ORIGINAL ARTICLE

Detection of Isocitrate Dehydrogenase (IDH-1), Epidermal Growth Factor Receptor (EGFR), P53 and C-erbB2/HER2 Mutation in Glial Tumour

Nusaibah Azman[1], Anani Aila Mat Zin[1,2], Sharifah Emilia Tuan Sharif[1,2], Ikmal Hisyam Bakrin[3]

[1] Department of Pathology, School of Medical Sciences, Universiti Sains Malaysia, Health Campus, Kubang Kerian, 16150 Kota Bharu, Kelantan, Malaysia.
[2] Hospital Universiti Sains Malaysia, Kubang Kerian, 16150 Kota Bharu, Kelantan, Malaysia.
[3] Department of Pathology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

ABSTRACT

Introduction: In the last decade, several molecular pathways in gliomagenesis have been discovered, with each involving a unique set of molecular alterations. IDH1 has become a diagnostic tool in the latest 2016 WHO Classification. The tumour protein, p53, is involved in the IDH-mutant arm, observed in astrocytoma and oligodendroglioma (grades II and III), and secondary glioblastoma. Meanwhile, EGFR and c-erbB2/HER2 were postulated to be expressed in higher-grade glioma as the disease progresses. **Methods:** A retrospective cross-sectional study was conducted to evaluate the association of IDH1, EGFR, p53 and c-erbB2/HER2 protein expression in astrocytic and oligodendroglial tumours with clinicopathological data in HUSM, Kota Bharu, Kelantan, Malaysia. This study examined 61 archived formalin-fixed paraffin-embedded (FFPE) tissue blocks of patients diagnosed with glioma. The immunohistochemistry (IHC) test was performed using antibodies, IDH1, EGFR, p53 and c-erbB2/HER2, and the protein expressions were evaluated microscopically. Finally, the association between IDH1, p53, EGFR and c-erbB2/HER with the clinicopathology variables were statistically analysed. **Results:** A total of 61 glioma cases consisting of 36 (59%) males and 25 (41%) females were included in this study. The IDH1 protein was positively expressed in 14 cases (23%), P53 was highly expressed in 26 cases (42.6%), and EGFR was substantially observed in 34 cases (55.7%). For glioblastoma cases, IDH1 was expressed in two cases (11.1%), EGFR in 14 cases (77.7%), p53 in 12 cases (66.7%) and c-erbB2 in 1 case (5.6%). Significant associations exist between IDH1, p53 and EGFR expressions in astrocytoma and oligodendroglial tumours with the histological types and WHO tumour grades. **Conclusion:** Recently, our knowledge regarding the genetics of central nervous system (CNS) tumors has expanded; hence, newer antibodies or molecular markers, which can be used in IHC, are continuously being developed. These antibodies, IDH1, p53 and EGFR markers are useful for diagnostic, prognostication and therapeutic. In addition, help to clarify the nature of cellular maturation, tissue differentiation, and tumor progression to be considered as an integral part of WHO classification of CNS tumours.
Malaysian Journal of Medicine and Health Sciences (2022) 18(SUPP21): 110-118. doi:10.47836/mjmhs18.s21.17

Keywords: IDH1, p53, EGFR, c-erbB2/HER2, astrocytoma, oligodendroglioma

Corresponding Author:
Anani Aila Mat Zin, MPath
Email: ailakb@usm.my
Tel: +609-7676957

INTRODUCTION

Primary brain tumours are a heterogenous group of tumours arising from cells within the central nervous system, which can be further divided into glial and non-glial cells. Gliomas are tumours arising from the glial cells, including diffuse astrocytoma, oligodendroglioma, glioblastoma and other astrocytic tumours, such as pilocytic astrocytoma. The diagnosis of primary brain tumours is determined based on clinical evaluation, neuroimaging, and histopathological assessment.

Tumours can be benign or malignant depending on the clinical behaviour and morphology, and can be graded using the World Health Organisation (WHO) Classification Grades I to IV. The 5th and latest edition of the WHO Classification of Tumors of the Central Nervous System (WHO CNS5) was published in 2021 (1), with a significant shift from the 4th edition published in 2016 with the inclusion of molecular characteristics into the classification of diffuse gliomas from the traditional histological-based diagnosis. The incorporation of molecular subtyping will aid pathologists in cases

with limited tissue biopsy and ambiguous or mixed morphologies. Additionally, the molecular classification offers meaningful data for prognosis and targeted treatments (2).

The global primary brain cancer incidence is approximately 3.9 per 100,000 males and 3.0 per 100,000 females, with 308,102 new cases (1.6% tumour burden) reported in 2020 (3). In Malaysia, the incidence is slightly lower at 1.8 per 100,000 males and 1.6 per 100,000 females, with 642 (2.9%) reported cases from 2012 to 2016 (4). Nevertheless, these numbers have been rising in adults and children in Malaysia (5), thus, the need for research on the diagnostic, prognostic, and therapeutics of primary brain cancer.

The most common malignant primary brain tumours in adults are glioblastomas (49.1%), while most benign tumours are meningiomas (54.5%) (6). Pilocytic astrocytoma is common in children 0 - 14 years of age, whereas pituitary tumours are common among adolescents and young adults (15 - 39 years old). Meanwhile, glioblastoma cases increase with age, particularly among patients between 75 and 84 years old (6). Symptoms of a primary brain tumour often vary between patients, depending on tumour growth rate and location. The symptoms include seizures (50-80%), headaches (30%) and increased intracranial pressure (15%) (7). In contrast, children with pilocytic astrocytoma experience more headaches (65.2%), visual problems (34.8%), and high intracranial pressure (28.3%) compared to seizures (8.7%) due to the reduced involvement of cerebral hemispheres (8).

Several molecular pathways in gliomagenesis have been discovered in the last decade, involving different sets of molecular alterations. Isocitrate dehydrogenase 1 (IDH1) has emerged as a major stratifier of infiltrating glioma with prognostic importance, distinguishing between IDH-mutant and IDH-wildtype groups (9). The IDH1 mutations are frequently observed in diffuse astrocytic and oligodendroglial tumours (grades II and III) and secondary glioblastoma. Furthermore, IDH1 mutations are considered an early mutation in gliomagenesis occurring before genetic modifications promote specific differentiation, such as TP53 and Alpha Thalassemia/Mental Retardation Syndrome X-linked (ATRX) mutations in astrocytomas or 1p/19q co-deletion in oligodendrogliomas (10). Patients with IDH mutations also have a better prognosis, contrary to primary glioblastoma cases that lack IDH mutation; thus, the latter may involve a different gliomagenesis pathway. Grade IV IDH-wildtype also demonstrates other molecular alterations such as EGFR, PTEN, NF1, RB1, CDKN2A and CDKN2B (9). The EGFR mutations have been described in the literature and have been observed in grade II (0 - 4%), grade III (0 - 33%) and grade IV (34 - 64%) astrocytoma (11).

Studies on c-erbB2/HER2 mutation in glioblastoma have increased following the discovery of EGFR gene amplification and overexpression in glioblastoma. These markers belong to the same receptor tyrosine kinase family, which includes EGFR (c-erbB1/HER1), c-erbB2/HER2, c-erbB3/HER3 and c-erbB4/HER4 (12). Currently, c-erbB2/HER2 is a prominent oncogene with significant diagnostic and prognostic value in multiple human cancers, particularly breast cancer. Despite that, the exact role of this protein in glioma remains uncertain (13). Most studies reported that c-erbB2/HER2 expression in primary brain tumours ranged from 0 to 90% (14).

Nonetheless, these studies are primarily conducted in Western countries; thus, the findings may not apply to the Malaysian population. Previous studies have shown concordance between immunohistochemistry (IHC) and DNA sequencing results with 94% sensitivity and 100% specificity (17). The IHC method can provide an alternative diagnostic method to molecular studies, particularly in small centres where molecular tests are not offered or available. Therefore, the current study investigated the association between IDH1, EGFR, p53, and c-erbB2/HER2 expressions in astrocytoma and oligodendroglial tumours using the IHC method as few studies have utilised this technique on brain tumours in Malaysia (15, 16).

MATERIALS AND METHODS

This single-centre, retrospective cross-sectional study was conducted in Hospital Universiti Sains Malaysia (HUSM), Kubang Kerian. The study protocol was approved by the Human Research Ethics Committee (HREC), Universiti Sains Malaysia (USM/JEPeM/19020157). The patients were selected using a convenient sampling method based on the inclusion and exclusion criteria. Only 61 glioma cases between January 2009 and December 2019 were included in this study due to the time constraint and other unforeseen circumstances. Cases excluded from this study were those with unavailable or missing formalin-fixed, paraffin-embedded (FFPE) tissue blocks, inadequate tumour tissue, and indeterminate diagnosis. The patients' demographic data were retrieved from the Lab Information System (LIS) system in HUSM.

Selected FFPE tissue blocks were sectioned at 2 - 5 µm thickness for the IHC staining performed at the Pathology Laboratory, HUSM. First, sections were deparaffinised, and epitope retrieval was performed using the EnVision Flex Target retrieval solution (high pH). All the slides were subsequently placed in the Squenza immunostainer and incubated for 30 minutes (except EGFR for 40 minutes) at room temperature with the following antibodies and dilutions: Anti-IDH1 R132H/DIA-H09 (Clone H09), 1:100 (Dianova, Switzerland), Anti-EGFR (phospho Y1068) [EP774Y], 1:200 (Abcam, United Kingdom

(UK)), Monoclonal Mouse Anti-Human p53 Protein (Clone DO-7), 1:100 (Dako, Denmark) and Polyclonal Rabbit Anti-Human c-erbB-2 Oncoprotein (Code A0485), 1:400 (Dako, Denmark). Diffuse astrocytoma, colon adenocarcinoma, cervical cancer, and breast cancer tissues served as external positive controls for IDH1, p53, EGFR, and c-erbB2/HER2, respectively. The antigen-antibody detection was achieved using the labelled polymer, EnVFLEX/HRP, and the complex was later visualised with substrate chromogen (Envision FLEX Substrate Working Solution).

The IDH1 expression was determined by assessing the proportion of positively stained cytoplasm/nucleus of tumour cells. Cases with ≥ 10% stained cells were rated positive, while those with < 10% stained cells were considered negative (18). The IHC scoring for EGFR and p53 were based on the staining of cytoplasmic and/or membranous distribution (EGFR) and nuclear distribution (p53) and the percentage of positive tumour cells based on the literature (19). The scoring system is presented as follows: Score 0 (no staining), score 1+ (staining in <10% of cells), score 2+ (staining in 10-50% of cells) and score 3+ (staining in >50% of cells).

Scores 0 and 1 + were classified as low expression, while scores 2 + and 3 + were classified as high expression for the statistical analysis. Meanwhile, c-erbB2/HER2 expression was determined by percentage and intensity of staining as follows: 0 (no staining), 1 + (low intensity and incomplete membrane staining in > 10% of cells), 2 + (low intensity and complete staining in > 10% cells) and 3 + (high intensity and complete membrane staining > 10% of cells) (20). The staining was evaluated under high power magnification (400 x) (Figure 1).

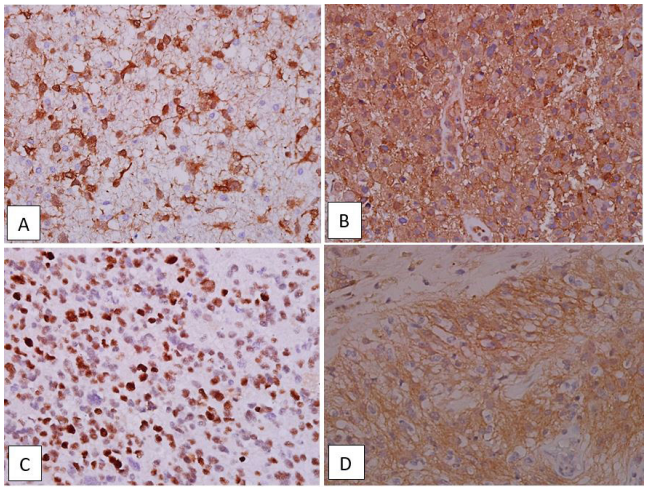

Figure 1: Staining pattern in immunohistochemistry test for IDH1, EGFR, p53 and c-erbB2/HER2 (x400 magnification)
(A) Positive staining pattern of IDH1 (cytoplasmic/weak nuclear) ≥10% of cells (B) Staining pattern for EGFR (cytoplasmic/membranous), Score 2+ (C) Staining pattern for p53 (nuclear), Score 3+ (D) Staining pattern for c-erbB2/HER2 (cytoplasmic/membranous), Score 2+.

Statistical analysis was performed using the SPSS version 25.0. Descriptive analysis was used to illustrate the frequency distributions of categorical variables and median distributions of continuous variables. Furthermore, the association between IDH1, p53, EGFR and c-erbB2/HER2 protein expressions with the clinicopathological variables was analysed using the Pearson's Chi-Square test or Fisher's Exact test. The level of significance was set at $p < 0.05$.

RESULTS

Clinicopathological data

Among the 61 patients in this study, 36 (59%) were males, while 25 (41%) were females. Their ages ranged from 8 – 67 years old, with a mean age of 38.84 (Table I). The age groups used in this research were based on a previous study (6). Resultantly, the most common age group was older adults, with 34 cases (55.7%). All cases were of Malay ethnicity.

Seven histological types of primary brain tumours according to the 2016 WHO classification were included in this study. The most common histological subtypes were glioblastoma with 18 cases (29.5%), followed by anaplastic astrocytoma with 12 cases (19.7%) and diffuse astrocytoma with 12 cases (19.7%). In addition, the cases were categorised into four WHO tumour grades (I - IV). The findings were as follows: 9 cases of

TABLE I: The demographic and clinicopathological parameters of astrocytoma and oligodendroglial tumors. (n=61)

VARIABLES	MEAN (SD)	FREQUENCY, n (%)
AGE (8 to 67 years old)		
0 to 14 years old	38.84 (16.21)	5 (8.2%)
15 to 39 years old		22 (36.1%)
40 to 70 years old		34 (55.7%)
GENDER		
Male		36 (59.0%)
Female		25 (41.0%)
HISTOLOGICAL TYPES		
Pilocytic astrocytoma		9 (14.8%)
Pleomorphic xanthoastrocytoma		5 (8.2%)
Diffuse astrocytoma		12 (19.7%)
Oligodendroglioma		1 (1.6%)
Anaplastic astrocytoma		12 (19.7%)
Anaplastic oligodendroglioma		4 (6.6%)
Glioblastoma		18 (29.5%)
WHO GRADES		
Grade I		9 (14.8%)
Grade II		18 (29.5%)
Grade III		16 (26.2%)
Grade IV		18 (29.5%)

grade I (14.8%), 18 cases of grade II (29.5%), 16 cases of grade III (26.2%) and 18 cases of grade IV (29.5%).

Expression of IDH1 protein

The association between IDH1 expression and the clinicopathological variables are shown in Table II. Positive IDH1 expressions were higher in the 40 - 70 age group (11/14, 78.6%) compared to the 15 - 39 age group (3/14, 21.4%). Meanwhile, none of the children's age group showed positive expression. Nevertheless, the results were not statistically significant ($p = 0.176$). In addition, IDH1 was positively expressed in 14 cases (23%) which include diffuse astrocytoma (5/12, 41.7%), anaplastic astrocytoma (3/12, 25%), anaplastic oligodendroglioma (4/4, 100%) and glioblastoma (2/18, 11.1%) (Table II). In contrast, all patients with pilocytic astrocytoma, oligodendroglioma and pleomorphic xanthoastrocytoma had negative IDH1 (Table II). There were significant associations between IDH1 expression with histological types ($p = 0.002$) and WHO tumour grades ($p < 0.044$).

Expression of EGFR protein

The association between the expression of EGFR and the clinicopathological variables are shown in Table II. This protein was highly expressed in most histological types, particularly glioblastoma (77.8%), followed by anaplastic astrocytoma (75%) and anaplastic oligodendroglioma (50%). There were significant associations between EGFR expression with the histological types ($p = 0.002$) and WHO tumour grades ($p = 0.005$). On the contrary, no association was observed between EGFR expression with age groups and gender.

Expression of P53 protein

High expression of p53 was observed in glioblastoma (66.7%), anaplastic astrocytoma (50%) and diffuse astrocytoma (33.3%) (Table II). There were significant associations between p53 protein expression with histological types ($p = 0.015$) and WHO grades ($p = 0.011$). Meanwhile, no association was observed between p53 expression with age groups and gender.

Expression of c-erbB2/HER2

Most cases did not exhibit any staining except for glioblastoma cases (4/61, 6.56%) with scores of 1 + and 2 + (Table II). No 3 + score was observed. No significant association was seen between c-erbB2 expressions with the clinicopathological variables.

DISCUSSION

The state of Kelantan has a population exceeding 1.9 million, consisting of 94.7% Malay Muslims with a male-to-female ratio of 1.00:0.98 (21). These numbers potentially explain why all the cases were Malay, and none were from other ethnicities. Previous studies on the pattern of brain tumours in HUSM from 2011 to 2014 found that meningioma (32.7%) was the most common adult primary brain tumour, followed by glioblastoma (7.8%). Nevertheless, the ethnicity of the study population was not revealed, and there was no gender predominance in brain tumour prevalence (5).

The current study on IDH1 expression in brain cancer is not a novel phenomenon in Malaysia. Previous research at HUSM explored IDH1 mutations using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), which was then confirmed using direct sequencing. Resultantly, IDH1 (R132H) mutation was present in 14/40 (35%) brain tumours, which was significantly associated with the histological type (16). Similarly, the present study findings corresponded with the literature, suggesting that IHC is a feasible alternative to molecular studies to determine IDH1 expression. On the other hand, another study that was conducted at HUSM examined the IDH status in 47 glioma cases using PCR-RFLP and DNA sequencing and reported that only 3/47 (6.4%) of the brain tumours tested positive for IDH1 mutations, two of which were IDH1 R132H and one IDH1 R132L (15). Possible lower IDH1 expression compared to the current study (15) include different locus of IDH1 mutation. Seven IDH1 mutations have been reported: IDH R132H, R132C, R132G, R132L, R132V and R132P. The IDH1 R132H gene mutation was found in approximately 90% of gliomas, while the remaining mutations were less common (22). This study utilised the anti-IDH1 R132H clone for the IHC protocol.

In the present study, IDH1 expression was found in 14/61 (23%) of glioma cases using the using IHC method, which was lower than an earlier study (40% - 70%) (10). Nonetheless, this finding almost similar to another study conducted in Malaysia, which reported 35% IDH1 expression (10). There are several explanations for this result, including mutation in other IDH isoforms such as IDH2 that occurs at amino acid R172, albeit less prevalent than IDH1 (9). This possibility can be confirmed via molecular testing to validate the current results in a future cohort. Notably, most cases in this research were glioblastoma (18/61), followed by diffuse astrocytoma (12/61), oligodendroglioma (1/61), anaplastic astrocytoma (12/61), and anaplastic oligodendroglioma (4/61). These findings suggested a possible bias as IDH1 was more prevalent in grades II and III astrocytomas, oligodendroglioma and secondary glioblastoma.

Only 2/18 (11.1%) glioblastoma cases were positive for IDH1, thus suggesting it's progression from lower grade astrocytoma, while negative IDH1 glioblastoma could be a primary glioblastoma that arises de novo (24). These findings are contradict with a previous study, which reported IDH1 mutation in 73% of secondary glioblastoma versus 3.7% in primary glioblastoma (25). Furthermore, most glioblastoma cases in the current study could be a primary glioblastoma. Histologically, primary and secondary glioblastoma are indistinguishable, but

TABLE II: The association between expression of IDH1, EGFR, P53 and C-erbB2/HER2 with its clinicopathological variables. (n=61)

VARIABLES	IDH1		P-VALUE	EGFR		P-VALUE	P53		P-VALUE	C-ERBB2/HER2			P-VALUE
	POSITIVE (n)	NEGATIVE (n)		HIGH EXPRESSION (n)	LOW EXPRESSION (n)		HIGH EXPRESSION (n)	LOW EXPRESSION (n)		0	1+	2+	
AGE													
0 to 14 years old	0 (0%)	5 (100%)	0.176**	3 (60%)	2 (40%)	0.457**	2 (40%)	3 (60%)	0.173**	5 (100%)	0 (0%)	0 (0%)	0.136**
15 to 39 years old	3 (13.6%)	19 (86.4%)		10 (45.5%)	12 (54.5%)		6 (27.3%)	16 (72.7%)		19 (86.4%)	3 (13.6%)		
40 to 70 years old	11 (32.4%)	23 (67.6%)		21 (61.8%)	13 (38.2%)		18 (52.95)	16 (47.1%)		33 (97.1%)	0 (0%)	1 (2.9%)	
GENDER													
Male	9 (25%)	27 (75%)	0.648*	23 (63.9%)	13 (36.1%)	0.124*	14 (41.7%)	21 (58.3%)	0.856*	35 (97.2%)	1 (2.8%)	0 (0%)	0.381**
Female	5 (20%)	20 (80%)		11 (44%)	14 (56%)		11 (44%)	14 (56%)		22 (88%)	2 (8%)	1 (4%)	
HISTOLOGICAL TYPES													
Pilocytic astrocytoma	0 (0%)	9 (100%)	**0.002****	5 (55.6%)	4 (44.4%)	**0.002****	0 (0%)	9 (100%)	**0.015***	9 (100%)	0 (0%)	0 (0%)	0.606**
Pleomorphic xanthoastrocytoma	0 (0%)	5 (100%)		3 (60%)	2 (40%)		2 (40%)	3 (60%)		5 (100%)	0 (0%)	0 (0%)	
Diffuse astrocytoma	5 (41.7%)	7 (58.3%)		1 (8.3%)	11 (91.7%)		4 (33.3%)	8 (66.7%)		12 (100%)	0 (0%)	0 (0%)	
Oligodendroglioma	0 (0%)	1 (100%)		0 (0%)	1 (100%)		0 (0%)	1 (100%)		4 (100%)	0 (0%)	0 (0%)	
Anaplastic astrocytoma	3 (25%)	9 (75%)		9 (75%)	3 (25%)		6 (50%)	6 (50%)		14 (77.8%)	0 (0%)	0 (0%)	
Anaplastic oligodendroglioma	4 (100%)	0 (0%)		2 (50%)	2 (50%)		1 (25%)	3 (75%)		26 (100%)	0 (0%)	0 (0%)	
Glioblastoma	2 (11.1%)	16 (88.9%)		14 (77.8%)	4 (22.2%)		12 (66.7%)	16 (45.7%)		31 (88.6%)	3 (16.75%)	1 (2.9%)	
Low grade	5 (19.2%)	21 (80.8%)	0.552*	10 (28.6%)	17 (65.4%)	**0.004***	7 (26.9%)	19 (73.1%)	**0.033***	26 (100%)	0 (0%)	0 (0%)	0.254**
High grade	9 (25.7%)	26 (74.3%)		25 (71.4%)	9 (34.6%)		19 (54.3%)	16 (45.7%)		31 (88.6%)	3 (8.6%)	1 (2.9%)	
WHO GRADES													
Grade I	0 (0%)	9 (100%)	**0.044****	5 (55.6%)	4 (44.4%)	**0.005***	0 (0%)	9 (100%)	**0.011***	9 (100%)	0 (0%)	0 (0%)	0.103**
Grade II	5 (27.8%)	13 (72.2%)		4 (22.2%)	14 (77.8%)		7 (38.9%)	11 (61.1%)		18 (100%)	0 (0%)	0 (0%)	
Grade III	7 (43.8%)	9 (56.3%)		11 (68.8%)	5 (31.3%)		7 (43.8%)	9 (56.3%)		16 (100%)	0 (0%)	0 (0%)	
Grade IV	2 (11.1%)	16 (88.9%)		14 (77.8%)	4 (22.2%)		12 (66.7%)	6 (33.3%)		14 (77.8%)	3 (16.7%)	1 (5.6%)	

p-value < 0.05 is significant.
*Analysis is done using Chi Square tests
**Fisher's exact test is applied when expected frequency is less than 5

Malaysian Journal of Medicine and Health Sciences (eISSN 2636-9346)

the IDH1 expression in both cases may vary, allowing clinicians to differentiate these cases. Nevertheless, the histological reports did not contain further classification into primary and secondary tumours as HUSM utilises the 2007 and 2016 WHO classification for diagnosis.

High p53 (12/18, 66.7%) and EGFR (14/18, 77.8%) expressions were evident in glioblastoma cases. These proteins were also strongly associated with histological types, contradicting an earlier study that reported p53 expression was inversely proportionate to EGFR (26). The genetic profile of primary glioblastoma demonstrated 42% EGFR expression and 23% p53 expression. Meanwhile, EGFR expression was 4%, and p53 expression was 74% in secondary glioblastoma (26). The reversed association could be explained by the differences in the role of the mutated proteins. For instance, TP53 is a tumour suppressor gene that prevents carcinogenesis via multiple cellular responses, including apoptosis of damaged cells, maintaining genetic stability, inhibiting angiogenesis and regulating cellular metabolism. Thus, p53 is commonly found in 85% of tumours and 28% of lower-grade glioma. It was reported that more than 90% of TP53 mutations were related to IDH mutations (27).

The TP53 mutations are common in grade II and III astrocytomas but not in oligodendrogliomas, where 1p/19q co-deletion is more common. The 1p/19q co-deletion is undetectable by IHC but is mutually exclusive with ATRX and p53 mutation in IDH mutant glioma. Thus, IDH1 could be a powerful diagnostic tool for distinguishing between the two histological types. Despite that, there is a lack of standardisation regarding the cut-off points used for p53 mutation. For example, Takano et al. used ≥ 10% as the cut-off point for positive immunostain, with a sensitivity of 78.8% and a specificity of 96.7% (28). Alternatively, another study examined the intensity of staining as 'strong staining only', 'weak staining only', or 'staining of any intensity (0 – 100%)', which was then confirmed with direct sequencing with 78.8% sensitivity and 96.7% specificity (29).

The present study was reported that 37% of astrocytoma patients who had a high p53 expression show a significant association with histological subtypes. It employs a scoring system adapted from a previous study done in India (19). Similarly, this study yielded a comparable percentage of p53 in diffuse astrocytoma (33.3%) and anaplastic astrocytoma (50%) with a significant association ($p = 0.015$) with histological types (Table II). Ultimately, any IHC study requires a validation step such as DNA sequencing. Nonetheless, IHC remains a moderately sensitive and highly specific test to predict TP53 mutation. The TP53 mutation alone has no prognostic value, but survival rates are improved when combined with IDH and ATRX mutation analysis. Patients with IDH1/CIC/FUBP1 genetic alterations (common in oligodendroglioma) had an overall survival of 96 months compared to those with IDH1/ATRX/TP53 (51 months) and patients with primary glioblastoma without both genetic alterations (13 months) (30).

The EGFR is an oncogene that can promote invasion, proliferation, survival, angiogenesis, and invasion when altered by overexpression, amplification, or deletion mutants. One of the most studied mutations is epidermal growth factor receptor variant III (EGFRvIII) due to the vaccine trials in the UK (31). The role of EGFR mutations in gliomagenesis and as prognostic, diagnostic, and therapeutics are still debated and researched (12). In this study, EGFR was highly expressed in high-grade histological types 25/34 (71.4%) (Table II), which is consistent with previous research that reported an increase in EGFR expression with WHO tumour grade (32). Additionally, the study found the highest EGFR expression in primary glioblastoma (70.3%), which aligned with the current study outcome at 14/18 or 77.8%. An ascending trend was also evident in the EGFR expression of different tumour grades with a significant association: 5/34 (14.7%) in Grade I, 4/34 (11.8%) in Grade II, 11/34 (32.5%) in Grade III, and 14/34 (41.2%) in Grade IV.

The EGFR gene mutations influence the development and progression of several human cancers, including non-small-cell lung, gastric, pancreatic, and nasopharyngeal carcinomas. A meta-analysis found that a high EGFR expression indicated poor prognosis in 12 studies, whereas the other five reported negative or uncertain findings (33). Furthermore, EGFR alterations can be identified using various methods, including IHC, fluorescent in situ hybridisation (FISH), and reverse-transcriptase polymerase chain reaction (RT-PCR). A glioblastoma study in the UK discovered high consistencies between IHC and FISH (96%) and IHC and RT-PCR (98%) as a diagnostic method. It was also noted that neither EGFRvIII nor EGFR are associated with overall survival (31). In the present study, the anti-EGFR antibody [EP774Y] from Abcam was utilised in the IHC protocol, targeting the phosphorylated form of activated EGFR within the cytoplasm. It was challenging to analyse the EGFR scoring pattern because the lesion has an irregular distribution, with intensity ranging from weak to strong. Moreover, the background staining was quite prominent in this study, which was also reported in a study conducted in Brazil (34). The issue was possibly caused by a pre-analytical factor, such as tissue fixation or processing method, or technical differences such as antibody exposure time and dilution ratio, which was reduced by extending the incubation time to 40 minutes.

Most cases in this study exhibited negative c-erbB2/HER2 expression except for four cases (1 + and 2 + expression. All positive expression were glioblastoma cases. This outcome aligned with an earlier study conducted in Cleveland, Ohio, where 49 glioblastoma cases were studied, and all tumours were negative for

Her2/neu protein by IHC or amplification by FISH (35). A recent IHC study in Egypt discovered 29 (58%) c-erbB2/HER2 positive astrocytomas cases, with a significant association between c-erbB2/HER2 expression and histological types. The scoring system for c-erbB2/HER2 expression was similar to that used in breast cancer research (36), which was adopted in the present study. Similarly, Ramezani et al. reported that 42.1% of primary brain cancers were HER2-positive (scores 2 and 3) (20). Previously, a mini review highlighted discrepancies between most glioma studies with a range of positive cases (0% to 90%) that might have been due to antibody dependence and differences in study design (14). Tissue handling, fixation, antigenic decay, antigen retrieval methods, antibody types, working dilution, incubation time, temperature, tumour heterogeneity, immunostaining assessment, and interpretation issues are other technical factors to consider when conducting IHC, particularly for diagnostic purposes.

Despite advances in early disease detection, surgical debulking by a trained neurosurgeon, radiation, and chemotherapy, the prognosis for glioblastoma patients remained poor, with a median survival of less than 15 months. Recent studies have examined the role of the c-erbB2/HER2 mutation in glioblastoma targeted immunotherapy using chimeric antigen receptor (CAR) T-cell therapy, as reviewed by Yu and Quail (37). This treatment is an adoptive T-cell therapy that targets the patient's T cells and genetically modifies them to express CAR, targeting cancer cells. Despite being proven effective in haematologic cancers such as acute lymphoblastic leukaemia (ALL) and diffuse large-B-cell lymphoma (DLBCL), translating this treatment into solid tumours has been challenging. The CARs targeting EGFRvIII have been tested in clinical trials and demonstrated a potent antitumor effect in vitro and in vivo (38). In glioblastomas, c-erbB2/HER2 is expressed at low levels and ineffectively recognised by trastuzumab/herceptin, thus resulting in non-responsive or resistance to treatment. Nevertheless, several clinical trials of CARs targeting proteins such as IL-13, HER2, and EGFRvIII in glioblastoma have yielded encouraging results. (39)

Numerous research on brain tumours has been spearheaded by HUSM and primarily managed at the tertiary healthcare centres. Furthermore, HUSM is one of the teaching hospitals in Malaysia with the capacity to conduct studies on IDH and other mutations using molecular testing such as PCR and DNA sequencing, which is highly accurate and specific. These analyses require extensive labour, trained personnel, and expensive equipment, which may not be available at every centre.

There are several limitations in this study. Firstly, this single-centre retrospective study was conducted on a small sample size of 61 patients. In addition, subsequent validation of the cases with the molecular study was not performed due to the lack of advanced technology in the laboratory. Therefore, a prospective study using molecular methods is warranted to validate IDH1, p53, EGFR and c-erbB2/HER2 expressions in astrocytoma and oligodendroglioma tumours. Future studies with a larger sample size are highly recommended by collaborating with other institutions from different states in Malaysia. Secondly, IHC is a semi-quantitative technique influenced by multiple factors, such as different antibody clones used in studies with varying sensitivity and a cut-off value of positivity. Standardisation of antibody clones and positivity values are essential to establish more significant findings.

CONCLUSION

Recently, our knowledge regarding the genetics of central nervous system (CNS) tumors has expanded; hence, newer antibodies or molecular markers, which can be used in IHC, are continuously being developed. These antibodies; IDH1, p53 and EGFR markers are useful for diagnostic, prognostication and therapeutic. In addition, help to clarify the nature of cellular maturation, tissue differentiation, and tumor progression to be considered as an integral part of WHO classification of CNS tumors. Furthermore, the IHC method is proven useful as an alternative diagnostic and prognostic tool, particularly in small centres where molecular testing is unavailable.

ACKNOWLEDGEMENTS

Special thanks to the staff of the Pathology Laboratory, HUSM, especially Ummi Atikah Ayub and Rosli Jusoh, for their excellent technical assistance. This study was funded by the USM Bridging Grant (304/PPSP/6316326) and Tabung Geran Penyelidikan Sarjana Perubatan (GPSP) 2022, School of Medical Sciences, USM (1001/PPSP/8070011).

REFERENCES

1. Louis DN, Perry A, Wesseling P, Brat DJ, Cree IA, Figarella-Branger D, et al. The 2021 WHO classification of tumors of the central nervous system: A summary. Neuro Oncol. 2021;23(8):1231–51. doi:10.1093/neuonc/noab106
2. Louis DN, Perry A, Reifenberger G, von Deimling A, Figarella-Branger D, Cavenee WK, et al. The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. Acta Neuropathol. 2016;131(6):803–20. doi:10.1007/s00401-016-1545-1
3. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021 May 1;71(3):209–49. doi:10.3322/caac.21660
4. Azizah A, Hashimah B, Nirmal K, Zubaidah AS,

Puteri N, Nabihah A, et al. Malaysia National Cancer Registry Report 2012-2016. Putrajaya: Ministry of Health Malaysia; 2019.
5. Dzali NBM, Zahary MN, Abu Bakar NH binti, Jaafar H bin, Taib WRW. Distribution pattern of brain tumour in a tertiary hospital in east coast, Malaysia. Malaysian J Public Heal Med. 2017;2017(Special Issue 2):41–8.
6. Ostrom QT, Patil N, Cioffi G, Waite K, Kruchko C, Barnholtz-Sloan JS. CBTRUS statistical report: Primary brain and other central nervous system tumors diagnosed in the United States in 2013-2017. Neuro Oncol. 2020 Oct 5;22(Supplement_1):IV1–96. doi:10.1093/neuonc/noaa200
7. Lapointe S, Perry A, Butowski NA. Primary brain tumours in adults. The Lancet 2018 p. 432–46. doi:10.1016/S0140-6736(18)30990-5
8. Mair MJ, Wuhrer A, Furtner J, Simonovska A, Kiesel B, Oberndorfer S, et al. Clinical characteristics and prognostic factors of adult patients with pilocytic astrocytoma. J Neurooncol. 2020;148(1):187–98. doi:10.1007/s11060-020-03513-9
9. Appin CL, Brat DJ. Molecular pathways in gliomagenesis and their relevance to neuropathologic diagnosis. Adv Anat Pathol. 2015;22(1):50–8. doi: 10.1097/PAP.0000000000000048
10. Watanabe T, Nobusawa S, Kleihues P, Ohgaki H. IDH1 mutations are early events in the development of astrocytomas and oligodendrogliomas. Am J Pathol. 2009;174(4):1149–53. doi:10.2353/ajpath.2009.080958
11. Wilson TA, Karajannis MA, Harter DH. Glioblastoma multiforme: State of the art and future therapeutics. Surg Neurol Int. 2014;5(Supplement). doi: 10.4103/2152-7806.132138
12. Saadeh FS, Mahfouz R, Assi HI. Egfr as a clinical marker in glioblastomas and other gliomas. Int J Biol Markers. 2018;33(1):22–32. doi: 10.5301/ijbm.5000301
13. Mei J, Wang T, Xu R, Chen D, Zhang Y. Clinical and molecular immune characterisation of ERBB2 in glioma. Int Immunopharmacol. 2021 May 1;94:107499. doi:10.1016/j.intimp.2021.107499
14. Waage IS, Vreim I, Torp SH. C-erbB2/HER2 in human gliomas, medulloblastomas, and meningiomas: A minireview. Int J Surg Pathol. 2013;21(6):573–82. doi:10.1177/1066896913492196
15. Goh WC, Idris B, Kandasamy R, Shamsuddin S, Jaafar H, Ahmad F. PCR-RFLP method enhance DNA sequencing of IDH1 somatic mutations detection in gliomas. Gulhane Med J. 2020;61(4):167–71. doi:10.26657/gulhane.00078
16. Yusoff AAM, Zulfakhar FN, Sul'Ain MD, Idris Z, Abdullah JM. Association of The IDH1 C.395G>A (R132H) Mutation with Histological Type in Malay Brain Tumors. Asian Pacific J Cancer Prev. 2016;17(12):5195–201.
17. Capper D, WeiЯert S, Balss J, Habel A, Meyer J, Jдger D, et al. Characterisation of r132h mutation-specific idh1 antibody binding in brain tumors. Brain Pathol. 2010;20(1):245–54. doi:10.1111/j.1750-3639.2009.00352.x
18. Takano S, Tian W, Matsuda M, Yamamoto T, Ishikawa E, Kaneko MK, et al. Detection of IDH1 mutation in human gliomas: Comparison of immunohistochemistry and sequencing. Brain Tumor Pathol. 2011;28(2):115–23. doi:10.1007/s10014-011-0023-7
19. Bhalala NK, Patel NA, Vora HH. Clinical significance of IDH1, EGFR, p53 and MIB1 in Astrocytoma Patients. Int J Sci Res Rev. 2019;08(04):233–53. doi:10.37794/ijsrr.2019.8417
20. Ramezani M, Siami S, Rezaei M, Khazaei S. Immunohistochemical Study of c-erbB2 / HER2 Tumor Marker in Primary Malignant Brain Tumors. Preprints. 2018 Dec 3;2(December):1–9.
21. Mohd Hatta H, Mohd Fuzi NMH, Mohd Zin ND, A Rahim AI, Zakria NM, Sulaiman S, et al. An Epidemiological Analysis of COVID-19 cases from Jan to July 2020 in Kelantan, Malaysia. Ulum Islam. 2021;33(July):149–65. doi:10.33102/uij.vol33no1.293
22. Agarwal S, Sharma MC, Jha P, Pathak P, Suri V, Sarkar C, et al. Comparative study of IDH1 mutations in gliomas by immunohistochemistry and DNA sequencing. Neuro Oncol. 2013;15(6):718–26. doi:10.1093/neuonc/not015
23. Yan H, Parsons DW, Jin G, McLendon R, Rasheed BA, Yuan W, et al. Mutations in Gliomas. N Engl J Med. 2009;360(8):765–73. doi:10.1056/NEJMoa0808710
24. Ichimura K, Pearson DM, Kocialkowski S, Bдcklund LM, Chan R, Jones DTW, et al. IDH1 mutations are present in the majority of common adult gliomas but rare in primary glioblastomas. Neuro Oncol. 2009;11(4):341–7. doi:10.1215/15228517-2009-025
25. Nobusawa S, Watanabe T, Kleihues P, Ohgaki H. IDH1 mutations as molecular signature and predictive factor of secondary glioblastomas. Clin Cancer Res. 2009;15(19):6002–7. doi:10.1158/1078-0432.CCR-09-0715
26. Oh JE, Ohta T, Nonoguchi N, Satomi K, Capper D, Pierscianek D, et al. Genetic Alterations in Gliosarcoma and Giant Cell Glioblastoma. Brain Pathol. 2016 Jul 1;26(4):517–22. doi: 10.1111/bpa.12328
27. Zhang Y, Dube C, Gibert M, Cruickshanks N, Wang B, Coughlan M, et al. The p53 pathway in glioblastoma. Cancers (Basel). 2018;10(9). doi:10.3390/cancers10090297
28. Takano S, Ishikawa E, Sakamoto N, Matsuda M, Akutsu H, Noguchi M, et al. Immunohistochemistry on IDH 1/2, ATRX, p53 and Ki-67 substitute molecular genetic testing and predict patient prognosis in grade III adult diffuse gliomas. Brain Tumor Pathol. 2016;33(2):107–16. doi:10.1007/

s10014-016-0260-x
29. Takami H, Yoshida A, Fukushima S, Arita H, Matsushita Y, Nakamura T, et al. Revisiting TP53 mutations and immunohistochemistry - A comparative study in 157 diffuse gliomas. Brain Pathol. 2015 May 1;25(3):256–65. doi:10.1111/bpa.12328
30. Jiao Y, Killela PJ, Reitman ZJ, Christopher MH. Frequent ATRX, CIC, FUBP1 and IDH1 mutations refine the classification of malignant gliomas. Oncotarget. 2012;3(July):710–22. doi: 10.12693/APhysPolA.116.261
31. Faulkner C, Palmer A, Williams H, Wragg C, Haynes HR, White P, et al. EGFR and EGFRvIII analysis in glioblastoma as therapeutic biomarkers. Br J Neurosurg. 2015;29(1):23–9. doi:10.3109/02688697.2014.950631
32. Mishra S, Rathi KR, Shelly D, Bharadwaj R. Immunohistochemical expression of IDH1R132H in Astrocytictumours and its association with histopathological grade, TP53 and EGFR protein expression. Ann Pathol Lab Med. 2017;4(5):A522–9. doi:10.21276/APALM.1458
33. Li J, Liang R, Song C, Xiang Y, Liu Y. Prognostic significance of epidermal growth factor receptor expression in glioma patients. Onco Targets Ther. 2018;11:731–42. doi:10.2147/OTT.S155160
34. Montgomery RM, Queiroz LDS, Rogerio F. EGFR, p53, IDH-1 and MDM2 immunohistochemical analysis in glioblastoma: Therapeutic and prognostic correlation. Arq Neuropsiquiatr. 2015;73(7):561–8. doi:10.1590/0004-282X20150059
35. Haynik DM, Roma AA, Prayson RA. HER-2/neu expression in glioblastoma multiforme. Appl Immunohistochem Mol Morphol. 2007 Mar;15(1):56–8. doi:1097/01.pai.0000213133.09160.da
36. Ali MY, El MA. Human epidermal growth factor receptor 2 neu and E-cadherin expression in primary astrocytic brain tumors as prognostic biomarkers. Al-Azhar Assiut Med J. 2020;(19):18–26.
37. Yu MW, Quail DF. Immunotherapy for Glioblastoma: Current Progress and Challenge. Front Immunol. 2021;12(May):1–12. doi:10.3389/fimmu.2021.676301
38. Yu S, Li A, Liu Q, Li T, Yuan X, Han X, et al. Chimeric antigen receptor T cells: a novel therapy for solid tumors. J Hematol Oncol. 2017;10(1):1–13. doi:10.1186/s13045-017-0444-9
39. Huang B, Zhang H, Gu L, Ye B, Jian Z, Stary C, et al. Advances in immunotherapy for glioblastoma multiforme. J Immunol Res. 2017; doi:10.1155/2017/3597613