Available online on 15.03.2023 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

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Research Article

Detection of C677T, rs75582903 and SNV64878785 in Methylenetetra hydrofolate Reductase Gene (*MTHFR*) in Meningioma among Sudanese patients

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Article Info:

Article History:

Received 13 Jan 2023
Reviewed 19 Feb 2023
Accepted 28 Feb 2023
Published 15 March 2023

Cite this article as:

Rhoma AA, Mohammed BA, Alfatih DM, Ebraheem FM, Ebrahim FZE, Abuzied MA, Adam OAA, Aldeaf SA, Gassoum E, Detection of C677T, rs75582903 and SNV64878785 in Methylenetetrahydrofolate Reductase Gene (*MTHFR*) in Meningioma among Sudanese patients, Journal of Drug Delivery and Therapeutics. 2023; 13(3):70-77

DOI: http://dx.doi.org/10.22270/jddt.v13i3.5763

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Abstract

Introduction: Meningiomas are commonly benign tumors come from the arachnoid cap cells, and the incidence is account 6 cases per 1000000 of population per year or even much higher in African countries, in Sudan during the period May 2005-May 2012 a total of 405 patients were operated upon and diagnosed as having cranial meningioma, regarding the etiologies of meningiomas are not yet known; it may be related to radiation exposure, genetic disorders (Neurofibromatosis type2), hormonal imbalance (progesterone) and single nucleotide polymorphisms (SNPs).

Aims of the study: This study aimed to amplify *MTHFR* gene in specific region using PCR, and to detect C677T, rs755829023 and SNV64878785 of *MTHFR* gene in meningioma among Sudanese patients.

Materials and Methods: This was a cross-sectional study conducted at the National Center for Neurological Sciences between May to August 2022, Khartoum Sudan, all meningioma patients attending the national center of neurological sciences during the period of the study was included. Demographic variables were included (age and gender), and the clinical data included (meningioma histology subtype and WHO grading), tissue and blood samples were collected, DNA extraction from the tissue and blood samples was done, polymerase chain reaction was made to amplify specific region of *MTHFR* gene. PCR products were sent for Sanger sequencing to Macrogen Europe Laboratory. Data was collected and analyzed by using Statistical Package for the Social Sciences (SPSS) version 19, and sequencing was analyzed using, BioEdit and Mutation taster.

Results: In this study the most affected age group was 41-50 years, female was the most common, WHO grade I meningioma constituted 70% of the cases mostly the fibrous subtype. C>T (g.10369 C>T) was detected in 100% of the cases and deletion C (g.10380-10380delC) was detected only in the cases 16.6%.

Conclusion: In this study, female was the most common 11(55%) with male to female ratio 1:2, the most affected age group was ranging from 41-50 years constituted 35%, WHO grade I meningioma was detected in 70% of the patients. C>T (g.10369 C>T) was detected in 100% of the cases and deletion C (g.10380-10380delC) was detected only in the cases 16.6%.

Keywords: Meningiomas, PCR, MTHFR, and Sanger.

INTRODUCTION

Meningioma described by Harvey Cushing, the term refers to a set of tumor that arises contiguously to the meninges, meningiomas commonly benign tumors come from the arachnoid cap cells [1].

It's the second most common type of primary intracranial tumor of the central nervous system compromising about 30% of all primary brain tumors with incidence rates 1.3 to 7.8 annually and mortality rate 0.3 deaths per 100.000 individuals [2].

The incidence is account 6 cases per 1000000 of population per year or eve much higher in African countries including Sudan [3].

Meningiomas can be either *NF2*-mutated or non-*NF2*-mutated, Previous studies reported the loss of q arm of chromosome 22 lead to individual suffer from meningioma. *NF2* gene located on 22q12.2 is related to Neurofibromatosis type 2 (NF2) which is a tumor predisposition syndrome. Due to the inactivation of the *NF2* gene about 50% of all *NF2* patients have more than one type of meningioma and in the case of non-*NF2* mutated meningioma mutations in other genes, such as *SMO, AKT1, KLF4,* and *PIK3CA* are involved especially in

grade I meningioma also missense mutations in *SMARCB1, SUFU, MTHFR, MTRR, BRIP1, SRSF2,* and *UNC13C* genes are associated with meningioma [4].

Meningiomas originate from meningothelial cells (arachnoid cap cells) and develop more frequently in areas where cap cells most numerous. Cap cells are especially concentrated in the arachnoid granulations which are a common site of origin meningiomas, mainly along the dural venous sinuses where villi of arachnoid granulations are clustered [5].

The etiologies of meningiomas are not yet known; it may be related to radiation exposure, genetic disorders (Neurofibromatosis type2), injuries in the skull and surrounding membranes, hormonal imbalance (progesterone) and single nucleotide polymorphisms (SNPs) are important molecular markers that occur in may site through whole genome; they are sufficient for disrupting a protein's structural and functional viability [4].

The World Health Organization (WHO) defines 15 subtypes of meningiomas and divides them into 3 grades of malignancy based on histopathological characteristics, the most common type of meningioma accounts for grade 1 (80%), atypical grade 2 (18%), and anaplastic grade 3 (2%). Addition to this, the histological subtypes of meningioma grouped by WHO grade to: grade I meningiomas which are includes: Meningothelial meningioma ,Fibrous (fibroblastic) meningioma ,Transitional (mixed) meningioma ,Psammomatous meningioma ,Angiomatous meningioma ,Microcystic meningioma ,Secretory meningioma ,Lymphoplasmacyte-rich meningioma ,and Metaplastic meningioma. Grade II meningiomas includes: Atypical meningioma, Clear-cell meningioma and Chordoid meningioma. Grade III meningiomas include the following: anaplastic (malignant) meningioma, rhabdoid meningioma and papillary meningioma [4, 6].

The human *MTHFR* gene is located in 1p36.3 containing about 11 extrons and 10introns and encoding a protein of 74.6kDa [7-8].

Methylenetetrahydrofolate reductase plays critical roles in the maintenance of DNA normal methylation, de novo synthesis of nucleotide, and DNA repair by modulating the folate metabolism. Decreased levels of MTHFR may be associated with increased risks for many diseases, including Alzheimer's disease, Down's syndrome, diabetes mellitus, cardiovascular diseases, ischemia, schizophrenia, depression, hypotonia, migraine, pregnancy complications, cleft palate, and various cancers, also the polymorphisms of the folate cycle enzymes are associated with tumorgenesis in different cancers sometimes the same polymorphism is associated with increased risk of some cancers and decreased risk of others [9].

Association of MTHFR C677T and GSTM1/ GSTT1 polymorphisms with brain tumor risk has been widely studied, one study done in Caucasian population and found that SNPs of MTHFR C677T are related to meningioma risk with ethnic differences [8].

Methylenetetrahydrofolate reductase (*MTHFR*) gene plays key roles not in folate metabolism only but also in carcinogenesis. The single nucleotide polymorphism *MTHFR* C677T indicated in the development of various tumors and associated with modify of the risk for brain tumors, particularly meningioma[10].

MATERIALS AND METHODS

This was a cross-sectional study conducted at the National Center for Neurological Sciences located in Khartoum the capital of Sudan between May-August 2022, all meningioma patients attending the national center of neurological sciences during the period of the study was included; in addition to that, blood samples from apparently healthy individuals was collected and processed as control, demographic variables were included (age and gender), and the clinical data included (meningioma histology subtype and WHO grading), Based on guideline of the research laboratory in the National Center for Neurological Sciences (NCNS) DNA was extracted from the tissue and blood samples was done, polymerase chain reaction was made to amplify specific region of *MTHFR* gene. PCR products were sent for Sanger sequencing to Macrogen Europe Laboratory. Data was collected and analyzed by using Statistical Package for the Social Sciences (SPSS) version 19, and sequencing was analyzed using, BioEdit, Ensemble, NCBI and Mutation taster.

This study was approved by the ethical committee at the faculty of medical Laboratory science (histopathology and cytology department), Al-neelain University. The participants sample were collected after writing agreement assigned with laboratory administrations in the National Center for Neurological Sciences, personal data were collected from database office at NCNS by using structured predesigned questionnaire as well as hospital and medical record.

DNA extraction from tissue

Genomic DNA was isolated from tissue samples according to the guanidine chloride extraction method; the meningioma tissue was placed into Petri dish and then cut into small pieces until becomes homogeneous, then the homogeneous tissue sample was transferred to eppindroff tube, and then 800µl of STE buffer, 100µl of 10% SDS and 20µl proteinase K were added, and the tubes were incubated at 65 °C for overnight. After incubation, the protein was precipitated by adding 300µl of 6 M NaCl and then the tubes were kept at 4 °C for 15 minutes, after this step the tubes were centrifuged at 18000rpm for 20 minutes. after centrifugation, 500µl of the supernatant was transferred to a new eppindroff tube, and then, 350µl of 8M guanidine chloride and 150µl l0.49M NH4 acetate were added, and the tubes were left at room temperature for 90 minutes, following this step, 500µl pre-chilled chloroform was added, and then the tubes were centrifuged at 12000rpm for 5 minutes. After that the upper layer was transferred to a new tube, and then the genomic DNA was precipitated by adding 800µl of cold absolute ethanol, after this the tubes was incubated at -20 °C for overnight, and then centrifuged at 12000rpm for 5 minutes. Then the pallet was washed with 400µl of 70% ethanol, then vortex and centrifuged at 7000rpm for 5 minutes, after centrifugation, the supernatant was discarded and left to drying. The last step, DNA was eluted in 100µl of ddH2O and kept at 4 °C for overnight and stored at -20 °C till use.

DNA extraction from blood

Genomic DNA was extracted from peripheral blood leukocytes by the standard phenol chloroform extraction method; about 3ml of blood was collected in EDTA tubes, then the blood samples was transferred into 15 ml falcon tube, then the RBCs were lysed by adding 7ml lysis buffer, this step was repeated till the WBC pallet appeared clean, to the pallet, 1ml of WBCs lysis buffer and 100 µl proteinase K were added, then the tubes were incubated at 65 °C for overnight, after incubation, 1ml of pre-chilled chloroform was added and the tubes were incubated at -20 °C for few minutes, and then the tubes were centrifuged at 6000 rpm, after centrifugation, the supernatant was transferred to a new eppindroff tube, then 5ml of cold ethanol was added and the tubes were incubated at -20 °C for overnight, after incubation the tubes were centrifuged at 6000 rpm, and the pallet containing DNA was washed in 70% ethanol, after washing, the tubes were centrifuged at 6000 rpm, and the supernatant was pour off and the tubes were left

to dry, after this step the DNA was eluted in 100 µl ddH_2O, then incubated in 4 °C for 15 minutes, and stored at -20 °C till use.

PCR protocol

The extracted DNA was amplified for specific sequence of *MTHFR* gene, using standard PCR protocol; in the PCR tube, 2µl of genomic DNA was added to 14µl distill water, 4µl master mix containing (1.5 buffer, nM MgCl2, 200µm of dNTPs and 0.5 units of Taq polymerase), 1µl from reverse and 1µl forward primers then was processed in PCR thermo-cycler machine.

MTHFR gene amplification

The *MTHFR* gene was amplified using PCR. The following primer sequences were used to obtain 494 bp fragments;

Forwards: 5- GGTCAGAAGCATACTAGTCATGAG -3

Reverse: 5- CTGGGAAGAACTCAGCGAACTCAG-3

The PCR was carried using thermal cycler (Swift™MaxPro SWT-MXP-BLC-4). The amplification steps consisted of an initial 15 minutes of denaturation at 95 °C, followed by 35 cycles of denaturation at 95 °C for 20 seconds, the primers were annealed at 68 °C for 50 seconds, then the elongation period was 30 seconds at 72 °C, the final elongation was adjusted for 10 minutes at 72 °C.

Demonstration of PCR product

The PCR amplification products were separated on 0.7% agarose gel (0.7g of agarose powder + 28 ml of DW + 7 ml of TE buffer this mixture was placed in microwave for 1 mint then was stained with 1 µl of ethedium bromide and mixed, after that poured on gel electrophoresis tray and left to dry).After drying, 4µl from each PCR amplification products was filled in the wells, then 1X TBE buffer was used as a running buffer .The voltage applied to the gel was 150 volt, with running time duration was 15 minutes. 100bp DNA ladder was used as molecular weight marker. Finally, the results were tarns-illuminated with UV light and visualized through gel documented system.

Sequencing

The PCR products were sealed with parafilm wax and sent for Sanger sequencing to Macrogen Europe Laboratory (Amsterdam1105 BA).

RESULTS

Demographic results

A total of 20 meningioma patients were included in the present study, males were 9 constituted 45% and females were 11 constituted 55%. In addition to this, female was the most common 11(55%) with male to female ratio 1:2 (Table 1).

There was different age groups participated in this study, the mean age was 45.45 years and distributed into six groups; group 1 ranging from 0-20 years constituted 5%, group 2 ranging from 21-30 years in 5%, group 3 ranging from 31-40 years represented 25%, group 4 ranging from 41-50 years constituted 35%, group 5 ranging from 51-60 years in 25% and group 6 >60 years constituted 5% (Table 2).

Histopathology and WHO grading results

In the present study, WHO grade I meningioma was the most frequent among the studied materials, and constituted 70% of the cases, the most common histological subtype in this grade was the fibrous.

The most common subtypes of meningioma grade I was the fibrous in (45%) of the samples, followed by meningothelial (15%) and mixed (10%). Grade II meningioma showed that, choroid subtype was detected in 10%, atypical (10%) and clear cell (5%). The papillary meningioma (grade III) was present in 5% of the samples as shown in (Table 3).

Molecular genetics results

In this study the gel electrophoresis of *MTHFR* gene showed positive PCR for all samples (Figure 1).

C>T(g.10369 C>T) was detected in 100% of the cases, in control group C>T was detected in 80%, moreover, C>G in the same position was only detected in control group 20%, in addition to that, T>G(g.10370) was present in the cases and control group in 100% and 80% respectively. At position g.10370 T>C was detected only in control group in 20%, at position g.10371 G>A was detected in 16.6% of the cases and G>C in 80% of control group, deletion G (g.103701-10371delG) was detected in 83% of the cases and 20% in control group, deletion C (g.10380-10380delC) was detected only in the cases 16.6%, furthermore, deletion A (g.10381-10381delA) was seen in 16.6% and 40% of the cases and control group respectively. A>G and A>C at position g.10381-10381 were detected only in control group 20%, insertion T (g.10816-10817insT) was seen in 50% of the cases and 60% of control group, in the cases only, G insertion (g.10816-10817insG) was observed in 16.6%, in addition to this, insertion A and C were seen in controls just and representing 20%, interestingly, G>T, T>A and insertion A were detected in 80% of control group, but not detected in the cases. Detail information was displayed in (Table 4) and (Figures 2 A, 2 B, 2 C, 3 and 4).

Table 1: Shows the frequency of gender in meningioma patients

Gender	Frequency	Percent
Male	9	45%
Female	11	55%
Total	20	100%

Table 2: shows frequency of age group

Valid	Frequency	Percent
0-20	1	5%
21-30	1	5%
31-40	5	25%
41-50	7	35%
51-60	5	25%
>60	1	5%
Total	20	100%

Table 3: Shows the histological subtypes and grades among study population

Subtypes	Frequency	Percent	WHO Grades
Fibrous	9	45%	I
Meningothelial	3	15%	I
Mixed	2	10%	I
Chordoid	2	10%	II
Atypical	2	10%	II
Clear Cell	1	5%	II
Papillary	1	5%	III

Table 4: Shows the mutations of *MTHFR* gene within the cases &control group

Mutations	Cases	Control group
C>T	100%	80%
C>G	-	20%
T>G	100%	80%
T>C	-	100%
G>A	16.6%	-
G>C	-	80%
DelG	83%	20%
DelC	16.6%	-
DelA	16.6%	40%
A>G	-	20%
A>C	-	20%
InsT	50%	60%
InsG	16.6%	-
InsA	-	20%
InsC	-	20%
G>T	-	80%
T>A	-	80%
InsA	-	80%

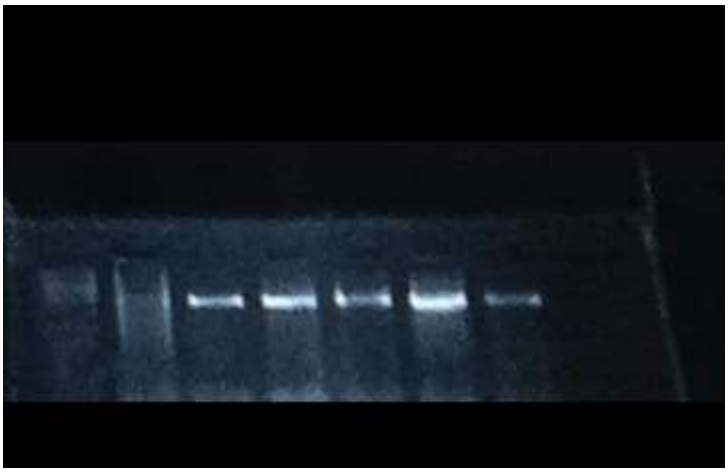

Figure 1: shows gel electrophoresis of *MTHFR* gene in meningioma.

Lane 1: DNA ladder (100bp), Lane 2, 3,4,5,6, 7: meningioma variant (494bp).

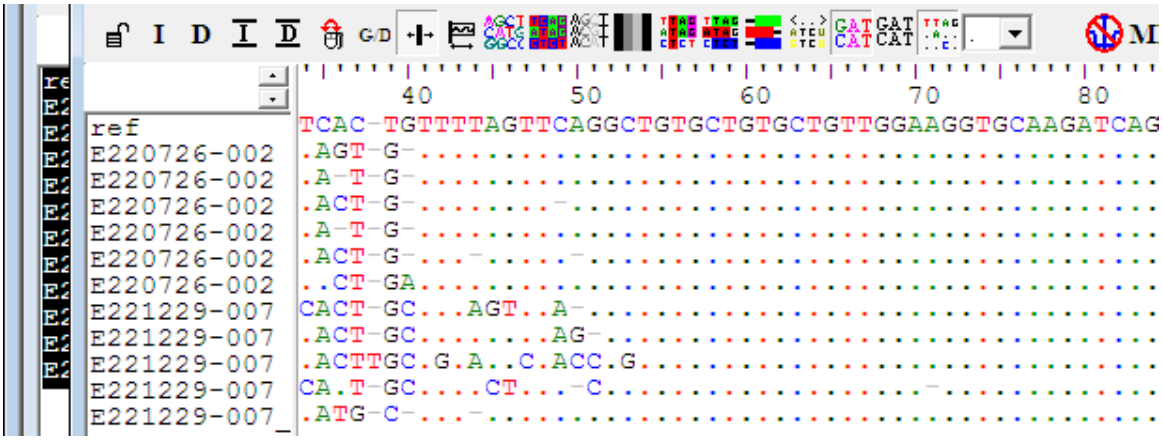

Figure 2 (A): shows C>T& C>G (g.10369), T>G& T>C (g.10370), Deletion G (g.10371-10371delG), G>A & G>C (g.10371), Deletion C (g.10380-10380delC) and Deletion A (g.10381-10381delA) in cases and control group.

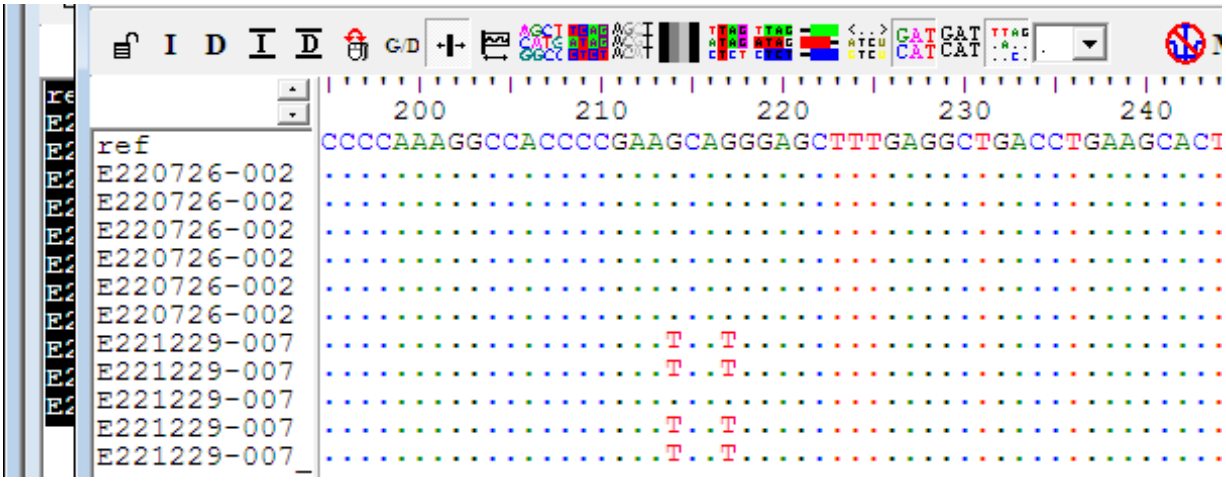

Figure 2 (B): shows G>T with in the control group

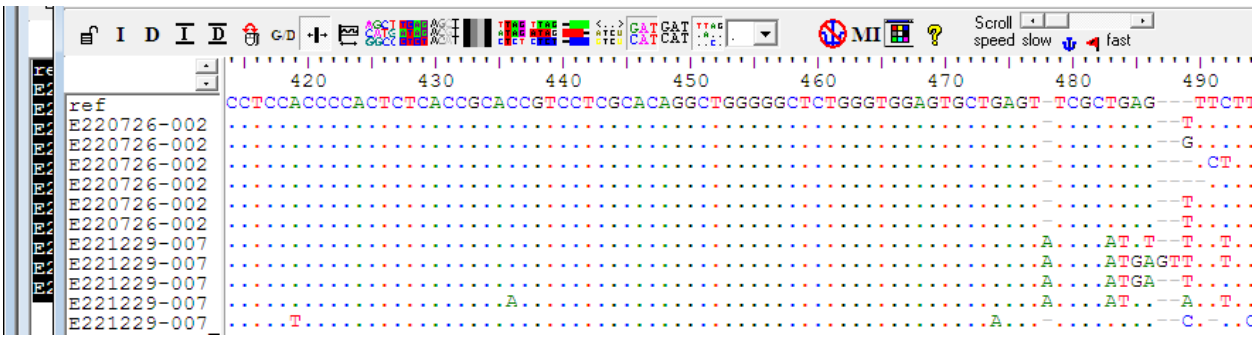

Figure 2 (C): shows insertion A, T >A and insertion T, G, A &C (g.10816-10817) in the cases & control group.

Prediction polymorphism

Summary

- **splice site changes**

analysed issue	analysis result
name of alteration	no title
alteration (phys. location)	chr1:11856609G>AN/A show variant in all transcripts IGV
HGNC symbol	MTHFR
Ensembl transcript ID	ENST00000376592
Genbank transcript ID	NM_005957
UniProt peptide	P42898
alteration type	single base exchange
alteration region	intron
DNA changes	g.10369C>T

Figure 3: Mutation taster analysis predicted C>T as polymorphism.

Prediction polymorphism

Summary

- **splice site changes**

analysed issue	analysis result
name of alteration	no title
alteration (phys. location)	chr1:11856598_11856598delG show variant in all transcripts IGV
HGNC symbol	MTHFR
Ensembl transcript ID	ENST00000376592
Genbank transcript ID	NM_005957
UniProt peptide	P42898
alteration type	deletion
alteration region	intron
DNA changes	g.10380_10380delC

Figure 4: Mutation taster analysis predicted deletion C as polymorphism.

DISCUSSION

Meningiomas represent the most common intracranial extra-axial neoplasia. Account for approximately 30 % of central nervous system (CNS) tumors in adults [11], in Sudan, during the period May 2005-May 2012 a total of 405 patients were operated upon and diagnosed as having cranial meningioma, the results of this above mentioned work, revealed that meningioma is the most common brain tumor [3].

In addition to this, the incidence of meningioma increases with age and shows a remarkable predominance in females particularly in the 3rd to 6th decade [12], in Sudan, study done by Gassoum et al., 2014 explained that the distribution of the age of the studied material is about 68.3% of patients were above the age of 40 years, this finding did not differ from the international incidence of intracranial meningioma among age group in male and females, in this current study, age group of 41-50 are the most affected. It is known that, and according to data from the Central Brain Tumor Registry of the United States (CBTRU) a more than twofold higher incidence among females (age-adjusted incidence rate per 100,000 person years of 8.36 and 3.61for females and males respectively [12], in

Sudan, Arbab et al., 2014 suggested that the most patients affected with meningioma categories are females suggested being hormonal dependent, our study provides that females are the most affect in having meningioma,11(55%) with male to female ratio 1:2.

Moreover, World Health Organization (WHO) meningiomas are categorized into 3 grades. Criteria include cell type, mitotic activity, cellularity, necrosis, and brain invasion. Benign meningiomas (WHO grade I) represent 90 % of all meningiomas and have several histological subtypes: Meningothelial, Fibrous (fibroblastic), Transitional (mixed), Psammomatous, Angiomatous, Microcystic, Secretory, Lymphoplasmacyte-rich, Metaplastic meningioma. Atypical meningiomas (WHO grade II) represent 5 %–7 % of all meningiomas and comprise Clear-cell or Chordoid variants. They show a mitotic index of ≥ 4 mitoses per 10 high-power fields (HPF), increased cellularity, and a high nucleus: cytoplasm ratio, sheet like growth and foci of spontaneous necrosis .Anaplastic meningiomas (WHO grade III, 3 % of all meningiomas) include Papillary and Rhabdoid variants, and show a mitotic index of ≥20 mitoses per 10 HPF and larger zones of spontaneous necrosis. In this current study, the fibrous subtype of meningioma constituted nearly halve of the samples, which agree with the published data from the national center of neurological Sciences [3].

Furthermore, Folate Is anionic, a water-soluble vitamin (vitamin B9) in nature present as reduced form, found in some foods (e.g., okra, broccoli, asparagus, lentils, and spinach) or in a synthetic form folic acid [13, 14]. This vitamin is a limiting factor in the methylation reactions of DNA, RNA and proteins. Plants and bacteria can synthesize folic acid, but not humans and other animals. Many proteins have been identified in folate transport; a specific anion transporter to the reduced form with ubiquitous tissue distribution, a glycosyl-phosphatidylinositol (GPI) carrier bound to high-affinity proton in an acidic pH environment and a transporter of high-affinity GPI anchored proteins [15]. In adding to this, biological activation of folic acid requires a reduction in the intermediate forms of dihydrofolate and tetrahydrofolate. Folate can also bind to the carbon units of methyl groups (CH3), methylene (CH2), formyl (–CHO–) or formimino (–CHNH–) at positions N5, N10, or both positions of the structure. This process imparts function to folate coenzyme in various enzymatic systems as a carbon carrier unit at different degrees of oxidation. A carbon donor unit function is essential for methylation reactions, nucleotide synthesis and DNA synthesis and repair [16, 17], and can influence carcinogenesis by adverse effects on global DNA methylation and/or promoter regions of specific CpG sites [18]. And the effects of methylation in DNA and possible subsequent damage to molecules, disturbances in the metabolism of carbon-1 cause DNA damage via effects on nucleotide synthesis. In folate deficiency, uracil is incorporated into DNA molecules; during repair, breaks may occur in DNA molecules by uracil glycosylase (UDG), causing damage and chromosomal translocations, a hallmark of genomic instability that may contribute to tumor progression [18, 19].

MTHFR (5, 10-methylene tetrahydrofolate reductase), MTR (methionine synthase) and MTRR (methionine synthase reductase) are key enzymes in the metabolism of folate. MTHFR occupies a central position while maintaining homeostasis between DNA synthesis and methylation, promoting irreversible conversion of 5, 10 methylene tetrahydrofolate to 5-methyltetrahydrofolate [20]. The substrate 5, 10 methylene tetrahydrofolate is used by thymidylate synthase to methylate dUMP to dTMP. The latter is the only required source of thymidine for DNA synthesis and repair, and the product functions as a methyl donor required for the remethylation of homocysteine to methionine. This reaction is catalyzed by the MTR enzyme and dependent on vitamin B12, which acts as a carrier of intermediate methyl groups. The oxidation of vitamin B12 acts as a cofactor that may inactivate MTR, which can again be functionally active when the reductive remethylation of vitamin B12 by MTRR occurs. The reduced activity of the MTHFR pathway inhibits the production of 5-methyltetrahydrofolate and can lead to accumulation of the substrate. Decreased levels of the MTHFR product can cause increased levels of homocysteine, decreased levels of blood folate and displacement means for folate synthesis and DNA repair. Circulating folate is a co substrate for the remethylation of homocysteine to methionine, and methionine is a precursor to S-adenosyl methionine (SAM), which is the main methyl donor. Low levels of folate and/or reduced enzyme activity of the main proteins involved in folate metabolism may result in limiting the substrate for methionine synthase, thus affecting the remethylation pathway and resulting in a high concentration of homocysteine in the plasma; high plasma levels of homocysteine have been linked to several types of human cancers [21, 22]

DNA methylation causes the covalent modification of genomic DNA that alters gene expression and changes in the patterns of DNA methylation are critical features in the process of carcinogenesis, of which is Breast tumors are characterized by a high incidence among women and are one of the main causes of mortality and brain tumors like Gliomas which are heterogeneous tumors and have high rates of recurrence and mortality [23, 24].

MTHFR is a key enzyme in the folate metabolism pathway that converts 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the primary circulatory form of folate, producing a methyl donor for the remethylation of homocysteine to methionine in a conversion catalyzed by MTR using vitamin B12 as a cofactor. Defects in the processing of folate due to decreased enzyme activity have been associated with reduced levels of circulating folate and may culminate in changes in the levels of DNA methylation and gene regulation. The occurrence of polymorphisms in enzymes encoding genes of the folate pathway provides a functional impact on metabolism [22, 25, 26, 27]. Nevertheless, there are two polymorphisms of the MTHFR gene described as associated with numerous pathologies C677T and A1298C [28, 29, 30].

In this study, Mutation taster analysis predicted C>T as polymorphism and found in 100% of the cases, this mutation has splice site effect and may alter the normal function of MTHFR gene, this alteration may further contribute negatively on the pathway responsible for folate metabolism. In addition to that, another mutation (deletion G) was detected in 83%, and deletion C was detected only in the cases and predicted as polymorphism with splice site effect.

The C677T polymorphism is an exchange at position 677 of the MTHFR gene whose exchange of nucleotide cysteine for thymine culminates in the replacement of alanine by valine in the MTHFR enzyme. This mutation is associated with reduced enzyme activity and increased thermolability of the enzyme already reported in vitro, leading to a decrease in 5-methyltetrahydrofolate and an increase in the accumulation of the substrate 5, 10- methylenetetrahydrofolate. The MTHFR A1298C polymorphism occurs in exon 7 and results in glutamate transformation into alanine at codon 429. This polymorphism is located in the regulatory domain S-adenosyl methionine (SAM) of the enzyme and causes conformational changes in the MTHFR enzyme that also reflect a reduction in enzyme activity but do not result in a thermolabile protein [29, 30].

Other studies have shown that folate deficiencies and the presence of polymorphisms in genes related to the folate pathway increase the incidence of cancer. MTHFR is directly involved in folate metabolism. Therefore; MTHFR polymorphisms may directly affect the incidence of cancer, likely by causing an imbalance in maintaining the epigenome. However, data regarding the association between the MTHFR polymorphism status and cancer risk have also been conflicting [31, 32]. Several types of tumors have been associated with the presence of polymorphisms in MTHFR. The MTHFR gene polymorphisms show a relationship with leukemia's and lymphomas with a more marked presence of the C677T polymorphism than A1298G in studies with significant results obtained [33,34]. In a meta-analysis increased risk of stomach and esophageal cancer in addition to an increased risk in Asian [35].

Our current study revealed that, most of *MTHFR* gene mutations among investigated samples showed splice site effect and may affect the pathway responsible for folate metabolism, *MTHFR* gene is one of the central gene in this pathway.

Acknowledgement:

The authors acknowledge the staff and researchers in the research laboratory at the National Center of Neurological Sciences for their helpful and support.

Conflict of interest:

No conflict of interest

Funding:

This research was self funded by the authors

Author contribution:

All authors were equally contributed

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