Pineoblastomas in Pediatric Patients: A Single Institutional Experience

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Abstract

Pineoblastomas are rare, malignant pineal parenchymal tumors encountered predominantly in pediatric patients. They are distinct from primitive neuroectodermal tumors (PNET) at other sites in that they exhibit photosensory differentiation including Flexner–Wintersteiner rosettes and fleurettes. Diagnosis can be challenging since they share morphologic and immunohistochemical features with other embryonal tumors and the developing pineal gland. Pineal anlage tumor is a rare variant of pineoblastoma defined by divergent neuroepithelial and ectomesenchymal differentiation without an endodermal component. To date the five published cases of anlage tumors behaved aggressively. We describe a case series which includes one patient with pineal anlage tumor and the clinical, radiological and pathological characteristics of pediatric pineoblastomas.

Keywords: pineoblastoma, immunohistochemistry, pediatric tumors, anlage tumors, primitive neuroectodermal tumors/PNET

1. Introduction

Pineoblastomas (PBs) are rare, highly malignant (grade IV in the 2021 World Health Organization (WHO) classification of the tumors of the central nervous system (CNS)) pineal parenchymal tumors (PPT) occurring mainly in the pediatric population. They are part of the broad category of central primitive neuro-ectodermal tumors (cPNET) and may exhibit photosensory differentiation including Flexner–Wintersteiner rosettes and fleurettes (2). Diagnosis can be challenging since they share morphologic and immunohistochemical features with other embryonal tumors and the developing pineal gland (2). Pineal anlage tumor is a rare variant of PB. It is defined by divergent neuroepithelial and ectomesenchymal differentiation without an endodermal component.
The few published cases of anlage tumors behaved aggressively (3-5).

We describe the clinicopathologic characteristics of thirteen pediatric cases of PBs and compare responses to therapy and prognosis to better understand the clinical course of PBs in the pediatric population.

2. Material and Methods

Pathology records from 1980 to 2012 were searched for PBs at a tertiary pediatric hospital (Children Hospital Wisconsin). Thirteen consecutive cases of PBs, consisting of 12 classical PBs and one anlage tumor were identified. Cases were reviewed and classified using the presence/absence of anlage/divergent differentiation component, rosette formation, pigment production, mitoses and necrosis. Radiology reports and clinical data were reviewed. Formalin fixed, paraffin-embedded tissue was sectioned and stained with hematoxylin and eosin. We assessed the histologic diagnoses according to the 2021 WHO classification of tumors of the CNS (1). Mitoses were counted in 10 high-power fields (HPF, 400x) and expressed as the number of mitoses per 10 consecutive HPFs. Immunohistochemistry was performed with the following commercially available antibodies: anti-synaptophysin (SY38, mouse, monoclonal, 1:100, catalog# IS776, Dako, Tokyo, Japan), glial fibrillary acidic protein (GFAP) (polyclonal, rabbit, 1:4000, catalog# Z0334, Dako, Glostrup, Denmark), vimentin (clone V9, mouse, monoclonal, 1:1000, catalog# M0725, Dako, Tokyo, Japan), anti-neurofilament (2F11, mouse, monoclonal, 1:50, catalog# M0762, Dako, Tokyo, Japan), antineuronal nuclear antigen (anti-NeuN; A60, 1:100, catalog# MAB377 Millipore, Billerica, Massachusetts, USA), and anti-Ki67 (MIB-1, mouse, monoclonal, 1:100, catalog# IS626, Dako, Tokyo, Japan). The MIB-1 indices represent rate of immunopositive cells, determined by examining more than 1000 tumor cells.

3. Results

Histopathology

Microscopic examination showed densely cellular neoplasms composed of sheets of haphazardly arranged, variably sized immature cells with scant eosinophilic cytoplasm. The smaller cells had small angulated to oval nuclei with hyperchromatic smudged nuclear chromatin and indistinct nucleoli. The larger cells had lobulated vesicular nuclei with small nucleoli and moderately abundant granular to eccentric glassy eosinophilic cytoplasm. Mitotic figures were present from rare mitoses (1-2/10HPF) to 40/10 HPF in one instance. Variable degrees of apoptosis, necrosis and karyorrhexis were noted. In areas the sheets of tumor cells were interrupted by interdispersed bands of fibrovascular stroma with scattered blood vessels. Rosette-like formations were variably present. The average MIB-1 index was 41% (median is best) (range 30-60%). The anlage tumor had areas of glioneuronal differentiation (Figure 1C) alternating with areas of high grade small round blue cells with focal/minimal necrosis (Figure 1B), morphologically similar to medulloblastoma. Homer Wright and Flexner-Wintersteiner rosettes were present. Melanin production, cartilaginous, and rhabdomyoblastic differentiation were also present (Figure 1A). Immunoperoxidase stains for synaptophysin was diffusely positive (Figure 1E), while glial fibrillary acidic protein (Figure 1D) is negative. The MIB1 index was 50% (Figure 1F).

Clinical characteristics

We report thirteen cases of biopsy-proven PBs including one anlage tumor (Table 1). The female to male ratio was 2.2 and the age ranged from 8 months to 17 years (median 10 years). The radiological findings by brain computer tomography (CT) scan and magnetic resonance imaging (MRI) showed heterogeneous enhancing, lobulated pineal gland lesions with dimensions ranging from 2.5 to 5 cm. Hydrocephalus was identified in nine (69%) patients. Five cases (38.4%) had spinal cord metastasis, three (23%) were negative for what (cerebrospinal fluid) CSF dissemination and no information was available in the remaining five cases (38.4%). Nine patients (69%) received a combination of surgical resection, irradiation (whole brain: 7, craniospinal: 3) and chemotherapy and one patient had a combination of resection and irradiation.
### Table 1

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Age at diagnosis</th>
<th>Gender</th>
<th>Therapy</th>
<th>Hydrocephalus</th>
<th>Outcome</th>
<th>Survival from initial Dx</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12 y</td>
<td>M</td>
<td>resection and XRT</td>
<td>Yes</td>
<td>Alive</td>
<td>19 y</td>
</tr>
<tr>
<td>2</td>
<td>11 y</td>
<td>F</td>
<td>NA</td>
<td>N/A</td>
<td>Alive</td>
<td>22 y</td>
</tr>
<tr>
<td>3</td>
<td>11 y</td>
<td>M</td>
<td>resection, XRT and chemo resection, XRT and chemo</td>
<td>Yes</td>
<td>Alive</td>
<td>6 y</td>
</tr>
<tr>
<td>4</td>
<td>10 y</td>
<td>M</td>
<td>resection, XRT and chemo resection, XRT and chemo</td>
<td>N/A</td>
<td>Alive</td>
<td>16 y</td>
</tr>
<tr>
<td>5*</td>
<td>11 mo</td>
<td>F</td>
<td>resection, XRT and chemo resection, XRT and chemo</td>
<td>Yes</td>
<td>Alive</td>
<td>12 mo</td>
</tr>
<tr>
<td>6</td>
<td>17 y</td>
<td>F</td>
<td>resection, XRT and chemo resection, XRT and chemo</td>
<td>Yes</td>
<td>Alive</td>
<td>12 y</td>
</tr>
<tr>
<td>7</td>
<td>8 mo</td>
<td>F</td>
<td>NA</td>
<td>N/A</td>
<td>Deceased</td>
<td>(3 yo)</td>
</tr>
<tr>
<td>8</td>
<td>4 y</td>
<td>M</td>
<td>resection, XRT and chemo resection, XRT and chemo</td>
<td>Yes</td>
<td>Deceased</td>
<td>(11 yo)</td>
</tr>
<tr>
<td>9</td>
<td>9 y</td>
<td>F</td>
<td>resection, XRT and chemo resection, XRT and chemo</td>
<td>Yes</td>
<td>Deceased</td>
<td>(13 yo)</td>
</tr>
<tr>
<td>10</td>
<td>5 y</td>
<td>F</td>
<td>resection, XRT and chemo resection, XRT and chemo</td>
<td>No</td>
<td>Alive</td>
<td>6 mo</td>
</tr>
<tr>
<td>11</td>
<td>13 y</td>
<td>F</td>
<td>resection, XRT and chemo resection, XRT and chemo</td>
<td>Yes</td>
<td>Deceased</td>
<td>(18 yo)</td>
</tr>
<tr>
<td>12</td>
<td>4 y</td>
<td>F</td>
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<td>Yes</td>
<td>Alive</td>
<td>15 y</td>
</tr>
<tr>
<td>13</td>
<td>11 y</td>
<td>F</td>
<td>resection, XRT and chemo resection, XRT and chemo</td>
<td>Yes</td>
<td>Alive</td>
<td>25 y</td>
</tr>
</tbody>
</table>

Legend:*- Anlage tumor; XRT- Radiation therapy; Dx- diagnosis

Two of these patients (15.4%) had third ventriculostomy due to severe headache and altered consciousness (secondary to hydrocephalus). No information was available regarding treatment of three (23%) patients. Nine patients (69%) are alive with survival times between 6 months and 25 years (median 13 years). Four patients (30.7%) died, with an average survival time from the diagnosis of 4.25 years (range 2-6 years). The mean duration of follow-up was 14.5 months (range: 6 months-25 years). The anlage tumor was diagnosed in a 11- month-old female. The MRI of the brain showed a focally enhancing, well circumscribed, lobulated pineal gland mass lesion (44.6 mm x 45.6 mm x 43.3 mm) with attenuated apparent diffusion coefficient (ADC) signal on diffusion-weighted imaging MRI and prominent calcification along its posterior right margin on CT (Figure 1). MRI of the cervical spine appeared unremarkable without evidence of tumor seeding. Adjuvant therapy was given using high dose methotrexate arm of ACNS0334 Phase III clinical trial with induction therapy to include 3 cycles of vincristine, high-dose methotrexate, etoposide, cyclophosphamide, and cisplatin and consolidation therapy to include 3 cycles of carboplatin and thiotepa with autologous stem cell rescue. This regimen has been chosen based upon its potential effectiveness towards the embryonal (PB) component of the tumor. The patient is free of disease 12 months after the diagnosis.
Figure 1: 11-month-old female with pineal anlage tumor. Sagittal, T1-weighted sequence without contrast shows a large, well-circumscribed, lobulated hypointense pineal region mass (left). Histologic sections show melanin production and cartilaginous differentiation (A, 100x), alternating with areas of high grade small round blue cells with focal/minimal necrosis, Homer Wright and Flexner-Wintersteiner rosettes (B, 200x) admixed with rhabdomyoblastic and glioneuronal differentiation (C, 400x). GFAP is negative (D, 200x), synaptophysin demonstrates diffuse positivity (E, 200x) while mitoses are common and the MIB1 index is approximated at 50% (F, 200x).

4. Discussion

Pineal parenchymal tumors (PPTs) are a subcategory of primary neoplasms of the pineal gland. They comprise less than 1% of all brain tumors and are most commonly diagnosed in pediatric patients or young adults. They include PBs, pineocytomas, and pineal parenchymal tumors of intermediate differentiation (PPTID). The 2021 WHO Classification of Tumors of the Central Nervous System added desmoplastic myxoid tumour of the pineal region and SMARCB1-mutant tumor. In light of the 2021 WHO classification, molecular subtyping of pineoblastomas is becoming increasingly important (1, 17, 45). Pineocytomas are well-differentiated, less aggressive tumors composed of cells with abundant eosinophilic cytoplasm and pineocytic rosette formation (1, 6). PPTID have features intermediate between those of well-differentiated PCs and undifferentiated PBs show a cellular milieu that is less dense but with limited differentiation or rosette formation (1). PBs are primitive small round blue cell tumors that resemble medulloblastomas, with a strong potential for leptomeningeal seeding (7, 8). In several published case series PBs were the most common type of PPTs, representing up to half of all PPTs (5, 9, 10). Extracranial metastasis to the peritoneal cavity can occur via ventriculoperitoneal shunts. Rare metastases to the bone (11) and lung (8) have been described. Similar to central nervous system primitive neuroectodermal tumors, the prognosis for patients with PBs is very poor, with 5-year survival rates of only 10% (5, 10, 12). Compared to other primary pineal tumors, anlage tumors have neuroepithelial and ectomesenchymal differentiation but lack endodermal differentiation. They are extremely rare and have been only recently mentioned as a provisional entity in the WHO classification system, but are not recognized as a separate entity (1, 13). Schmidbauer et al. (4) first described this tumor in 1989, reporting a 9-year-old female with a primitive pineal tumor. Only a few cases have since been reported and showed that pineal anlage tumors include immature cartilage, skeletal muscle, and melanin pigmented cells as well as Homer Wright and Flexner-Wintersteiner rosettes (31). Most reported patients presented with hydrocephalus, but some had spasticity and ataxic gait (9, 14, 15). Patients with pineal anlage tumors have previously been given a prognosis similar to classical PBs (3, 16). Including our case, only 6 cases have been reported thus far to the best of our knowledge, so the significance, prognosis, and treatment of pineal anlage tumors are uncertain.

On brain CT imaging PBs appear as large, lobulated mass lesions and usually show
heterogeneous contrast enhancement (6,14). Hydrocephalus is often present and the tumors show variable degrees of cystic changes. On MRI, PBs are hypo- to isointense on T1-weighted images and hyperintense on T2-weighted images with heterogeneous contrast enhancement (6,14,15,17). The interface between the tumor and surrounding structures is sometimes poorly defined (6,14).

On gross pathological examination PBs are pink-tan soft, gelatinous tumors, occasionally showing diffuse infiltration of surrounding tissues (7). Hemorrhage and necrosis are often encountered (7–9,18–20). Microscopically they appear as patternless sheets of small cells with scant cytoplasm, hyperchromatic nuclei, high nucleocytoplasmic ratios, and scant cytoplasm (3,21). Lobular architecture is absent (6). Necrosis is common but mitotic activity is variable. Frequent Homer-Wright rosettes (Figure 1) and occasional Flexner-Wintersteiner rosettes are identified.

Pineocytomatous rosettes are absent. Rare bundles of cytoplasmic processes (fleurettes) showing bulbous expansion at their distal portion can be seen and indicate photoreceptor differentiation (21,22). Silver staining highlights the scant cytoplasm and few cellular processes. Melanin production as well as cartilaginous and rhabdomyoblastic differentiation are encountered in pineal anlage tumors (4,23).

PBs show expression of neuronal and photoreceptor markers. Immunolabeling of neuron-specific enolase and synaptophysin is weak and diffuse compared to other pineal parenchymal tumors. Labeling for neurofilaments, class III β-tubulin, chromogranin A, and retinal S-antigen is focal (19,24–28). Rare cases show focal positivity for glial fibrillary acidic protein αB-crystallin, warranting careful exclusion of entrapped reactive astrocytes (2,13). Flexner-Wintersteiner rosettes and fleurettes are strongly positive for retinal S-antigen. Hence, PBs have common morphological and immunohistochemical features with photoreceptor cells of the developing pineal gland and retina. The average MIB-1 labeling index is significantly higher in PBs than in other PPTs, with average values of 24% to 27% (29–31).

The ultrastructure of PBs is similar to other poorly differentiated neuroectodermal tumors and shows no recognizable neuronal structures. Tumors are composed of sheets of small cells with round to oval nuclei which are often irregularly indented, with scant cytoplasm, containing few organelles as annulate lamellae, endoplasmic reticulum (smooth and rough), membranous whorls and occasional microtubules, intermediate filaments and lysosomes (25,32–35). Junctional complexes are usually inconspicuous. Compared to pineocytomas, PBs have rare dense core vesicles (33,34). Poorly formed, short cell processes containing microtubules and a few dense core vesicles can be seen (6,34). In some instances of photoreceptor differentiation, such as synaptic ribbons, microtubular sheaves, and club-shaped giant cilia with a 9 + 0 configuration is observed (32–35).

Molecular and genetic alterations underlying the formation of PPTs have not been well defined. Cytogenetic studies have suggested that distal monosomy 12q and partial deletion or loss of chromosome 11 are related to tumor progression (36–38). Monosomy of chromosomes 20 and 22, and trisomy of chromosome 14 have also been described (24). Two studies reported INI1 gene (22q11) mutations (39,40). Patients with RB1 gene abnormalities PBs have a significantly worse prognosis compared to sporadic cases (41). PBs develop in patients with familial bilateral retinoblastomas, an occurrence termed ‘trilateral retinoblastoma syndrome’ (17,42), and have also been reported in patients with familial adenomatous polyposis (43). One study using RT-PCR analysis identified a higher expression of four genes (PRAME, CD24, POU4F2, and HOXD13) in PBs and PPTID, in contrast to pineocytomas (37). Patients with signs of elevated intracranial pressure (ICP) (papilledema, decreased visual acuity) and obstructive hydrocephalus should be admitted for urgent CSF diversion.

Given the propensity of certain pineal tumors to metastasize to the spinal column (PB, ependymoma, or germinoma), a baseline contrast enhanced MRI of the entire spinal axis should be obtained. In addition to further imaging, the presence of a pineal tumor should
also prompt sampling of Germ-Cell Tumors (GCT) markers (\( \beta \)-human chorionic gonadotropin or \( \beta \)-fetoprotein) from serum and/or CSF. Since most patients with pineal tumors present with symptoms related to hydrocephalus, a shunt. Either procedure allows for both CSF sampling and alleviation of obstructive hydrocephalus, which is the most pressing concern during acute management of patients with PB. Patients with CSF positive for GCT markers should then receive adjuvant therapy without the need for further surgical intervention. The treatment of choice for most PPTs, including PBs, remains open surgical resection. Open surgery allows for adequate tissue sampling and improved diagnostic accuracy, which is critical for effective management of pineal lesions. In addition, aggressive surgical resection is associated with improved outcome in both pineocytoma (gross total resection is often curative, obviating the need for adjuvant therapy) and PB (gross total resection results in prolonged survival). Standard adjuvant therapy for pathology-confirmed PB after maximal surgical resection includes fractionated radiotherapy and chemotherapy. Typical radiotherapy protocols for PB are 5500 cGy to the tumor region and 3500 cGy to the spinal axis (2 Gy fractions). Chemotherapy protocols are less consistent among centers but usually include 2 to 3 agents selected from vincristine, cisplatin/carboplatin, cyclophosphamide, etoposide, and CCNU (lomustine). In addition to these standard therapies, more recent modalities such as Gamma Knife radiotherapy have been suggested as an adjunct to conventional radiotherapy or as a substitute for surgical resection, but definitive data are lacking. Several promising experimental therapies are being evaluated for the treatment of PB, including vorinostat (a histone deacetylase inhibitor), retinoic acid, and high-dose chemotherapy with autologous stem cell rescue (44).

5. Conclusions

PBs are uncommon pediatric tumors. Diagnosis can be difficult as the surgical biopsy material is often limited in quality and quantity and the tumor lacks a distinctive immunophenotype. In our study the majority of patients (69%) did well with appropriate therapy. Adequate follow up in order to detect the possibility of recurrence or dissemination is warranted. Further studies are needed to better classify pineal tumors and, in light of the latest 2021 WHO classification molecular subtyping of these unusual pediatric brain tumors is highly recommended.

Abbreviations:
2F11 - anti-neurofilament antibody
ADC - apparent diffusion coefficient
Anti-NeuN - antineuronal nuclear antigen antibody
CCNU - lomustine
CD4 - cluster of differentiation 4
CNS - Central Nervous System
CSF - cerebrospinal fluid
cPNET - central primitive neuroectodermal tumors
GCT- Germ - Cell Tumors
GFAP- glial fibrillary acidic protein
HOXD13 - homeobox B13
ICP - intracranial pressure
PBs - pineoblastomas
PNETs - primitive neuroectodermal tumors
POU4F2 - POU Class 4 Homeobox 2
PPTs - pineal parenchymal tumours
PPTID - pineal parenchymal tumors of intermediate differentiation
PRAME - preferentially expressed antigen in melanoma
MIB-1 - mindbomb antibody
MRI - magnetic resonance imaging
SMARCB1 - SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily b, member 1
SY 38 - anti-synaptophysin antibodies
WHO - World Health Organisation

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