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Research Article

The Role of TNF Gene RS18715293, SNV101403225 and SNV69305309 in Gliomas Tumors Among Sudanese Patients.

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Abstract:

Background:Gliomas are the most prevalent primary tumors of the brain and spinal cord which represent about 33 percent of all brain tumors. This study was designed to detect the association between TNF gene and gliomas among Sudanese patients.
Materials and Methods: This study was descriptive cross sectional study, conducted at the research laboratory of the National Center for Neurological Sciences(NCNS), Khartoum, Sudan during the period from June to September 2022. Demographical data (gender, age, residence, histology and WHO grading). DNA extraction was done from tissue of all operated patients with gliomas tumors. PCR for TNF was done and thus Sanger sequencing.
Results: The PCR results showed positive PCR for TNF gene in all samples. And sequencing result showed base bare exchange in TNF gene C to T polymorphism.
Conclusion:TNF gene polymorphism was detected and might be associated with gliomas tumors among Sudanese patients.

Keywords: TNF gene, PCR, sequencing, and polymorphism.

Supplementary information The online version of this article (https://doi.org/xx.xxx/xxx.xx) contains supplementary material,which is available to autho-rized users.

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Introduction

The first recorded reports of gliomas were given in British scientific reports, by Berns in 1800 and in 1804 by Abernety[1] .Gliomas are the most prevalent primary tumors of the brain and spinal cord. About 33 percent of all brain tumors are gliomas. Histologically, they share characteristics of normal glial cells that surround and support neurons in the brain and are generally named according to these similarities, including astrocytes, oligodendrocytes and ependymal cells. Gliomas are called intra-axial brain tumors because they grow within the substance of the brain and often mix with normal brain tissue [2]Animal modeling of glioma suggests that these three cell types have the capability to be the origin of glioma, and we have reason to believe that, depending on the initiating cell type, prognosis and response to therapy may be significantly different [3]. primary brain tumors incidence occurs in people between 35 and 44 years of age, and there is an increased prevalence among white people and men[4].

They have been traditionally classified as astrocytic, oligodendroglial or ependymal tumors based on cellular classification. Furthermore, World Health Organization (WHO) classified these tumors taking the molecular behaviour into account. And the grades ranging from grade I–IV, which indicate different degrees of malignancy. In addition to this, remarkable progress in genomic, transcriptomic and epigenetic profiling has resulted in new concepts of classifying and treating gliomas [5]. subtypes of glioma show different molecular and genetic profiles. According to the most recent classification (WHO 2016), histopathological analysis should always be accompanied by genetic screening. This represents a substantial change towards integrating the concept of morphological and genetic classifications when translating specific cell tumor pathology into clinical practice [6].

Tumor necrosis factor alpha (TNF-α) is a proinflammatory cytokine which identified in the late1970s as a cytokine produced by immune cells having a capacity to suppress tumor cell proliferation and induce tumor regression with an important role in the pathogenesis of several diseases [7]. Its encoding gene is located in the short arm of chromosome 6 in the major histocompatibility complex class III region. Most of the TNF-α gene polymorphisms are located in its promoter region and they are thought to affect the susceptibility and severity of different human diseases [8]. recent reports have shown that TNF is involved in all aspects of carcinogenesis as cellular transformation, survival, proliferation, invasion, angiogenesis, and metastasis[9]. TNF genes determine strength, effectiveness, and duration of local and systemic inflammatory reactions, as well as repair and recovery from infectious and toxic agents also implicated in the pathogenesis of several diseases, including cancer, sepsis, rheumatoid arthritis, diabetes and inflammatory bowel disease [10]. Mitogen-activated protein kinase (MAPK) pathways are evolutionarily conserved kinase modules that link extracellular signals to the machinery that controls fundamental cellular processes such as growth, proliferation, differentiation, migration , apoptosis and play a critical role in the development and progression of cancer[11] .

Materials and Methods

This study was descriptive cross sectional study which included Sudanese patients arrived at the National Center for Neurological Sciences (NCNS) (Khartoum state) with brain glioma during the period from June to September 2022.The study was done in 21 operated patients histologically, diagnosed as glioma tumors. The tissue samples were obtained from glioma tumors and stored in -20 °C till processed for DNA extraction, PCR and sequencing.

The data were coded in master sheets checked entered and analyzed using statistical package for social science (SPSS), P value <0.05 was considered as statistically significant, in addition to that, PCR findings was analyzed using different bioinformatics tools and online programs(BioEdit, NCBI, Ensemble and mutation Taster).

Ethical approval was obtained from Al-Neelain ethical committee; furthermore, from each participant verbal and written consent was obtained.

Genomic DNA was isolated using genomic extraction kit. Primers were designed using prime3 software. The forward primer for TNF was designed as (5'ATCCCAGGTTTCGAAGTGGT 3') and the reverse as (5'TTTGGGAAACTTTGGAGGGCTAGG 3')

Polymerase Chain Reaction (PCR):

Depending on the National Center for Neurological Sciences (NCNS) criteria and guidelines the target sequence of TNF gene was amplified using polymerase chain reaction. Following standard protocol; in a PCR tube 2ul of DNA, 14 µl of distill water, 4 µl of master mix, 1µl from reverse primer and1µl forward primer was added, then the PCR tube was placed in the PCR machine (MaxPro Thermal Cycler Base: SWT-MXP). And according to primers specifications (annealing temperature and GC contents), the PCR machine was programmed as follow: initial pre-heat temperature was adjusted to 95 °C for 15 minutes, followed by 72 °C for 30 minute, and 60 °C for 30 seconds, this cycle was repeated for 35 times (cycles) except the pre-heat step.

Sequencing analysis:

The sequencing results were analyzed using different bioinformatic software and tools. The obtained sequences were aligned using BioEdit software with a reference sequence retrieved from Gene Bank (National Center for biotechnology information).

Results

Demographical results:

The distribution of residency presented as follow: Al-jazirah state represented the highest frequency of residency followed by Sinnar and Khartoum states, Gender in this study showed that, male were 12 represented57.2%, while female were 9 (48.8%). And the age group 31-40 years represented 28,6% of the study population followed by the age group 21-30 years in 23,8%. (Figure 1, 2, and 3).

Clinical data:

As shown in figure (4), the most common glioma type was grade Iastrocytoma in57.1% of the patients followed by GBM and grade II astrocytoma in 14.3% each; pilocytic astrocytoma had the lowest frequency (9.5%). As shown in table (1) the frequency of astrocytoma was dominated in the study population by 17 (11 males, 6 females), followed by GBM with frequency 3 (2 females, 1 male) and 1 female pilocytic case.

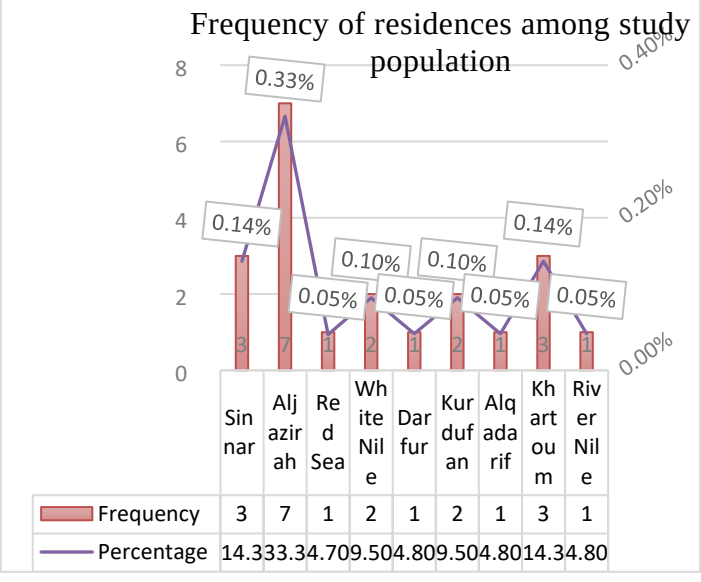

Figure (1): The Distribution of residency among glioma tumors.

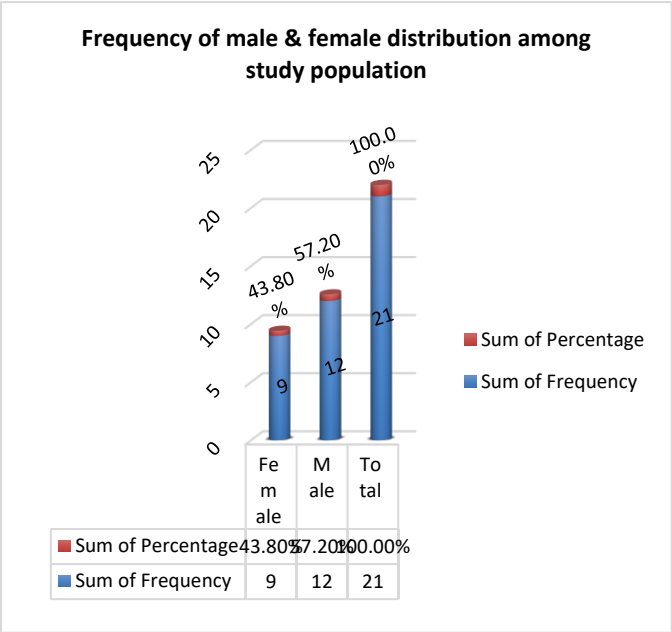

Figure (2): Distribution of gender within glioma tumors.

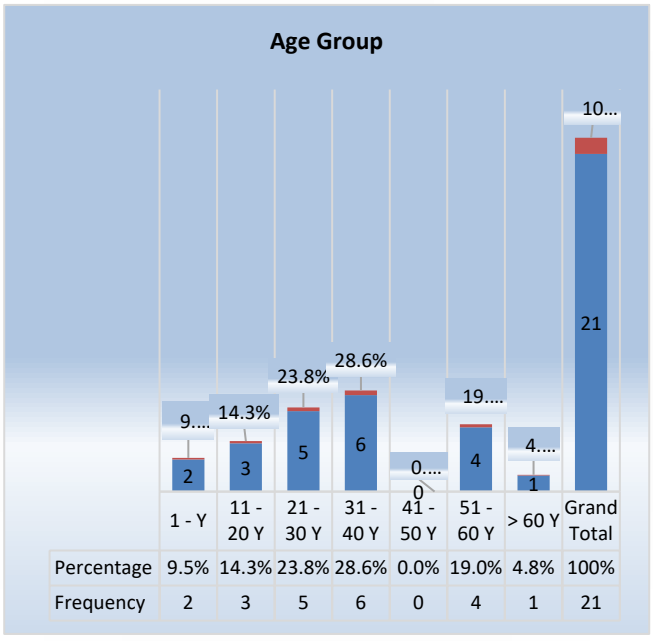

Figure (3): The Distribution of age groups within glioma tumors.

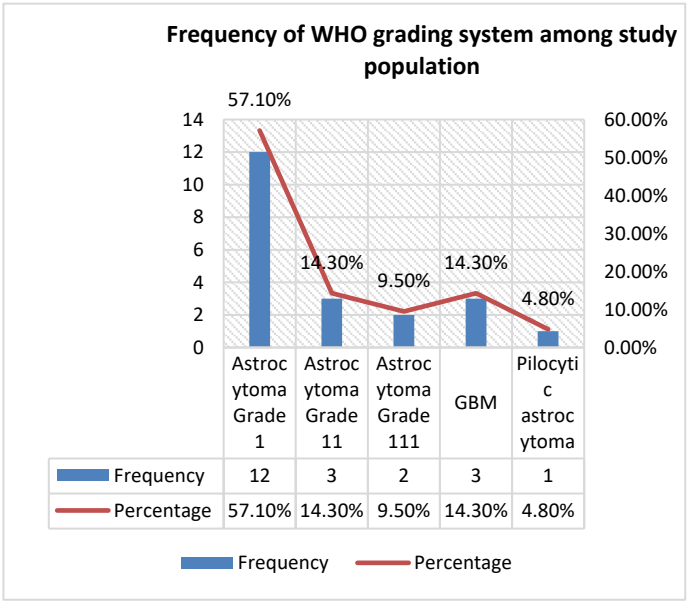

Figure (4): Distribution of WHO grading frequency.

Table 1: The correlation between histopathology and gender

Grade	Male	Female	Total
Astrocytoma	11	6	17
Pilocytic astrocytoma	0	1	1
GBM	1	2	3
Total	12	9	21

Molecular results

Electrophoresis result

Image in figure (5) show electrophoresis of TNF gene (PCR product) after resolution on an agarose gel and staining with ethidium bromide.

Sequencing result

The frequency of mutations among study samples was found as Single base mutations displayed in figure 6. Mutation from C>T was dominated with frequency 4 which represent 23.6% followed by G>A, insertion C with frequency 3 which represent 17.6%(table 2).Grade II astrocytoma had the highest number of mutations (n=6) comparing to the other grades followed by grade III and GBM (n=3) (table 3).

(G>A) rs13306710 mutation was predicted as polymorphism

Figure (5): Gel electrophoresis of TNF gene inglioma samples. Lane 1=100 bp ladder, lane 2-6 TNF gene 872 bp

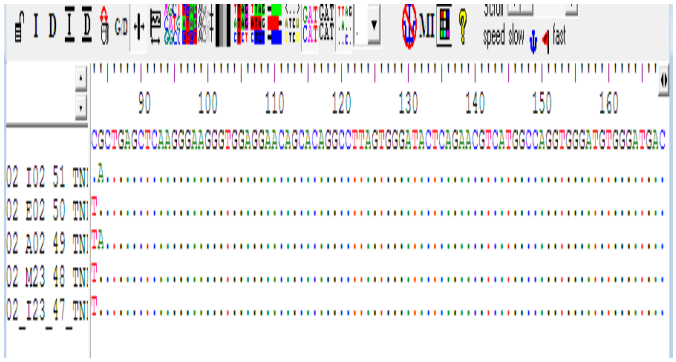

Figure (6):Single base mutation from G to A at position (g.1352 G>A). C to T in position 83

Table(2) :The frequency of TNF gene mutations among study population.

Mutation type	Frequency	Percentage
C>T	4	23.6%
G>A	3	17.6%
C>G	1	5.9%
A>C	1	5.9%
G>T	2	11.7%
A>T	1	5.9%
G>C	1	5.9%
Insertion C	3	17.6%
Deletion G	1	5.9%
Total	**17**	**100%**

Table (3): The correlation between grading and mutations:

Mutation Grading	C>T	A>C	G>T	A>T	G>C	G>A	C>G	Ins C	Del G
Grade 1	___	___	___	___	___	*	___	*	___
Grade 2	*	*	*	*	*	___	*	___	___
Grade 3	*	___	___	___	___	*	___	*	___
GBM	*	___	___	___	___	___	___	*	*
Pilocytic	*	___	___	___	___	*	___	___	___

Table key:

___: Absent

*: Present

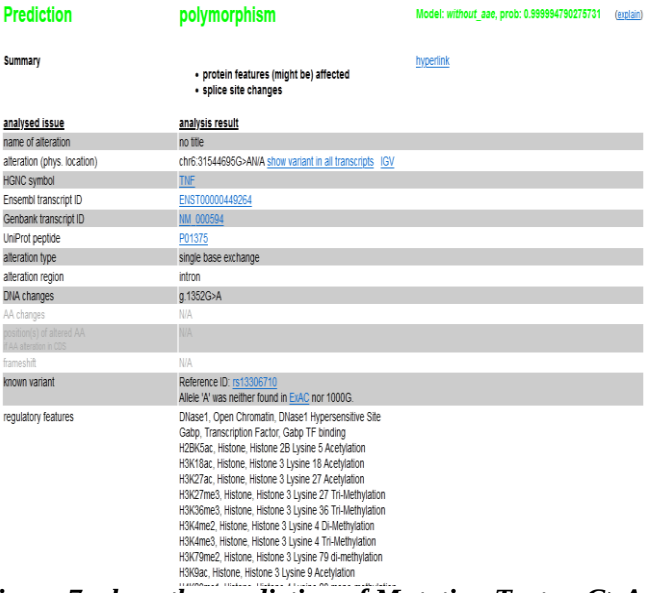

Figure 7: show the prediction of Mutation Taster, G>A (rs13306710) was predicted as polymorphism

Discussion

Occurrence of gliomas variesbetween age, sex, and race or ethnicity among different population, in general incidence is highest among nonHispanic whites. Glioma incidence also varies globally, with the highest rates in

the United States, Canada, Australia, and Northern Europe. Lifetime risk of developing a malignant brain tumor is more than twice as high among non-Hispanic whites compared with blacksAmerican and is 25% higher compared with Hispanic whites. Survival rates after a diagnosis of malignant brain tumor also vary by race or ethnicity[12].However in this study our patients are coming from different Sudanese states but the most common patients are coming from aljazirah and Khartoum states.

In this current study,gliomas are more common in men than women. This finding is in agreement with other study[13] that showed men are more likely to develop glioma than women, with the exception of pilocytic astrocytoma, which occurs at similar rates in men and women.Few studies have shown that blacks Americans are less prone, and incidence of glioblastoma multiform (GBM) is higher in other ethnic groups including Asians, Latinos and Whites[14].In addition to this, one studyshowed that gliomas can occur*in people of all ages*[15]. This beside, there is increased incidence occurs in people between 35 and 44 years of age low grade glioma (LGG) is the most common brain tumor in children, while high grade glioma (HGG) is the most frequent brain tumor in adults, our results in this study glioma are more common in the middle age.

Some studies revealed that gliomas occur at different ages are clinically and molecularly distinct, suggesting that the tumorigenic potential of the cells that give rise to them is different, primary GBMs tend to occur in older patients, whereas lower-grade gliomas and secondary GBMs (which progress from lower-grade gliomas) tend to occur in younger patients, and studies have suggested, that the age-related changes may result from alterations in growth factor responsiveness and tumor suppressor expression. It is also likely that developmentally related changes in the brain environment, such as age-related changes in immune function and progenitor recruitment, could have effects on the patterns of gliomagenesis[16].

Furthermore, gliomas have been classified on the basis of histopathological features, with reference to their proposed relationship to specific glial lineages. Other gliomas (called oligodendrogliomas) show similarities to cells of the oligodendrocyte lineage. Gliomas are further classified on the basis of tumor grade, with less aggressive astrocytomas and oligodendrogliomas designated as WHO grade II, more aggressive forms designated as WHO grade III and the most aggressive ones as GBM (WHO grade IV). Our results providing in this study showed that grade I astrocytoma is the most common type of glioma in the study population, this finding is disagreed with other studies [14].

Cytokines, which are inflammatory mediators, playing physiological role in changing the state of cells and the way they respond to environmental stimuli. This feature has been referred to as ‘memory’ or ‘training’, and includes enhanced responses to subsequent challenges (priming) or refractoriness to stimulation (desensitization or tolerance). Accumulating evidence suggests that TNF modifies cellular responses to other stimuli. The pro-inflammatory cytokine TNF-α (tumor necrosis factor-α) controlsinflammatory cell populations and mediating many of the other aspects of theinflammatory process.

Also it is important in early events in tumors, regulating a cascade ofcytokines, chemokines, adhesions, and pro-angiogenic activities. Thus, TNF-α is one of the ways in which inflammation acts as a tumor promoter and blocking antibodies thathave significant therapeutic efficacy in some inflammatory diseases may have applicationsin therapy in cancer[17].In addition to this, IL-1, TNF-a, and IL-6 are 3 master proinflammatorycytokines produced primarily by

microglia, astrocytes, and infiltrating leukocytes during CNS inflammation[18].

Molecular mechanisms underlying TNF-induced priming and tolerization have been revealed. Additionally mechanism that potentially contributes to TNF-induced cell priming or tolerization is the effect of TNF on signaling events. In addition,prolonged exposure to TNF increases the intracellular reservoir of signal transducer and activator of transcription 1 (STAT1. Moreover, in macrophages, exposure to TNF induces the expression of signaling brakes, , which potentially restrict upstream signaling upon secondary stimulation with lipopolysaccharides (LPS). This beside, several studies suggest that the homeostatic and pathogenic activities of TNF are mediated by distinct molecular and cellular pathways. TNF receptor which is expressed on regulatory T cells, oligodendrocytes and astrocytes, mediates immunoregulation, neuronal survival and re-meyelination; TNFR, by contrast, induces CNS inflammation and neuronal demyelination. Selective inhibitors of the neurotoxic TNFR pathway, which preserve the neuroprotective TNFR pathway, are being developed and could be a new avenue in therapeutics for patients with multiple sclerosis[19].

Moreover, TNF's tumor promoting role has also been demonstrated in various mouse tumor models and there is mounting evidence indicating that pathophysiological concentrations of endogenous TNF promote tumor genesis and growth. In a colitis-associated colon carcinogenesis mouse model, mice lacking TNFR or blocking TNF function with the neutralizing antibody markedly induced colonic inflammation and attenuated subsequent tumor formation [7].

In cancer, tumor necrosis factor alpha-induced proteins (TNFAIP) is caused by TNF-α and interleukin-6 (IL-6) in umbilical vein endothelial cells, which is high expression in normal cell lines and low expression in tumor cell lines. Studies found that TNFAIP was significantly overexpression in osteosarcoma. TNFAIP knockout could inhibit proliferation and induce apoptosis in osteosarcoma cells, and was associated with malignant tumors and septic shock, esophageal squamous cell carcinoma (ESCC), gastric cancer, head and neck squamous cell carcinoma (SCCHN), lymphoma and breast cancer. NPC is a metastable epithelial carcinoma, which is closely related to Epstein–Barr virus (EBV) infection. Studies found that TNFAIP as cell transfer promoter protein was a correlation with invasion and metastasis, and it was overexpressed in NPC samples than adjacent normal tissues[20].

In gliomas, most TNF family members were related to the overall survival of patients. TNF family based-signature which related to clinical, molecular, and genetic characteristics of patients with gliomawas developed, patients were divided into low-risk group and high-risk group according to the median risk score we found significantdifferences in clinical and pathological characteristics between the two groups. Patients in the high-risk group were older than those in the low-risk group, Immunological analysis showed the immuno-suppressive status in the high-risk group[9].our results concerning mutation in this gene C>T represented nearly quarter of the sample, this mutation has been found in the spleen, kidney, lymph nodes, and duodenum of piglets. moreover, mutations in the *TNF*-α promoter and to determine the mRNA expression levels for *TNF*-α in order to assess the feasibility of using the −791(C > T) mutation as a genetic marker and provide some theoretical and experimental basis for disease-resistance breeding based on the *TNF*-α gene. In this current study, this mutation was found in all glioma subtypes, this may explain the similarity of resistanceimmune -system to

react against the glioma cells , however, mega data will be needed to study this mutation[21].

Conclusion:

TNF gene polymorphism was detected and might be associated with gliomas tumors among Sudanese patients.

Abbreviations:

PNET: Primitive Neuroectodermal Tumor

DNA: Deoxyribonucleic Acid

HLA: Human Leukocyte Antigens

SRF: Somatotropin

MAPK: Mitogen-Activated Protein Kinase

IL: Interleukin

DSS: Dextran Sulfate Sodium

EBV: Epstein–Barr virus

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Conflict of interest:

The author's declare that there are no conflicts of interest.

Author Contribution

All authors similarly contributed to this manuscript, cover wrote, corrected and authorized this manuscript.

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