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Primary Rhabdomyosarcoma of Brain and Cerebellum

Report of Four Cases in Infants: An Immunohistochemical Study

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Summary. Tumors of the central nervous system (CNS) composed of pure mesenchymal derivatives with both embryonal and mature striated muscle cells devoid of neuroblastic elements should be considered rhabdomyosarcomas. Some 13 cases have been reported, and here we study four additional cases in infancy under 3 years of age which represent 0.82% of 483 intracranial tumors studied by us at the Children's Hospital in the last 12 years. Two cases were localized in the temporal lobes, and two were in the cerebellar vermis. All of them were typical embryonal rhabdomyosarcomas at various stages of differentiation including undifferentiated mesenchymal cells, embryonal cells, and rhabdomyoblasts. Tumor cells achieved a higher degree of differentiation in the cerebellum, as shown by readily detectable immature muscle fibers which were consistently absent in tumors involving the brain. Myoglobin [peroxidase-antiperoxidase (PAP) technique] was positive throughout in rhabdomyoblasts and in immature muscle cells, whereas glial fibrillary acidic protein was negative in all four tumors.

In spite of the well differentiated appearance of the cerebellar tumors, their behavior was highly malignant with extensive infiltration of brainstem leptomeninges in one case, and all patients survived for only a short time after surgery.

These tumors may be observed in the midline structures of the posterior fossa and in the brain, but we suspect their true incidence might be higher if immunohistochemical techniques were applied.

Key words: Brain – Cerebellum – Infancy – Rhabdomyosarcoma

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Introduction

The studies of Wolbach [38] and Stout [35] have provided histopathologic criteria allowing for the identification of some pleomorphic rhabdomyosarcomas even in the absence of cross striation. Other tumors, such as certain undifferentiated round or spindle-shaped cell neoplasms, have been considered as alveolar rhabdomyosarcomas by Horn and Enterline [9].

The prognosis of rhabdomyosarcoma is more related to its location and to the possibility of a complete surgical removal than to its histopathologic characteristics [11]. Thus, the practical significance of a classification based on its histopathologic features is questionable at the very least.

Rhabdomyosarcomas are of pure mesenchymal derivation with both embryonal cells and rhabdomyoblasts. Russell and Rubinstein [32] have postulated that these tumors should be devoid of neuroblastic elements. If we apply this criterion, primary rhabdomyosarcoma of the CNS is a very rare tumor indeed and must not be confused with medullomyoblastoma or teratoma.

This report presents four cases; two in the temporal lobes and two in the cerebellum. We describe the light microscopy observations, the results of the PAP technique for glial fibrillary acidic protein (GFAP) and myoglobin as well as ultrastructural findings in case 4.

Case reports

Case 1

In February 1973, a girl aged 3 years and 9 months was admitted to the Neurosurgical Unit of the Children's Hospital where a fronto-parieto-temporal tumor was diagnosed. The tumor was subtotally excised, and the specimen measured 12 cm × 8 cm × 3.5 cm. It was firm in consistency, greyish, with small foci of necrosis and myxoid areas. The girl received Cobalt 60 teletherapy, but her general condition deteriorated and she died 1 month after surgery.

Case 2

In January 1975, a girl aged 2 years and 9 months was admitted to the Neurosurgical Unit of the Children's Hospital and found to have a left temporo-parietal tumor. At surgery the tumor was subtotally excised an the specimen found to measure 7 cm × 4 cm × 2 cm. It was soft and greyish, with small necrotic foci. The patient died immediately after surgery.

Case 3

In May 1975, a 4-year-old boy was submitted to surgery in another hospital for a posterior fossa tumor of the inferior vermis, adherent to the fourth ventricle floor, that was totally resected. The specimen was 2 cm in diameter, firm, with soft areas. As hydrocephalus was present, a ventriculo-peritoneal shunt was carried out. The child received chemotherapy and Cobalt 60 teletherapy. A year later, showing signs of intracranial hypertension and a left cerebellum syndrome, he was admitted to our hospital where a tumor occupying the cerebellar vermis and projecting into the fourth ventricle was subtotally removed. The specimen measured 6 cm × 8 cm × 4 cm. It was soft and greyish with myxoid areas. There was some neurologic improvement after surgery, but he died 2 months later.

Case 4

In January 1982, a 3-year-old boy was admitted to the Neurosurgical Unit of the Children's Hospital where a tumor occupying the cerebellar vermis and protruding into the fourth ventricle was diagnosed. At surgery, it was subtotally removed and the specimen found to consist of several fragments, greyish in color, and firm in consistency. Due to progressive obstructive hydrocephalus a ventriculo-peritoneal shunt was carried out. Following Cobalt 60 teletherapy (2,500 rads) there was clinical deterioration and leukopenia, and he died 2 months later.

Autopsy (Case 4)

The brain weighed 1,100 g, and there was diffuse basal leptomeningeal thickening particularly surrounding the optic tracts, the chiasm, the cerebellum, the base of the pons, the medulla, and the cervical cord. Both lateral ventricles, the third ventricle, the Sylvian aqueduct, and the fourth ventricle all exhibited severe dilatation. Findings were confined to the CNS.

Material and methods

Four cases of rhabdomyosarcoma were diagnosed at the Children's Hospital, Buenos Aires, in the last 12 years, accounting for 0.82% of 483 intracranial tumors in infancy. All specimens were surgical biopsies, and in one case the autopsy was available.

Histologic-Immunohistochemical and Ultrastructural Procedures

All specimens were fixed in 10% formalin, embedded in paraffin, and stained by conventional techniques. An ultrastructural study was carried out on material embedded in Epon 812. Immunohistochemical studies for glial fibrillary acidic protein (GFAP) and myoglobin with Sternberger's PAP method [34] were carried out on paraffin-embedded sections. Antisera were diluted in phosphate-buffered saline (PBS) as follows: anti-myoglobin serum (DAKO Myoglobin, Denmark) 1:100 and 1:500, anti-GFAP serum 1:500, anti-rabbit IgG goat serum (Cappel Lab., Cochranville, PA, USA) 1:30 and soluble PAP complex (DAKO Immunoglobulin, Denmark) 1:50. No specific staining was observed in control sections incubated with normal rabbit serum nor with PBS instead of specific antiserum.

Results

Microscopic Findings

Histologically, two basic patterns could be discerned. There were undifferentiated mesenchymal cells, closely packed areas of embryonal cells but few rhabdomyoblasts in the supratentorial cases (1 and 2), and there was focal necrosis in case 2. In the infratentorial cases (3 and 4), in addition to the above features, there were more differentiated areas with rhabdomyoblasts and immature muscle fibers arranged in fascicles. Astrocytic hyperplasia and calcium deposits were readily seen within the infiltrated nervous tissue. GFAP was strongly positive in the surrounding reactive astrocytes but negative within the tumor itself. Case 4 featured massive leptomeningeal invasion by rhabdomyoblasts at autopsy. Undifferentiated mesenchymal cells (Fig. 1) had oval nuclei with loose homogeneous chromatin and stellate or spindle-shaped cytoplasm, interspersed within Alcian blue-positive substance. Embryonal cells (Fig. 2) showed rounded, vesicular nuclei and conspicuous nucleoli, sccanty cytoplasm with ill-defined boundaries.

Rhabdomyoblasts (Fig. 3) were identified as cells having rounded, spindle-shaped or tadpole eosinophilic cytoplasm and eccentric nuclei with prominent nucleoli. PAS-positive granules were also found in some of these cells as well as cytoplasmic cross striations.

Immature muscle fibers, some resembling myotubes with multiple central nuclei in tandem (Fig. 4) and others with peripheral nuclei, were arranged in fascicles (Fig. 5). Cross-striations were observed in hematoxylin-eosin (HE)- and PTAH-stained sections (Fig. 6). PAP technique for myoglobin was positive in rhabdomyoblasts (Fig. 7) and immature muscle fibers (Fig. 8), but this protein could not be found in either embryonal or undifferentiated mesenchymal cells.

Ultrastructural Findings (Case 4)

Although formalin fixation was hardly satisfactory in this study, structure preservation made it possible to observe rhabdomyoblasts with randomly oriented thin filaments in their cytoplasm as well as glycogen granules, swollen mitochondria, and dilated endo-

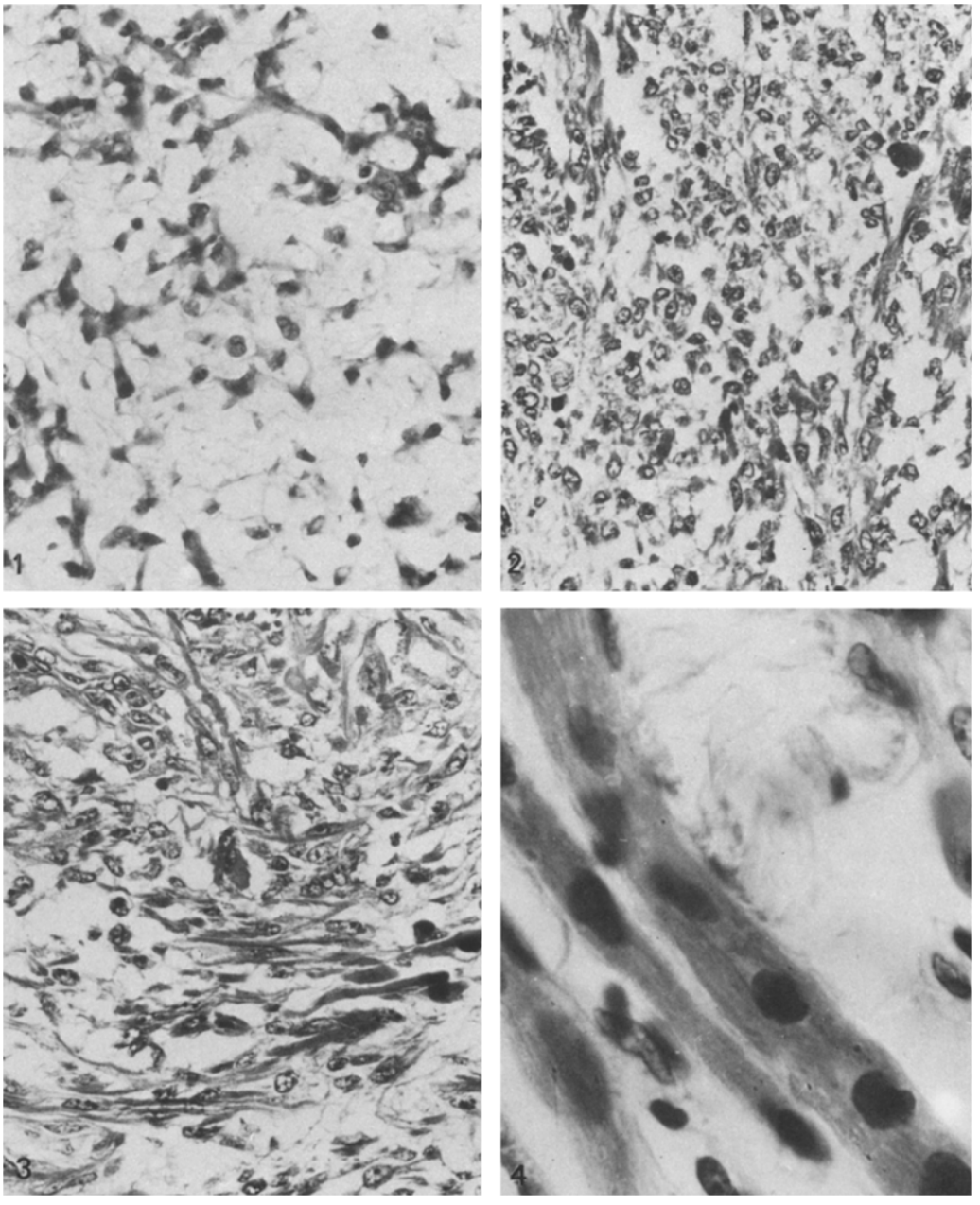


Fig. 1. Loose area with stellate undifferentiated mesenchymal cells (case 1). HE; ×400

Fig. 2. Embryonal cells with vesicular nuclei, conspicuous nucleoli, scanty cytoplasm with ill-defined boundaries. Some rhabdomyoblasts are intermingled with them (case 2). PAS (McManus); ×400

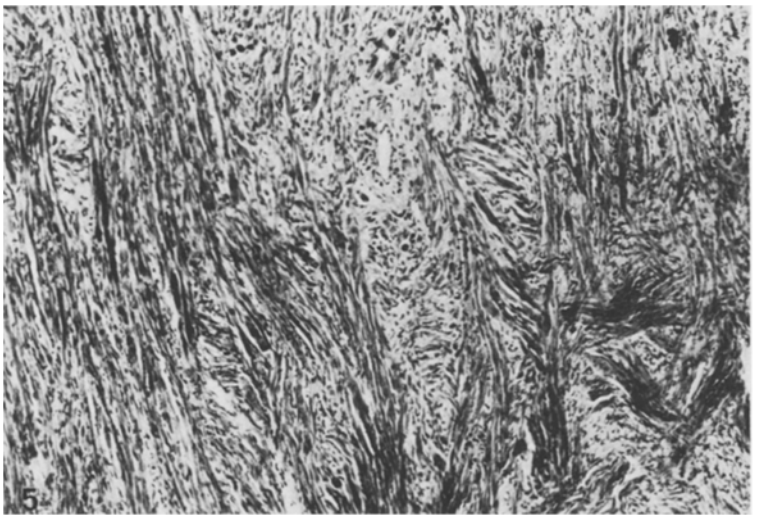
Fig. 3. Rhabdomyoblasts with spindle-shaped or tadpole cytoplasm is a prominent feature in this field (case 3). HE; ×400

Fig. 4. Myotubular fiber with multiple central nuclei in tandem (case 4). HE; ×1,000

plasmic reticulum. Immature muscle fibers had both thick and thin filaments well organized in myofibrils similar to normal muscle cells, with sarcomeres and Z bands aligned in register. Basement membrane was clearly observed in the periphery of these cells outside of the plasma membrane.

Discussion

Cases reported here met the histological criteria essential for the diagnosis of primary rhabdomyosarcoma. Thus, tumors were made up by mesenchymal cells, embryonal cells, rhabdomyoblasts, and imma-



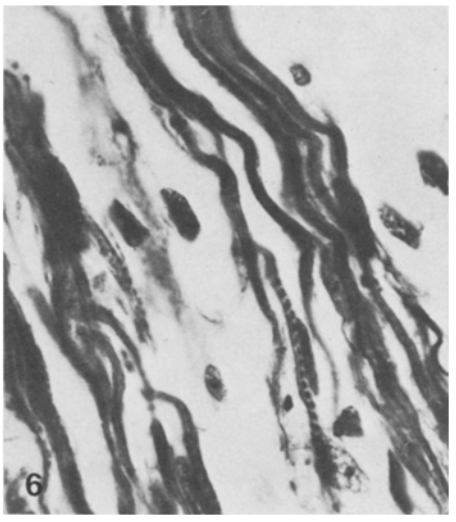


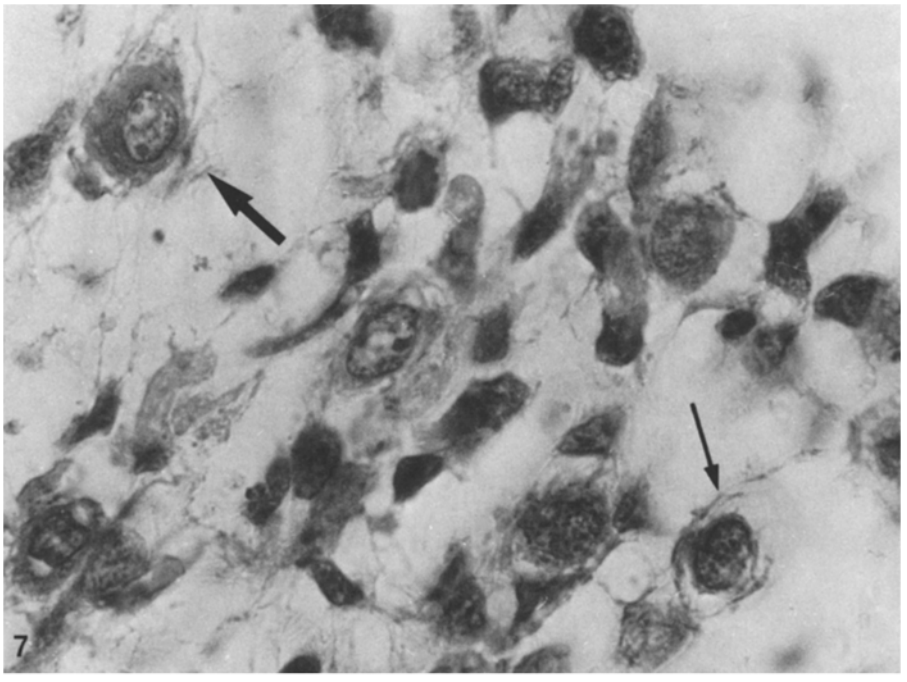
Fig. 5. Rhabdomyoblasts and immature muscle fibers arranged in fascicles (case 4). HE; ×100

Fig. 6. Elongated immature muscle fibers arranged in fascicles with prominent cross-striations (case 3). PTAH; ×750

ture striated muscle cells. A secondary origin of these tumors, or extension from a soft tissue, rhabdomyosarcoma was carefully ruled out on the basis of clinical and radiologic findings, as well as an autopsy in case 4. Both a teratoid tumor and a medullomyoblastoma were also ruled out due to the absence of neuroectodermal cells or other tissue. Histogenesis of primary CNS rhabdomyosarcomas has been a matter of controversy.

Hoffman and Rorke [7] found well developed striated muscle fibers in leptomeninges in a newborn infant with 13–15 trisomy defect and congenital anomalies. They also commented on Marsden's unpublished observation of striated muscle in the leptomeninges adjacent to the medulla in an individual affected by Klippel-Feil syndrome with hydrocephalus. Viragh et al. [37] found heterotopic striated muscle on the surface of normal brain, and Ambler [1] in cerebral dysplasia. Pluripotent mesenchymal cells which could develop into striated muscle fibers found in leptomeninges, or even rhabdomyosarcomas [20, 25, 33] have been described in perivascular areas, particularly in the pia arachnoid [3]. A neural crest origin has been advanced for the mesenchyme (ectomesenchyme) of the head. The trunk crest, including the cervical crest, gives rise to pigment cells, sensory and autonomic neuroblasts, Schwann cells, and also makes some contribution to meninges. Autonomic and sensory neurons and supportive or connective tissue, mostly skeletal elements of the head, as well as muscles of the head region also originated in the cranial crest [5, 10].

Although most of the embryologic experiments on migration and differentiation of neural crest cells have been carried out in lower vertebrates, some tumors, such as the so-called ectomesenchymoma [12], gangliorhabdomyosarcoma [8], and ganglioneuroblastoma associated with malignant mesenchymoma [26] strongly suggest that ectomesenchyme also exists in humans. Research on tissue cultures from nitrosourea-induced rat tumor provided evidence that striated muscle cells can arise from neuroectodermal derivatives, a view supported by surface cell antigen studies [2, 4, 17–19, 30]. The presence of rhabdomyoblasts in medullomyoblastomas lends further strength to these contentions. All 13 cases reported previously [14–16, 20–23, 25, 27, 28, 31, 33, 39] have been considered as true primary CNS rhabdomyosarcomas on clinical and pathologic grounds, besides complying with the stated diagnostic criteria. Ten of the 13 patients with primary CNS rhabdomyosarcomas were already dead when reported. Three patients survived surgery, but no follow-up was available. Four autopsy studies, including our case 4, have been described in the literature [25, 27, 33]. In all four necropsies there was diffuse leptomeningeal spread of the tumor, mainly at the base of the brain, midbrain, pons, medulla, optic tract sheath, and chiasm. Of the 17 cases reviewed, including ours, six were adults and 11 children. The adult cases consisted of four supratentorial and two infratentorial tumors, and the childhood cases were four supratentorial and seven infratentorial tumors. All the cases reported included well differentiated areas. In our two brain cases there were mesenchymal cells, embryonal cells, but few rhabdomyoblasts. In the two cerebellar cases there were more differentiated areas with immature muscle fibers resembling the so-



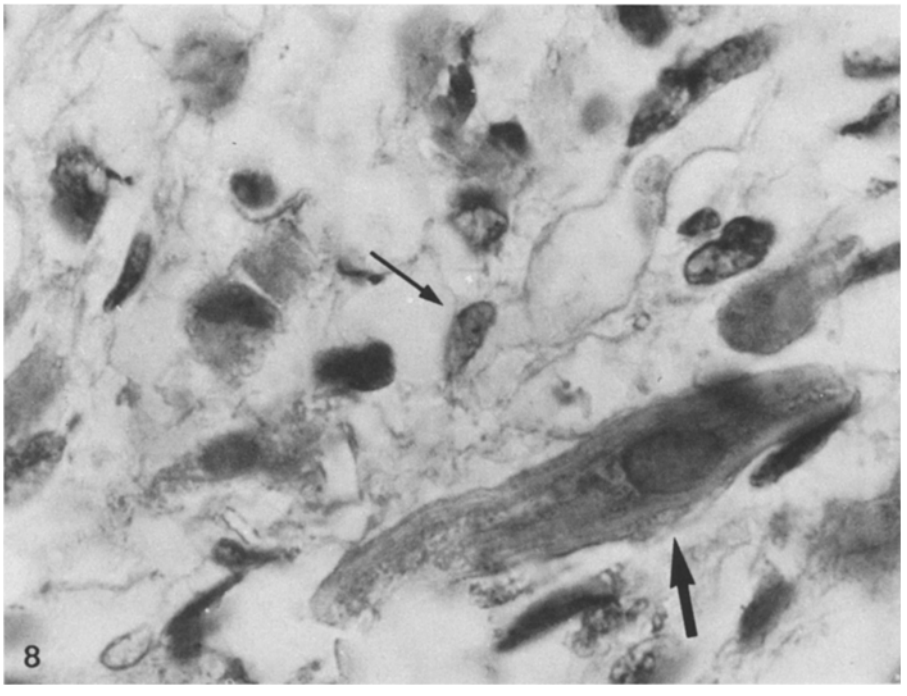


Fig. 7. Rhabdomyoblasts containing myoglobin in their cytoplasm (*thick arrow*) and embryonal cells without myoglobin (*thin arrow*) (case 2). PAP-myoglobin; ×1,000

Fig. 8. Immature muscle fibers strongly stained for myoglobin (*thick arrow*) and negative undifferentiated mesenchymal cells (*thin arrow*) (case 4). PAP-myoglobin; ×1,000

called soft tissue fetal rhabdomyoma [6], but their infiltrative character as well as the clinical behavior, correspond to a malignant neoplasm. Myoglobin was present in the immature muscle fibers and in most rhabdomyoblasts but could not be detected in embryonal or mesenchymal cells. GFAP was absent throughout the tumor. Ultrastructural microscopy of case 4 showed thin filaments in rhabdomyoblasts and thick and thin filaments in more differentiated cells. Sarcomeres and Z lines, clearly aligned in register,

Table 1. Summary of cases of primary rhabdomyosarcoma of brain

Author (year)	Age (yr)	Sex	Location, extension	Histology	Treatment	Follow-up	Status
1. López (1934)	18	F	Cerebellopontine angle strictly localized	Striated muscle, stellate cells	None	Several weeks	Dead
2. Lopes de Faria (1957)	52	F	Cerebellar hemisphere, well circumscribed	Striated muscle, undifferentiated mesenchymal cells	None	5 months	Dead
3. Koide and Ishizone (1957)	15	M	Cerebral hemisphere, sharply demarcated, later infiltrative	Striated muscle, undifferentiated mesenchymal cells	Decompressive surgery + irradiation	5 months	Dead
4. Ozeki (1960)	29	M	Cerebral hemisphere, encapsulated	Pleomorphic spindle cells with cross striation	None	1.5 months	Dead
5. Legier and Wells (1967)	3.5	M	Vermis, infiltrative and subarachnoid spread	Rhabdomyoblast, undifferentiated mesenchymal cells	Excision + irradiation	2 years	Dead
6. Shuangshoti et al. (1968)	4	F	Vermis, subarachnoid spread	Striated muscle, undifferentiated mesenchymal cells	None	1 month	Dead
7. Leedham (1972)	45	F	Cerebral hemisphere infiltrative	Striated muscle, undifferentiated mesenchymal cells	Excision	10 months	Dead
8. Matsukado et al. (1975)	3.5	F	4th ventricle, encapsulated, later infiltrative	Striated muscle undifferentiated mesenchymal cells	Excision + irradiation	2 years	Dead
9. Pasquier et al. (1975)	8	F	Brain stem	Striated muscle undifferentiated mesenchymal cells	None	3 months	Dead
10. Min et al. (1975)	48	M	Right frontotemporal lobes, well circumscribed, later infiltrative	Rhabdomyoblast, undifferentiated mesenchymal cells	Excision + irradiation + chemotherapy	8 months	Dead
11. Yagishita et al. (1979)	51	F	Right cerebral hemisphere, attached to the falx	Embryonal cells, rhabdomyoblast, undifferentiated mesenchymal cells	Excision + irradiation + chemotherapy	?	?
12. Roy et al. (1980)	10	M	Vermis and 4th ventricle, well demarcated	Rhabdomyoblast, embryonal cells, undifferentiated mesenchymal cells	Excision + irradiation	3 months	?
13. Masuzawa et al. (1982)	2	M	Left basal ganglia	Immature muscle fibers, rhabdomyoblast, embryonal cells, undifferentiated mesenchymal cells	Excision + irradiation	3 months	?
14. Taratuto et al. (1984)	$3^{9}/_{12}$	F	Left temporal lobe infiltrative	Embryonal cells, rhabdomyoblast, undifferentiated mesenchymal cells	Excision + irradiation	1 month	Dead
15. Taratuto et al. (1984)	$2^{9}/_{12}$	F	Left temporal and parietal lobes, infiltrative	Embryonal cells, rhabdomyoblast, undifferentiated mesenchymal cells	Excision	Dead immediately after surgery	
16. Taratuto et al. (1984)	5	M	Vermis, infiltrative	Immature muscle fibers, rhabdomyoblast, embryonal cells, undifferentiated mesenchymal cells	Excision + irradiation + chemotherapy	1 year, 2 months	Dead
17. Taratuto et al. (1984)	3	M	Vermis, brain, stem, subarachnoid spread	Immature muscle fibers, rhabdomyoblast, embryonal cells, undiffferentiated mesenchymal cells	Excision + irradiation	3 Months	Dead

were seen in immature muscle cells. The degree of differentiation was similar to that described for soft tissue rhabdomyosarcomas [29] as well as human myogenesis prior to innervation [36] and explanted human tissue in vitro [24].

Primary sarcomas of the brain are rare tumors, accounting for barely 3% of all intracranial tumors, according to Kernohan [13]. Highly specific immunohistochemical techniques, such as those for GFAP and myoglobin, have enabled us to diagnose confidently four cases of primary CNS childhood rhabdomyosarcomas, accounting for 0.82% of the intracranial tumors in infancy in our series of 483 patients. It seems, therefore, that these reliable procedures may readily be extended to determine the true incidence of this type of tumor in even larger series.

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