Brain swelling in pediatric cerebral malaria

1. 5T MRI to Investigate Potential Etiologies of Brain Swelling in Pediatric Cerebral Malaria

Abbreviations:

Red Blood Cell (RBC)

Parts per billion (ppb)

Cerebral malaria (CM)

Blood brain barrier (BBB)

Abstract

Objective:

Cerebral malaria (CM) remains a common cause of death in African children. The pathologic hallmark of pediatric CM is sequestration of parasitized red blood cells in the cerebral microvasculature. Recent Malawi-based research utilizing a 0. 35T MRI has established that severe brain swelling is associated with fatal CM, but the etiology of brain swelling remains unclear. Autopsy and clinical studies suggest several potential etiologies, but technical limitations of 0. 35T MRI precluded optimal investigations into swelling pathophysiology. A 1. 5T MRI in Zambia allowed for further investigations including susceptibility weighted imaging (SWI). SWI is an ideal sequence for identifying regions of sequestration and microhemorrhages given the ferromagnetic properties of hemozoin and blood.

Methods:

Using 1. 5T MRI, Zambian children with retinopathy-confirmed CM underwent imaging with SWI, T2, T1 pre- and post-gadolinium, DWI with ADC and T2/FLAIR sequences.

Results:

Sixteen children including two with moderate/severe edema were imaged. All survived. Gadolinium extravasation was not seen. Cerebral perfusion was intact with DWI abnormalities sparing the gray matter. SWI findings consistent with microhemorrhages and parasite sequestration co-occurred in white matter regions where DWI changes consistent with vascular congestion were seen. Findings consistent with posterior reversible encephalopathy syndrome as a cause of swelling were also present.

Interpretations:

High field MRI findings indicate that vascular congestion associated with parasite sequestration, local inflammation from microhemorrhages and autoregulatory dysfunction contribute to brain swelling in CM.

Keywords: sequestration; venous congestion; hemozoin

Introduction:

Pediatric cerebral malaria (CM), defined as *P. falciparum* peripheral parasitemia and unarousable coma with no other coma etiology evident, primarily affects children in sub-Saharan Africa [1]. Although antimalarial agents provide rapid parasite clearance, mortality rates remain high (8-25%) [2, 3]. The pathological hallmark of pediatric CM at autopsy is intravascular

sequestration in which parasitized red blood cells (RBCs) adhere to the endothelium of cerebral microvessels.

Although malaria causes almost a million deaths per year, neuroimaging capacity is typically limited in malaria-endemic regions. Only one large MRI case series from Malawi using a 0. 35T MRI has provided insights into the *in vivo* structural abnormalities associated with pediatric CM [4]and CM mortality [5]. Other studies and case reports using higher field MRIs have been performed on adults [6, 7], but adult CM appears to represent a different disease syndrome [8]. In adult CM, coma onset largely occurs some days after illness onset in the setting of multisystem organ failure often including hepatic dysfunction, renal failure and gross electrolyte abnormalities. As such, the coma of adult CM is clinically dominated by the effects of a toxic, metabolic encephalopathy. In contrast, in pediatric CM coma onset occurs very early in the malaria illness, often as one of the first signs of the illness, with very limited hepatic or renal involvement and no evident systemic cause for coma. MRI insights gained from imaging pediatric CM to date have been limited to low field MRI technology.

The recent pediatric CM MRI study used 0. 35T technology to establish that increased intracranial pressure due to increased brain volume is the cause of death in CM [9], but the low field MRI technology was unable to further evaluated the potential etiologies of brain swelling in pediatric CM, so the underlying cause(s) of cerebral edema in CM remains unclear. Further study delineating the underlying cause(s) of swelling is needed to develop appropriate interventions. Potential etiologies suggested by autopsy and clinical studies include any/all of the following: (a) blood brain barrier (BBB)

breakdown with resultant vasogenic edema [10, 11]; (b) impaired perfusion resulting in cell death with cytotoxic edema [12]; (c) vascular congestion due to occlusion at the post-capillary venules [13]; (d) hyperemia with auto-regulatory dysfunction due to endothelial injury and CM-associated seizures, anemia and hyperpyrexia [14, 15] , [16]; and (e) diffuse cerebral microhemorrhages (i. e. ring hemorrhages) [11].

Hemozoin is an iron-rich breakdown product of the parasite's metabolism of hemoglobin [5]. Hemozoin is present primarily in mature, sequestered parasites. Thus, susceptibility weighted imaging (SWI) [17], which is extremely sensitive to the magnetic field inhomogeneity caused by ferromagnetic substances, is an ideal imaging sequence for identifying regions of parasite sequestration. SWI also offers the ability to identify small hemorrhages on the order of several μg of blood per gram of tissue [18] , [19].

We hypothesized that imaging retinopathy-confirmed pediatric CM with a 1. 5T MRI including DWI, SWI and gadolinium enhanced sequences would identify pathophysiological mechanisms underlying cerebral edema in pediatric CM and undertook an imaging study of CM in Zambia where 1. 5T MRI is available specifically seeking evidence of blood brain barrier breakdown, impaired perfusion, parasite sequestration, autoregulatory dysfunction and microhemorrhages.

Material and Methods:

Subjects and Recruitment

During the malaria seasons (Jan-June) in 2012-2014, comatose children with retinopathy-confirmed [20] CM underwent brain MRI on the 1. 5T MRI scanner (Siemens Magnetom Essenza using Syngo MR 200 4A version software, Germany) at the Cancer Diseases Hospital in Lusaka, Zambia within 24 hours of admission. Inclusion criteria were: (1) admission to the pediatric high care unit of the University Teaching Hospital, (2) a Blantyre Coma Score of â‰¤ 2 [21], (3) *P. falciparum* infection as determined by a Paracheck Rapid Diagnostic Test (RDT), (4) the presence of malarial retinopathy, and (5) no other evident etiology for coma. A thick peripheral blood smear to identify parasitemia was also obtained prior to recruitment, but was not immediately available and was not required for inclusion. All children received standard antimalarial treatment, anticonvulsants, antipyretics, antibiotics and blood transfusions, as clinically indicated and in accordance with national treatment guidelines. As per present treatment standards, no steroids were given. Children with comorbid meningitis as determined by cerebrospinal fluid analysis were excluded from enrollment. Written consent was obtained from the child’s parent or guardian. Children with impaired renal function (creatinine â‰¥2. 0) did not receive gadolinium. This study was approved by the Institutional Review Boards at the University of Zambia, Michigan State University and the University of Rochester.

Imaging

Gadolinium (Magnevist) doses were determined by individual patient weight and administered intravenously (0. 2 mL/kg, 0. 1 mmol/kg) by hand injection. The scanning protocol is provided in an appendix. Apparent diffusion

coefficient (ADC) calculations were provided by the standard Siemens software algorithms [22]. SWI phase images were collected unfiltered and post-processed with a 64×64 high pass filter then viewed using SPIN (signal processing in nMR) software. SWI was also collected with a shorter echo time (15ms) for some subjects to avoid potential aliasing [17].

Interpretation

Images were reviewed independently by two radiologists (MJP; neuroradiologist, and SDK; MRI fellowship trained radiologist) and data were managed using NeuroInterp, a web-based program that allows radiographic findings to be entered into a searchable and quantified database [23]. Reader discrepancies, determined in advance of the analysis, were reevaluated by the two radiologists to develop a consensus interpretation.

Increased brain volume, the imaging finding associated with fatal cerebral malaria, was rated on a scale from 1-8 with 3 being no edema, 1 and 2 indicating atrophy. An edema score of 4-5 indicated minimal-mild edema, with no loss of sulcal markings. Grade 6 (moderate edema) was defined as loss of some sulcal markings. An edema score of 7 represented moderate/severe edema with diffuse sulcal and cisternal effacement universally evident but without herniation present, and the severe edema score of 8 required sulcal and cisternal effacement with evidence of herniation.

MRI findings coded within the NeuroInterp database that could plausibly be associated with the five potential pathogenic mechanisms of brain swelling in CM were then reviewed. Specifically, (a) to evaluate diffuse BBB

breakdown causing vasogenic edema, we looked for evidence of gadolinium enhancement [24], (b) to assess for impaired perfusion and subsequent cytotoxic edema we looked for gray matter diffusion weighted imaging (DWI) abnormalities [25], (c) evidence for vascular congestion or venous micro-occlusion was sought by looking for white matter DWI abnormalities [26], (d) autoregulatory dysfunction was evaluated by looking for focal regions of symmetric hemispheric edema of varying confluence in regions susceptible to autoregulatory vulnerabilities [9, 27], and (e) SWI abnormalities were assessed clinically and quantitatively based upon effective voxel susceptibility with the anticipation that these would be located in the same anatomical regions as ring hemorrhages and sequestration have been identified in prior autopsy studies [28]. Given the small anticipated sample size (<20 subjects) and the lack of a normal control group, no statistical analyses or comparisons were planned.

Results:

Patient Characteristics and Data Acquisition

Twenty three children met study inclusion criteria during the enrollment period. Parents declined participation for 2 children and 5 children were deemed too ill to transfer for imaging or died before imaging could be performed, so 16 subjects were imaged–5 (31%) were male and the mean age was 6. 4 years (range 1-15). Table 1 provides demographic data and admission clinical characteristics from the 16 subjects who were imaged.

Of the 5 consented children who were not imaged, 3 died. Among the 16 subjects imaged, the scans for one patient was non-diagnostic on the SWI

sequence due to movement artifact. Renal function could not be ascertained on two children, so these subjects did not receive gadolinium. There were no fatalities among the imaged study subjects and none had clinical sequelae evident at discharge. Table 2 provides the frequencies of the 1. 5T MRI findings identified and captured in NeuroInterp.

MRI Findings

Increased brain volume: None of the subjects had severe (grade 8) edema. Moderate/severe (grade 7) edema was present in 2/16 (13%); moderate (grade 6) edema in 4/16 (25%); minimal/mild (grade 4 & 5) edema in 7/16 (44%) and no edema in 3/16 (19%).

T2 signal changes: The total number of cases exhibiting white matter increased T2 signal was 12/16 (75%), and two distinct patterns were observed: primarily subcortical (10/12, or 83%) and primarily periventricular/peritrigoneal (2/12, 17%) (Figure 1). These generally occurred in isolation; only 2 cases had both findings.

Gadolinium enhancement : The expected normal physiological intravascular and circumventricular organ enhancement was evident in all subjects on the post-contrast images (Figure 2). A small region of subtle focal cortical enhancement was seen in one subject with positive SWI signal and no associated T2 abnormalities consistent with a capillary telangiectasia. There was no evidence of gadolinium extravasation in the other 13 patients who received contrast.

Cortical findings: Cortical swelling and increased T2 signal was seen in 10/16 (63%), but these signal abnormalities were relatively mild in extent, confluent, and without associated cortical DWI findings. Increased cortical T2 signal was generally diffuse, with only 2/16 (13%) having a posterior predominant pattern [4]. DWI showed restricted water diffusion in the subcortical white matter in 10/16 (63%) which was confirmed by accompanying ADC maps.

Basal Ganglia and Thalamus Abnormalities : The structures in the basal ganglia had different levels of involvement. T2/FLAIR signal abnormalities were present in the globus pallidus and putamen in 10/16 (63%), and the caudate in 9/16 (56%). While frequently involved simultaneously, there was generally a region of predominance (Figure 3). Regional differences were also illustrated in the DWI images. Fifty six percent of subjects had DWI abnormalities in the globus pallidus, 13% in the putamen and none in the caudate.

Pontine and Brainstem Signal Abnormalities : This was assessed at two levels, within the pons at the level of the middle cerebellar peduncle and within the brainstem at the level of the substantia nigra. Pontine involvement was seen in 9/16 (56%) and brainstem in 11/16 (69%). Abnormalities were usually diffuse, and consisted of generalized increase in T2 signal. However, focal areas of involvement were also seen.

Corpus callosum : Showed increased T2 signal and thickening in 10/16 (63%) with 6/10 having associated positive DWI findings as confirmed by ADC

maps. The splenium was the primary site of involvement in 9/10 (90%) of cases.

SWI Findings : Decreased signal is defined as a positive SWI finding as it localizes to areas of magnetic field inhomogeneity caused by the presence of a ferromagnetic substance (Figure 4). SWI findings were noted along the regions of the venules of both the superficial and deep venous systems corresponding to areas of parasite sequestration and ring hemorrhages. SWI resolution did not allow distinction between gray and white matter involvement in the cerebellum. One SWI dataset was not interpretable due to severe motion artifact. In the remaining cases, 7/15 (47%) showed abnormal paramagnetic signal within the following regions of the parenchyma: corpus callosum (7/15, 47%), sub-cortical white matter (6/15, 40%), cerebellum (5/15, 33%), lenticulae striate (5/15, 33%), and periventricular white matter (2/15, 13%). In two subjects, both the internal capsule and optic radiation had abnormal paramagnetic signal.

The susceptibility of heavily infected red blood cells is ~1880 parts per billion (ppb) relative to water [18]. The effective voxel susceptibilities in the corpus callosum and junction of the cortical gray and white matter was 50 ppb relative to water in SWIM. As distributed within the voxel, this represents a 1/38 th decrease in susceptibility. Given the voxel size of 0. 5 x 0. 5 x 2. 0 mm ^{3} , this represents 1/78 th µL. Assuming the capillary volume is ~5% (or 1/20 th of the pixel) [29], this indicates that ~half of the capillaries are filled with hemozoin.

The combination of moderate to severe symmetrical cortical swelling (edema score of 6 or 7), with corresponding underlying subcortical white matter changes with associated DWI and ADC findings was evident in 4/16 (25%) of cases (Figure 5) with two of the four showing a predominantly posterior distribution.

Table 3 summarizes the MRI findings seen using 1. 5T in 16 Zambian children with CM in the context of the proposed mechanisms for brain swelling in CM and the 1. 5T MRI findings anticipated for each mechanism.

Discussion:

MRI findings using a 0. 35T MRI have shown that death from pediatric CM occurs due to increased brain volume [9] but low field MRI was unable to further delineate the etiology for the brain swelling. Interventions studies aimed at reducing or preventing cerebral edema in CM would ideally target the underlying mechanism of swelling. Existing clinical and autopsy data suggest at least five potential etiologies for brain swelling in CM. In this study, we describe what the MRI findings associated with each of these potential etiologies would be and then used 1. 5T MRI in children with retinopathy-confirmed CM to identify the presence or absence of findings consistent with each of the five proposed etiologies. As such, the results of this study can be subdivided into evidence both for and against these specific potential origins of brain swelling in pediatric CM.

Decreased SWI signal was evident on the brain MRIs of children with CM and furthermore these changes were seen in regions where autopsy studies have shown microhemorrhages (Figure 6) as well as in the regions where

sequestration is common. Since the SWI signal effectively identifies blood and hemozoin, both sequestration and ring hemorrhages were likely identified. Marked T2/DWI abnormalities were evident in the subcortical brain regions most sensitive to venous outflow obstruction. If perfusion is obstructed in regions with SWI signal changes, then blood flow to the tissue would decrease by ~50% which is consistent with what is seen in an animal model of malaria where blood flow was found to be reduced to 53% +/- 12% [29].

In the setting of the sequestration-associated SWI abnormalities and intact large venous drainage systems (i. e. no venous thrombosis), the T2/DWI findings are strongly suggestive of a venous obstruction phenomenon in the capillary bed system. Much of what is known about pediatric cerebral malaria has been learned from autopsy studies, so it is reassuring to see that the distribution of microhemorrhages and parasite sequestration found in prior autopsy studies are very similar in distribution to the microhemorrhages and parasite sequestration identified in living children who survived CM.

Vasogenic edema was demonstrated by increased T2 signal in the white matter. Cytotoxic edema has a similar appearance, but is accompanied by restricted water motion identified by increased DWI signal. Both were evident in this cohort, with cytotoxic being more common. This tended to be significant and diffuse. None of these children died and there were no clinical sequelae at discharge, suggesting that the process is reversible, and may represent early cytotoxic edema rather than tissue infarction.

MRI findings of symmetrical cortical swelling with underlying white matter changes were seen, consistent with posterior reversible encephalopathy syndrome (PRES) and suggestive of autoregulatory dysfunction. Pediatric CM is congruent with many other clinical conditions associated with PRES. Specifically, pediatric CM generally involves a rapid neurologic deterioration, usually in the setting of seizure, followed by relativity prompt full recovery in most patients. Radiographically, brain swelling with underlying vasogenic edema associated with positive DWI findings is the hallmark of both CM and PRES [4, 16]. Autoregulatory dysfunction as a result of the primarily endothelial process associated with parasite sequestration in CM may result in vasoconstriction coupled with hypoperfusion causing vasogenic edema and associated brain swelling. This is the favored theory for the etiology of the radiographic findings seen in PRES [27].

We found no evidence of cortical cytotoxic edema and there was no radiographic evidence of gadolinium enhancement although gadolinium was clearly seen within the vessels and in circumventricular organs. Gadolinium, as a contrast agent, is chelated by a range of very small molecules (Magnevist 0. 54kDa)[30]. These agents are all hydrophobic, so they do not cross the intact BBB. At autopsy in CM, areas of sequestration show fibrinogen (340kDa)[24] leakage and ring hemorrhages which require sufficient BBB breakdown to allow a deformable, non-parasitized blood cells (7 μM) to escape. The SWI imaging in this study identified ring hemorrhages so some BBB breakdown associated with their presence must have occurred, but if there was associated gadolinium extravasation, the quantity and concentration of gadolinium was insufficient to be visually evident on MRI.

Gross BBB breakdown indicative of severe vasogenic edema was not evident in this small series of non-fatal pediatric CM.

This study is limited by the small sample size, less severe disease spectrum, and lack of a comparison group. In Zambia, children felt to be at risk of imminent death were not imaged since transport for imaging there requires ambulance transportation to an adjacent facility. The small number of subjects prevented meaningful quantitative analyses despite the use of NeuroInterp. Although no a prior analyses were planned, we did conducted a post-hoc comparison to determine if the edema score or the presence of SWI, DWI, or focal cortical abnormalities was associated with age, coma duration prior to admission or the seizures prior to admission. No associations were found (all p’s > 0. 05). The absence of subjects with severe brain swelling or fatal disease may have impacted our findings, as florid BBB breakdown might not occur to a significant degree in less severe CM. Normal MRIs on a similar aged comparison group were not available. In the Zambian setting, most imaging is obtained on advanced disease with normal images being uncommon. Acquisition of imaging in an age-comparable group of healthy children was not feasible given the risk of sedation, particularly in this environment. Finally, more quantitative MRI analyses would have allowed more optimal assessments, but the power injections equipment required to obtain perfusion studies and/or dynamic contrast enhanced studies, which could detect contrast influx too small to be visually evident, is prohibitively expensive and was not available in this resource limited setting.

Conclusions:

Pediatric CM brain MRI findings in non-fatal cases using 1. 5T technology suggest that vascular congestion, autoregulatory dysfunction, and microhemorrhages likely contribute to brain swelling pathogenesis.

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