A Pediatric Case of Diffuse Glioma Diagnosed at Autopsy

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ABSTRACT

Sudden death from an undiagnosed primary intracranial neoplasm is extremely uncommon and even rarer in the pediatric population. Gliomatosis cerebri (GC) represents a growth pattern demonstrable by a variety of gliomas, predominating in adults. Herein we present a rare occurrence of diagnosis of a pediatric glioma with a GC pattern of infiltration at autopsy and compare the immunohistochemical results and molecular characteristics in this tumor to the small amount of published knowledge available about pediatric diffuse gliomas with widespread brain invasion. *Acad Forensic Pathol.* 2017 7(4): 657-666

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INFORMATION

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INTRODUCTION

The forensic pathologist is charged with investigating sudden and/or unexpected deaths. Sudden natural death in all age groups can result from diseases or conditions arising in any organ system, although conditions affecting the heart, lungs, and brain are the most likely cause of the demise. The decedent's past medical history and symptomatology before death may prove invaluable in interpreting the autopsy findings and in identifying a cause of death.

Sudden death from an undiagnosed primary intracranial neoplasm is exceptionally rare, with reported frequencies in the range of 0.02% to 2.1% in medicolegal autopsy series (1-3). As a result of widespread access to modern imaging technology, such as computed tomography (CT) and magnetic resonance imaging (MRI), sudden death due to undiagnosed intracranial neoplasms accounts for even fewer cases diagnosed at autopsy. Radiological diagnosis remains difficult in view of the nonspecific findings. A head CT may either appear normal or show subtle hypodensity or diffuse cerebral edema (4). Before the era of CT, the diagnosis of most reported cases was based on autopsy findings. Magnetic resonance imaging is superior at identifying the majority of intracranial neoplasms. However, slight changes in signal intensity with loss of the gray-white matter interface might be the only sign of diffuse neoplastic infiltration.

Gliomatosis cerebri (GC) is a term formerly used to describe a glial tumor that infiltrates the central nervous system extensively without greatly altering the gross neural architecture. The macroscopic appearance has been described as enlargement of the involved structures, flattening of gyri, swelling, and poor demarcation between affected and normal areas. Prior to the 2016 World Health Organization (WHO) *Classification of Tumors of the Central Nervous System* (5), GC was considered a distinct entity, defined as a diffuse glioma involving at least three cerebral lobes, frequently bilaterally, and often extending to infratentorial structures and even the spinal cord (6). Gliomatosis cerebri has been removed from the most recent 2016 WHO Classification as a separate entity

and is instead considered a growth pattern demonstrable by a variety of gliomas, including IDH-mutant astrocytic and oligodendroglial tumors as well as IDH-wild type glioblastomas (7, 8). Such gliomatosis cerebri-like tumors predominate in adults, however multiple case reports and a few small series have been described in children (9-12). The most common presenting complaint is poorly controlled seizures. Other symptoms may include hemiparesis, lethargy, altered mental status, cranial nerve deficits, developmental delay, intention tremor, headache, and emesis (9).

This case report describes the rare occurrence of diagnosis of such an entity at autopsy and compares the immunohistochemical results and molecular characteristics in this tumor to the small amount of published knowledge available about pediatric diffuse gliomas with widespread brain invasion.

Case Report

The decedent was a morbidly obese, 16-year-old Hispanic female with a history of migraine headaches diagnosed seven months prior to her death. Other symptoms included photosensitivity, a "lazy eye," and difficulty hearing in the left ear. A CT done at that time was reported as normal; an MRI was not performed. Two months prior to her death, she experienced severe headache and neck pain aggravated by movement and was diagnosed with a neck muscle spasm and given pain medication. Her terminal events included headache with nausea/vomiting and she was then found unresponsive on the couch. She was taken to the hospital and diagnosed with hypoxic/ischemic brain injury, diffuse cerebral edema with herniation, and multiorgan failure. Death was pronounced 24 hours after arriving at the hospital.

Autopsy Findings

The 1350 g brain was diffusely edematous with evidence of mild, bilateral uncal and cerebellar tonsillar herniation. Serial sectioning of the brain revealed patchy blurring of the gray-white junctions and diffuse softening. There were no obvious tumor masses; however, there was significant, near-symmetrical

Image 1: Gross brain at autopsy without obvious tumor masses. Instead, there is near-symmetrical enlargement of the cerebral hemispheres and deep gray nuclei, most predominantly involving the thalami.

enlargement of the cerebral hemispheres and deep gray nuclei, most predominantly involving the thalami (**Image 1**). The lateral ventricles were compressed, but slightly enlarged in the anterior and posterior regions, secondary to gross enlargement of the deep gray nuclei. The left basal ganglia were slightly larger than the right. The thalami were enlarged, had a firm texture, a mottled gray-white appearance, and an expanded midline connection. The hippocampi were asymmetrical; the left hippocampus was enlarged and had a mottled, gray-white appearance. The posterior aspect of the right hippocampus was displaced laterally, secondary to enlargement of the right thalamus. Sectioning of the cerebellum and brainstem revealed parenchymal softening and patchy blurring of the gray-white junctions.

The brain showed microscopic evidence of diffuse hypoxia/ischemia (with hypereosinophilic neurons with pyknotic nuclei) and parenchymal edema. Additionally, it harbored a diffusely infiltrating glioma composed of tumor cells with irregularly-shaped nuclei and scattered mitotic figures. The tumor involved the basal ganglia, thalami, hippocampi, brainstem structures (midbrain, pons, medulla and 3rd cranial nerves), cerebellum, and cervicomedullary junction/upper cervical spinal cord, as well as the cerebral hemispheres. The thalami (**Images 2 and 3**), pons, and cerebellum (**Images 4 and 5**) had the most extensive tumor infiltration. Tumor cells diffusely infiltrated white and gray matter and sometimes exhibited perineuronal, perivascular, or subpial tumor collections. From a morphologic standpoint, the tumor consisted of a mi-

Image 2: Thalamus with hypercellularity due to infiltrating glial tumor cells with irregularly-shaped nuclei. Extensive perineuronal satellitosis is seen throughout this field (H&E, x100).

totically active, infiltrative astrocytoma (anaplastic astrocytoma), lacking any evidence of microvascular proliferation or palisading necrosis. Tumor cells were positive for glial fibrillary acidic protein (GFAP) and had rare p53 nuclear positivity. The mitotic index (Ki-67/MIB-1) was approximately 8-10%, with an average mitotic figure count of one to two per high power field in regions of highest cellularity (**Image 6**). Mutant-specific immunohistochemical stain targeting histone H3-K27M (Millipore, 1:800, ABE419) was negative (**Image 7**). Additional mutant-specific immunohistochemical assays for BRAF-V600E (Ventana, predilute, clone VE1) and IDH1-R132H (Dianova, 1:200, clone H09) were also negative. ATRX (Abcam,

1:500) expression was retained, though PDGFR α (Santa Cruz, 1:150, clone C20) immunohistochemical stain was negative. Fluorescence *in situ* hybridization (FISH) evaluation for amplifications of *MYCN* or *PDGFR α* ; deletions involving *CKDN2A* (*p16*);

duplication/translocation involving *BRAF*; or translocations involving *MYBL1*, *FGFR1*, *MYB*, or *NTRK2* were all negative. Chromosomes 1p and 19q were intact/non-codeleted.

Image 3: Thalamus at high-power with infiltrating glial tumor cells with irregularly-shaped nuclei and scattered mitotic figures (indicated with arrow) (H&E, x400).

DISCUSSION

Gliomatosis cerebri has been removed from the new 2016 World Health Organization *Classification of Tumors of the Central Nervous System* as a distinct entity and is instead a descriptive pattern of widespread infiltration that several gliomas can display. A newly defined group of tumors primarily occurring in children (but sometimes in adults) is characterized by K27M mutations in the histone H3 gene *H3F3A*, or less commonly in the related *HIST1H3B* gene. These tumors have a diffuse growth pattern and a midline location (thalamus, brainstem, and spinal cord) (13, 14). The new entity, termed diffuse midline glioma, *H3* K27M-mutant, includes the majority of those tumors previously called diffuse intrinsic pontine glioma (DIPG). A GC-like pattern of diffuse infiltration is not uncommon in these tumors. Approximately 50% of pediatric infiltrative gliomas arising in deep gray structures will harbor Histone *H3* K27M mutation, while approximately 80% of DIPGs will qualify for this specific diagnosis as well. In the current case, although the tumor predominantly involved the deep gray structures (thalamus/basal ganglia), cerebellum, and brainstem, the lack of staining with mutant-specific *H3* K27M immunohistochemical stain disqualifies this tumor from being diagnosed as diffuse midline glioma, *H3* K27M-mutant.

Image 4: Pons with modest hypercellularity due to infiltrating glial tumor cells. Note the prominent perivascular condensation of tumor cells in the upper aspect of this image (H&E, x100).

We present a case of pediatric diffuse glioma with gliomatosis cerebri-like pattern of involvement and rapid demise diagnosed at autopsy with molecular correlation. The molecular testing performed on this tumor revealed the absence of a variety of mutations/alterations. The presence of intact/non-codeleted chromosomes 1p and 19q portends a poor prognosis, especially in the pediatric population. The tumor in this case had only rare p53 nuclear positivity, most consistent with wildtype *TP53* status. Our results are comparable with published literature that describes the frequent occurrence of few alterations in infiltrating diffuse gliomas formerly classified as gliomatosis cerebri. The involvement of p53 mutation or nuclear protein accumulation described in some cases of GC has linked the astrocytic phenotype of these diffusely infiltrating gliomas to p53 alterations frequently found in common infiltrating astrocytomas. However, the frequency of these alterations overall is low, and likewise other molecular genetic changes have been only rarely identified (15). The alterations commonly found in high-grade astrocytomas and glioblastomas are usually missing in GC-like tumors. The mechanism(s) responsible for the enhanced infiltrative potential of GC-like tumors thus remains in question.

Very little has been published about the molecular characteristics of pediatric diffuse glioma with a GC-like pattern of infiltration (10, 16-18). Some studies have shown that these pediatric diffuse gliomas, previously called pediatric GC, harbor mutations, oncogene amplifications, and particularly, a methylation profile that corresponds to other pediatric high-grade gliomas (19-22). A published analysis of methylation array data by Broniscer et al. from 32 pediatric GC cases classified the tumors as IDH (17%), G34 (22%), mesenchymal (17%), and RTK I '*PDGFRA*' (44%). No tumors were classified as K27M subgroup in that study, and *PDGFRA* was the most commonly amplified oncogene. Other amplified oncogenes included *MDM4* (n=2), *CDK4* (n=1), *MYC* (n=1), and *MYCN* (n=1). *CDKN2A* deletion was observed in two of 22 cases (9%). *H3F3A* p.G34 occurred in all cases classified as G34. Two of three cases in the IDH subgroup had *IDH1* p.R132H. No *H3F3A* p.K27M, *IDH2* p.R172, or *BRAF* p.V600E mutations were observed (7).

The molecular features of pediatric GC-like tumors appear to be distinct from their adult counterparts, despite some minor overlap. Whereas the IDH subgroup is fairly uncommon in children, it accounts for approximately half the cases of adult GC-like tumors (8, 17, 23). The K27 methylation subgroup, which

accounts for the largest group of pediatric high-grade gliomas (19, 20), is sometimes found in pediatric GC-like tumors (in the context of diffuse midline glioma, *H3 K27M* mutant tumors with widespread brain involvement). This finding may not seem intuitive since the definition of GC-like tumors is based on tumor

Image 5: Cerebellum at high-power with loss of architecture due to marked infiltration by glial tumor cells (H&E, x200).

involvement of cerebral lobes while *H3F3A p.K27M* predominantly affects midline tumors (20-22). However, children with GC-like tumors commonly have involvement of deeper brain structures, including the thalami, in addition to the cerebral lobes.

In the literature, the most common primary brain tumors responsible for sudden death are glioblastomas (3). Other tumors that have been reported to present this way include anaplastic astrocytoma, oligodendroglioma, medulloblastoma, lymphoma, teratoma,

Image 6: Ki67 immunohistochemistry with scattered nuclear staining consistent with a cell proliferation rate of approximately 8% (MIB-1; cell proliferation marker, x200).

and pituitary adenoma (24). Mechanisms of death include seizures, acute hemorrhage, and herniation due to mass effect. Possible mechanisms for sudden death in the current case include tumor involvement of the respiratory center nuclei of the medulla, diffuse cerebral edema with downward herniation, and/or acute

hydrocephalus due to tumor obstruction of the ventricular system.

Despite the use of aggressive therapy, GC-like tumors occurring at any age portend one of the poorest prognoses of all central nervous system cancers, probably

Image 7: Negative staining results for Anti-Histone *H3 K27M* immunohistochemistry, x200.

only surpassed by DIPG (which itself may present as a GC-like tumor) (25). Studies have shown that patients with bithalamic involvement had worse outcomes. Survival typically ranges from weeks to some years (uncommonly) after the manifestation of symptoms.

CONCLUSION

It is important for the forensic pathologist to consider brain tumors as a possible cause of sudden death in both adults and children and to look carefully at the brain for subtle gross findings. This case of diffuse glioma appeared as a poorly defined lesion causing enlargement of the affected structures, often with blurring of the gray-white junctions. Some cases may lack any visible macroscopic pathology, except for a diffuse swelling of the brain (26).

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