RESEARCH ARTICLE

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Expression of CD133 Cancer Stem Cell Marker in IDH-Mutant and IDH-wildtype (Isocitrate Dehydrogenase) Astrocytoma

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Abstract

Objective: This study evaluated the differences between IDH1-R132H and CD133 expression in different categories of astrocytoma. **Material and methods:** This study used a cross-sectional design. Sixty-seven paraffin embedded block of Diffuse Astrocytoma (DA), Anaplastic Astrocytoma (AA) and Glioblastoma (GB) were assessed using using the monoclonal antibody IDH1-R132H and Rabbit polyclonal antibody CD133. **Results:** It was found that there was a significant relationship between the expression of IDH1-R132H and CD133 in DA, AA and GB ($p<0.001$). Astrocytoma with IDH-mutant molecular status will express more markers of cancer stem cell CD133 than IDH-wildtype. **Conclusion:** The IDH1-R132H and CD133 can provide predictive value on treatment success, disease prognosis, recurrence and can be considered as target combination therapy with chemotherapy.

Keywords: IDH1-R132H- IDH-mutant- IDH-wildtype- CD133- astrocytoma

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Introduction

Brain cancer accounts for about 85-90% of all central nervous system cancers. The incidence of malignant primary brain tumors is significantly lower in East Asia, Southeast Asia, and India. The highest incidence is found in Europe, Canada, the United States, and Australia. Data from Globocan 2018 shows the worldwide incidence of brain and nervous system tumors is 308,000 (1.6%) with a mortality rate of 251,000. (Globocan Observatory, 2020). Anaplastic astrocytomas and glioblastomas account for about 38% of all primary central nervous system tumors, and 27% are meningiomas and other mesenchymal tumors. The other primary brain tumors are including pituitary tumors, schwannomas, CNS lymphomas, oligodendrogliomas, ependymomas, low-grade astrocytomas, and meduloblastomas.In Indonesia, there is no clear national epidemiological data regarding primary brain tumors, especially astrocytomas (KPKN, 2017; Leece et al., 2017).

Astrocytoma is the most common primary intracerebral tumor in adults and causes high morbidity and mortality. Most patients with astrocytomas, especially diffuse gliomas, have a poor prognosis. (Rasmussen et al., 2017) Astrocytomas are classified into subtypes according to glial cell histology and are divided into grades 1 to 4 based on morphology and malignant behavior, and using molecular information as determined in the World Health Organization (WHO) 2016 classification. High grade glioma (HGG) or also called malignant glioma has rapid tumor growth although rarely metastasize outside the CNS. Glioblastomas are included in the WHO grade 4 HGG, with an incidence about 75% of all HGG. (Rasmussen et al., 2017)

The change in classification by including molecular parameters based on genotype creates new challenges with respect to testing and reporting of glioma diagnoses. This 2016 classification updates a molecular parameters into the classification of diffuse gliomas, and this shift has affected the classification in several ways. All astrocytic tumors were previously grouped together. At present, however, all diffuse gliomas (astrocytic origin or not) are grouped together, on the basis of not only their growth pattern and behavior, but also on the expression of genetic status IDH1 and IDH2 (Isocitrate dehydrogenase 1 and 2) (Louis D et al., 2016).

In this new classification, the category of diffuse glioma includes WHO grade 1 and 3 astrocytic tumors, grade 2 and 3 oligodendroglioma, grade 2 and 3 oligoastrocytoma, grade 4 glioblastoma, and associated diffuse gliomas (in childhood). This approach separates astrocytomas that have a more restricted growth pattern, lack the IDH gene, and occasionally have BRAF mutations (pilocytic astrocytomas, pleomorphic xanthoastrocytomas, and subependymal giant cell astrocytomas) from diffuse gliomas. The presence of IDH1 and IDH2 mutations is found in almost all glioblastomas that develop from astrocytomas (secondary glioblastomas).

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Clinically, primary glioblastoma with IDH1 mutations can be misclassified and may actually be an asymptomatic low-grade glioma that has developed and then become symptomatic after becoming a glioblastoma. Thus, the IDH1 mutation is a molecular marker that can be used to separate groups of glioblastomas that may be clinically or histopathologically identical to the secondary type (Louis, 2016; Louis D et al., 2016).

Recent genetic and epigenetic studies have shown that mutations in the IDH gene play an important role in the pathogenesis and prognosis of gliomas so that identification of IDH1 mutations in the sample population can be an important marker, not only to help diagnose and determine the prognosis of these gliomas, but also for the development of targeted therapies such as chemotherapy (Louis, 2016).

It is known that targeted therapy can result in partial regression which is generally followed by the appearance of new tumor clones that develop from the existing Cancer Stem Cells (CSC) population. Therefore, identifying and targeting CSCs is thought to be a promising and effective alternative in cancer treatment. It has been reported that targeting CSCs results in more pronounced tumor regression. Stable CSCs are generally divide slowly and responsible for the development of tumor resistance to conventional cancer therapies (Louis D et al., 2016).

Cancer stem cells also play a role in radioresistant and chemoresistant gliomas by increasing the capacity for DNA repair through a checkpoint activation response to DNA damage. CD133 has been identified as a marker for identifying CSC. CD133 with Wnt and Notch signaling pathway can lead to cell proliferation. The collaboration between Shh and Wnt has also been proven as a regulator of self-renewal and cancer growth in stem cells. In addition, CD133 inhibits apoptosis and increases FLICE-inhibitory protein (FLIP) which causes chemotherapy resistance. Therapy targeting CD133 may be an efficient strategy for the tumor shrinkage (Barzegar Behrooz et al., 2019).

Based on the importance in clinical application and their role in biological concepts, this research was first conducted specifically by using samples in Makassar to see the expression of the CSC CD133 marker in astrocytoma based on the 2016 WHO classification using molecular characteristic parameters through immunohistochemical examination.

Materials and Methods

In this study, we collected 67 paraffin block samples of patients with the diagnosis of Diffuse Astrocytoma (DA), Anaplastic Astrocytoma (AA) and Glioblastoma (GB) from the Anatomical Pathology Laboratory, Faculty of Medicine, Hasanuddin University, from October 2020 to August 2021.

Unstained slides were made from paraffin blocks and IDH1-R132H and CD133 immunohistochemistry staining was performed. In each case, slides were made from paraffin blocks then cut with a 3 µm thick microtome. The cut in the water bath was taken using a poly-L-lysinie slide, then deparaffinized. Immunohistochemical staining using monoclonal IDH1-R132H and polyclonal CD133.

Assessment of IDH1-R132H and CD133 expression were assessed on membrane and cytoplasm of tumor cells using a light microscope with 400x. Assesment by two pathologists who were blinded to clinical information and outcomes.IDH expressions was divided into 2 groups: IDH-mutant if IDH1 expression was 10% stained on the membrane and cytoplasm of tumor cells and IDH-wildtype if IDH1 expression <10% stained on the membrane and cytoplasm of tumor cells. (Malueka et al., 2017)

CD133 immunoexpression was calculated using semiquantitative analysis by assessing the intensity and percentage of stained area, then the overall score was calculated by the H-score for each case by multiplying the staining intensity by the percentage of positive cells. The intensity of CD133: uncolored : 0/negative, weak (visible at 400x magnification): +1, moderate (visible at 100x magnification): +2, strong (seen at 40x magnification): +3. The extensibility (area of the colored area at 400x magnification): no area is colored 0%, colored area <10%, colored area 10-50%, colored area >50%.

The data in this study were processed using SPSS 20 for Windows software. Descriptive statistical techniques used to describe the characteristic of the basic data obtained in the form of frequency distribution. Chisquare test were used to determine CD133 in each Astrocytoma classification.

Results

Of the 67 samples, distribution of astrocytoma samples based on diagnoses that are in line with histopathological grading, gender, age, tumor location based on clinical or radiological data, and the molecular status of IDH1 and CD133 are shown in Table 1.

Distribution of samples based on histopathological diagnosis, 22 samples (32.80%) of DA cases were obtained, 15 samples of AA (22.40%) and 30 samples of GB (44.80%) were found. From this total sample, there were 40 samples of male (59.70%) and 27 samples of female (40.30%). Where in the male sample, 13 samples were diagnosed with DA, 6 samples were diagnosed with AA and 21 samples were diagnosed with GB, then with immunohistochemical staining there were 28 samples showing the IDH-mutant molecular status and 12 samples with IDH-wildtype molecular status. Meanwhile, in the female gender sample, 9 samples were diagnosed as DA, 9 samples were diagnosed as AA and 5 samples as GB, and there were 18 samples with IDH-mutant molecular status and 9 samples with IDH-wildtype molecular status.

In the age category 0-19 years, there were 9 samples (13.40%), for the age category 20-39 years, there were 24 samples (35.80%), for the age category 40-59 years, there were 24 samples (35.80%) and the age category >59 years obtained 10 samples (14.90%). Based on table 2, the average age is 39 years, with a minimum age of 5 years and a maximum age of 70 years.

For the location of the tumor, it was categorized into 2 location: supratentorial with 55 samples (82.10%) and infratentorial with 12 samples (17.90%).

The results of the examination using IDH1-R132H

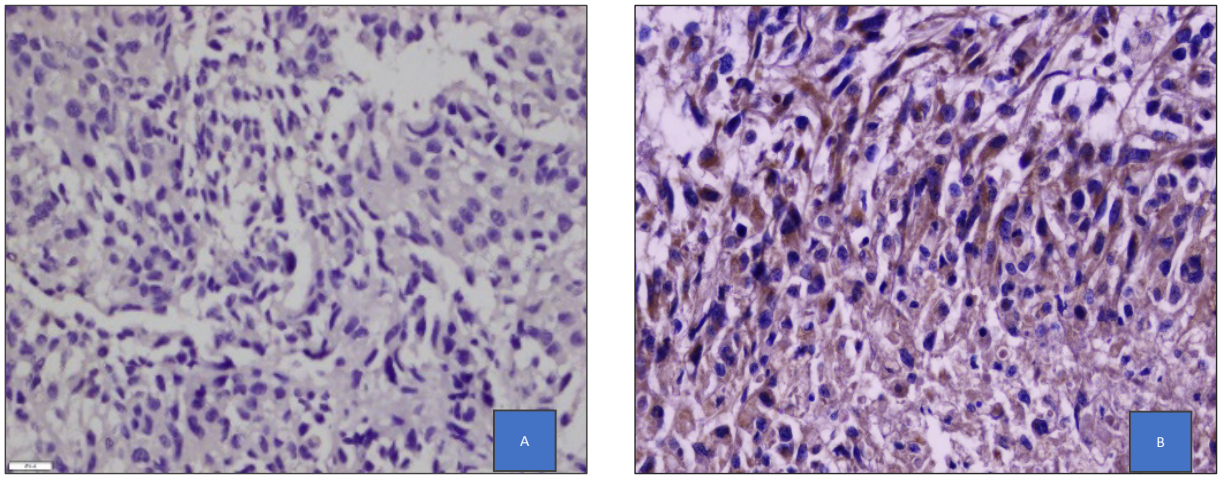

Figure 1. Expression of IDH1-R132H (Glioblastoma). A. Negative IDH1-R132H expression at 400x magnification. B. Expression of IDH1-R132H positively stained on the membrane and cytoplasm of tumor cells at 400x magnification.

Table 1. Sample Distribution based on Demographics and Clinical Characteristics

Characteristics	Total	Percentage
Diagnosis		
Diffuse Astrocytoma (DA)	22	32.8
Anaplastik Astrocytoma (AA)	15	22.4
Glioblastoma (GB)	30	44.8
Gender		
Male	40	59.7
Female	27	40.3
Age		
0-19 years old	9	13.4
20-39 years old	24	35.8
40-59 years old	24	35.8
> 59 years old	10	14.9
Location		
Supratentorial	55	82.1
Infratentorial	12	17.9
IDH1-R132H		
> 10% (positive)/ mutant	46	68.7
< 10% (negative)/ wild-type	21	31.3
CD133		
High expression	33	49.3
Low expression	34	50.7

immunohistochemical staining, obtained 46 samples (68.70%) expressing IDH1-R132H >10% (positive/ IDH-mutant) and 21 samples (31.20%) expressing IDH1-R132H <10 % (negative/ IDH-wildtype). On CD133 staining, 33 samples (49.30%) with high expression and 34 samples (50.70%) with low expression were obtained which were determined based on the median Hscore value of 90 (minimum value 0 and maximum value 270). IDH1-R132H and CD133 expressions in astrocytoma can be seen in Figure 1 and Figure 2 respectively.

From Table 2, the results of the correlation analysis test between age and astrocytoma diagnosis using the Chi-Square test show that p value = 0.847 (p>0.05) which means that there is no significant relationship between age and astrocytoma diagnosis. In the age category 0-19 years, from a total of 9 samples, there were 3 samples (33.3%) with a diagnosis of diffuse astrocytoma, 1 sample (11.1%) with a diagnosis of anaplastic astrocytoma and 5 samples (55.6%) with a diagnosis of glioblastoma. In the age category 20-39 years, from a total of 24 samples, there were 10 samples (41.7%) diagnosed as diffuse astrocytoma, 5 samples (20.8%) diagnosed as anaplastic astrocytoma and 9 samples (37.5%) diagnosed as glioblastoma. In the age category of 40-59 years, from a total of 24 samples, 6 samples (25.0%) were diagnosed with diffuse astrocytoma, 7 samples (29.2%) were diagnosed with anaplastic astrocytoma and 11 samples (45.8%) were diagnosed with glioblastoma. In the age category >59 years, from a total of 10 samples, 3 samples (30.0%) were diagnosed with diffuse astrocytoma, 2 samples (20.0%) were diagnosed with anaplastic astrocytoma and 5 samples (50.0%) were diagnosed with glioblastoma.

In Table 3, the results of the correlation analysis test between the diagnosis of astrocytoma and the location of

Table 2. Characteristics of Astrocytoma Diagnosis Based on Age

Age (years old)	Diagnosis			Total (%)	p-value
	DA (%)	AA (%)	GB (%)		
0-19	3 (33.3)	1 (11.1)	5 (55.6)	9 (100)	
20-39	10 (41.7)	5 (20.8)	9 (37.5)	24 (100)	0,847*
40-59	6 (25.0)	7 (29.2)	11 (45.8)	24 (100)	
>59	3 (30.0)	2 (20.0)	5 (50.0)	10 (100)	

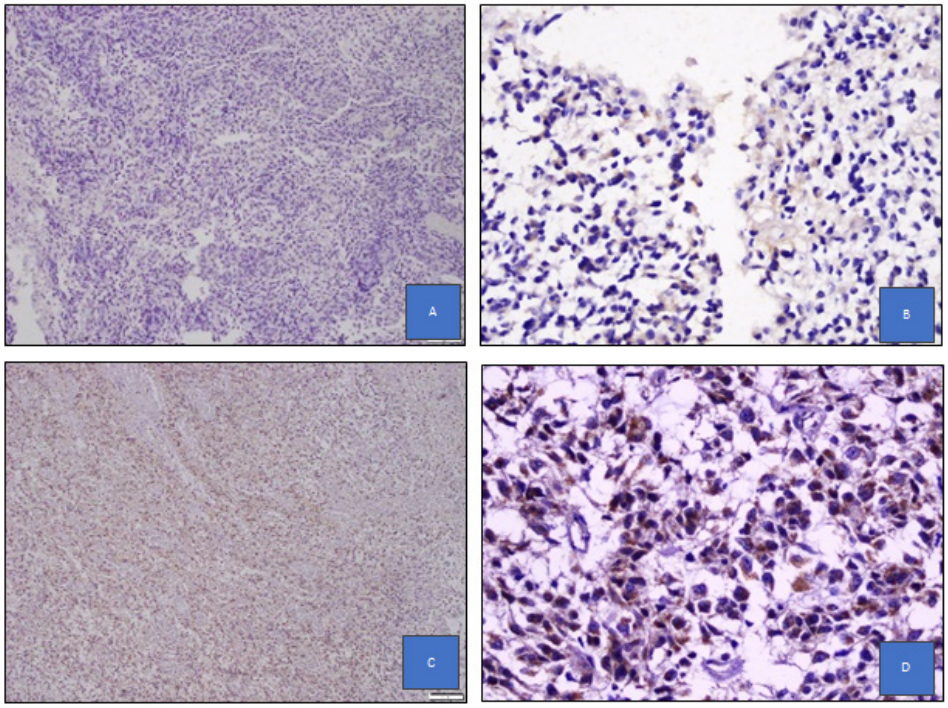

Figure 2. Intensity Expression of CD133 (Glioblastoma). A. Negative CD133 expression (100x). B. CD133 (+1) weak expression (400x). C. CD133 (+2) moderate expression (100x). D. CD133 (+3) strong expression (400x).

Table 3. Characteristics of Astrocytoma Diagnosis Based on Tumor Location

Diagnosis	Location (%)		Total (%)	p-value
	Supratentorial	Infratentorial		
DA	13 (59.1)	9 (40.9)	22 (100)	
AA	12 (80.0)	3 (20.0)	15 (100)	0.001*
GB	30 (100.0)	0 (0.0)	30 (100)	

the tumor using the Chi-Square test were obtained which showed p value = 0.001 ($p<0.05$), which means that there was a significant relationship between the diagnosis of astrocytoma and the location of the tumor. For tumor samples with a diagnosis of diffuse astrocytoma, the most commonly found in supratentorial locations were 13 samples (59.1%). Similarly, for tumor samples with a diagnosis of anaplastic astrocytoma, most of them were found in supratentorial locations (80.0%). For glioblastoma tumor samples, all samples were located

Table 4. Relationship between Astrocytoma Diagnosis and Expression of IDH1-R132H

		IDH1-R132H expression (%)			
		<10% (negative) IDH-wildtype	>10% (positive) IDH-mutant	Total (%)	p-value
Diagnosis	DA	12 (54.5)	10 (45.5)	22 (100)	
	AA	1 (6.7)	14 (93.3)	15 (100)	0.007*
	GB	8 (26.7)	22 (73.3)	30 (100)	
Total (%)		21 (31.3)	46 (68.7)	67 (100)	

Abbreviation: DA, Diffuse Astrocytoma; AA, Anaplastic Astrocytoma; GB, Glioblastoma; IDH, Isocitrate dehydrogenase; *Chi-Square Test

Table 5. Relationship between Astrocytoma Diagnosis and Expression of CD133

		CD133 expression (%)		Total (%)	p Value
		Low	High		
Diagnosis	DA	18 (81.8)	4 (18.2)	22 (100)	
	AA	8 (53.3)	7 (46,7)	15 (100)	<0.001*
	GB	8 (26.7)	22 (73,3)	30 (100)	

Abbreviation: DA, Diffuse Astrocytoma; AA, Anaplastic Astrocytoma; GB, Glioblastoma; *Chi-Square Test

Table 6. Relationship between IDH1-R132H and CD133 Expression

IDH1-R132H expression	CD133 expression (%)		Total (%)	p -value
	Low	High		
<10% (negative) IDH-wildtype	18 (85.7)	3 (14,3)	21(100)	<0.001*
>10% (positive) IDH-mutant	16 (34.8)	30 (65.2)	46 (100)	

Abbreviation: IDH, Isocitrate dehydrogenase; *Chi-Square Test

Table 7. Relationship between each Molecular Diagnosis and CD133 Expression

Molecular diagnosis	CD133 expression		Total	p Value
	Low	High		
DA IDH-wildtype	11	1	12	0.190*
DA IDH-mutant	7	3	10	
AA IDH-wildtype	1	0	1	0.333*
AA IDH-mutant	7	7	14	
GB IDH-wildtype	6	2	8	<0.001*
GB IDH-mutant	2	20	22	
Total	33	34	67	

Abbreviation: DA, Diffuse Astrocytoma; AA, Anaplastic Astrocytoma; GB, Glioblastoma; IDH, Isocitrate dehydrogenase; *Chi-Square Test

in supratentorial (100%) and no samples were located in Infratentorial.

Based on Table 4, the results of the correlation analysis test between the diagnosis of astrocytoma and the expression of IDH1-R132H using the Chi-Square test showed p value=0.007 ($p<0.05$), which means that there is a significant relationship between the diagnosis of astrocytoma and the molecular status of IDH1-R132H. Astrocytoma with negative IDH1-R132H expression was classified as IDH-wildtype while astrocytoma with positive IDH1-R132H expression was classified as IDH-mutant. The prevalence of astrocytoma with negative IDH1-R132H expression (IDH-wildtype) is lower by 21 samples (31.3%) compared to astrocytoma with positive IDH1-R132H expression (IDH-mutant) which is 46 samples (68.7%).

In the table 4, it can be seen that from the total 22 samples of diffuse astrocytoma, there were 12 samples (54.5%) with negative IDH1-132H expression (IDH-wildtype diffuse astrocytoma) and 10 samples (45.5%) with positive IDH1-132H expression (IDH-mutant diffuse astrocytoma). From a total of 15 samples of anaplastic astrocytoma, there was only 1 sample (6.7%) with a negative IDH1-132H expression (IDH-wildtype anaplastic astrocytoma) and 14 samples (93.3%) with a positive IDH1-132H expression (IDH-mutant anaplastic astrocytoma). For the diagnosis of Glioblastoma, more IDH-mutant samples were obtained when compared to IDH-wildtype samples. Of the total 30 samples of Glioblastoma, there were 8 samples (26.7%) with negative IDH1-132H expression (IDH-wildtype Glioblastoma) and 22 samples (73.3%) with positive IDH1-132H expression (IDH-mutant Glioblastoma).

In the Table 5, the results were obtained between the diagnosis of astrocytoma (histopathology) and the expression of CD133 using the Chi-Square test which showed p- value <0.001 ($p<0.05$), which means that there was a significant relationship between the diagnosis of astrocytoma and CD133 expression. Table 5 shows that from a total of 22 samples of Diffuse Astrocytoma, 18 samples (81.8%) showed a low CD133 expression and 4 samples (18.2%) showed a high CD133 expression. In the case of Anaplastic Astrocytoma, from a total of 15 samples, 8 samples (53.3%) showed low CD133 expression and 7 samples (46.7%) showed high CD133 expression. In Glioblastoma, from a total of 30 samples, 8 samples (26.7%) showed low CD133 expression and 22 samples (73.3%) showed high CD133 expression scores.

Based on table 6, the results of the analysis between IDH1-R132H expression and CD133 expression using the Chi-Square test showed a p value <0.001 ($p<0.05$), which means that there is a significant relationship between IDH1-R132H expression and CD133 expression. In the table, from 21 samples of astrocytoma with negative IDH1-R132H expression, 18 samples (85.7%) showed low CD133 expression and 3 samples (14.3%) showed high CD133 expression.While, from a total of 46 samples of astrocytoma with positive IDH1-R132H expression, 16 samples (34.8%) showed low CD133 expression and 30 samples (65.2%) showed high CD133 expression.

Statistically, the results of the analysis in Table 7 show that there is no significant relationship between the molecular diagnosis of Diffuse Astrocytoma IDH-wildtype and IDH-mutant with a CD133 expression. Similarly, the IDH-wildtype and IDH-mutant anaplastic astrocytoma group did not show a significant relationship with the CD133 expression. While, the IDH-wildtype and IDH-mutant glioblastoma groups actually showed a significant difference $p<0.001$ ($p<0.05$) with a CD133 expression. In table 7, it can be seen that from a total of 8 samples of IDH-wildtype glioblastoma, there were 6 samples with low CD133 expression and 2 samples with high CD133 expression. While, from a total of 22 IDH-mutant glioblastoma samples, there were 2 samples with low CD133 expression and 20 samples showing high CD133 expression.

Discussion

Astrocytoma is a primary brain tumor, derived from astrocyte cells with the most frequent and aggressive incidence with a poor prognosis. In Indonesia, apart from surgery, other therapies have not been developed. In addition, tumor cells remaining after surgery are generally resistant to conventional therapies such as radiotherapy and chemotherapy so that astrocytoma patients show high therapeutic recurrence. (Gargini et al., 2020)

In the recent developments have added several molecular characteristics to the classification of astrocytoma. Although astrocytoma is a very heterogeneous disease, this molecular classification of astrocytomas may reflect their biologic behaviour suggesting that tumors with similar expression profiles exhibit similar gene alterations and signaling pathways. The identification of mutations in isocitrate dehydrogenase 1/2 (IDH1/2), which suggests astrocytomas with a better prognosis regardless of the histopathological grade. One hypothesis is that mutated IDH1 converts α--ketoglutarate to 2-hydroxyglutarate, which in turn blocks various enzymes, thereby contributing to tumor development (Malueka et al., 2017).

Resistancy and recurrence of Astrocytoma are classic features associated with cancer stem cells, which constitute a minor population of cancer cells, are pluripotent, quiescent and self-renewing. This unrestricted proliferation maintains tumors and the low mitotic activity of CSCs protects them from treatment approaches directed against actively dividing cells. Thus, CSCs can survive treatment and cause relapse. (Biserova et al., 2021) The CD133+ subpopulation of GSC (Glioma Stem Cell) showed a more malignant behavior, the frequency of CD133+ expressing cells was shown to increase with increasing tumor grade, and the frequency was associated with tumor recurrence. Therefore, CD133+ plays an important role in GSC resistance to chemotherapy and radiotherapy. (De Almeida Sassi et al., 2012)

In table 1, the most samples we obtained were glioblastoma (43.10%) followed by diffuse astrocytoma (33.80%) and then anaplastic astrocytoma (23.10%). From this whole sample, the highest incidence is in male compared to female with a ratio of 1.5:1. These data are in line with several previous studies which found that the incidence of glioblastoma was highest compared to other types of astrocytoma and was most common in males. (Metellus et al., 2011; Omer et al., 2018; Rasmussen et al., 2017)

In the age category, we found the highest prevalence of astrocytoma was in the age group of 20-59 years and this result is in line with other studies. This shows that gliomagenesis occurs at a younger age in the 2nd to 5th decades with an average age of 39 years. (Omer et al., 2018) In the anaplastic astrocytoma and glioblastoma, the frequency increased with age and decreased over the age of 59 years (Table 2). This may be due to the low survival rate of astrocytoma patients. However, we did not find a statistically significant relationship between age and astrocytoma diagnosis.

Based on the tumor location (Table 3), in this study there was a significant relationship between astrocytoma diagnosis and tumor location. The most common location was supratentorial (81.50%) compared to infratentorial location (18.50%). This is in line with research conducted by Omer et al, (Omer et al., 2018) who said that this could be due to the supratentorial location containing the most glial cells. Neural stem cells (NSCs) can be found in several locations in the adult brain including the subventricular zone (SVZ), the dentate gyrus of the hippocampus, and the subcortical white matter. This SVZ is thought to be the origin for most of these cells and has been proposed as the origin of gliomas and other brain tumors. Louis et al (Louis D et al., 2016), also mentioned that Diffuse astrocytoma can occur throughout the CNS but is usually located supratentorially and has an intrinsic tendency to develop into anaplastic astrocytoma and eventually to glioblastoma.

In this study, we used a specific monoclonal antibody to detect IDH1-R132H mutations. Based on several previous studies, the accuracy of immunohistochemical staining and sequencing for IDH1 mutation detection ranged from 88% to 100%. Several studies have even shown that immunohistochemical staining is more sensitive in detecting IDH1 mutations than DNA sequencing, especially in tumor samples that are so small. However, several other studies have shown that DNA sequencing is a more sensitive test because the antibodies used can only detect the R132H mutation, while other mutations such as R132C, R132L, R132S, and R132G cannot be detected by immunohistochemical staining. (Ichimura, 2012; Loussouarn et al., 2012)

We found that IDH1-R132H was positively expressed (IDH-mutant) in 44 cases (67.70%) of astrocytomas. Our results are in line with studies conducted by Myung et al and Wagn et al. The important point of these results is that in this study and several other studies, more secondary glioblastoma samples were included in the study resulting in higher positive IDH1-R132H expression, while the primary glioblastoma samples included had lower rates. This explains the importance of differentiating secondary glioblastoma from primary glioblastoma and also confirms that this IDH1 expression pattern may indicate that secondary glioblastoma has a longer survival rate. There are also different results of other studies in America, Australia and Europe, where the expression of negative IDH1 (IDH-wildtype) is higher (about 90%) compared to positive IDH1 (IDH-mutant). These different results are caused by different epidemiology and polymorphisms or the different methods used for IDH1 mutation detection (Omer et al., 2018; Sriwidyani et al., 2020; Yao et al., 2018).

Table 4 shows that there is a significant relationship between the diagnosis of astrocytoma and the molecular status of IDH1-R132H. These results prove that IDH1-R132H plays a role in the occurrence and development of astrocytoma Huang et al., (2019). Loussouarn et al., (2012) revealed that IDH1 mutations give rise to new gene functions, decreasing the production of α α-KG and increasing the production of D-2-Hydroxyglutarate (D-2HG). D-2HG has a similar structure to α-KG, acts as an oncometabolic and competitively inhibits the activity

of various dioxygenase enzymes. In addition, this IDH1 mutation produces high levels of 2-hydroxyglutaric acid (2-HG), thereby inhibiting the differentiation of glioma stem cells. At the same time, IDH1 mutations can increase vascular endothelial growth factor (VEGF) which plays a role in the formation of the tumor microenvironment. In addition, IDH1 mutations can also induce HIF-1α to trigger glioma invasion. Ultimately, these changes will lead to the development and pathogenesis of astrocytoma. (Huang et al., 2019)

In Table 4 we can see that the frequency of astrocytomas with a positive IDH1-R132H molecular status (IDH-mutant) will be higher in line with the increase in tumor grade when compared to astrocytomas with negative IDH1-R132H molecular status (IDH-wildtype). IDH1 mutations were lowest in astrocytoma grade 2 which correlated with slow tumor growth and good survival. IDH1 mutations are highest in grade 4 gliomas or glioblastomas that correlate with rapid tumor growth and poor survival. In early 2008, Parsons et al found an association between astrocytoma and IDH mutations in exon sequencing. Parsons found the IDH1 gene in 1/5 of the tumor samples. Further studies have found that the IDH1-R132H mutation is the most frequent mutation in astrocytoma, whereas the IDH2-R172H gene also undergoes a similar mutation but the frequency of the mutation is relatively low. (Huang et al., 2019)

Table 5 shows a significant relationship between astrocytoma diagnosis and CD133 expression. Based on table 5, it can be seen that the higher the degree of astrocytoma, the higher the expression of the CD133 cancer stem cell marker. Recent reports have also demonstrated a rise in cd133 expression in other tumor entities, with CD133 expression increasing as the histopathological grade worsens (Ikram et al., 2021). These results are in line with the analytical studies conducted by (Rehfeld et al., 2021) which stated that diffuse astrocytic neoplasms (diffuse astrocytoma, anaplastic astrocytoma, and glioblastoma) had a percentage of CD133-positive cells that correlated with an increase in tumor grade. Thus high expression of CD133 is an aggressive astrocytic tumor marker and an important factor for tumor development. These data suggest that CD133 CSCs play a significant role in carcinogenesis.

An experimental study conducted by Brescia et al. showed that CD133 expression was strongly influenced by hypoxic conditions and was associated with changes in mitochondrial function. And this is in line with the results of our study which is shown in table 7, that Glioblastoma (WHO grade 4) has a significant relationship with an increase in CD133 expression. (Brescia et al., 2013)

The expression of IDH1-R132H with the CSC CD133 marker shown in Table 6 shows a significant relationship. This proves that IDH1-R132H can affect the presence of CSC CD133. In this study, the IDH1-R132H mutation will increase CD133 expression. Cell differentiation will be inhibited during tumor evolution, IDH1 mutations will continue to recur with increasing tumor grade and aggressiveness so that the fraction of differentiated tumor cells decreases while CSC proliferation and population increases. These results confirm the theory that IDH1 mutations are associated with an epigenetic defect caused by the IDH1-mutant oncometabolic 2HG formation. Epigenetic regulation of CSCs occurs through DNA methylation, changes in chromatin architecture caused by post-translational histone modifications or activation of polycomb group proteins as well as modified miRNA spectra. Abnormal DNA methylation involves the promoter region (CpG island) thereby affecting gene expression. Promoter hypermethylation can suppress tumor suppressor genes such as Tp53, whereas hypomethylation can activate oncogenes. Post-translational histone modifications involve methylation, acetylation, phosphorylation and other reactions that result in epigenetic changes close to the promoter or enhancer regions of the gene that then affect the transcription of the genes involved. Suva et al, reported that transcription factors were able to convert non-stem glioblastoma cells into GSCs. These four important transcription factors are SOX3, SALL2, OLIG2 and POU3F2. (Biserova et al., 2021)

The Wnt/β-catenin pathway also plays important functions in CSC self-renewal and differentiation. DNA methylation is associated with aberrant activation of the Wnt/β-catenin pathway through increased promoter methylation and silencing of various WNT inhibitors such as WIF-1, AXIN2, SFRP-1 and DKK1. EMT activation causes tumor cells to have CSC properties and tumor-inducing properties and loss of membrane protein E-cadherin are hallmarks of EMT. DNA methylation of the E-cadherin promoter has been shown to recruit HDACs, leading to histone deacetylation and transcription factor silencing. Likewise, the promoter CD44, CD133 and Musashi-1 exhibited a hypomethylated state that was associated with high expression of CSC markers in TNBC. (Kagara et al., 2012)

Extrinsically, GSCs are regulated by growth factors as well as cell-cell and extracellular matrix (ECM) interactions. GSC behavior is continuously influenced by external signals from the niche, including tumor stromal, immune, and neighboring non-stem cells. Such signals will trigger the intrinsic pathways described above and will thus regulate the function and properties of CSCs. Several of these extrinsic pathways are well described: signal transducer and transcription activator 3 (STAT3), a member of the STAT transcription factor family, important in Glioblastoma, tumorigenesis, central nervous system development, and embryonic stem cell (ESC) biology. STAT3 is activated by a wide variety of cytokines and growth factors. The STAT3 target gene regulates many cellular processes, including proliferation and apoptosis, and constitutive activation of STAT3 has been observed in many human cancers. Hypoxia is an important feature of the microenvironment in glioblastoma. Hypoxia supports GSC self-renewal and even increases the GSC pool. Malignant changes in neural stem cells and can be driven by replication stress. Non-stem cell dedifferentiation of glioblastoma cells reaches a dynamic equilibrium with GSCs, and can also develop (Brescia et al., 2013).

Different results were obtained by other researchers, as reported by Yao et al., (2018) through invitro study, where the results showed that the high expression of

GSCs in patients with IDH-mutant was significantly less than in patients with IDH-wildtype. A comprehensive examination of the cellular properties of in vitro transfected GSCs showed that overexpression of IDH1-R132H leads to decreased proliferation, increased differentiation, induces apoptosis and reduces the ability to migrate. At the molecular level, these data from Yao et al., (2018) suggest that the IDH1-R132H mutation triggers a reduction in Wnt/β-catenin signaling activity. A study conducted by Auffinger et al, 2014 of recurrent Glioblastoma samples that had been given repeated chemotherapy would increase the CSC population through an amplification process resulting in a phenotypic shift from non-stem cells to stem cells. These findings provide the first evidence that glioma cells exposed to a chemotherapeutic agent (Temozolamide) are continuously capable of interconverting between non-GSCs and GSCs, thereby replenishing the original tumor population, leading to a more infiltrative phenotype and increased chemoresistance (Auffinger et al., 2014; Yao et al., 2018).

Temozolamide is an alkylating agent that causes DNA methylation and then DNA damage and apoptosis. Because cancer cells have a lot of DNA for repair mechanisms so that they can quickly provide resistance to therapeutic agents. The repair enzyme in this glioblastoma is MGMT [O-(6)-methylguanine-DNA methyltransferase]. MGMT helps cancer cell survival by removing methyl groups in cancer cell DNA. The presence of mutations in IDH1 will cause methylation in the promoter region of the MGMT enzyme which results in enzyme silencing which then reduces DNA repair activity in glioma tumor cells. So the repair of this blocked DNA damage makes treatment with Temozolamide effective. The opposite mechanism occurs in the IDH-wildtype so that it can explain that astrocytoma without IDH mutations is difficult to treat (Bhavya et al., 2020).

In conclusion, there is a significant difference in the expression of IDH1-R132H in Diffuse Astrocytoma, Anaplastic Astrocytoma and Glioblastoma, where the proportion of IDH-mutant status is higher than IDH-wildtype and IDH1 mutation status has a significant relationship with increasing tumor grade.There is also a significant difference in the expression of cancer stem cell marker CD133 in Diffuse Astrocytoma, Anaplastic Astrocytoma and Glioblastoma, while higher tumor grade shows higher CD133 expression. There is a significant relationship between IDH1-R132H expression and CD133 in Diffuse Astrocytoma, Anaplastic Astrocytoma and Glioblastoma.

Author Contribution Statement

ODS, CK, and UAM were involved in the method's conceptualization and design; ODS, AZ, NKS and MHC were involved in data curation, analysis, and interpretation. CK and UAM gave it a thorough conceptual and editing evaluation; The final version of the essay was revised and approved by all authors.

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Study Approval

This work was permitted by the research committee of the Faculty of Medicine, Hasanuddin University.

Ethical approval

The Faculty of Medicine's Ethics Committee waived informed consent for this study (Protocol #UH21020104 – Registry No. 128/H4.6.4.5.31/PP36/2021).

Availability of Data

On reasonable request, the associated author will release the datasets used in this work.

Conflict of Interest

All authors state that they have no conflicting interests.

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