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Adenomatous Polyposis Coli Gene Polymorphisms in Gliomas

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Abstract

Background: Gliomas are the most common brain neoplasms in adults, accounting for about 70% of primary neoplasms of the central nervous system (CNS). *Adenomatous polyposis coli* (*APC*) is a tumor suppressor gene and one of the key players of the Wnt signaling pathway. The Wnt pathway has repeatedly been implicated in brain tumor genesis.
Objective: To investigate *APC* exon 15 polymorphism in gliomas.
Materials and methods: DNA was extracted from 61 tumors followed by PCR to amplify fragments of APCs exon 15. Samples showing amplified products were further sequenced.
Results: Two polymorphisms {rs41115; G>A at position cDNA. 4859 G>A (5:112175770) leading to codon change of T1493T, and rs456899; G>A at position cDNA. 6260 G>A (5:1121777171)} were detected at the mutation cluster region in 10% of the cases.
Conclusion: *Adenomatous polyposis coli* gene 's exon 15 polymorphism has significant association with carcinogenesis of glioma neoplasms (particularly astrocytomas).

Keywords: Glioma, DNA, *APC* exon 15 gene, Polymorphisms.

Introduction

Gliomas are the most common primary tumor of the central nervous system. Gliomas include ependymomas, oligodendrogliomas, mixed gliomas and astrocytomas. Astrocytomas is the largest group of gliomas (>75%), followed by ependymomas (13%), oligodendrogliomas (5-6%) and mixed gliomas (2%). Astrocytomas form the largest group of gliomas (>75%), and glioblastoma multiforme (GBM) is the most popular glioma, accounting for approximately 70% of astrocytomas and 15% of all intracranial neoplasms. 90% of GBMs primarily affecting elder people (average age 62 years), have rapid evolution (less than 3 months)[1].
Secondary GBMs affect younger individuals (average age 45 years) and progress slowly from a lower degree of diffuse astrocytoma. The two forms of GBM have a poor prognosis. In spite of the fact that histological analysis defines the type of an astrocytic neoplasm, diagnostic difficulties may encounter due to the heterogeneity of the tumor, morphological overlap with

other gliomas, or partial sampling of the lesion. Recently, several studies have used molecular techniques to find biomarkers with diagnostic and/or prognostic relation, which may facilitate the identification of such markers and lead to a significant increase in knowledge of the pathogenesis of gliomas and identification of potential targets for new therapeutic approaches[2].
APC (ID: 324) gene located on chromosome 5, consists of 8535 bp spanning 21 exons, and encodes a 2843-amino acid protein in its commonest isoform. Exon 15 comprises >75% of the coding sequence of APC and is the most common target for both germline and somatic mutations. It encodes a multi-domain protein that plays a major role in tumor suppression by antagonizing WNT signaling pathway; as the inappropriate activation of this pathway through mutation of APC gene contributes to cancer progression. APC protein has many cellular functions: as a component of adherence junctions, and as a component of the cytoskeleton stabilization. Mutation analysis of the *APC* gene revealed more than 400 different germline mutations responsible for familial adenomatous polyposis coli (FAP), but the overall number of detected mutations, germline and somatic, is more than 700 according to the Human Gene Mutation Database[3].
The majority of detected mutations result in a truncated (shorter) protein product. Novel mutational reports reveal a great variety of private mutations, and also single-base substitutions that result in silent DNA variants having a role in RNA splicing regulation. Mutations in *APC* gene have been reported in many studies to be one of the important acquired genetic changes in gliomas. The most common type of mutation either non-sense or frame shift mutation commonly occurring in the mutation cluster region (MCR) between codons 1286-1513 of *APC* gene; that contains β-catenin binding sites (the 20 amino acid repeats) hence mutation in the gene causes loss of domains required for β-catenin binding to APC to initiate its subsequent degradation, the integral role in tumor suppressor activity of *APC* gene. Immunoprecipitation experiments identified interactions between APC and alpha and beta- catenin[4] .
These experiments suggest that APC might interfere indirectly with cadherins, proteins that mediate cell-cell interactions. Therefore, APC might be involved in cell adhesion. Wnt proteins and other components of the Wnt signaling cascade, like beta-catenin and axin regulate critical developmental processes of normal central nervous system (CNS) development. Although *APC* has been thought of primarily as a colon specific tumor suppressor gene, its high expression in the CNS, and its critical involvement in particular syndromes, like the Tourcot syndrome, which includes the development of primary brain tumors such as medullo-blastomas and gliomas[5].

Materials and methods

A total of 61 surgical specimens were obtained from patients diagnosed and treated for gliomas at the National Center for Neurological Sciences (NCNS) during the period from January 2017 to December 2019. Gnomic DNA was extracted according to the guanidine chloride method. Each tumor tissue was minced using surgical blade into small pieces, then incubated in a mixture of 20µl of proteinase K, 100 µl of 10% SDS, and 800 µl of STE buffer at 37ºC overnight. Protein was precipitated by 6 M sodium chloride at 4ºC for 15 minutes and centrifuged at 18000 rpm for 20 minutes. To each 500 µl of the supernatant 350 µl of 8 M guanidine chloride and 150 µl of ammonium acetate were added and incubated at room temperature for 90 minutes then 500µl of

chilled chloroform was added and centrifuged at 12000 rpm for 5 minutes. The upper layer (containing the DNA) was collected in a clean tube, then 800µl of chilled ethanol was added and incubated at -20ºC over night to precipitate DNA. Next day the tube was centrifuged at 12000 rpm for 5 minutes. The supernatant was discarded and washed with 400µl of 70% ethanol and centrifuged at 7000 rpm for 5 minutes. DNA was dried by air and 50 µl of deionized water was added to elute DNA. PCR was performed to amplify sequences of APC gene at exon 15 using the following primers sequences[6] .

* For codons (1286-1513) (751bp):

f-GAAATAGGATGTAATCAGACG and r-CATTCCCATTGTCATTTTCC.

* For each 2 µl of DNA, 4 µl PCR master mix, 1.5 µl from each primer, 0.2 µl magnesium chloride, 0.2 µl taq polymerase and 14 µl distilled water were added and PCR reaction was initiated by denaturation step at 95ºC for 10 min followed by 30 cycles of 95ºC for 30 sec, 58ºC for 30 sec, 72ºC for 1.5 min and final elongation step at 72ºC for 7 min. The PCR products were then separated in 2.5 % agarose gel electrophoresis and visualized under UV light. To amplify codons 2016-2196 (542bp) the following primers sequences were used[7].

f - ATGATGTTGACCTTTCCAGGG

r - CTTTTTTGGCATTGCGGAGCT

In a volume of 22 µl that contained 2 µl of DNA, 4 µl PCR master mix, 1.5 µl from each primer, 0.2 µl magnesium chloride, 0.2 µl taq polymerase and 14 ml distilled water. The PCR reaction was initiated by denaturation for 5 min at 95ºC then 30 second at 95ºC followed by annealing at 57ºC for 30s then initial extension at 72ºC for 1.75 min and final extension at 72ºC for 7 min in a total of 30 cycles. PCR products were then visualized under UV light on 2.5 % agarose gel.

The samples which showed no amplification of the PCR products were further stained by immunohistochemical stain to confirm APC protein expression.

Latter 12 samples were selected based on their histological grades, and two amplified PCR products for each sample were sequenced to investigate single nucleotide polymorphisms in the two fragments of the gene.

Results

Of the 61 patients investigated, 52.5% were males and 47.5% were females. Age range of all patients was 1-80 years old. The most significant affected age group was that aged more than 45 years accounting for 24.6%.

According to the affected brain sites, the commonest significantly affected site was the supratentorial site (80.30%), as compared to infratentorial site (19.7%).

The most significantly frequent variant of glioma tumours was astrocytoma II (35.6%), followed by astrocytoma I (33.9%), glioblastoma multiforme (16.9%), and astrocytoma III (13.6%). Frequency rate glioma types was astrocytoma (96.7%), ependymoma (1.6%), and pleomorphic xanthoastrocytoma (1.6%).

The majority (96%) of the tumor tissues showed amplified PCR reaction for a*denomatous polyposis coli* (APC) gene (Fig. 1).

All the tumors' tissues (4%) lacking amplified PCR products showed absence of APC protein expression by immunohistochemical staining indicating total deletion of the gene. Sequencing

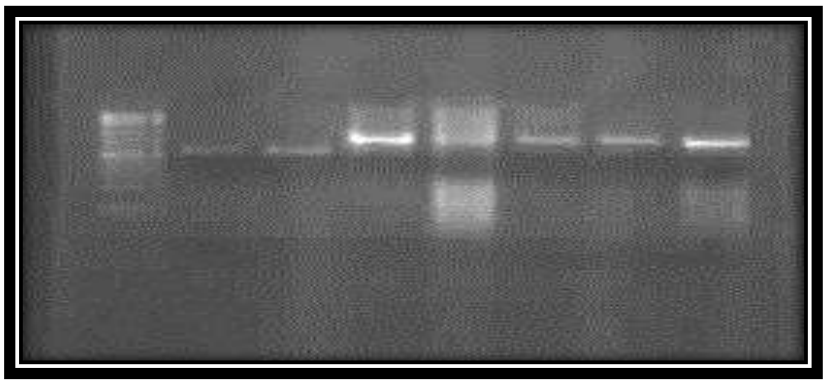

Fig. (1): PCR amplified products of APC exon 15 fragments.
Left to Right: Lanes 1& 2 (542 bp); Lanes 3 to 7 (751bp)

analysis revealed two missense mutations (rs41115, rs456899) in 6 samples (3 samples: astrocytoma I, 2 samples: astrocytoma II and GBM) with overlapping in two cases of astrocytoma II and GBM. The two polymorphisms {rs41115; G>A at position cDNA. 4859 G>A (5:112175770) leading to codon change of T1493T, and rs456899; G>A at position cDNA. 6260 G>A (5:1121777171)} were detected at the mutation cluster region (Fig. 2, 3, 4).

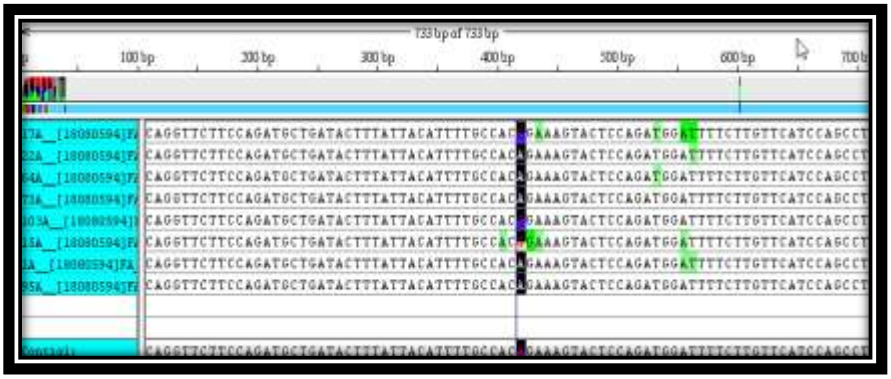

Fig. (2): Sequence alignment of APC mutation (rs 41115)

Discussion

In spite of the fact that APC gene has been thought to be primarily a colon-specific tumor suppressor gene, its association with certain brain tumors and its expression in the CNS suggests that it performs important functions in these tissues. Genes involved in formation and acquisition of full metastatic potential of specific tumors are not only those responsible for cell survival and proliferation but are also genes responsible for the control of cell adhesion and cell motility. Perego and his co-workers demonstrated disorganization of cadherin mediated junction (in which APC is included) is required to promote migration and invasiveness in glioblastoma tissue[8]

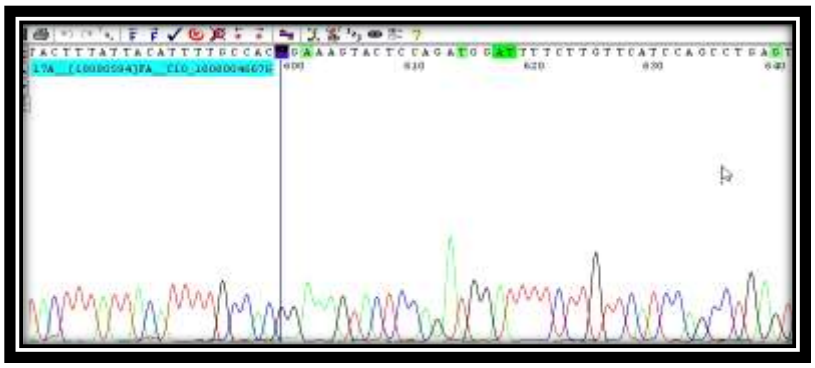

Fig. (3): rs 41115; G>A at position cDN.A4859G>A (5:112175770)

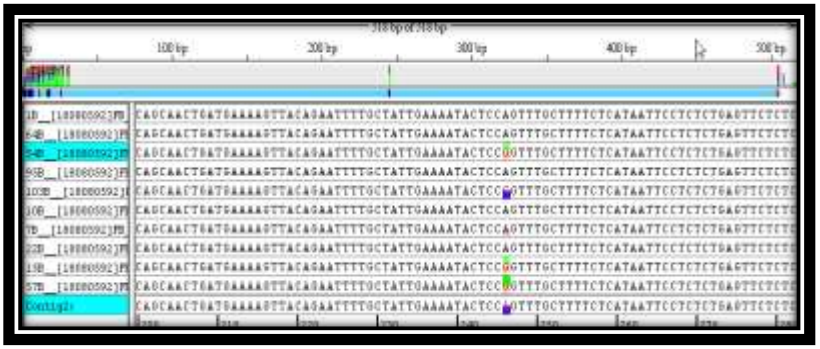

Fig. (4): Sequence alignment of APC mutation (rs 465899)

Most of the tumors (80%) were located in the supratentorial site of the brain. Despite the fact that pathologies in the supratentorial areas remain silent for prolonged periods due to their slow growth and delayed presentation, yet their earlier diagnosis is enhanced after utilization of MRI. The most frequent subtype of astrocytoma (34.4%) was astrocytoma II which was not consistent with the finding of pilocystic astrocytoma in one patient. Pilocystic astrocytoma is the most common type (62.1%) of glioma, followed by glioblastoma (31.2%)[8].

APC's polymorphism (rs 41115 (4479 G>A polymorphism/p.Thr1493Thr) was found in a variety of tumors, especially in FAP families. The in coding region of *APC* at position 4479 substitution G>A is a neutral polymorphism, and is known as silent polymorphism. Little is known about the clinical significance of *APC* 4479 G>A polymorphism which does not lead to amino acid replacement. However, GA heterozygous genotype at the position 4479 was associated with significantly decreased colorectal cancer risk, and the wild genotype (G/G) appeared to have a protective effect that decreases the colorectal risk to a moderate/severe disease[6].

Palacio-Rúa and his colleagues[9] reported that the *APC* single nucleotide polymorphism (SNP) rs41115 was significantly associated with stomach and colorectal cancers.

Jiun-Hung and his co-authors[10] found that rs41115 is significantly associated with time to progression in prostate cancer patients receiving androgen deprivation therapy.

Also Crabtree and his co-workers[11] reported that rs41115 is associated with severity of the disease in colonic familial adenomatous polyposis patients.
Oana and his co-workers[12] reported the association of rs456899 with colorectal cancer; the rs456899 polymorphism has introduced a pro change (1960 pro) and was classified as a missense gene and was predicted to affect a splicing site in exon/intron boundary.
Conclusion: *Adenomatous polyposis coli* gene 's exon 15 polymorphism has significant association with carcinogenesis of glioma neoplasms (particularly astrocytomas).

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