



Expression of Anaplastic Lymphoma Kinase in Astrocytic Tumors (Histopathological and Immunohistochemical Study)

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Abstract

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BACKGROUND: Astrocytic tumors are the most common primary brain tumors. Glioblastoma is the most common astrocytic tumor representing the highest World Health Organization (WHO) grade (WHO grade IV) with poor prognosis and short survival time. Anaplastic lymphoma kinase (ALK) has a role in embryonic central nervous system development. ALK receptor is thought to contribute to nervous system function, repair, and metabolic homeostasis and is expressed in high-grade tumors like anaplastic large cell lymphoma that makes it a potential target for therapeutic intervention.

AIM: This work aimed to examine the immunohistochemical expression of ALK in astrocytic tumors and its correlation with age, sex, clinical presentation, location, laterality, recurrence, and WHO grade to implicate possible therapeutic potential.

METHODS: This retrospective study was conducted on sixty cases of archived, formalin-fixed, paraffin-embedded tissue blocks that included different subtypes and grades of astrocytic tumors. Immunohistochemistry using ALK monoclonal antibody was performed using a standard avidin-biotin-peroxidase system.

RESULTS: Of the sixty cases, 57 (95%) cases were negative for ALK, while three (5%) cases are positive for ALK; all showed the strong intensity of expression. No statistically significant association was found between ALK expression and astrocytic tumors in addition to other clinical variables of the studied tumors.

CONCLUSIONS: Most cases of astrocytic tumors showed negative ALK expression apart from three positive cases seen in higher WHO grades, especially gliosarcoma. The high number of negative cases for ALK in our study group suggests that ALK expression is not associated with a prognostic significance toward astrocytic tumors whatever its grade.

Introduction

Astrocytic tumors are the most common primary tumor of the brain [1]. The World Health Organization (WHO) classification system divides them into four grades according to their biological behavior; the least aggressive are WHO grade I tumors and the most aggressive represent WHO grade IV tumors [2]. The recent update in WHO classification of astrocytic tumors was based on incorporating molecular parameters besides histopathologic examination [3]. Glioblastoma (GBM) is the most lethal and most common primary malignant brain tumor [4]. The exact cause of astrocytic tumors was unknown, but cell phone use was a recent potential risk factor for the development of many gliomas [5].

Astrocytic tumors are more common with increasing age, male gender and white race [6]. Low-grade astrocytic tumors account for 30% of all astrocytic tumors [7]. High-grade astrocytic tumors are very common among the elderly [8]. Astrocytic tumors were the most commonly diagnosed central nervous system (CNS) tumor in the Delta region in Egypt followed by

meningioma [9]. In Egypt, astrocytomas account for about 54.85% of CNS tumors diagnosed at NCI, Cairo University during the period 2000–2011 [10].

The WHO scheme is based on the appearance of certain characteristics: atypia, cellularity, mitoses, endothelial proliferation, and necrosis. These features reflect the malignant potential of the tumor in terms of invasion and growth rate. Astrocytic tumors have four WHO grades as follow: Astrocytic tumors without any of these features are classified as WHO grade I. This includes pilocytic astrocytoma and subependymal giant cell astrocytoma. Astrocytic tumors with cytological atypia alone are considered WHO grade II. This will include diffuse astrocytoma, pleomorphic xanthoastrocytoma, and pilomyxoid astrocytoma. Astrocytic tumors with anaplasia and high mitotic activity in addition to cytological atypia are considered WHO grade III. This will include anaplastic astrocytomas and anaplastic pleomorphic xanthoastrocytoma. Astrocytic tumors exhibit marked anaplasia, high mitotic activity, atypical mitoses as well as microvascular proliferation and/or necrosis are considered WHO grade IV, representing the most aggressive type, GBM [2]. 2016 WHO update contains numerous differences from the 2007 CNS

WHO classification. The current update breaks with the century-old principle of diagnosis based entirely on microscopy by incorporating molecular parameters into the classification of CNS tumor entities [11].

The biopsy is the gold standard in the diagnosis of astrocytic tumors [12]. Treatment modalities include surgery, followed by observation, and chemotherapy or radiotherapy, depending on histological characteristics and extent of tumor resection [13], [14]. Therapeutic resistance is due to poor drug delivery [15]. The majority of astrocytic tumors will recur after surgery [16] due to single tumor cell infiltration leading to incomplete surgical removal and high recurrence rate [17].

The prognosis for astrocytic tumors is highly dependent on their WHO grade, being extremely poor for WHO grade IV tumor while there is long-term survival for WHO grade I tumor [18] and less than 1 year for patients with GBM [19]. Age is a significant prognostic factor for low-grade astrocytoma as survival benefits may be confined to those under age 50 years [20].

Anaplastic lymphoma kinase (ALK) gene is located at the short arm of chromosome 2 that encodes ALK receptor tyrosine kinase [21]. ALK plays an important role in cell proliferation and cell differentiation [22]. Many translocations involving ALK gene have been seen in cases of anaplastic large cell lymphoma [23]. In cases of non-small-cell lung cancer, there is a fusion of ALK gene to *EML4* gene [24].

Activated ALK causes increased VEGF secretion in anaplastic large-cell lymphoma [25]. Recent report describes an alternative transcription initiation site leading to the detection of an oncogenic ALK isoform in 11% of melanomas [26]. Mucosal melanoma which expresses a novel *EML4-ALK* fusion is highly sensitive to ALK inhibitors [27]. Recently, high-grade glioma of infancy revealed a novel PPP1CB-ALK fusion protein [28]. Some hypothesized that ALK signaling may contribute to tumor growth through hypoxia-independent neovascularization in GB [29], [30]. ALK-positive cells are linked with neovascularization features including vascular co-option and vascular mimicry in GB [31]. Overexpression of ALK induces an enhancement of the HIF-1 α /VEGF-A-axis [32].

ALK has a role in embryonic CNS development. ALK receptor is thought to contribute to nervous system function, repair, and metabolic homeostasis. ALK receptor activity in cancer can be mediated by amplification, overexpression, ligand binding, mutations in the intracellular domain of the receptor and by the activity of the receptor tyrosine phosphatase. Overexpression of ALK, its smaller homolog leukocyte tyrosine kinase, and the ligands PTN and MK in cancer tissues from patients correlate significantly with worse outcomes of the tumor [33].

ALK rearrangements have been reported in many brain tumors [34]. ALK and its ligand, pleiotrophin, are highly expressed during embryonic

brain development and in high-grade brain tumors such as GBM compared to normal brain tissue [35], [36]. ALK and cMET protein expression were seen in a significant number of GBM samples, with 55% having concurrent ALK gene amplification along with cMET overexpression and these data suggest a role for cMET and/or ALK in the pathogenesis of GBM [37]. There is promising potential for new chemotherapeutics targeting ALK and cMET to improve the prognosis of GBM [38].

Materials and Methods

This retrospective study was carried out on 60 cases of randomly chosen formalin-fixed paraffin-embedded tissue blocks of patients diagnosed with astrocytic tumors from the archives of the Pathology department of Cairo University of Medicine from the period from January 2015 till January 2019. We included only astrocytic tumors with adequate biopsies and excluded those with extensive hemorrhage and necrosis. Clinicopathological data of all cases were recorded from the patient records and tabulated. Ethical clearance was obtained by the institutional review board before the study.

Each paraffin block was cut by rotator microtome at 5 μ thick sections which were then mounted on glass slides to be stained by hematoxylin and eosin for histopathological re-evaluation by two pathologists and on charged glass slides for immunostaining process.

The slides were deparaffinized in xylene. Then, they were treated for antigen retrieval (using a microwave oven for 30 min) in an automated Omnis DAKO immunostainer at a high pH of 8. After this, the sections were treated with antibodies using avidin-biotin-peroxidase 3% (Thermo Scientific) for 30 min. Diaminobenzidine tetrahydrochloride was used as a substrate and chromogen. Hematoxylin (Biogenex) was used as a counterstain. The primary antibody was a mouse monoclonal antibody for ALK (clone 5A4, Newcastle, United Kingdom) was used at a dilution of 1:30 and incubated at 42°C for 2 h.

The WHO scheme is based on the appearance of certain characteristics: atypia, mitoses, endothelial proliferation, and necrosis. These features reflect the malignant potential of the tumor in terms of invasion and growth rate. Astrocytic tumors have four WHO grades as follows; Astrocytic tumors without any of these features are classified as WHO grade I. Astrocytic tumors with cytological atypia alone are considered WHO grade II. Astrocytic tumors with anaplasia and high mitotic activity in addition to cytological atypia are considered WHO grade III. Astrocytic tumors exhibit marked anaplasia, high mitotic activity, atypical mitoses as well as microvascular proliferation and/or necrosis are considered WHO grade IV representing the most aggressive type, GBM [2].

Tumor tissue sections were examined and scored under Leica DM500 microscope at low power than high power magnification by two independent pathologists who were not informed of the histological diagnosis. Assessment of ALK expression in the tumor cells was designated as brownish cytoplasmic staining in more than 10% of tumor cells (threshold point of positivity). The intensity of cytoplasmic staining was subcategorized into: Negative; absence of cytoplasmic staining, weak; faint cytoplasmic staining, moderate intensity; moderate cytoplasmic staining and strong intensity; intense cytoplasmic staining. Five categories on the basis of the proportion of immunopositive cells, as follows: Score 0; all tumor cells are negative, Score 1; < 25% of tumor cells are positive, Score 2; 25%–<50% of tumor cells are positive, Score 3; 50%–75% of tumor cells are positive, Score 4; >75% of tumor cells are positive [30].

The positive control was from CD30 positive anaplastic large cell lymphoma (ALK positive). ALK positivity in the background stroma served as a positive internal control. The results of ALK immunostaining in tumor cells were correlated with multiple prognostic factors including (age, sex, site, side, tumor recurrence, clinical presentation, and WHO grade of astrocytic tumor).

Microsoft excel 2010 was used for data entry and the statistical package for social science (SPSS version 21) was used for data analysis. Simple descriptive statistics (arithmetic mean and standard deviation) used for the summary of quantitative data and frequencies used for qualitative data. The bivariate relationship was displayed in cross-tabulations and comparison of proportions that were performed using the Chi-square test. The T-independent test was also used to compare normally distributed quantitative data. The Chi-square test was used to assess differences between qualitative variables, while t-test and analysis of variance tests were used for quantitative variables. The significance of the results was assessed by determining probability factor “p-value”. $p < 0.05$ was considered statistically significant.

All slides were screened on Leica DM500 microscope and all photos were imaged by HD digital microscope camera, named Leica ICC50 HD, connected to the same microscope.

Results

This study included 60 cases of astrocytic tumors, in which the ages of the patients ranged 13 years to 72 years with mean age 43.1 (± 15.63 years) (Figure 1). Statistical analysis showed that the peak incidence of astrocytic tumors was found in the seventh decade followed by fifth decade of life. As regards to the

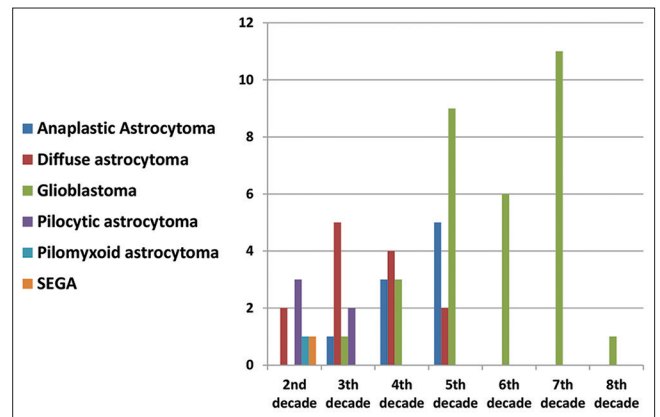


Figure 1: Age distribution among studied cases

gender, male preponderance was noted; as we have 40 males (66.7% of studied cases) and 20 females (33.3% of studied cases) with a female to male ratio of 1:2.

As regards the histological diagnoses as well as WHO grades (Figure 2) of astrocytic tumors among the studied cases; 6 (10%) cases were classified as WHO grade I astrocytic tumors including five cases of pilocytic astrocytoma and one case of SEGA. Fourteen (23.3%) cases were classified as WHO grade II astrocytic tumors including 13 (21.7%) cases of diffuse astrocytoma as well as one (1.6%) case of pilomyxoid astrocytoma. Nine (15%) cases were classified as anaplastic astrocytoma, WHO grade III. Thirty-one (51.7%) cases were classified as GBM, WHO grade IV.

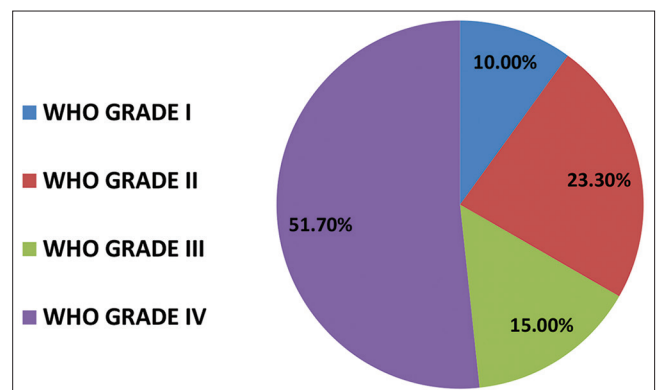


Figure 2: World Health Organization grade distribution among studied cases

Astrocytic tumors showed preference for cerebral hemisphere in our study as 48 (80%) cases showed a cerebral hemisphere location (Figure 3).

We found a left sided preponderance in our study, as 34 (56.7%) cases were left sided, 20 (33.3%) cases were right sided and 6 (10%) cases were midline in location. As regards the clinical presentation among the studied cases; 25 (41.7%) cases presented by seizures, 21 (35%) cases presented by headache, 14 (23.3%) cases are presented by hemiparesis.

ALK expression was not established in 57 (95%) cases which showed a negative intensity,

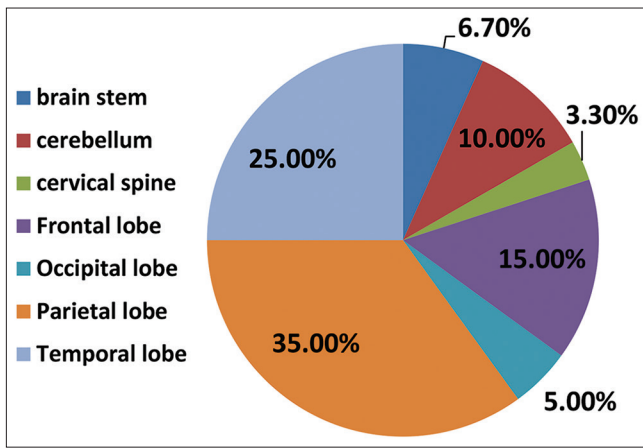


Figure 3: Anatomical distribution among studied cases

(score 0) while three cases (5%) cases had strong cytoplasmic intensity; two cases were score 4 and the remaining case a score of 1 (Figure 4). The two strongly positive cases were GBM, WHO grade IV, gliosarcoma variant and both cases were left sided; one located in the occipital lobe while the other located in the temporal lobe. The remaining strongly ALK-positive case was score 1 detected in a case of right-sided anaplastic astrocytoma, WHO grade III, located in the parietal lobe. Correlation between ALK expression and WHO grade as well as tumor laterality were non significant ($p = 0.366$, $p = 0.344$), respectively. In addition to the remaining correlations between age, gender, and tumor location also displayed lack of statistical significance.

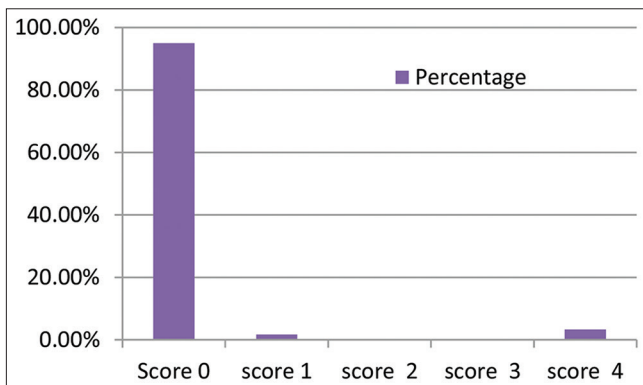


Figure 4: ALK expression among studied cases

On the other hand, there was a statistically significant correlation seen between age of patients and gender among the study group ($p = 0.001$) with females tending to present with astrocytic tumors at an older age than males. With a similar pattern, the correlation between the age of patients and WHO grade of the astrocytic tumors among the study group was statistically significant ($p = 0.001$) implying an increase in WHO grade with older age groups. Another correlation between the gender of patients and tumor recurrence was seen ($p = 0.001$), implying a more tumor recurrence among males.

The gender of the patients showed multiple significant correlations including WHO grade of the tumors, clinical presentation, and tumor location and recurrence.

WHO grade I tumors were commonly located in the cerebellum while WHO grade II tumors as well as WHO grade III tumors were commonly located in the parietal lobe. The WHO grade IV tumors were commonly located in the parieto-temporal region. The location of the tumor and the WHO grade showed statistically significant correlation ($p = 0.001$).

In addition, the tumor location and clinical presentation (Figure 5) was statistically significant ($p = 0.006$). Tumors that affect the brain stem and cervical spine are presented by hemiparesis. Tumors that affect the cerebral hemisphere are commonly presenting by seizures. Cerebellar tumors are commonly presented by headaches. Regarding the WHO grade of the tumors, the higher the WHO grade, the higher the possibility of tumor recurrence ($p = 0.001$). The relationship between clinical presentation and WHO grade of the astrocytic tumors showed a significant finding ($p = 0.001$) implying that the higher the grade, the more frequent the clinical presentation.

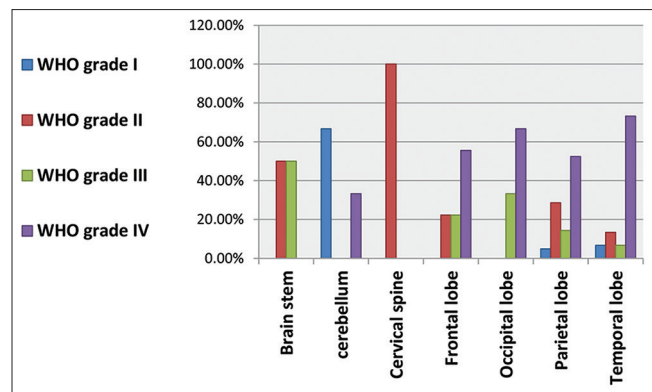


Figure 5: World Health Organization grade distribution with correlation to anatomical site among studied cases

Histopathological and findings of different cases of astrocytic tumors are presented on Figures 6-20.

Discussion

Astrocytic tumors are the most frequent primary brain tumors. They originate in a particular type of glial cells known as astrocytes. GBM is a highly aggressive astrocytic tumor and is reported to be the most common and the most lethal primary brain tumor [39]. ALK contributes to gliomagenesis via multiple mechanisms including growth stimulation, anti-apoptotic pathways, self-renewal of glioma stem cells and angiogenesis [40].

As regards the expression of the ALK among our study group; we noticed that ALK was strongly expressed in High-grade astrocytic tumors especially gliosarcoma cases. In our study we had 57 (95%) cases having negative staining, score 0 while three (5%) cases had strong cytoplasmic intensity; two were score 4 and one case was score 1.

Karagkounis *et al.* found that ALK overexpression in up to 70% of GBM without a significant correlation with any underlying ALK gene amplification and they used different immunohistochemical techniques by using two anti-ALK antibodies [41].

In our study, we find the two strongly positive cases, score 4 were left-sided GBM, WHO grade IV, gliosarcoma variant; one located in the occipital lobe while the other located in the temporal lobe. The remaining strongly ALK-positive case was score 1 detected in a case of right-sided anaplastic astrocytoma, WHO grade III, located in the parietal lobe. Chiba *et al.*, 2017 showed ALK immunopositivity in nine of 51 WHO grade II tumors, five of 29 WHO grade III tumors, and 29 (49.2%) of 52 GBM cases with a higher immunohistochemical scores in GBM as compared to those of lower grade astrocytic tumors [30]. It may be due to the larger sample size as we used a similar anti-ALK antibody (5A4). Genetic or environmental factors may play role in such differences.

In our study group, two females showed positive ALK expression while negative ALK expression was demonstrated in 18 females. One male showed positive ALK expression while negative ALK expression were demonstrated in 39 males. Two positive cases presented with hemiparesis while the third positive case presented with seizures. Correlation between ALK expression and age of patients was statistically nonsignificant with mean age 42.8 (\pm 15.9 years) for score 0 cases while mean age was 41 for the score 1 case as well as mean age was 52.5 for score 4 cases.

There is no statistically significant correlation between ALK expression on one hand and other variables on the other hand including; age, gender, clinical presentation, location, laterality, recurrence and the WHO grade of the astrocytic tumors ($p = 0.285, 0.285, 0.289, 0.750, 0.344, 0.723, 0.366$, respectively). The high number of negative cases for ALK in our study group suggests that ALK expression is not associated with a prognostic significance toward astrocytic tumors whatever its grade similar.

To the results stated by Karagkounis *et al.*, 2017 who stated that despite of being highly expressed ALK in the GBM cases, ALK overexpression did not show a correlation with prognosis in their study [41]. Recently and on a molecular base ALK variation was an independent indicator of poor prognosis in gliomas and IDH-WT-GBM evidenced by the presence of only germline ALK variants in 12 out of 99 cases (12.12%) [42]. These finding is a possible important clue to our immunohistochemical results in our study in high-grade astrocytic tumors, especially in GB cases.

Our results are close to Junca *et al.*, 2017 who stated that no significant dysregulation of ROS1 or ALK in GSCs and the associated tumors. Neither amplification nor polysomy of ALK was observed in GSC as well as no ALK mutation was found by Sanger's

direct sequencing [43]. This is recently encouraged by Niklas *et al.*, 2019 who stated that the majority of GBM cases showed no copy number aberrations of ALK gene [44]. However Chmielecki *et al.*, 2017 stated that despite negative immunohistochemistry, novel genetic fusions can be detected through molecular studies in some astrocytic tumors like PPP1CB-ALK fusion [45].

The age of patients in our study ranged from 13 years to 72 years with mean age 43.1 (\pm 15.63 years) which was near what was reported by Parvin and Fatemeh, 2019 whose mean age was 46.53 (\pm 1.81 years) [46] and away from what reported by Chiba *et al.*, 2017 who find the mean age of the patients in their study was 54.3 years [30].

The mean age of the patients with WHO grade IV tumors, GBM, ranged from 30 years to 72 years in our study with mean age of 54 (\pm 11.1 years) close to that stated by Karagkounis *et al.*, 2017 who found the mean age among GBM cases was 59.5 \pm 12.4 years [41]. Statistical analysis showed that the peak incidence of astrocytic tumors was found in the seventh decade followed by the fifth decade of life that is reported by Raverto *et al.*, 2010 who stated that astrocytic tumors, especially the most common and most aggressive tumor termed GBM, can be developed at any age, but its incidence rate is reported to be the highest at 45–75 years of age [47] while Zalata *et al.*, 2011 showed that the sixth decade followed by the fifth decade are the peak incidence for astrocytic tumors in Delta in Egypt and this difference may be due to the large sample size (452 cases) [9].

We found a male preponderance in our study with forty cases were males (66.7% of studied cases) as well as twenty females (33.3% of studied cases) matching the results of Karagkounis *et al.*, 2017 who found 33/51 cases were males (65 % of studied cases) [41]. Also Zalata *et al.*, 2011 found a male preponderance (about 63 % of studied cases) [9].

Astrocytic tumors showed a preference for the cerebral hemisphere in our study as 48 (80%) cases showed a cerebral hemisphere location, while 12 (20%) cases were located outside the cerebral hemisphere. The astrocytic tumors located within the cerebral hemisphere among our studied cases as follows; 21 (35%) cases located in the parietal lobe representing the most common location for astrocytic tumors, 15 (25%) cases located in the temporal lobe, 9 (15%) cases in the frontal lobe as well as 3 (5%) cases affect occipital lobe. However, Jonas *et al.*, 2018 reported that the commonest location for the astrocytic tumor was the frontal lobe (29.4%) and this difference may be due to the presence of many cases had multifocal locations in their study (31.4%) [48].

Frontal lobes were most frequently involved (more than 50% of all cerebral sites) followed by the temporo-parietal region as stated by Zalata *et al.*, 2011 and Karagkounis *et al.*, 2017 [9], [41]. Furthermore,

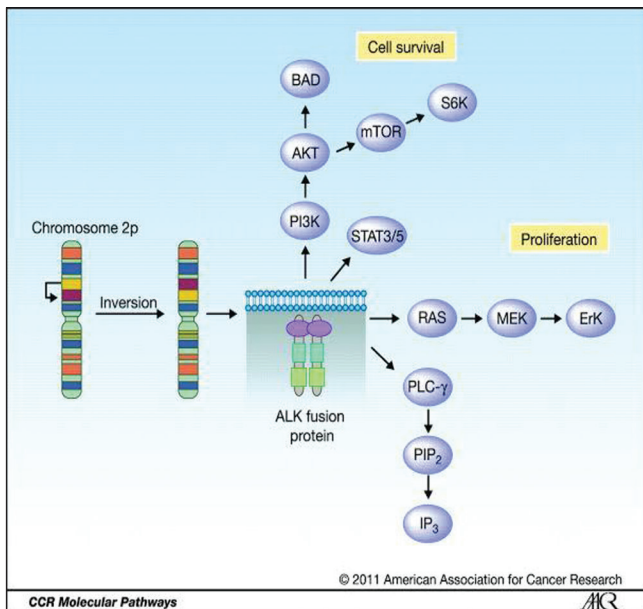


Figure 6: Role of the anaplastic lymphoma kinase as oncogene (American Association for Cancer Research, 2011)

frontal lobe is the commonest location for astrocytic tumors representing (37% of cases) as stated by Ze-Lin Sun *et al.*, 2015 possibly due to its sample concentrated on WHO grade II and III tumors [49]. The temporal lobe is the second most common location in their study, as we found in our study, representing 27% of cases which is very close to our results (25% of cases). Karagkounis *et al.*, 2017 also found temporal lobe location was the second most common location for astrocytic tumors in 37% of cases [41].

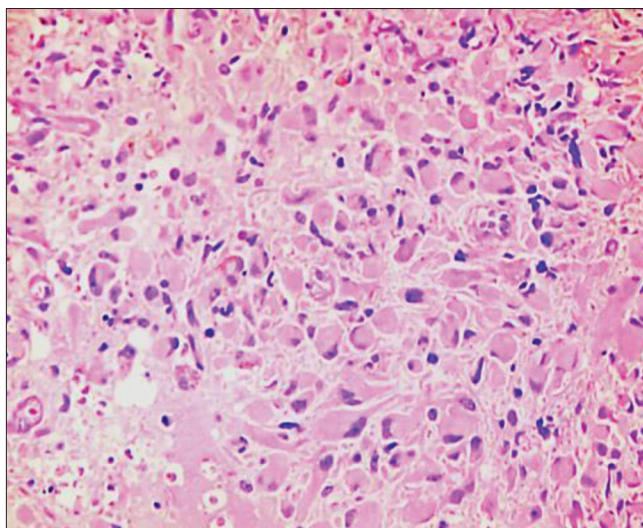


Figure 7: A case of SEGA, World Health Organization grade I, formed of large cells exhibiting abundant eosinophilic cytoplasm and eccentric nuclei with scattered spindle cells (hematoxylin and eosin $\times 400$)

The astrocytic tumors that are located outside the cerebral hemisphere including six (10%) cases located in cerebellum, four (6.7%) cases located in brain stem and two (3.3%) cases located in cervical spine. Zalata *et al.*, 2011 showed 78.8% of astrocytic tumors having cerebral hemisphere location while 9% having cerebellar

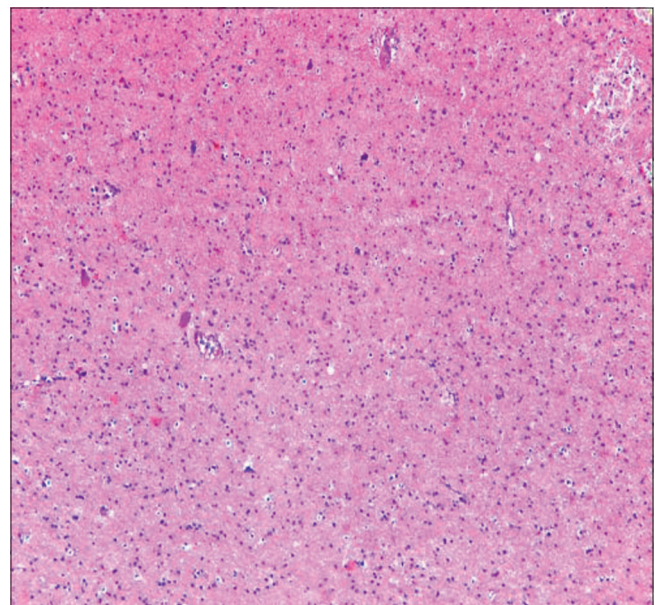


Figure 8: A case of diffuse fibrillary astrocytoma, World Health Organization grade II, showing mild cellularity with fibrillary background (hematoxylin and eosin $\times 100$)

location as well as 5.5% having brain stem location and 3% having the spinal location in Delta in Egypt [9] which very close to our study results and this possibly due to similar racial, socioeconomic and geographic factors.

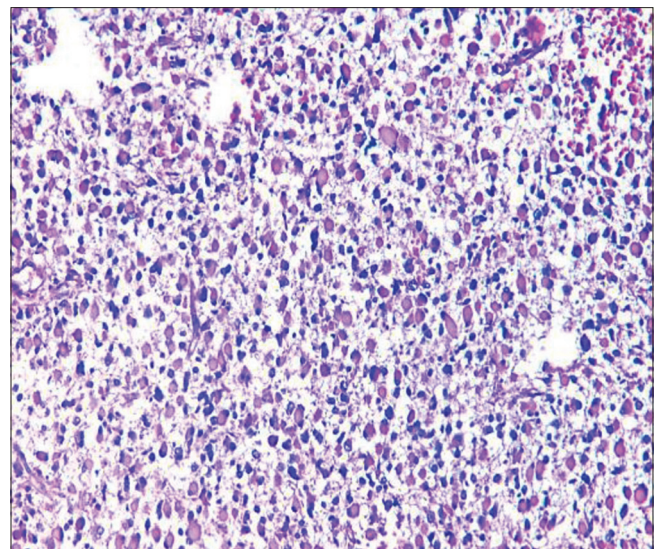


Figure 9: A case of diffuse gemistocytic astrocytoma, World Health Organization grade II, (hematoxylin and eosin $\times 100$)

We find a left-sided preponderance in our study, as 34 (56.7%) cases were left sided, 20 (33.3%) cases were right sided and 6 (10%) cases were midline in the location which are agreed with the results of Jonas *et al.*, 2018 who showed 54.9% of the sample were left sided while 35.3% were right-sided cases [48].

As regards the clinical presentation among our studied cases; the most common clinical presenting symptom is seizures followed by headache agreed with Sánchez and Lodenkemper, 2017 [50]. We found in our study; 25 (41.7%) cases presented with seizures,

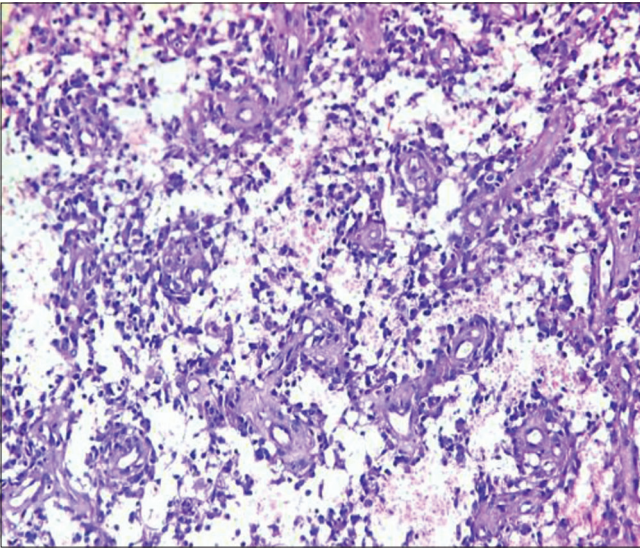


Figure 10: A case of pilomyxoid astrocytoma, World Health Organization grade II, showing angiocentric arrangement of the tumor cells with formation of pseudopapillary structures in a myxoid matrix (hematoxylin and eosin $\times 400$)

21 (35%) cases presented with headache, 14 (23.3%) cases are presented with hemiparesis. However, Rasmussen *et al.*, 2017 and Dobran *et al.*, 2018 results showed that focal neurological deficit was the most frequent presenting symptom mainly motor deficit, followed by hemianopsia and sensory symptoms. Seizures were the second most common presenting symptom [51], [52].

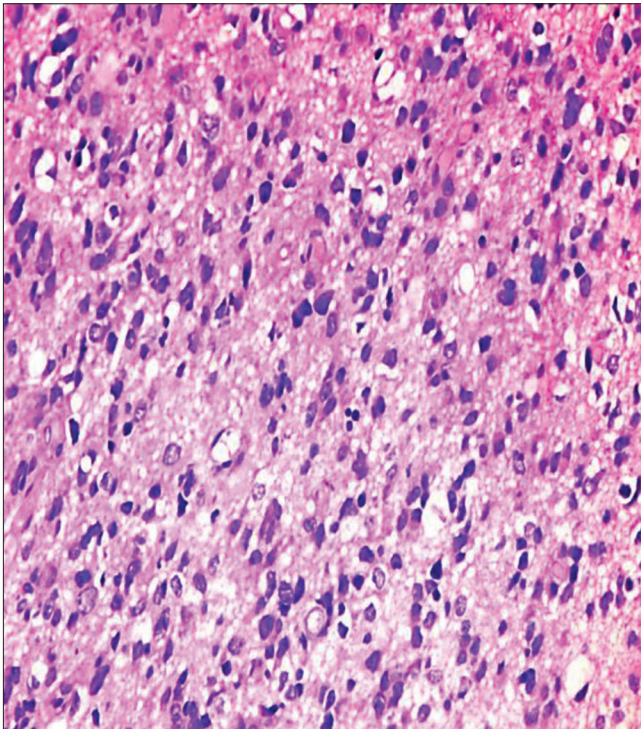


Figure 11: A case of anaplastic astrocytoma, World Health Organization grade III, showing extreme cellularity and marked pleomorphism (hematoxylin and eosin $\times 400$)

Keogh and Henson, 2012 stated that clinical presentation of intracranial astrocytic tumors are usually variable and referable to the anatomic area

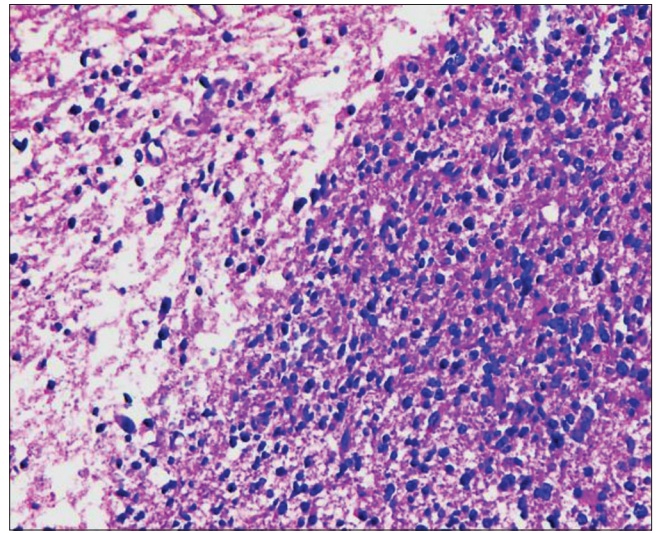


Figure 12: A case of recurrent astrocytic tumor diagnosed as anaplastic astrocytoma, World Health Organization grade III, showing extreme cellularity and marked pleomorphism (to the right). A component of mild cellularity and mild pleomorphism was seen (to the left) (hematoxylin and eosin $\times 400$)

of the brain involved that sometimes may allow the tumor to reach substantial size while remaining clinically silent until present too late by hemiparesis. In contrast, small lesions in critical areas are more likely to clinically present too early by seizures [53]. Tumor recurrence were observed in six (10%) cases in our study that agreed by Jinquan *et al.*, 2016 who stated that recurrence and progression to higher grade lesions are key biological events and characteristic behaviors in the evolution process of gliomas especially astrocytic tumors [54].

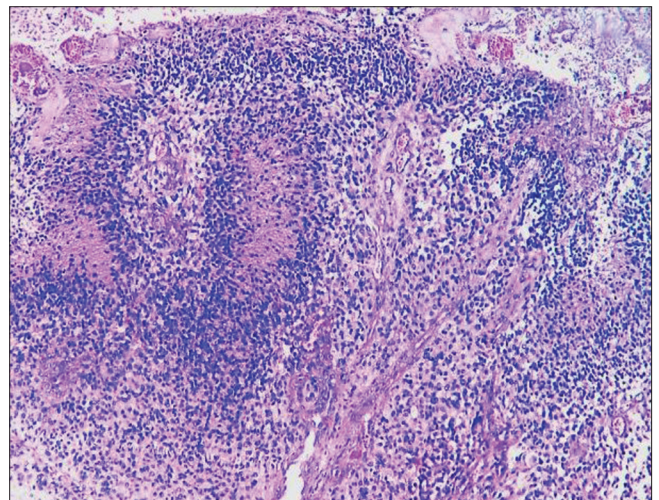


Figure 13: A case of glioblastoma, World Health Organization grade IV, showed endothelial vascular proliferations and palisaded necrosis (hematoxylin and eosin $\times 200$)

In our study, we found six (10%) cases of WHO grade I astrocytic tumors including five cases of pilocytic astrocytoma and one case of SEGA while fourteen (23.3%) cases were classified as WHO grade II astrocytic tumors including 13 (21.7%) cases were diffuse astrocytoma together with one (1.6%)

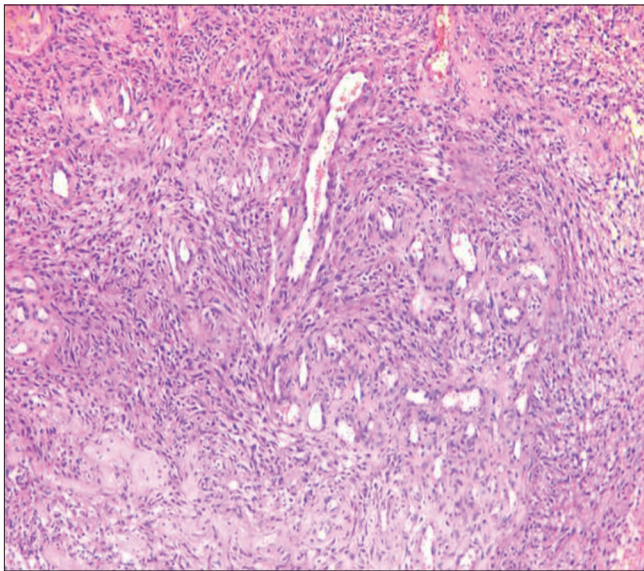


Figure 14: A case of glioblastoma, gliosarcoma variant, World Health Organization grade IV, showing extensive endothelial vascular proliferations (hematoxylin and eosin ×200)

case diagnosed as pilomyxoid astrocytoma which are similar to what is stated by Parvin and Fatemeh, 2019 whose results showed WHO grade I astrocytic tumors

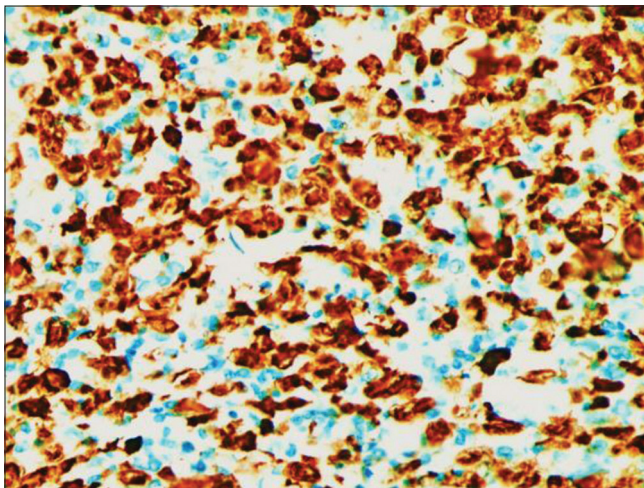


Figure 15: Strong diffuse ALK positivity in a case of anaplastic large cell lymphoma as a control (IHC ×400)

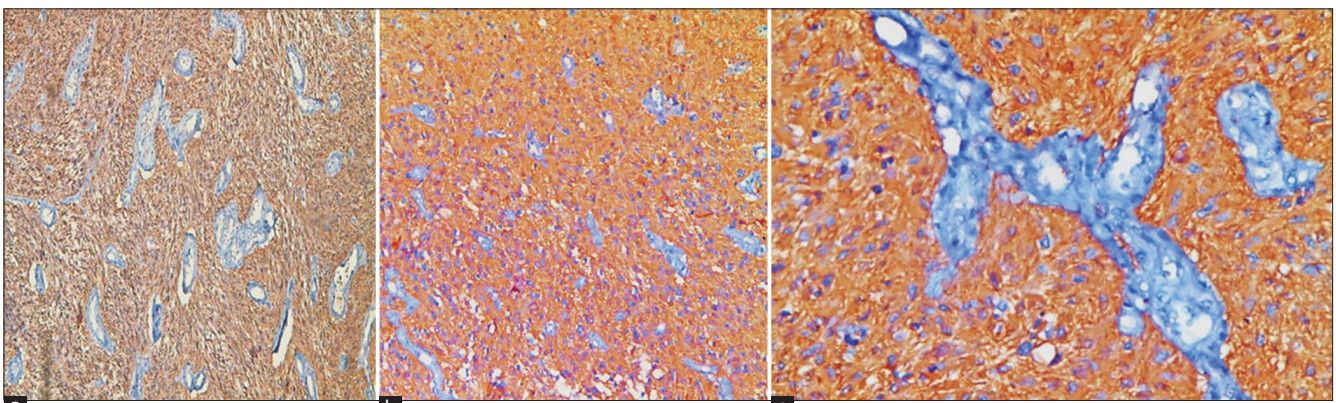


Figure 16: A case of glioblastoma, gliosarcoma variant, World Health Organization grade IV, showed strong cytoplasmic ALK expression in most of the tumor cells, score 4 (a); (IHC ×100), (b): ×200, (c): ×400

are 10% while WHO grade II astrocytic tumors are 25% of their sample [46]. Chiba *et al.*, 2017 revealed 37% of the cases were WHO grade II tumors [30].

Nine (15%) cases were classified as anaplastic astrocytoma, WHO grade III, in our study that is agreed by Ann Mari *et al.*, 2017 [55] while Chiba *et al.*, 2017 revealed 25% of cases in their study were WHO grade III tumors [30] and higher than what is reported by Parvin and Fatemeh, 2019 who found it represents 5% of their sample [46].

The most common astrocytic tumor in our study was GBM as we have 31 cases classified as WHO grade IV astrocytic tumors, GBM, that is very close to what stated by Soomro *et al.*, 2017 and Schneider *et al.*, 2018 who found 50% of gliomas in adulthood are classified as GBM [56], [57] and our results not very far from what reported by Parvin and Fatemeh, 2019 who found that GBM is representing 60% of their studied astrocytic tumors [46]. A lower percentage was reported by Giedrius *et al.*, 2019 who found it 36% of their sample while Chiba *et al.*, 2017 found 37% of cases were WHO grade IV tumors [30].

Ostrom *et al.*, 2018 stated that GBM was the most common astrocytic tumor that commonly present in the seventh decade with the median age at diagnosis of 65 years with male-to-female ratio; 1.6:1, accounting for 50–60% of all astrocytic tumors [58]. This data is matching to a great extend to what we concluded in our study as we find GBM was the most common astrocytic tumor commonly present in the seventh decade of life with median age at diagnosis of 54 years with a male-to-female ratio 2:1, accounting for 51.7% of all astrocytic tumors in our sample.

Correlation between age of patients and gender among our study group showed statistically significant correlation ($p = 0.001$) with females tending to present with astrocytic tumors at an older age than males but Robinson and Kleinschmidt, 2017 stated tumors affect older males than older females [59]. This is maybe due to possible genetic

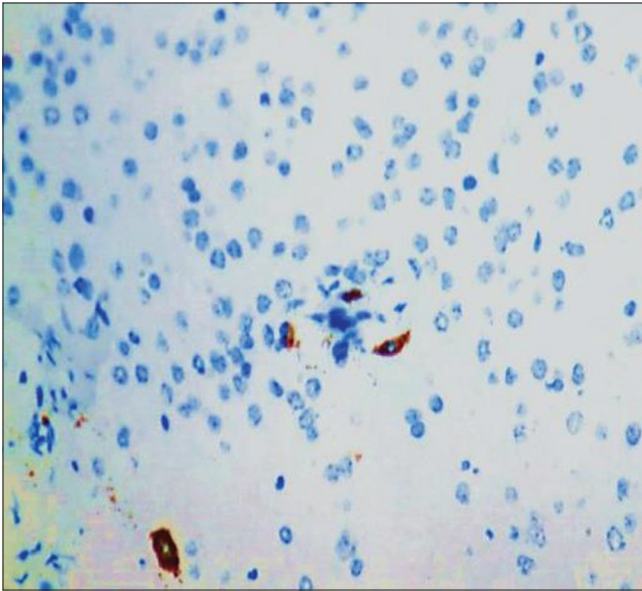


Figure 17: A case of anaplastic astrocytoma, World Health Organization grade III, showed strong cytoplasmic ALK expression in less than 25% of the tumor cells, score 1 (IHC x 400)

or environmental factors in addition to their large sample size (578 cases). Correlation between age of patients and WHO grade of the astrocytic tumors among our study group was statistically significant ($p = 0.001$) implying an increase in the WHO tumor grade with older age groups similar to what is stated by [51].

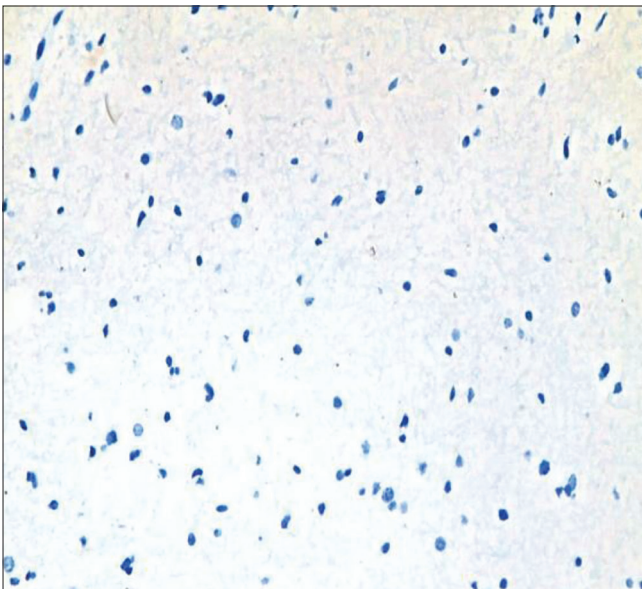


Figure 18: A case of pilocytic astrocytoma, World Health Organization I, showed negative ALK expression, score 0 (IHC x 400)

Furthermore, the correlation between the gender of patients with tumor location as well as tumor laterality was statistically significant ($p = 0.0001$ and 0.001 , respectively) implying that tumor location tends to be more in the cerebellum, cerebrum, and cervical spine among males as well as the left-sided tumors are more common in males against what is reported by Elena *et al.*, 2016 that found a nonsignificant correlation

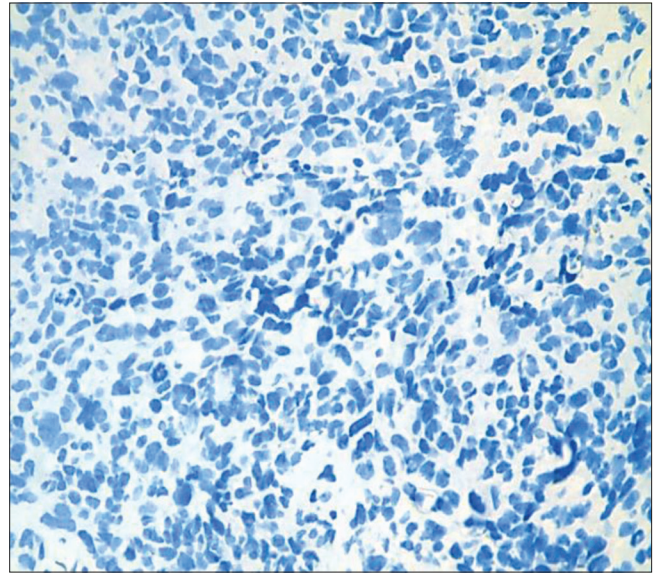


Figure 19: A case of glioblastoma, World Health Organization grade IV, showed negative ALK expression, score 0 (IHC x 400)

possibly due to large sample size that are conducted on one tumor type only; diffuse astrocytomas [60].

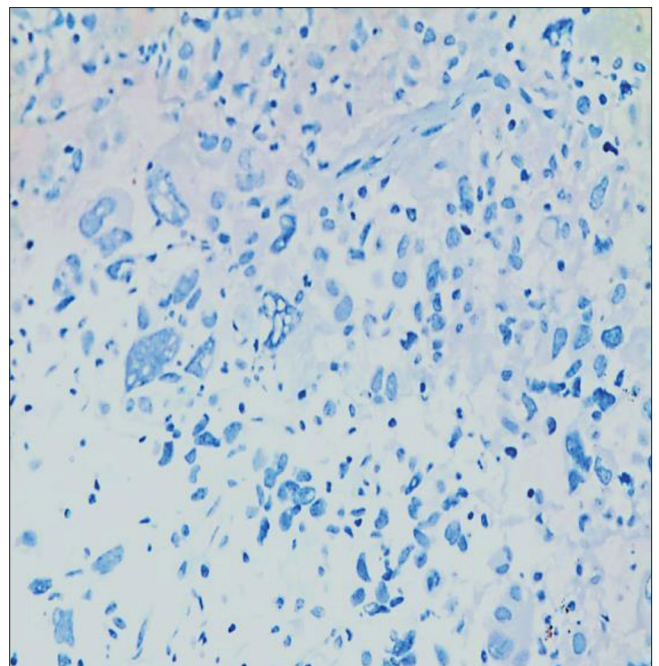


Figure 20: A case of glioblastoma, World Health Organization grade IV, showed negative ALK expression, score 0 (IHC x400)

Correlation between tumor recurrence and WHO grade as well as clinical presentation of the astrocytic tumors (Tables 1 and 2) was statistically significant ($p = 0.001$ and 0.003 , respectively) that signify recurrent cases will have a higher WHO grade that will be mostly presented by seizures as documented by Cai and Sughrue, 2018 who found WHO grade IV astrocytic tumors were following a previous low-grade astrocytic tumor [61].

Correlation between clinical presentation with tumor location and WHO grade of astrocytic tumors was statistically significant in our study ($p = 0.006$ and

Table 1: Relationship between ALK and clinicopathological variables

Factors	ALK expression		p-value	Significance
	Negative n = 57	Positive n = 3		
Age (years) Mean±SD	42.75 ± 15.9	48.67 ± 7.09	0.528	Insignificant
Sex				
Male	39	1	0.209	Insignificant
Female	18	2		
Laterality				
Right	19	1	0.830	Insignificant
Left	32	2		
Midline	6	0		
Tumor location				
Occipital	2	1	0.394	Insignificant
Parietal	20	1		
Frontal	9	0		
Temporal	14	1		
Spinal	2	0		
Brain stem	4	0		
Cerebellum	6	0		
Histological types				
Pilocytic	5	0	0.863	Insignificant
Pilomyxoid	1	0		
Diffuse	13	0		
Anaplastic	8	1		
GBM	29	2		
SEGA	1	0		
Recurrence				
Yes	6	0	0.554	Insignificant
No	51	3		
WHO				
G1	6	0	0.594	Insignificant
GII	14	0		
GIII	8	1		
GIV	29	2		
Clinical picture				
Seizures	24	1	0.157	Insignificant
Headache	21	0		
Hemiparesis	12	2		

0.001 respectively). Tumors that affect brain stem and cervical spine are presented by hemiparesis while tumors that affect the cerebral hemisphere are commonly presented with seizures and cerebellar tumors are commonly presented with headache. Correlation between tumor laterality on one hand and tumor location as well as the clinical presentation of astrocytic tumors among our study group, on the other hand, was statistically significant ($p = 0.001$)

Table 2: Relationship between WHO grade and clinicopathological variables

Factors	WHO grade				p-value	Significance
	GI n = 6	GII n = 14	GIII n = 9	GIV n = 31		
Age (years) Mean±SD	19.3 ± 5.24	30.64 ± 8.6	40.56 ± 6.76	53.97 ± 11.1	0.000	Significant
Sex						
Male	3	4	5	28	0.000	Significant
Female	3	10	4	3		
Laterality						
Right	0	7	3	10	0.001	Significant
Left	6	3	4	21		
Midline	0	4	2	0		
Tumor location						
Occipital	0	0	1	2	0.001	Significant
Parietal	1	6	3	11		
Frontal	0	2	2	5		
Temporal	1	2	1	11		
Spinal	0	2	0	0		
Brain stem	0	2	2	0		
Cerebellum	4	0	0	2		
Recurrence						
Yes	0	1	1	4	0.778	Insignificant
No	6	13	8	27		
Clinical picture						
Seizures	3	7	4	11	0.601	Insignificant
Headache	3	3	2	13		
Hemiparesis	0	4	3	7		

implying all midline cases are having brain stem and cervical spine locations presented by hemiparesis. Left-sided tumors are mostly having cerebellar and

parieto-temporal locations while right sided tumors are commonly affecting the frontal lobe. Both left and right-sided tumors are commonly presented by seizures followed by headache.

There is also a significant relationship between tumor laterality and WHO grade of the astrocytic tumors among our study group ($p = 0.001$) implying the more the left and right-sided tumors with the higher the WHO grade. Tumor laterality and tumor locations must be taken in our considerations as advised by Salo *et al.*, 2002 who stated that tumor laterality and tumor location among cases with brain tumor can affect the quality of life evidenced by brain tumors that located in the right hemisphere seemed to have a poorer quality of life than left-sided tumors based on regular postoperative follow-up [62].

As for a correlation between tumor location and WHO grade of the astrocytic tumors among the study group was statistically significant ($p = 0.001$). WHO grade I tumors were commonly located in the cerebellum that agreed by Bornhorst *et al.*, 2016 who stated that pilocytic astrocytoma, the most common pediatric brain tumor, is most commonly located in the cerebellum [63]. WHO grade II and WHO grade III tumors were commonly located in the parietal lobe, while WHO grade IV tumors were commonly located in the parieto-temporal region identical to results of Dobran *et al.*, 2018 [52], however, results of Zalata *et al.*, 2011 and Ze-Lin Sun *et al.*, 2015 stated that frontal lobe is the most common location [9], [49].

Conclusions

GBM and gliosarcoma are highly aggressive tumors. Recent advances in molecular and pathological diagnosis have a great impact on the prediction of prognosis and recurrence. Expression of ALK was found to be abundant among high-grade like anaplastic large cell lymphoma and some aggressive cases of NSCLC and they benefit greatly from targeted ALK chemotherapeutics. In a similar approach, more studies and experiments should also be attempted on high-grade astrocytic tumors. In this study, three positive cases were seen in higher grade astrocytic tumors, particularly gliosarcoma. Therefore, larger studies on a wider scale of GBM patients especially gliosarcoma variant are needed to elucidate the exact role of ALK in this high-grade astrocytic tumor as so far most studied concluded no significant role. Other markers are recommended to be used in association with ALK in such studies on GBM to improve test sensitivity like EGFR, Cyclin D1, ROS, PTEN and cMET. Molecular studies may be used in combination with immunohistochemical studies to overcome the effect of the underexpression of ALK protein in tumors that have ALK gene mutations.

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