwave for 5 minutes. The molten agarose was allowed to cool to 60°C and stained with 3µl of 0.5 g/ml ethidium bromide (which absorbs invisible UV light and transmits the energy as visible orange light). A comb was inserted into the slots of the casting tray and the molten agarose was poured into the tray. The gel was allowed to solidify for 20 minutes to form the wells. The 1XTAE buffer was poured into the gel tank to barely submerge the gel. Two microliters (2 l) of 10X blue gel loading dye (which gives colour and density to the samples to make it easy to load into the wells and monitor the progress of the gel) was added to 4µl of each PCR product and loaded into the wells after the 100bp DNA

ladder was loaded into well 1. The gel was electrophoresed at 120V for 45 minutes visualized by ultraviolet trans-illumination and photographed. The sizes of the PCR products were estimated by comparison with the mobility of a 100bp molecular weight ladder that was run alongside experimental samples in the gel.

Purification of Amplified Product

After gel integrity, the amplified fragments were ethanol purified to remove the PCR reagents. Briefly, 7.6 µl of Na acetate 3M and 240 µl of 95% ethanol were added to each about 40µl PCR amplified products in a new sterile 1.5 µl tube Eppendorf, mix thoroughly by vortexing and keep at -20°C for at least 30 min. Centrifugation for 10 min at 13000 g and 4°C followed by removal of supernatant (invert tube on trash once) after which the pellet was washed by adding 150 µl of 70% ethanol and mix then centrifuge for 15 min at 7500 g and 4°C (Mandrekar, 2016). Again, remove all supernatant (invert tube on trash) and invert tube on a paper tissue and let it dry in the fume hood at room temperature for 10-15 min. then re-suspend with 20 µl of sterile distilled water and kept at -20oC before sequencing. The purified fragment was checked on a 1.5% Agarose gel ran on a voltage of 110V for about 1hour as previously, to confirm the presence of the purified product and quantified using a nanodrop of model 2000 from Thermo-scientific (Mandrekar, 2016).

Sequencing

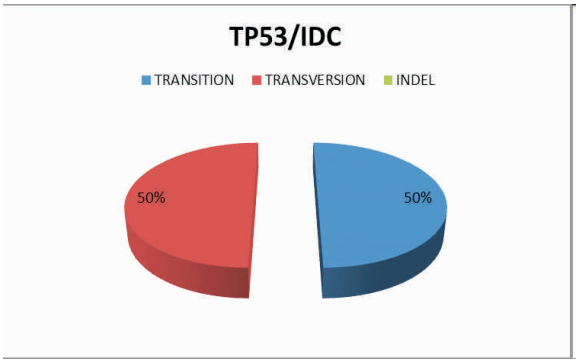
The sequencing procedure was similar to the procedure used by Njoko *et al.* (2020). Whereby the amplified fragments were sequenced using a Genetic Analyzer 3130xl sequencer from Applied Biosystems using the manufacturers' manual while the sequencing kit used was that of BigDye terminator v3.1 cycle sequencing kit. Bio-Edit software and MEGA 6 were used for all genetic analyses.

Results

Summary table showing the effect of mutation along the TP53 Gene.

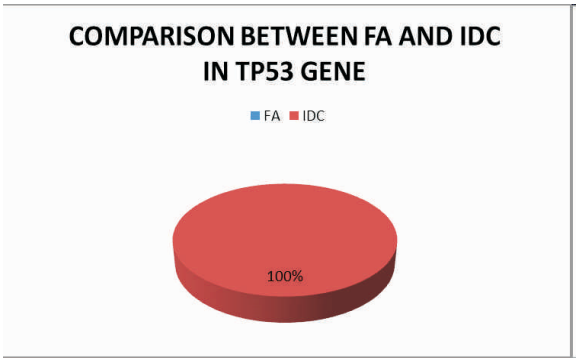
DESCRIPTION	LOCATION	SPECIMEN	GENE TYPE	MUTATION TYPE	MUTATION TYPE DESCRIPTION
G: A	24(7:3)	IDC	TP53	Transition	Missense mutation changing Glycine to aspartate.
G: C	64(9:1)	IDC	TP53	Transversion	Missense mutation changing Arginine to serine.

A Pie chart showing the Freq uency of S NP Mutation Freq uency of Tp53 Gene in Finroadenoma.



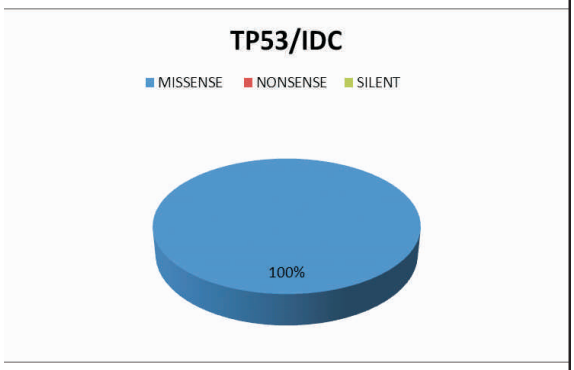
This chart shows the result of TP53 single nucleotide polymorphism mutation in Invasive ductal carcinoma, there are 50% transition mutation and 50% transversion mutation in Invasive ductal carcinoma.

A Pie-Chart of Comparison between FA and IDC Mutation Frequency in The TP53 Gene.



This chart shows the comparison between fibroadenoma and invasive ductal carcinoma mutation frequency in the TP53 gene, this shows that there is 100% mutation in IDC and none in FA.

A Pie chart showing the Frequency of Functional Mutation in TP53/IDC

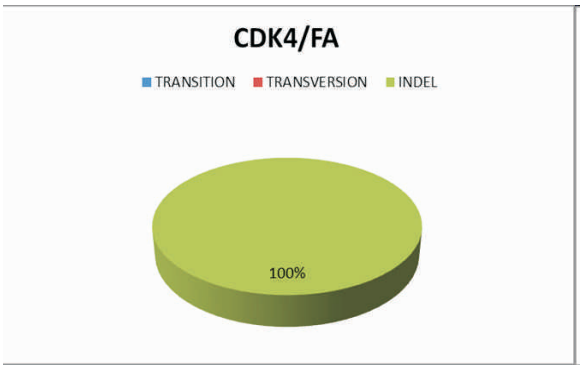


The Pie chart demonstrates the value frequency of functional mutation in IDC of TP53 gene which is 100% missense mutation and 0% 0f silent and 0% nonsense mutation.

Summary table showing the effect of mutation along the CDK4 gene

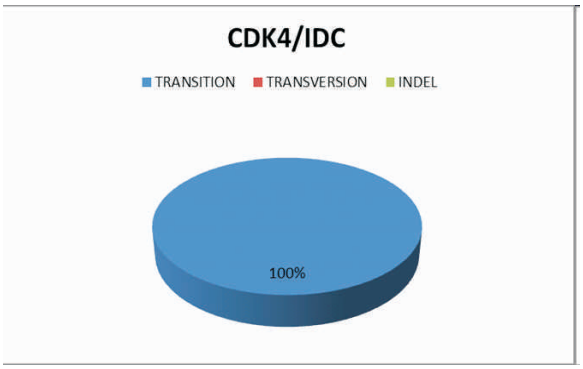
GENE TYPE	DESCRIPTION	LOCATION	SPECIMEN	MUTATION TYPE	MUTATION TYPE DESCRIPTION
CDK4	C	95(9:1)	FA	Indel	Deletion of Alanine.
CDK4	C:T	248(9:1)	IDC	Transition	Missense mutation changing Serine to phenylalanine.

A Pie Chart Showing the Frequency of SNP Mutation of CDK4 in Fibroadenoma.



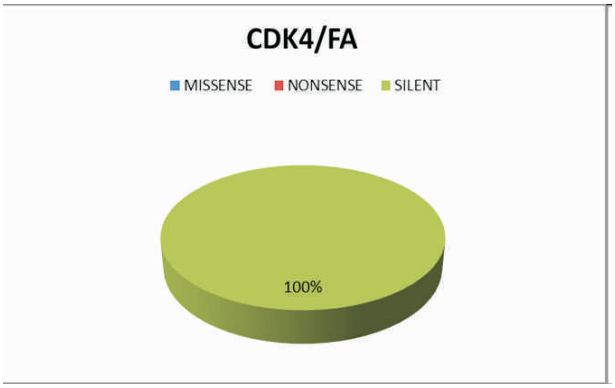
This chart shows the result of CDK4 single nucleotide polymorphism mutation, there is a 100% Indel mutation in Fibroadenoma.

A Pie Chart Showing the Frequency of SNP Mutation of CDK4 in invasive Ductal Carcinoma.



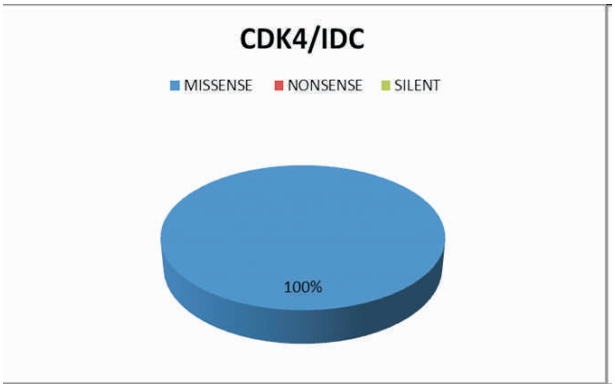
This chart shows the result of the CDK4 gene demonstrating 100% Missense mutation in Invasive ductal carcinoma.

A P ie Char t Showing the Frequency of Functional Mutation in CDK4/FA

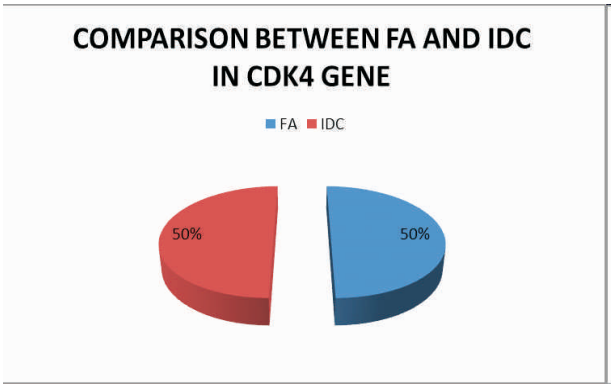
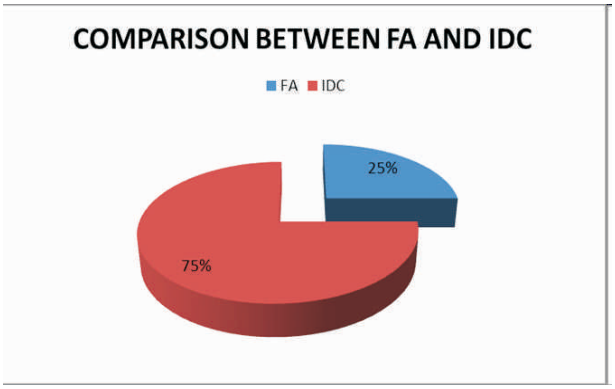


The Pie chart demonstrates the value frequency of function mut ation in FA of CDK4 gene which is 0% missense mutation and 100% 0f silent and 0% nonsense mutation.

A Pie Chart Showing the Frequ ency of Functional Mutation in CDK4/IDC.



The Pie -chart demonstrates the value frequency of function mutation in IDC of CDK4 gene which is 100% missense mutation and 0% 0f silent and 0% nonsense mutation.

A Pie Chart of Comparison Between F A And I DC Mutation Frequency in CDK4 Gene.	A Pie Chart of Comparison Between FA and IDC Mutation Frequency.
	
This chart shows the comparison between fibroadenoma and invasive ductal carcinoma mutation frequency in CDK4 genes, this shows that there is a 50% mutation in IDC and a 50% mutation in FA.	This chart shows the comparison between fibroadenoma and i nvasive ductal carcinoma mutation frequency between two genes of interest, this shows that there was more mutation in IDC than in FA.

Discussion

Using Polymerase chain reaction (nuclei acid amplification technique) in the study and diagnosis of benign and malignant lesions has been studied and serves as an efficient mode in providing clinical information about tumors. Breast lesions can be classified into either benign or malignant lesions, benign or malignant lesions in the breast are the two most important terms to know for understanding breast injuries (Mohiyuddin *et al*., 2022). In benign lesions, cells form lumps but do not lead to malignancy (Stachs *et al*., 2019). They occur in a vast majority of the breast but are often neglected because they are not as dangerous as malignant lesions. A malignant lesion is cancerous and can be threatening to the health after a biopsy (Punitha *et al*., 2018). They are characterized by progressive and uncontrolled growth. Breast cancer is a global condition with a yearly incidence of over 1. 3 million accounting for over 25% of all malignancies (Finn, 2016).

Ten (10) cases were recruited prospectively for this research; among the 10 cases include 5 cases of Fibroadenoma (FA) and 5 cases of Invasive Ductal Carcinoma (IDC). The 10 cases were analyzed to study the gene mutation in TP53 and Cyclin-dependent kinase 4 (CDK4) genes. In the TP53 gene, the Fibroadenoma showed no mutation for both Single Nucleotide Polymorphisms (SNPs) and Functional mutation. However, in Invasive ductal carcinoma cases, the evaluation reported G – A 50% transition and A- G 50% transversion gene mutation. This is in agreement with Begona et al. (2019) which showed the base transition (purine - pyrimidine and pyrimidine – purine) was more frequent than transversion (pyrimidine-pyrimidine and purine – purine) in 34.5% (10/29) exploring clinicopathological variants such as N stage and hormone receptor states associated with the detectability of tumour-derived mutations in blood and somatic mutations. Receptors are proteins in cells that attach certain substances in the blood. Hormone receptors (estrogen and progesterone) attach themselves to receptors and stimulate cancer to grow thus, this may be a result of exposure to ultraviolet radiation or certain chemicals. These cancers are then called hormone receptor-positive or hormone receptor-negative. This is at variance with an investigation carried out by Rogoża-Janiszewska *et al*. (2021) which showed the prevalence of germline pathogenic variants in TP53 among early-onset breasts with a parent,

and it indicated 4/100 TP53 pathogenic variant onset in breast tumor patient with a positive malignancy family history. None of the four variants appeared to be a recurrence. No large insertion and deletion were formed and thus were found to be rare. A genetic alteration that increases an individual's susceptibility or predisposition to cancer. When such a variant is inherited, there's the development of symptoms is more likely, but not certain. Also in the IDC case, a functional mutation was detected, the analysis listed a 100% missense mutation, changing glycine to aspartate. The record agrees with Abeer *et al*. (2011). Of which 40 of 119 individuals were (33.67%), 28 (59.57%) recorded missense mutation, 6(12.77%) were silent, 5 (10.64%) were nonsense (stop) mutation and 3 (6.38%) deletions were discovered in the intron-exon intersection. This variation in p53 mutation in breast cancer may be because of factors such as the ethio-geographically dispersed population's examined exposure to different carcinogens, size of the examined population, lifestyle and dietary habits. Highlighting G: C - A: T is caused by spontaneous deamination of methylene cytosine and G: C - AT can be produced by numerous carcinogens in particular oxidizing agents and alkylating groups agents such as N – nitride compounds. This study is at variance with Xuerui *et al*. (2020). It aimed to delineate the variation rates and characteristics of TP53 through NGS in a large cohort comprising 411 Chinese breast cancer cases and compared it with the data in a METABRIC cohort. 19 mutations were detected in Chinese cases, clustered into the DNA binding region of TP53 (exon 5-8) and nearly 20% of the variations detected in this cohort were identified in the coding region beyond exons 5–8. It suggested an association of TP53 anomalies with the prognostic status of cancer patients. Compared with the missense mutations of TP53, the nonsense mutations displayed a stronger association with poor prognosis in the cases. The TP53 mutations were associated with endocrine resistance. The estrogen receptor (ER) pathway plays a pivotal role in breast cancer development and progression. Mutations of ER, as well as crosstalk with bypass pathways, cause endocrine resistance.

In CDK4 gene, FA cases, the sequence was analyzed, and SNPs recorded 100% indel inserting of C. This agrees with a study carried out by Condorelli *et al*., (2018). This study identified 3 cases that had pre- and post-genotype have in tissue and peripheral blood samples after receiving CDK4/6 inhibitors,1 substitution, 19 deletions and 3 insertions were recorded in cases highlighting the emergence of somatic mutation in RB after exposure to Palbociclib or ribocicilib. A deletion mutation occurs when part of an a DNA molecule is not copied during DNA replication. This uncopied part can be as small as an entire chromosome. The loss of this DNA during replication can lead also lead to a genetic disorder. Insertion is the addition of one or more nucleotide base pairs into a DNA sequence. This can lead to an effect in microsatellite regions due to DNA polymerase slipping.

In cdk4, FA cases recorded functional mutation as 100% silent alteration deletion of alanine. This agrees with a study by Farma *et al.* (2022). which demonstrated in 200 individuals the alterations in retinoblastoma gene targeting exon a harbouring acetylate residues it record 11 mutations present in Exon 19 were reported mutation at g.46521 (T>C) were found to be a T>G alteration. The exon-19 mutations were more frequent in well-differentiated (GIII) and moderately well-differentiated (GII) tumors. ˜37% (74/200) of breast tissues and ˜35.5% (71/200) of blood samples were found mutated for this exon, which is alarmingly high. Statistically, the difference in mutations was found significant ($p < 0.0001$) among both blood and tumour tissues compared to their ANCT controls with an odds ratio of 15.18 and 22.91 respectively. This is at variance with Abid et al. (2016). In this study,1 silent mutation in 200 individuals demonstrating cyclin D1 and cdk4 genes was recorded to evaluate their association with breast cancer. In the CDK4 gene, IDC cases were analyzed, and it recorded 100% transition C: T. This supports Mahjabeen *et al*. (2022) which reported G> T in 3.68% and C >t IN 3.78% in 400 individuals (200 healthy control and 200 individuals). This study is in an argument with Taji *et al*. (2022). Which links understanding the human genome and cdk4/6. However, both studies start with cyclin D1 and CDK4 mutation

are associated with an increased risk of breast cancer. However, in the CDK4 gene, IDC recorded functional mutation with a frequency range of 100% missense mutation, changing serine to phenylalanine. This agrees with the study done by Qaiser *et al*. (2022). The study highlighted that Exon-21 was highly mutated in the Rbl2 gene.94 out of 200 samples that account for 47% of the study cohort (blood) were found mutated for this exon, whereas 50% (100/200) tissue samples were also positive for this exon mutation. This highlights the significance of this mutation in determining the overall prognosis of the disease which is in variance with a study done by Ayesha et al. (2019). Studies focus on the role of CDKN2A in breast cancer.42.8% were entire gene deletions, while 24.2% were missense mutations. The deletion/malfunctioning of CDKN2A in different tumors including breast cancer has recently led to the discovery of many clinical CDK inhibitors. The missense mutation of the IDC case was a result of germline mutations and 2 hit hypotheses were two alleles are inactivated either through mutations or through epigenetic silencing to cause a phenotypic change.

This study was also designed to correlate between TP53 and CDK4 in Fibroadenoma and Invasive ductal carcinoma, invasive ductal carcinoma gene mutation is a prevalence of 75% and in fibroadenoma is 25% prevalence.

This study further revealed that the TP53 gene is more susceptible to missense mutation which is more susceptible to cancer. Our findings also demonstrated that a complication of one gene can lead to another. This agrees with a study by Albiruni *et al*. (2020) which demonstrated MDM2 contributes to transformation and support the role of CDK4 in opposing TP53 function and Dhivya & Rajesh, (2022) which showed that genes in breast cancer have the potential to become a useful target for diagnosis in breast cancer.

Conclusion

This investigation demonstrated Missense mutation is more recorded in both genes compared to the sequence of Fibroadenoma and Invasive ductal carcinoma. This research found that the correlation between TP53 and CDK4 implied that a complication to one can lead to another. The data generated from this research has highlighted the importance of carrying out further studies in assessing both genes to understand their involvement in the etiology of carcinogenesis which will help in clinical information about breast cancer.

Recommendations

In line with the findings from this study, there is an urgent need to understand carcinogenesis, which may serve in the early diagnosis and understanding of breast lesions and further help prevent or delay the onset of chronic complications and reduce morbidity and mortality rates to improve and assure the health of individuals.

Conflict of Interest

None declared.

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APPENDIX

Components used in DNA Extraction

S/N	COMPONENTS	NEW PROTOCOL
1	Nacl	1.5M
2	B- marcaptoethanol	Excluded
3	Sodium metabi-sulfate	0.5%(w/v)
4	Polyethyleneglycol	10%(w/v)
5	Polyvinylprolidore	1-2%(w/v) [1]

Gene Sequences for CDK4 and TP53

Gene name	Primer name	Primer sequence	PCR Profile
P53	P53F	TGGAAGAAATCGGTAAGAGGTG	An initial denaturation at 94c for 5 minutes, followed by a 30 cycles consisting of 94^0c for 30 seconds, 53c for 30seconds and 72c for 1 minute, and a final termination at 72c for 10 minutes.
		CATCTTGGGCCTGTGTTATCT	
	P53R		
CDK4	CDK4F	CTACATAA GGATGAA GGTAA TCCGGAGTGA	An initial denaturation at 94c or 5 minutes, followed by 30 cycles consisting of 94c for 30 seconds , 51c for 30 seconds and a final termination at 72c for 10 minutes.
		GGAAAGGGACAAGAGGGGAACATAC	
	CDK4R		

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