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Subependymal Giant Cell Astrocytoma – a Clinicopathological Study of 23 Cases with Special Emphasis on Histogenesis

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Subependymal giant cell astrocytomas (SEGAs) are relatively rare tumors but occur commonly in the setting of the familial syndrome of tuberous sclerosis complex (TSC). In view of its varied morphology, i.e. resemblance to astrocytic and ganglion cells, its histogenesis remains controversial. We studied 23 cases of SEGA, 19 from our own institute and 4 from NIMHANS, Bangalore. These 19 cases of SEGAs were collected over a period of 23 years (1979 to 2001), and accounted for 0.16% of intracranial tumors and 0.51% of all gliomas reported at our center. The majority of patients presented with visual disturbances (19/23, 82.6%) in the form of decreased vision (60.8%) and blindness (21.7%), generalized tonic clonic seizures (43.4%) and focal motor seizures (4.37%). Age ranged from 4 to 37 years (mean 13.2 years) with male predominance (M:F 2.2:1), and the duration of symptoms varied from 1 month to 96 months (mean 17.2 months). Lateral ventricular involvement was the most common site (91.3%), followed by the third ventricle (8.6%). Nine patients (39.1%) had stigmata of tuberous sclerosis (6 at the time of diagnosis and 3 in the follow-up period). Two patients died due to surgical complications, while the rest were alive and well in the follow-up period ranging from 3 to 264 months (mean 37.1 months). Two patients experienced recurrences, one two years and another 22 years after surgery. Microscopic examination showed varied histology consisting of sweeping bundles of spindle cells, gemistocyte and ganglion-like cells with interspersed inflammatory cell component. The inflammatory cell component on special staining turned out to be an admixture of mast cells and T lymphocytes. Six cases showed areas of necrosis and/or mitosis, but were not indicative of aggressive nature of this tumor. Immunoreactivity for GFAP, NF, S-100, NSE and synaptophysin indicates that this is a hybrid tumor with glial and neuronal differentiation. None of the tumors was immunopositive for HMB-45. The significance of the presence of T lymphocytes and mast cells is not clear. It could be related to tumor immunology and may indicate a favorable prognosis. (Pathology Oncology Research Vol 10, No 4, 219–224)

Keywords: tuberous sclerosis, SEGA, epilepsy, lateral ventricle tumor, immunohistochemistry

Introduction

Subependymal giant cell astrocytoma (SEGA) is a tumor that typically occurs in the lateral ventricle near the foramen of Monro and rarely in the third ventricle. The association of this tumor with tuberous sclerosis complex (TSC) is well known,[1] but cases in its absence are also reported in literature.[2,3] Shepherd et al[1] reported 6% incidence of SEGA in TSC patients. In tuberous sclerosis this tumor is thought to evolve from the enlargement of the hamartomatous subependymal nodule.[4,5]

The histogenesis of this tumor is poorly understood. Previous studies have reported glial (astrocytic or rarely ependymal), neuronal or mixed glial-neuronal differentiation. These hypotheses are based upon studies of small numbers of cases, except for a few reports that studied a

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slightly larger number of cases.[3,6] The astrocytic nature is based on reactivity for glial fibrillary acidic protein (GFAP) and S-100 protein, as well as ultrastructural demonstration of intermediate filaments, presence of Rosenthal fibers and junctional complexes with capillary basement membrane.[3,7-10] Ependymal differentiation is based on perivascular rosetting and intracytoplasmic lumina.[8,11] The presence of large ganglion-like cells and their immunoreactivity for neuron-specific enolase (NSE), neurofilaments (NF),[4,12,13] and ultrastructural features like neurosecretory granules or synapse-like structures indicate the neuronal nature of this tumor.[7,14] However, immunopositivity for synaptophysin has not been reported. Recently, a number of other tuberous sclerosis-associated lesions, including pulmonary and uterine lymphangiomyomatosis,[15,16] renal angiomyolipoma[17] and cardiac rhabdomyoma[18] were shown to stain with monoclonal antibody HMB-45, an antibody directed against melanosomal matrix protein gp100, suggesting a common origin of these tumors.

Hence this study was undertaken to study the immunohistochemical profile of this tumor along with the characteristics of the accompanying inflammatory component.

Materials and Methods

All cases of intraventricular tumors diagnosed as subependymal giant cell astrocytomas were reviewed in the Department of Pathology of our institute. Nineteen patients were clinically worked up and operated upon at the Department of Neurosurgery of this hospital. Four patients were operated and followed up in another institute (NIMHANS, Bangalore) in South India. All specimens had been fixed in neutral buffered formalin, routinely processed into paraffin and assessed on hematoxylin and eosin (HE) stained sections to confirm the diagnosis of SEGA. Histological sections were examined independently as well as together by three pathologists (MCS, CS and AMR). Five-µm-thick sections were cut from paraffin blocks and immunohistochemical staining was performed using streptavidin-biotin peroxidase method with antisera against glial fibrillary acidic protein (GFAP, 1:1000), synaptophysin (1:50), neurofilament 68 kDa (NF) (prediluted), chromogranin (1:100), neuron-specific enolase (NSE), S-100 protein (1:100) and HMB-45 (prediluted). To characterize the accompanying inflammatory component, immunostaining for leukocyte common antigen (LCA, 1:100), B-cell marker (CD20, 1:100) and T-cell

Table 1. **Clinical features of SEGAs**

Age (years)/ sex	*Site*	*Duration of symptoms (months)*	*Symptoms*	*Assoc. TS*	*Type of surgery*	*Follow-up (months)*	*Necrosis/ mitosis*
14/M	RLV/FM	84	GTC, B/L blindness	+	Total	12	–/–
8/M	FM, 3rd ventricle	4	Decreased vision	–	Total	12	–/–
20/M	RLV	24	Decreased vision	–	Subtotal	Died	–/–
10/F	LLV	18	GTC, decreased vision	+ at FU	Subtotal	20	–/–
14/F	RLV	6	B/L blindness decreased vision	–	Subtotal	Died	–/–
4/F	RLV/FM	8	GTC	+	Total	36	+/+
11/M	RLV/FM	96	FMS, decreased vision	+ at FU	Total	66	–/–
10/M	RLV/FM	3	GTC, decreased vision	–	Subtotal	48	–/–
13/M	LLV/FM	3	Decreased vision	–	Total	60	–/–
10/M	RLV/FM	6	Decreased vision	–	Subtotal	72	–/–
12/M	RLV/FM	18	GTC, decreased vision	+ at FU	Total	18	–/–
37/F*	LLV	4	GTC	–	Subtotal	264	–/+
7/F	LLV	12	GTC, decreased vision	+	Subtotal	3	–/–
6/M	LLV	4	B/L blindness	–	Subtotal	24	+/–
15/F	LLV	12	Decreased vision	–	Subtotal	12	–/–
21/M	RLV	30	Decreased vision	–	Subtotal	30	–/+
7/F	RLV	24	GTC, Lt. eye blindness	+	Subtotal	24	–/–
11/M	RLV	2.5	B/L blindness	–	Total	30	–/–
19/M	RLV	1	Decreased vision	–	Total	3	–/–
26/M	LLV	6	Decreased vision	–	Subtotal	20	–/–
10/M	LLV	8	GTC	+	Subtotal	48	–/+
4/M*	LLV	10	Decreased vision	–	Total	30	+/+
15/M	RLV	15	GTC	+	Total	24	–/–

* Recurrence, *M*: Male, *F* : Female, *RLV*: Right lateral ventricle, *LLV*: Left lateral ventricle, *TS*: Tuberous sclerosis, *GTC*: Generalized tonic clonic seizure, *FU*: Follow-up, *FM*: Foramen of Monro, *FMS*: Focal motor seizures, *B/L*: Bilateral

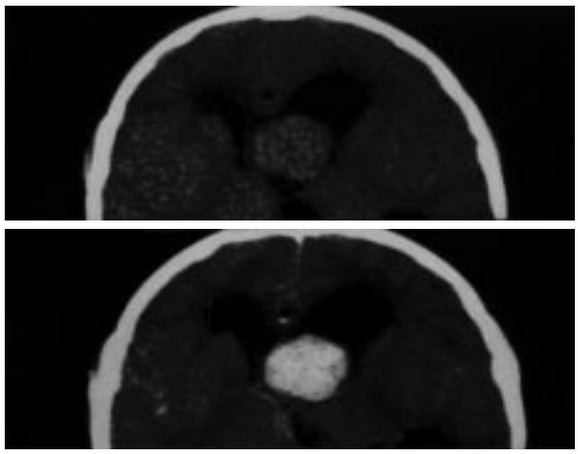

Figure 1. *CT scan showing isointense mass in the lateral ventricle, which is homogeneously enhancing on contrast injection.*

marker (CD3, 1:100) was done. All antibodies were obtained from Dako, Denmark. Appropriate positive and negative controls were made for each immunolabeling. Microwave processing was used for retrieval of synaptophysin antigen. For mast cells, Giemsa and toluidine blue stains were used.

Results

Clinical features

During the period of 23 years (1978 to 2001), a total of 11,437 intracranial tumors were diagnosed at the Department of Pathology of this Institute, of which 3670 were intracranial gliomas and 19 were subependymal giant cell astrocytomas (excluding 4 referral cases). SEGAs comprised 0.16% of all intracranial tumors and 0.51% of all gliomas.

Clinical features are shown in *Table 1*. Age ranged from 4 to 37 years (mean 13.2 years) with male preponderance (2.2:1). All patients were in the first and second decades of life except 3 patients. The duration of symptoms varied from 1 month to 96 months (mean 17.2 months). The most common symptoms were visual disturbance (19/23, 82.6%) in the form of decreased vision (60.8%) and blindness (21.7%), followed by generalized tonic clonic seizures (43.4%) and focal motor seizures (4.37%). Five patients (21.7%) were blind at the time of presentation. Nine patients (39.1%) were diagnosed to have tuberous sclerosis; six (26%) at the time of presentation and three (13%) during the follow-up period at 20, 65 and 120 months, respectively. Four out of 9 patients with tuberous sclerosis (44.4%) had mental retardation. Adenoma sebaceus were observed in 4 patients. The most common location was lateral ventricle (21 cases, 91.3%) (*Figure 1*). Right lateral ventricle was involved in 12 cases and left lateral ventricle in 9 cases. In two cases, the tumor was predominantly in the third ventricle (8.6%). In 2 patients who presented before CT scan availability, only angiography was performed, but the rest of the patients had either CT scan or MRI. CT scan showed isodense mass with uniform enhancement on contrast injection (*Figure 1*). MRI scan revealed isointense mass lesion on T1WI, which were hyperintense on T2WI. Cortical tubers were observed in 3 patients and subependymal nodules (candle gutterings) in 5 patients at the time of diagnosis of SEGA.

Follow-up

Two patients died in the immediate postoperative period. For the rest, follow-up ranged from 3 to 264 months (mean 37.1 months). Two patients had recurrence at 2 years and 22 years after first operation, respectively.

Pathological examination

Histological features in all cases were similar irrespective of their association with tuberous sclerosis. Microscopic examination showed mainly three components. Some areas showed sweeping bundles of fibrillated spindle-shaped cells without merging onto the blood vessels. The other component was composed of swollen gemistocytic cells with eosinophilic cytoplasm and distinct cellular boundaries. Admixed with these components were ganglion-like giant cells with coarse chromatin and vesicular nuclei (*Figure 2*). Blood vessels were thickened and hyalinized in 11 cases but none of them showed endothelial proliferation. Small areas of necrosis were seen in 3 cases and mitoses in the range of 1-3 per 10 hpf were observed in 4 cases. Thus, mitosis and/or necrosis were seen in 5 cases. Amidst these cells were inflammatory mononuclear cells. Immunohistochemical staining for GFAP and S-100 protein showed positivity in all cases, varying from focal to diffuse positivity. It was more strongly positive in the spindle cells than in other components. NSE and NF positivity was observed in 15 cases each. It was observed in all three components. Synaptophysin positivity was observed in 3 cases in the ganglion-like cells, which were also GFAP positive. All tumors were negative for chromogranin and HMB-45. Some of the inflammatory cells showed metachromatic granules on Giemsa and toluidine blue staining, indicating that these cells were mast cells, which were seen in all cases (100%). The other inflammatory cells were positive for LCA and were predominantly of T-immunophenotype (*Figure 3*).

Discussion

SEGA is the most common intracranial tumor found in TSC,[1] others being renal angiomyolipoma, cardiac rhabdomyoma, lymphangiomyomatosis of the uterus and lung,

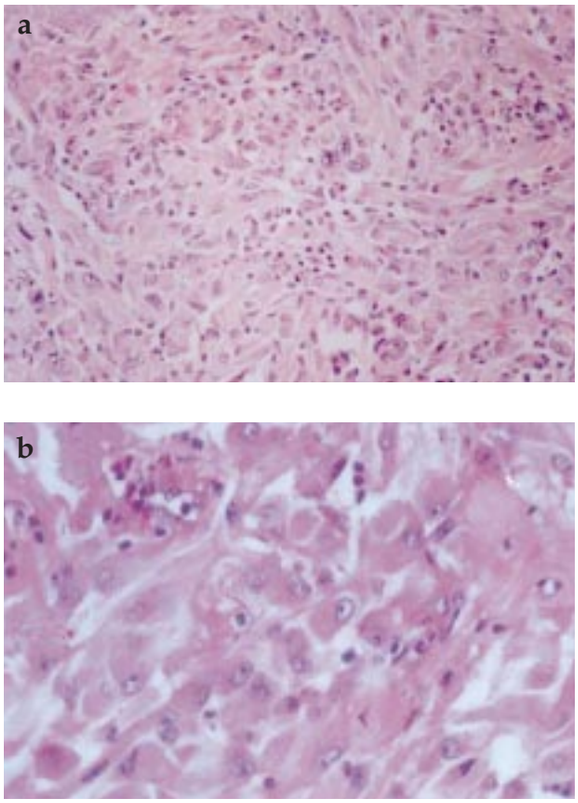

Figure 2. (a) *Photomicrograph showing admixture of fibrillary astrocyte, gemistocyte and ganglion-like cells with interspersed inflammatory component (HE, x40).* ***(b)*** *Higher magnification showing large cell with eosinophilic cytoplasm and vesicular nucleus with prominent nucleolus and sprinkling of inflammatory cells (HE, x200).*

and facial angiofibroma. Rare malignant tumors that occur in TS are renal cell carcinoma (RCC), malignant angiomyolipoma, and glioblastoma multiforme.[19]

In a large series of 345 patients from Mayo Clinic, Shepherd et al reported 6.1% incidence of this tumor in TSC.[1] In a series of 15 patients reported by Kingsley et al,[20] CT scan done in 9 cases for raised intracranial pressure showed SEGA in 6 cases (40%), thereby suggesting higher incidence of SEGA in TSC patients. In a series of 22 cases of SEGA reported by Bonnin et al only 5 cases (22.7%) were associated with tuberous sclerosis.[3] In contrast, 9 of our patients (39.1%) were associated with tuberous sclerosis, 6 at the time of initial diagnosis of the tumor and 3 in the follow-up period. One case developed stigmata of TS as late as 10 years after the initial diagnosis of the tumor, thereby suggesting that SEGA is the *forme fruste* of TS.

Mean age at presentation was 13.2 years, and the predominant lateral ventricle involvement near the foremen of Monro was similar to that reported in the literature.[1,6] Rarely these tumors present in the neonatal period,[21] but none of the cases in our series presented in this period. However, male predominance, long duration of symptoms (mean 17.2 months) and marked visual disturbances and blindness at presentation are some of the unusual features in the series under discussion. This may be attributed to the delay in arrival of these patients to the outpatient clinic which very often happens in this institute as it is a tertiary care center.

Seizures, especially of myoclonic type, are common presenting symptoms in TS and were seen in half of the cases in the present series. Raised intracranial pressure, seizures and visual disturbances were the commonest presenting symptoms in this series. Radiologically, intraventricular space-occupying lesions were seen in all cases. In addition, subependymal calcified nodules and cortical tubers were observed in 5 and 3 cases, respectively.

Of the 19 patients who were operated upon and followed up in this Institute, 2 died immediately after operation due to surgical complications. Of the 17 patients who survived, 7 received postoperative radiotherapy, all were alive and well in the follow-up period ranging from 3 to 264 months (mean 37.1 months). Only one case recurred 22 years after the first operation. This case was earlier misdiagnosed as intraventricular meningeal sarcoma at the time of the first surgery. Of the 4 referred cases from another institute, one recurred two years after surgery.

Microscopically SEGAs have varied morphology ranging from resemblance to astrocytic, gemistocytic and ganglion cells. However, sweeping bundles of spindle-shaped cells with fibrillary background not merging onto the blood vessels, ganglion-like cells and perivascular inflammatory cells are highly characteristic of SEGA. The inflammatory component is usually an admixture of mast cells and T lymphocytes. Presence of mitosis and necrosis was not indicative of malignancy in this tumor, as has also been observed earlier.[1,2] Rarely SEGAs with frank features of malignancy have been recorded in the literature.[22] Sporadic case reports of high-grade glioma in TSC patients are also on record.[23,24]

Immunohistochemical staining for glial and neuronal markers revealed that both glial and neuronal proteins are expressed in SEGAs, and the results are similar to that reported by Lopes et al.[6] The degree of GFAP positivity showed no difference between the tumors with and without TSC, and this is in contrast to the observation made by Bonnin et al, reporting GFAP negativity in the tumors associated with TS.[3] However, in the present series, GFAP positivity was expressed in all three components at variable intensity. Neuronal markers NF and NSE were positive in 15/23 cases (65.2%).

The positivity for synaptophysin in the ganglion-like cells was observed in 3 cases (12.1%) in the present series,

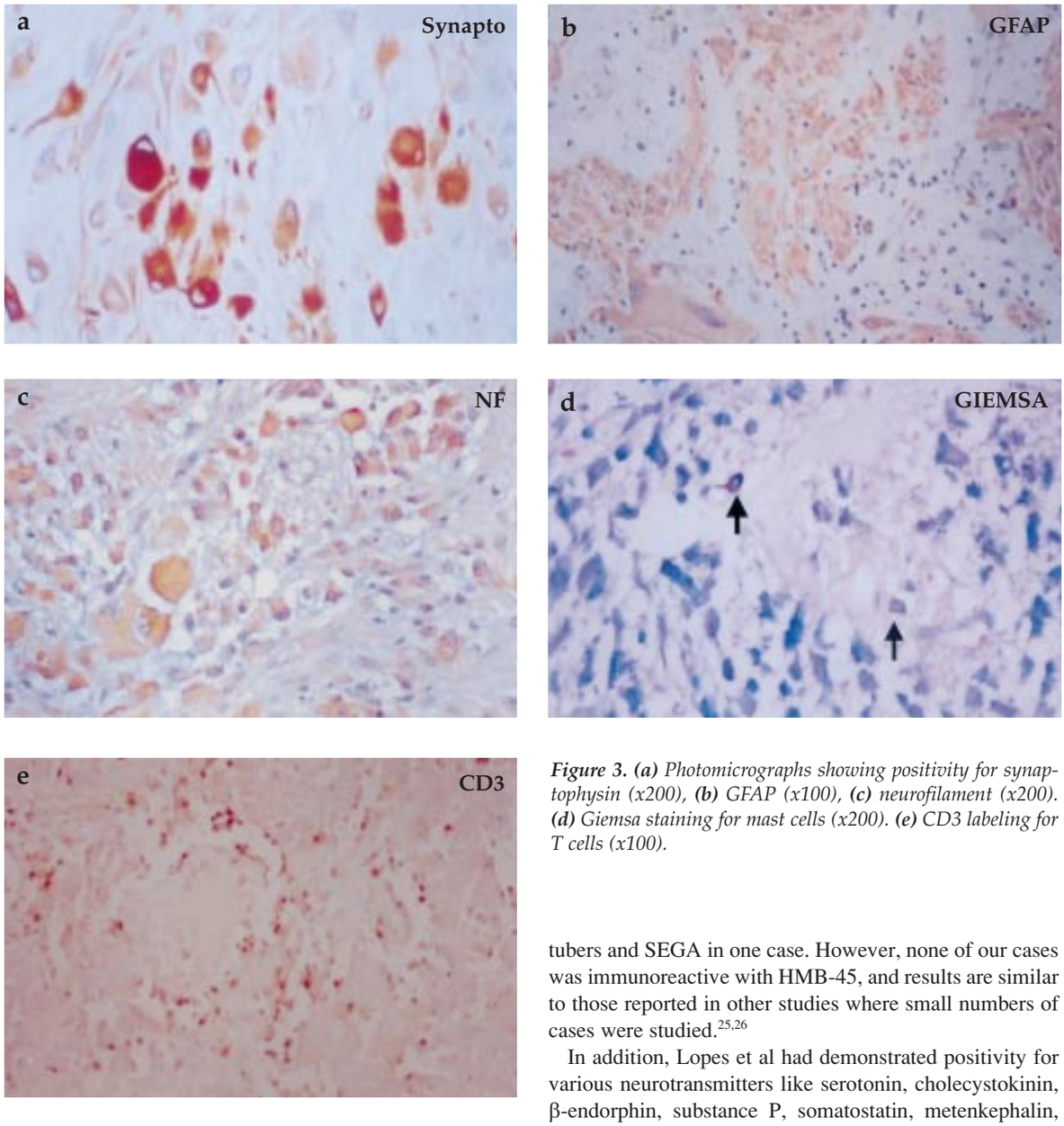

Figure 3. ***(a)*** *Photomicrographs showing positivity for synaptophysin (x200),* ***(b)*** *GFAP (x100),* ***(c)*** *neurofilament (x200).* ***(d)*** *Giemsa staining for mast cells (x200).* ***(e)*** *CD3 labeling for T cells (x100).*

and this has not been reported earlier in the literature. This positivity was not observed in spindle cells and gemistocyte-like cells. These ganglion-like cells also co-expressed GFAP. Positivity for NSE and S-100 protein was seen both in the ganglion-like component and astrocytic component, indicating that these are not definitive neuronal markers.

Recently, lesions associated with tuberous sclerosis like pulmonary and uterine lymphangiomyomatosis, renal angiomyolipoma and rhabdomyoma have been shown to be positive for HMB-45 in smooth muscle cells.[15-18] Week et al[18] reported weak positivity for HMB-45 in cortical tubers and SEGA in one case. However, none of our cases was immunoreactive with HMB-45, and results are similar to those reported in other studies where small numbers of cases were studied.[25,26]

In addition, Lopes et al had demonstrated positivity for various neurotransmitters like serotonin, cholecystokinin, β-endorphin, substance P, somatostatin, metenkephalin, neuropeptides and vasoactive intestinal polypeptide (VIP), 28 kDa neuron-associated calcium protein-calbindin in addition to GFAP, neurofilaments, Class III β-tubulin and microtubule-associated protein, thereby indicating neuroendocrine differentiation of this tumor.[6] Therefore, we tend to agree with the hypothesis put forward by Lopes et al that SEGAs represent proliferation of cell lineages with capacity to undergo divergent glioneuronal as well as neuroendocrine differentiation to a greater extent than other glial neuronal neoplasms.

The prognosis of this tumor is excellent and surgery is the treatment of choice. Seven cases in the present series

who were operated in 1980s received radiotherapy and one of them recurred after 22 years. Of the cases that did not receive radiotherapy, recurrence was noted in one case, 2 years after surgery. Therefore, the role of radiotherapy in this tumor is questionable. The presence of mast cells and T lymphocytes may suggest immunological mechanisms influencing the prognosis of this tumor.

Therefore, SEGA is a low-grade tumor with favorable outcome, if surgically removed. It occurs in the background of TS and many of these patients develop stigmata of TS in the follow-up period, supporting the theory that it represents a *forme fruste* of tuberous sclerosis.

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References

1. *Shepherd CW, Scheithauer BW, Gomez MR, et al:* Subependymal giant cell astrocytoma: a clinical, pathological and flow cytometric study. Neurosurg 28: 868-864, 1991
2. *Chow CW, Klug GL, Lewis EA:* Subependymal giant cell astrocytoma in children: An unusual discrepancy between histological and clinical features. J Neurosurg 68: 880-883, 1988
3. *Bonnin JM, Rubinstein LJ, Papasozomenos SC and Marangos PJ:* Subependymal giant cell astrocytomas. Significance and possible cytogenetic implications of an immunohistochemical study. Acta Neuropathol 62: 185-193, 1984
4. *Fuziwara S, Takaki T, Hikita T, Nishio S*: Subependymal giant cell astrocytoma associated with tuberous sclerosis: do subependymal nodules grow? Child's nervous system 5: 43-44, 1989
5. *Morimoto K, Mogami H:* Sequential CT study of subependymal giant cell astrocytoma associated with tuberous sclerosis: case report. J Neurosurg 65: 874-877, 1986
6. *Lopes MBS, Altermatt HJ, Scheithauer BW, et al:* Immunohistochemical characterisation of subependymal giant cell astrocytomas. Acta Neuropathol 91: 368-375, 1996
7. *Bender BL, Yunis EJ*: Central nervous system pathology of tuberous sclerosis in children. Ultrastruct Pathol 1: 287-299, 1980
8. *Halmagyi GM, Bignold LP, Allospi JP:* Recurrent subependymal giant cell astrocytoma in the absence of tuberous sclerosis. J Neurosurg 50: 106-109, 1979
9. *Sima AAF, Robertson DM:* Subependymal giant cell astrocytoma. Case report with ultrastructural study. J Neurosurg 50: 240-245, 1979
10. *Trombley IK, Mirra SS*: Ultrastructure of tuberous sclerosis: cortical tuber and subependymal tumor. Ann Neurol 9: 174-181, 1981
11. *Bancel B, Belin MF, Meiniel A, et al:* Contribution a l'etude de l'histogenese des gliomes sous ependymaires de la sclerose tubereuse de Bourneville. Ann Pathol 10: 109-116, 1990
12. *Iwasaki Y, Yoshikawa H, Sasaki M, et al:* Clinical and immunohistochemical studies of subependymal giant cell astrocytoma associated with tuberous sclerosis. Brain Dev 12: 478-481, 1990
13. *Stefanssan K, Wollmann RL:* Distribution of neuronal specific protein 14-3.2 in central nervous system lesions of tuberous sclerosis. Acta Neuropathol (Berl) 53: 113-117, 1981
14. *Nakamura S, Tsubokawa T:* Ultrastructure of subependymal giant cell astrocytoma associated with tuberous sclerosis. J Clin Electron Microscope 20: 5-6, 1987
15. *Bonetti F, Chiodera PL, Pea M, et al:* Transbronchial biopsy in lymphangiomyomatosis of the lung: HMB- 45 for diagnosis. Am J Surg Pathol 17:1092-1102, 1993
16. *Gyure KA, Hart WR, Kennedy AW:* Lymphangiomyomatosis of the uterus associated with tuberous sclerosis and malignant neoplasia of the female genital tract: a report of two cases. Int J Gynecol Pathol 14: 344-351, 1995
17. *Pea M, Bonetti F, Zamboni G, et al:* Melanocyte marker HMB-45 is regularly expressed in angiomyolipoma of the kidney. Pathology 23: 185-188, 1991
18. *Weeks DA, Chase DR, Malott RL, et al:* HMB-45 staining in angiomyolipoma, cardiac rhabdomyoma, other mesenchymal processes and tuberous sclerosis associated brain lesions. Int J Surg Pathol 1:191-198, 1994
19. *Al-Saleem T, Wessner LL, Scheithauer BW, et al:* Malignant tumors of the kidney, brain, and soft tissues in children and young adults with the tuberous sclerosis complex. Cancer 83: 2208-2216, 1988
20. *Kingsley DPE, Kendall BE, Fitz CR:* Tuberous sclerosis: A clinico-radiological evaluation of 110 cases with particular reference to atypical presentation. Neuroradiology 28: 38-46, 1986
21. *Boesel CP, Paulson GW, Kosnik EJ, Earle KM:* Brain hamartomas and tumors associated with tuberous sclerosis. Neurosurgery 4: 410-417, 1979
22. *Padmalatha C, Harsuff RC, Ganick D, Hafez GR:* Glioblastoma multiforme with tuberous sclerosis. Arch Lab Med 105: 645-650, 1980
23. *Brown JM:* Tuberous sclerosis with malignant astrocytoma. Med J Austr 1:811-814, 1975.
24. *Medhkour A, Traul D, Husain M:* Neonatal subependymal giant cell astrocytoma. Ped Neurosurg 36: 271-274, 2002
25. *Gyure KA, Prayson RA:* Subependymal giant cell astrocytoma: A clinicopathologic study with HMB-45 and MIB-1 immunohistochemical analysis. Mod Pathol 10: 313-317, 1997
26. *Bacchi CE, Bonetti F, Pea M, et al:* HMB-45: a review. Appl Immunohistochem 4: 73-85, 1996