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Diagnostic Cocktail of Adult Type Diffuse Gliomas: Analysis of Atrx, Gfap and Olig2 Protein Expression

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Abstract

The development of molecular profiles has radically changed the diagnosis and classification of adult type diffuse gliomas and aims to improve the selection and administration of appropriate therapy. This study aims to determine the appropriate diagnostic algorithm in cases of adult type diffuse gliomas, especially to differentiate astrocytoma and oligodendroglioma. An analytical observational study for diagnostic development with a cross-sectional analityc study design was undertaken since May 2024 - May 2025 at the Anatomical Pathology Laboratory of Prof. Dr. dr. Mahar Mardjono Jakarta Hospital. Inferential analysis to analyze the differences in ATRX, GFAP, OLIG2 expression between astrocytoma and oligodendroglioma using the Fisher Exact Test. The diagnostic values were analyzed using the MedCalc calculator. All data were analyzed using SPSS version 25.0. This study found differences in ATRX expression (p = 0.000) and OLIG2 (p = 0.001) but no differences in GFAP (p = 0.878) between astrocytoma and oligodendroglioma patients. The diagnostic value (sensitivity, specificity and accuracy) of each diagnostic biomarker in differentiating the diagnosis of astrocytoma and oligodendroglioma were ATRX positive (100%, 70.3% and 78.9%); GFAP> 8.5 (89.2%, 13.33% and 67.31%); and OLIG2> 7.5 (86.7%, 43.2% and 55.8%). It can be concluded that in differentiating astrocytoma and oligodendroglioma diagnostics, ATRX (+) protein expression has the best sensitivity, specificity and accuracy compared to OLIG2 and GFAP.

Keywords: Elementary School, Learning Outcomes, Psychological Aspects, Science Learning Difficulties.

Introduction

Diffuse gliomas are primary brain tumors that grow infiltratively in the Central Nervous System (CNS) and are broadly categorized based on their cell of origin (1, 2). These neoplasms arise from glial cells and are characterized by their diffuse growth pattern, which makes complete surgical resection challenging and contributes to high rates of recurrence. The biological behavior of diffuse gliomas varies widely, ranging from relatively indolent low-grade tumors to highly aggressive high-grade malignancies. Their clinical presentation is often nonspecific, including seizu-res, headaches, cognitive decline and focal neurological deficits, depending on tumor location and size. The incidence of diffuse glioma varies from 1.9 to 9.6 per 100,000 people, depending on age, sex, ethnicity and geographic location (3, 4). Epidemiological data also indicate that incidence rates are generally higher in adults compared to children, with certain subtypes demonstrating age-specific predilections. Such variability underscores the importance of accurate diagnostic stratification to ensure appropriate management (5).

Over the past decade, the development of molecular profiling has radically changed the diagnosis, classification and management of various types of cancer, including primary brain tumors (6, 7). Advances in genomics, epigenomics and transcriptomics have provided deeper insights into the molecular heterogeneity of gliomas, enabling more precise tumor categorization beyond conventional histopathological assessment. The integration of molecular parameters into diagnostic criteria has improved reproducebility and reduced interobserver variability among neuropathologists. In the context of diffuse gliomas, molecular markers such as IDH mutation status and 1p/19q co-deletion have become central to classification systems. The importance of classifying diffuse gliomas in adults, especially in

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differentiating astrocytomas from oligodendrogliomas based on molecular alterations, the goal is to enhance the selection and delivery of suitable treatments, expand the available therapeutic options and predict patient prognosis (8, 9). This paradigm shift reflects a movement toward precision medicine, where therapeutic decisions are guided by tumor biology rather than morphology alone. Consequently, molecular stratification has direct implications for patient survival, treatment response and long-term disease monitoring (10).

A chromatin remodeler protein (ATRX) is recurrently mutated in grade 2/3 adult diffuse glioma IDH mutation (11). ATRX plays a crucial role in chromatin remodeling, telomere maintenance and genomic stability and its inactivation is associated with the alternative lengthening of telomeres phenotype. In the current WHO CNS5 framework, ATRX status serves primarily as a lineage-associated marker. ATRX loss supports astrocytic differentiation, while retained ATRX expression is generally seen in oligodendroglioma when accompanied by 1p/19q co-deletion. Thus, ATRX should not be interpreted as a standalone diagnostic marker but rather as a complementary tool within integrated molecular classification and if ATRX is negative and IDH mutations are present, the tumor is classified as an astrocytoma type 6. This integrative approach reflects the recognition that no single biomarker can fully capture the biological complexity of diffuse gliomas. Instead, a panel of molecular and immunohistochemical markers is necessary to achieve diagnostic accuracy. The interpretation of ATRX immunohistochemistry requires careful correlation with clinical, radiological and additional molecular findings to avoid misclassification (12).

Other IHC examinations that can be used are Glial Fibrillary Acidic Protein (GFAP) and Oligodendrocyte Lineage Transcription Factor 2 (OLIG2) (13, 14). GFAP is an intermediate filament protein expressed in astrocytes and is widely utilized as a marker of glial differentiation. The use of GFAP to determine glial cell differentiation, significantly higher GFAP expression is found in astrocytomas, when compared to normal brain tissue (14, 15). Elevated GFAP expression in astrocytomas reflects their astrocytic lineage and reactive gliosis-like features, although staining intensity may vary depending on tumor grade and cellular differentiation. Meanwhile, strong OLIG2 expression is found in all oligodendroglial components, although it can also be expressed by astrocyte cells with weak expression (16, 17). OLIG2 is a transcription factor critical for oligodendrocyte development and is frequently expressed in diffuse gliomas irrespective of subtype. However, differences in staining patterns and intensity may provide supportive information in distinguishing tumor lineages. The combined evaluation of GFAP and OLIG2 expression patterns can therefore contribute to refining histopathological interpretation, particularly in diagnostically challenging cases (18).

Previous investigations have predominantly examined markers reflecting distinct molecular or epigenetic pathways, such as p53 and H3K27me3 (19). While these markers have provided valuable insights into tumor biology and prognostic stratification, they do not directly address the comparative diagnostic utility of lineage-associated immunohistochemical markers in routine practice. Research directly comparing ATRX, GFAP and OLIG2 biomarkers in differentiating astrocytomas and oligodendrogliomas, especially as an integrated immunohistochemical panel, has not been widely performed. The limited availability of comprehensive comparative studies leaves a gap in understanding how these markers perform collectively rather than individually. In many pathology laboratories, particularly in resource-constrained settings, access to comprehensive molecular testing may be limited by infrastructure, technical expertise and cost considerations. Advanced molecular techniques such as next-generation sequencing and fluorescence in situ hybridization may not be routinely available in all institutions (20).

As most pathology laboratories in Indonesia routinely perform immunohistochemistry, there is a practical need for reliable IHC-based strategies to support tumor classification.

Immunohistochemistry offers a relatively accessible, cost-effective and technically feasible approach that can be implemented in diverse healthcare settings. The proposed diagnostic approach was therefore developed to provide a cost-conscious and accessible method for distinguishing astrocytoma from oligodendroglioma, which is clinically important given the

differences in therapeutic strategies and prognostic implications between these entities. Astrocytomas and oligodendrogliomas differ not only in molecular characteristics but also in responsiveness to chemotherapy and radiotherapy, as well as in overall survival outcomes. Accurate differentiation is thus essential for optimal patient management. Therefore, this study aims to provide a complementary diagnostic perspective by evaluating the integrated performance of these lineage-associated biomarkers. By assessing ATRX, GFAP and OLIG2 collectively within an immunohistochemical framework, this research seeks to strengthen diagnostic confidence and support evidence-based neuropathological practice.

Methodology

An observational analytical study for diagnostic development with a cross-sectional analytical study design was undertaken from May 2024 to May 2025 in the Anatomical Pathology Laboratory of Prof. Mahar Mardjono Hospital, Jakarta (approximate GPS coordinates' range: 7°30′N–8°10′N; 4°00′E–5°05′E). All paraffin blocks from astrocytoma and oligodendroglioma patients aged > 19 years, male gender, tumor location in the cerebrum and IDH 1/2 mutations were used as research samples. The restriction to male patients was implemented to maintain cohort homogeneity and reduce potential biological variability, allowing for a more controlled evaluation of biomarker performance. Similarly, the focus on cerebellar tumors was chosen to reduce anatomical variability. However, these limitations may affect the generalizability of the findings to female patients or gliomas located outside the cerebrum. While the study evaluates immunehistochemical markers, it should be noted that 1p/19q co-deletion analysis and next-generation sequencing (NGS) remain the established reference standards for molecular diagnosis of gliomas and our research focuses on the potential role of immunohistochemical markers as complementary tools.

Samples were re-evaluated on HE slides and diagnosed based on the latest classification based on the WHO 2021 CNS, then IHC staining was performed for ATRX, GFAP and OLIG2. This study received ethical approval from the Institutional Ethics Committee of Rumah Sakit Pusat Otak Nasional Prof. Dr. Mahar Mardjono, Jakarta (approval number: DP.04.03/D.XIII/9046/2024). As this research was conducted using archived formalin-fixed paraffin-embedded (FFPE) tissue blocks without direct patient involvement, the requirement for informed consent was waived by the ethics committee. All procedures were conducted in accordance with institutional guidelines and the principles of the Declaration of Helsinki. The minimum sample size was calculated based on the expected sensitivity of each biomarker derived from prior studies. For biomarkers without previously reported sensitivity, an expected value of 85% was assumed. The calculated minimum sample sizes were 32 for ATRX, 46 for GFAP, 49 for OLIG2 and 34 for IDH1R132H. The largest required sample size (49 cases) was adopted as the minimum overall sample size for the study and the final cohort met/exceeded this requirement. This approach ensured adequate precision for estimating the diagnostic performance of the primary biomarkers. The diagnostic performance of each biomarker was evaluated using MedCalc software. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated based on the reference standard classification of tumors with and without 1p/19q co-deletion. Receiver operating characteristic (ROC) curve analysis was performed to determine the optimal cut-off values for GFAP and OLIG2 expression. The area under the curve (AUC) and 95% confidence intervals were calculated to assess discriminatory performance. Statistical significance was set at $p < 0.05$. IHC staining of ATRX, GFAP and OLIG2 biomarkers used primary antibodies Rabbit polyclonal, clone N/A (Biocare, ACR3251A) with a dilution of 1:100 for ATRX; Mouse monoclonal, clone GA-5 (Biocare, CM 065 A, C) with a dilution of 1:100 for GFA; and Rabbit monoclonal, clone EP112 (Cell Marque, CMC 38731020) with a dilution of 1:50 – 1:200 for OLIG2. Secondary antibodies used Starr Trek Universal Link (Biocare Medical).

Brain tumor diagnostic samples were assessed based on morphological features into adult type diffuse glioma, non-otherwise specified (NOS) to determine the group of astrocytoma or oligodendroglioma tumor cells. The diagnosis of astrocytoma was confirmed by finding cell morphology such as elongated, irregular nuclei while the diagnosis of oligodendroglioma was

confirmed by finding cell morphology with round, uniform nuclei, cytoplasm forming a honey-comb/fried-egg image. All of these findings were obtained in histopathological preparations, HE smears, which were viewed under a light microscope with 100x magnification.

Inferential analysis to analyze differences in ATRX, GFAP and OLIG2 expression between astrocytoma and oligodendroglioma using the Fisher Exact Test. The diagnostic value was then analyzed using the MedCalc calculator.

Results

Sample Characteristics

Sample In this study, the mean age of adult type diffuse gliomas patients was 41. 12 years with a median of 40 years and an age range of 17 to 71 years. Patients with astrocytoma were found more (71.2%) than oligodendroglioma patients (28.2%) (21). There was no difference in age between astrocytoma and oligodendroglioma patients.

ATRX, GFAP and OLIG2 Protein Expression

Adult type diffuse gliomas patients with positive ATRX were found as many as ATRX negative. Patients with strong GFAP expression were found the most with a percentage of 94.2% with an average of 10.4 (range 4 - 12). Likewise, patients with strong OLIG2 expression were found as many as 65.4% with an average of 7.63 (range 3 - 13). Differences in ATRX expression were found between astrocytoma and oligodendroglioma patients (p = 0.000), all oligodendroglioma patients had positive ATRX while astrocytoma patients with positive ATRX were only found in 29.7%. This study also found differences in OLIG2 between astrocytoma and oligodendroglioma patients (p = 0.001). Oligodendroglioma patients had a significantly greater mean OLIG2 than astrocytoma patients. However, despite these differences, the cut-off analysis revealed that OLIG2's diagnostic performance was moderate, with an AUC of 0.771 and limited specificity. These findings suggest that while OLIG2 can differentiate between astrocytoma and oligodendroglioma to some extent, it lacks the diagnostic accuracy required for it to serve as a reliable standalone discriminator for molecular subtypes. Despite a higher mean GFAP expression in astrocytoma patients, no significant difference was observed between astrocytoma and oligodendroglioma patients (p = 0.878). The poor specificity and non-significant AUC of GFAP (AUC = 0.508) indicate its inadequacy as a discriminative marker. These findings suggest that GFAP alone lacks sufficient accuracy for distinguishing between astrocytomas and oligodendrogliomas.

Table 1: Expression of ATRX, GFAP and OLIG2 Proteins in Astrocytoma and Oligodendroglioma Patients (Analysis Using Fisher Exact Test and Pearson Chi-Square Test)

Biomarkers	Total (n = 52)	Astrocytoma (n = 37)	Oligodendroglioma (n = 15)	p-value
ATRX, n(%)				
Positive	26 (50.0)	15 (100)	11 (29.7)	0.000*a
Negative	26 (50.0)	0 (0)	26 (70.3)	
GFAP, n(%)				
Strong	34 (65.4)	20 (54.1)	14 (93.3)	0.026*b
Medium	16 (30.8)	15 (40.5)	1 (6.7)	
Weak	2 (3.8)	2 (5.4)	0 (0)	
GFAP				
Mean ± SD	11.40±1.57	11.49±1.33	11.20±2.11	0.878c
Median	12	12	12	
Min-Max	6-12	8-12	6-12	
OLIG2, n(%)				
Strong	50 (96.2)	37 (100)	13 (86.7)	0.079*a
Medium	2 (3.8)	0 (0)	2 (13.3)	
OLIG 2				
Mean ± SD	8.15±2.21	7.49±2.02	9.80±1.78	0.001*c
Median	9	9	9	
Min-Max	3-12	3-12	6-12	

Note: The p-value calculated using the Fisher Exact Test shows a significant difference in ATRX expression with a p-value of 0.000. Pearson Chi-Square Test was used for the analysis of GFAP expression, with a p-value of 0.026. The p-values for comparisons between astrocytoma and oligodendroglioma for GFAP and OLIG2 are also included. [a]Fisher Exact Test, *p < 0.005, [b]Pearson Chi Square Test, *p < 0.005, [c]Mann Whitney Test, *p < 0.005, Normally distributed data uses mean±SD, Non-normally distributed data uses median (min-max)

Table 1 presents the comparative expression of ATRX, GFAP and OLIG2 in astrocytoma (n = 37) and oligodendroglioma (n = 15) patients. A statistically significant difference was observed in ATRX expression between the two groups (p = 0.000), with ATRX loss exclusively identified in oligodendroglioma cases. GFAP expression demonstrated a significant categorical difference (p = 0.026), whereas OLIG2 showed a significant difference in quantitative expression levels (p = 0.001). These findings indicate distinct immunohistochemical expression patterns between astrocytoma and oligodendroglioma.

ROC Curve Analysis

Table 2 presents the cut-off points for OLIG2 and GFAP, which are used to differentiate between astrocytoma and oligodendroglioma. The cut-off point for OLIG2 is set at 0.75, with an AUC of 0.771, indicating good diagnostic performance in distinguishing between the two tumor types. The 95% confidence interval (CI) for OLIG2 ranges from 0.636 to 0.906, suggesting reliable estimation. The p-value for OLIG2 is 0.002, highlighting a statistically significant difference between the expression of OLIG2 in astrocytoma and oligodendroglioma.

Table 2: Cut-off Points of OLIG2 and GFAP

Biomarkers	Astrositoma vs Oligodendroglioma			
	Cut Off Points	AUC	IK95%	p-value
OLIG2	0.75	0.771	0.636-0.906	0.002
GFAP	0.85	0.508	0.330-0.687	0.928

In contrast, for GFAP, the cut-off point is 0.85, with a much lower AUC of 0.508, indicating poor diagnostic performance. The CI95% for GFAP ranges from 0.330 to 0.687, showing greater uncertainty in the results. The p-value for GFAP is 0.928, suggesting no significant difference in GFAP expression between astrocytoma and oligodendroglioma. Overall, OLIG2 shows better diagnostic performance compared to GFAP, making it more reliable for distinguishing between these two tumor types, while GFAP has significant limitations as a standalone biomarker.

Figure 1 demonstrates the diagnostic performance of safety protocol effectiveness and enforcement frequency using ROC curve analysis. Panel (A) shows that effectiveness of health and safety protocols achieved an AUC of 0.771 (95% CI: 0.636–0.906; p = 0.002) with a cut-off value of 0.75, indicating good discriminatory ability. In contrast, panel (B) presents an AUC of 0.508 (95% CI: 0.330–0.687; p = 0.928) with a cut-off value of 0.85, reflecting no significant discriminatory performance.

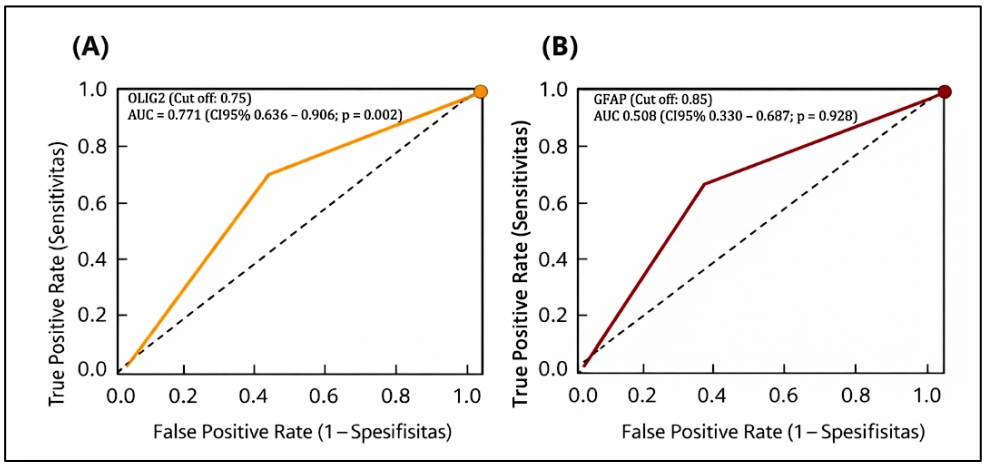

Figure 1: ROC Curve of Cut-Off Point: (A) OLIG2, (B) GFA

GFAP and OLIG2 Cutoff Points

The cutoff points of these two biomarkers were analyzed using ROC curves. The cutoff values of GFAP and OLIG2 with the best sensitivity and specificity were 8.5 and 7.5. The GFAP cutoff point had an AUC value of 0.508 (95% CI 0.330 – 0.687; p = 0.928), as shown in Table 2 and Figure 1A. While the OLIG2 cutoff point had an AUC value of 0.771 (95% CI 0.636 – 0.906; p = 0.002), as shown in as shown in Table 2 and Figure 1B.

Diagnostic Value of ATRX, GFAP and OLIG2 in Astrocytoma and Oligodendroglioma Diagnosis

ATRX protein expression (+) had a sensitivity of 100%, a specificity of 70.3% and good accuracy (78.9%) in differentiating the diagnosis of astrocytoma and oligodendroglioma. Meanwhile, GFAP protein expression > 8.5 has a sensitivity of 89.2%, specificity of 13.33% and good accuracy (67.31%) in differentiating the diagnosis of astrocytoma and oligodendroglioma. In addition, it was reported that OLIG2 protein expression > 7.5 had a sensitivity of 86.7%, specificity of 43.2% and moderate accuracy (55.8%) in differentiating the diagnosis of astrocytoma and oligodendroglioma, as described in Table 3.

Table 3 presents the cut-off points for the biomarkers OLIG2 and GFAP, used to differentiate astrocytoma from oligodendroglioma. For OLIG2, the cut-off point is set at 0.75, with an AUC of 0.771, indicating that it has good diagnostic performance. The 95% confidence interval (CI) for OLIG2 is between 0.636 and 0.906, showing that the diagnostic performance is reliable. The p-value of 0.002 indicates a statistically significant difference between the two tumor types. For GFAP, the cut-off point is 0.85, but its AUC is much lower at 0.508, showing weak diagnostic performance. The CI95% for GFAP ranges from 0.330 to 0.687, which indicates greater uncertainty in its diagnostic value. The p-value of 0.928 indicates no statistically significant difference between astrocytoma and oligodendroglioma based on GFAP expression.

Table 3: Cut-off Points of OLIG2 and GFAP

Biomarkers	Astrositoma vs Oligodendroglioma			
	Cut Off Points	AUC	IK95%	p- value
OLIG2	0.75	0.771	0.636-0.906	0.002
GFAP	0.85	0.508	0.330-0.687	0.928

Table 4: Diagnostic Value of ATRX, GFAP and OLIG2

Biomarkers	Astrocytoma vs Oligodendroglioma					
	Cut off	Sensitivity	Spesificity	PPV	NPV	Accuracy
ATRX (+)	-	100%	70.27	0.577	1.000	78.9%
GFAP	8.5	89.2%	13.3%	0.712	0.333	67.3%
OLIG2	7.5	86.7%	43.2%	0.382	0.889	55.8%

Table 4 provides the diagnostic values for ATRX, GFAP and OLIG2 in distinguishing astrocytoma from oligodendroglioma. For ATRX, the sensitivity is 100%, meaning it successfully identifies all oligodendroglioma cases, while its specificity is 70.27%, which indicates that it can exclude oligodendroglioma in about 70% of cases. PPV (positive predictive value) is 0.577, suggesting moderate reliability in positive test results, while NPV (negative predictive value) is 1.000, indicating a very high reliability in identifying true negatives. ATRX has an accuracy of 78.9%, showing good overall diagnostic performance. For GFAP, with a cut-off of 8.5, sensitivity is 89.2%, indicating that it detects most of the astrocytoma cases, but the specificity is very low at 13.3%, meaning it often misidentifies oligodendroglioma cases as astrocytomas. The PPV is 0.712, while the NPV is 0.333, reflecting that GFAP is not very reliable in terms of accurately excluding oligodendrogliomas. The overall accuracy is 67.3%, suggesting that GFAP has limited utility as a standalone diagnostic marker. Finally, OLIG2, with a cut-off point of 7.5, shows sensitivity of 86.7%, which is quite high, but its specificity of 43.2% is moderate, meaning it correctly identifies oligodendrogliomas only half the time. The PPV is 0.382, indicating that OLIG2 has moderate accuracy in predicting positive cases, while the NPV of 0.889 is high, showing that it is effective at excluding astrocytoma cases. The overall accuracy of OLIG2 is 55.8%, indicating its moderate performance in distinguishing the two tumor types.

Figure 2 illustrates the morphological and immunohistochemical differences between astrocytoma and oligodendroglioma at 100× magnification. On hematoxylin and eosin (H and E) staining, astrocytoma (Figure 2A) demonstrates diffusely infiltrating glial cells with irregular, elongated nuclei and a fibrillary background. In contrast, oligodendroglioma (Figure 2B) shows relatively uniform round nuclei with a clearer perinuclear halo and a more delicate microcystic stromal pattern.

Immunohistochemical staining for IDH1R132H reveals cytoplasmic positivity in tumor cells of

both astrocytoma (Figure 2C) and oligodendroglioma (Figure 2D), confirming mutant protein expression. ATRX staining demonstrates loss of nuclear expression in astrocytoma (Figure 2E), while retained nuclear expression is observed in oligodendroglioma (Figure 2F).

OLIG2 immunostaining shows strong nuclear expression in both tumor types, with diffuse nuclear positivity in astrocytoma (Figure 2G) and oligodendroglioma (Figure 2H). GFAP expression is more intense and diffuse in astrocytoma (Figure 2I), whereas oligodendroglioma (Figure 2J) exhibits comparatively weaker staining intensity. Overall, Figure 2 highlights the contrasting morphological features and differential immune-histochemical expression patterns of ATRX, GFAP and OLIG2 between astrocytoma and oligodendro-glioma.

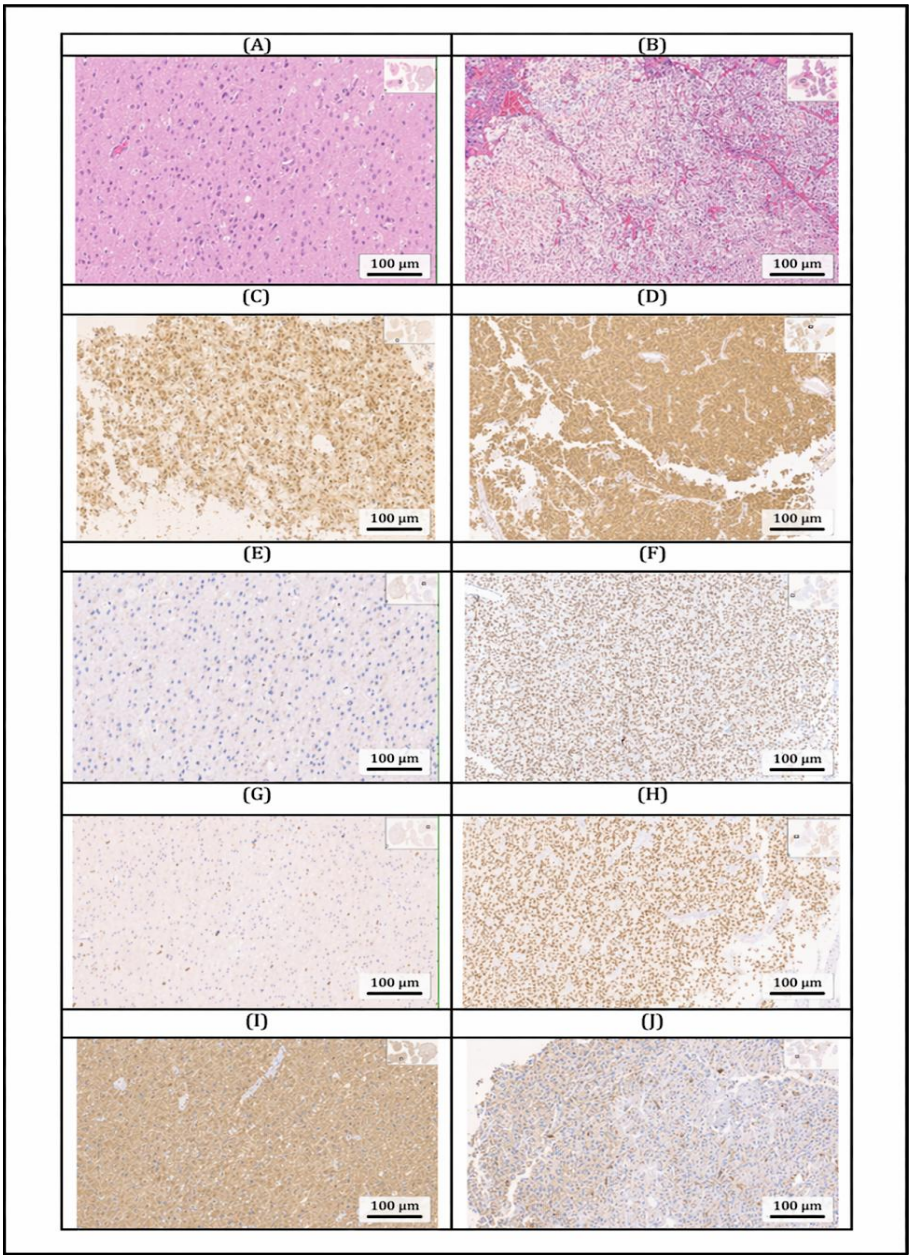

Figure 2: Morphological and Immunohistochemical Comparison Between Astrocytoma and Oligodendroglioma (100×) - HE Staining: (A) HE Astrocytoma, (B) HE Oligodendroglioma; IDH1R132H Expression: (C) IDH1R132H Astrocytoma (D) IDH1R132H Oligodendroglioma; ATRX Loss in Astrocytoma and Retention in Oligodendroglioma: (E) ATRX Astrocytoma, (F) ATRX Oligodendroglioma; OLIG2 Nuclear Expression: (G) OLIG2 Astrocytoma, (H) OLIG2 Oligodendroglioma; GFAP Expression: (I) GFAP Astrocytoma, (J) GFAP Oligodendroglioma.

Discussion

Adult type diffuse gliomas are one of the parts of gliomas, glioneuronal tumors and neuronal tumors based on CNS WHO 2021. This tumor is infiltrative, attacking normal brain parenchyma and is the most common tumor (22, 23). Despite advances in molecular diagnostics, the classification of adult-type diffuse gliomas remains challenging, particularly in settings where comprehensive molecular testing may not be readily available. The proposed diagnostic algorithm enhances existing methodologies by integrating immunohisto-chemical markers, such as ATRX, GFAP and OLIG2, into a streamlined, cost-effective approach for glioma subtype classification. This algorithm offers an accessible alternative to molecular testing, enabling more widespread and accurate diagnosis, especially in resource-limited environments, while maintaining alignment with established molecular classifications (24, 25).

The incidence of adult type diffuse gliomas increases with age. As many as 2.74% of individuals aged 20-34 years suffer from adult type diffuse gliomas and increase to 18.82% in individuals aged 75-84 years. 12 Men are 1.6 times more likely to be diagnosed with glioma than women (26). In this study, the average age was 41.12 years with a median of 40 years and an age range of 17 to 71 years (21). Research conducted by Wang *et al.*, in 2022 reported that patients with adult type diffuse gliomas had a median age of 45 years with a range of 22 to 81 years and the majority were <50 years old.14 Another study reported that the median age of adult-type diffuse glioma patients was 45 years with a range of 23 to 65 years (27).

In this study, it was found that the percentage of astrocytomas was higher than oligodendro-gliomas. This is no different from studies that also reported a higher percentage of astrocytomas compared to oligodendrogliomas. Astrocytoma has an average annual incidence of 1.21 per 100,000 while oligodendroglioma has a smaller annual incidence of 0.48 per 100,000 (28).

A chromatin remodeling protein (ATRX) is recurrently mutated in IDH mutation grade 2/3 adult type diffuse glioma. The results of IHK interpretation of ATRX staining are believed to be representative in determining 1p/19q codeletion, if the ATRX IHK result is positive and there is an IDH mutation, it can be ascertained that the tumor is classified as an oligodendroglioma type and if ATRX is negative and there is an IDH mutation, then the tumor is classified as an astrocytoma type (29).

While ATRX demonstrated strong diagnostic performance, combining ATRX with OLIG2 and GFAP may provide even greater diagnostic accuracy. The integration of these three biomar-kers into a single diagnostic panel could help mitigate the limitations of individual markers, such as GFAP's poor specificity. This coupled biomarker strategy could improve sensitivity and specificity, particularly in distinguishing astrocyto-mas from oligodendrogliomas and may serve as a more robust diagnostic tool, especially in resource-limited settings. Future studies should investigate the diagnostic efficacy of this combined approach to further validate its role in glioma classification. This finding aligns with existing literature suggesting that GFAP is expressed variably in both astrocytomas and oligodendrogliomas, thereby limiting its specificity (30). Consequently, GFAP should be interpreted as part of an integrated diagnostic approach, combined with other lineage-oriented markers, for accurate glioma subtype classification. ATRX protein expression has a sensitivity of 100%, meaning that all patients with ATRX (+) are diagnosed with oligodendroglioma. ATRX has a specificity of 70.3%, meaning that 70 out of 100 patients with ATRX (-) are not oligodendroglioma. ATRX (+) can diagnose oligodendroglioma by 100% and ATRX (-) can exclude oligodendroglioma by 70.3%. The accuracy of ATRX in this study was 78.9%, meaning that ATRX has good agreement compared to anatomical pathology results and can be considered as a diagnostic tool to differentiate astrocytoma and oligodendroglioma.

Conclusion

In conclusion, ATRX demonstrated the highest diagnostic performance among the evaluated immunohistochemical biomarkers, showing excellent sensitivity and acceptable specificity in differentiating astrocytomas from oligodendro-gliomas. Its integration into a diagnostic algorithm alongside GFAP and OLIG2 enhances the accuracy of glioma subtype classification, providing a prac-tical, cost-effective alternative to comprehensive molecular testing, particularly in resource-limited

settings. While ATRX alone shows strong discriminatory power, the combination of multiple biomarkers may mitigate individual limitations, offering a more robust approach for clinical diagnosis. However, this study is limited by its primary focus on subtype differentiation without stratifying by tumor grade, as well as the relatively small and heterogeneous sample, which may affect generalizability. Therefore, these results should be interpreted as supportive evidence for the complementary role of immunohistochemistry rather than a replacement for molecular classification. Future research involving larger, more homogeneous cohorts and incorporating additional molecular parameters is warranted to further validate the utility of combined immunohistochemical panels, explore potential grade-related variations and optimize diagnostic algorithms for adult-type diffuse gliomas. Overall, the findings highlight the important contribution of ATRX within an integrated immune-histochemical framework, reinforcing its value in the accurate and accessible classification of glioma subtypes.

Abbreviations

ATRX: Alpha Thalassemia/mental Retardation Syndrome X-linked, AUC: Area Under the Curve, CNS: Central Nervous System, GFAP: Glial Fibrillary Acidic Protein, HE: Hematoxylin and Eosin (staining method), IDH: Isocitrate Dehydrogenase, NPV: Negative Predictive Value, OLIG2: Oligodendrocyte Lineage Transcription Factor 2, PPV: Positive Predictive Value, ROC: Receiver Operating Characteristic.

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Author Contributions

Dwi Sri Rejeki: conceptualization, data curation, methodology, writing – original draft, I Ketut Sudiana: supervision, methodology, writing – review, editing, Gondo Mastutik: conceptualization, methodology, data analysis, writing – review & editing, Rita Cempaka: data collection, formal analysis, writing – review, editing, Indri Safitri Mukin: investigation, data curation, writing – review, editing.

Conflict of Interest

The authors declare no conflict of interest related to this study.

Data Availability

The data that support the findings of this study are available from the corresponding author.

Declaration of Generative AI and AI-Assisted Technologies in the Writing Process

The authors declare the authors declare that no generative AI or AI-assisted technologies were used in the writing process of this manuscript.

Ethics Approval

This study was conducted in accordance with ethical guidelines and all necessary approvals were obtained from the relevant ethical review board (Ref. number: DP.04.03/D.XIII/9046/2024).

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