

# Intraoperative Squash Cytologic Features of Subependymal Giant Cell Astrocytoma

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## ABSTRACT

Subependymal giant cell astrocytoma (SEGA) is a low grade (WHO Grade I) tumor, usually seen in patients with tuberous sclerosis complex and commonly occurs at a lateral ventricular location. Intraoperative squash cytologic features can help in differentiating SEGA from gemistocytic astrocytoma (GA), giant cell glioblastoma and ependymoma, in proper clinical context and radiological findings, which may alter the surgical management. Here, we present a case of SEGA with squash cytologic findings and a review of cytology findings of SEGA presently available in the literature. Loose cohesive clusters of large polygonal cells containing an eccentric nucleus, evenly distributed granular chromatin, distinct to prominent nucleoli, and moderate to the abundant eosinophilic cytoplasm in a hair-like fibrillar background are the key cytologic features of SEGA. Other important features are moderate anisonucleosis and frequent binucleation and multinucleation. The absence of mitoses, necrosis, and vascular endothelial proliferation are important negative features. Other consistent features are cellular smears, few dispersed cells, few spindly strap-like cells, rare intranuclear cytoplasmic inclusion, and perivascular pseudorosettes.

**Key words:** Squash cytology, subependymal giant cell astrocytoma, tuberous sclerosis

## INTRODUCTION

Squash cytology is now well established as an intraoperative diagnostic technique for brain tumors and also an important adjunct to the frozen section.<sup>[1-4]</sup> Cytologic smears are better to visualize the fine nuclear and cytoplasmic details, which may be difficult to appreciate on frozen sections due to freezing artefacts.<sup>[1,2,5]</sup> The extent of surgery depends on squash cytologic diagnosis.<sup>[1-4]</sup> Cytologic features of subependymal giant cell astrocytoma (SEGA) have not been described sufficiently due to the relative rarity of the tumor. To the best of our literature search, we found only six literature describing the cytologic findings of SEGA in 19 cases.<sup>[1-6]</sup> Intraoperative diagnosis of SEGA is important, particularly if other clinical signs/symptoms of tuberous sclerosis

complex (TSC) are absent or overlooked.<sup>[1-6]</sup> Here we describe the squash cytologic finding of SAGE along with a relevant review of literature and discuss the differential diagnosis.

## CASE REPORT

A 12-year-old-male presented with a headache, generalized tonic-clonic seizures, and weakness. Physical examination revealed few pigmented spots on the forehead and reddish spots on cheek and nose. Brain magnetic resonance imaging, revealed a 3.5 cm × 3 cm × 3 cm sized well-defined abnormal signal intensity lesion in the frontal horn of left lateral

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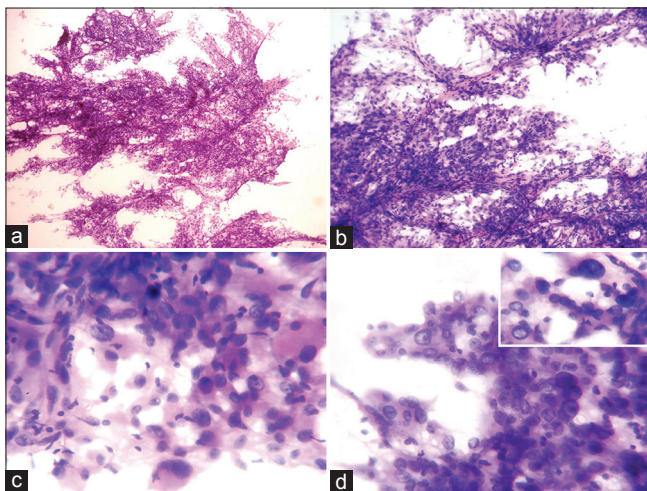
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ventricle with mass effect. The mass lesion appeared hypointense in the T1-weighted image with homogenous postcontrast enhancement. Possibilities of central neurocytoma, ependymoma, and SEGA in a TSC have been raised. The patient was operated to relieve pressure symptoms of the tumor.

The specimen was sent for intraoperative squash cytology. Hematoxylin and eosin stained cytology smears showed predominantly loosely cohesive clusters of tumor cells, arranged around capillary vessels and few dispersed cells in a faint thin hair-like fibrillar background in the direction of smear. Large pleomorphic tumor cells showed large round to elongated eccentrically located nuclei, even granular chromatin, occasional distinct nucleoli, and moderate to the abundant well-defined granular eosinophilic cytoplasm, mimicking ganglionic/gemistocytic appearance. Occasional tumor cells showed parallel located nucleus. Few scattered spindly cells showed elongated nucleus looks like strap cells. Few tumor cells were giant bizarre in appearance but with even nuclear chromatin. Few binucleation and multinucleation in tumor cells were seen. Occasional intranuclear inclusions were seen. Mitoses, necrosis or endovascular proliferation was not evident in the smear [Figure 1]. In view of clinical details and radiological findings, diagnosis of SEGA was made.

Subsequent histopathological examination of the remaining tissue showed predominantly sheets of large polygonal cells with moderate to the abundant

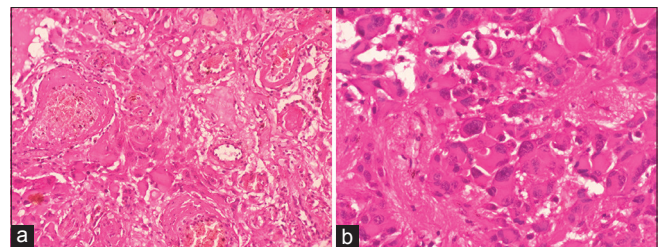


**Figure 1:** Cytology (H and E): (a) Loose cohesive cell clusters with many thin vascular capillaries and few dispersed cells (×40). (b) Variable sized tumor cells aligned to the vessels with the meshing of thin hair-like processes, along the direction of squash smear (×100). (c) Pleomorphic tumor cells show eccentric large atypical nuclei, fine granular chromatin, occasional distinct nucleoli and abundant granular eosinophilic cytoplasm (×400). (d) Few binucleation and multinucleation in tumor cells are seen. Inset figure highlights a giant bizarre tumor cells and intranuclear inclusion (×400)

well-defined cytoplasm with many variable sized blood vessels in a fibrillar background. The nucleus of the cell showed even granular chromatin and occasional prominent nucleoli. Frequent giant bizarre cells, binucleation, and multinucleation in tumor cells are seen. Mitoses, necrosis or endovascular proliferation was not evident [Figure 2]. Histologic findings confirm the diagnosis of SEGA.

## DISCUSSION

SEGA is a slow growing WHO Grade I tumor; that is classically seen in TSC.<sup>[2,4,5,7,8]</sup> SEGA mostly occurs in the first two decades of life, with a slight male preponderance.<sup>[1,7,9,10]</sup> It typically arises from the medial portion of lateral ventricle near the foramen of Monro. Rarely, the tumor may arise from the third ventricle.<sup>[2-6,8]</sup> Patients commonly present with seizures and symptoms of raised intracranial pressure such as a headache, vomiting, and visual disturbances.<sup>[1,5-11]</sup> Though SEGA is typical of TSC, sometimes it may be initially presenting neoplasm or often clinical findings may be overlooked or even SEGA may occur in the absence of TSC.<sup>[1,6,8,12]</sup> In such instances, pathologist needs to be aware of the cytologic features of SEGA and its differential diagnosis. SEGA is generally curative with complete resection.<sup>[7-9,11]</sup> Cytologic findings of the present case and its comparison with previously reported cases have been presented in Table 1.<sup>[1-6]</sup> Loose cohesive clusters of large polygonal cells containing an eccentric nucleus, distinct to prominent nucleoli and moderate to abundant eosinophilic cytoplasm in a thin/thick hair-like fibrillar background are the key features of SEGA. Other important features are moderate anisonucleosis but evenly distributed granular nuclear chromatin and frequent binucleation and multinucleation. The absence of mitoses, necrosis, and vascular endothelial proliferation are important negative features. Other consistent features are cellular smears, few dispersed cells,



**Figure 2:** Histology (H and E): (a) Large tumor cells are closely intermingled with variable sized blood vessels (×200). (b) Tumor cells are large, round to polygonal, displaying eccentric pleomorphic nuclei, fine granular chromatin, occasional prominent nucleoli and abundant eosinophilic cytoplasm with a well-defined border. Binucleation and multinucleation in tumor cells with fibrillary areas are seen (×400)

**Table 1: Cytologic findings of the present case and its comparison with previously reported cases of subependymal giant cell astrocytoma**

Cytologic features	Present case	Takei <i>et al.</i> <sup>[1]</sup> (7 cases)	Jaiswal <i>et al.</i> <sup>[2]</sup> (4 cases)	Ahluwalia and Chandrasoma <sup>[3]</sup> (1 case)	Kim <i>et al.</i> <sup>[4]</sup> (3 cases)	Azarpira <i>et al.</i> <sup>[5]</sup> (1 case)	Altermatt and Scheithauer <sup>[6]</sup> (3 cases)
Cellularity in smears	Cellular	Hyper-cellular	Cellular	NM	NM	Hyper-cellular	Cellular
Cohesiveness							
Dispersed cells	Few +	NM	Some +	Few +	NM	NM	NM
Loosely cohesive cell clusters	++	+	++	+	+	+	+
Perivascular cells arrangements	+	+ Occasional rosette formation	NM	NM	NM	NM	+ Occasional rosette formation
Background							
Fibrillar	+	+	-	NM	NM	NM	NM
Thin/thick hair like processes from cell border	+	+	+	NM	+ (in direction of smear)	+	+
Shape of cells							
Large round to polygonal	++	++	++	NM	++	++	++
Spindly strap-like cells	Few +	Some +	Few +	Some +	+	Few +	+
Nuclear: Cytoplasmic ratio	Low	Low	Low	NM	Low	NM	1:1-1:3
Cytoplasm							
Eosinophilic granular	+	+	+	+	+	+	+
Moderate to abundant	+	+	+	+	NM	+	+
Well defined cell borders	+	NM	+	NM	NM	NM	+
Nucleus							
Eccentric round to oval nucleus	+	+	+	+	+	+	+
Nuclear membrane	Smooth and regular	Smooth and regular	NM	NM	NM	NM	Thickened
Even granular chromatin	+	+	+	NM	+	+	+
Nucleoli	Distinct to prominent in some cells	Single or multiple small prominent	Conspicuous to prominent	NM	Distinct	NM	Single or multiple
Binucleation and multi nucleation	+	+	+	+	+	NM	+
Anisonucleosis	++	++	+	+	+	+	+
Other features							
Mitoses	-	Rare but not atypical	-	-	Rare	-	Rare
Necrosis	-	-	-	NM	NM	-	-
Endothelial proliferation	-	-	-	-	NM	NM	-

NM: Not mentioned, -: absent, +: present, ++: predominantly present

few spindly strap-like cells, rare intranuclear cytoplasmic inclusion, and perivascular pseudorosettes.<sup>[1-6]</sup>

SEGA should be differentiated from GA, giant cell glioblastoma, and ependymoma on cytologic ground.<sup>[1,2,4,5]</sup> GA usually occurs in the brain parenchyma in contrast to the intraventricular location of SEGA. Common age for presentation of GA is the fourth decade rather than a first or second decade of SEGA. GA shows nuclear atypia, mitoses, circumferential hair-like processes (rather than straps), vertical arrangement of the nucleus in relation to the cytoplasm and absence of cohesiveness. The tumor also lacks striking cell to cell variability and multinucleation of SEGA.<sup>[2-6]</sup> Giant cell glioblastoma can be reliably differentiated from SEGA based on its cytological features such as lack of cohesiveness, pronounced nuclear pleomorphism, atypia, mitoses, necrosis, and vascular endothelial proliferation.<sup>[1,4,6]</sup> Ependymoma may mimics SEGA based on clinical details like patient's age (first or second decade), intraventricular location, and cytologically by generating

cohesive clusters of tumor cells. However, cytologic features of ependymal cells are completely different from SEGA. Ependymoma shows small sized tumor cells with the absence of multinucleation and characteristic true/pseudorosette with the eosinophilic fibrillar area in between capillary lumen and neoplastic cells.<sup>[1,6]</sup> Rarely, SEGA can show worrisome/anaplastic features such as necrosis, microvascular proliferation, and high mitotic activity, which may mimic malignant gliomas and better termed as atypical SEGA.<sup>[7,8,10,11]</sup> Atypical SEGA should not equate with aggressive behavior.<sup>[10]</sup> However, some atypical SEGA can show more aggressive behavior and requires longer follow-up.<sup>[7,8,11]</sup> Cytologic features of atypical SEGA are not described in the literature till date. In our opinion, smear cytology probably unable to distinguish atypical SEGA from malignant gliomas. Rare SEGA may show recurrence after surgical excision.<sup>[10]</sup> Occasional recurrence composed of predominantly blood vessels.<sup>[9]</sup> Rare cases may show intracerebral and spinal cord metastasis.<sup>[11]</sup> On immunohistochemistry, SEGA shows dual positivity for a neuronal and astrocytic

markers such as synaptophysin, S-100, neurofilament, neuron-specific enolase, and glial fibrillary acidic protein.<sup>[8,10,13]</sup> In spite of this, in the recently revised WHO classification of the central nervous system tumors, SEGA has been still included in the group of astrocytomas.<sup>[13]</sup> High frequency of BRAF<sup>V600E</sup> in SEGAs may be useful in the differential diagnosis, and also offers a potential specific treatment targeting BRAF<sup>V600E</sup>.<sup>[14]</sup> Thyroid transcription factor-1 and epithelial membrane antigen expression are seen in SEGA.<sup>[15]</sup> Ki-67 index is usually low (ranged from 0% to 8%). P53 immunoreactivity in nucleus ranged from 1% to 7.3%.<sup>[8,10]</sup> Testing for the mutation of TSC1 and TSC2 genes may be helpful when a patient does not fulfill criteria for the definite diagnosis.<sup>[8]</sup>

### CONCLUSION

SEGA can be reliably differentiated from its close mimickers based on cytology, even when clinical findings of TSC are not present or just subtle. Loose cohesive clusters of large polygonal cells containing an eccentric nucleus, distinct or prominent nucleoli and moderate to the abundant eosinophilic cytoplasm in a hair-like fibrillar background are the key features of SEGA. Other important features are moderate anisonucleosis but evenly distributed granular nuclear chromatin and frequent binucleation and multinucleation. The absence of mitoses, necrosis, and vascular endothelial proliferation are important negative features. Other consistent features are cellular smears, few dispersed cells, few spindly strap-like cells, rare intranuclear cytoplasmic inclusion, and perivascular pseudorosettes.

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### Conflicts of interest

There are no conflicts of interest.

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