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Prognostic Significance of EGFR in Various Glioma with Special Reference to Glioblastoma Multiforme

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Abstract

Background: *Epidermal growth factor receptor (EGFR) is a transmembrane protein. That is a receptor for members of epidermal growth factor family (EGF family) of extracellular protein ligand. The EGFR is a member of the erb family of receptor, a subfamily of four closely related receptor tyrosine kinase - EGFR (erb B-1), Her2/neu(erb B-2), Her3 (erb B-3) and Her4 (erb B-4). Over expression of EGFR is commonly noted in breast, bladder and lung carcinoma. Amplified EGFR gene has also been observed in a number of high grade brain tumour, glioblastoma multiforme (Grade IV) in particular.*

Material and Methods: *A retrospective study of four years (2012 October to 2016 September) and one and half years prospective (2016 October to 2018 April) study was carried out in IMS & SUM Hospital in collaboration with Neurosurgery department and the follow up was done for a period of 1 year. All CNS tumours were subjected to squash and frozen section followed by histo-pathologic biopsy. The inclusion criteria were all the glioma cases with the patients' age ranging from 5 to 85 years. This was further subjected to IHC study of EGFR.*

Results and Observation: *It was found that EGFR expression was negative in low grade glioma like pilocytic astrocytoma, diffuse astrocytoma, oligodendroglioma and ependymomas, whereas EGFR is was positive in high grade gliomas (grade III & IV) like anaplastic astrocytoma and glioblastoma. Amongst the GBM, 80% were primary which showed 100% EGFR positivity where as only 20% of secondary GBM showed EGFR positivity and amongst the variants of GBM, gliosarcoma showed EGFR negativity in contrast to other subtypes.*

Conclusion: *EGFR can be used as a prognostic marker in gliomas, especially to distinguish between low grade type and high grade type and primary with secondary GBM. But therapeutic success of tyrosine kinase inhibitor that target EGFR has produced encouraging results.*

Study type and design: *Hospital based cross sectional study and an observational study.*

Keywords: *EGFR, Glioma, CNS, GBM, Tyrosine kinase inhibitors.*

Introduction

Immunohistochemistry (IHC) was originally described by Coons et al in 1940, however this method become popular in 1900s in surgical pathology.[1] Epidermal growth receptor (EGFR) is a transmembrane protein, which is a receptor

for epidermal growth factor family (EGF family) of extracellular protein ligand. The EGFR is a member of the erb family of receptor, a subfamily of four closely related tyrosin kinase receptor, EGFR (erb B-1), Her2/neu(erb B-2), Her3 (erb B-3) and Her4 (erb B-4).[2] Amplified EGFR signaling induce uncontrolled cell growth and malignant potential.[3][4]

Gliomas are the most common primary brain tumours in adultsare divided in to four grades[5][6][7]. they are subclassified as astrocytoma, oligodendroglioma, ependymoma. Oligodendroglioma and ependymoma are usually grade 2 tumours where as anaplastic oligodendroglioma, ependymoma are grade 3 tumours. The astrocytoma are sub divided in to grade 1 (pilocytic astrocytoma, grade 2 diffuse astrocytoma, grade 3 anaplastic astrocytoma and grade 4 glaioblastoma). Glioblastoma is again divided in to classical, epithelioid, giant cell, small cell and gliosarcoma type. Glioblastoma being the most malignant tomour diognosed by pleomorphism, mitosis, microvascular proliferation and necrosis. The mean age of survival of glioblastoma is 16 months to 24 months.[8] It is categorized as primary tumours seen in older individuals and secondary with a past history of low grade astrocytoma. The latter usually occur in younger age group. [9] The primary glioblastoma often have amplified mutated EGFR gene where as secondary type usually have p53 mutation and MDM2 amplification. [10]

Material and Methods

A 4 years retrospective (2012 October to 2016 September) and one half year prospective (2016 October to 2018 April) study was conducted in IMS & SUM Hospital in collaboration with Neurosurgery department with a follow up of 1 year period. Inclusion criteria were all Glioma within the range of 5-85 years and exclusion criteria were post-chemotherapy, post-radiotherapy, stereotactic biopsy and markedly necrotic tumours. The tissue was processed and stained by hematoxylin and eosin stain and histological diagnosis was made with clinico-radiological correlation. Past history and surgery of gliomas were elucidated and glioblastoma was categorized into primary and secondary groups and different histologic types (Giant cell, epithelioid, sarcoma and classical type).

This was further subjected to IHC for EGFR.[11] The interpretation was made according to staining pattern. No staining (O), weak membranous staining (1+). Intermediate staining (2+) and strong staining (3+), which was clearly visible using 4x objective lens. EGFR score scale ranged from 0-300 and calculated using following formula:- 1x (percentage of weakly staining cells, 1+) + 2x(percentage of moderately stained cells, 2+) + 3x (percentage of strongly stained cells, 3+). High and low scores of EGFR expression were defined using 200 as threshold. [12]

Observation and Results

Total number of Glioma cases included in our study were 50, amongst which WHO grade I were five (10%), grade II were ten (20%), grade III were thirteen (26%) and grade IV were twenty two cases (44%). Ependymoma was least recorded (1 in number). The commonest age of presentation was between 36-45 years with male predominance (3:2) [Table 1]. It was observed that grade I tumours occurred in younger ager group with lower expression of EGFR (average score of 134), where as high grade gliomas occurred in older age group with higher EGFR expression (average score of 250.45) [Figure 1a & b]. Amongst the 22 cases of glioblastoma, 17 were primary and rest 5 was secondary. Primary glioblastoma showed 100% EGFR positivity (score >200), where as one out of 5 cases was EGFR positive (20%).

The positive secondary glioblastoma was epithelioid type, previously diagnosed as diffuse grade II astrocytoma 2 years back. The mean age of presentation of primary glioblastoma (56.94) is higher than secondary glioblastoma (47:4) [Table 2]. Amongst the variants of glioblastoma, giant cell glioblastoma and epithelioid glioblastoma

showed more EGFR score (295 and 290 respectively), whereas gliosarcoma was associated with older patients (average 60 years) with a negative EGFR score of 180 [Table 3] [Figure 2a & b; 3a & b].

Table 1: Incidence of gliomas in comparison with age and sex of study population

Sl. No	Age distribution	Number of cases	%	Male	%	Female	%
1.	5-15	4	8	2	50	2	50
2.	16-25	3	6	3	100	0	0
3.	26-35	5	10	4	80	1	20
4.	36-45	13	26	7	53.5	6	46.5
5.	46-55	12	24	9	75	3	25
6.	56-65	11	22	3	27.5	8	72.5
7.	66-75	1	2	1	100	0	0
8.	76-85	1	2	1	100	0	0

Table 2: Mean age and mean EGFR score in primary and secondary glioblastoma

Clinical cases of glioblastoma	Mean age	Mean EGFR score
Primary glioblastoma	56.94	275.88
Secondary glioblastoma	47.4	164

Table 3: Mean age and mean EGFR score in variants of glioblastoma

	Mean age	Mean EGFR score
Glioblastoma	53.07	250.71
Giant cell glioblastoma	55	295
Gliosarcoma	60	180
Epitheliod glioblastoma	54.33	290

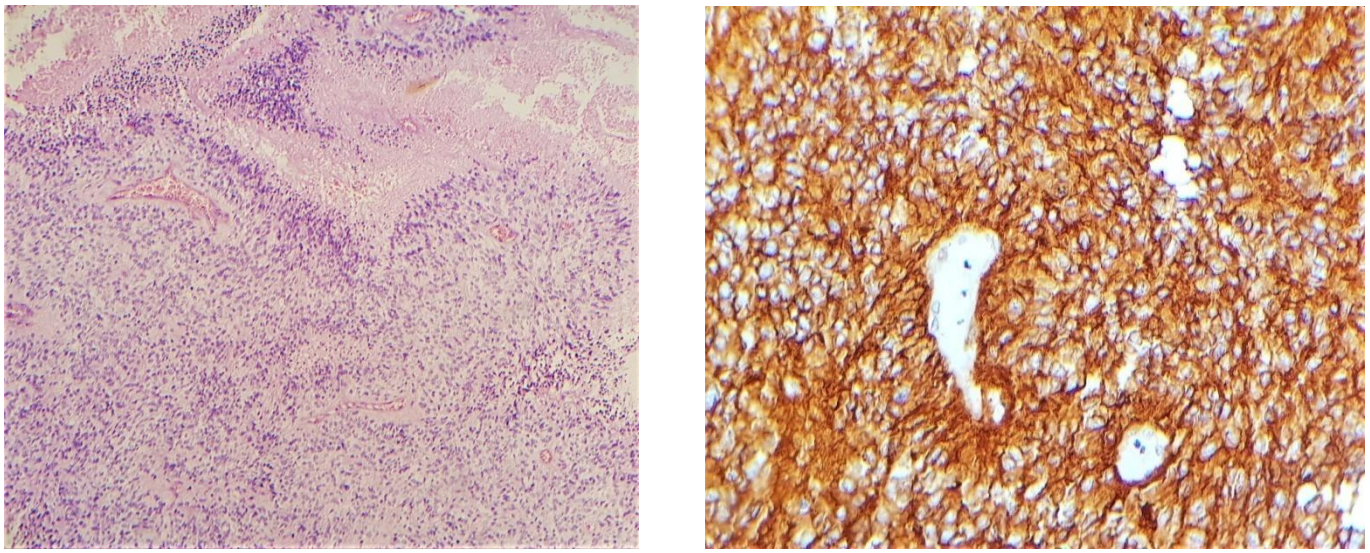

Fig 1: Microphotograph of classical Glioblastoma showing neoplastic glial cells with areas of necrosis (a) [H&E 800x]; strong and diffuse EGFR positivity (b) [IHC 800x].

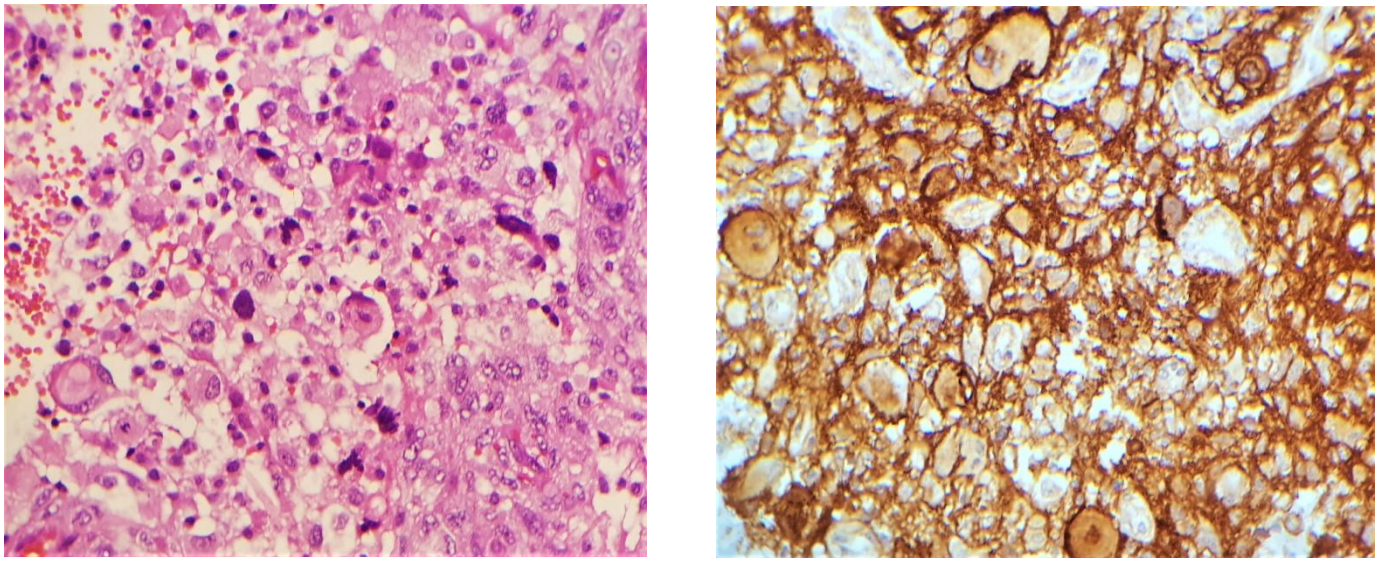

Fig 2: Microphotograph of giant cell glioblastoma showing multiple tumour giant cells with neoplastic glial cells (a) [H&E 800x]; EGFR strong positivity (b) [IHC 800x].

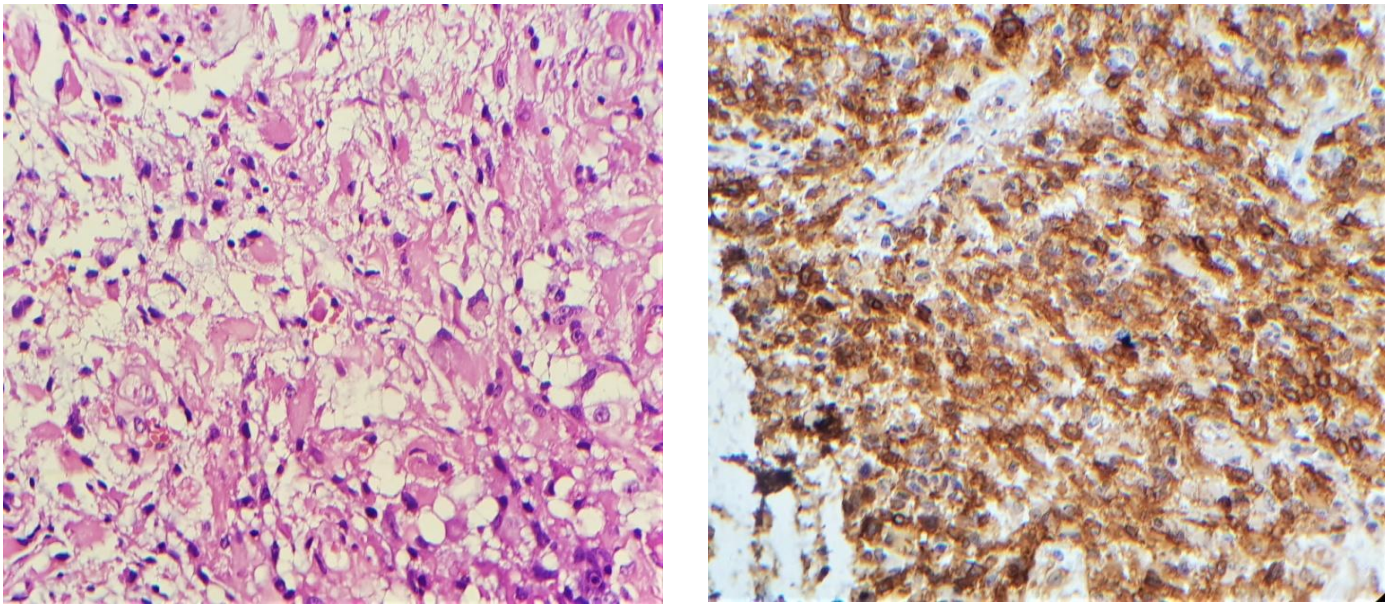

Fig 3: Microphotograph of epithelioid glioblastoma showing gemistocytic component (a) [H&E 800x]; strong and diffuse positivity of EGFR (b) [IHC 800x].

Discussion

In our study it was found that out of total 50 cases of Glioma, the commonest age of presentation was 36-45 years (26%) followed by 46- 55 years (24%). Males were more in 46-55 age group, where as female presented in higher age group 56-65 years (72.5%) tumors were glioblastoma being the commonest group (44%) followed by grade III tumor. Primary glioblastoma were 17 (77.5%) and secondary were 5(22.5%). This was in concordance with Ohgaki H et al. (2007) who concluded 90% of glioblastoma as primary.[9] Feng Liu et al. (2018) showed that 26.15% of glioblastoma showed EGFR amplification.[13]

In our study, 77.5% of glioblastoma showed EGFR positivity. According to Hongsheng Xu et al. (2017) EGFR over expression is found in 60% primary glioblastoma versus only 10% secondary glioblastoma.[14] In our study, EGFR expression was 100% in primary where as 20% in secondary astrocytoma.

As per Hongsheng Xu et al. classical glioblastoma are synonymous with focal EGFR amplification (95%); whereas mesenchymal, neural and

proneural glioblastoma are associated with reduced rates of EGFR amplification (29%, 67% and 17%) respectively.[14] According to their study, gliosarcoma presented with negative EGFR score which was correlated with our study (score of 180). Median overall survival times for patients having negative EGFR expression, over expression or mutant expression (EGFR VIII) were 0.96, 0.98 and 1.07 years respectively. According to Kyu Sang Lee et al. (2013), the age of onset of secondary glioblastoma are younger that primary glioblastoma, and the medial survival of secondary ones is 7.8 months, significantly longer than that of primary glioblastoma (4.7 months).[15] In our studies, out of 17 cases of primary glioblastoma 10 (58.8%) were dead at the end of 1 year of follow up whereas 2 out of 5 cases of secondary glioblastoma (40%) were dead at the end of follow up period which is in concordance with Kyu Sand Lee et al. (2013).[15]

Conclusion

Several markers have come out for prognostication of CNS tumours, gliomas in particular. IDH-1 tops the list based on which gliomas are categorized as mutant/wild type, which further helps in successful therapeutic approach especially in high grade gliomas. EGFR amplification is an ongoing research subject which has been found to have prognostic significance in high grade gliomas. But the EGFR as a predictive (therapeutic) marker is yet under study which in future will come out with great therapeutic revolution.

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