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Original Article

Significance of Nestin and CD133 as cancer stem cell markers in diffuse glioma and association with p53 expression and IDH status

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Abstract: Background: Recent evidence suggests that the tumor stem cells that are responsible for the pathogenesis of gliomas have similar properties to those of neural stem cells. We have studied two of the most consistently expressed stem cell markers in gliomas, i.e., CD133 and Nestin, and compared them with respect to p53 expression and IDH status. Objectives: To assess the level of expression of Nestin and CD133, and identify a correlation among various grades of diffuse glioma with IDH status and expression of p53. Materials and Methods: A cross-sectional retrospective study with 102 subjects for the expression of cancer stem cell markers; CD133 and Nestin and the correlation of their expression with that of p53 and IDH1 status in adult diffuse glioma. The study was conducted in the Departments of Pathology and Neurosurgery. The expression was assessed by immunohistochemistry on formalin-fixed paraffin-embedded sections. The scoring of expression of CD133 and Nestin was adapted from *Zhang et al.* The scoring for p53 was adopted from *Aruna et al.* Results: The diffuse gliomas were graded based on WHO into grade II (30.3%), grade III (28.4%), and grade IV (41.3%). Among WHO grade IV, 59.4% were primary, and 40.4% were secondary glioblastomas. 73% of the diffuse gliomas were IDH mutant, and p53 showed an overall expression of 76.4%. The expression of CD133 and Nestin were compared with the increasing grades of diffuse gliomas, which, when plotted on ROC curves, had AUCs of 0.6806 and 0.6119, respectively. Their expression showed a positive correlation with the IDH status of the tumor. Conclusions: Cancer stem cell markers CD133 and Nestin are expressed in diffuse glioma and have a higher expression with increasing WHO grade of malignancy. These cancer stem cell markers have shown significant association with the IDH-1 mutant status of diffuse gliomas. Hence, it can be inferred that diffuse gliomas with a higher expression of CD133 and Nestin have a poorer prognosis. Further, these cancer stem cell markers may be used as therapeutic targets in the future.

Keywords: Diffuse glioma, cancer stem cell markers, neural stem cell markers, CD133, Nestin, p53, IDH-1, prognosis of diffuse gliomas

Introduction

Glial tumors are the most common primary tumors of the central nervous system within most grades of malignancy. In recent years, studies have led to drastic progress in diagnosing and treating glioma. However, the prognosis of glioma patients is poor [1]. The ability of these tumors to evade novel chemotherapeutic and radiotherapeutic agents is attributed to tumor heterogeneity [2]. The foremost treatment of glioma is surgery to diagnose the tumor and remove it from the brain. This is followed by chemoradiation; chemotherapy which can be concurrent, adjuvant, or concurrent and adjuvant; and radiotherapy which can be proton beam therapy, intensity modulated radiation therapy, three-dimensional conformal radiation therapy, or stereotactic radiosurgery. The plan of treatment depends on the age, performance status, tumor type, grade radiologically, and location of the tumor.

The Classification of Tumors of the Central Nervous System (CNS) given by the 2021 World Health Organization (WHO) classifies diffuse gliomas as adult and pediatric gliomas. Adult gliomas are further classified broadly as follows

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[3]: WHO grade II and grade III astrocytic tumors, WHO grade II and III oligodendrogliomas, WHO grade IV glioblastomas.

Evidence suggests that the tumor stem cells that are responsible for the pathogenesis of gliomas have similar properties to those of neural stem cells [4]. The terms 'stem cells' and 'cell of origin' have been used interchangeably.

Based on the cancer stem cell theory, special cells are responsible for the existence of tumors which have been called "cancer stem cells" (CSC). CSCs have similar characteristics to stem cells of embryonic origin, in particular self-renewal, uncontrolled proliferation, and multi-directional differentiation. Hence, when genetic mutations accumulate, any normal stem cell can be transformed into a cancer stem cell. In addition, anti-chemotherapeutic and anti-radiotherapeutic properties are also exhibited by cancer stem cells. These characteristics of cancer stem cells are the ones that are responsible for the evasion of a tumor to traditional treatments of chemotherapy and radiation. Consequently, CSCs are not eliminated effectively, leading to continuous proliferation and differentiation, resulting in residual tumor tissue and causing tumor recurrence [5].

A wide variety of immunohistochemical (IHC) markers are used to isolate and identify CSC in glioma patients. Of these, CD133 and Nestin are the most commonly used two markers with consistent expression in diffuse gliomas. They are, however, widely expressed in various tumors arising from glial tissue, liver, ovary, colon, and lung [2].

CD133, also known as Prominin-1, is encoded by the PROM1 gene. CD133 is a 5-transmembrane glycoprotein located mainly in the membrane of neural progenitor cells and human hematopoietic cells. It acts mainly by two pathways: AKT pathway and JNK signaling, which in turn leads to an increase in the ability of the cells to resist chemotherapy and stimulates cancer cell growth aiding in its self-renewal property, respectively [6].

Nestin is an intermediate filament (IF) protein that is expressed in the developmental stages of the embryo and fetus in a variety of tissues. In the cells of mature central nervous system cells, nestin expression is down-regulated. However, its expression is again observed in gliomas. Nestin, when expressed in gliomas, has been related to the dedifferentiation status, improved cell motility, invasive potential, and increased grade of malignancy [7]. However, they were not expressed in metastatic carcinomas. Moreover, with increasing grades of malignancy, the expression of Nestin showed an increasing trend [8].

p53 is a tumor suppressor gene with a protein mass of 53 KDa, popularly known as "the guardian of the genome" because of its property of prevention of genomic mutation. This gene is situated in the short arm of chromosome 17 and is composed of 11 exons. Almost all gliomas show a loss of p53 and are involved in both the initiation and recurrence of glioblastomas. Their expression, along with ATRX mutation, is attributed to the diagnosis of astrocytic tumors but not oligodendrogliomas. The prognosis of gliomas can be predicted based on the immunohistochemical expression of p53, which has been reviewed in a metanalysis by *Jin et al.* [9].

Zhang et al. proved that the expression of CD133 and Nestin concomitantly have stronger prognostic value than individual markers. The main reason for this is because these markers identify the level of differentiation of the tumor [4].

Further, our study also involves the correlation of these cancer stem cell markers, CD133 and Nestin, with the IDH-1 status of the glioma, which is an integral part of the molecular classification of diffuse gliomas.

Materials and methods

A prospective cross-sectional comparative study involving human tissue was conducted in the Departments of Pathology and Neurosurgery, JIPMER, from January 2016 to December 2019, after obtaining approval from the PG Research Monitoring Committee and Institute Human Ethics Committee. The study population is all cases of diffuse glioma diagnosed in the Department of Pathology during the study period fulfilling the WHO 2016 criteria for grades II, III, and IV.

Study procedure

A total of 102 cases were studied during the study period. All the cases of diffuse gliomas

Table 1. Scoring of p53 staining in tumor cells

	Staining	Interpretation
Score 0	No staining	Negative
Score 1+	<10% nuclear staining	Weak positive
Score 2+	10-30% nuclear staining	Moderately positive
Score 3+	>30% nuclear staining	Strong positive

Table 2. Reporting pattern of IDH-1

	Reported as	Interpretation
IDH-1 (Cut off-10%)	Positive	IDH mutant
	Negative	IDH wildtype

that were diagnosed in the Department of Pathology and treated in the Department of Neurosurgery at JIPMER, Puducherry were included in the study. We excluded pediatric diffuse gliomas, the cases that were given chemotherapy/radiotherapy, and the non-availability of blocks for immunohistochemistry.

Clinical parameters

Demographic data (age and gender), clinical symptoms, and radiologic data for the location of the tumor and characteristics of the tumor were taken from the database.

Laboratory data

Histopathologic examination

Tissue was received in 10% neutral buffered formalin. The tissue was subjected to standard processing protocols and embedded in paraffin. There were a few occasions where we received outside blocks for review, which were verified for formalin fixation and paraffin embedding. Sections 4 μm thick were cut using a rotary microtome and were then stained with routine hematoxylin and eosin.

Sections exhibiting increased cellularity with astrocytic morphology and mild to moderate nuclear pleomorphism, with no necrosis or microvascular proliferation were designated as astrocytoma. Tumors having increased cellularity with oligodendroglial morphology, i.e., round to oval nucleus with clear halo, fried egg appearance, presence of chicken wire capillaries, calcification with or without microvascular proliferation, and necrosis, were designated as oligodendroglioma. Tumors having morphology with overlapping features were classified as diffuse glioma on histology. The final diagnosis for these tumors was given only after a complete immunohistochemical panel. Tumors with moderate to marked nuclear pleomorphism with increased mitosis, microvascular proliferation, and palisading necrosis were designated as glioblastoma.

Immunohistochemistry (IHC)

Sections on silane-coated slides were used for immunohistochemistry with Ki67, IDH-1, p53, CD133, and Nestin. IHC was performed manually. Each slide was stained with primary antibody of anti-Ki67 (MIB-1; Dako, prediluted), anti-p53 (D07; thermo-scientific, prediluted), anti-IDH-1 (H09 (R132H); Dianova, prediluted), anti-CD133 (MD49R; Medyasis, prediluted), anti-Nestin (EP287; Medyasis, prediluted) as per the manufacturer's recommended protocol. Anti-CD133 and anti-nestin were utilized for analysis only after standardization in control tissue as well as test tissue. We used fetal brain tissue as the control for CD133 and nestin.

Interpretation of IHC markers

Ki67: It is a marker of proliferation calculated in the hotspots as a percentage of tumor cells showing nuclear positivity. One of the parameters of WHO grading depends on this index: (1) Grade II tumors - ≤4%; (2) Grade III tumors - 5-15%; (3) Grade IV tumors - ≥15%.

P53: It is an overexpression type of mutation seen in the TP53 gene. Immunohistochemical marker surrogates for this genetic mutation. The expression of p53 is scored based on nuclear positivity in the tumor cells as described in **Table 1** taken from *Aruna et al.* [10].

IDH-1: Mutation in the R132H region is detected by this antibody. Tumor cells having the mutation show cytoplasmic positivity. It is interpreted as positive when >10% of the tumor shows positivity as described in **Table 2**.

CD133: It is a cancer stem cell marker expressed in the tumor cells. The expression of CD133 is scored based on membranous positivity in the tumor cells as described in **Table 3** taken from Z*hang et al.* [4].

Nestin: It is a cancer stem cell marker expressed in tumor cells. The expression of CD133 is

Table 3. Scoring of CD133 staining in tumor cells

	Staining	Interpretation
Score 0	No staining	Negative
Score 1+	<30% membranous staining	Weak positive
Score 2+	30-60% membranous staining	Moderately positive
Score 3+	>60% membranous staining	Strong positive

Table 4. Scoring of Nestin staining in tumor cells

	Staining	Interpretation
Score 0	No staining	Negative
Score 1+	<30% cytoplasmic staining	Weak positive
Score 2+	30-60% cytoplasmic staining	Moderately positive
Score 3+	>60% cytoplasmic staining	Strong positive

scored based on membranous positivity in the tumor cells as described in **Table 4** taken from *Zhang et al.* [4].

Statistical analysis

The distribution of categorical variables such as gender, IHC markers, and site of biopsy was expressed as frequency and percentage. The continuous variables, such as age, were expressed as mean with standard deviation. The comparison of IHC markers with other categorical variables and histomorphology was carried out by using the Fisher exact test. The diagnostic accuracy of the IHC marker for subtyping the glioma in comparison with histomorphology was carried out by estimating sensitivity and specificity along with ROC curves plotted for each of the IHC markers. All the statistical analysis was carried out at a 5% level of significance, and $P<0.05$ was considered significant. We used the software Stata, version 17 for analysis.

Results

In the study period of three years, we received 117 cases of diffuse glioma, which were diagnosed based on histology and immunohistochemistry, of which 15 cases were excluded. Fifteen cases were excluded as they were pediatric gliomas [4], post-radiotherapy/post-chemotherapy [3], or due to non-availability of tissue blocks in old cases [8]. Histomorphology was studied for 102 cases, following which IHC was performed in these cases of diffuse gliomas. The clinicopathological characteristics of diffuse gliomas in our study and compiled in **Table 5**.

Sociodemographic data

Among glioblastomas, 17 (40%) were primary, and 25 (60%) were secondary glioblastomas. Most of the cases were in the age group 30-50 with the median age being 43 years. The grade 4 tumors were identified to be peaking in the age group 60-70 years. Male preponderance was noted with a male-to-female ratio of 1.75:1. Astrocytomas and glioblastomas had male preponderance, while oligodendrogliomas had an almost equal incidence in both genders.

Tumor location

Tumor location was determined by radiology. Most of the diffuse gliomas were located supratentorial, with the commonest location being the frontal lobe (41.8%). Glioblastomas were found to have a higher occurrence in the parietal lobe (19.1%), oligodendrogliomas were frequently encountered in the frontal lobe (51.6%); astrocytomas were frequently found in the frontal lobe and parietal lobe (29.1% each).

Ninety-four of the one hundred and two cases were single lesions that predominantly involved the right side (50.9%). Multiple lesions at presentation were encountered in about 8% of the diffuse gliomas, which were predominantly glioblastomas.

Immunohistochemistry: CD133 (Prominin-1), Nestin, IDH-1 (Isocitrate Dehydrogenase-1), p53

Overall, expression of CD133 was identified in about 54% of cases, and we arrived at a cut-off of score >1+ as significant ($P<0.001$). The overall expression of Nestin was positive in 95.1% of cases, and we arrived at a cut-off of score >1+ as significant ($P<0.001$). The overall expression of IDH-1 in our study was 71.5%. Glioblastomas, on the other hand, had a higher proportion of IDH-1 wild type (56.6%), which are classified under primary glioblastomas, and the IDH-1 mutant cases were accounting for about 40% were secondary glioblastomas. Expression (scoring) of CD133 and Nestin respectively in different WHO grades of diffuse gliomas and compared with IDH-1 status is depicted in the graphs of **Figure 1**. The ROC curve showing the

Table 5. Clinical and immunohistochemical findings in various grades of diffuse glioma

	WHO Grade II Diffuse glioma	WHO Grade III Diffuse glioma	WHO Grade IV Glioblastoma	WHO Grade IV Astrocytoma
Number of cases	31/102 (30.3%)	29/102 (28.4%)	25/102 (24.5%)	17/102 (16.6%)
Mean age at diagnosis (years)	41.63	44.01	54.54	49.88
Male-to-Female ratio	1.8:1	1.9:1	1.5:1	2.4:1
Histologic type of tumor	Astrocytoma - 17/31 (54.8%) Oligodendroglioma - 0/31 (32.2%) Others - 4/31 (9.6%)	Astrocytoma - 7/29 (24.1%) Oligodendroglioma - 21/29 (72.4%) Others - 2/29 (6.8%)	Glioblastoma - 25/25 (100%)	Astrocytoma - 17/17 (100%)
Clinical presentation (most common)	Headache and seizures	Hemiparesis	Hemiparesis	Headache and vomiting
IDH-1 status (>10% expression)	30/31 (96.7%)	26/29 (89.6%)	Negative (0/25)	Positive (17/17)
P53 expression (Score >2+)	16/31 (51.6%)	12/29 (41.3%)	15/25 (60%)	14/17 (82.3%)
CD133 expression (Score >1+)	8/31 (25.8%)	17/29 (58.6%)	23/25 (92%)	7/17 (41.2%)
Nestin expression (Score >1+)	29/31 (93.5%)	27/29 (93.1%)	25/25 (100%)	16/17 (94.1%)

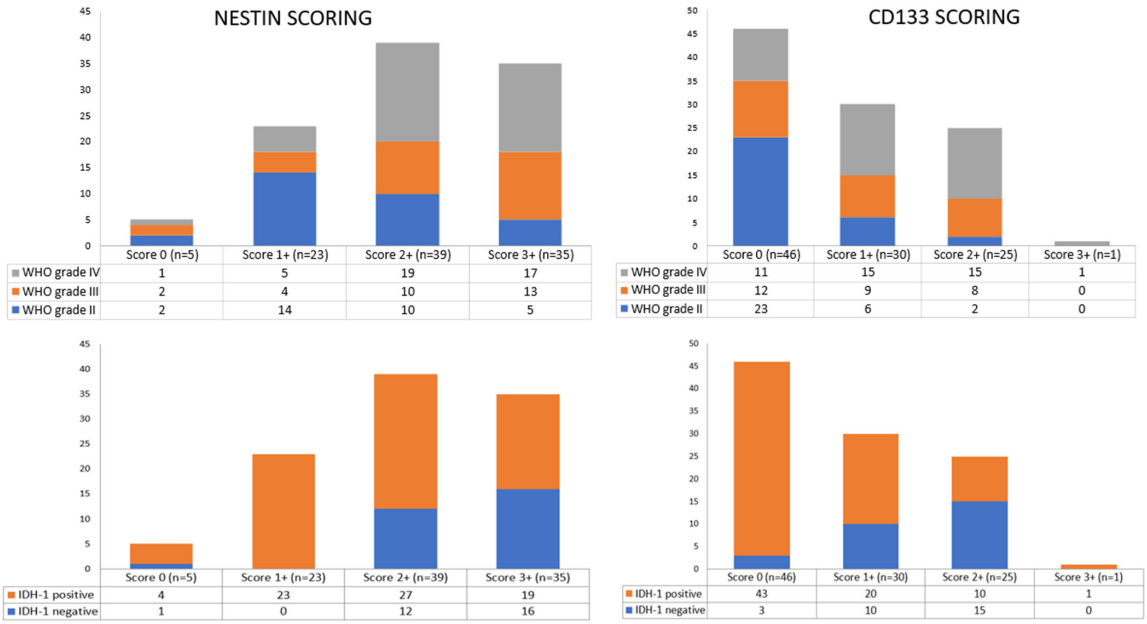

Figure 1. Expression (scoring) of CD133 and Nestin respectively in different WHO grades of diffuse glioma (n=102) and comparison with IDH-1 Status.

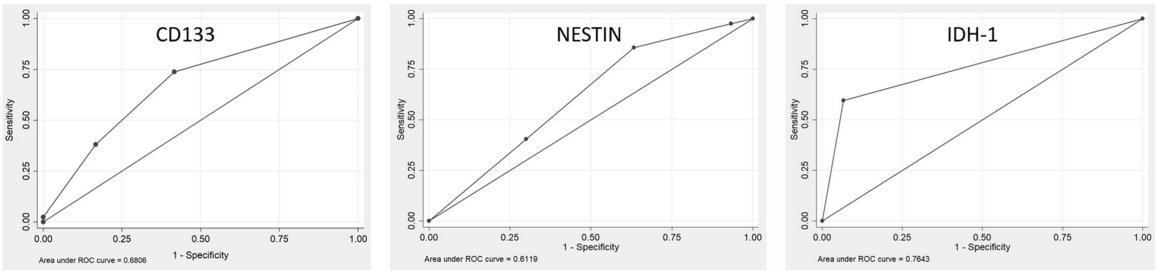

Figure 2. ROC curve showing the significance of CD133, Nestin expression, and IDH-1 status with WHO grade IV diffuse glioma.

significance of CD133, Nestin expression, and IDH-1 status with WHO grade IV diffuse gliomas is depicted in **Figure 2**. Nuclear localization of p53 had a significant association with tumors of astrocytic lineage. The overall expression of p53 was 76.4% (78/102). The cut-off score was 2+, which was expressed in more than 10% of tumor cells. The correlation of p53 expression with expression CD133 and Nestin was found not to be statistically significant. However, its expression showed statistical significance with an increasing WHO grade of malignancy.

Discussion

Cancer stem cell markers have been hypothesized to be the origin of diffuse glioma, which is why they has been recently studied extensively and have been mentioned in the recent 2021 WHO CNS tumors. The concept of cancer stem cell theory is depicted in **Figure 3**. The quoted study under the cell of origin in the chapter of glioblastoma about CD133 positive cells which have shown higher expression in primary glioblastoma than in secondary glioblastoma [7, 8].

Diffuse glioma is a heterogeneous tumor with unique gene expression profiles and defining cytogenetic events. It is important to stratify these tumors into subgroups to determine the treatment, prognosis, and overall survival. Various studies have shown molecular alterations such as IDH mutation and 1p/19q co-deletion, which have a better prognosis compared to tumors with IDH wildtype and non-co-deleted 1p/19q. There are several methods

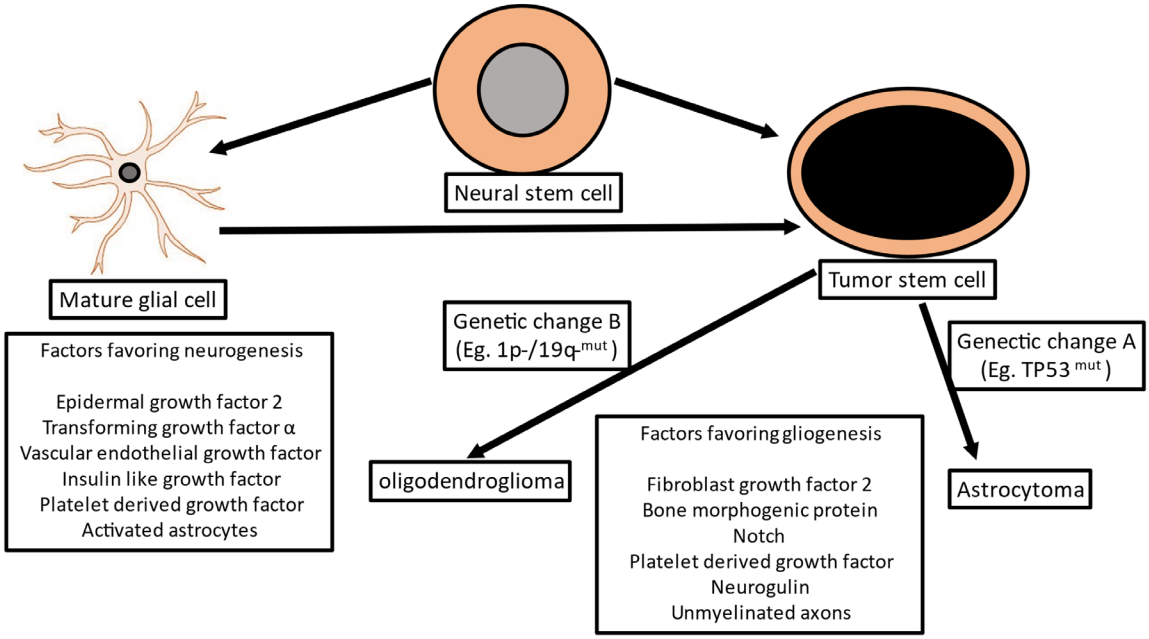

Figure 3. Cancer stem cell theory.

employed to categorize diffuse gliomas, which comprise FISH, IHC, and NGS. In our study, we used IHC to categorize these tumors.

The panel of IHC markers performed on the 102 cases collected was CD133, Nestin, IDH1, and p53. They were analyzed and compared with different WHO grades of diffuse gliomas, and WHO grade IV was considered to be the truth to all the different scores of CD133, Nestin, p53, and the IDH status. Following this, a ROC curve was plotted to represent the different scores of IHC markers with that of WHO grade IV diffuse glioma that had a significant area under the curve (AUC).

Almost all the patients had overlapping clinical presentations. Patients with glioblastoma had the most common presentation of headache (52.3%) and hemiparesis (38.1%). The most common presentations in oligodendroglioma were seizures (40.6%) and headache (43.7%), and hemiparesis and seizures were common in astrocytomas (39.1% each). The data relating to the distribution of age, gender, and location of tumors have been provided in the results. This data corroborated the distribution of diffuse gliomas in a population-based study done by *Larjavaara et al*. [11].

IDH-1 is a crucial molecular alteration in tumorigenesis described in various solid and hematologic malignancies. Our understanding of IDH and its role in the development of glioma has drastically improved over the years. It is pivotal in the diagnosis and prognosis of grade II and grade III diffuse glioma and for its role as a therapeutic target for which drugs like AG-120 and AG-122 are in clinical trials [12]. In-depth genomic analysis is seldom available in all institutions; hence immunohistochemistry is the method of choice for the detection of IDH. The frequency of IDH mutation is variable, ranging from 54% to 95%, as described by different studies [13-15]. Of these IDH-1 mutations, R132H is the most frequent and constitutes about 80-90%. We studied IDH-1 mutation by IHC in 102 cases. Overall positivity was 71.5%, with astrocytoma showing 91.6% and oligodendroglioma with 93.5% positivity. The expression in GBMs was only 40.4% which was classified under secondary GBMs. It was also noted that as the tumor grade increased, the positivity of IDH reduced, i.e., grade II (96.7%), grade III (89.6%), and grade IV (40.4%). As stated by Ruess et al., most of the IDH wildtype gliomas can be resolved into other tumor entities such as glioblastoma [16]. This can also be linked to the fact that IDH wild-type tumors have a poor prognosis. In such cases, evaluation of TERT promoter mutation will solve the problem since it is exclusive to GBM. An ROC curve was plotted with IDH mutant status of diffuse glioma

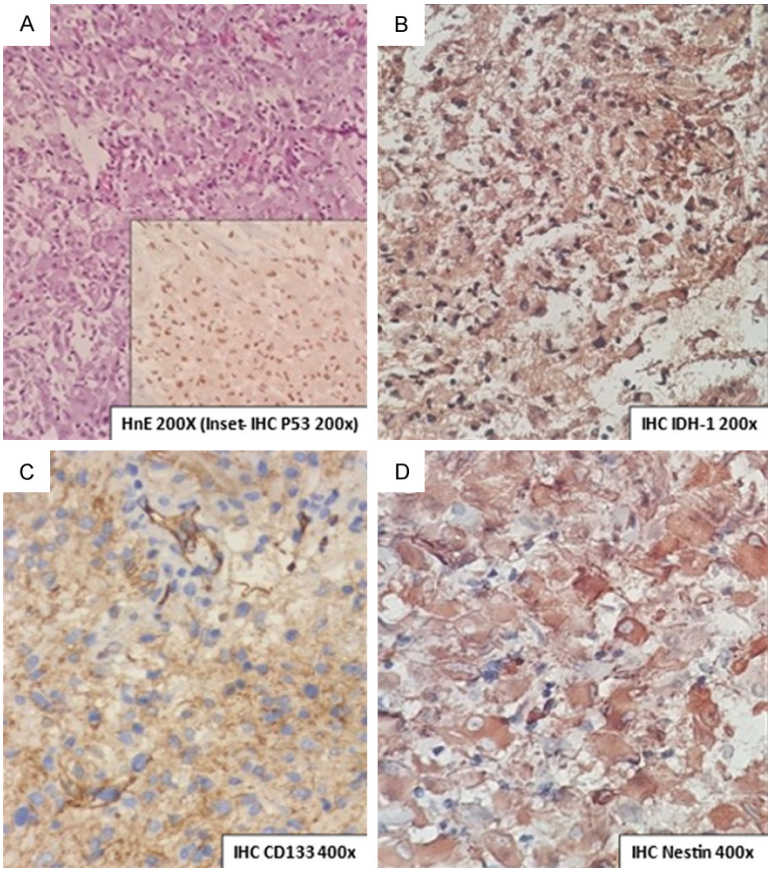

Figure 4. Case of gemistiocytic astrocytoma, WHO CNS grade 3. A. Biopsy showing >20% gemistiocytes with inset of p53 nuclear expression of >30% (positive). B. IHC with IDH-1 showing cytoplasmic positivity. C. IHC with Nestin showing 3+ cytoplasmic positivity. D. IHC with CD133 showing 2+ membranous positivity.

and WHO grade IV, which showed significance with an AUC of 0.7643. This proves the diagnostic utility of the test with a specificity of 93.3%.

Primary glioblastoma occurs *de novo*, and these tumors do not harbor IDH mutation. Distinctively secondary GBM occurs in the background of low-grade glioma, and these are usually IDH-positive. Three cases in our study were diagnosed as GBM after deeper sectioning of tissue and after immunohistochemistry. Two cases were primary GBM, and one case was diagnosed as secondary GBM (IDH-1 positive). We would like to highlight the importance of examining multiple sections with a focus on identifying areas of necrosis, particularly pseudo-palisading and microvascular proliferation. Further, IHC markers, i.e., p53 and our stem cell marker CD133, can be of value in differentiating primary and secondary GBMs.

After confirming the diagnosis of diffuse glioma, another marker for astrocytic lineage is the p53 mutation, which is an overexpression type of mutation. However, there is heterogeneity in the staining of p53, which can lead to misinterpretation. While it is specific for astrocytic lineage, a significant percentage of oligodendroglioma can also show p53 overexpression. In the literature, we found various cut-offs being described for overexpression of p53 ranging from 10 to 50% [17-19]. In our study, the overall expression of p53 was 76.4%, with a maximum expression at a score of 3+ (48.7%). At a score of 1+, which is a positivity of 10%, there was high sensitivity, accounting for 88.6%. However, the specificity was only 31.6%. The expression of p53 was higher in secondary glioblastoma (82.3%) compared to primary glioblastoma (60%).

The ROC curve was plotted with different scores of p53 and WHO grade IV diffuse gliomas and gave a significant AUC of 0.6141. Following this, we arrived at a cut-off of score 2+, which is a positivity of 10-30% that had a sensitivity of 69.5% and specificity of 53.03%, and a *P*-value of <0.001. Hence, we inferred that the p53 expression increased with an increasing grade of malignancy and showed a higher expression in secondary glioblastoma.

IHC markers for cancer stem cell markers were performed for CD133 and Nestin as they have been suggested as the most significant and consistent cancer stem cell markers compared to the other 27 CSC markers reviewed by Dahlrot et al. [20]. The examples of WHO CNS grade 3 and 4 tumors are shown in **Figures 4** and **5** respectively.

Recently, a study done by *Peng et al.* studied CD133 and SOX2 in glioblastomas in brain resection specimens and studied different

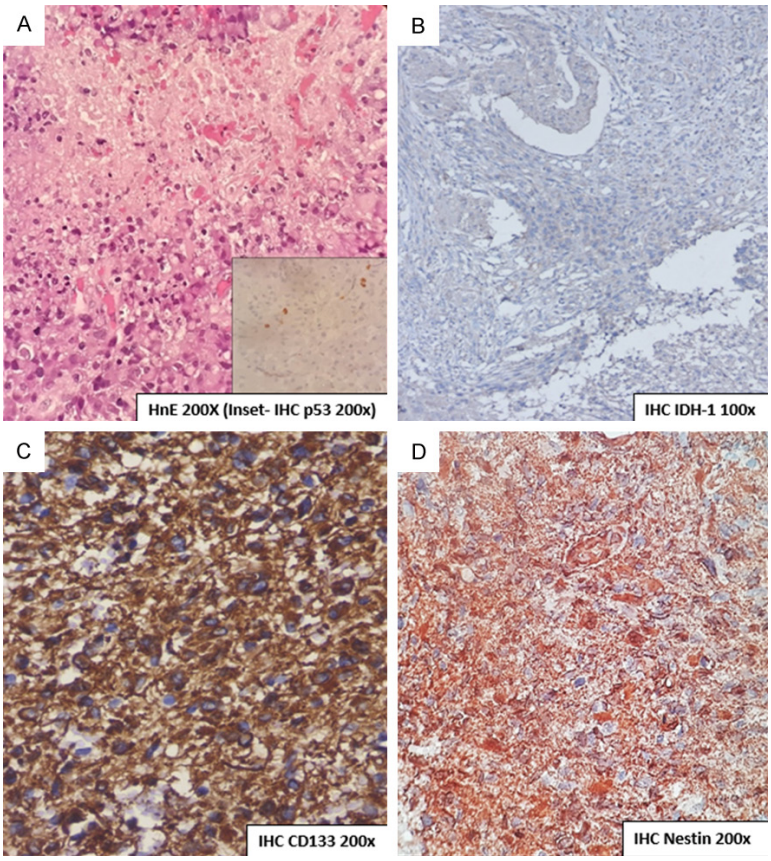

Figure 5. Glioblastoma, IDH wildtype, WHO CNS grade 4. A. Biopsy showing features of glioblastoma with pseudo-palisading necrosis inset of p53 nuclear expression of <10% (negative). B. IHC with IDH-1 is negative. C. IHC with Nestin showing 3+ cytoplasmic positivity. D. IHC with CD133 showing 3+ membranous positivity.

areas around the tumor for two patients. The results of the infiltrating tumor edge in patient 1 were 22.07±1.62 and 14.5±0.78% respectively of CD133 positive and SOX2 positive cells, respectively. The same in that of patient 2 were 16.03±1.29 and 11.24±0.76%, respectively [21]. This proved that the expression of CD133 is correlated with the increase in tumor invasion. Our study showed an increased expression in CD133, as the WHO grades of diffuse gliomas were increasing. The expression of CD133 in grades II, III, and IV were 25.8%, 58.6%, and 73.8%, respectively. The expression in astrocytoma was 33.3%, oligodendroglioma was 51.6%, and glioblastoma was 73.8%. Maximum expression was seen in glioblastoma. There was also a higher expression in primary glioblastomas (92%) compared to primary glioblastomas (41.2%).

The expression of CD133 was correlated with the expression of p53 and IDH-1 status to assess the association with overexpression of p53 and IDH-1 mutant status. A Fisher exact test performed between CD133 and IDH-1 had a strong power of correlation with a *P*-value of <0.001. However, the power of correlation done by Fisher's exact test did not show significance between CD133 and p53 expression with a *P*-value of 0.057. Hence, it can be inferred that CD133 increases as the grade of malignancy increases and will show a poorer prognosis. IDH-1 mutant cases (IDH-1 positive cases) have shown a significant correlation with CD133 expression. The expression of CD133 in glioblastoma also showed increased expression in primary glioblastoma (92%), which in turn again infers a poorer prognosis.

The latest studies on the expression of Nestin in diffuse glioma, of which one was done by *Hussam et al.,* showed the expression of Nestin in all diffuse gliomas to differentiate astrocytomas from oligodendrogliomas. They studied 16 astrocytomas and 12 oligodendrogliomas, including both WHO grades II and III, and inferred that the expression of Nestin was higher in tumors of astrocytic lineage, with the highest expression in glioblastoma [22]. This expression of Nestin was corroborated by our study showing the strongest intensity of expression in glioblastoma (97.6%). The intensity and expression of Nestin in diffuse glioma with increasing WHO grades, was grade II (93.5%), grade III (93.1%), and grade IV (97.6%). The expression of the highest intensity of score 3+, which is an expression of more than 60%, was found to be highest in grade IV diffuse gliomas accounting for 48.5%. Nestin expression in astrocytomas accounted for 96.7%, and that of oligodendrogliomas accounted for 91.6%. This also corroborated the previous studies.

The expression of Nestin in the fetal brain is replaced by intermediate filaments like GFAP as

neural differentiation happens [23]. However, during gliogenesis, the expression of Nestin is re-established and intensifies with higher grades of malignancy. Our study shows an overall expression of 95.1% of all the cases, along with showing an increasing trend with increasing grades of malignancy. This has also allowed us to use Nestin as a possible addition to the glioma panel as an adjunct to GFAP.

As previously discussed, ground truth, which was taken as WHO grade IV diffuse glioma, was compared with different scores for expression of Nestin, and a ROC curve was plotted for the same, which showed the statistical significance of an AUC of 0.6119 (p value =0.001). At a score of 1+, i.e., expression of <30%, the sensitivity was 97.62% but with a specificity of only 6.67% but with a score of 2+, i.e., expression of 30-60%, the sensitivity was 85.71% and specificity was 36.67%. Hence, we took a score of 2+ as the cut-off value with an expression of anything more than 30%. Nestin expression as scores was compared with the expression of p53 as scores and the IDH-1 status in the 102 cases of diffuse glioma. Fisher's exact test showed the power of correlation of expression of Nestin with p53 was not statistically significant with a p-value of 0.38. However, the power of correlation of Nestin with that of IDH-1 mutant cases was significant with a p-value of <0.001. Hence, it can be inferred that the expression of Nestin increases with an increasing grade of malignancy and has a significant association with the IDH-1 mutant status in diffuse glioma.

Hence, cancer stem cell markers used in our study have shown a significant correlation between increasing grade of malignancy and the IDH-1 status of diffuse gliomas. Based on this, we can infer a poorer prognosis for diffuse gliomas with higher expression of CD133 and Nestin.

Conclusion

Cancer stem cell markers CD133 and Nestin are expressed in diffuse glioma and have a higher expression with increasing WHO grade of malignancy. This study utilized a minimum panel of IHC markers IDH-1, p53, Ki67, CD133, and Nestin. The CSC markers, CD133 and Nestin, have shown significant associations with the IDH-1 mutant status of diffuse gliomas. However, they did not show significance when correlated with p53 overexpression. Hence, it can be inferred that diffuse gliomas with the higher expression of CD133 and Nestin have a poorer prognosis.

Further, these cancer stem cell markers have the potential to be used as therapeutic targets in the future [24-26].

Disclosure of conflict of interest

None.

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