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| **Original Article** |

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| **Mediator Complex in Fibro-adenoma and Phyllodes Tumour of Breast** |

**Role of Mediator Complex Subunit 12 Mutation in Fibro-adenoma and Phyllodes Tumour of Breast**

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**ABSTRACT**

**Objective:** To determine the role of mediator complex subunit 12 mutation in fibro-adenoma and phyllodes tumour of breast.

**Study Design:** Retrospective observational

**Place and Duration of Study:** This study was conducted at the department of Pathology, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre, from January, 2016 to December, 2020.

**Materials and Methods:** A total of 52 cases of fibro-adenoma and 5 cases of phyllodes tumour of breast were received and evaluated for mediator complex subunit 12 gene mutations by using polymerase chain reaction method. The results were analyzed using SPSS version 22.

**Results:** Out of 52 cases of fibro-adenoma only 17 (32%) found to be positive in gene mutation whereas 3(60%) cases out of 5 of phyllodes tumour were positive. The phyllodes tumour was divided into benign, borderline and malignant. Cases of borderline and malignant phyllodes tumour could not be retrieved in our study

**Conclusion:** Present study concludes that MED12 gene exon 2 mutation in fibro-adenomas and benign phyllodes tumors of breast is not substantial.

**Key Words:** Mediator Complex Subunit 12 (Med 12), Fibro-adenoma, Phyllodes tumour, Polymerase Chain Reaction (PCR)

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**INTRODUCTION**

MED12 is a gene located on chromosome X at position q13. It is believed that MED12 protein is involved in early development of cells and chemical signaling pathways within the cells (Kämpjärvi, Mäkinen et al. 2012).1 MED12 gene gives instruction to form a protein called mediator complex subunit 12. This protein makes one subunit of mediator complex, which is a group of 25 proteins which work together for gene regulation. The mediator complex links transcription factor with an enzyme called polymerase II.

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Once the transcription factors are attached, the enzyme initiates gene transcription (Kämpjärvi, Mäkinen et al. 2012).1 MED12 along with MED13, Cyclin C (CycC), and either CDK8 or CDK19 forms the kinase part that reversibly links with the core Mediator. MED12 starts the kinase action of CDK8 by linking the contact between MED13 and CycC-CDK8. The kinase-exciting activity of MED12 depends on its direct contact with CycC. CycC is an extremely conserved cyclin family member which consists of a negatively charged surface groove mediating its CDK8 attachment in addition to a CycC precise surface for MED12 attachment. MED12 binds to CycC through its N-terminus determined largely by exons 1 and 2 where the hotspot mutations commonly occur in hormone dependent tumors (Kämpjärvi, Mäkinen et al. 2012) 1 (Zhang, O’Regan et al. 2020)2 (Alkutbi, Ameen et al. 2021).1

It is believed that MED12 protein is involved in early development of cells and chemical signaling pathways within the cells, such as cell growth, cell movement and cell differentiation MED12 gene (Kämpjärvi, Mäkinen et al. 2012)1. Mutations in MED12 genes lead to various conditions such as schizophrenia, FG syndrome, Lujan syndrome and Ohdo syndrome which are characterized by intellectual disability, behavioral problems, hypotonia, imperforate anus, tall stature and distinctive facial features (Vulto-van Silfhout, De Vries et al. 2013).4

Fibro-epithelial tumors of breast include fibro-adenoma and phyllodes tumor. They are biphasic tumors and arise from epithelial and stromal components of breast. Fibro-adenoma is the most common benign tumor of breast. Phyllodes tumors are occasional fibro-epithelial lesions (Piscuoglio, Murray et al. 2015).5 Based on histological features which include margins (pushing or infiltrating), stromal cellularity and atypia, stromal overgrowth (absent, slight, or severe), and the number of mitosis per high power field, phyllodes tumor can be classified into benign, borderline and malignant groups (Tan, Acs et al. 2016)6.

MED12 mutations are commonly seen in estrogen-dependent benign growths and in several malignant lesions, proposing that mutation of MED12 could be a tumor beginning incident(Lim, Ong et al. 2014).7 Somatic mutations in MED12 genes are found in uterine leiomyomas and leiomyosarcomas (Nagasawa, Maeda et al. 2015)8. The present study was designed to evaluate MED12 mutation in fibro-adenoma and phyllodes tumor of breast. Since MED12 gene is involved in cell development, somatic mutation in this gene may be involved in the development of these tumors of breast.

**MATERIALS AND METHODS**

The present study is an observational, retrospective study carried out at the department of Pathology, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Center Karachi from 1st January 2016 to 31st December 2020. Non probability purposive sampling technique was used to select cases. Paraffin embedded tissue blocks and slides were retrieved of cases diagnosed as fibroadenoma, phyllodes tumor received at the department of Pathology, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Center Karachi. All cases were anonymized before selection. Total 57 diagnosed cases were selected which include 52 cases of fibroadenoma and 5 cases of benign phyllodes tumor with the help of senior pathologist. Selected cases were evaluated for MED12, exon 2 gene mutation by using polymerase chain reaction. The DNA was extracted from formalin fixed paraffin embedded tissue blocks. The DNA purification from tissue was carried by using Epicenter Kit (MCD 85201) and the protocol was followed accordingly. PCR was performed in a tube containing 200µl of reaction mixture made up of the following components: 20pmol of each primers (Forward and reverse) 500µm of four deoxynucleotides, 2.5 U of Taq polymerase (Promega), 10 X PCR buffer containing and 1.5Mm MgCl2. Primer pair that amplifies a 278bp fragment encompassing exon 2 of the MED12 gene was employed

Forward 5’-TGTTCTACACGGAACCCTCCTC-3’ 278 bp

Reverse 5’-CTGGGCAAATGCCAATGAGAT-3

The amplified product was compared with 100-bp DNA ladder (GibcoBRL, Life Technologies) and mutation was detected and compared with control positive and negative cases.

**RESULTS**

**Table No.1: Frequency of Med 12 Mutation in Selected Cases Subjected To PCR** (n= 57)

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| MED12 | Fibro-adenoma(n=52) | Phyllodes Tumor(n=5) |
| n (%) | n (%) |
| Positive | 17 (32.7) | 3 (60) |
| Negative | 35 (67.3) | 2 (40) |

Pearson Chi Square value 1.49, p = 0.228

Out of 52 cases of fibro-adenoma 17 (32.7%) were positive for MED12 exon 2 mutation and 35 (67.3%) were negative. Out of 5 cases of benign phyllodes tumor 3 (60%) were positive for MED12 mutation and 2 (40%) were negative. Pearson Chi Square test did not give any significant association of MED12 with subject to PCR (p=0.228).



**Figure No.1: Association of MED 12 Mutation in selected cases subjected to PCR**

**DISCUSSION**

This study showed 32.7% cases of fibro-adenoma positive for MED12 exon 2 mutation and 67.3% cases negative for mutation. (Piscuoglio, Murray et al. 2015)5, (Lim, Ong et al. 2014)7 and (Nagasawa, Maeda et al. 2015)8 reported 65%, 59% and 67% cases positive for mutation.

MED 12 exon 2 mutation respectively. MED12 exon 2 mutation have been reported in uterine leiomyomas by (Mittal, Shin et al. 2015)9, (Wu, Zou et al. 2017)10 and (Lee, Cheon et al. 2018)11 suggested that MED12 gene is a gene which interacts with estrogen receptors and its mutation is associated with dysregulated estrogen signaling and may be responsible in the development of fibro-adenoma and uterine leiomyomas. (Firdaus, Agrawal et al. 2021)12 reported that uterine leiomyomas possibly will not have a clonal origin but variants in exon-2 of MED-12 may perhaps be responsible in its development. (da Silva, Beca et al. 2022)13 reported stromal mutation of MED12 exon 2 in 17% cases of complex fibro-adenoma cases. (Je, Kim et al. 2012)14 reported that MED12 mutation appeared to be ethnically different. Mediator is a big macromolecular complex with multipurpose roles having at least 31 subunits. Hence any change in MED12 disturbing the kinase module can have negative effects on its controlling functions. Both exons 1 and 2 encode the cyclin C binding domain of MED12. Therefore, mutations in these exons disturb MED12 cyclin C binding and effect in decreased affinity for cyclin C-CDK8 and loss of mediator-associated CDK function. Therefore proper exon sequence is cruical for the protein’s performance (Alkutbi, Ameen et al. 2021)3 (Klatt, Leitner et al. 2020)15. This explains the possible pathogenesis of the cases that were negative for MED12 mutation. However, the exact cause for the pathogenesis of fibro-adenoma is still not known.

Cases for benign phyllodes tumor were 05 which were included in this study. Cases of borderline and malignant phyllodes tumor were not included. The reason was that borderline and malignant phyllodes are rare tumors therefore the cases were not found in the archives of department of Pathology, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Center Karachi. Our study showed 60% cases of benign phyllodes tumor positive for MED12 exon 2 mutation. (Nagasawa, Maeda et al. 2015)8 reported 45% positivity in benign phyllodes tumor. (Yoshida, Sekine et al. 2015)16 determined that MED12 exon 2 mutation was common among phyllodes tumor regardless of tumor grade. In contrast (Piscuoglio, Murray et al. 2015)5 and (Garcia-Dios, Levi et al. 2018)17 reported higher percentage of MED12 mutation in benign phyllodes tumor than in borderline and malignant phyllodes tumor. (Mishima, Kagara et al. 2015)18 also reported higher frequency 74.1% positivity in benign phyllodes tumor. We could not analyze borderline and malignant phyllodes tumor due to its non-availability. (Mishima, Kagara et al. 2015)18 found fibro-adenomas with polyclonal stroma and showing a focal monoclonal overgrowth of stroma. They found MED12 mutation in secondary phyllodes tumor, which originated in one case out of three cases of metachronous multiple tumors of fibro-adenoma. MED12 gene mutation was not found in primary fibro-adenoma of that case. This finding suggests that secondary phyllodes tumor was related to the primary fibro-adenoma for its pathogenesis.

**CONCLUSION**

Present study concludes that MED12 gene exon 2 mutation in fibro-adenomas and benign phyllodes tumors of breast is not substantial.

**Recommendations:**

A large sample size should be studied which include

1. Multiple fibro-adenomas from same patient, in same breast or both breasts.
2. Borderline and malignant phyllodes tumor.
3. Clinical history of the patients.

Other exons of MED 12 gene could be studied along with exon 2 in order to come to a strong conclusion

**Author’s Contribution:**

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| Data Analysis: | Muhammad Mansoor Iqbal |
| Revisiting Critically: | Ayesha Iftikhar, Mohammad Ahmed |
| Final Approval of version: | Jawed Iqbal |

**Conflict of Interest:** The study has no conflict of interest to declare by any author.

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