



A STUDY OF VESSEL MORPHOLOGIES FOR GRADING OF ASTROCYTIC TUMOURS IN INTRAOPERATIVE SQUASH SMEAR PREPARATIONS

Pathology

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ABSTRACT

Introduction: Astrocytoma cause significant morbidity due to impairment of brain functions in all ages and with equal sex predilection. A simple procedure of intraoperative smear preparations leads us to diagnosis as well as grading of astrocytic tumors. Vascular features along with other cytological characteristics of tumor helps us to differentiate between low grade and high-grade astrocytoma. **Aims and Objectives:** To study the vessel morphologies in the cytological squash preparations of astrocytoma for intra operative diagnosis and grading and to study the morphological characteristics of astrocytoma on cytology. **Materials and Methods:** This was an observational study of 50 cases of astrocytoma. We studied the vessel morphologies along with cytological features on squash smear preparation (stained by hematoxylin and eosin) for diagnosis and grading of astrocytoma and correlated with histopathological diagnosis. **Observations and Results:** There were 29 cases of low-grade astrocytoma and 21 cases of high grade astrocytoma classified histologically according to WHO 2016 classification system. Mean vessel diameter and multiple branching of vessel showed significant difference between low grade and high grade astrocytoma (p value- 0.000). **Conclusion:** Vascular features along with other cytological features are helpful in grading of astrocytic tumor on intraoperative squash smear preparations.

KEYWORDS

Astrocytoma, Vessel Morphologies, Squash Preparations

INTRODUCTION

Gliomas, as the most common group of primary brain tumors, consist for about 30% of all tumors. They include astrocytoma, oligodendroglioma and ependymomas. One-third of all gliomas are astrocytoma.¹ Histological grading of astrocytoma ranges from WHO grade I to grade IV according to WHO classification 2016. Grade I and grade II are considered as low-grade astrocytoma and grade III and grade IV are considered as high grade astrocytoma.² The general cytological features of astrocytic tumors on squash smear are presence of cells with cytoplasmic processes with characteristics fibrillary background.³ Microvascular proliferation is a feature of high grade astrocytoma and it has been shown that vessels with more than 3 branches and mean vessel diameter more than 20µm can be assessed in intraoperative squash preparation in high grade astrocytoma cases.¹ The morphological analysis of other cytological features like nuclear shape, nuclear atypia, chromatin, nuclear enlargement, nuclear size, nucleolus, mitotic figures, necrosis and cell density also aid help in differentiating between low grade and high grade astrocytoma on squash preparations.¹

In our study we differentiated the high grade astrocytoma from low grade astrocytoma on the basis of vascular morphologies in intra operative squash preparation along with other cytological features.

MATERIALS AND METHODS

This study was conducted in the Department of Pathology, SMS Medical College and Attached hospital, Jaipur. Intra operative squash smear preparations of all the cases of astrocytomas and gliomas were examined and correlated with histopathological diagnosis on subsequent biopsy.

Intra operative squash smear was prepared in each case and hematoxylin and eosin staining was done.

Small 2-3mm sized tissue bits were taken on one end of the glass slide. Pressure was applied over tissue to crush it with the help of another glass slide. 3-4 smears were prepared for each case. The smears are then immediately immersed in 95% alcohol for fixation. Rapid hematoxylin and eosin staining was done. The fixed smears were dipped in hematoxylin for 3 minutes, washed in running water for bluing (approximately 30-40 seconds), 3 dip in eosin, dip in acetone, 2 dip in xylene and mounted with DPX.

The vessel morphologies along with cytological features on squash smear were noted.

Study of vessel morphologies included number of branching and mean vessel diameter. Vessel diameter(µm) was measured with the help of Motic live panthera series software at 10x10X magnification and mean was calculated using the maximum and minimum diameters of the vessels.

Statistical Analysis:

Quantitative variables were summarized as mean and standard deviation while nominal/categorical variables were presented as proportions (%). Parametric tests like student test and ANOVA were used for analysis of quantitative variables whereas Chi-square test/Fischer Exact test was used for nominal/categorical variables. p-value <0.05 was considered as significant. Data entered in MS excel and Epiinfo version 7.2.2.6 software used for all statistical calculation.

RESULTS

In our study 50 patients were included, 29 (58%) were diagnosed as low grade astrocytoma and 21 (42%) as high grade astrocytoma.

Vessels seen in the smears were analyzed and their diameter and branching was noted. The vessel diameter in astrocytoma ranged from 2.83µm to 89.05µm. Branching was also seen at 10x10X magnification. Vessels with atleast 3 branches were considered as multibranched vessels.

Table 1: Characteristics of tumor vessels

Tumour Vessel Characteristics	Low grade n (29)	High grade n (21)	Chi square value	p-value
Mean vessel diameter $\geq 20\mu\text{m}$	3	16	22.414	0.0001
Vessel branches ≥ 3	6	15	12.872	0.0003

Mean vessel diameter was significantly high in high grade astrocytoma as compared to low grade astrocytoma. We observed that in low grade astrocytoma, it was 14.7 µm and in high grade, it was 26.2 µm. Mean vessel diameter ≥ 20 µm was more prevalent in high grade than in low grade astrocytoma. In high grade astrocytoma, 15 cases out of 21 cases (71%) showed vessels with more than 2 branching whereas low grade astrocytoma did not show significant branching. There is statistically significant difference in branching of a vessel on squash smear in low grade and high grade astrocytic tumors. (p value- 0.0003). (Table 1)

Table 2: Discrepancy in cytology and histopathological grading of the cases

Cytological grading	Histopathological grading		Total
	Low grade	High grade	
Low grade	26	1	27
High grade	3	20	23
Total	29	21	50

$$\text{Accuracy} = \frac{\text{TP} + \text{TN}}{\text{TOTAL}} \\ = \frac{26 + 20}{50} = \frac{46}{50} = 92\%$$

Table 2 shows that 26 cases of low grade tumours were correctly

diagnosed in cytological findings. Similarly, 20 cases of high grade tumour were correctly diagnosed in cytological findings. Only 1 case of high grade was classified as low grade on cytology and 3 cases of low grade were diagnosed high grade on cytology. On calculation accuracy was 92%.

DISCUSSION:

In the present study, we studied cytology of 50 cases of astrocytoma with respect to characteristics of vessels along with other cellular features.

Squash preparation demonstrated the difference in tumor vessel characteristics between low grade and high grade astrocytoma.

In our study there is significant difference in branching of a vessel on squash smear in low grade and high grade astrocytoma (p value-0.000). S. Sato et al. study also found a significant difference between branching of vessels and astrocytic tumor grades (p value- <0.001).⁴

Fujita et al. study also revealed that the multiple branching was helpful to differentiate between high grade and low grade astrocytoma. (p value-<0.01).¹

We also studied the use of mean diameter of tumor vessels to distinguish low grade and high grade astrocytoma. Mean vessel diameter with a cut off of 20µm differed significantly between the both grades (p value- 0.000). S. Sato et al and Fujita et al study found a significant difference between mean vessel diameter and astrocytic tumor grades (p value- <0.001).^{1,4}

They also proposed that for a practical and quick intraoperative diagnosis, mean vessel diameter should be estimated whether it is >2.5 times the size of a red blood cell.¹

Histologic analysis of astrocytic tumors has demonstrated that vascular area and branches counts are increased in anaplastic astrocytoma and/or glioblastoma.^{5,6}

In our study, we were able to give accurate diagnosis in 92% cases. However, 3 cases of low grade astrocytoma were erroneously reported as high grade astrocytoma.

Low grade astrocytoma are mostly showing thin walled and without multiple branched vessels but pilocytic astrocytoma are highly vascular tumor having hyalinized, ectatic and glomeruloid blood vessels which leads to misinterpretations of these cases.⁷

CONCLUSION:

Vessel morphologies observed in cytological squash smear preparations are useful for grading astrocytoma. Specifically, the presence of multibranched (>3 branches) vessels and the presence of tumour vessels with a mean diameter more than 20µm, which is more than 2.5-fold the diameter of red blood cell, are useful distinguishing high grade from low grade astrocytoma, and thus cytological evaluation of vessels on squash preparations will greatly aid the intraoperative diagnosis of high grade astrocytoma with fair sensitivity.

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Conflict of Interest

There is no financial conflict of interest to declare for any of the authors in association with the publication of this manuscript.

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Author Contributions

All authors had access to the study data. contributed to the study concept and design, data collection, data analysis and interpretation, and drafting of the manuscript. Dr Sonu contributed to the data collection, data analysis and interpretation, and drafting of the manuscript. All authors reviewed and approved the final version of the manuscript before submission.

Supplementary Data-No other supplementary data is available.

Photographs

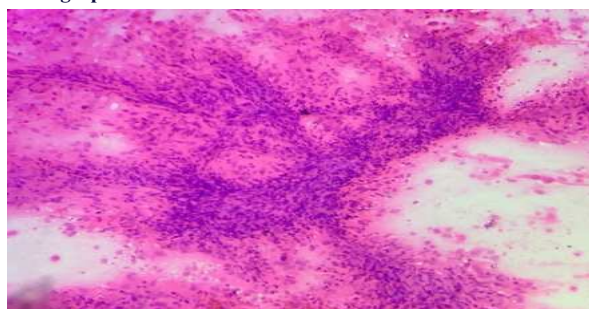


Figure 1: High grade astrocytoma showing thick vessel with multiple branching on squash smear preparation (10x10X)

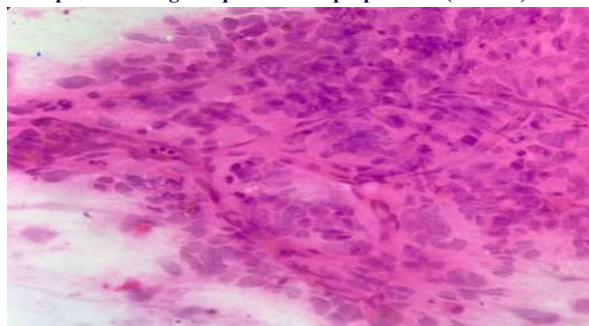


Figure 2: High grade astrocytoma showing multiple branching of a vessel on squash smear preparation (40x10X).

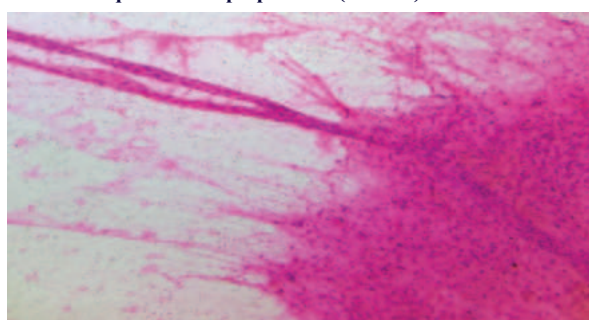


Figure 3: Squash smear preparation of low grade astrocytoma showing two branches of a vessel at (10x10X)

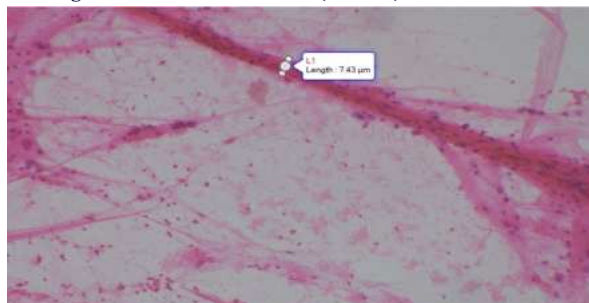


Figure 4: Picture showing vessel diameter at 10x10X magnification on squash smear preparation in a case of low grade astrocytoma.

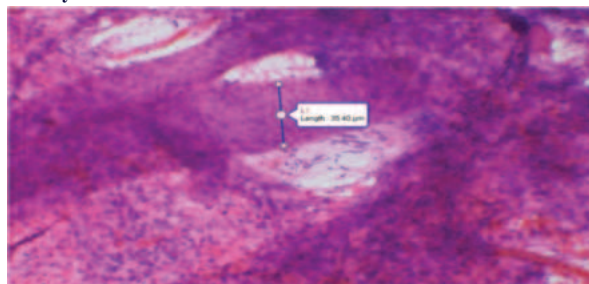


Figure 5: Picture showing vessel diameter in a case of high grade astrocytoma at 10x10X.

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