

Case Report

Hyaline eosinophilic cortical astrocytic inclusions in a child with intractable epilepsy

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Abstract: The presence of hyaline eosinophilic inclusions in protoplasmic astrocytes of cerebral cortex is a rare finding that is mostly associated with history of intractable epilepsy and cases of Aicardi syndrome. Here, we describe a 17-year-old male who presented with a longstanding history of intractable epilepsy. Microscopic examination of the excisional biopsy of the epileptic focus showed hyaline eosinophilic inclusions within cortical astrocytes. In addition, we discuss the most recent evidence with regards to their pathogenesis.

Keywords: Hyaline, astrocyte, inclusion, epilepsy

Introduction

The presence of hyaline eosinophilic inclusions in protoplasmic astrocytes of cerebral cortex is a rare finding that has been documented mostly in cases of intractable epilepsy and Aicardi syndrome. The etiology and pathogenesis of such a finding is currently not well established. A case of intractable epilepsy with hyaline eosinophilic astrocytic inclusions is discussed, followed by a review of the current literature on the subject.

Case report

This 17 year-old male presented with a long history of epilepsy starting at the age of 7 months, when he had the first seizure attack, a generalized tonic-clonic seizure. Shortly after that, he suffered seizures with frequency around 20 times per day as sudden staring spells with unresponsiveness followed by left arm clonic movements, that lasted for a few minutes followed by left arm weakness and deep sleep. He was started on phenobarbital and clonazepam. At the age of 3 years, he experienced seizures

of complex partial type during sleep, described as waking up and walking during sleep with sudden staring and talking nonsense. These episodes lasted from 10 to 45 minutes and occurred with an average frequency of once per 2 weeks. Consequently, carbamazepine and lamotrigine were added to the previous therapy. As the patient was still having seizures, topiramate and levetiracetam were added at the age of 7. The longest seizure free period was 2 months.

He was born full-term by uncomplicated spontaneous vaginal delivery. There was no history of febrile convulsions or head trauma prior to the seizure episodes. He had a family history of seizure disorder in his maternal uncle. His parents are not relatives. He has 4 siblings with no health problems. He has short stature since early childhood. On mental status examination, the patient had attention-deficit hyperactivity disorder (ADHD), and borderline IQ.

Investigations showed blood counts, and electrolytes that were within normal. Antinuclear antibodies (ANA) and rheumatoid factor were

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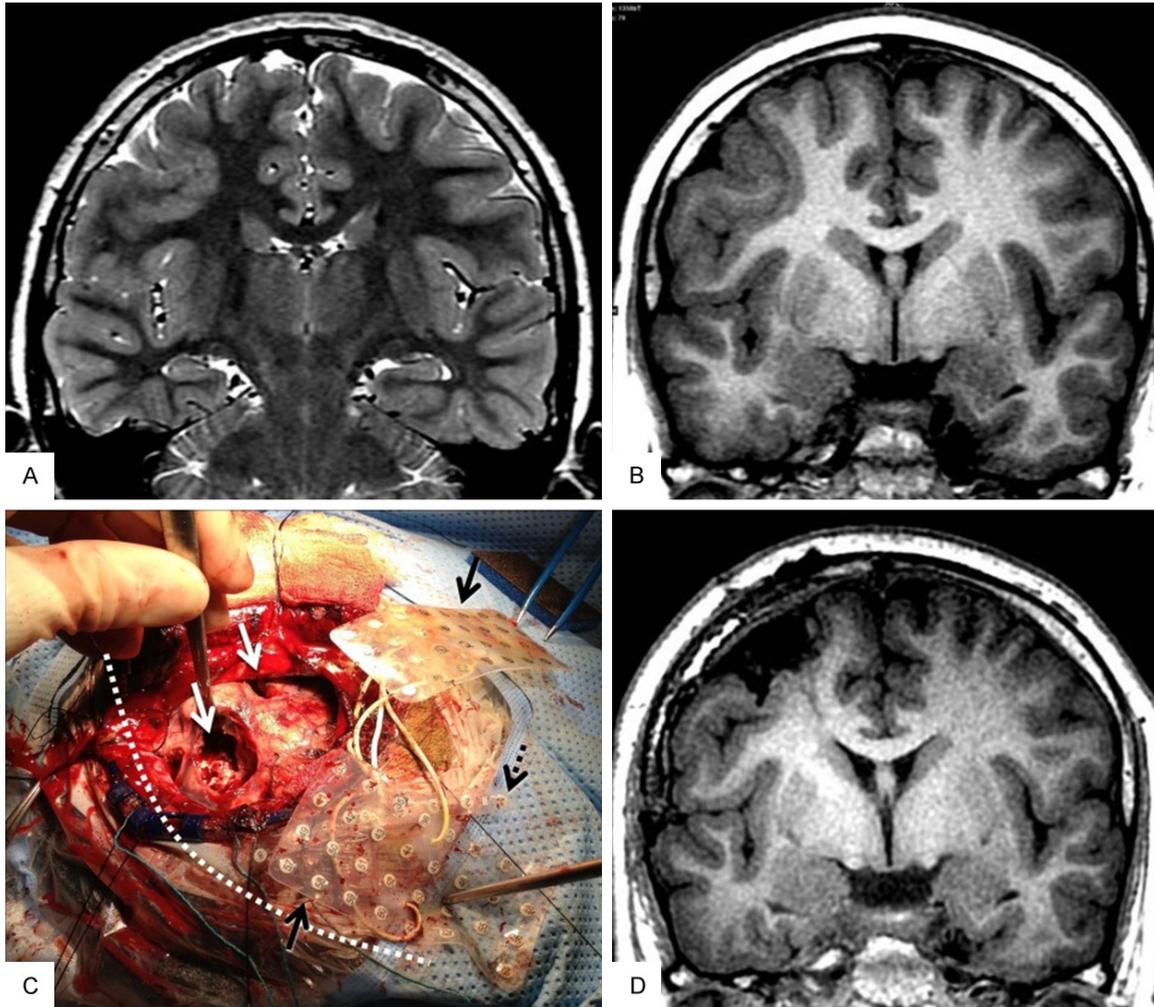


Figure 1. A, B. Preoperative coronal T2-weighted and coronal 3D T1-weighted images show diffuse polymicrogyria involving the right frontal lobe and right perisylvian region. C. Intraoperative photograph showing resection cavities (White arrows) the resection was tailored according to electrocortical recording using two (4×8) grids (solid black arrows) and one (8) strip (dotted black arrow). The midline is indicated by the white dotted line. D. Post-operative coronal 3D T1-weighted image demonstrates partial resection of the right frontal polymicrogyria.

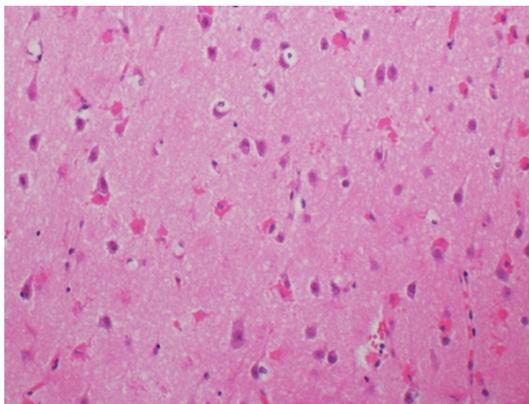


Figure 2. Hyaline refractile astrocytic inclusions, mostly in astrocytes close to neurons (H&E stain, 200× magnification).

negative. HIV, hepatitis B and hepatitis C antigen serology were negative. Karyotype analysis was normal, and testing for Fragile X-syndrome was negative.

Magnetic Resonance Imaging (MRI) of the brain revealed focal malformation of cortical development in the form of polymicrogyria involving the right middle and inferior frontal gyri and the right perisylvian region with cortical infolding and deepening sulci. In addition, the gray-white matter junction is blurred and the volume of the underlying white matter structure is reduced (Figure 1A, 1B).

He underwent video electroencephalography (EEG) monitoring which demonstrated indepen-

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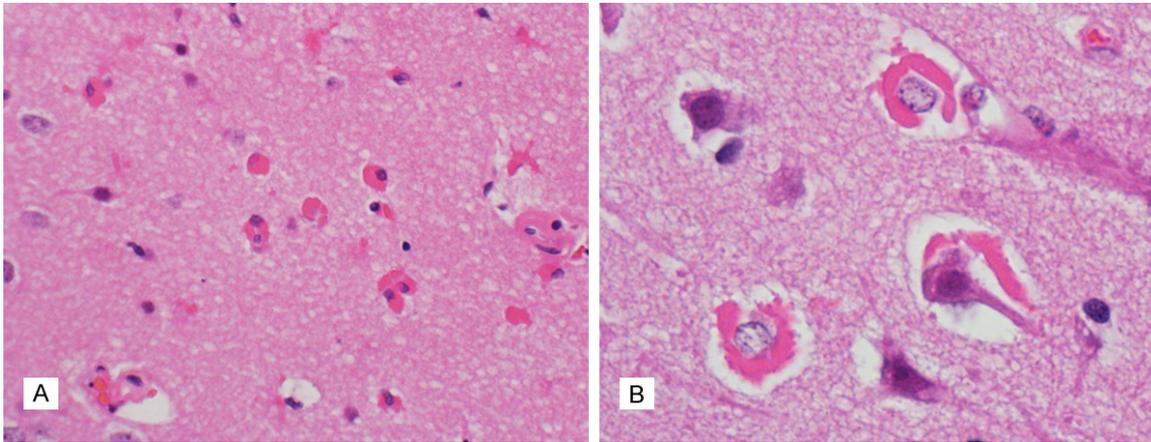


Figure 3. A. The inclusions are mostly close to the nucleus with few scattered in the background neuropil (H&E stain, 400× magnification). B. The inclusions frequently have a horseshoe shape (H&E stain, 1000× magnification with oil).

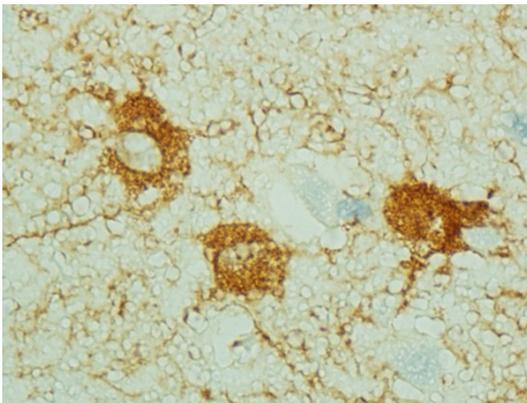


Figure 4. Inclusions are positive for GFAP in this case (GFAP immunohistochemistry, 1000× magnification with oil).

dent spike and wave activity more in the right than the left frontal lobe, causing right frontal complex partial epilepsy. This was further assessed by inserting intracranial grid and strip electrodes for telemetry monitoring for one week. This also assisted in functionally localizing the motor cortex.

The patient was elected for a craniotomy and excision of the epileptic focus under electrocorticographic monitoring. Two right frontal non-contiguous resections were tailored according to the corticographic information available before and after the resection. Intraoperative MRI and neuronavigation guidance were utilized to improve and document the degree of surgical resection (**Figure 1C**). Post-operative MRI imaging shows 2 adjacent resection cavities in the right frontal lobe (**Figure 1D**).

On microscopic examination of excised tissue, fragments of cortex and white matter were present. The cortex showed normal layering with sharp demarcation from white matter. The white matter was normal with no increased cellularity. The cortical astrocytes contained multiple intracytoplasmic eosinophilic and highly refractile inclusions that had an amorphous to granular quality, mostly close to the nucleus, and often had a horseshoe or ring appearance. They were evenly distributed throughout the layers with no involvement of the white matter. Few of the inclusions were free in neuropil background and close to the cortical neurons (**Figures 2, 3**). These inclusions were negative for periodic acid-Schiff (PAS) and Congo red. Immunohistochemistry showed that the inclusions were positive for glial fibrillary acidic protein (GFAP) (**Figure 4**), vimentin, and IgG, weakly positive for S100, and negative for tau, synaptophysin, amyloid-P, neurofilament protein, ubiquitin, and IgA.

The post-operative course was uneventful. The patient had 4 months of seizure freedom. The last documented follow-up was one year after the surgery. The patient's seizures recurred with similar frequency to what he had pre-operatively.

Discussion

Cytoplasmic inclusions in the nervous system are rarely confined to astrocytes. The differential diagnosis for astrocytic inclusions includes Rosenthal fibers (RF), eosinophilic granular bodies (EGB), amyloidosis, and Lafora bodies.

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Table 1. Collection of case reports of hyaline astrocytic inclusions

No.	Year	Author	Age	Sex	Clinical Presentation
1	1970	Miyakawa et al [11]	32	M	Mental retardation from early childhood
2	1992	Minagawa et al [1]	20	M	Severe Physical & Mental Retardation, epilepsy
3	1992	Abe et al [7]	5	F	Aicardi syndrome, moderate psychomotor retardation
4	1994	Špaček et al [10]	71	M	Rapid mental & psychomotor retardation in a previously mentally & physically fit patient
5	1994	Minamitani et al [12]	17	F	Severe mental & physical retardation
6	1996	Buchino JJ et al [8]	13	F	Aicardi syndrome
7	2003	Horoupian et al [2]	17	F	Pediatric onset intractable seizures, seizure free post-surgery
8	2004	Van den Veyver et al [9]	7	F	Aicardi syndrome
9	2004	Van den Veyver et al [9]	17	F	Aicardi syndrome
10	2008	Hazrati et al [3]	5	M	Pediatric onset intractable seizures, developmental delay
11	2008	Hazrati et al [3]	6	F	Pediatric onset intractable seizures, developmental delay
12	2008	Hazrati et al [3]	9	F	Pediatric onset intractable seizures, developmental delay
13	2008	Hazrati et al [3]	4	M	Pediatric onset intractable seizures, developmental delay
14	2008	Hazrati et al [3]	8	M	Pediatric onset intractable seizures, developmental delay
15	2009	Hedley-Whyte et al [4]	14	F	Pediatric onset seizures with developmental delay
16	2009	Hedley-Whyte et al [4]	7	M	Pediatric onset seizures, developmental delay
17	2009	Hedley-Whyte et al [4]	26	M	No data
18	2009	Hedley-Whyte et al [4]	14	F	Pediatric onset seizures, developmental delay
19	2009	Hedley-Whyte et al [4]	4	M	Pediatric onset seizures, developmental delay
20	2010	Adam J. et al [5]	16	F	Pediatric onset intractable seizures, seizure free post-surgery
21	2010	Adam J. et al [5]	9	F	Pediatric onset intractable seizures with mental retardation, partially improved by surgery
22	2013	Wong et al [6]	6	M	Pediatric onset intractable seizures with developmental delay, seizure free 3 months post-surgery

Rosenthal fibers (RF) occur in various neoplastic and nonneoplastic conditions. Examples of neoplasms harboring RF are pilocytic astrocytoma, pleomorphic xanthoastrocytoma (PXA), and ganglioglioma (GG). Nonneoplastic conditions such as trauma and Alexander disease may show abundant RF. Our case does not show an increased cellularity enough to put it in the neoplastic category. In addition, there is no evidence of pleomorphism, xanthomatous change or dysplastic ganglion cells to suggest PXA or GG. Alexander disease is a demyelinating disorder characterized by microcephaly & hydrocephalus. The brain shows features of demyelination of the white matter which is not present in our patient. Amyloidosis appears as an extracellular hyaline material and usually shows perivascular distribution, and is Congo red positive. Our patient shows granular cytoplasmic inclusions that are Congo red negative. Lafora bodies are PAS-positive, diastase-resistant, spherical inclusions that are found in the perikarya of neurons in Lafora disease, which usually presents with myoclonic epilepsy, ataxia and dementia. The inclusions we describe are within the astrocytes and are PAS negative.

The presence of hyaline eosinophilic inclusions in neocortical astrocytes has been reported in

the literature in scattered case reports (**Table 1**). Most of these occurred in the context of pediatric-onset epilepsy not responding to medications, often associated with psychomotor retardation [1-6]. In addition to Aicardi syndrome [7-9] and cerebral autosomal dominant arteriopathy with subcortical infarcts and leukodystrophy (CADASIL) [10].

The specific nature of these inclusions has not been clearly defined. Recently, Van den Veyver et al identified the presence of filamin A in these inclusions in 2 patients with Aicardi syndrome, [9] followed by Hazrati et al in 5 patients with infant-onset epilepsy, [3] whom proposed that these cases should fall under the spectrum of "Filaminopathies". Hedley-Whyte et al [4] did an extensive immunohistochemical study on 5 cases and found that in addition to filamin A, these inclusions are immunopositive for glutamate transporter 1 (GLT1) and cytoglobin. In our case, the inclusions were immunopositive for GFAP which is unusual but has been reported in 2 cases by Hedley-Whyte et al.

Ultrastructural analysis of the inclusions reveal that they are composed of electron-dense granular and amorphous non-membrane bound material, and do not contain filamentous material as in Rosenthal fibers [1, 2, 3, 7, 9].

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Proteomic analysis of brain tissue containing the inclusions have been attempted [13] and showed that expression of carbonic anhydrase I and catalase is increased in comparison to normal brain tissue, but not filamin A.

Post-operative follow-up in cases treated with resection of the affected areas showed varying results ranging from no effect on seizure frequency as in our case, to complete control of seizures.

In conclusion, the presence of hyaline neocortical astrocytic inclusions is a rare finding associated mostly with cases of pediatric-onset intractable epilepsy and Aicardi syndrome. Recent evidence suggests that it could represent a filaminopathy. Further research is needed to understand their nature and pathogenesis.

Disclosure of conflict of interest

None.

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