Original article

The study of CD117 expression in glial tumors and its relationship with the tumor-type and grade

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Background: CD117 is a thyrosin kinase receptor encoded by c-kit proto-oncogene. It is expressed during normal development in some tissues and also in a subset of neoplasia especially gastrointestinal stromal tumors (GISTs). Treatment with thyrosin kinase inhibitors (e.g., Imatinib) is useful in CD117- positive GISTs. The goal of this study is to investigate the expression of CD117 in glial tumors as a potential diagnostic marker and target for therapy. **Materials and Methods:** in this descriptive-analytical study, paraffin-embedded tissue blocks from 50 cases of glial tumors (various histological types and grades) were selected in a convenience sampling for the CD117 immunhistochemical study including expression of the marker, staining intensity, and percentage of the stained cells. The results were analyzed by Chi-square and Mann–Whitney tests. **Results:** CD117 expression was detected in about 76% of glial tumors but the frequency of the expression showed no statistically significant relationship with the tumor type ($P = 0.829$). Although CD117 immunoreactivity was more frequent in high-grade tumors (84%) compared to the low-grade ones (68%), no statistically significant relationship was found between the CD117 expression and grade of the tumor ($P = 0.09$). Staining intensity and percentage of stained cells in high-grade tumors were significantly more than in low-grade tumors (P values of 0.046 and 0.023, respectively). **Conclusion:** according to the statistically significant difference in the staining intensity and percentage of the stained cells between the low-grade and high-grade glial tumors, these two parameters may be useful for making distinction between various grades of these tumors. Moreover, according to the prominent expression of CD117 in high-grade gliomas, these tumors may be potential candidates for treatment with thyrosin kinase inhibitors.

Key words: CD117, glial tumors, immunohistochemical staining, tumor grade

INTRODUCTION

The protooncogene c-kit, located on chromosome 4q11-21, encodes one of the growth factor receptors known as CD117.[1] This 145 KD receptor is a transmembrane glycoprotein from type-III subfamily of the tyrosine kinase receptors.[1-4] The ligand for CD117 is called the stem-cell factor (SCF) and stimulation of CD117 with its ligand initiates a signaling cascade resulting in the cell growth.[5]

It has been demonstrated that CD117 expression is necessary for the normal development of mast cells, melanocytes, some hematopoietic cells, and germ cells. The role of CD117 in normal hepatocyte maturation and regeneration of injured liver cells has also been shown.[6,7]

Overexpression of this receptor has been observed in some types of tumors, including lung, breast, skin, uterus, bladder and ovarian cancers, leukemias, germ cell tumors, Ewing sarcoma, and gastrointestinal stromal tumors (GISTs).[8-12]

Among tumors, GISTs show CD117 expression as a highly specific diagnostic marker. Moreover, CD117 positive GISTs have been treated with Imatinib, a tyrosine kinase inhibitor.[13-15] Successful treatment of GISTs with Imatinib has encouraged the investigators to study the expression of CD117 in other tumor types including gliomas.

Glioma as the most frequent family of the central nervous system tumors embraces astrocytoma, oligodendroglioma, oglioastrocytoma, and ependymoma, as well as their various subtypes and combinations.[16] According to the WHO grading system, glial tumors are classified as either low grade (I, II) or high grade (III, IV).[17]

The studies on the expression of CD117 in glial tumors are limited and most data are obtained from astrocytic tumors.[18-20] Limited data, frequency of glial tumors, inadequacy of surgical treatment especially in high-grade tumors, and successful results reported from the treatment of these tumors with Imatinib[17,21-24] made us investigate the expression of CD117 in glial tumors and determine if there is any dramatic difference in the CD117 immunoreactivity between the various types and grades of the glial tumors.

MATERIALS AND METHODS

In this descriptive-analytical study, formalin-fixed and

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paraffin-embedded tissue specimens of 50 glial tumors with unequivocal diagnosis including four pilocytic astrocytomas (WHO grade I), seven diffuse fibrillary astrocytomas (WHO grade II), seven anaplastic astrocytomas (WHO grade III), 12 glioblastomas (WHO grade IV), five oligodendrogliomas (WHO grade II), five anaplastic oligodendrogliomas (WHO grade III), five oligoastrocytomas (WHO grade II), one anaplastic oligoastrocytoma (WHO grade III), and four ependymomas (WHO grade II) were retrieved in a convenience sampling from the pathology archive of the Al-Zahra Hospital, Isfahan, Iran. Microscopic slides stained with hematoxylin and eosin were reviewed by a neuropathologist to confirm the diagnoses according to the criteria of the World Health Organization for central nervous system tumors.[17] Tumor blocks with insufficient sample for IHC staining were deleted.

A tissue block of a documented CD117 positive GIST specimen was used as the control and stained in each work run to validate the results. In this specimen, tumor cells were expected to act as positive control while the smooth muscle cells of the tumor vessels were expected to the slides were studied by light microscopy to determine the presence (+) or absence (−) of CD117 immunoreactivity, intensity of expression (weak: light staining in cytoplasm; moderate: heavy staining in cytoplasm; and strong: cytoplasmic staining with accentuation of the membrane), be the internal negative control.[13] Although La Rosa and colleagues have shown automated immunohistochemical staining procedures superior to the manual techniques,[25] we used a manual procedure in this study. Since the reliable results are achieved only by optimized staining protocols and careful interpretation, we have tried to minimize the errors by following closely the manufacturer recommendations for appropriate duration, PH and temperature of each step, using appropriate positive and negative controls, and careful interpretation of the results. CD117 immunohistochemical staining (IHC) was done manually in the Avidin-Biotin Complex (ABC) method (EnVision type) according to the proposed manufacturer protocol. Tissue sections measuring 3 μm were prepared and placed on the glass slides, coated with poly-l-lysine, and deparaffinized by immersion in xylene and alcohol. The following steps included immersion in citrate buffer (pH 6.0), incubation with 3% hydrogen peroxide, incubation with the primary antibody (Rabbit anti-human c-kit polyclonal antibody, A4502, DAKO) at 1:800 dilution, washing in phosphate buffer saline (PBS), polymer envision, and incubation with diaminobenzidine (DAB) in the chromogen solution. Finally, the sections were counterstained with Mayer's hematoxylin and mounted.[26]

and percentage of stained cells (0: 0%; 1 +: 1 − 25%; 2 +: 26 − 50%; 3 +: 51 − 75%; 4 +: > 75%).[24] The data were collected and analyzed with Chi square and Mann–Whitney tests using SPSS software (version 18, Chicago, USA).

RESULTS

Thirty-eight out of 50 glial tumors (76 % of the cases) were immunoreactive with CD117 marker regardless of the tumor type and grade, intensity of staining and percentage of stained cells [Table 1]. Except for anaplastic oligoastrocytoma that included only one case that was positive for the marker, the highest frequency of CD117 expression was observed in fibrillary astrocytoma and anaplastic astrocytoma (86% in each group), followed by glioblastoma (83%) and anaplastic oligodendroglioma (80%). The chi-square test revealed no statistically significant relationship between the glial tumor type and CD117 immunoreactivity (*P*-value = 0.829).

CD117 expression was detected in 68% and 84% of low-grade and high-grade glial tumors, respectively [Table 2] and the difference between the two groups was not found to be statistically significant by the chi-square test (*P*-value = 0.09).

Table 1: Immunohistochemical expression of CD117 in glial tumors

Tumor type	CD117 Expression number of cases (%)		Total (%)
	Yes	No	
Pilocytic astrocytoma	3 (75)	1 (25)	4
Diffuse astrocytoma	6 (86)	1 (14)	7
Anaplastic astrocytoma	6 (86)	1 (14)	7
Glioblastoma	10 (83)	2 (17)	12
Oligodendroglioma	3 (60)	2 (40)	5
Anaplastic oligodendroglioma	4 (80)	1 (20)	5
Oligoastrocytoma	3 (60)	2 (40)	5
Anaplastic oligoastrocytoma	1 (100)	0 (0)	1
Ependymoma	2 (50)	2 (50)	4
Total	38 (76)	12 (24)	50 (100)

Table 2: CD117 expression in high-grade and low-grade glial tumors

Tumor grade	CD117 Expression number of cases (%)		Total
	Yes	No	
Low grade	17 (68)	8 (32)	25 (100)
High grade	21 (84)	4 (16)	25 (100)
Total	38 (76)	12 (24)	50 (100)

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Data revealed that the negative results and weak staining were more frequently seen in low-grade glial tumors while moderate and strong intensities of staining were mainly observed in high-grade tumors. Strong intensity was seen in 2 (8%) of low-grade and 5 (20%) of high-grade tumors. Seventeen cases (68%) of low grades showed negative results and weak staining but these results were found in 12 (48%) cases of high-grade glial tumors [Table 3] [Figures 1 and 2]. The Mann–Whitney test showed that the staining intensity of CD117 is statistically more significant in high-grade glial tumors (*P*-value = 0.046).

It was also found that the highest percentages of stained cells (3+ and 4+) were observed among high-grade tumors. In 18 cases (72%) of low-grade tumors less than 50% of tumoral cells were stained (1+ and 2+) and none of them were 4+ whereas seven cases (28%) of high grades were 4+ [Table 4]. The difference in the percentage of positive cells between the low-grade and high-grade tumors was shown to be statistically significant by the Mann–Whitney test (*P*-value = 0.023).

DISCUSSION

CD117 is a transmembrane tyrosine kinase receptor that shows different biological functions. The expression of this receptor has been confirmed in some normal cells and some tumoral tissues. CD117 distribution and its functions have been studied over the last 20 years and many scientists have encouraged the study of this receptor and its encoding gene in human tumors as a useful therapeutic target for the tyrosine kinase inhibitors such as Imatinib.[6,27] Three mechanisms of CD117 activation in tumoral cells have been recognized including (i) stimulation of the receptor by the stem-cell factor (SCF); (ii) cross-activation by other kinases and/or loss of regulatory phosphatase activity; and (iii) activating mutations that show a key role in the pathogenesis of GISTs. It has been demonstrated that successful treatment with tyrosine kinase inhibitors has been experienced only in mutant receptors.[28]

Table 3: CD117 staining intensity in glial tumors

Intensity of staining	Low-grade tumors Number of cases (%)	High-grade tumors Number of cases (%)
No staining	8 (32)	4 (16)
Weak	9 (36)	8 (32)
Moderate	6 (24)	8 (32)
Strong	2 (8)	5 (20)
Total	25 (100)	25 (100)

Table 4. Percentage of cells showing CD117 immunoreactivity in glial tumors

Percentage of stained cells	Low-grade tumors Number of cases (%)	High-grade tumors Number of cases (%)
0	8 (32%)	4 (16%)
1–25	3 (12%)	2 (8%)
26 – 50	7 (28%)	8 (32%)
51–75	7 (28%)	4 (16%)
76–100	0 (0%)	7 (28%)
Total	25 (100%)	25 (100%)

Considering the limited data on immunohistochemical expression of CD117 in glial tumors, the current study was designed to determine CD117 expression in these tumors and compare the intensity of staining and the percentage of stained cells in glial tumors of various histopathological grades.

As mentioned previously, the paraffin block of a tissue specimen with the definite diagnosis of the CD117-positive gastrointestinal stromal tumor (GIST) was used to validate the results of immunohistochemical staining. In this specimen, the stromal tumor cells and interstitial cells of Cajal were intensely stained with CD117 antibody whereas normal smooth muscle cells (in muscularis mucosa, muscularis propria, and vascular wall) and fibroblasts were not immunoreactive with the antibody. These unstained elements in GIST served as internal negative control.[24,29]

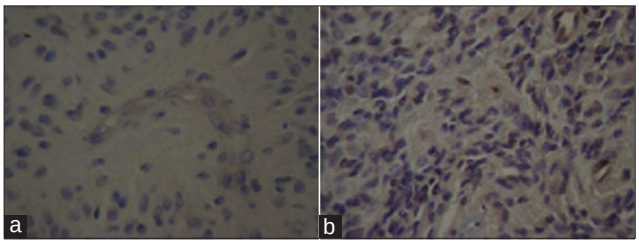

Figure 1: Two cases of *Ependymoma*. (a) No immunoreactivity with CD117; (b) Weak CD117 immunoreactivity

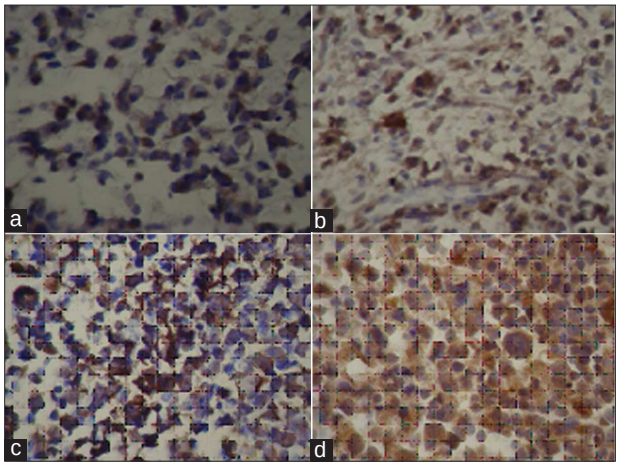

Figure 2: CD117 immunostaining in tumoral cells. Moderate CD117 immunostaining in a case of oligodendroglioma (a); strong CD117 immunoreactivity in anaplastic astrocytoma (b), glioblastoma (c), and oligodendroglioma (d).

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To avoid false positive interpretations, we have ignored areas of the specimens adjacent to the sections margins, since these areas commonly show artifactual staining. Moreover, wherever possible, we have evaluated those areas of the tumors that have been far from the tumor margins since reactive gliosis in these marginal zones may result in a false positive interpretation.

According to the results of our study, 76% of brain glial tumors were CD117 immunoreactive. This shows a remarkable frequency of CD117 expression in these tumors. Cetin *et al.* have also reported CD117 immunoreactivity in 75% of glial tumors.[24] Although various types of glial tumors showed CD117 expression in our study, the frequency of CD117 expression showed no statistically significant relationship with the tumor type. CD117 immunoreactivity was observed in 84% and 68% of high-grade and low-grade gliomas, respectively. However, the relationship between the frequency of CD117 expression and tumor grade was not statistically significant. Kristt *et al.*,[18] Went *et al.*,[30] Cetin *et al.*,[24] and Skardelly *et al.*[20] have shown that this marker is expressed in high-grade tumors more frequently than low-grade ones, but the study of Cetin *et al.* was the only one that obtained a statistically significant difference between the two groups.[24]

We realized that the intensity of staining in high-grade tumors is significantly higher than low-grade ones. This finding is similar to the obtained results from the studies of Kristt *et al.*,[18], Went *et al.*,[30] Cetin *et al.*,[24] and Mennel *et al.*[19]

Since high-grade glial tumors have poor prognosis despite progression in surgical procedures and adjuvant treatments, an important question is that whether or not patients with CD117 immunoreactive glial tumors benefit from targeted therapy with tyrosine kinase inhibitors (e.g., Imatinib). Successful treatment with tyrosine kinase inhibitors has been proven in some tumors such as GIST, acute myeloid leukemia (AML), and mast cell tumors in which the CD117 receptor has been activated by somatic mutations.[28] It should be considered that the CD117 immunoreactivity does not necessarily result from mutations in the coding gene. There is evidence of CD117 expression not related to gene mutations in thymic and gastrointestinal neuroendocrine carcinomas. Treatment with Imatinib has not been useful in these cases.[31,32] The same finding has been observed in malignant endocrine tumors with CD117 expression not related to gene mutations.[33]

CD117 receptor activation mechanisms in brain glial tumors have not been identified exactly and further researches should take place focusing on the molecular pathology aspects. Moreover, further studies with larger sample sizes are necessary to validate the results concerning the CD117 expression in various types and grades of glial tumors.

CONCLUSION

Although CD117 is frequently expressed in glial tumors, it does not seem to be a helpful marker in making distinction between various types of gliomas. Moreover, the frequency of CD117 expression does not show a statistically significant difference between the low-grade and high-grade gliomas. However, the intensity of CD117 expression as well as the percentage of stained cells shows difference between the low-grade and high-grade glial tumors, suggesting them as helpful parameters in making distinction between the low-grade and high-grade gliomas when determination of grade is not straightforward. According to the prominent expression of CD117 in high-grade gliomas, these tumors may be potential candidates for treatment with thyrosin kinase inhibitors.

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Authors' Contributions

PM designed the study and supervised it throughout the work. MJ provided assistance in the design of the study. The authors have actively participated in interpreting the results and preparing the manuscript.

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