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RESEARCH ARTICLE

DISTRIBUTION AND IMAGING CHARACTERISTICS OF HIGH-GRADE GLIOMAS ACCORDING TO METHYLATION STATUS OF MGMT GENE PROMOTER

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Abstract

Background: Many molecular investigations have identified that the methylation status of the O-6-methylguanine-DNA methyl transferase (MGMT) gene promoter in patients with high grade glioma is associated with a improved prognosis and survival when treated with temozolamide (TMZ).

Aims and Objective: The aim of the study was to find out the distribution of methylation status and imaging characteristics in patients with High Grade Gliomas.

Method: The study included forty consecutive patients who presented with neurological symptoms and were subsequently diagnosed as having a primary brain tumour on conventional MRI scan. MR-perfusion imaging was performed to determine the grade of the tumor which was subsequently compared with histopathological grade of the tumor after brain biopsy. The biopsy or surgical specimens were further analysed for determining the MGMT promoter methylation status using Methylation-specific PCR (MSP).

Results: The mean age of patients was 50.3 years in the methylated group and 49.1 years in the unmethylated group (p value = 0.671). Out of 40 patients, 18 had methylated MGMT promoters while 22 had unmethylated MGMT promoters. We found that ring-like enhancement was seen more frequently in the unmethylated tumours (72.7%) than in methylated ones (61.1%). The presence of T_2 heterogeneous signal intensity was seen more in the methylated group (77.8%) than unmethylated group (68.2%). Severe necrosis (affecting >50% of the tumour) was seen more in the methylated tumours (61.1%) than in the unmethylated tumours (40.09%). However, these differences were found to be statistically insignificant.

Conclusion: The distribution and imaging characteristics may provide some information about methylation of high-grade gliomas but a multicentric large data maybe required to reach a consensus about its reliability when biopsy of the methylation status of the tumour is not available.

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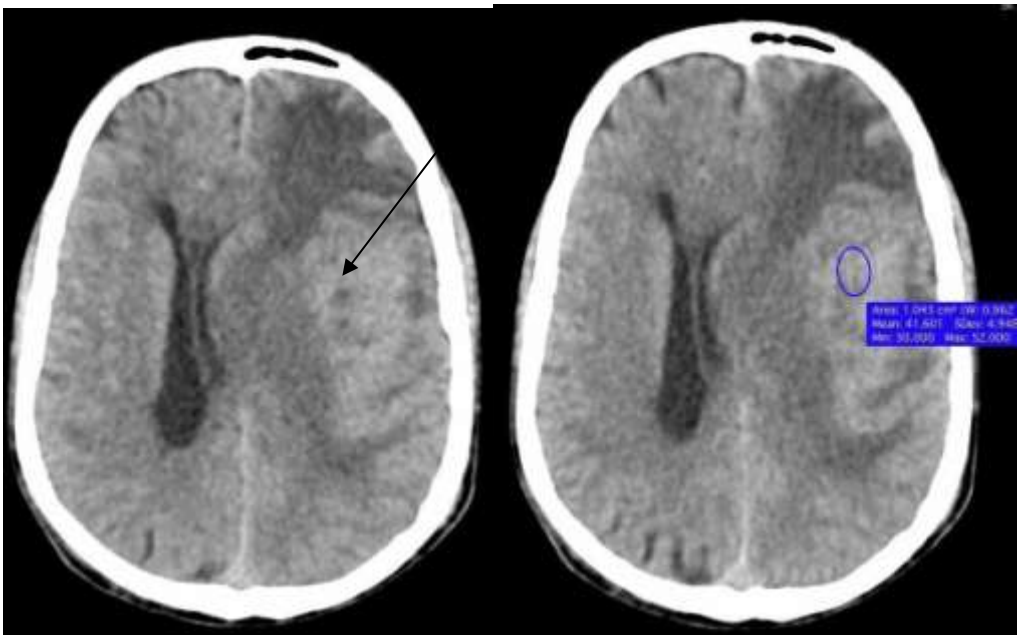
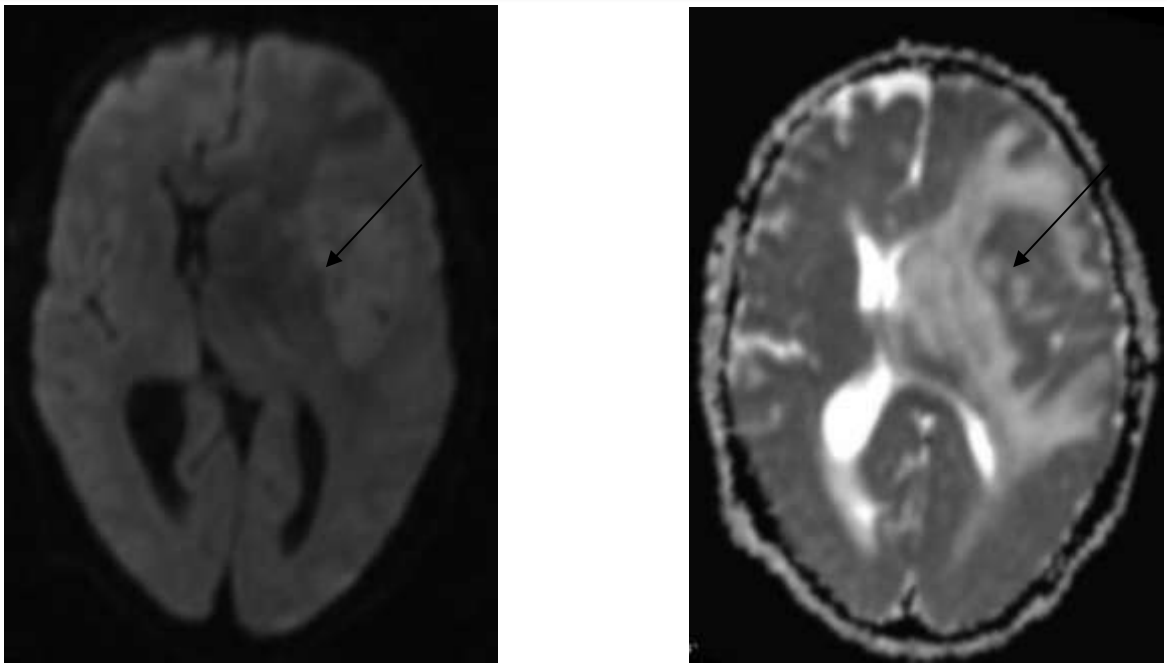
Introduction:-

For patients with high grade gliomas (HGGs) treated with temozolamide (TMZ), methylation status of the O-6-methylguanine-DNA methylation transferase (MGMT) genes promoters is related with a good prognosis and prolonged survival [1,2,3]. The MGMT promoter's gene is located in band q26 on chromosome 10. At the molecular level, methylation of the MGMT promoter gene causes decreased levels of MGMT proteins, an important DNA repair enzyme. DNA damage and cell death that follows are carried on by this low level of MGMT proteins [4,5]. Accordingly, methylation of the MGMT promoter at the microscopic level renders HGG more susceptible to chemotherapy. As per reports, MGMT promoter methylation is evident somewhere between 30% and 53% of glioblastoma multiforme (GBM) and between 50% and 84% of anaplastic gliomas (AG)[3,8]. The methylation pattern of the MGMT promoter is only determined by glancing at the operative or tissue sample, regardless of the fact that it is considered to serve as an important prognostic marker for patient's outcome having HGGs and a yield consistent result of the responses to alkylating chemotherapy [2,6,9]. Consequently, unguided intraoperative biopsy is prone to sampling error due to the complexity of HGGs, that could contribute to incomplete inclusion of the targeted area and undergrading in up to 30% of cases [10,11]. The methylation status of the MGMT promoter can also be erroneously inferred in a similar manner. The MGMT repair gene promoter's methylation suppresses MGMT transcription, raises sensitivity to TMZ, and facilitates better prognosis [12]. A non-invasive method of determining the state of MGMT promoter methylation has been studied using tumor characteristics collected from conventional and advanced MRI. For instance, non-methylated MGMT promoter is linked to ring enhancement [13]. The left cerebral hemisphere was shown to have more MGMT promoter methylation tumors than the right in a study of 358 de-novo GBMs [14]. However, a comparable study using a somewhat lower sample size of patients (N.=72) was unable to demonstrate a geographical preference for these tumors. Additionally, it has been hypothesized that tumors with MGMT promoter methylation have minimal edema and have a better prognosis [15]. Traditional MRI sequences including T1-weighted (T1W), T2-weighted (T2W), fluid attenuated inversion recovery (FLAIR), T2*W gradient echo, and post-contrast T1W images are frequently utilized to assess intracranial cancer. The use of a gadolinium-based contrast agent in this technique enables for the identification of regions where the blood-brain barrier is permeable, and these sequences reveal precise anatomic information.

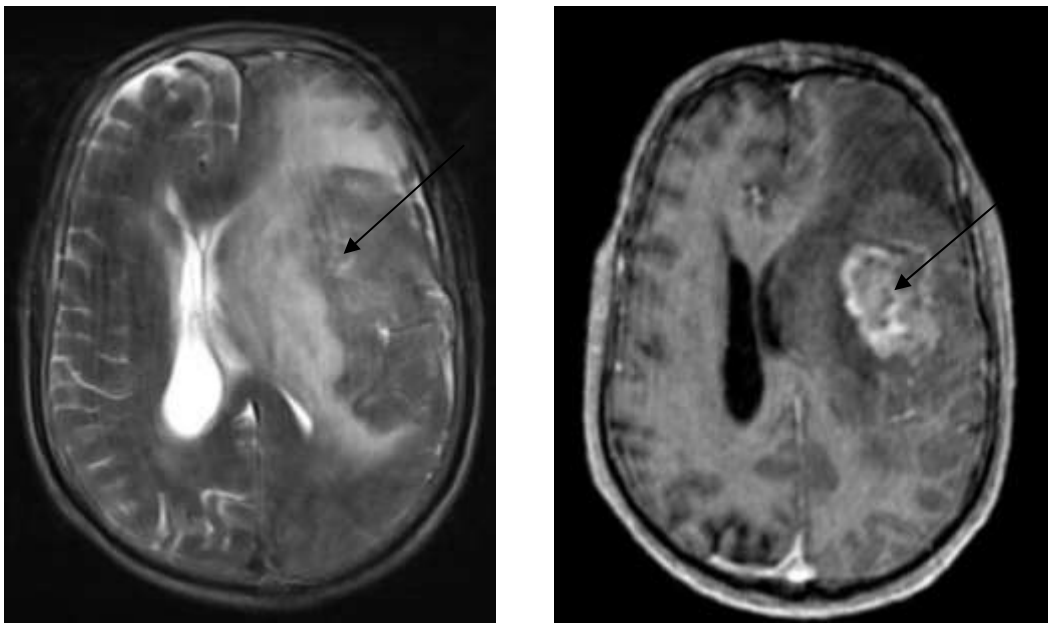
Gliomas typically have a hypointense T1W signal and a hyperintense T2W signal. The probability of a high-grade glial neoplasm is raised by contrast enhancement, necrosis, bleeding, poorly defined infiltration of the surrounding brain, and significant peritumoral edema, which are frequently regarded as imaging hallmarks of aggressive lesions. There is a lot of research on the relationship between contrast enhancement and tumor grade. Additionally, MGMT promoter methylation has been predicted using sophisticated MR imaging techniques. [fig 1a-f]



a. b.



c. d.



e. **f.**

Figure1(a-f). HighgradegliomawithanunmethylatedMGMTpromoter.
aand**b**Axialnon-contrastCT, **caxialT**2WI, **daxialpost-**contrastT1WI
e and**f**DWimageswithADCmap

Aims and Objectives:-

The study was aimed to find out the distribution of methylation status and imaging characteristics in patients with High Grade Gliomas.

Material and Methods:-

From May 2019 to May 2021, patients referred by the Department of Neurosurgery at SKIMS were studied in the Department of Radiodiagnosis & Imaging at SKIMS, Soura. Forty consecutive patients who first reported with neurological symptoms and were later determined to have a primary brain tumor on a traditional MRI scan were included in the trial during a two-year period. Following a brain biopsy, the tumor's histological grade and its grade as determined by MR-perfusion imaging were compared. Utilizing methylation-specific PCR, the status of MGMT promoter methylation was further examined in the biopsy or surgical tissues (MSP). DNA was extracted for MSP using standard procedures. By chemically converting unmethylated but not methylated cytosines to uracil, methylation patterns on the CpG island of MGMT were identified. Using primers made specifically for methylation or unmethylated DNA, MSP was carried out.

Criteria for inclusion and removal Patients with claustrophobia and secondary brain tumors, such as metastatic brain tumors, were excluded from the research. Patients whose conventional MRI scans were tentatively classified as High-Grade Gliomas (HGGs) were also included.

Statistical Methods:

The collected data was put into a Microsoft Excel spreadsheet and exported to the data editor of SPSS Version 20.0. (SPSS Inc., Chicago, Illinois, USA). The expression for continuous variables was MeanSD. Comparing continuous variables was done using the Mann-Whitney U-test or Student's independent t-test. For comparing categorical variables, the Fisher's exact test or the chi-square test was used. Statistical significance was defined as a p-value 0.05.

Ethical Clearance:
Before the study started, the institutional ethics committee at the Sher-e-Kashmir Institute of Medical Sciences (SKIMS), Soura, was contacted.

Results:-

Table 1:- Age distribution of study patients according to methylation status.

Methylation status	Number of Patients	Mean Age (in years)	SD	Range	p-value
Methylated	18	50.3	11.22	27-65	0.671
Unmethylated	22	49.1	7.74	29-63	

The mean age of patients was 50.3 years in the methylated group and 49.1 years in the unmethylated group (p value 0.671).

Table 2:- Gender distribution of study patients according to methylation status.

Gender	Methylated		Unmethylated		P-value
	No.	%age	No.	%age	
Male	9	50.0%	12	54.5 %	0.775
Female	9	50.0%	10	45.5%	
Total	18	100%	22	100%	

Out of 18 patients in the methylated group, 9 were men and 9 were women while in the unmethylated group, 12 were men and 10 were women. There no statistically significant difference in gender distribution.

Table 3:- Imaging characteristics of study patients according to methylation status.

		Methylated		Unmethylated		P-value
		No.	%age	No.	%age	
WHO Grade	Grade 3	7	38.9	5	22.7	0.267
	Grade 4	11	61.1	17	77.3	
Cyst	Present	3	16.7	4	18.2	0.901
	Absent	15	83.3	18	81.8	
Side	Right	8	44.4	11	50.0	0.768
	Midline	3	16.7	2	9.1	
	Left	7	38.9	9	40.9	
Enhancement	Patchy	3	16.7	2	9.1	0.693
	Ringlike	11	61.1	16	72.7	
	Nodular	4	22.2	4	18.2	
Edema	Absent	2	11.1	3	13.6	0.971
	Mild	10	55.6	12	54.5	
	Severe	6	33.3	7	31.8	
Heterogeneous T2 signal intensity	Present	14	77.8	15	68.2	0.749
	Absent	4	22.2	7	31.8	
Necrosis	Absent	0	0.0	2	9.1	0.257
	Mild	7	38.9	11	50.0	
	Severe	11	61.1	9	40.9	
Tumor crossing midline	Yes	14	77.8	18	81.8	0.751
	No	4	22.2	4	18.2	
Multifocal	Yes	5	27.8	4	18.2	0.732
	No	13	72.2	18	81.8	

Out of 40 patients,18 had methylated MGMT promoters while 22 had unmethylated MGMT promoters. In the methylated group, WHO grade 3 tumours were seen in 7 patients while 11 patients had WHO grade 4 tumours. In the unmethylated group, only 5 patients had WHO grade 3 tumours while grade 4 tumours were seen in 17 patients.

Discussion:-

Gender, age, and WHO tumor grade did not significantly differ between the methylation and unmethylated groups. Patients in the methylation group were on average 50.3 years old, whereas those in the unmethylated group were 49.1 years old (p value 0.671). There were 40 patients, 18 of whom had methylated MGMT promoters and 22 who did not. 7 patients in the methylated group had WHO grade 3 tumors, whereas 11 individuals had WHO grade 4 tumors. Only 5 individuals in the unmethylated group had WHO grade 3 tumors, compared to 17 who had grade 4 tumors. There were 18 patients, 9 males and 9 men in the methylation group, 12 men and 10 women in the unmethylated group. We determined that, among other common imaging characteristics, ring-like enhancement was more commonly observed in unmethylated tumors (72.7%) than methylated ones (61.1%). Similar to this, methylated cells showed greater T2 heterogeneous signal intensity (77.8%) than unmethylated cells (68.2%). The methylation tumors (61.1%) exhibited more severe necrosis (affecting >50% of the tumor) than the unmethylated tumors (40.09%). But it was shown that these differences were statistically insignificant. (Table 3). Conventional imaging methods have been used in several investigations to show the imaging capabilities of MGMT promoter methylation gliomas. While some studies found that MGMT promoter methylated glioblastoma displayed less edema than the unmethylated one [16,17], another study found that MGMT promoter methylated glioblastoma significantly more frequently exhibited mixed-nodular enhancement [18], and other studies found that ring enhancement was more frequently linked to unmethylated glioblastoma. [18,19] According to a study's finding, glioblastoma lateralized to the right hemisphere in unmethylated cases and the left hemisphere in methylated cases. [16] The comparison of tumor location and MGMT promoter methylation indicated that GBM was associated with the parietal and occipital lobes in one research [20], the temporal lobe and left hemisphere in another, and that MGMT promoter methylation is not reliant on tumor location in a third study [21, 22]. However, it is well acknowledged that the tumor's location is a significant picture characteristic [23] connected to a patient's genetics and prognosis [24]. According to a different study, glioblastomas with high levels of the MGMT promoter protein expression were less necrotic than those with lower levels of the protein expression or negative results. [25] Han Y et al.'s [26] study.'s found that only the location of the tumor and its degree of necrosis were connected to the methylation status of the MGMT promoter and that methylated MGMT promoters were more commonly linked to severe glioblastoma necrosis. Furthermore, it has been shown that as compared to lymphoma, highly cellular tumors like High Grade Gliomas tend to have a more diverse texture. In comparison to unmethylated gliomas, methylated gliomas may have a more heterogeneous or less cellular tumor texture, which may be indicated by a lower CT attenuation value. It is well established that the methylation of the MGMT promoter in HGGs results in lower MGMT activity and, as a result, prevents the repair of DNA damage after chemotherapy and radiation.

Conclusion:-

The outcome of patients with high grade gliomas is significantly influenced by MGMT promoter methylation. When a biopsy of the methylation status of the tumor is unavailable, the distribution and imaging features may offer some information about the methylation of high-grade gliomas, but a multicentric big data set may be necessary to come to an agreement regarding its validity.

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