REVIEW ARTICLE

# GENETIC RISK FACTORS ASSOCIATED WITH GALLBLADDER CARCINOMA

**Saara Ahmad, Jibran Sualeh Muhammad*, Asra Khan, Muhammad Rizwan Khan**, Kulsoom Ghias**

**Department of Biological and Biomedical sciences, The Aga Khan University, Stadium road, Karachi, *Department of Basic Medical Sciences, College of Medicine, University of Sharjah, Sharjah, UAE, **Department of Surgery, The Aga Khan University, Karachi, Pakistan**

Gallbladder cancer (GBC) is an aggressive biliary tract cancer with wide geographic diversity. Various genetic modifications of GBC, including mutations in *p53, KRAS, p16* and retinoblastoma (*RB*) gene. Mutations in *p53* lead to carcinoma, while point mutations in *KRAS* leads to hyperplasia. *KRAS* mutations are often found in both pancreatico-biliary ducts junction and in neoplastic loci in gallbladder polyps. The survival rate for overall 5-year of GBC patient is less than 1%. GBC is crucial for diagnostic and prognostic markers and potential drug targets. Ovid-MEDLINE, PubMed, CINHAL and Google Scholar databases were searched using keywords gallbladder carcinoma, neoplasia, tumour, tumor, adenocarcinoma, biliary tract carcinoma, gene mutations, *KRAS*, *p53*, *RB,* and *p16*. Ten out of 470 research articles were finally included. It was observed that loss of heterogeneity and mutations of *KRAS*, *p53*, *p16* and *RB* involve in the disruption of cell cycle leading to continuous cell division and cancer.

**Keywords**: Gallbladder carcinoma, genetic mutations, *KRAS*, *p53*, *p16*, retinoblastoma, *RB*

**Pak J Physiol 2019;**15(4):66–70

## INTRODUCTION AND BACKGROUND

Gallbladder carcinoma (GBC) is a lethal malignancy with a range of latitude and gender inequalities, and 5-year survival for GBC is <1%.[1] The commonest symptoms of GBC are vomiting, abdominal pain and jaundice. Complete resection of the gallbladder is sometimes the only cure for the disease. However, because of a huge disparity in signs and symptoms, the disease usually spreads to other organs, such as the liver, at the time of diagnosis and is beyond any intervention.[2] Even in patients in whom surgical resection is feasible, the anatomical complexity of the portal hepatic system as well as the morbidity and mortality associated with resection and the risk of tumour metastasis are secondary to manipulation deterrents. Recurrence rates are also high in postoperative resections.[3]

With the advent of genomics, a mounting understanding of the molecular basis of different tumours is under way. However, studies in GBC are still limited. Early diagnosis of the disease is not possible and conceivable despite advances in imaging modalities.[4] To date, no conclusive biomarker has been identified for diagnosis or prognosis of GBC, as recognition is restricted by the availability of biological material for profiling. However, a variety of genetic changes are recognized to be involved in gallbladder cancer.[5] In this review, we focused on the most frequently reported mutations in *p53*, *KRAS*, *p16*, *retinoblastoma (RB)*, or protein (*pRB*) gene modifications and their clinical implications.

### Gallbladder carcinogenesis

Studies have identified three different pathways of pathogenesis in GBC; these are (i) *de novo* growth, (ii) carcinoma adenoma formation, (iii) progression of hyperplastic carcinoma associated with abnormal pancreatic-biliary duct transition (AJCPD).[6]

The mechanism of GBC occurs via two main pathways. These two paths are: one is the carcinoma sequence through dysplasia and the second is the carcinoma sequence of the adenoma formation.

#### *Dysplasia-carcinoma sequence*

In the carcinoma sequence of dysplasia, normal wild type cells are mutated by changes in normal cell genes that convert proto-oncogenes into oncogenes. This results in a step-by-step progression from normal GBC to invasive carcinoma over a period of several years. The *RAS* family (*HRAS, NRAS* and *KRAS*) of the proto-oncogene is often mutated. The most notorious gene mutation on codon 12 in the *KRAS* is generally associated with human GBC formation.[7] This review involved the collection as well as selection of comprehensive review data from past 30 years about gall bladder carcinoma and data analysis (Figure-1).

### Genetic mutations

#### *KRAS*

As already described the *KRAS* is a member of the *RAS* gene family with *HRAS* and *NRAS*.[7] Its role in the development of human carcinoma conditions has widely been accepted in the field of human oncology. *KRAS*, in particular, is considered to be one of the most frequently-affected genes in a variety of tumours[8], with mutations leading to *KRAS* wild-type amplification and triggering oncogenic conversions in the gallbladder; ovary, stomach, uterus, lung and colorectal carcinomas.[9] Table-1 shows the collective information of previous studies done on different mutation related with GBC.

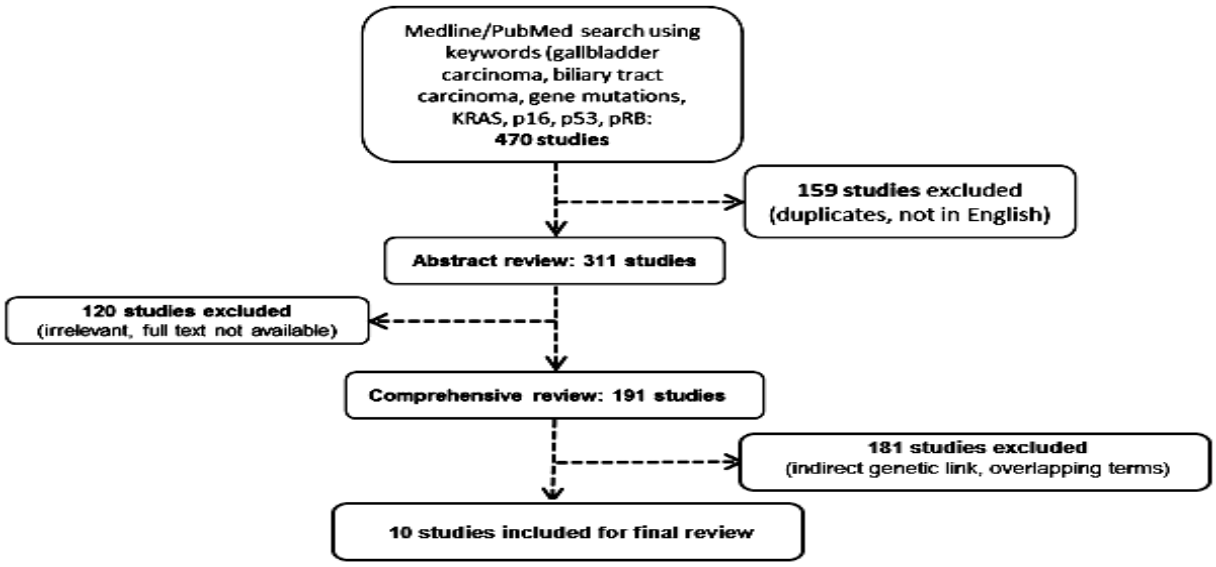


**Figure-1: Flow chart for literature search of past 30 years**

**Table-1: Genetic patterns of gallbladder carcinoma worldwide**

| Studied gene | Chromosome section | Type of study | Expression pattern | Studied population | Reference No. |
|---|---|---|---|---|---|
| *K-RAS* | 3p21 | Mutation at codon-12 (8%) | Cell signalling, regulating cell growth, differentiation and apoptosis. (0−80%) | India, Chile, Japan | [12−14] |
| | | Mutation at codon 25 exon 1 | | | [15] |
| *P53* | 17p13 | Mutation, overexpression, LOH | Expression (20−70%) | Greece, Japan, Chile, Slovenia, Chile | [16−17] |
| | | Mutation at exons 5−8 | Missense mutation | | [18] |
| | | Mutation at exon 3 of beta catenin | 62.5% (10 of 16) of adenomas | | [19] |
| *Non-Small Cell gallbladder Carcinoma (NSCGBC)* | 18q | Mutation, deletion p16 | Over expression | Japan | [20] |
| *RB* | 13q | Mutations at 2p24, 21q22, 14q22 | | | [21−22] |
| | 2p, 8q, 14p, 16p and Xp | >50% allelic loss | | | [21−22] |
| | 4q, 4p, 8q, 9q, 10p, 14q, 14 p, 19p and 21p | Equal to 50% allelic loss | | | [21−22] |

## RESULTS AND DISCUSSION

In GBC is reported that the incidence of *KRAS* mutation at codon 12 in exon 2 (GGT> GAT) ranges from zero to 80 percent.[10] This wide range can be attributed to insensitivity of use or to ethnic and geographical differences in the populations studied.[11] Also it is observed that the incidence of codon 12 mutation is significantly higher in GBC where there is a synchronous state of anomalous junction of pancreatico-biliary duct (AJPBD) than in other types.[12,13] This suggests that *KRAS* mutation detection is a useful molecular diagnostic marker for the early stage of carcinogenesis in the gallbladder with AJPBD.[14] A new *KRAS* polymorphism is detected in codon 25 (CAG> CAT; Gln25His) in exon 1. This is present both in the germ line as a GBC woven tissue and is therefore, a known pathogenic variant.[15] Research in patients with AJBPD for this polymorphism may facilitate early diagnosis of GBC.[15] However, other mutations are also identified in GBC [16−22], with KRAS predominantly in South Asian populations.[8]

*KRAS* mutations are also detected in de novo carcinomas and all other reported mutations that are associated with over expression of the *p53* gene.[23] KRAS mutations are not observed in the cases of adenomas.[24,25]

The normal protein product pathway of the *KRAS* gene is through the GTPase pathway that is involved in cell signalling mechanisms. Subsequent to mutation, the *KRAS* mutant results in the activation of mitogen-activated protein kinase (MAPK), which progresses the cell cycle through cell proliferation, cell survival, invasion, and metastasis.[26]

Interestingly, the *RASSF1A* gene on chromosome 3p21, which has also been recently classified as a tumour suppressor gene, shows to have a positive association with the recurrent allelic losses in GBC.[27] The *RASSF1A* protein has a strong homology with the *Nore1* mouse *RAS* protein which likewise acts via a GTP-dependent method and is required for receptor activation.[6,28,29]

***Adenoma-carcinoma sequence***

The carcinoma sequence through the adenoma formation includes mutations in tumour suppressor genes (TSGs) with loss of heterogeneity (LOH) on polymorphic loci flanking TSG.[30] This have been documented as features of cancer formation. In GBC, LOH often occurs on a large number of chromosome sections, including *1p, 3p, 5q, 8p, 9p, 9q, 13q, 16p, 16q* and *17p*.[31] We are interested here in the most frequent genetic changes in GBC, particularly in *p53* at the *17p13* locus[32] and in the *retinoblastoma (RB)* and *p16* genes.[33]

***p53***

The most frequently transformed tumour suppressor gene *p53* is located on chromosome 17p13[34] and leads to gallbladder dysplasia. Immunohistochemistry (IHC) shows over expression of *p53* in the initial phase of GBC.[33] A significant feature of *p53* is its predisposition to remain dormant in normal gallbladder cells. Unlike the normal protein product or *p53*, the aberrant *p53* protein is likely to accumulate in cell nuclei and can be detected by mutations in the IHC gene.[34] *p53* leading to LOHs noted in more than 50% of the GBC cases.[17] *p53* mutations are similarly observed in de novo carcinoma regardless of size and depth of invasion, and in GBC with AJPBD, but have not been documented in adenocarcinomas.[35] Studies to date have focused on mutations in exons 5 to 8.[18] Point mutations result in alterations in phosphorylation in mutated *p53*, results in altered β-catenin characteristics or cell adhesion in GBCs causing metastasis and invasion of cancer cells.[19]

***Retinoblastoma (RB)***

Mutations in the tumour suppressor RB gene are associated with GBC formation and identified as a prognostic factor for the diseased condition.[36] The changes in this gene on chromosome 13q have been documented in the consequence of GBC formation. The detection of this modification is done by the deletion of the *RB* locus on chromosome 13, which leads to the abnormal formation of the *RB1* Protein (*PRB1*). The abnormal generation of the abnormal retinoblastoma protein results in its localization in the nucleus altering the translation of enzymes production for DNA synthesis and therefore cell replication and proliferation.[21] The complete process occurs by *PRB1* binding to the elongation factors (E2F), while phosphorylation of *PRB1* allows the release of E2F driving the cell to the S phase of the cell cycle. This process of cell cycle is known to be driven by cyclin D, in combination with CDK4 and CDK6, and cyclin E in combination with CDK2.[22] GBC linked to AJPBD is known to have the highest allelic loss and the same is correspondingly observed with *RB* gene mutation. The loss of functional *pRB* is also related to *p16* mutations which may induce the dysregulation of cell cycle and malignancies. This is conversed far ahead in the review for better understanding about *p16* mutations.

***p16***

The loss of manifestation of the protein in tumour suppressor *p16* gene also known to be the cyclin-dependent kinase inhibitor encoded by *p16* gene and *SMAD4/DPC4* can lead to the formation of non-small cell lung carcinoma, gallbladder or biliary tract non-small cell carcinoma (NSCGBC).[20] The frequency of *SMAD4/DPC4* loss is higher with peri-hilar and proximal-hepatic tumours of the biliary tract. The loss of *the SMAD4/DPC4* protein also causes GBC in situ, suggesting that inactivation may occur in early GBC lesions.[37] The appearance of *SMAD4/DPC4*-protein loss in these tumours may indicate the loss of heterozygosity on chromosome *18q*, previously identified in allele typing studies of gallbladder carcinoma. The tumour suppressor genes that are involved in gallbladder carcinogenesis are in 5–59% of subjects with established AJBPD[15], as *p16* normally plays a vital role in cell cycle control, the cyclin-dependent kinase inhibitor, it inhibits the production of cells from G1 phase to S phase.[32]

**Clinical implications**

There is little research on GBC-related genetic mutations known to be the third most common digestive cancer in the South Asian sub-continent, in which Pakistan is also located.[38] The only curative intervention in GBC is complete surgical resection of the gall bladder. However, this only offers benefits for people with localized disease. Limited treatment options are available for patients.[39] with progressive and non resectable gallbladder cancer due to a shortage of identified molecular targets. In recent years, research has focused on the molecular mechanisms of disease in order to identify treatment goals, in addition to diagnosis and prognosis. The importance of these genetic mutations in *KRAS, p53, p27, p16* and *pRB* may help clinicians and surgeons identify them as GBC markers for detection and prognosis, as well as potential therapeutic targets for the treatment and healing. It is useful to recognize the importance of *KRAS* and *p53*, because it is an important potential are presented as independent prognostic markers, and may also be useful for identifying early stages of lesions that may develop in cancers malignant. In addition, it is important to

identify new therapeutic agents which contain antiproliferative effects in cancer cells.[37]

Molecular markers of cancer thus identified will be useful not only for cancer recognition and predictive effects, but correspondingly for the construction of the management program for patients with GBC. Previous studies with the above said genetic alteration are known to have shown a high potential to become autonomous predictive markers of GBC. Therefore, their role in the efficacy of therapeutic targets for the treatment of the disease is absolutely essential. They can also be useful for identifying precancerous lesions that can lead to malignant cancers, particularly in the case of *p53*. GBCs have also repeatedly shown *p53* gene mutations as well as *KRAS*, and *RB* gene mutation. Consequently, these mutations may also be useful in understanding the mechanism and extension as well as prolongation of the GBC pathogenesis.

## CONCLUSIONS

This review focuses on the common genetic changes that occur in GBC. LOH and mutations affecting *KRAS, p53*, *p16*, and *RB* play a role in the progression of GBC. The accumulation of these genetic changes results in disruption of the normal cell cycle leading to continuous cell division and cancer. Limited studies on genetic mutations have been reported in South Asian GBC patients. The absence of this literature in this review underscores the need for detailed studies in larger cohorts to identify new molecular targets that can be used for GBC diagnosis, treatment and prognosis, particularly in the Asian population where the disease is common.

## REFERENCES


1. Kanthan R, Senger JL, Ahmed S, Kanthan SC. Gallbladder Cancer in the 21st Century. J Oncol 2015;2015:967472.
2. Shukla SK, Singh G, Shahi KS, Bhuvan, Pant P. Staging, Treatment, and Future Approaches of Gallbladder Carcinoma. J Gastrointest Cancer 2018;49(1):9–15.
3. Goetze TO. Gallbladder carcinoma: Prognostic factors and therapeutic options. World J Gastroenterol. 2015;21(43):12211–7.
4. Sharma A, Sharma KL, Gupta A, Yadav A, Kumar A. Gallbladder cancer epidemiology, pathogenesis and molecular genetics: Recent update. World J Gastroenterol 2017;23(22):3978–98.
5. Hundal R, Shaffer EA. Gallbladder cancer: epidemiology and outcome. Clin Epidemiol 2014;6:99–109.
6. Takahashi T, Shivapurkar N, Riquelme E, Shigematsu H, Reddy J, Suzuki M, *et al*. Aberrant promoter hypermethylation of multiple genes in gallbladder carcinoma and chronic cholecystitis. Clin Cancer Res 2004;10(18 Pt 1):6126–33.
7. Saetta A, Lazaris AC, Davaris PS. Detection of ras oncogene point mutations and simultaneous proliferative fraction estimation in gallbladder cancer. Pathol Res Pract 1996;192(6):532–40.
8. Kazmi HR, Chandra A, Nigam J, Noushif M, Parmar D, Gupta V. Prognostic significance of K-ras codon 12 mutation in patients with resected gallbladder cancer. Dig Surg 2013;30(3):233–9.
9. Romano D, Maccario H, Doherty C, Quinn NP, Kolch W, Matallanas D. The differential effects of wild-type and mutated K-ras on MST2 signaling are determined by K-ras activation kinetics. Mol Cell Biol 2013;33(9):1859–68.
10. Goldin RD, Roa JC, Gallbladder cancer: a morphological and molecular update. Histopathology 2009;55(2):218–29.
11. Singh MK, Chetri K, Pandey UB, Kapoor VK, Mittal B, Choudhuri G. Mutational spectrum of K-ras oncogene among Indian patients with gallbladder cancer. J Gastroenterol Hepatol 2004;19(8):916–21.
12. Hanada K, Tsuchida A, Iwao T, Eguchi N, Sasaki T, Morinaka K, *et al.* Gene mutations of K-ras in gallbladder mucosae and gallbladder carcinoma with an anomalous junction of the pancreaticobiliary duct. Am J Gastroenterol 1999;94(6):1638–42.
13. Kai K. Organ-specific concept and controversy for premalignant lesions and carcinogenesis of gallbladder cancer. Hepatobiliary Surg Nutr 2016;5(1):85–7.
14. Masuhara S, Kasuya K, Aoki T, Yoshimatsu A, Tsuchida A, Koyanagi Y. Relation between K-ras codon 12 mutation and p53 protein overexpression in gallbladder cancer and biliary ductal epithelia in patients with pancreaticobiliary maljunction. J Hepatobiliary Pancreat Surg 2000;7(2):198–205.
15. Pramanik V, Sarkar BN, Kar M, Das G, Malay BK, Sufia KK, *et al.* A novel polymorphism in codon 25 of the K-RAS gene associated with gallbladder carcinoma patients of the eastern part of India. Genet Test Mol Biomarkers 2011;15(6):431–4.
16. Wistuba II, Albores-Saavedra J. Genetic abnormalities involved in the pathogenesis of gallbladder carcinoma. J Hepatobiliary Pancreat Surg 1999;6(3):237–44.
17. Roa I, Villaseca M, Araya J, Roa J, de Aretxabala X, Melo A, *et al.* p53 tumour suppressor gene protein expression in early and advanced gallbladder carcinoma. Histopathology 1997;31(3):226–30.
18. Roa I, Melo A, Roa J, Araya J, Villaseca M, de Aretxabala X. P53 gene mutation in gallbladder cancer. Rev Med Chil 2000;128(3):251–8.
19. Ghosh M, Sakhuja P, Singh S, Agarwal AK. p53 and beta-catenin expression in gallbladder tissues and correlation with tumor progression in gallbladder cancer. Saudi J Gastroenterol 2013;19(1):34-9.
20. Parwani AV, Geradts J, Caspers E, Offerhaus GJ, Yeo CJ, Cameron JL, *et al*. Immunohistochemical and genetic analysis of non-small cell and small cell gallbladder carcinoma and their precursor lesions. Mod Pathol 2003;16(4):299–308.
21. Wistuba II, Sugio K, Hung J, Kishimoto Y, Virmani AK, Roa I, *et al.* Allele-specific mutations involved in the pathogenesis of endemic gallbladder carcinoma in Chile. Cancer Res 1995;55(12):2511–5.
22. Ante S. Lundberg, Robert A. Weinberg. Functional Inactivation of the Retinoblastoma Protein Requires Sequential Modification by at Least Two Distinct Cyclin-cdk Complexes. Mol Cell Biol 1998;18(2):753–61.
23. Itoi T, Watanabe H, Ajioka Y, Oohashi Y, Takel K, Nishikura K, *et al*. APC, K-ras codon 12 mutations and p53 gene expression in carcinoma and adenoma of the gall-bladder suggest two genetic pathways in gall-bladder carcinogenesis. Pathol Int 1996;46(5):333–40.
24. Hershkovitz D, Simon E, Bick T, Prinz E, Noy S, Sabo E, *et al*. Adenoma and carcinoma components in colonic tumors show discordance for K-RAS mutation. Hum Pathol 2014;45(9):1866–71.
25. Kim YT, Kim J, Jang YH, Lee WJ, Ryu JK, Park YK, *et al*. Genetic alterations in gallbladder adenoma, dysplasia and carcinoma. Cancer Lett 2001;169(1):59–68.
26. Kumari N, Corless CL, Warrick A, Beadling C, Nelson D, Neff T, *et al*. Mutation profiling in gallbladder cancer in Indian population. Indian J Pathol Microbiol 2014;57(1):9–12.
27. Riquelme E, Tang M, Baez S, Diaz A, Pruyas M, Wistuba II, *et al.* Frequent epigenetic inactivation of chromosome 3p candidate tumor suppressor genes in gallbladder carcinoma. Cancer Lett 2007;250(1):100–6.

28. Vavvas D, Li X, Avruch J, Zhang XF. Identification of Nore1 as a potential Ras effector. J Biol Chem 1998;273(10):5439–42.
29. Kee SK, Lee JY, Kim MJ, Lee SM, Jung YW, Kim YJ, *et al.* Hypermethylation of the Ras association domain family 1A (RASSF1A) gene in gallbladder cancer. Mol Cells 2007;24(3):364–71.
30. Roa I, Aretxabala XD, Araya JC, Roa J. Preneoplastic lesions in gallbladder cancer. J Surg Oncol 2006; 93(8):615–23.
31. Jain K, Mohapatra T, Das P, Misra MC, Gupta SD, Ghosh M, *et al.* Sequential occurrence of preneoplastic lesions and accumulation of loss of heterozygosity in patients with gallbladder stones suggest causal association with gallbladder cancer. Ann Surg 2014;260(6):1073–80.
32. Srivastava V, Patel B, Kumar M, Shukla M, Pandey M. Cyclin D1, retinoblastoma and p16 protein expression in carcinoma of the gallbladder. Asian Pac J Cancer Prev 2013;14(5):2711–5.
33. Misra S, Chaturvedi A, Goel MM, Mehrotra R, Sharma ID, Srivastava AN, *et al.* Overexpression of p53 protein in gallbladder carcinoma in North India. Eur J Surg Oncol 2000;26(2):164–7.
34. Washington K, Gottfried MR. Expression of p53 in adenocarcinoma of the gallbladder and bile ducts. Liver 1996;16(2):99–104.
35. Hanada K, Itoh M, Fujii K, Tsuchida A, Ooishi H, Kajiyama G. K-ras and p53 mutations in stage I gallbladder carcinoma with an anomalous junction of the pancreaticobiliary duct. Cancer 1996;77(3):452–8.
36. Ma HB, Hu HT, Di ZL, Wang ZR, Shi JS, Wang XJ, Li Y. Association of cyclin D1, p16 and retinoblastoma protein expressions with prognosis and metastasis of gallbladder carcinoma. World J Gastroenterol 2005;11(5):744–7.
37. Chuang SC, Lee KT, Tsai KB, Sheen PC, Nagai E, Mizumoto K, *et al*. Immunohistochemical study of DPC4 and p53 proteins in gallbladder and bile duct cancers. World J Surg 2004;28(10):995–1000.
38. Talat TJ, Sarwar AJ. Risk Factors for Gallbladder Cancer in Karachi. J Ayub Med Coll Abbottabad 2003;15(3):16–8.
39. Bizama C, García P, Espinoza JA, Weber H, Leal P, Nervi B, *et al.* Targeting specific molecular pathways holds promise for advanced gallbladder cancer therapy. Cancer Treat Rev 2015;41(3):222–34.



## Address for Correspondence:

**Dr. Saara Ahmad,** Assistant Professor, Department of Biological and Biomedical Sciences, The Aga Khan University, Stadium Road, Karachi, Pakistan. **Cell:** +92-334-3448258

**Email:** saara.muddasira@aku.edu, saara_ahmad@hotmail.com


**Received: 7 Nov 2019** **Reviewed: 12 Dec 2019** **Accepted: 26 Dec 2019**