MTA-1 Expression in Glial Tumors and İts Relation with the IDH1/2 Mutation and other Diagnostic and Prognostic Factors

Running head: MTA-1 expression in glial tumors

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ABSTRACT

Objective: There is limited data on the effect of Metastasis-associated proteins (MTA1) gene expression could predict prognosis or might be a therapeutic target or diagnostic marker in glial tumors. In this study, we aimed to identify MTA1 genetic expression in glial tumors and compare different levels of expression in terms of the types and histopathological, immunohistochemical, and clinical characteristics of the tumors.

Methods: MTA-1 gene expression levels were calculated from the tissue samples of 38 patients operated for glial tumors. The living tissue samples of ten patients operated with the diagnosis of mesial temporal lobe epilepsy were used as the control group. A correlation study was performed using the data on the length of the mass lesion, lobar location, and surrounding edema on preoperative magnetic resonance imaging and histopathological diagnoses, grades (according to WHO 2, 3, 4), Ki67, and isocitrate dehydrogenase enzyme mutation (IDH 1/2) status (+,-) on postoperatively obtained pathological specimens.

Results: In our cases of glial tumors, the mean tissue MTA-1 gene expression value (164.351) was found to be significantly higher than the control group values (1.000), (Z:-4.845, p<0.001). The expression level of tissue MTA-1 in patients with a positive IDH1/2 status (24.098) was significantly higher than that in patients with a negative IDH1/2 (271.360), (Z:-4.743, p<0.001).

Conclusion: Owing to the higher levels of MTA-1 expression in glial tumors and the presence of an inverse correlation with IDH1/2 mutation, it seems that this molecular biomarker has the potential to be a good

candidate for diagnostic studies, prediction of prognosis, and possible targeted immunological treatment strategies.

Key words: Metastasis-associated proteins; MTA-1; expression; glial tumors; IDH1/2 mutation
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INTRODUCTION

Glial tumors are among the most common brain tumors, and despite the optimized treatment protocols, no satisfactory results can be obtained in high-grade glial tumors [30]. This might be possibly a consequence of the limited knowledge of the nature of glial tumors [8]. Molecular studies in human tumors provide essential novel information on complex genetic, chromosomal, and epigenetic changes that accompany the formation of the gliomas [25]. These data made the World Health Organization design an integrated classification system based on tumor morphology and molecular changes in 2016 [19]. Considering the molecular and genetic discoveries within the last decade, this integration has become more important to improve the prognostic prediction and form the treatment [3, 25. There are various suggested molecular pathways in the pathophysiology of glial tumors [31].

Metastasis-associated proteins are a group of core regulatory proteins originating from three different genes (MTA-1, MTA-2, and MTA-3) [4]. Studies have shown that MTA-1 is one of the irregular oncogenes highly found in human cancers, and high expression levels of this gene is associated with aggressive tumor behavior, metastasis, and adverse clinical outcomes in cancer patients [35].

MTA-1 was first identified in animal and then in human malignant tissues [26, 32]. Studies have demonstrated that MTA-1 is expressed in many types of cancer [7, 20, 27, 37]. Moreover, it was also evaluated as a target molecule for therapeutic agents in some cancer types [33, 34].

In this study, we investigated the relationship of MTA-1 gene expression levels to clinical and other genetic factors in an initial attempt to evaluate that whether it could serve as a therapeutic target or could predict prognosis in glial tumors in the future.

MATERIALS AND METHODS

This study was planned prospectively and in accordance with the Helsinki Declaration with the permission of the Ethics Committee of our hospital (2018/204). The study population consisted of patients who were operated with the preliminary radiological and clinical diagnosis of glial tumors in our Neurosurgery Clinic between April 2018 and September 2019. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

Exclusion criteria were postoperative diagnosis of any other tumor than the glial tumor, those lost to clinical, pathological, or radiological follow-up, failure to obtain any specimen, the presence of MTA-1 receptor in the specimen for any reason, accompanying malignant diseases, renal dysfunction, hyperproliferative disease, severe psoriasis, and those who refused to participate in the study.

Thirty-eight patients were included in the study. The study consisted of two groups as Group 1 (Control) and Group 2 (Tumor). The control group was was formed from ten samples of living tissue of patients who underwent surgery for mesial temporal lobe epilepsy and did not fulfill the criteria for exclusion.

MTA-1 expression levels were evaluated in brain tissue samples obtained from patient and control groups. The demographic data of the patients were collected for age, gender, and smoking status. The preoperative radiological images were recorded based on the lobar location of the tumor (Figure 1) and the maximum length of the mass lesion measured in the sagittal coronal or axial plane (Figure 2). Edema levels surrounding the tumoral tissue were classified as minimal, smaller than the mass lesion size, close to the mass lesion size, and larger than the

mass lesion size (Figure 3). Histopathological diagnosis, grades (WHO-defined 2, 3, 4), Ki67, and isocitrate dehydrogenase enzyme mutation (IDH 1/2) (+,-) was recorded, as determined by a single-center pathology unit.
Forty-eight tissue samples of 38 glioma patients and 10 control subjects were stored in dry tubes in a -80 ºC refrigerator. Tissues were transferred to another institution genetic laboratory under appropriate conditions for the experimental study.

Demonstration of MTA1 gene expression in tissue samples

A three-step procedure was followed to demonstrate genetic expression of MTA-1 on 38 glioma samples obtained from patients with glial tumors and 10 normal brain tissues obtained from patients undergoing anterior temporal lobectomy: RNA isolation from tissue samples, synthesis of cDNA from RNA by reverse transcriptase, and finally a demonstration of genetic expression by real-time polymerase chain reaction (PCR).

RNA isolation from tissue samples

RNA isolation was performed from frozen tumor tissues using Trizol Reagent. 1 ml of TRIzol™ reagent was added to 50-100 mg of the tissue sample and the tissue samples were homogenized. The homogenized sample was incubated at room temperature for 5 minutes. The sample was transferred to a new 1.5 ml microcentrifuge tube. The sample was centrifuged at 12-16,000xg for 10 minutes to remove insoluble particles. The transparent supernatant was transferred to a new 1.5 ml microcentrifuge tube (RNase-free), onto which 200 µl chloroform was added. The microcentrifuge tube was shaken vigorously for 10 seconds. For phase separation, the sample was centrifuged at 12-16,000 x g for 15 minutes at 4°C. The upper aqueous phase was transferred to a new 1.5 ml microcentrifuge tube and proceeded to the RNA precipitation step. 1 volume of isopropanol was added to the aqueous phase and then the tube was mixed by inverting several times. The sample was incubated at room temperature for 10 minutes. The sample was centrifuged at 12-16,000 x g for 10 minutes at 4°C to form the RNA pellet. The supernatant was carefully removed and discarded. To wash the RNA pellet, 1 ml of 70% ethanol was added and vortexed briefly. The sample was centrifuged at 4ºC for 5 minutes at 12-16,000 x g, and the supernatant was removed with a pipette. The RNA pellet was dried at room temperature for 5-10 minutes. RNase-free water of 20-50 µl was added to re-suspend RNA pellet. It was incubated at 55-60°C for 10-15 minutes. Isolated RNAs were stored in the -80°C refrigerator.

RNA quantification

Absorbances of isolated RNAs were measured at 260 nm and 280 nm for the determination of their quantification and purity. For this purpose, 200 µl dH2O and 195 µl dH2O and corresponding RNA were added to quartz cuvettes for blank value and RNA measurements, respectively. Acquired absorbance values and purities were calculated through the equations: RNA quantity = A260 x 40 x 40 ng/µl, Purity = A260/A280.

cDNA synthesis (RT-PCR)

For cDNA synthesis, 1 µg of total RNA isolated was used. Briefly, 1 µg of total RNA, 1 µl of random primary and distilled water were mixed into a total volume of 10 µl; the mixture was incubated at 65°C for 5 minutes and then taken on the ice. To reach a final volume of 20 µl, 5X Reaction buffer, 0,5 µl RNAse inhibitor, 2 µl dNTP mix, and 2 µl of reverse transcriptase was added and placed to PCR device at 420C for 60 minutes and 700 C for 5 minutes.

Real-time polymerase chain reaction

After cDNA synthesis, PCR was performed under optimized conditions for MTA1 and human β-2-microglobulin. These studies were performed with Applied Biosystem 7500 Fast. 5 µl of Master mixture, 1 µl cDNA, 0.5 µl of forward and reverse primers at a concentration of 100 ng/µl, and 3 µl of distilled H2O were put to reach a

final volume of 10 µl; and each cDNA was studied at least three times with MTA-1 and human β-2-microglobulin genes. The latter gene was used for normalization.

Primers

The primers used in this study were MTA1 and human β-2-microglobulin primers. Human β-2-microglobulin was used as control. These primers were purchased from TaqMan (ThermoScientific).

Statistical analysis

Real-time PCR results were evaluated according to Livak method. Each of the Ct values obtained for MTA1 was subtracted from the mean of the human β-2-microglobulin gene and the obtained value was exponentiated to the power of two, which were divided by the control group value. Results were obtained in multiples of 1 and statistical evaluation using one-way ANOVA test was conducted with Minitab (Minitab, Ltd., UK). Study data were analyzed through SPSS 22.0 software (IBM Corp., San Diego, USA). Nonparametric tests were used considering the skewness-kurtosis values and the sample size. Mann Whitney U test was used for pairwise comparisons, Kruskal Wallis test for more than two groups, and Spearman correlation analysis for correlation analysis test. Statistical significance of the data was inferred by a level of $p<0.05$.

RESULTS

The demographic characteristics of 38 patients and 10 controls were summarized in Table 1. The mean gene expression level of MTA-1 in glial tumoral tissues (164.351) was significantly higher than that of the control tissues (1), (Z: -4.845, $p<0.001$), (Table 2).
We did not detect a statistically significant difference in the mean MTA-1 tissue genetic expression while we categorized the patients in terms of gender, smoking status, predominant lobar localization of the tumor, peritumoral edema level, or histopathological diagnosis of the tumor ($p>0.05$). The evaluation of IDH1/2 mutation status was performed in 31 patients. We detected that patients with IDH1/2 mutation had significantly diminished tumoral genetic expression of MTA-1 (24.098) compared to those without the IDH1/2 mutation (271.360), (Z:-4.743, $p=0.000$), (Table 3).

We did not find a significant correlation between MTA-1 tissue gene expression levels and patients' age, maximal tumor length, and ki67 values ($p>0.05$). The determination of ki67 levels was not possible in twelve patients (Table 4).

DISCUSSION

Our results showed that MTA-1 expression levels in glial tumors were significantly higher than the expression in normal parenchymal tissue. Moreover, analyses of tumor tissue revealed a negative correlation of MTA-1 gene expression to the presence of IDH1/2 gene mutation. However, no significant relationship was observed between MTA-1 expression levels and tumor size, tumor location, edema level, histopathological type, Ki67 level, and WHO grade classification as well as patients' age, gender, and other demographic parameters.

The survival of patients with glial tumors varies from several months to 20 years, depending on the WHO grade (I-IV) whereas WHO grade IV tumors are highly and rapidly mortal [2, 14]. WHO switched to a multilayered diagnostic method in 2016 and added molecular features as a final layer as a variable of histopathological diagnosis [13]. Studies showed that IDH, ATP-dependent helicase (ATRX), Telomerase Reverse Transcriptase (TERT), Capicua Transcriptional Repressor (CIC), and Far upstream element-binding protein-1 (FUBP1) were introduced as some of the possible biomarkers in terms of diagnostic, prognostic and targeted treatment options in glial tumors [2].

There are limited number of studies evaluating MTA-1 gene expression in glial tumors. Therefore, our study seems to be the first in the literature in terms of methods and patient groups. Our findings have suggested the presence of a significantly high MTA1 expression value in glial tumors, whereas expression levels of tissue samples

taken from temporal lobe parenchyma were significantly lower in the control group of patients with mesial temporal sclerosis.

The important role of MTA-1 in the regulation of transcription has been verified in recent years. The protein modulates stable protein states by regulating the addition of ubiquitin, triggering a DNA injury response [4]. MTA-1 belongs to NuRD (nucleosome repair and histone deacetylation) complex associated protein family (MTA-1, MTA-2, and MTA-3) of three different genes, and functions has through histone deacetylation and chromatin remodeling for transcriptional regulation [15, 22, 33]. Another critical role of MTA-1 was emphasized in the epithelial-mesenchymal transitions, DNA injury response, oncogenesis, transcription-induced and -dependent inflammation [18]. The MTA-1 gene was mapped at the center of 14q 31.2 and encodes a 715 amino acid protein with a molecular weight of 80.8 kDa [9, 22, 36]. As a vital regulator, MTA-1 has been found to be abnormally expressed in various types of human malignant tumors. The expression levels of MTA-1 have been associated with the spreading and metastasis potential of cancers [17]. It was reported to be associated with angiogenesis, invasion, and metastasis in many aggressive malignant tumors such as breast adenocarcinoma, endometrial adenocarcinomas, non-small cell lung cancer, and gastrointestinal cancer [1, 12, 23, 28]. These suggest MTA-1 protein as an important tool for the clinical diagnosis and treatment of various tumors [4]. An in vivo experimental study reported that resveratrol and its naturally occurring analog pterostilbene are potential inhibitors of MTA-1, and these agents could inhibit the growth and spread of prostate cancer [16].

To our knowledge, there is not a current study directly evaluating MTA-1 expression in glial tumors. The study by Chen et al. investigated MTA-1 expression in medulloblastoma cells, and reported increased gene expression as well as the diminished capacity of adhesion, migration, and invasion of the tumor cells while MTA-1 destruction was induced [4]. Another study by Wang et al. reported increased MTA-1 gene expression in pituitary adenomas and further stated a correlation between these tumors' invasion to adjacent bony tissues and their endocrine functions [34]. Cheng et al. in their study regarding glial tumors reported increased expression of MTA-2 gene, another gene of protein in MTA family [5]. On the other hand, Shan et al. reported decreased expression of MTA-3 in glial tumors compared to the normal tissues and a negative association with the glial tumor histology, WHO grade, and prognosis [29].

IDH genes normally encode isocitrate dehydrogenase which catalyzes isocitrate to alpha-ketoglutarate as part of the Krebs cycle [24]. IDH1/2 gene mutations are found in secondary glioblastoma multiforme (85%), primary glioblastoma multiforme (3-7%), and low-grade glial tumors (70%-80%) [6, 11]. Our findings showed an inverse correlation between the presence of an IDH1/2 mutation and MTA-1 gene expression while IDH1/2 (-) tumor tissues had higher MTA-1 genetic expression, and IDH1/2 (+) tumors had lower expression levels. The IDH1/2 mutation has been identified as an important prognostic and diagnostic biomarker in the 2016 WHO classification [13]. It has also been reported as a predictive factor in response to chemoradiotherapy in some glial tumors [11]. Also, the presence of an IDH1/2 mutation has been shown to be related with a relatively prolonged patient survival for glioma and glioblastoma in various studies, and in vitro studies with cancer cell cultures revealed an increase in the sensitivity of tumor cells to radiation and chemotherapy following a genetically induced synthetic or silencing of the wild-type IDH1/2 [11]. On the other hand, IDH1/2 mutations were suggested to be associated with the progression of glioma from grade II to grade III [6]. Several reports suggested a role for IDH1/2 mutation presence in down-regulation of leukocyte chemotaxis, hence repressed macrophages, microglia, monocytes, and neutrophils response to the tumor tissue [11].

This negative correlation between MTA-1 gene expression and IDH1/2 mutation suggests that increased MTA-1 gene expression could be associated with poor prognosis. However, we did not evaluate the prognosis of the patients in the context of this study. Thus, prognostic studies regarding the disease-free and overall survival durations of the patients are rquired for further conclusion.

CONCLUSION

In conclusion, we could not detect a positive evidence that the MTA-1 gene expression level could be used for histopathological diagnosis and WHO grading of glial tumors. Nevertheless, this molecular biomarker might be a good candidate in the diagnostic workup, prognostic determination, and possible targeted therapies due to the high levels of MTA 1 in glial tumors compared to normal brain tissue and its negative correlation with the presence of IDH1/2 mutation. The outcomes of this study need to be further verified by more detailed studies with larger sample sizes and subsequent measures of MTA-1 protein levels during the course of the disease.

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Table 1: The characteristics of the patient and the control groups.

n=48	**n**	**%**
Group		
Patient	38	79.2
Control	10	20.8
Patient group		
Female	18	47.4
Male	20	52.6
Control group		
Female	5	50
Male	5	50
PATIENT GROUP		
Localization		
Frontal	13	34.2
Occipital	2	5.3
Parietal	7	18.4
Temporal	16	42.1
IDH1 mutation status		
Absent	15	48.4
Present	16	51.6
Smoking status		
No	27	71
Yes	11	29
Grade		
2	14	36.8
3	1	2.6
4	23	60.5
Pathological grade		
Low grade astrositoma	8	21.1
Oligodendroglioma	7	18.4
GBM	23	60.5
Level of edema		
Regional minimal	12	21.6
Smaller than the mass lesion size	13	34.2
Close to the mass lesion size	11	28.9
Larger than the mass lesion size	2	5.3

Table 2. Comparison of patient and control groups in terms of MTA1 tissue genetic expression levels

N=48				
		Mean ± SD	Range	Z* P-value
MTA-1 expression	N			
Patients	38	164.351±167.269	3.53-653.03	-4.845 **<0.001**
Control group	10	1±0	1-1	
Total	48	130.319±162.851	1-653.03	
*Z: Mann Whitney U test value				

Table 3. Comparison of MTA1 tissue parameter levels by patients' baseline and clinical characteristics

N=58	**MTA1 expression**		
	Mean ± SD	**Range**	**X^2 or Z P-value**
Sex			
Female	152.012±161.938	3.96-653.03	-0.269 0.788
Male	175.456±175.35	3.53-523.24	
Smoking			
Yes	136.428±155.231	3.53-653.03	-1.207 0.227
None	225.863±191.956	5.23-523.24	
IDH1/2 mutation			
No	271.360±157.090	112.43-653.03	-4.743 **0.000**
Yes	24.098±24.780	3.53-85.00	
Localization			
Frontal	119.654±143.563	3.53-375.42	2.585 0.460
Occipital	206.01±-	206.1-206.1	
Parietal	113.27±70.118	45.4-242.58	
Temporal	217.326±210.568	3.96-653.03	
Tumor histology			
Low grade astrocytoma	110.935±182.515	3.96-458.49	5.435 0.066
Oligodendroglioma	255.625±150.395	113.46-523.24	
Glioblastoma multiforme	155.151±162.821	3.53-653.03	
Edema degree			
Surrounding minimal	197.338±177.247	3.96-458.49	2.449 0.485
Smaller than mass lesion size	133.63±149.853	3.53-523.24	

Near mass lesion size	200.096±190.536	12.21-653.03	
Larger than mass lesion size	33.23±39.597	5.23-61.23	
Kruskal-Wallis or Mann Whitney U tests were used for comparing multiple or two groups, respectively (X^2 or Z).			

Table 4. Correlation analysis of MTA1-tissue genetic expression to patient's age, maximal tumoral length, and ki67 values.

		Age	Maximal tumor length	Ki67 level	MTA-1 expression
Age	r	1.000			
	p	.			
	N	48			
Maximal tumor length	r	.019	1.000		
	p	.909	.		
	N	38	38		
ki67	r	.011	-.083	1.000	
	p	.957	.687	.	
	N	26	26	26	
MTA-1 expression	r	.147	.308	-.317	1.000
	p	.317	.060	.115	.
	N	48	38	26	48
r: Spearman correlation coefficient					

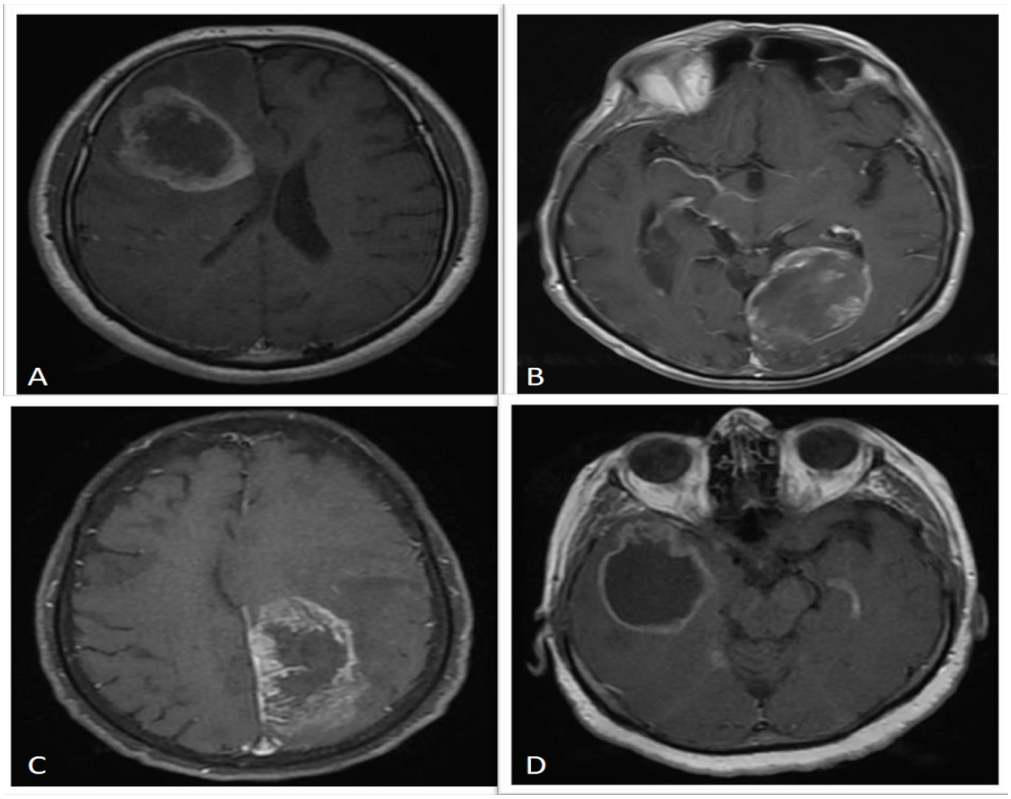

Figure 1. Classification of tumor by its predominant lobar localization. Predominant lobar localization by combined evaluation of T2, T1, contrasted T1, and flair sequences in contrasted cranial magnetic resonance imaging of patients was classified as 1a, frontal; 1b, occipital; 1c, parietal; or 1d, temporal.

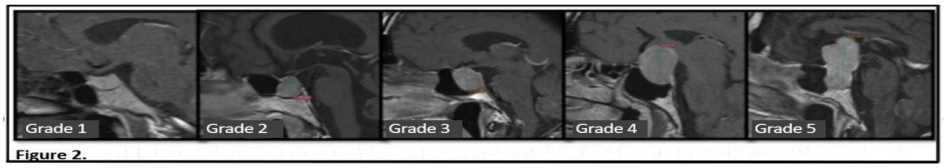

Figure 2. Figure 2. Measurement of maximal tumor length. Maximal length of tumor was measured in coronal, sagittal, and axial planes by combined evaluation of T2, T1, contrasted T1, and flair sequences in contrasted cranial magnetic resonance imaging of patients.

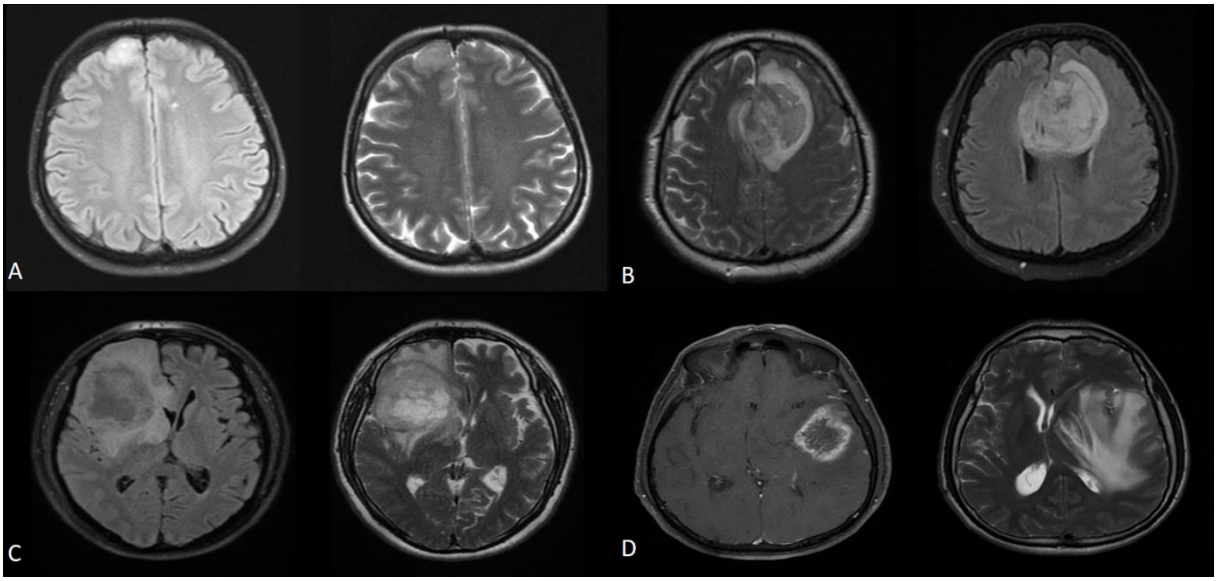

Figure 3. Classification of edema level surrounding the tumor tissue. Edema pattern surrounding the solid mass regardless of a definite measuring or volume system by combined evaluation of T2, T1, contrasted T1, and flair sequences in contrasted cranial magnetic resonance imaging of patients was classified as 3a, surrounding minimal; 3b, smaller than mass lesion size; 3c, near mass lesion size; or 3d, larger than mass lesion size.

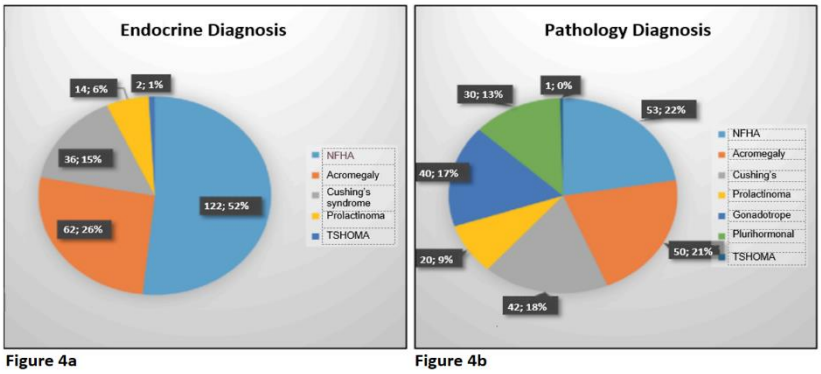

Figure 4. Endocrine and Pathology Diagnosis