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IDH1(R132H) Immunoexpression in Glioma

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Abstract

Background and objectives: Gliomas are the most common type of primary brain tumors. They result from accumulation of multiple genetic alterations. Isocitrate dehydrogenase 1 mutations are detected in gliomas. This study aimed to assess IDH1 immunoexpression in different types of gliomas and to correlate the results with patient's age, gender, tumor site, tumor type and grade. **Methods:** During the period from January 2015 –January 2017, 97 cases of gliomas were collected from Rizgary Teaching Hospital and private laboratories in Erbil city. Typing and grading of tumors were done according to World Health Organization classification of brain tumors 2007.Immunohistochemical staining was done for IDH1 using monoclonal Antibody IDH1R132. **Results:** Out of 97 cases of gliomas; 16 were grade I, 27 were grade II, 10 were anaplastic gliomas grade III and 44 cases were glioblastomas; grade IV. Positive IDH1 immunoexpression was observed in 36 (37.1%) cases of gliomas. The highest prevalence of IDH1 was found in the age group 30-44 years (52.8%), in supratentorial tumors (44.3%) and in grade II and III astrocytoma (72.2% and 75%).Highly significant correlation was found between Isocitrate dehydrogenase 1 expression and age of the patients, tumor type and grade while a significant correlation was found between Isocitrate dehydrogenase 1 expression and tumor site. **Conclusions:** Our study concluded that Isocitrate dehydrogenase 1 is 36 (37.1%) expressed in gliomas and it is significantly associated with patient's age, tumor site, histological type and grade, thus it may act as a good diagnostic and prognostic marker for gliomas.

Keywords: Glioma; IDH1; Immunohistochemistry

Introduction

Gliomas are the most common type of primary brain tumors and constitute about 40-50% of all central nervous system neoplasms[1]. They arise from glial cells and depending on their origin; there are mainly of three types: astrocytomas, oligodendrogliomas, and ependymomas. The WHO has classified gliomas to Grade I to IV on the basis of clinicopathological criteri as[1, 2]as follow:

WHO grade I gliomas have an indolent growth, usually benign, generally with complete surgical resection and are curable[2]. While WHO grade II or III are invasive, progress to higher-grade lesions, and having a poor outcome. WHO grade IV tumors; glioblastoma multiforme(GBM) is the commonest and biologically it's the most aggressive type with dismal prognosis and it is classified into two groups: primary and secondary, the primary GBM present de novo as advanced tumors with no evidence of precursor lesions, whereas secondary GBM are tumors that have clinicopathological and radiologic evidence of malignant progression from lower grade tumors[3-5].

Isocitrate dehydrogenase 1; belongs to IDH gene family, is localized on 2q33, in the cytoplasm and in peroxisoms, and encodes for the cytosolic NADP+ dependent isocitrate dehydrogenase, the product protein catalyzes the cytosolic oxidative decarboxylation of isocitrate to alpha-ketoglutarate, and resulting in the production of reduced form of NADP+ (NADPH) which is main function as anti- oxidant and promotes resistance to apoptosis, gene mutation alters the enzymatic property of IDH1and leads to increase conversion of alpha-ketogluterate to 2-hydroxyglutarate metabolite and decreased production of NADPH, and accordingly reduced glutathione. These alterations may raise the oxidative stress level in mutant IDH1cells and acting as an oncogen[6-10].

In the routine pathologic evaluation of glioblastomas and lower grade diffuse gliomas; IDH1mutant protein is increasingly performed because of its prognostic significance, and it has been mentioned that about80% of all WHO grade II–III infiltrating/diffuse gliomas and grade IV secondary glioblastomas show mutations in IDH1, these mutations correlated with better outcomes[6,11].

Expression of IDH1 mutant protein has established to be diagnostically useful in the distinction of diffuse low-grade gliomas from reactive astrocytosis[12]. Also, immunopositivity of IDH1mutant protein may be useful in distinguishing diffuse gliomas from other primary brain tumors and metastatic malignancies which are typically negative[13, 14].

The aims of this study were to determine the frequency of IDH1 immunoexpression in gliomas in Erbil city and correlate the IDH1immunoexpression with some clinicopathological parameters like: Age, sex, tumor site, histological type and tumor grade.

Materials and methods

This is a retrospective study, done in a period from January 2015- January 2017, in which 97 cases were enrolled. All types of intracranial gliomas were collected including both sexes and all age groups. The cases were registered at the department of histopathology in Rizgary Teaching Hospital as well as private laboratories in Erbil city/ Kurd-

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istan region - Iraq. Clinical data including age, sex and the site of tumor were collected from histopathology reports.
Paraffin-embedded blocks were sectioned on three micron thickness and stained with hematoxylin and eosin. Tumors then classified and graded according to 2007 WHO classification of the CNS tumors. Ethical Approval was obtained from the regional Committee of Ethics in the Kurdistan Board for Medical Specialties.
Immunohistochemical technique:
Additional 3 micron thickness slides from the tumor tissue were deparaffinized and rehydrated. Antigen retrieval was carried out by autoclaving at 97 C0 for 20 minutes using antigen retrieval solution (citrate buffer 10 mmol/L, pH 6.0). Sections then allowed for cooling at room temperature, followed by washing 3 times, each for 3 minutes, in phosphate buffered saline (PBS). Endogenous peroxidase activity was blocked by dipping sections in 3% hydrogen peroxidase blocker (Dako USA) for 10 minutes and washed in 3 changes of PBS. After the initial processing step, sections were incubated overnight with 1:20 diluted primary antibodies anti-human IDH1 R132H (USA, Mouse Monoclonal Antibody) at room temperature. This was followed by incubation with supersensitive nonbiotin HRP detection system for 35 minutes. Finally, the sections were counterstained with hematoxylin, dehydrated and mounted.
Positive result showed strong cytoplasmic and weak nuclear staining which appear only in the tumor cells and negative controls are prepared simultaneously for all 97 samples by replacing the primary antibody with distilled water Expression of IDH1was determined by semiquantitative assessment of the proportion of the positively stained tumor cells. Cases with ≥10% stained cells were labeled as positive, and cases with <10% stained cells were labeled as negative. A weak diffuse staining and staining of macrophages were scored as negative[10, 13, 14].
Statistical analysis was performed by using Statistical package for social sciences (SPSS) version 20.Data were interpreted in form of frequencies, percentages and Mean Standard deviation of quantitative variables . A Chi square test and Fisher exact test were used to associate the IDH-1status and different study variables. Statistical significance was achieved when the p-value was less than or equal to 0.05.

Results

Out of total of 97 cases, 59 were females and 38 were males with a female to male ratio of 1: 0.64. The patients' age ranged from 3 months to 84 years with a mean age of 37.075±20 years and a median of 38 years.
In the current study positive IDH1staining was observed in 36 (37.1%) cases of gliomas while negative IDH1 staining was observed in 61(62.9%) cases.
The highest prevalence of IDH1 was found in the age group 30-44 years (52.8%) followed by age group 15-29 years (22.2%) and age group 45-59(16.7%). A highly significant correlation was found between IDH1 expression and patient's age (p-value<0.001), Figure 1.

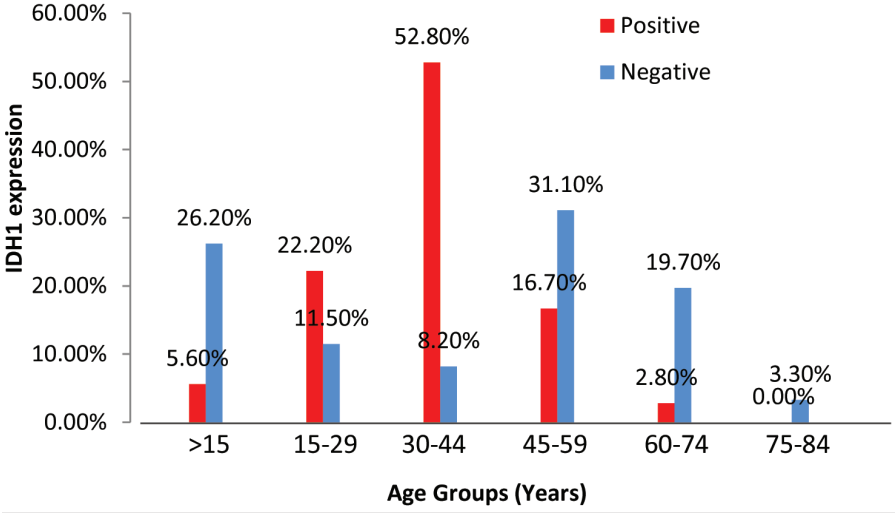

Figure (1): Patients Age and IDH1 expression

Regarding gender, out of 38 males; 11 were positive while among 59 females; 25 were positive for IDH1and the association of IDH1 expression with gender was statistically not significant (p-value<0.182), Figure2.

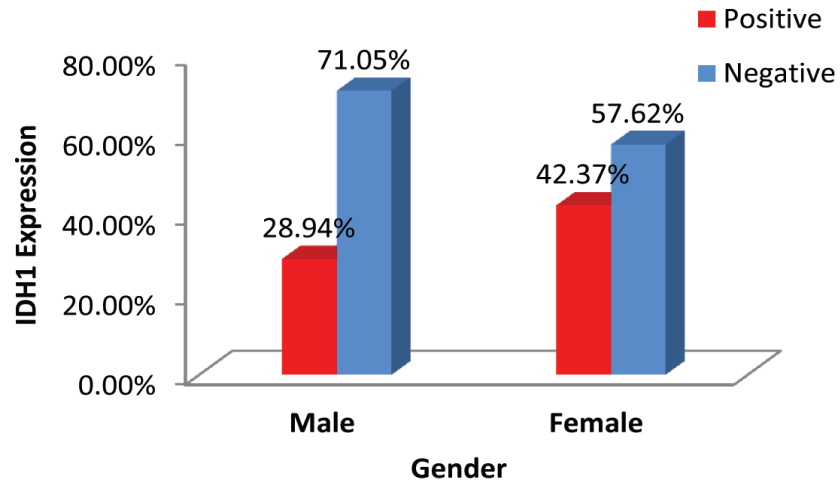

Figure (2): Patients Gender and IDH1 expression

As far as tumor site; majority of tumors were supratentorial 79 cases(81.4%), among these; 35 cases were positive for IDH1, while the reminder 18 cases were infratentorial in location and among them one case was positive and the association of IDH1 expression with tumor site was statistically significant (p-value=0.002),Figure 3.

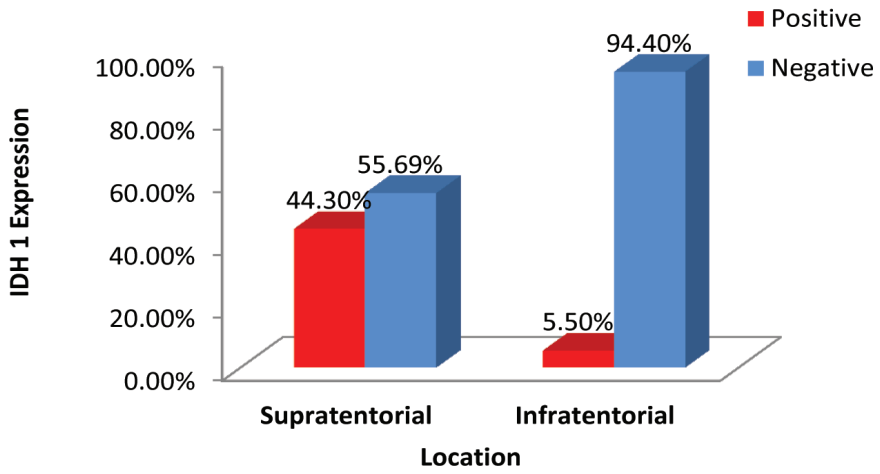

Figure (3): Tumor Site and IDH1 expression

Concerning the tumor type, astrocytic tumors were the predominant type of glioma in this study, including 44 glioblastomas (GBM) (one case was gliosarcoma), 18 diffuse astrocytomas (DA), 15 pilocytic astrocytomas, 4 anaplastic astrocytomas (AA), 2 pleomorphic xanthoastrocytomas, 1 subependymal giant cell astrocytoma, also there were 3 oligodendroglioma/oligoastrocytoma, 2 anaplastic oligodendroglioma, 4 conventional ependymomas and 4 anaplastic ependymomas. Among astrocytomas; DA and AA showed the highest IDH1 expression (72.2% and 75% respectively), followed by GBM (34.09%).While all pilocytic astrocytomas were negative for IDH1 expression and the association of IDH1 expression with tumor type was statistically highly significant (p-value<0.001),table 1.

Table (1): IDH1 expression with tumor type

No.	Histologic_type	IDH-1		Total no. (%)
		Positive no. (%)	Negative no. (%)	
1	Pilocytic astrocytoma	0(0)	15(100)	15(15.46)
2	Subependymal gaint cell astrocytoma	0(0)	1(100)	1(1.03)
3	Diffuse astrocytoma	13(72.2)*	5 (27.3)	18(18.55)
4	Oligodendroglioma	2(100)	0(0)	2(2.06)
5	Oligoastrocytoma	1(100)	0(0)	1(1.03)
6	Pleomorphic xanthoastrocytoma	1(50)	1(50)	2(2.06)
7	Ependymoma	0(0)	4(100)	4(4.1)
8	Anaplastic astrocytoma	3(75)	1(25)	4(4.1)
9	Anaplastic oligodendroglioma	1(50)	1(50)	2(2.06)
10	Anaplastic ependymoma	0(0)	4(100)	4(4.1)
11	Glioblastomamultiforme	15(34.88)*	28(65.2)	43(44.3)
12	Gliosarcoma	0(0)	1(100)	1(1.03)
	Total	**36(37.1)**	**61(62.9)**	**97(100)**

*p value <0.001 Fisher's exact Test

Regarding tumor grade; all 16 (100%) grade I gliomas were negative for IDH1 expression, while 17/27 (62.96%) of grade II(Figure5A) and 4/10 (40%) in grade III glioma (Figure 5C) were positive for IDH1, the predominant grade was grade IV representing44 cases and among them 15 cases (34.09%) were positive (Figure 5E), and the association between IDH1 expression and grade of gliomas was statistically highly significant (p-value<0.001),Figure 4.

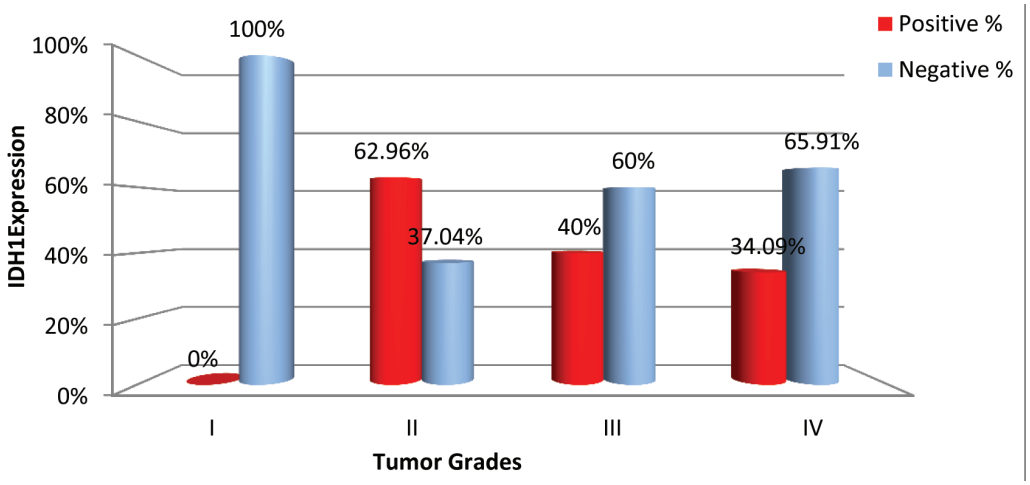

Figure (4): Tumor grade and IDH1expression

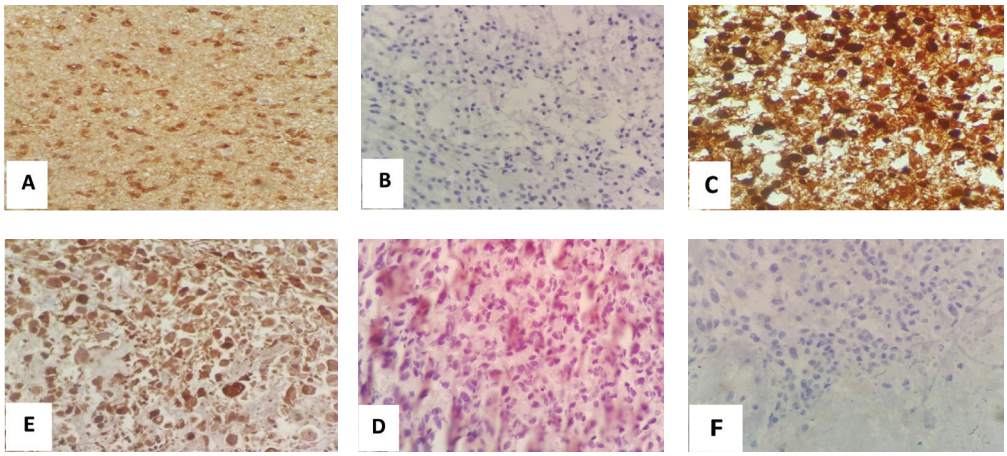

Figure (5): Immunohistochemical expression of IDH1-R132H in different grades of astrocytoma. A, Diffuse astrocytoma, WHO grade II positively stained and B, negatively stained. C, Anaplastic astrocytoma, WHO grade III positively stained and D, negatively stained. E, Glioblastoma, WHO grade IV, positively stained, and F, negatively stained (A- F, ×400).

Discussion

Worldwide brain tumors are considered one of the most dramatic and rapidly fatal of all human cancers[16, 17]. Multiple oncogenic events have been recognized in the progression of the brain tumor cells involving several defects in signalling pathways with variety of genes. Among these genes cytosolic IDH1located on chromosome 2q33.3 has emerged as a major diagnostic and prognostic biomarker for gliomas[2].

The present study is the first in our region to detect IDH1 R132 H immunoexpression in gliomas and correlatesIDH1 expression with various clinico pathological parameters by using antibody specific for IDH1 mutation, as studies also found that immunohistochemistry for IDH1 protein expression was sensitive and specific in detecting IDH1 R132 H mutation[2, 8]. In the current study IDH1 was expressed in 36(37.1%) cases of gliomas, our result is in close alignment with what has been observed by others as shown in Table 2.

Study(year)	Location	Sample (n)	IDH1 positive (n)	IDH1 positive (%)
Sonoda et al.(2009)[18]	Japan	125	39	31.2
Qi et al.(2011)[19]	China	203	75	36.9
Myung et al.(2012)[20]	Korea	134	72	53.7
Thota et al.(2012)[8]	India	74	31	41.9
Mukasa et al.(2012)[21]	Japan	250	73	29.2
Qi et al.(2014)[22]	China	193	111	57.5
Saeed MS(2014)[10]	Mosul-Iraq	109	38	34.86
Wagn et al.(2016)[23]	China	811	488	55.2
Current study(2018)	Erbil-Iraq	97	36	37.1

Table (2): IDH1 immunoexpression in different studies

In the present study highest IDH1 expression was observed in age group(30-44)constituting 52.8%of the positive cases and the correlation was statistically highly significant (p value<0.001), and this result was in agreement with other studies[2, 8,16,24,25] thus suggesting that IDH1 mutation occurs as an early event in gliomagenesis specifically in 3rd and 4thdecades.

IDH1 expression was observed more in females (42.37%) than males with no significant statistical correlation, however other studies found a higher prevalence of IDH1 expression in males than females[17, 22, 26]. This discrepancy is probably due to the predominance of female gender in our study forming 60.8% of cases and limited sample size.

The majority of IDH1 positive tumors were supratentorial in location and forming (44.3%) with statistically significant correlation (p-value=0.002) In agreement with Yan et al result[26] and this could be explained by the fact that majority of astrocytic tumors which showed IDH1 immunoexpression were supratentorial in location.

The frequency of IDH1 expression in DA was (72.2%), and in AA was (75%); which are in agreements with others who mentioned high expression of IDH1 in both DA and AA.[2, 8, 27, 28]

In GBM,IDH1 expression was positive in 15/44 (34.09%) which probably all represent secondary GBM as they are, in contrast to primary GBM, well known for high IDH1 expression, although a full clinical history was not available in all our cases.our figure was within the range that has been reported in other studies (from 12.5% up to 64.5%)[8, 17, 29]; a point to be mentioned here is that in some studies more secondary GBM were included so they got higher IDH1 expression[10,17], while in others more primary GBM were included so they reported lower figures8. This clarifies the importance of differentiating secondary glioblastoma from primary glioblastoma confirming that; this pattern of IDH1 expression may indicate secondary GBM and associated with longer overall survival rate of patients.30
Also in our study there was a highly significant correlation between IDH1 expression and the tumor type Table1 (P-value <0.001) which was in agreement with Yusoff et al[17].
In agreement withseveral previous studies that reported a high frequency of IDH1 expression in grade II gliomas ranging from (52%-83.5%),[18,24,30,31] which were in close alignment with our result (62.96%), while in grade III gliomas IDH1 expression was found in(40%) which was lower than other studies that their expression was ranging from 52%-92.8%[8,24,28-32] and this probably due to limited sample size in our study (only 10 cases) in addition among these 10 cases there were 4 anaplastic ependymomas which are well known to be negative for IDH1 expression and probably have other pathways for tumorogenesis. In Grade IV gliomas 15/44 (34.09%) cases were positive and our results fall within the range that has been reported by others (from 12.5% up to 64.5%)[8,17, 29] . This variability may be due to differences in ethnicity, cut off value for IDH1 expression and type of antibody clones used, regarding the correlation of IDH1 with tumor grade; it was statistically highly significant(p-value <0.001) which was in agreement with other studies[17,33].
Another observation in our study was that IDH1 expression not identified in all pilocytic astrocytoma 15(100%) grade I, all grade II ependymoma 4(100%), and all anaplastic ependymoma 4(100%) grade III; a result which was similar to other studies[2,17] confirming that, these tumors are genetically distinct, arising through different mechanisms and may be derived from different type of glial progenitor cells.

Conclusions

Our study revealed that IDH1 is frequently expressed in different types of gliomas, particularly in grades II – III astrocytomas and in younger age group, more in females and in supratentorial tumors and it is significantly associated with patient's age, tumor site, histological type and grade, thus it may act as a good diagnostic and prognostic marker for gliomas.
Conflict of interest:
The authors declare that there was no conflict of interest.

References

1. Louis DN, Ohgaki H, Wiestler OW, Cavenee WK, Burger PC, Jouvet A, et al.The2007 WHO Classification of Tumors of the Central Nervous System. Acta Neuropathol. 2007; 114(2):97–109.
2. Sipayya V, Sharma Ira, Sharma KC, Singh A. Immunohistochemical expression of IDH1in gliomas: A tissue microarray-based approach. J of Cancer Res and Therap.2012;8(4):598-601.
3.Stupp R, Mason WP, Van den Bent MJ, Weller M,FisherB,Taphoorn M. J.B, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med.2005; 352:987-96.
4. Wen PY, Kesari S. Malignant gliomas in adults.NEngl J Med.2008;359:492-507.
5.Ohgaki H, Dessen P, Jourde B, Horstmann S, Nishikawa T, DiPatre PL, et al. Genetic pathways to glioblastoma: a population-based study. Cancer Res. 2004;64: 6892-9.
6. Guo C, Pirozzi CJ, Lopez GY, H Yan. Isocitrate dehydrogenase mutations in gliomas: mechanisms, biomarkers and therapeutic target. Curr Opin Neurol. 2011; 24:648–52.
7.Toedt G, Barbus S, Wolter M, Felsberg J, Tews B, Blond F, et al. Molecular signatures classify astrocyticgliomas by IDH1 mutation status. Int J Cancer. 2011;128:1095–1103.
8.Thota B, Shukla SK, Srividya MR, Shwetha SD, Arivazhgan A, Thennarasu K, et al. IDH1 mutations in diffusely infiltrating astrocytomas: grade specificity, association with protein expression, and clinical relevance. Am J ClinPathol. 2012;138:177–84.
9. Thompson CB.Metabolic enzymes as oncogenes or tumor suppressors. N Engl J Med. 2009;360:813–15.
10.Saeed MS. IDH1 Mutation in Gliomas in Mosul City – IraqMacedJ-MedSci. 2015,15; 3(2):250-55.
11.Bigner SH, Matthews MR, Rasheed BKA, Wiltshire RN, Friedman HS, Friedman AH, et al. Molecular genetic aspects of oligodendrogliomas including analysis by comparative genomic hybridization. Am J Pathol 1999;155:375-86.
12.Horbinski C, Kofler J, Yeaney G, Camelo-Piragua S, Venneti S, Louis DN, et al. Isocitrate dehydrogenase 1 analysis differentiates gangliogliomas from infiltrative gliomas. Brain Pathol 2011;21: 564–74.
13.Piragua SC, Jansen M, Ganguly A, Kim JC, Louis DN, Nutt CL. Mutant IDH1-specificimmunohistochemistry distinguishes diffuse astrocytoma from astrocytosis. Acta Neuropathol 2010;119: 509–11.
14.Bleeker FE, Lamba S, Leenstra S, Troost D, Hulsebos T, Vandertop WP, et al. IDH1 mutations at residue p.R132 (IDH1(R132) occur frequently in high-grade gliomas but not in other solid tumors. Hum Mutat 2009;30:7–11.
15.Joseph NM, Phillips J, Dahiya S, Felicella M, Tihan T, Brat DJ, Perry A.IDH1 and OLIG2 expression in glioblastomavariants. Mod Pathol. 2013; 26:315–26.
16. Parsons DW, Jones S, Zhang X, Cheng-Ho Lin J, Leary RJ, Angenendt P, et al. An integrated genomic analysis of human glioblastomamultiforme. Science 2008;321:1807–12.
17. Yusoff A, 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 Pac J Cancer Prev. 2016; 17(12): 5195–201.
18.Sonoda Y, Kumabe T, Nakamura T, Saito R, Kanamori M, YamashitaY, Suzuki H, Tominaga T, et al. Analysis of IDH1 and IDH2 mutations in Japanese gliomapatients. Cancer Sci. 2009;100:1996-8.
19.Qi ST, Yu L, Lu YT, Ou YH, Li ZY, Wu LX,et al. IDH mutations occur

frequently in Chinese glioma patients and predict longer survival but not response to concomitant chemoradiotherapy in anaplastic gliomas. Oncol Rep. 2011;26:1479–85.

20.Myung JK, Cho HJ, Park CK, Kim SK, Phi JH, Park SH. IDH1 mutation of gliomas with long-term survival analysis. Oncol Rep. 2012;28:1639–44.

21.Mukasa A, Takayanagi S, Saito K, Shibahara U, Tabei Y, Furuya K, et al. Significance of IDH mutations varies with tumor histology, grade, and genetics in Japanese glioma patients. Cancer Sci. 2012;103:587–92.

22.Qi S, Yu L, Li H, Ou Y, Qiu X, Ding Y,et al. Isocitrate dehydrogenase mutation is associated with tumor location and magnetic resonance imaging characteristics in astrocytic neoplasms. Oncol Lett. 2014;7:1895–1902.

23. Wang HY, Tang K, Liang TY ,Zhang WZ, LiJ Y, Wang W, et al. The comparison of clinical and biological characteristics between IDH1 and IDH2 mutations in gliomas. J Exp Clin Cancer Res. 2016;35:86.

24. Watanabe T, Nobusawa S, Kleihues P, Ohgaki H. IDH1 mutations are early events in the development of astrocytomas and oligodendrogliomas. Am J Pathol.2009; 174:1149-53.

25. Robinson C, Kleinschmidt-DeMasters BK. IDH1-Mutation in Diffuse Gliomas in Persons Age 55 Years and Over. J Neuropathol Exp Neurol. 2017 Feb 1;76(2):151-54.

26. Yan W, Zhang W, You G, Bao Z, Wang Y, Liu Y, et al. Correlation of IDH1 mutation with clinicopathologic factors and prognosis in primary glioblastoma: a report of 118 patients from China. PLOS One. 2012;7:30339.

27.Fouad EM, Saba EEI, Rashed MEH. Abdelwahab RM. Evaluation of the role of mutatant, EGFR and BCL2 protien expression in distinguishing diffuse astrocytoma from astrocytosis. ZUMJ.2014. 20(3),404-13.

28.Capper D, Weißert S, Balss J, Habel A, Meyer J, Jäge D, et al. Characterization of R132H Mutation-specific IDH1 Antibody Binding in Brain Tumors. Brain Pathol.2010; 20;245-54.

29.Agrawal 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-Oncology 2016, 15(6);718-26.

30. Ichimura K, Pearson DM, Kocialkowski S, Backlund LM, Chan R, Jones DT, et al. IDH1 mutations are present in the majority of common adult gliomas but are rare in primary glioblastomas. NeuroOncol. 2009; 11:341-47.

31. Hartmann Ch, Hentschel B, Wick W, Capper D, Felsberg J, Simon M, et al. Patients with IDH1 wild type anaplastic astrocytomas exhibit worse prognosis than IDH1-mutated glioblastomas, and IDH1 mutation status accounts for the unfavorable prognostic effect of higher age: implications for classification of gliomas. Acta Neuropathol.2010; 120(6); 707-18.

32.Nobusawa S, Watanabe T, Kleihues P, Ohgaki H. IDH1 Mutations as Molecular Signature and Predictive Factor of Secondary Glioblastomas. AACR journal.2009;15(19):6002-7.

33. Dwianingsih EK, , Bawono RG , Rhamadianti AF, Bayuangga HF, Malueka RG. Correlation between grading and IDH1 mutation in Indonesian glioma. Jour of NeurolSci. 2017;381:386–87.