RESEARCH ARTICLE

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Prognostic Value of Immunohistochemical Expression of MTAP and *AKIP1* in *IDH1* Mutant Astrocytoma

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Abstract

Background: Definite treatment for glioma is not exist, and with increased drug resistance, more effort should be paid to identify new prognostic biomarkers and molecular targets for therapy for glioma patients. **Aim:** The current study aimed to evaluate the immunohistochemical (IHC) expression of MTAP and A-Kinase Interacting Protein 1 (*AKIP1*) in astrocytoma and to investigate their association with the clinicopathological characters of these cases. **Methods:** Totally 66 cases of astrocytoma patients involved in this study. Cases underwent tumor resection and tissue sections were stained with MTAP, *AKIP1* and *IDH1* by IHC and evaluated in different grades of astrocytoma and their association with survival and response to therapy was investigated. **Results:** High *AKIP1* expression was positively correlated with treatment resistance and progressive disease. Positive IDH and retained MTAP expressions had shown better treatment response rather than negative IDH and lost MTAP. High AKIP, negative IDH and loss of MTAP expressions were significantly associated with poor survival outcome. **Conclusion:** Irrespective to grade and IDH status, the loss of MTAP immunoreactivity and high *AKIP1* expression are predictive factors in astrocytoma, and they may be used as a biomarker for guiding astrocytoma management and prognosis surveillance.

Keywords: MTAP- *AKIP1*- Isocitrate dehydrogenase1- astrocytoma- immunohistochemistry- survival

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Introduction

Glioma is the most common primary neoplasm of CNS worldwide. According to Yehia et al., in Egypt, gliomas account for 37.3% of primary CNS tumor. Among all its subtypes, astrocytoma is the most frequent glial tumors (Yehia et al., 2018). The new WHO classification of CNS tumor introduces major changes in form of integration of molecular characteristics in addition to histologic features in the classification and prediction of the outcome of astrocytoma patients (Louis et al., 2021).

Direct DNA sequencing is the major competitor to immunohistochemistry in diagnosis and risk stratification of tumors, but it is unavailable at most medical centers. Accurate molecular tests need sufficient tissues, that is unavailable in most cases as all the small biopsies placed immediately in formalin. Concordance between IHC and molecular testing of IDH is about 88% to 99% and some studies revealed that IHC detected mutations more than sequencing (Zouet al., 2015). That is why searching for immunohistochemical markers that replace molecular testes and can reflect the nature and prognosis of the disease will be valuable. According to the mutations in isocitrate dehydrogenase (IDH), astrocytoma is classified into 3 subtypes: IDH mutant, IDH wild type, and not otherwise specified. The IDH mutation subtype has the potential to progress to glioblastoma multiforme (GBM) (Nishikawa et al., 2022).

CDKN2A HD is one of the markers that is assessed using molecular procedures as genomic hybridization, or multiplex ligation-dependent probe amplification (Brat et al.,2020) and (Jeuken et al., 2006). The finding of Satomi et al., (2021), work supports that the loss of MTAP (5'-methylthioadenosine Phosphorylase) immunoreactivity represents an acceptable predictive surrogate for CDKN2A HD. Homozygous deletion of MTAP is frequently associated with many types of tumors due to loss of its tumor suppression function through its role in metabolism of polyamine and purines (Tang et al., 2018). A-kinase interacting protein 1 (*AKIP1*) contributes to the activation of p65 and NF-kappaB. It also has a role in the process of different diseases such as cancer and inflammatory diseases. *AKIP1* may have a role in the development, progression, and drug resistance of astrocytoma (Shen and Yao 2021).

The aim of this work is to evaluate the IHC expression

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of MTAP and *AKIP1* in astrocytoma and to investigate their association with the clinicopathological characters of these cases.

Materials and Methods

From January 2018 to January 2022, sixty-six formalin-fixed, paraffin-embedded tissue blocks of astrocytoma were gathered from the pathology departments' archives at the Faculty of Medicine at Zagazig University. The following cases are among those involved: There are 12 cases of grade 2 diffuse astrocytoma, 28 cases of anaplastic astrocytoma (G3), 26 cases of glioblastoma (G4). clinicopathological information was extracted from the hospital's medical records.

Age above 18 and under 80 at the time of surgery, a histologic diagnosis of primary low- or high-grade astrocytoma, and the availability and suitability of tumor tissues removed after surgery for immunohistochemistry (IHC) detection were all inclusion criteria. Grade 1 astrocytoma and cases with insufficient biopsy were excluded. Grading of astrocytoma was done based on the 2016 WHO classification (Louis et al., 2016). The Ethics Committee of Faculty of Medicine Zagazig university had approved this study by number (#9447) in accordance with Declaration of Helsinki.

According to feasibility, patients at the neurosurgery department underwent gross total resection or subtotal maximum safe resection or stereotactic biopsy. The patients were treated at Zagazig University's clinical and medical oncology departments. The patients received their treatment at clinical Oncology Department at Zagazig University, Radiotherapy (Rth) was delivered by three dimensional conformal radiotherapy in incomplete resected or surgically inaccessible, symptomatic low grade glioma (G2), dose 50 – 54 Gy/ 25-27 fractions. The patients with karnofsky scale (KPS) ≥ 60 diagnosed by WHO G3,4, standard radiotherapy was delivered 60 Gy/30 fractions with concurrent and adjuvant temozolomide (TMZ), while the patients with poor KPS (< 60), Rth was delivered in hypo fractionation schedule 40 Gy/15 fractions ± concurrent or adjuvant temozolomide. MRI brain with gadolinium-enhancement was requested from 2 – 8 weeks after Rth then every 3 – 6 months, other radiological evaluation performed to exclude the metastatic spread. Data was collected from patient's files. Hematoxylin and eosin-stained histological sections of tumors were reviewed by 3 expert pathologists.

Immunohistochemistry

Using the standard streptavidin–biotin labeling technique and the LSAB kit (Dako, Glostrup, Denmark) with appropriate positive and negative controls, immunohistochemistry was done. From the paraffin-embedded tumor tissue specimens, sections of 4mm were cut, then deparaffination. Using xylene, graded ethanol and microwave heating, rehydration and antigen retrieval for the sections were performed. After blocking the peroxidase activity, the sections were incubated with primary antibody.

1- MTAP rabbit monoclonal antibody EPR6893 (Abcam, Cambridge, UK, 1:1000).

2- *AKIP1* Polyclonal Antibody (Invitrogen, Waltham, 1:30).

3- *IDH1* R132H monoclonal antibody (DIA-H09, Dianova, Hamburg, dilution .1: 100).

The sections in the next day were incubated with Goat anti-Rabbit IgG (H+L). Secondary Antibody, HRP (1:10,000, Invitrogen, Waltham, MA) at room temperature for 1 hour. Finally, the staining and counterstaining of the sections were carried out using diaminobenzidine (Sigma-Aldrich, Louis, MO, USA) and hematoxylin (Sigma-Aldrich, Louis, MO).

Any nuclear and cytoplasmic positivity for *IDH1* was considered IHC-positive and for scoring cases divided into negative, no tumor cells stained; partly positive, presence of stained and not stained tumor cells; and completely positive, all identifiable tumor cells stained and for statistical evaluation positive cases were categorized as IDH mutant and negative cases as IDH wild type (Preusser et al., 2011).

MTAP immunohistochemistry identified as a cytoplasmic staining with or without nuclear staining in normal cells (as endothelial cells). The loss of immunoreactivity to MTAP is defined as absence of cytoplasmic staining with keeping the reactivity to the internal positive controls; tumors that showed cytoplasmic MTAP immunoreactivity were considered to retain MTAP staining (Chapel et al.,2020).

Cases that failed to stain positive for internal positive controls were considered uninterpretable.

The evaluation of *AKIP1* expression done depending on staining intensity and extent with score ranging from 0 to 12 and considered as low expression (IHC score 0–3), mild expression (IHC score 4–6), moderate expression (IHC score 7–9) and high expression (IHC score 10–12) (Shen and Yao, 2021).

Results

Clinicopathological finding and immunohistochemistry

Resume of clinicopathological features of involved cases represented in Table 1. Studying IDH status shows that there was a statistically significant association between wild type IDH and higher grade (P< 0.02). IDH mutation was noticed among cases with retained MTAP (P=0.04) and lower *AKIP1* expression (P=0.001). The IDH mutant astrocytoma has a better prognosis than wild type. There was a statistically significant increase in median of progression free survival (P=0.006) and overall survival (P=0.002) among mutant cases (Table 2, Figure 1).

According to MTAP expression, cases classified into retained 28 /66 (42.4%) and lost 38/66 (57.6%). There was a significant association between retained MTAP expression and high *AKIP1* expression (P< 0.001). It was found that median of progression free survival and overall free survival decreased among lost MTAP cases (P< 0.001). Loss MTAP is a strong predictor of short progression free survival and overall free survival (Table 2, Figure 2). Table 3, Figure 3, shows a significant association between *AKIP1* expression and histological grade of astrocytoma (p=0.01). Also, it was found that

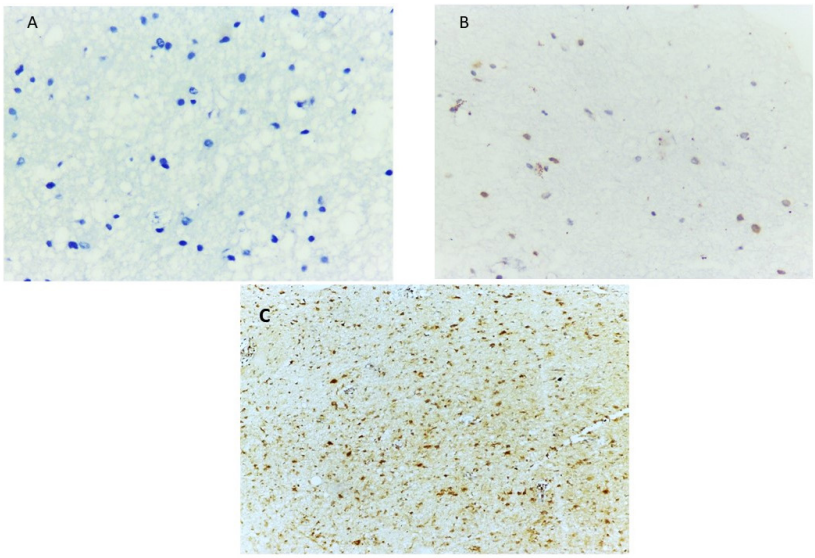

Figure 1. 1 *IDH1* Immunohistochemistry: a- a case of anaplastic astrocytoma all cells are not stained (x400). b- diffuse astrocytoma IDH stains scattered cells and most cells of the tumor are not labeled (x400). C. diffuse astrocytoma showing IDH positive immunostaining in all tumor cells immunostaining in all tumor cells (x200).

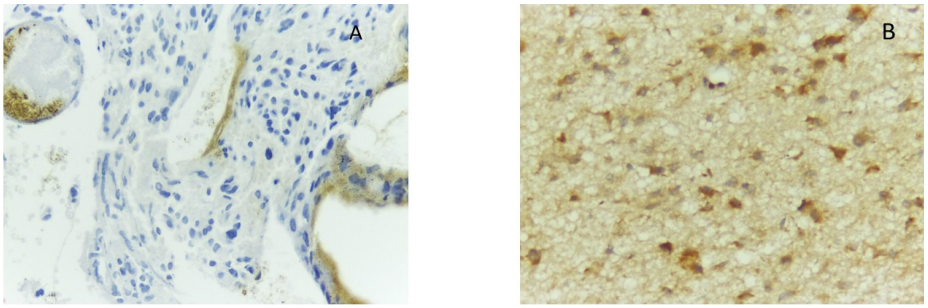

Figure 2. a. MTAP immunohistochemistry demonstrating the loss of cytoplasmic MTAP staining while the internal control is positively labelled. b. A case of IDH-mutant astrocytoma retaining cytoplasmic MTAP staining (x400).

the higher *AKIP1* expression was significantly associated with the progression of disease (P=0.001). Cases with low and mild expression of *AKIP1* had better overall survival.

2. Treatment outcome, survival, and progression analysis (Figure 4):

High *AKIP1* expression was positively correlated with

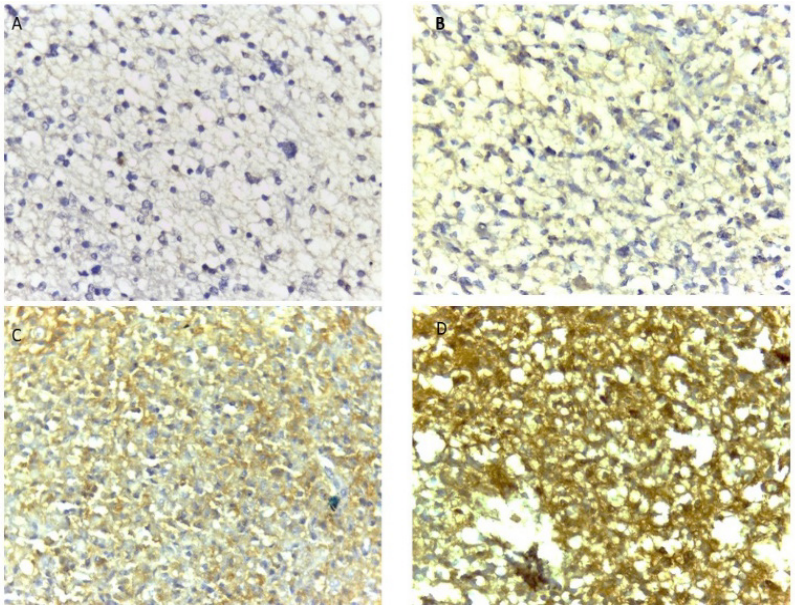

Figure 3. A-kinase Interacting Protein 1 (*AKIP1*) Expression in Glioma. The representative image of AKIP1 a. negative, b. mild expression, c. moderate and d. high expression in astrocytoma (x400).

Table 1. Demographic and Clinical Data of the Studied Cases

Variable		(n=66)	
		No	%
Age	<55	28	42.4
	≥55	38	57.6
Sex	Male	43	65.2
	Female	23	34.8
Tumor site	Frontal	31	47
	Temporal	19	28.8
	Parietal	16	24.2
Type of biopsy	Surgical resection	49	74.2
	Stereotactic	17	25.8
Size	≤ 3	38	57.6
	> 3	28	42.4
Histological type & WHO grading	Diffuse Astrocytoma (G2)	12	18.2
	Anaplastic Astrocytoma (G3)	28	42.4
	Glioblastoma (G4)	26	39.4
IDH mutation	Wild type	26	39.4
	Mutant	40	60.6
MTAP expression	Lost	38	57.6
	Retained	28	42.4
AKIP1expression	Low	12	18.2
	Mild	12	18.2
	Moderate	23	34.8
	High	19	28.8
Chemotherapy	No	12	18.2
	Yes	54	81.8
Radiotherapy	No	5	7.6
	Yes	61	92.4
Progression	No	14	21.2
	Yes	52	78.8
Out come	Live	24	36.4
	Died	42	63.6
Response	follow up (obseravation)	5	7.6
	CR	9	13.6
	PR	12	18.2
	SD	15	22.7
	PD	25	37.9

treatment resistance and a progressive disease, while low expressed patients had achieved a complete response, as well as patients with positive IDH and retained MTAP expressions had shown better treatment response rather than negative IDH, lost MTAP expressed patients.

MTAP and AKIP expressions are significantly associated with both OS and PFS independent of IDH status (Table 4).

Relation between progression free survival and overall survival based on IDH, MTAP and AKIP1 expression in relation to WHO grading among the studied cases represented in Table 5&6.

Discussion

Till now a definite treatment for astrocytoma has not existed. The increased incidence of drug resistance and restricted therapeutic options for astrocytoma requires more effort to identify prognostic biomarkers and new molecular targets for therapy to improve the long-term prognosis in astrocytoma patients.

The histopathological assessment of lower-grade astrocytoma in patients with IDH-mutation is particularly challenging, despite the revolution caused by the discovery of the IDH mutation. Homozygous deletions of the CDKN2A/B have a great prognostic value in IDH-mutant astrocytoma and incorporated in its updated classification. The adjacent location of *MTAP* gene to CDKN2A exposes it to frequent co-deleted (Marjon et al., 2016), explaining the good concordance between CDKN2A and the lack of MTAP staining. According to Satomi et al., (2021), MTAP immunohistochemistry was used as a proxy for CDKN2A homozygous deletion. Furthermore, loss of MTAP may increase the stemness of GBM and make it more susceptible to purine deprivation (Hansen et al., 2019). That is why we investigated the expression of MTAP in the studied group and evaluated its prognostic value irrespective to IDH status.

A-kinase interacting protein 1 (*AKIP1*), is one of the molecules that is suspected in initiation and progression of astrocytoma. It has a regulatory role in protein kinase A catalytic subunit and P65 interaction with subsequent activation of NF-kappaB cascade inducing resistance to treatement and astrocytoma stem cell differentiation (Liu et al., 2019) and (Feng et al., 2019). Silencing of *AKIP1* associated with suppression of the tumor cell progression, opening door for the trial of its implication in cancer treatment (Chen et al., 2022). In the current work, IDH mutation was detected in low grade astrocytoma more frequently than in high grade astrocytoma and represents a good prognostic factor independent on the tumor grade, similar finding was reported by previous studies of Hartmann et al., (2010). According to Le et al., (2020), IDH mutation was valuable molecular predictor of response to Temozolomide in GBM and, its mutation was positively correlated with the presence of O6-Methylguanine-DNA methyltransferase.

In agreement with previous reports of Von Deimling (Von Deimling et al., 2018), there is a poor PFS and OS in patients of wild IDH in comparison to mutated IDH patients. In this study, IDH mutation was significantly associated with MTAP retained expression (p =0.04), and wild IDH associated with *AKIP1* high expression (p= 0.001). As far as we know no previous work investigated this relation. The current work reported that MTAP expression is retained frequently in lower grade astrocytoma. On the other hand, loss of its expression observed in higher grade astrocytoma. These findings agree with Li et al., (2021), finding that concluded that copy number loss of MTAP, was found to be significantly enriched in GBM. Also, according to Becker et al., (2015) MTAP expression was still present in 85% of low grade pilocytic astrocytoma.

In terms of the relationship between MTAP expression and survival, our research showed that maintaining MTAP expression is associated with a favorable prognosis regardless of tumor grade and IDH status whereas losing

Table 2. Relation between IDH and MTAP Immunohistochemical Expression and the Clinicopathological Characteristics of the Studied Cases

Variable			IDH				P	MTAP				p
		No	Wild type (n=26)		Mutant (n=40)			-lost (n=38)		Retained (n=28)		
			No	%	No	%		No	%	No	%	
Age	<55	28	10	35.7	18	64.3	0.60 #	16	57.1	12	42.9	0.95 #
	≥55	38	16	42.1	22	57.9	NS	22	57.9	16	42.1	NS
Sex	Male	43	18	41.9	25	58.1	0.58#	27	62.8	16	37.2	0.24#
	Female	23	8	34.8	15	65.2	NS	11	47.8	12	52.2	NS
Size	≤ 3	38	13	34.2	25	65.8	0.32#	23	60.5	15	39.5	0.57#
	> 3	28	13	46.4	15	53.6	NS	15	53.6	13	46.4	NS
WHO grading	Diffuse Astrocytoma	12	1	8.3	11	91.7	0.0045#	4	33.3	8	66.7	
	Anaplastic Astrocytoma	28	9	32.1	19	67.9	**	17	60.7	11	39.3	0.16128#
	Glioblastoma	26	16	88.9	10	11.1		17	65.3	9	34.7	NS
Grade:	Low grade (G2)	12	1	8.30%	11	91.70%	0.02*	12	4	33.30%	8	0.104
	High grade (G3&4)	54	25	56.80%	29	43.20%		54	34	63%	20	NS
MTAP expression	Lost	38	19	50	19	50	0.04*#					
	Retained	28	7	25	21	75						
AKIP1 expression	Low	12	1	8.3	11	91.7		0	0	12	100	
	Mild	12	1	8.3	11	91.7	0.001*#	4	33.3	8	66.7	<0.001#
	Moderate	23	12	52.2	11	47.8		17	73.9	6	26.1	**
	High	19	12	63.2	7	36.8		17	89.5	2	10.5	
Progression	No	14	2	14.3	12	85.7	0.03*#	1	7.1	13	92.9	<0.001#
	Yes	52	24	46.2	28	53.8		37	71.2	15	28.8	**
Out come	Live	24	4	16.7	20	83.3	0.004*#	5	20.8	19	79.2	<0.001#
	Died	42	22	52.4	20	47.6		33	78.6	9	21.4	**
Response	CR	9	0	0	9	100		1	11.1	8	88.9	
	PR	12	4	33.3	8	66.7	0.04* #	3	25	9	75	<0.001#
	SD	15	8	53.3	7	46.7		9	60	6	40	**
	PD	25	12	48	13	52		25	100	0	0	
Progression free survival	Median (month)		16		24		0.006* $		12		30	<0.001 $**
Overall free survival	Median (month)		22		30		0.002* $		20		28	<0.001 $**

#, Chi square test ($\chi 2$); $, Log Rank (Mantel cox) test; NS, Non-significant (P>0.05); *, Significant (P<0.05); **, Highly significant (P<0.001)

MTAP expression is associated with a poor prognosis in different grades of astrocytoma. Additionally, Satomi et al., (2021), showed that MTAP deficiency was linked to a trend toward shorter PFS and a significantly shorter OS. According to Singh et al., (2021) MTAP reduction promotes temozolomide resistance. In agreement with the finding of Shen and Yao (2021) the current work revealed that *AKIP1* is positively associated with higher WHO grade in astrocytoma patients.

According to previous report (Shen and Yao, 2021) and in accordance with our observations, overall survival (OS) was lowest in patients with *AKIP1* higher expression, and greatest in patients with *AKIP1* low expression. Furthermore, high *AKIP1* expression was positively connected with treatment resistance and a progressing condition, whereas low expression associated with full responses. In other words, high *AKIP1* immunohistochemical expression is linked to decreased overall survival and its high level predicts poor survival regardless of the tumor's grade, with a statistically significant rise in median PFS and OS among cases with low and mild *AKIP1* expression compared to cases with moderate and high expression in all grades. This association is reasonable as Liu et al., (2020), found that the cytotoxicity of the chemotherapeutic agents can be blocked by the action of *AKIP1* that enhances the ability of astrocytoma stem renewal. Moreover, Zhang et al., (2018) found that knockdown of the gene expressing *AKIP1* inhibits cell cycle propagation, and arrest tumor growth as well as angiogenesis through deactivating of chemokines CXC motif ligand.

While Feng et al., (2019), reported that *AKIP1* high expression not only protects tumor cells against the cytotoxic effect of chemotherapy (temozolomide), but also enhances the self-renew capacity of astrocytoma cells. It also increases the tumor recurrence rate and the drug

Table 3. Relation between Clinicopathological Data of the Studied Cases and *AKIP1* Expression

Variable			Low		Mild		Moderate		High		P
		No	(n=12)		(n=12)		(n=23)		(n=19)		
			No	%	No	%	No	%	No	%	
Age	<55	28	6	21.4	7	25	8	28.6	7	25	0.51 #
	≥55	38	6	15.8	5	13.2	15	39.5	12	31.6	NS
Sex	Male	43	6	14	7	16.3	17	39.5	13	30.2	0.51#
	Female	23	6	26.1	5	21.7	6	26.1	6	26.1	NS
Size	≤ 3	38	8	21.1	6	15.8	12	31.6	12	31.6	0.75#
	> 3	28	4	14.3	6	21.4	11	39.3	7	25	NS
Histological type & WHO grading	(G2)	12	6	50	3	25	3	25	0	0	
	(G3)	28	5	17.9	7	25	7	25	9	32.1	0.0034*#
	(G4)	26	1	3.8	2	7.7	13	50	10	38.5	
Grade:	Low grade (G2)	12	6	50%	3	25%	3	25%	0	0%	0.001**
	High grade(G3&4)	54	6	11.10%	9	16.70%	20	37%	19	35.20%	
Progression	No	14	10	71.4	4	28.6	0	0	0	0	<0.001#
	Yes	52	2	3.8	8	15.4	23	44.2	19	36.5	**
Out come	Live	24	11	45.8	9	37.5	4	16.7	0	0	<0.001#
	Died	42	1	2.4	3	7.1	19	45.2	19	45.2	**
Response	CR	9	7	77.8	2	22.2	0	0	0	0	
	PR	12	2	16.7	4	33.3	6	50	0	0	<0.001#
	SD	15	0	0	3	20	10	66.7	2	13.3	**
	PD	25	0	0	1	4	7	28	17	68	
Progression free survival	Median (month)		35		30		18		10		<0.001 $**
Overall free survival	Median (month)		35		32.5		26		16		<0.001 $**

#, Chi square test ($\chi 2$); $,Log Rank (Mantel cox) test; NS, Non-significant (P>0.05); *, Significant (P<0.05); **, Highly significant (P<0.001)

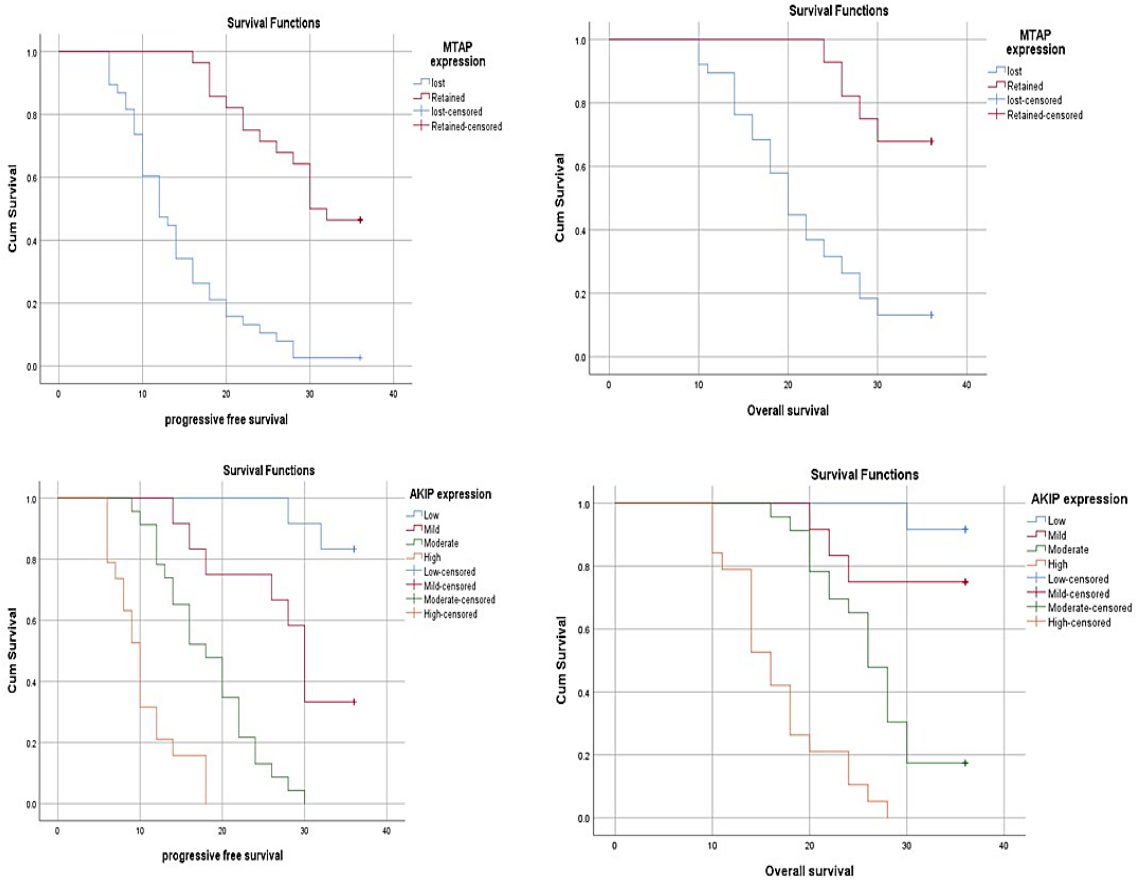

Figure 4. Kaplan Meier Plots, Left panel: progression free survival, right panel :overall survival stratified by *MTAP* and *AKIP* expression.

Table 4. Relation between Survival and AKIP and MTAP1 Expression according to IDH expression:

IDH	AKIP1	N	OS	P$	PFS	P$	MTAP expression	No	OS	P$	PFS	P$
Wild type	Low	1		0.004*		<0.001**	Lost	19	18	0.009*	18	0.009*
	Mild	1										
	Moderate	12					Retained	7	28		28	
	High	12										
Mutant	Low	11	35.5	<0.001**	35	<0.001**	Lost	19	20	<0.001	20	<0.001**
	Mild	11	32.2		30					**		
	Moderate	11	28		18		Retained	21	34		34	
	High	7	14		8							

OS, Overall Survival; PFS, Progressive Overall Survival; $, Log Rank (Mantel cox) test; NS, Non significant (P>0.05); *, Significant (P<0.05); **, Highly significant (P<0.001)

Table 5. Relation between Progression Free Survival and Overall Survival of IDH base and MTAP Expression According to WHO Grading among the Studied Cases

WHO Grade	IDH base	N	OS	P$	PFS	P$	MTAP expression	No	OS	P$	PFS	P$
G2	Negative	1	36	---	22	<0.001**	Lost	4	36	---	25	<0.001**
	Positive	11	36		34		Retained	8	36		36	
G3	Negative	9	22	0.003*	13	<0.001**	Lost	17	22	<0.001**	13	<0.001
	Positive	19	30		24		Retained	11	36		30	
G4	Negative	16	23	0.004*	16	0.85	Lost	17	16	<0.001**	10	<0.001**
	Positive	10	28		17	NS	Retained	9	28		21	

OS, Overall Survival; PFS, Progressive Overall Survival; $, Log Rank (Mantel cox) test; NS, Non significant (P>0.05); *, Significant (P<0.05); **, Highly significant (P<0.001)

Table 6. Relation between Progression Free Survival and Overall Survival of and *AKIP1* Expression According to WHO Grading among the Studied Cases

WHO Grade	*AKIP* expression	No	OS Mean	P$	PFS Mean	P$
G2	Low	6	36		36	
	Mild	3	36	----	36	0.01*
	Moderate	3	36		24	
G3	Low	5	36		36	
	Mild	7	36	<0.001	28	<0.001
	Moderate	7	28	**	14	**
	High	9	18		12	
G4	Low	1	30		28	
	Mild	3	28	0.001	25	<0.001
	Moderate	13	24	**	16	**
	High	10	12.5		8	

OS, Overall Survival; PFS, Progressive Overall Survival; $, Log Rank (Mantel cox) test; NS, Non significant (P>0.05); *, Significant (P<0.05); **, Highly significant (P<0.001)

resistance in astrocytoma patients, resulting in patients with *AKIP1* high expression having a worse prognosis compared to cases with low *AKIP1* expression.

Concerning its role in cancer progression, *AKIP1* plays an important role in epithelial mesenchymal transformation and metastasis through the carcinogenic process. It stimulates the stemness and increases the invasive capacity of malignant cells by different pathways as β-catenin and HIF-1α pathways (Luo et al., 2022).

To conclude, independent on *IDH1* status, *MTAP* and *AKIP1* are prognostic markers in astrocytoma that is why we recommend their evaluation in all cases of astrocytoma even low-grade cases.

Author Contribution Statement

All authors have read and agreed to the final version of this manuscript. Mona Mostafa Ahmed, Amira Elwan, Enas M Fouad, Hanim M Abdelnour and Asmaa Abdullatif developed the study conception and design. Mohammed A.Lateef Amira Elwan, Dalia Hamouda Elsayed, Hanim M Abdelnour and Asmaa Abdullatif performed acquisition of patient data, analysis and interpretation of patient data. Mona Mostafa Ahmed and Asmaa Abdullatif performed critical revision of the manuscript.

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The Ethics Committee of Faculty of Medicine Zagazig university had approved this study by number (#9447).

Availability of data

Available upon request from the corresponding author.

Competing interests

No competing interests.

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