

P53 Gene Mutation in Low Grade Astrocytoma Among Sudanese Patients

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Abstract

Background: Low grade glioma (astrocytoma) represents 15% of the primary brain tumors diagnosed in adult per year, and it has risk of malignant transformation into high grade. The plan of its management consists of extensive surgical resection followed by chemotherapy and radiotherapy to reduce the possibility of recurrence of tumor or progression. Risk factor for such type of brain tumor isn't so clear. TP53 is a well-known tumor suppressor gene in cancers in general; and its mutation is found to occur in 50% rendering it as the most common genetic alteration in cancer, and in glioma accounts for 19%, the most detected mutations are missense type (75%). In neurosurgery, studying glioma by histology alone provides a limited understanding about its behavior and progression (which occurs in all glioma) or risk of recurrence; so molecular study is required for better understanding natural history of the disease with better management and prognosis.

Objectives: To detect P53 gene mutations in low grade astrocytoma in Sudanese patients using polymerase chain reaction (PCR) and to correlate molecular findings with glioma histopathology variants.

Material and methods: This is a hospital based cross sectional research study conducted in the research lab at the National Center for Neurological Sciences (NCNS) during the period from January 2021 to September 2021. This study included 20 glioma tissue samples, 5 were sent to Macrogen Company for Sanger sequencing.

Results: The result of this study showed that, 12 patients were female, the most frequent age group ranging from 30-14 years in 41.7%, 8 patients were male, 75% of them were detected within the age group 20-25 years. Sanger sequencing showed C > G (rs1042522) in 60% of the patients, and D48E in 40%. The correlation between samples histopathology and P53 gene mutation showed, (C > G rs1042522) was associated with 2 samples of grade I and one of grade II, in addition to that, D48E mutation was associated with one sample grade II. One sample of grade I associated with insertion type of mutation.

Conclusion: The mutations of TP53 gene in our patients may considered as one of the causes of glioma tumorigenesis, small sample size in this study may be not conclusive, so further broad genetic study with larger sample size is required and recommended.

Keywords: P53 Gene Mutation, Astrocytomas, mutations, glioma tumorigenesis

Introduction

Astrocytomas are type of cancer found in the brain and spinal cord in a cells that support the nerve cells called astrocytes. They are belong to a family of brain tumors named as low grade gliomas to differentiate it from the other type which is named high grade gliomas with each type has its own subtypes; criteria and presentation. Low grade glioma account for 15% of the primary brain tumors diagnosed in adults each year and they have a histological subtypes which are astrocytomas, oligoastrocytomas and oligodendrogliomas and they are all classified as WHO grade 2 (1).

Low grade glioma affects younger adult aged 17 to 44 years with median overall survival ranging from 7 to 13 years. LGG accounts for 19% of all gliomas (2).

Patients with low grade glioma (including astrocytoma) usually present symptoms and signs related to the direct parenchymal infiltration, local tumor effect by edema, hemorrhage or tumor mass or intracranial hypertension caused by the mass effect or the ventricular obstruction. In 80% of cases seizure is the most common presentation despite that the onset can be subtle and insidious (1). It is usually diagnosed by MRI scan of the brain with its significant featuring in T1 weighted images as homogeneously iso-intense to hypo intense and hyper intense on T2 weighted images. The common plan of management consists of extensive surgical resection which gives more favorable life expectancy by influencing the risk of malignant transformation of the tumor into higher grade. Adjuvant treatment like chemotherapy and radiotherapy is usually given following surgery to achieve the overall favorable outcome but its determination is still challenging and is based mainly on the best definition of the prognostic factors (1).

Astrocytoma has no direct cause associated with the pathogenesis but has a known association with previous exposure to ionizing radiation and also hereditary factors are seen to be involved such as neurofibromatosis type 1 and Li Fraumeni syndrome (1).

TP53 is a well-known tumor suppressor gene that has been extensively studied over the past decades in cancers. TP53 encodes P53 which is a transcription factor regulating cell cycle to prevent proliferation of genetically damaged cells with oncogenic properties (TP53 AND P53 statuses). P53 gene is the gene involved in a number of crucial cellular processes such as cell cycle arrest, differentiation, apoptosis and DNA damage repair thereby protecting the cell from tumorigenesis. TP53 mutations occur in 50% of all human cancers, rendering it the most common genetic alteration in cancer (2).

Somatic TP53 alterations are frequent in most human cancers, ranging between 5-80% depending on type and stage of tumors and supposed to represent an early stage in development of the tumor. The gene is located at band p13.1 of chromosome 17 consists of 1 exons of which exon 1 is noncoding. TP53 codes for the 393 amino acids residues long P53 protein that plays an essential role in DNA damage induced cell cycle arrest and apoptosis. Most of the evolutionarily conserved region of TP53 lies within exons 5 to 8 containing the DNA binding domain of P53 (8;13;18;25) studies to date have generally been restricted to the analysis of this region (3).

Most of these alterations are missense mutations (75%) leading to complete or partial loss of P53 function. Mutation of the P53 tumor suppressor gene is a genetic hallmark of human astrocytic neoplasms, but their predictive role in glioma progression is still poorly understood.

The majority of studies dedicated to TP53 in gliomas were focused on mutational hotspots located in exons 5-8. Recent studies have suggested that TP53 is also mutated outside the classic mutational hotspots reported in gliomas.

The importance of studying genetics in low grade glioma for neurosurgeons that all LGG transforms and its histological diagnosis provide a limited understanding of the behavior and so the genetic markers improve the diagnosis and help understanding prognosis and lead to better therapeutic options.

So, the focus of this study will as a wet lab based study to identify the TP53 gene mutation in cases of a Sudanese patients diagnosed with astrocytoma and to identify the type of this mutation with specification of the mutational exons.

Literature review

In a paper published in 2014; P53 is studied and its clinical impact in diffuse low grade glioma it denoted that TP53 has important role in the pathogenesis and it is frequently mutated especially in astrocytoma's, with the results showed that TP53 is mutated in 52.4 % of cases. TP53 mutations outside mutational hotspots (exons 4-8) are rare (2.6 %) supporting targeted TP53 sequencing in LGG. TP53 mutations are associated with astrocytes phenotype, younger age and p53 overexpression. In contrast, they are mutually exclusive with 1p/19q codeletion (4).

In 2004 a study is conducted about the alterations of P53 protein in adult astrocytoma with the results showed Overall 52% of supratentorial astrocytic tumors showed p53 immunopositivity with no correlation to the histological grade. In contrast, all the infratentorial tumors were p53 negative except for one brainstem glioblastoma (5).

Other study published in 2002 and was about the TP53 mutation prognosis and overexpression in the astrocytoma and it concluded that TP53 mutations are frequent and early events in the pathogenesis of WHO grade II astrocytomas/ oligoastrocytomas, and most of the univariately detected overall prognostic impact of the TP53 status must be related to the influence of the gemistocytic subtype. In nongemistocytic astrocytomas, a hot spot codon 175 TP53 mutation indicates a worse prognosis in terms of time to progression and malignancy (6).

Other studies went to speak about the possibility of using TP53 mutation as a prognostic factor even in low grade glioma through the effect of that in the efficacy of the chemotherapy.

This is showed in a study been conducted in October 2021 which is summarized that there are no established biomarkers currently in low grade glioma that may associate with the chemo sensitivity and it identified tumor protein 53 hotspot mutations in TP53 codon 273 in 33% of astrocytoma tissues and retrospectively found that these tumors were associated with significant improvement in clinical outcome with the chemotherapy and it concluded that TP53 codon 273 mutations can be an indicator of chemotherapeutic efficacy in astrocytoma and so useful in astrocytoma treatment decision making (2).

Other famous study conducted in February 2014 and is considered the largest cohort study conducted for low grade glioma TP53 and P53 statuses and this study concluded that TP53 is mutated in 52.4 % of cases. TP53 mutations outside mutational hotspots (exons 4–8) are rare (2.6 %) supporting targeted TP53 sequencing in LGG. Interestingly, seven novel TP53 mutations have been discovered in LGG. TP53 mutations are associated with astrocytic phenotype, younger age and p53 overexpression. In contrast, they are mutually exclusive with 1p/19q codeletion. Using a threshold of 10 % of p53-positive tumor cells, p53 expression is a good surrogate marker of missense TP53 mutation. However, it should be used with caution since it misses *20 % of TP53-mutated tumors (4). Regarding the local data in Sudan dealt with glioma there was a study conducted in the national center of neurological sciences (NCNS) and published in 2020 and it dealt with pathogenesis of glioma in Sudanese and specifically detection of human cytomegalovirus DNA among Sudanese glioma patients with the results showed low percentage of glioma were infected by human cytomegalovirus (7). Other locally conducted study and titled as in silico prediction of pathogenic mutations in glioma brain tumor and it was aimed to detect non synonymous mutations in genes related to glioma with the result showed 13 SNPs out of 66 missense variants had deleterious effect, and only 8 SNPs were located within the core of the protein, and it concluded that SNPs with ID numbers (er121913500, rs121913499, rs637511110,rs118101777, rs104894104, and rs1870377) were detected among genes associated with glioma (8).

Rational

P53 is an important gene in the development of low grade astrocytoma and is frequently found to be mutated (in about 50% of cases), in Sudan glioma tumor was poorly investigated. Several studies from the literature found significant association between TP53 gene mutations with the different types of glioma brain tumor. This is the first study that explored TP53 gene in low grade glioma, such mutation if present and correlated with histopathology of Low grade glioma may set a simple marker for prognosis and treatment direction.

Objectives

General

To study P53 gene mutations in low grade astrocytoma among Sudanese patients

Specific

1. To detect the P53 gene mutations in low grade in Sudanese patients using polymerase chain reaction (PCR)
2. To correlate molecular findings with histopathology of astrocytoma

Materials and methods

Study design

This is a hospital based cross sectional study

Study area

Conducted in the research lab at the National Center for Neurological Sciences (NCNS)

Study period

During the period from January 2021 to September2021

Study population

Astrocytoma tissue samples from patients diagnosed with low grade glioma.

Sample size and technique

Total number of patients during the above mentioned period of the study was 20 selected by simple random technique
The number of patients was obtained according to the following formula:

$$X = \frac{z^2 * p * q}{d^2}$$

Confidence interval 95%, Margin error 5%, Population proportion 50%, Population size 25, Calculated sample size 53

Study limitation

Small sample size (20); with (17) sample with adequate DNA and three without available DNA; and for sequencing only 5 samples were randomly selected and sent.

DNA extraction

DNA was extracted by using guanidine chloride method, according to the international protocol. Tissues sample were cut till it became homogenous then 800µl STE buffer, 100µl 10%SDS, 20µl proteinase K were added. Then the mixture was incubated at 65°C for 3hrs. After that, protein was precipitated by adding 300 µl of 6M NaCl and kept at 4°C for 15 minutes, after which it centrifuged at 18000 rpm for 20 minutes. Then 500 µl of supernatant was transferred to a new eppendroff tube, then 350 µl of 8M guanidine chloride and 150 µl of 49 M NH₄ acetate were added. Following this step the mixture was incubated at room temperature for 90 minutes, then after incubation 500 µl pre chilled chloroform was added and centrifuged at 12000 rpm for 5 minutes. After centrifugation the upper layer was transferred to a new tube and then 800 µl of cold absolute ethanol was added. Following this DNA was incubated at - 20 °C for 2 hours, after which another centrifugation was done at 12000 rpm for 5 minutes. The pellet was washed with 400 µl of 70% ethanol followed by vortex and then centrifuged at 7000 rpm for 5 minutes, after which the supernatant was pour off and the pellet was left to dry. The last step was the elution of DNA in ET buffer or ddH₂O and kept at 4°C cover night and then the DNA was stored at -20°C.

PCR

Was done according to the manufacturer instructions and protocol .Preparation of the already extracted DNA samples in rag; then Preparation of the PCR special tubes and Preparation of the suitable pipette for suitable volume sampling .Distilled water volume was 14 µl ; Master Mix 4 µl ;Forward primer 1 µl ; Reverse primer 1 µl and DNA sample 2 µl With total reaction volume was 22 µl with the mixture taken for PCR (**escohealthcare | swift.maxpro**) with setting adjusted according to the machine protocol and (30) cycle of PCR was done.

Preparation of gel electrophoresis

Gel electrophoresis of the samples was done by preparing the agarose gel first. (0.79 gram) of agarose powder taken with electrophoresis buffer (T-E 7ml+D.W 28ml) with ethidium promid 1 µl added then the mixture was heated on high temperature in a microwave till the powder is melted. The mixture was drained into a gel casting tray and a comb was placed at one end to make wells for the sample to be pipetted into, then it left to cool and became solid with notice the color changed to became more opaque after which the comb is removed. The gel is placed in electrophoresis tank and a running buffer(400 ml (396D.W+4 ml TE) is also added to the tank till the surface of the gel is totally covered and function of this buffer was to conducts the electric current (which is applied for about 45 minute).

A dye is added to the DNA sample to increase viscosity and so prevent the sample from floating out of the wells and so the sample migration seen. DNA marker (DNA ladder) is placed in the first well of the gel (2 µl) after which the DNA samples are pipetted into the remaining wells of the gel then the tank was adjusted so as the gel orientation and both electrodes are in the right position and the electrophoresis process done.

The result of the process is seen by staining the gel with fluorescent dye that will bind to the DNA and after which is visualized by ultraviolet Tran's illuminator and result is recorded (figure1).

Demographic Analysis

Study showed that, 12 patients were female, the most frequent age group ranging from 30-14 years in 41.7%, 8 patients were male, 75% of them were detected within the age group 20-25 years. Five samples from the total twenty underwent sequencing, four patients were female and only one male. Histopathology result of five patients were low grade astrocytoma, three were grade I astrocytoma and two were grade II astrocytoma.

Molecular analysis

Done by bioinformatics programs; mutation taster, ensemble, bio edit uniport and hope mutation.

Results

The result of this study showed that, 12 patients were female, the most frequent age group ranging from 30-14 years in 41.7%, 8 patients were male, 75% of them were detected within the age group 20-25 years. Sanger sequencing showed C > G (rs1042522) in 60% of the patients, and D48E in 40%. The correlation between samples histopathology and P53 gene mutation showed, (C > G rs1042522) was associated with 2 samples of grade I and one of grade II, in addition to that, D48E mutation was associated with one sample grade II. One sample of grade I associated with insertion type of mutation.

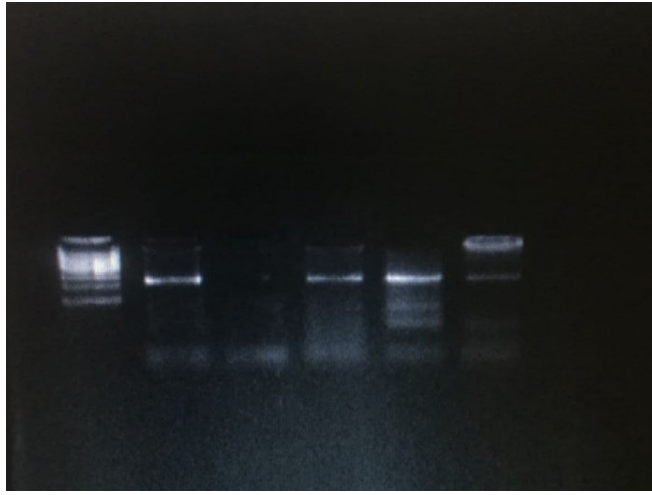


Figure:1 shows, gel electrophoresis of P53 gene (rs). Lane 1/ladder, lane 2-6/samples/band size 135.

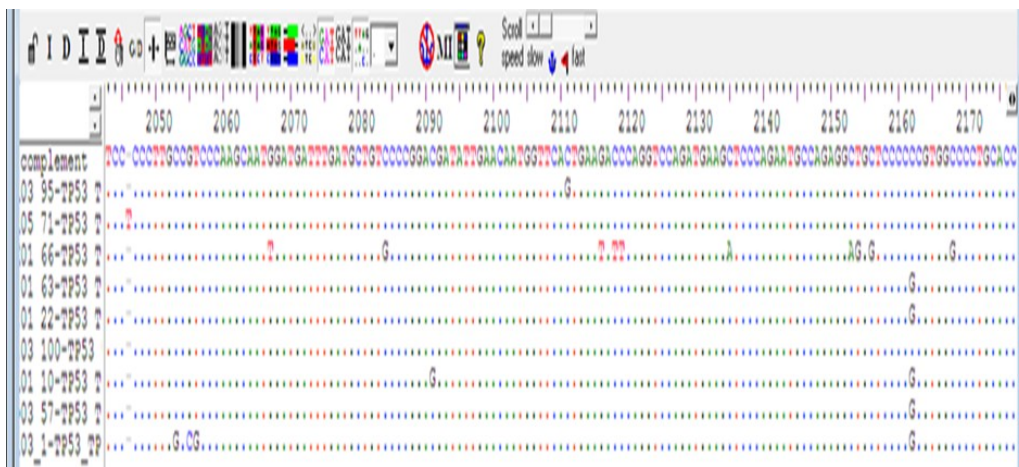


Figure 2A: The alignment of the p53 sample sequences with the reference sequence of p53 gene obtained from NCBI database.

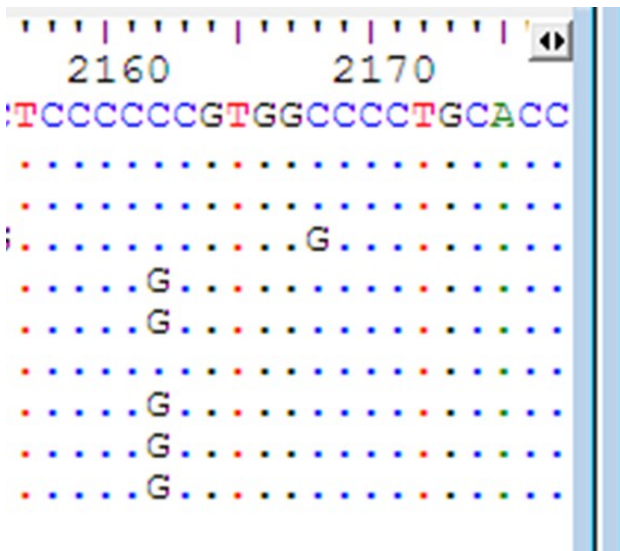


Figure 2B: Magnified chromo gram showed C>G mutation.

Prediction

Summary

analysed issue

name of alteration: no title

alteration (phys. location): chr17:7579472G>C [show variant in all transcripts](#) [IGV](#)

HGNC symbol: TP53

Ensembl transcript ID: ENST00000269305

Genbank transcript ID: NM_000546

UniProt peptide: P04637

alteration type: single base exchange

alteration region: CDS

DNA changes: c.215C>G, cDNA.405C>G, g.11385C>G

AA changes: P72R Score: 103 [explain score\(s\)](#)

position(s) of altered AA if AA alteration in CDS: 72

frameshift: no

known variant: Reference ID: [rs1042522](#)

polymorphism

- amino acid sequence changed
- homozygous in TGP or ExAC
- known disease mutation at this position (HGMD CM961374)
- protein features (might be) affected
- splice site changes

analysis result

| database | homozygous (C/C) | heterozygous | allele carriers |
|----------|------------------|--------------|-----------------|
| 1000G | 791 | 1137 | 1928 |
| ExAC | 27306 | -21845 | 5461 |

Figure 3A: Mutation taster analysis of C>G mutation (rs1042522) of the first sample.

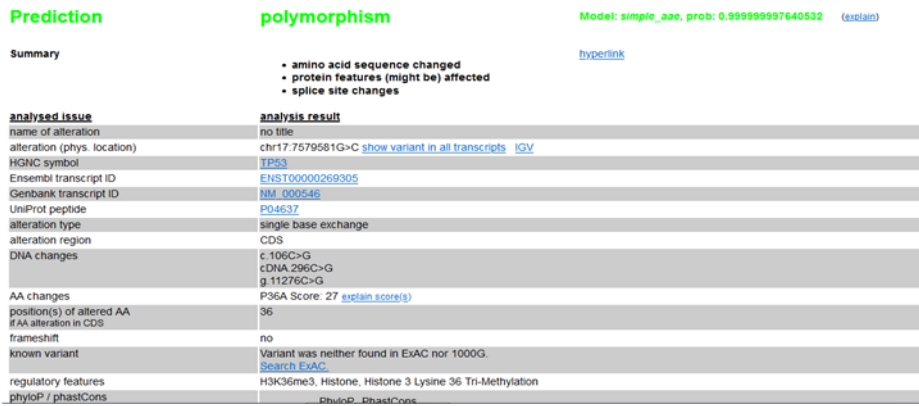


Figure 3B: Shows Mutation taster analysis of C>G mutation (rs1042522) of other two samples.

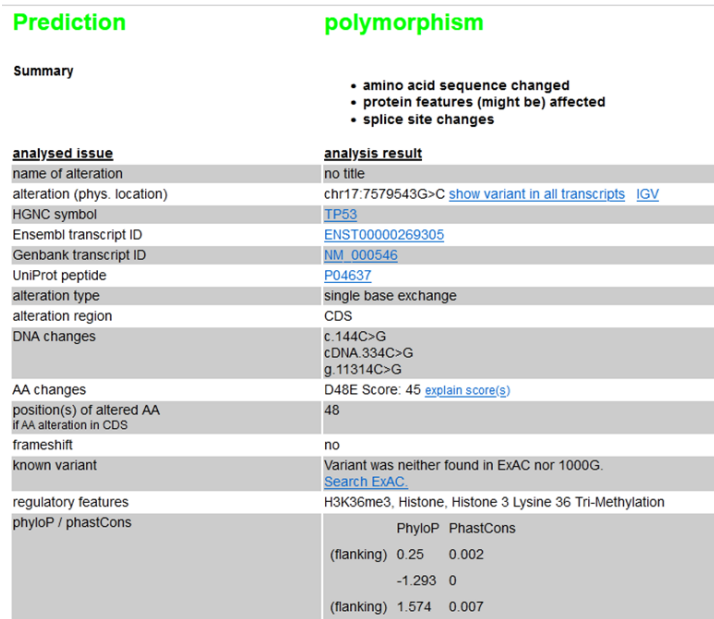


Figure 4: Shows Mutation taster analysis of second sample sequencing result G >G /D48E.

Prediction disease causing

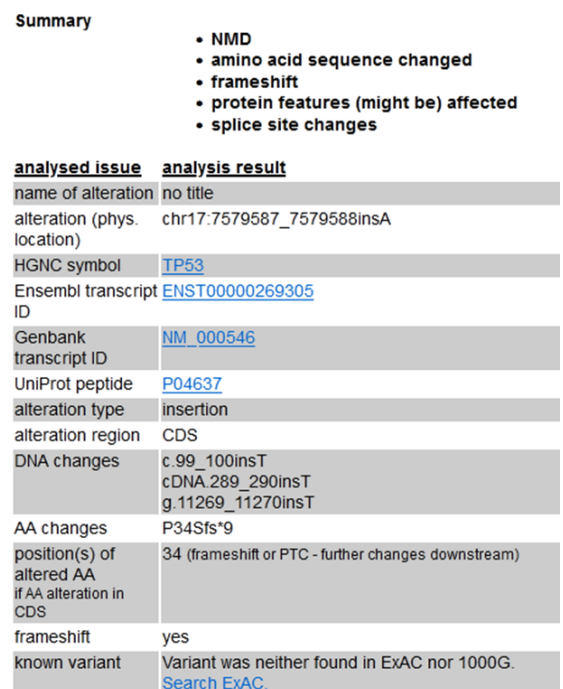


Figure 5: Shows Mutation taster analysis of fifth sample (cDNA.289_290insT|P34Sfs*9).

Table 1: Shows the correlation between the histopathology grades of samples with the resulting mutation type.

| Sample number | Histopathological type and grade | Type of mutation |
|---------------|----------------------------------|------------------|
| 1 | Astrocytoma Grade 1 | C>G rs1042522 |
| 2 | Astrocytoma Grade 2 | D48E |
| 3 | Pilocytic astrocytoma Grade 1 | C>G rs1042522 |
| 4 | Astrocytoma Grade 2 | C>G rs1042522 |
| 5 | Astrocytoma Grade 1 | Insertion |

Discussion

Our study result showed female predominance in 60% of patients and male in 40%; with dominant age in female between 15-30 year in 41.7% of patients and in male between 20-25 year in 75% of patients in literature low grade glioma has incidence of 15% of all primary adult brain tumors diagnosed each year and commonly affect younger age (fourth and sixth decade of life) (9) in relevant study published on 2018 mentioned that incidence of glioma varies significantly by sex and this is according to population based studies, and 30-50% incidence higher in male with this male preponderance of glial tumors increases with age (10). So, our study showed contradiction with literature and relevant studies with regard to age and sex occurrence of low grade glioma and this can be due to the small sample size of our study. The same previously mentioned study concluded that sex and other demographic differences in cancer susceptibility can provide important clues to etiology and these differences can maximally be used for discovery in genetic association studies. Regarding our study sequence results; TP53 gene revealed several mutations located at different locations on chromosome 17, these mutations were affecting protein structure, function and cause instability at splice sites of the gene. The most common mutation encountered in our samples was C>G rs1042522. this type of mutation is known as transversion type of mutations from purine to pyrimidine.

Sanger sequencing mainly showed C > G (rs1042522) in 60% of the patients, and D48E 40% in relevant study conducted in 1990 about the effect of TP53 mutations in LGG with the effect of that in the prognosis and it showed significant association, and it emphasized on the role of TP53 mutation in the malignant progression of low grade glioma especially grade II into higher grades. Also it concluded that there is a significant association between the presence of TP53 mutation with shorter progression free survival (PFS) ($P<0.05$) (2). The noted that in our study most of the mutation's variant were not found in the 1000g which make them to be considered as novel variant. Regarding the correlation between the mutations and the histological type of the tumor; our study showed predominance of grade I astrocytoma. In the previously mentioned paper the higher association with the malignant progression was found in grade II astrocytoma or oligo astrocytoma also the study talked about TP53 status in primary and recurrent tumors and it showed that TP53 status in the primary tumor was always predictive of TP53 status in the recurrent tumor with no cases recorded with absent TP53 in recurrent tumor but present in the recurrence (2).

In our study the type of mutation found in the first sample was point mutation in which P in position 72 replaced with R and it has a known variant in 1000G database.

Second sample sequencing result showed point mutation at position D48E with effect of this on the resulting protein and so splice site changed and it is also not found in the 1000G database third and fourth samples showed similar results of point mutations in position P35A but the mutation variant is not recorded in 1000G database or any others. Fifth sample sequencing result showed insertion type at CDS region position 34 with resulting frameshift and also it is not as mutation variant in 1000G database. The effect of these mutations revised and they were associated with a wide genetic related cancers and diseases, such as glioma of course and li-fraumeni syndrome and many others.

Conclusion

The mutations of TP53 gene in our patients may considered as one of the cause of glioma tumorigenesis, small sample size in this study may be not conclusive, so further broad genetic study with larger sample size is required and recommended.

Conflict of Interest

The authors declare no conflict of interest.

References

1. FELICIA WUISAN FR& L. . Vol. 16, *Angewandte Chemie International Edition*, 6(11), 951–952. 1967. 119–141 p.
2. Noor H, Briggs NE, McDonald KL, Holst J, Vittorio O. Tp53 mutation is a prognostic factor in lower grade glioma and may influence chemotherapy efficacy. *Cancers (Basel)*. 2021;13(21).
3. Hayes VM, Dirven CMF, Dam A, Verlind E, Molenaar WM, Mooij JJA, et al. High frequency of TP53 mutations in juvenile pilocytic astrocytomas indicates role of TP53 in the development of these tumors. *Brain Pathol*. 1999;9(3):463–7.
4. Gillet E, Alentorn A, Doukouré B, Mundwiller E, Van Thuij H, Reijneveld JC, et al. TP53 and p53 statuses and their clinical impact in diffuse low grade gliomas. *J Neurooncol*. 2014;118(1):131–9.
5. Friedman AH, McLendon RE, Signer DD, Bigner SH. Alterations of the TP53 Gene in Human Gliomas. *Cancer Res*. 1994;54(5):1324–30.
6. Peraud A, Kreth FW, Wiestler OD, Kleihues P, Reulen HJ. Prognostic impact of TP53 mutations and P53 protein over-expression in supratentorial WHO grade II astrocytomas and oligoastrocytomas. *Clin Cancer Res*. 2002;8(5):1117–24.

7. Idriss FA, Gassoum A, Daeaf S Al, Abdelraheem NE, Elmula IF. Detection of Human Cytomegalovirus DNA among Sudanese Glioma Patients in Khartoum State. 2020;5(2):1–7.
8. Glioma bioinformatics.
9. Nikmatuzaroh R. dan NM. 濟無. Skripsi. 2019.
10. Ostrom QT, Kinnersley B, Wrensch MR, Eckel-Passow JE, Armstrong G, Rice T, et al. Sex-specific glioma genome-wide association study identifies new risk locus at 3p21.31 in females, and finds sex-differences in risk at 8q24.21. *Sci Rep.* 2018;8(1):1–15.

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