HISTOPATHOLOGICAL PATTERN AND EXPRESSION OF Ki-67, EGFR IN CENTRAL NERVOUS SYSTEM TUMOURS

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ABSTRACT

BACKGROUND

Central Nervous system (CNS) tumours constitute only about 1-2% of all neoplasms, but they show a varied histopathological spectrum. A WHO grading scheme is followed in the reporting of these tumours which play a key role in deciding the choice of therapies. Immunohistochemical markers like Ki-67 and EGFR are found to have a significant role in predicting the behaviour of these tumours. Hence in tumours where proper grading becomes difficult, the usage of these markers will be extremely helpful. In this study, the histopathological pattern, grading and expression of Ki-67 and EGFR in CNS tumours, received during the study period of 18 months, are described. Thereafter the relationship between the expression of these markers and the WHO grades is also evaluated.

MATERIALS AND METHODS

A descriptive study was conducted to describe the histopathological pattern and the expression of Ki-67 and EGFR in CNS tumours received in the Department of Pathology, Government Medical College, Kottayam for a period of 18 months (June 2017 -November 2018). The relationship between the expression of these markers and the histological grades was also evaluated. Statistical analysis was done with available software.

RESULTS

Among the 80 cases of CNS tumours studied, 48 cases (60%) were Meningiomas. Most of the cases had a Ki 67 value between 0-5 % (50 cases, 62.5%). It was found that in all types of CNS tumours studied, Ki-67 increased with increase in WHO grade. Among the glioma cases studied, 70.8% of them had an EGFR score of 3+ and among meningiomas, 62.5% had an EGFR Immunoreactive score (IRS) between 11 and 15. By statistical analysis, it was found that both WHO grade and EGFR expression in these tumours are associated. But in case of gliomas, increase in WHO grade improves our prediction for EGFR to be high (3+) and among meningiomas, increase in WHO grade improves our prediction for EGFR to be low (0-5). In case of remaining CNS tumours, majority of them showed EGFR negativity.

CONCLUSION

In our study, Meningiomas were the most common histological type. Ki-67 values in all types of CNS tumours increased with increase in WHO grades. All gliomas and meningiomas showed expression of EGFR. A significant association exists between WHO grades and EGFR expression of these tumours.

KEY WORDS

Histopathological Pattern; CNS Tumours; Ki67; EGFR.

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BACKGROUND

Central Nervous system is responsible for the co-ordinated functioning of different organ systems in our body. As compared to other organ systems, central nervous system only contributes to 1-2% of all neoplasms. Even though the proportion is too small, the histopathological patterns and pathogenesis show extreme diversity.

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CNS tumours have a histological grading scheme as per WHO guidelines. This provides a means of predicting the biological behaviour of a neoplasm and influence the choice of therapies Assessment of the histological pattern helps policy making for tumour screening and early treatment.

There are a large number of immunohistochemical markers in use for CNS tumours. These serve as both diagnostic and prognostic tools. One group among these markers include those which determines the proliferative activity. Ki-67 and Epidermal Growth Factor Receptor (EGFR) are two such markers.

Ki-67 protein expression is strictly associated with cell proliferation. The antigen can be detected within the nucleus during interphase. It is present during all active phases of the cell cycle (G(1), S, G(2), and mitosis), but it is absent in resting cells (G-0). Hence it makes an excellent marker for determining proliferative activity of a neoplastic population. It has been widely documented for various human tumours,

including CNS tumours and is very helpful in predicting the biological behaviour of the tumours.

Epidermal growth factor receptor is expressed in a large number of tumours which include meningiomas, gliomas, cancers of the lung, colon, breast and ovary. It acts through its cognate receptor resulting in cell proliferation and evasion from apoptosis. Targeting of EGFR has been proposed as a possible therapeutic strategy in case of many CNS tumours especially gliomas.

Several studies have shown conflicting results regarding the histopathological pattern of CNS tumours and role of Ki-67 labelling index and EGFR in predicting their biological behaviour. Hence this study is to be conducted to investigate the histopathological pattern and expression of Ki-67 and EGFR in CNS tumours.

MATERIALS AND METHODS

Type of Study

Descriptive study.

Study Period

18 months (June 2017-November 2018).

Study Setting

Department of Pathology, Government Medical College, Kottayam.

Sample Size

Sample size N= $(4pq)/ d^2$

p= prevalence/proportion in previous study

q= 100-p

d= precision/allowable error.

The primary objective of this study is to describe the histopathological pattern of CNS tumours received in the department of Pathology, Government Medical College, Kottayam during a period of 18 months. Hence the sample size is calculated based on a similar study done by Chawla. N et al.[1]

Proportion of astrocytomas in previous study= 55.8%[1.]

So, p= 55.8, q= 100-55.8= 44.2, d= 20% of p= 11.2

Sample size, N= $(4pq)/ d^2 = (4\times55.8\times44.2)/ (11.2)^2 = 78.7$

Taking sample size as 80

Inclusion Criteria

First 80 histologically diagnosed cases of Central Nervous System tumours were included.

Exclusion Criteria

Cases without proper data, inadequate specimen, tumours of peripheral nervous system were excluded.

Study Procedure

Clinical details of each case were recorded first, followed by gross examination of the specimen. All specimens were fixed in formalin and embedded in paraffin. 4 microns thick sections were stained with H & E for routine histological examination. Once the diagnosis and grading were done with H & E stained sections, immunohistochemical staining were performed using mouse monoclonal antibody for Ki-67 and rabbit monoclonal antibody for EGFR.

Immunohistochemical Evaluation of Ki-67 in CNS Tumours

The sections stained with immunohistochemical markers were first scanned using a 40× objective. An eye grid was used for the areas with the highest density of labelled tumour cells (hot spots). At least 1000 tumour cells were examined. All the immunoreactive tumour cell nuclei were counted. The Ki-67 proliferation index was defined as the percentage of immunoreactive tumour cell nuclei among the total number of cells.

Immunohistochemical Evaluation of EGFR in Gliomas

The presence of EGFR is determined using the percentage of immuno-stained cells per 200 cells in 5 fields. Both membrane and cytoplasmic positivity were taken into consideration. The EGFR scoring system is based on the number of positive cells. It is as follows: negative (−), no positive cells observed in the random fields; weak positive (+), <25% positive cells; moderately positive (++), 25–50% positive cells; and strongly positive (+++), >50% positive cells.

Immunohistochemical Evaluation of EGFR in Meningiomas

Both intensity score and percentage score were assessed. Staining intensity (intensity score) was scored on a scale of 0 to 3. “0” stands for absent staining, “1” for weak staining, “2” for moderate staining and “3” for strong staining of the tumor specimen. The percentages of immunoreactive cells (Percentage score) was scored from 1 to 5. 1 (< 20% of the sample exhibiting staining); 2 (21-40% of the sample exhibiting staining); 3 (41-60% of the sample exhibiting staining); 4 (61-80% of the sample exhibiting staining), and 5 (81-100% of the specimen stained). From these values, an immunoreactive score (IRS) was calculated by multiplying the percentage score and intensity score.

Data Management and Analysis

The data was entered in Microsoft excel and further statistical analysis was done using SPSS software (version 25). The statistical methods used were:

1. Mean, frequency, standard deviation and proportion for:
   - Age distribution
2. Frequency and proportion for:
   - Gender distribution.
   - Location of CNS tumours.
   - Histological types and subtypes.
   - WHO grades, ki-67 & EGFR in CNS tumours.
3. Chi-square test to find out
   - Association between WHO grades and expression of EGFR in gliomas and meningiomas.
4. Somers’ D, Kendall’s tau-b and Gamma measures to evaluate the degree and direction of association.

RESULTS

The mean age of the study group was 52.25±13.67 years with female to male ratio of 1.1:1.

The majority of the tumours were located in the frontal region (25 cases, 31.3%) followed by spinal cord (12 cases, 15%) and temporal region (9 cases, 11.3%).

The most frequent histological type was found to be Meningiomas (48 cases, 60%), followed by Astrocytomas (20

cases, 25%). Transitional meningioma (20 cases, 41.7%) was the most frequent subtype among meningiomas and Glioblastoma (12 cases, 50 %) was the most frequent subtype among gliomas.

Most of the tumours were of grade I (50 cases, 62.5%) followed by grade II and grade IV (12 cases, 15% each). Among the 24 cases of gliomas studied, most of them (12 cases, 50%) belonged to Grade IV category. Among the 48 cases of meningioma studied, majority of them were of grade I category (42 cases, 87.5%).

Most of the cases had a Ki-67 value between 0-5% (50 cases, 62.5%). Majority of the grade I tumours (94%) had their ki-67 values between 0-5%, while grade II tumours (50%) had their ki-67 values between 6-10%. In case of grade III tumours, majority (83%) had ki-67 values more than 15% and majority of grade IV tumours (83%) had their ki-67 values more than 20%.

Among the 24 glioma cases studied, the single grade I case had EGFR expression of 2+ score. In case of Grade II tumours, 50% of them had a score of 2+ and remaining 50% had a score of 3+. 60 % of the grade III tumours had a score of 3+. Majority of the grade IV cases (91.7%) had a score of 3+.

Majority of the Grade I meningioma cases (69%) had an IRS between 11-15. In case of Grade II meningioma cases, 60% had their IRS less than 10. Only one case of Grade III meningioma was studied with an IRS of 5.

The association between WHO grades and EGFR expression in case of gliomas and meningiomas were checked separately using a Chi-square test.

	Gliomas		Meningiomas	
	Value	p Value	Value	p Value
Pearson Chi-Square	16.894	.010	28.594	.000
Likelihood Ratio	15.418	.017	17.209	.002
No. of Valid Cases	24		48	

In both instances, p value was found to be <0.05, which means there is association between these tumours and their respective WHO grades. The degree and direction of association were assessed by using Somers' D, Kendall's tau-b and Gamma measures.

		Gliomas		Meningiomas	
		Value	p Value	Value	p Value
Somers' D	WHO Grade Dependent	.488	.005	-.297	.022
	EGFR Dependent	.341	.005	-.677	.022
Kendall's tau-b		.408	.005	-.449	.022
Kendall's tau-c		.328	.005	-.227	.022
Gamma		.612	.005	-.870	.022

Here a positive association was noted with p value 0.005 in case of gliomas and a negative association was noted in case of meningiomas with p value 0.022. This means the association is significant and it is helpful in predicting the grade of the tumours from the EGFR expression.

H & E Sections of Different CNS Tumours

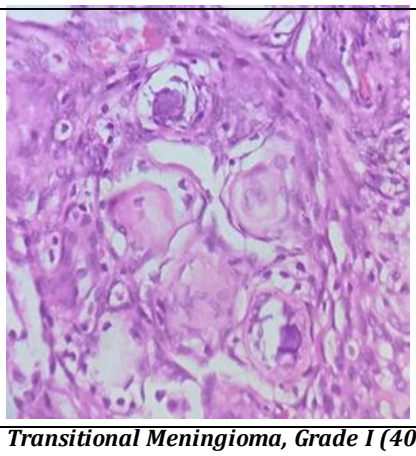


Figure 1. Transitional Meningioma, Grade I (40x) (H&E)

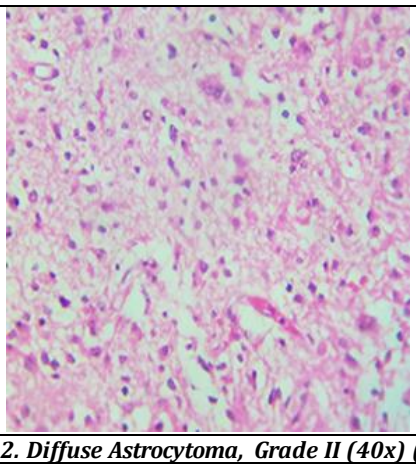


Figure 2. Diffuse Astrocytoma, Grade II (40x) (H & E)

Immunohistochemical Staining Among different Grades of CNS Tumours

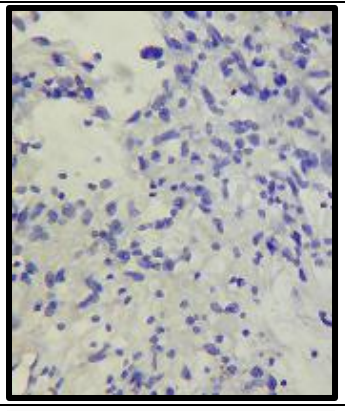
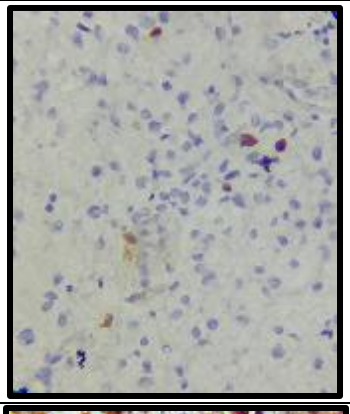
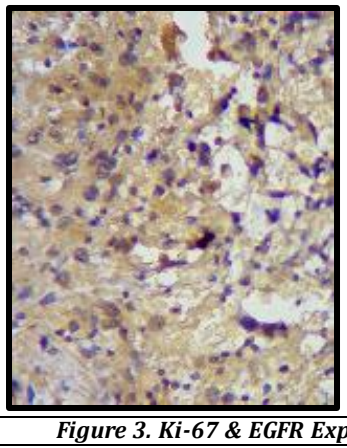
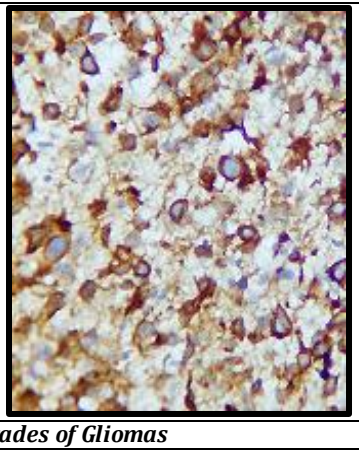
Grades	I	II
Ki-67		
EGFR		

Figure 3. Ki-67 & EGFR Expression in Different Grades of Gliomas

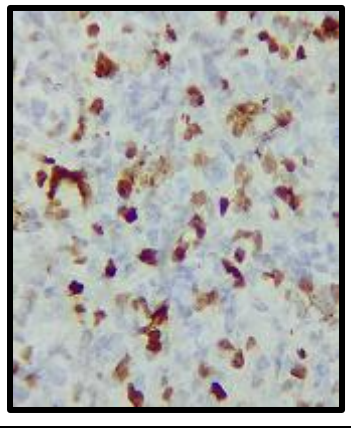
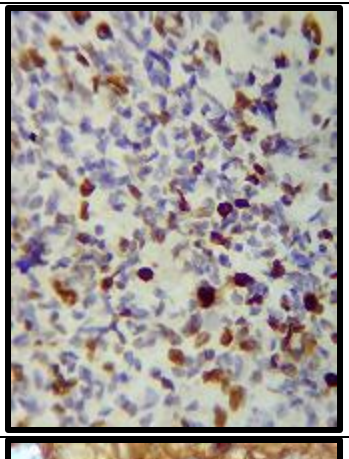
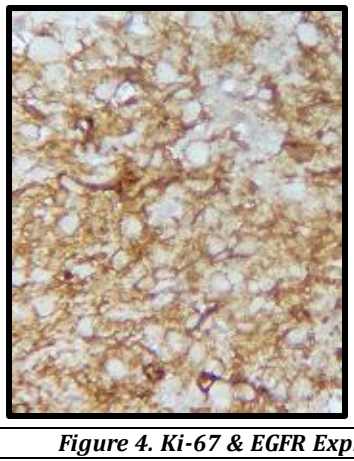
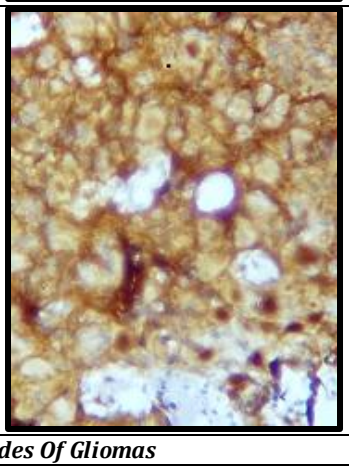
Grades	III	IV
Ki-67		
EGFR		

Figure 4. Ki-67 & EGFR Expression in Different Grades Of Gliomas

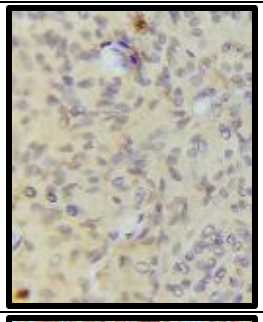
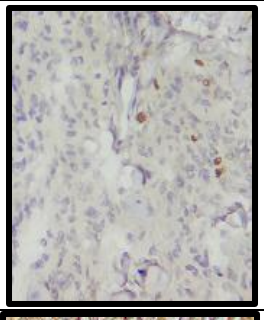
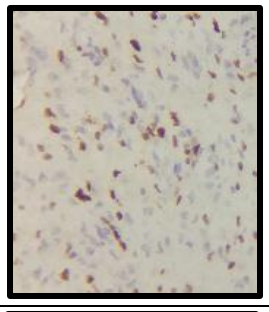
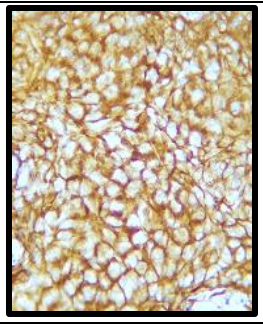
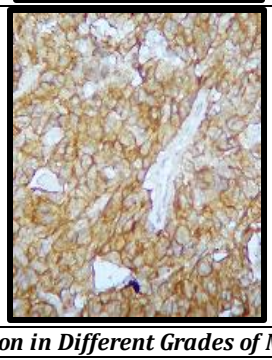
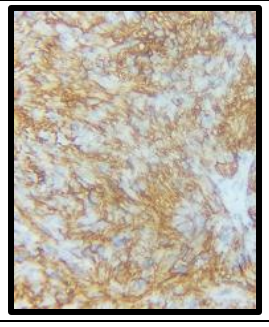
Grades	I	II	III
Ki-67			
EGFR			

Figure 5. Ki-67 & EGFR Expression in Different Grades of Meningiomas

DISCUSSION

The present study was conducted on 80 cases of Central Nervous System tumours received in the Department of Pathology, Govt. Medical College, Kottayam between June 2017 and November 2018.

The mean age of the study population was 52.25 ± 13.67 years which was comparable with a study conducted by Majjid N et al[2] in 2013 which was 45 ± 5 years.

The Female: Male ratio in the present study was 1.1:1 which was comparable to a study by Sumathi V et al[3] in which the female to male ratio was 1.2:1.

The specific location of each tumour was noted, and it was found that most of the tumours were located in the frontal region (25 cases, 31.3%) followed by spinal cord (12 cases, 15%) and temporal region (9 cases, 11.3%). This was comparable with a study conducted by Masoodi T et al[4] in which frontal lobe (20.7%) was the commonest intracranial location of tumours.

Among the 80 cases studied, Meningiomas (48 cases, 60%) was the predominant histological type followed by Astrocytomas (20 cases, 25%). This was comparable with a study conducted by Nagnath Kanthikar S et al[5] in which meningeal tumours formed the predominant category (40%). Another study done by Ghanghoria S et al[6] also had similar observation with meningeal tumours forming the predominant category (41.5%).

Study	Year of Study	No. of Cases	Commonest Histological Type
Nagnath Kanthikar S et al[5]	2017	38	Meningiomas (40%)
Ghanghoria S et al[6]	2014	65	Meningiomas (41.5%)
Present Study	2018	80	Meningiomas (60%)

Table 1. Comparison of Histological Type of Study Group with Other Studies

In the study population, each tumour was categorised into different histological subtypes. Among astrocytic tumours, Glioblastomas (12 cases, 15%) formed the predominant category. This was comparable to a study done by Gupta D et al, [7] where Glioblastoma (13.5%) formed the predominant category. Similar observation was made by Ahsan J et al[8] where Glioblastomas (22.6%) formed the most frequent astrocytic tumour.

Study	Year of Study	No. of Cases	Commonest Astrocytic Sub Type
Gupta D et al[7]	2017	59	Glioblastomas (13.5%)
Ahsan J et al[8]	2015	761	Glioblastomas (22.6%)
Present Study	2018	80	Glioblastomas (15%)

Table 2. Comparison of Histological Subtype of Astrocytomas of Study Group with Other Studies

Among the meningeal tumours, Transitional meningioma (20 cases, 25%) was the predominant one. This was comparable to a study done by Babu S et al.[9]

Tumours in our study population were graded according to WHO 2016 guidelines. Among the 80 cases studied, most of the tumours are of grade I (50 cases, 62.5%). This was comparable with a study done by Gupta D et al[7] in which 47.4% were of Grade I. Another study by Sumathi V et al[3] also had similar observation.

Study	Year of Study	No. of Cases	Commonest Grade
Gupta D et al[7]	2017	59	Grade I (47.4%)
Sumathi V et al[3]	2016	83	Grade I (37.2%)
Present Study	2018	80	Grade I (62.5%)

Table 3. Comparison of Commonest Grade Of CNS Tumours of Study Group with Other Studies

Most of the cases had their ki-67 value between 0-5 % (50 cases, 62.5%). It was found that higher grades of tumours had higher values of ki-67. EGFR showed varied expression in gliomas and meningiomas. In most of the cases studied, EGFR positivity was membranous. It is comparable to a study done by Hatanpaa K et al.[10] A difference in positivity was noted in Grade I glioma where the positivity was predominantly

cytoplasmic. But none of the studies referred had observed such a difference in EGFR expression. It is noted that there is an increase in expression of EGFR as we go from lower to higher grades of gliomas. But in case of meningiomas, an inverse relation is noted. ie, the score of EGFR decreased as we go from lower to higher grades of meningioma.

By statistical analysis, it was found that there is significant association between EGFR expression and WHO grades in case of both gliomas and meningiomas. The relation is positive in case of gliomas and negative in case of meningiomas. Hence if we get a higher EGFR expression for gliomas, it can be predicted that the corresponding WHO grade will be higher and the reverse for meningiomas. This is comparable to studies done by Xinhua Hu et al[11] & Dalia. A. Elsers[12] respectively.

CONCLUSION

The most common CNS tumour in this study is Meningioma followed by Astrocytoma. Both Ki-67 and EGFR expression in CNS tumours are related to WHO grades. Ki-67 increased with increasing grades. EGFR in gliomas showed positive correlation with WHO grades, whereas in Meningiomas, they showed negative correlation.

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